

# The EBMT Handbook

Hematopoietic Cell  
Transplantation and Cellular  
Therapies

Anna Sureda  
Selim Corbacioglu  
Raffaella Greco  
Nicolaus Kröger  
Enric Carreras  
*Editors*



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Anna Sureda • Selim Corbacioglu  
Raffaella Greco • Nicolaus Kröger  
Enric Carreras  
Editors

Isabel Sánchez-Ortega  
Project Leader

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Hematopoietic Cell Transplantation  
and Cellular Therapies



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EBMT

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## Preface

Hematopoietic cell transplantation (HCT) has become a well-established life-saving treatment procedure for many patients with hematological malignancies, inborn errors, or bone marrow failure syndromes. Starting more than 60 years ago as an “ultima ratio” option, HCT is now integrated as an essential part in many treatment concepts and protocols. The rapid evolution in this exciting field of medicine with innovative treatment concepts and the increasing number of long-term survivors require a continuous education of physicians, nurses, and healthcare providers who are involved in HCT and cellular therapies.

This thoroughly revised second edition of *the EBMT Handbook* with its new format is a major part of a broader educational strategy of the EBMT. This Handbook addresses the most recent developments and innovations in HCT and cellular therapy, presented by more than 200 authors, known as experts and well-recognized authorities in the field. In 94 chapters, all types of hematopoietic cell and bone marrow transplantation including haplo-identical and cord blood transplantation, indication for transplantation and management of complications as well as the new rapidly evolving field of cellular therapies are covered. Other important issues such as quality management and JACIE accreditation, stem cell collection, conditioning, donor selection, HLA typing, graft manipulation, ethical issues, psychological support, and quality of life are also thoroughly addressed.

The aim of this Handbook is, as the name implies, not an in-depth knowledge base like a textbook but rather a hands-on and concise source of information at bedside to present the state of the art and improve our practice skills. Major key points are summarized at the end of each chapter for a quick reference.

The EBMT Board wants to express their great gratitude to the strong effort of the Working Party chairs and all authors in planning and writing the chapters and the tremendous work of the project leader Isabel Sánchez-Ortega and

the secretarial work of the EBMT executive office in Barcelona but also to Karthik Periyasamy from Springer for the continuous support.

On behalf of the EBMT board, we hope this *EBMT Handbook* will be of help in your daily practice.

Barcelona, Spain

Regensburg, Germany

Milan, Italy

Catalunya, Barcelona, Spain

Hamburg, Germany

Anna Sureda

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### About the Editors



**Anna Sureda, MD, PhD** is nowadays the Head of the Clinical Hematology Department of Institut Català d'Oncologia—Hospitalet, Barcelona and Associate Professor of the University of Barcelona. She had previously been a Senior Consultant in Hospital de la Santa Creu i Sant Pau, Barcelona (from January 1991 to December 2010) and a Senior Consultant focused in lymphomas and hematopoietic cell transplantation (HCT) at Addenbrookes—Cambridge University Hospital, UK (December 2010–December 2012). Anna Sureda has

focused her career on clinical investigations into the treatment of Hodgkin's lymphoma, non-Hodgkin's lymphoma, and multiple myeloma patients evaluating novel therapies such as immunotherapy combined with HCT. Anna Sureda was appointed Chairperson of the Lymphoma Working Party (LWP) of the European Group for Blood and Marrow Transplantation (EBMT) from March 2004 to March 2010 and Secretary of the same organization from March 2010 to March 2016. She was elected co-chair of the Lymphoma Committee of the Center for International Blood and Marrow Transplant Research (CIBMTR) and has served the organization in this position from February 2015 to February 2019. She was subsequently appointed as a member of a large non-US Transplant Center in the Advisory Committee of the CIBMTR (from February 2019). Anna Sureda is President of the Spanish Society of Hematopoietic Stem Cell Transplantation and Cellular Therapy (GETH-TC) and, from March 2022, President of the EBMT. Anna Sureda is a regular reviewer of several peer-reviewed journals (*Blood*, *Annals of Oncology*, *Bone Marrow Transplantation*, *The Hematology Journal*, *The European Journal of Hematology* y *Annals of Hematology*) and has co-authored more than 400 manuscripts.



**Selim Corbacioglu** is Professor and Chair of the Department of Hematology, Oncology and Stem Cell Transplantation at the Children's Hospital in Regensburg, Germany. His major research interest is focused on curative options for hemoglobinopathies and transplant-related systemic endothelial complications. As the PI of several multicenter interventional trials in children, he is the PI for a prospective international trial to evaluate haploidentical HCT in sickle cell disease. He was also one of the lead investigators of the Crisp/Cas9-based gene editing trial in sickle cell disease and thalassemia, where this new approach was successfully applied first-in-man in a patient with thalassemia in his institution. The recipient of the Van Bekkum Award of the EMBT in 2010 for his work on defibrotide prophylaxis in children post-transplant is

author of numerous peer-reviewed articles published in the *New England Journal of Medicine*, *Lancet*, *Blood*, *Leukemia* and *Bone Marrow Transplantation* among others. During his term as the scientific council chair and the Chair of the Pediatric Disease Working Party of the EBMT, one of his major contributions was the inauguration of the Hemoglobinopathy Working Party (HGP) of the EBMT and the Dietrich-Niethammer Award for outstanding scientific achievements in pediatric HCT.



**Raffaella Greco** Senior Physician in the Blood and Marrow Transplant (BMT) Unit of the IRCCS San Raffaele Hospital in Milano, Italy. Hematologist involved in HCT and cellular therapies in all spectrum of hematological cancers and non-malignant indications. Her expertise in this field encompasses allogeneic and autologous HCT (for malignant and non-malignant diseases), cellular therapies (CAR-T cells, Treg cell-based therapies), immune reconstitution, biomarkers, transplant complications (i.e., graft-versus-host disease, infections). Her career has been focused on several clinical research projects on HCT. She has (co-)authored many research articles in peer-reviewed journals as well as reviews, book chapters, and best-practice guidelines in the field. She has been significantly involved with the EBMT, as Working

Party Chair of the Autoimmune Diseases Working Party (ADWP), Scientific Council Representative with the Education Portfolio, active member of the Cellular Therapy and Immunobiology Working Party (CTIWP), co-chair of the Harmonization Committee.



**Enric Carreras** Degree in Medicine and Surgery (1975), becoming specialist in Internal Medicine and Hematology-Hemotherapy, and Doctor of Medicine (1984) in the University of Barcelona. From 1993 to 2010, he was Director of the HCT Program at the Hospital Clínic in Barcelona and, from 2010 to 2022, Director of the Spanish Bone Marrow Donors Registry at the Josep Carreras Foundation in Barcelona, Spain. During these years, his main clinical/research fields of interest have

been focused in early complications and endothelial dysfunction after HCT, HCT in autoimmune diseases, and at-home HCT. He is also founder of the Barcelona Endothelium Team at the Hospital Clínic campus of the Josep Carreras Leukemia Research Institute and the author of more than 400 peer-reviewed international publications mainly focused on HCT. As a member of the EBMT since 1980, he participated actively in the Chronic Leukemia Working Party, ADWP, and Late Complications Working Party. He was Chair of the EBMT/ESH training course in 12 editions and Chair of the EBMT Educational Committee. He is also editor of more than 12 books focused on hematology and HCT, including the four last editions of the EBMT Handbook. In 2018, he was nominated Honorary Member of the EBMT. In 2021, he was awarded with the IACH H.J. Khoury Prize.



**Nicolaus Kröger** is Professor of Medicine and Medical Director of the Department of Stem Cell Transplantation at the University Medical Center Hamburg-Eppendorf, Germany. He was the President of the EBMT from 2018 to 2022 and Chairman of the German Stem Cell Working Group (DAG-KBT) and Past Chair of the Chronic Malignancies Working Party and of the Scientific Council of EBMT. He is also member of the Scientific Program

Committee and the Editorial Board of the European Hematology Association (EHA) and member of the Scientific Committee of the European School for Hematology (ESH). His research interests are on stem cell biology and stem cell transplantation, the detection and treatment of minimal residual disease by adoptive immunotherapy or novel drugs, the



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impact of NK-cell alloreactivity, optimizing the outcome with HLA-mismatched donor, improving conditioning regimen, and prevention and treatment of acute and chronic graft-versus-host disease. For his research, he received several awards including the prestigious EBMT Van Bekkum Award in 2015. Prof. Kröger has published extensively in his area of expertise and has contributed to more than 850 publications in peer-reviewed journals such as *The New England Journal of Medicine (NEJM)*, *The Lancet*, *Journal of Clinical Oncology (JCO)*, *Journal of the National Cancer Institute (JNCI)*, *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, *Blood*, and *Leukemia* among others.

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## Abbreviations

AA	Aplastic anemia
Ab	Antibody
ADA	Adenosine deaminase
ADR	Adriamycin
ADV	Adenovirus
Ag	Antigen
aGVHD	Acute graft-versus-host disease
AID	Autoimmune disease
AIHA	Autoimmune hemolytic anemia
AKI	Acute kidney injury
AL	Amyloid light-chain
ALEM	Alemtuzumab
ALG	Antilymphocyte globulin
ALL	Acute lymphoblastic leukemia
Allo-BMT	Allogeneic BMT
Allo-HCT	Allogeneic HCT
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
APL	Acute promyelocytic leukemia
Ara-C	Cytosine arabinoside
ARDS	Acute or adult respiratory distress syndrome
ASBMT	American Society for Blood and Marrow Transplantation
ATG	Antithymocyte globulin
ATRA	All-trans-retinoic acid
AUC	Area under the curve
Auto-BMT	Autologous BMT
Auto-HCT	Autologous HCT
BAL	Bronchoalveolar lavage
BCNU	1,3-Bis(2-chloroethyl)-1-nitrosourea (carmustine)
BM	Bone marrow
BMDW	Bone Marrow Donors Worldwide
BMF	Bone marrow failure
BMI	Body mass index
BMT	Bone marrow transplantation
BO	Bronchiolitis obliterans
BOOP	Bronchiolitis obliterans organizing pneumonia
BOR	Bortezomib

BOS	Bronchiolitis obliterans syndrome
BSA	Body surface area
BU	Busulfan
BUN	Blood urea nitrogen
BW	Body weight
CAR	Chimeric antigen receptor
CB	Cord blood
CBT	Cord blood transplantation
CBU	Cord blood unit
CC	Complete chimerism
CCI	Charlson Comorbidity Index
CFU	Colony-forming unit
CGD	Chronic granulomatous disease
cGVHD	Chronic graft-versus-host disease
CHF	Congestive heart failure
CI	Comorbidity index
CIBMTR	Center for International Blood and Marrow Transplant Research
CKD	Chronic kidney disease
CLL	Chronic lymphoid/lymphocytic leukemia
CML	Chronic myeloid/myelogenous leukemia
CMML	Chronic myelomonocytic leukemia
CMV	Cytomegalovirus
CMV-IP	CMV-associated interstitial pneumonia
CNI	Calcineurin inhibitor
CNS	Central nervous system
COP	Cryptogenic organizing pneumonia
CR	Complete remission
CR1	First complete remission
CRS	Cytokine release syndrome
CSA	Cyclosporine A
CSF	Cerebrospinal fluid
CT	Computed tomography
CTN	Clinical Trials Network
CVC	Central venous catheter
CVD	Cardiovascular disease
CY	Cyclophosphamide
d	Days
DAH	Diffuse alveolar hemorrhage
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DEX	Dexamethasone
DFS	Disease-free survival
DIC	Disseminated intravascular coagulation
DLBCL	Diffuse large B-cell lymphoma
DLCL	Diffuse large cell lymphoma
DLCO	Diffusion capacity of the lung for carbon monoxide
DLI	Donor lymphocyte infusion

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DLT	Dose-limiting toxicity
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSA	Donor-specific antibody
EBMT	European Society for Blood and Marrow Transplantation
EBNA	Epstein–Barr (virus) nuclear antigen
EBV	Epstein–Barr virus
ECG	Electrocardiogram
ECIL	European Conference on Infections in Leukemia
ECP	Extracorporeal photopheresis
EEG	Electroencephalogram
EFS	Event-free survival
ELISA	Enzyme-linked immunosorbent assay
ELN	European LeukemiaNet
EN	Enteral nutrition
EORTC	European Organisation for Research and Treatment of Cancer
EPO	Erythropoietin
ET	Essential thrombocythemia
EWOG	European Working Group
FA	Fanconi anemia
FACS	Fluorescence-activated cell sorter
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 s
FFP	Fresh frozen plasma
FFS	Failure-free survival
FISH	Fluorescence in situ hybridization
FL	Follicular lymphoma
FLIPI	Follicular Lymphoma International Prognostic Index
FLU	Fludarabine
FVC	Forced vital capacity
G-CSF	Granulocyte colony-stimulating factor
GF	Graft failure
GFR	Glomerular filtration rate
GI	Gastrointestinal (tract)
GM	Galactomannan
GM-CSF	Granulocyte–macrophage colony stimulated factor
GNB	Gram-negative bacilli
GVH	Graft-versus-host
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
h	Hours
HAART	Highly active antiretroviral therapy
HADS	Hospital Anxiety and Depression Scale
hATG	Horse ATG
HAV	Hepatitis A virus
HBV	Hepatitis B virus

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HC	Hemorrhagic cystitis
HCT	Hematopoietic cell transplantation
HCT-CI	HCT-Comorbidity Index
HCV	Hepatitis C virus
HDAC	High-dose Ara-C
HDT	High-dose therapy
HEPA	High-efficiency particulate air
HEV	Hepatitis E virus
HHV	Human herpesvirus
HIB	Haemophilus influenzae type B
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
HPV	Human papillomavirus
HR	Hazard ratio
HRCT	High-resolution chest tomography
HRT	Hormone replacement therapy
HSC	Hematopoietic stem cell
HSV	Herpes simplex virus
HTLV	Human T-cell lymphotropic virus
HU	Hydroxyurea
HUS	Hemolytic uremic syndrome
HVG	Host-versus-graft
IA	Invasive aspergillosis
IBW	Ideal body weight
ICU	Intensive care unit
IDM	Infectious disease markers
IFI	Invasive fungal infection
IFN	Interferon
Ig	Immunoglobulin
IgG	Immunoglobulin G
IL	Interleukin
IMiD	Immunomodulatory drug
IND	Investigational new drug
INR	International normalized ratio
IP	Interstitial pneumonia
IPI	International Prognostic Index
IPS	Idiopathic pneumonia syndrome
IPSS	International Prognostic Scoring System
IRB	Institutional Review Board
IS	Immunosuppressive
IST	Immunosuppressive therapy
ITT	Intent-to-treat
IV	Intravenous
IVIg	Intravenous immunoglobulin
JACIE	Joint Accreditation Committee of ISCT-Europe and EBMT
JCV	JC virus

JMML	Juvenile myelomonocytic leukemia
KIR	Killer immunoglobulin-like receptor
KM	Kaplan–Meier
KPS	Karnofsky Performance Score
L-asp	L-asparaginase
LAF	Laminar air flow
LBL	Lymphoblastic lymphoma
LDH	Lactate dehydrogenase
LENA	Lenalidomide
LFS	Leukemia-free survival
LN	Lymph node
LPS	Lipopolysaccharide
LVEF	Left ventricular ejection fraction
M protein	Monoclonal protein
MA	Myeloablative
MAC	Myeloablative conditioning
MCL	Mantle cell lymphoma
MDS	Myelodysplastic syndrome
MEL	Melphalan
Mesna	Sodium 2-mercaptoethanesulfonate
methylPRD	Methylprednisolone
MF	Myelofibrosis
MFD	Matched family donor
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MIC	Minimum inhibitory concentration
min	Minutes
MIPI	Mantle Cell Prognostic Index
miRNA	Micro-RNA
MLC	Mixed leukocyte culture
MM	Multiple myeloma
MMF	Mycophenolate mofetil
MMRD	Mismatched related donor
MMSD	Mismatched sibling donor
MMUD	Mismatched unrelated donor
MoAb	Monoclonal antibody
MODS	Multiple-organ dysfunction syndrome
MOF	Multiorgan failure
MPN	Myeloproliferative neoplasm
MRC	Medical Research Council
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
MSD	Matched sibling donor
MTX	Methotrexate
MUD	Matched unrelated donor

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NAT	Nucleic acid amplification test
NC	Nucleated cell
NCI	National Cancer Institute
NGS	Next-generation sequencing
NHL	Non-Hodgkin lymphoma
NIH	National Institutes of Health
NIMA	Non-inherited maternal antigen
NK	Natural killer (cell)
NMA	Non-myeloablative
NMDP	National Marrow Donor Program
NRM	Non-relapse mortality
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
OS	Overall survival
PAM	Pretransplant assessment of mortality
PB	Peripheral blood
PBSC	Peripheral blood stem cell
PBHCT	Peripheral blood HCT
PCR	Polymerase chain reaction
PERDS	Peri-engraftment respiratory distress syndrome
PET	Positron emission tomography
PFS	Progression-free survival
PFT	Pulmonary function test
Ph	Philadelphia (chromosome)
PHQ-9	Patient Health Questionnaire-9
PICC	Peripherally inserted central venous catheter
PID	Primary immunodeficiency disease
PJP	Pneumocystis jirovecii pneumonia
PK	Pharmacokinetic
PMF	Primary myelofibrosis
PMN	Polymorphonuclear neutrophil
PN	Parenteral nutrition
PNH	Paroxysmal nocturnal hemoglobinuria
PO	Per os
POEMS	Polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes
PR	Partial remission or partial response
PRCA	Pure red cell aplasia
PRD	Prednisone
PRES	Posterior reversible encephalopathy syndrome
PT-CY	Post-HCT cyclophosphamide
PTLD	Post-transplant lymphoproliferative disorder
PUVA	Psoralen–ultraviolet A irradiation
PV	Polycythemia vera
QLQ	Quality of Life Questionnaire
QOL	Quality of life
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
QW	Once weekly

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RA	Refractory anemia
RAEB	Refractory anemia with excess blasts
RAEB-T	Refractory anemia with excess blasts in transformation
RARS	Refractory anemia with ringed sideroblasts
RBC	Red blood cell
RCMD	Refractory cytopenia with multilineage dysplasia
RFS	Relapse-free survival
Rh	Rhesus
RI	Relapse incidence
RIC	Reduced-intensity conditioning
R/R	Relapsing/resistant
RR	Relapse rate/Relative risk
RRT	Regimen-related toxicity
RSV	Respiratory syncytial virus
RT-PCR	Real-time polymerase chain reaction
RTx	Radiotherapy
RTX	Rituximab
SAA	Severe aplastic anemia
SARS	Severe acute respiratory syndrome
SC	Subcutaneous
SCD	Sickle cell disease
SCF	Stem-cell factor
SCID	Severe combined immunodeficiency syndrome
SD	Standard deviation
SE	Standard error
SIR	Sirolimus
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
SOS	Sinusoidal obstruction syndrome
SOT	Solid organ transplantation
SPECT	Single-photon emission computed tomography
SR	Standard risk
SS	Sézary syndrome
SSc	Systemic sclerosis
SSOP	Sequence-specific oligonucleotide probe
t-AML	Therapy-related acute myeloid leukemia
t-MDS	Therapy-related myelodysplastic syndrome
TA-GVHD	Transfusion-associated GVHD
TAC	Tacrolimus
TAI	Thoracoabdominal irradiation
TAM	Transplant-associated microangiopathy
TBI	Total body irradiation
TCD	T-cell depletion
TED	Thromboembolic disease
TGF- $\beta$	Transforming growth factor beta
THAL	Thalidomide
TKI	Tyrosine kinase inhibitor

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TLC	Total lung capacity
TLI	Total lymphoid irradiation
TLR	Toll-like receptor
TLS	Tumor lysis syndrome
TM	Thalassemia major
TMA	Thrombotic microangiopathy
TMP–SMX	Trimethoprim–sulfamethoxazole
TNC	Total nucleated cell
TNF	Tumor necrosis factor
TNF- $\alpha$	Tumor necrosis factor $\alpha$
TPN	Total parenteral nutrition
TRALI	Transfusion-related acute lung injury
TREC	T-cell receptor excision circles
Treg	Regulatory T (cell)
TREO	Treosulfan
TRM	Transplant-related mortality
TRT	Transplant-related toxicity
TT	N-Triethylenethiophosphoramidate, thioTEPA
TTP	Thrombotic thrombocytopenic purpura
Tx	Therapy/Treatment
UC	Umbilical cord
UCB	Umbilical cord blood
UCBT	Umbilical cord blood transplant
URD	Unrelated donor
UV	Ultraviolet
VCR	Vincristine
VEGF	Vascular endothelial growth factor
VGPR	Very good partial remission
VIN	Vinblastine
VIND	Vindesine
VINO	Vinorelbine
VOD	Veno-occlusive disease
VP	Etoposide
VRE	Vancomycin-resistant <i>Enterococcus</i>
vWF	von Willebrand factor
VZIg	Varicella zoster immune globulin
VZV	Varicella zoster virus
WBC	White blood cell
WBMT	Worldwide Network for Blood and Marrow Transplantation
WMDA	World Marrow Donor Association
Wt	Wild-type
X-ALD	X-linked adrenoleukodystrophy
ZAP-70	Zeta-associated protein



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**Part I**

**Introduction**

**Topic Leaders: Anna Sureda and Jane Apperley**



# HCT: Historical Perspective

# 1

Noa Granot and Rainer Storb

## 1.1 Introduction

HCT has evolved from a field that was declared dead in the 1960s to the amazing clinical results obtained today in the treatment of otherwise fatal blood disorders. This chapter will reflect upon how HCT has progressed from the laboratory to clinical reality.

## 1.2 Early Enthusiasm and Disappointment

Research efforts on how to repair radiation effects resulted from observations on radiation damage among survivors of the atomic bomb explosions in Japan (reviewed in van Bekkum and de Vries 1967). In 1949, Jacobson and colleagues discovered protection of mice from TBI by shielding their spleens with lead. Two years later, Lorenz and colleagues reported radiation protection of mice and guinea pigs by infusing marrow cells. Initially, many investigators, including Jacobson, thought that the radiation protection was from some humoral

factor(s) in spleen or marrow. However, by the mid-1950s, this “humoral hypothesis” was firmly rejected, and several laboratories convincingly demonstrated that the radiation protection was due to seeding of the marrow by donor cells.

This discovery was greeted with enthusiasm because of the implications for cell biology and for therapy of patients with life-threatening blood disorders. The principle of HCT was simple: high-dose radiation/chemotherapy would both destroy the diseased marrow and suppress the patient’s immune cells for a donor graft to be accepted. Within 1 year of the pivotal rodent studies, Thomas and colleagues showed that marrow could safely be infused into leukemia patients and engraft, even though, in the end, the leukemia relapsed. In 1958, Mathé’s group attempted the rescue, by marrow transplantation, of six nuclear reactor workers accidentally exposed to TBI. Four of the six survived, although donor cells persisted only transiently. In 1965, Mathé and colleagues treated a leukemia patient with TBI and then marrows from six relatives, absent any knowledge of histocompatibility (Mathé et al. 1965). A brother’s marrow engrafted. The patient went into remission but eventually succumbed to complications from GVHD. Following up on early observations by Barnes and Loutit in mice, Mathé coined the term “graft-vs.-leukemia effect.” In 1970, Bortin summarized results of 200 human marrow grafts reported between 1957 and 1967 (Bortin 1970). All 200 patients died of

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either graft failure, GVHD, infections, or recurrence of leukemia.

These transplants were performed before a clear understanding of conditioning regimens, histocompatibility matching, and control of GVHD. They were based directly on work in inbred mice, for which histocompatibility matching is not absolutely required. In 1967, van Bekkum and de Vries stated, "These failures have occurred mainly because the clinical applications were undertaken too soon, most of them before even the minimum basic knowledge required to bridge the gap between mouse and patient had been obtained." Clinical HCT was declared a total failure and prominent immunologists pronounced that the barrier between individuals could never be crossed.

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### 1.3 Back to the Laboratory: Focus on Animal Studies

Most investigators left the field, pronouncing it a dead end. However, a few laboratories continued animal studies aimed at understanding and eventually overcoming the obstacles encountered in human allogeneic HCT. Van Bekkum's group in Holland used primates, George Santos at Johns Hopkins chose rats, and the Seattle group chose outbred dogs as experimental models. One reason behind using dogs was that, besides humans, only dogs combine unusual genetic diversity with a widespread, well-mixed gene pool. Also, dogs share spontaneous diseases with humans, such as non-Hodgkin lymphoma and X-linked SCID, among others. In addition to determining the best ways to administer TBI, new drugs with myeloablative or immunosuppressive qualities were introduced, including cyclophosphamide, ATG, and BU (Santos 1995). These agents improved engraftment and provided for tumor cell killing similar to TBI. Based on the mouse histocompatibility system defined 10 years earlier, *in vitro* histocompatibility typing for dogs was developed. Studies from 1968 showed that dogs given grafts from dog leukocyte antigen (DLA)-matched littermates or unrelated donors survived significantly longer than their DLA-mismatched

counterparts, even though typing techniques were very primitive and the complexity of the genetic region coding for major antigens was far from understood (Epstein et al. 1968). Serious GVHD was first described in H-2 mismatched mice and in randomly selected monkeys. However, the canine studies first drew attention to fatal GVHD across minor histocompatibility barriers.

These pivotal observations drove the search for posttransplant drug regimens to control GVHD. The most promising drug was the folic acid antagonist, MTX (Storb et al. 1970). Further work in canines showed that transfusion-induced sensitization to minor antigens caused rejection of DLA-identical grafts (reviewed in Georges and Storb 2016). Subsequent canine studies eventually led to ways of understanding, preventing, and overcoming transfusion-induced sensitization, especially in patients with SAA. Next, mechanisms of graft-host tolerance were investigated. It turned out that IS could often be discontinued after 3–6 months, and donor-derived T lymphocytes were identified that downregulated immune reactions of other donor T cells against GVHD targets. Immune reconstitution was found to be complete in long-term canine chimeras, enabling them to live in an unprotected environment. Techniques for isolating transplantable stem cells from peripheral blood were refined in dogs and primates. Importantly, studies in pet dogs with non-Hodgkin lymphoma showed cures, in part due to graft-vs.-tumor effects.

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### 1.4 Resuming Clinical Transplantation: 1968–1980s

The second half of the 1960s saw the refinement of high-intensity conditioning regimens, including fractionated TBI and maximally tolerated doses of CY or BU (Santos 1995). Histocompatibility matching was confirmed to be of utmost importance for reducing both graft rejection and GVHD (Thomas et al. 1975). However, even when donor and recipient were well matched, GVHD was a problem unless

post-grafting MTX was given, which slowed donor lymphocyte replication. Rapid progress in understanding the molecular nature of the major human histocompatibility complex—HLA—improved matching of donor recipient pairs.

By 1968, the stage was set to resume clinical trials. The first successful transplants were for patients with primary immune deficiency disorders. A 5-month-old boy with “thymic aplasia and agammaglobulinemia” was not perfectly matched with his sister (Gatti et al. 1968). Marrow and peripheral blood cells were infused intraperitoneally without conditioning. After a booster infusion several months later, the patient fully recovered with donor hematopoiesis and is well. A patient with Wiskott-Aldrich syndrome received a first unsuccessful marrow infusion from an HLA-identical sister without conditioning (Bach et al. 1968). A second transplant following CY conditioning resulted in full T- and B-cell recovery, but thrombocytopenia persisted.

During the first 7 or 8 years, most clinical studies were for patients with advanced hematological malignancies and SAA, who were in poor condition and presented tremendous challenges in supportive care (Thomas et al. 1975). They required transfusions and prophylaxis or treatment of bacterial, fungal, and viral infections. Therefore, in addition to discoveries made in marrow transplantation, these early trials stimulated advances in infectious diseases and transfusions (reviewed in Forman et al. 2016). The longest survivors from that era are patients with SAA who are approaching their 52nd anniversary from HCT with fully recovered donor-derived hematopoiesis and leading normal lives. Chronic GVHD emerged as a new problem among long-term survivors.

The initial studies saw GVHD among approximately half of the patients, despite HLA matching and despite receiving methotrexate. This stimulated further research in the canine system. Major improvements in GVHD control and patient survival were made when combining MTX with CNI inhibitors such as CSA or TAC (Storb et al. 1986). Combinations of drugs have

remained a mainstay in GVHD prevention. GVHD treatment with PRD was introduced.

Early results with marrow grafts from HLA-identical siblings after CY for SAA showed 45% long-term survival (reviewed in Georges and Storb 2016). The major cause of failure was graft rejection as expected from canine studies on transfusion-induced sensitization to minor antigens. Canine studies identified dendritic cells in transfusions to be the key element in sensitization. Depleting transfusions of white cells, therefore, reduced the rejection risk. Further canine studies generated a clinical conditioning regimen that alternated CY and ATG, which greatly reduced the rates of both graft rejection and chronic GVHD (Storb et al. 1994). Finally, irradiation of blood products with 2000 cGy in vitro almost completely averted sensitization to minor antigens. Consequently, graft rejection in transplantation for AA has become the exception, and current survivals with HLA-identical sibling and HLA-matched unrelated grafts range from 90% to 100%. First successful grafts for thalassemia (Thomas et al. 1982) and sickle cell disease were reported.

For patients with leukemia and other malignant blood diseases, disease relapse after HCT has remained a major problem. Attempts to reduce relapse by increasing the intensity of systemic conditioning regimens have met with success, but this benefit was offset by higher non-relapse mortality. Reports by Weiden and the Seattle group in 1979/1981 firmly established the existence of graft-vs.-leukemia (GvL) effects in humans (Weiden et al. 1979). DLI to combat relapse were introduced by Kolb and colleagues in 1990 (Kolb et al. 1990) (see Chap. 59).

Some investigators have removed T cells from the marrow as a means of preventing GVHD (reviewed in Soiffer 2016). Early studies showed high incidences of graft rejection, relapse of underlying malignancies, and infections. More recent studies showed that relapse seemed a lesser problem in patients with acute leukemia. Others have used T-cell depletion with close disease monitoring and treating recurrence with DLI in hopes of initiating GvL responses without causing GVHD. Most recently, younger patients

have been given high-intensity conditioning for grafts which were depleted of naïve T cells with a resulting decrease in GVHD (Bleakley et al. 2015).

The late 1980s saw the introduction of G-CSF-mobilized PBSC (reviewed in Schmitz and Dreger 2016). These were equivalent to marrow as far as engraftment and survival were concerned; however, they seemed to increase the risk of chronic GVHD. For patients with nonmalignant diseases, marrow has therefore remained the preferred source of stem cells in order to keep the rate of chronic GVHD low.

Only approximately 35% of patients have HLA-identical siblings. Therefore, alternative donors have been explored, predominantly HLA-matched unrelated volunteers. The first successful unrelated transplant for leukemia was reported in 1980. In order to expand the donor pool, national registries were established, with currently more than 40 million HLA-typed unrelated volunteers (reviewed in Confer et al. 2016). The likelihood of finding suitable unrelated donors is approximately 80% for Caucasians, although this percentage declines dramatically for patients from minority groups. A second, important alternative stem cell source has been unrelated cord blood (Gluckman et al. 1989), not requiring complete HLA matching and resulting in encouraging outcomes among patients with malignant blood diseases. First attempts with yet another donor source have included TCD megadose CD34+ cell grafts from related HLA-haploidentical donors to treat acute leukemia (Aversa et al. 1998).

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## 1.5 Moving Ahead: The 1990s and Beyond

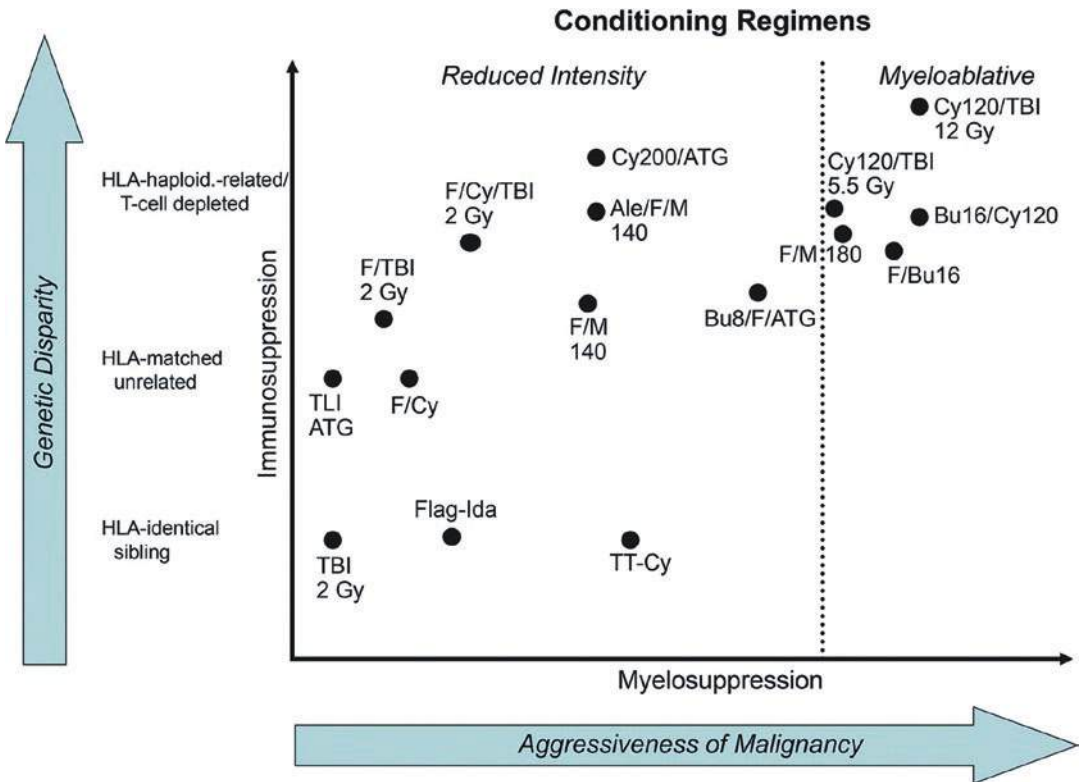
To allow the inclusion of older (highest prevalence of hematological malignancies), medically infirm, or very young immunodeficiency patients, less intensive conditioning programs have been developed. In patients with malignancies, these rely less on high-dose chemoradiation therapy and more on graft-vs.-tumor effects. One outpatient transplant strategy combines FLU and

2–3 Gy TBI conditioning with posttransplant IS using an inhibitor of purine synthesis MMF and CSA or TAC. Figure 1.1 illustrates the spectrum of current conditioning regimens (reviewed in Storb and Sandmaier 2016). High-intensity regimens carry the risk of short- and long-term toxicities, the latter including subsequent malignancies (Baker et al. 2019). The associated short-term toxicities restrict the therapy to younger, medically fit patients (as reviewed in Granot and Storb 2020). One outpatient transplant strategy combines FLU and 2–3 Gy TBI conditioning with posttransplant IS using an inhibitor of purine synthesis, MMF, combined with either CSA or TAC. Figure 1.1 illustrates the spectrum of current conditioning regimens (reviewed in Storb and Sandmaier 2016).

A transplant regimen combining fludarabine and 2 Gy TBI conditioning with additional cyclophosphamide before and after HCT has encouraged widespread use of unmodified HLA-haploidentical grafts (Luznik et al. 2008). It is well tolerated with low incidences of graft rejection and of acute and chronic GVHD, but relapse remains a problem. Strategies addressing relapse have included infusion of donor lymphocytes or NK cells. Retrospective multicenter analyses show comparable outcomes after HLA-matched vs. HLA-haploidentical HCT.

While reduced-intensity regimens have been well tolerated, relapse and GVHD need improving. Adding targeted radioimmunotherapy against host hematopoietic cells, using anti-CD45 or anti-CD20 antibody coupled to beta- and alpha-emitting radionuclides to minimal-intensity conditioning, has the potential to decrease the pretransplant tumor burden, thereby lessening the relapse risk (Mawad et al. 2014; O'Steen et al. 2019; Chen et al. 2012; Pagel et al. 2009). Encouraging results with maintenance therapy after HCT have been reported in AML patients treated with sorafenib (Xuan et al. 2020) and bortezomib in high-risk MM (Green et al. 2017).

As for GVHD prevention, emerging therapies target alloreactive T cells, alloreactive and autoreactive B cells through direct depletion from stem cell grafts (e.g., posttransplant Cy is in vivo



**Fig. 1.1** Spectrum of current conditioning regimens. (Reproduced with permission from Sandmaier, B.M. and Storb, R. Reduced-intensity allogeneic transplantation regimens (Ch. 21). In Thomas’ Hematopoietic Cell

Transplantation, fifth edition (ed. by Forman SJ, Negrin RS, Antin JH, & Appelbaum FR) 2016, pp. 232–243. John Wiley & Sons, Ltd., Chichester, UK)

depletion, posttransplantation cyclophosphamide, CD34 selection, IL-2 and IL-17 therapy), in vivo depletion (e.g., rituximab, ofatumumab, obinutuzumab), and signal inhibition (e.g., ITK, JAK 1/2, ROCK-II, BTK, SYK inhibition), as reviewed in depth by Cutler et al. (2017) and MacDonald et al. (2017).

As for GVHD, a recent phase III randomized trial convincingly demonstrated that a triple combination of MMF/cyclosporine/sirolimus significantly reduced both acute GVHD and non-relapse mortality and improved survival (Sandmaier et al. 2016).

Survival of patients with primary immune deficiency diseases given NMA conditioning before HLA-matched and HLA-mismatched grafts between 1998 and 2006 has stabilized at 82% (Moratto et al. 2011).

In the future, better understanding of hematopoietic cell-specific polymorphic minor histocompatibility antigens might result in ways of directing donor immune cells toward hematopoietic targets, thereby controlling relapse without inducing GVHD. Another major research target is containment of chronic GVHD.

**Key Points**

- Radiation protection of rodents by shielding the spleen or marrow infusion.
- First human transplants all failed.
- Allogeneic HCT called a total failure.
- HCT studies in large animals: histocompatibility matching; MTX for GVHD



prevention; CY, ATG, and BU; rejection from transfusion-induced sensitization; PBSC; graft-vs.-lymphoma effect.

- Fractionated TBI.
- HCT for patients with immunodeficiency diseases, aplastic anemia, leukemia, hemoglobinopathies.
- Advances in infection prophylaxis and treatment.
- Graft-vs.-leukemia effects.
- Donor lymphocyte infusions.
- ATG conditioning.
- Unrelated donors.
- Cord blood transplants.
- Mega CD34+ HLA-haploidentical grafts.
- MTX/CNI GVHD prophylaxis.
- Reduced and minimal-intensity conditioning.
- Outpatient transplantation.
- PT-CY GVHD prophylaxis.
- Targeted radioimmunotherapy.

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# The EBMT: History, Present, and Future

# 2

Anna Sureda, Nicolaus Kröger, Jane Apperley, and Alois Gratwohl

## 2.1 Introduction

“Only he/she who knows the past has a future” is a proverb attributed to Wilhelm von Humboldt (1767–1835), a great historian, scientist, and philosopher (Spier 2015). It appears as an ideal introduction to a chapter on the history of EBMT. The context by which HCT evolved in the middle of the last century fits with modern views on history. The novel “big history” concept attempts to integrate major events in the past, beginning with the “big bang” up to today’s industrial revolution number IV (Spier 2015). According to this model, nothing “just happens.” Progress occurs when the conditions fit, at the right time and at the right place. Such circumstances are called

“Goldilocks conditions,” according to the novel by Robert Southey ([https://en.wikipedia.org/wiki/Goldilocks\\_and\\_the\\_Three\\_Bears](https://en.wikipedia.org/wiki/Goldilocks_and_the_Three_Bears). Accessed November 6, 2018). They hold true for the formation of galaxies, suns, and planets, for the appearance of life on earth, or for the evolution of mankind. They apply specifically to the latter: as the one and only species, *Homo sapiens* managed to create “Goldilocks conditions” by him- or herself. They allowed man to fit religion, art, or beliefs in such ways to master society. In our perspective, big history thinking helps to understand the development of HCT and EBMT and to view it in a broader framework. It provides as well a caveat for the future.

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## 2.2 The Past: Development of HCT and EBMT

The use of bone marrow (BM) for healing purposes dates back long in history, and BM from hunted animals might have contributed as rich nourishment to the evolution of *Homo sapiens* (McCann 2016). Its recognition as a primary hematopoietic organ in adult life with a hematopoietic stem cell as source of the circulating blood cells began in the middle of the nineteenth century (Schinck 1920). It did result in some early recommendations on the potential therapeutic use of BM (JAMA 1908; Osgood et al. 1939), but with no broader application. All

changed after the explosions of atomic bombs in Hiroshima and Nagasaki in World War II, when survivors of the immediate exposure died from BM failure (Van Bekkum and De Vries 1967). Research was directed to find ways to treat this lethal complication. It led to the discovery that BM-derived stem cells from a healthy donor could replace hematopoiesis after total body irradiation (TBI); it provided at the same time, a tool, TBI, to eradicate aberrant hematopoiesis (Van Bekkum and De Vries 1967; Jacobson et al. 1949; Lorenz et al. 1951; Ford et al. 1956). The concept of HCT was born, and “the conditions were right.” It is to no surprise that the first clinical BMT centers in Europe started in hospitals with close links to radiobiology research institutes in the UK, the Netherlands, France, and Germany. Funding of radiobiology fostered basic research and stimulated clinical application. In the first series of patients reported in the NEJM in 1957 by the late Nobel Prize winner ED Thomas, all six patients died but two of them with clear signs of donor chimerism (Thomas et al. 1957). And BMT “saved” accidentally irradiated workers of a radiation facility in Vinca, a town in former Yugoslavia (Mathé et al. 1959). Hence, the clinical results confirmed the “proof of principle” obtained in mice: TBI could eradicate normal and malignant BM cells, and the infusion of healthy donor BM cells could restore the recipient’s depleted hematopoiesis with functioning donor cells. In reality, of more than 200 patients reported by Bortin for the IBMTR, all patients with leukemia had died, many of them free of their disease. Three patients survived, all with congenital immune deficiency and transplanted from HLA-identical sibling donors (Bortin 1970). Despite the dismal results, Goldilocks conditions prevailed. Armed forces were convinced of the need for a rescue tool in the event of a nuclear war, physicians viewed BMT as an instrument to treat hitherto incurable blood disorders, and patients envisioned a cure of their lethal disease.

In order to improve outcome, the “believers” joined forces. They met each other, openly reviewed their cases and charts one by one, exchanged views on hurdles and opportunities,

spent time together on the slopes in the Alps, and became friendly rivals: EBMT was born. Goldilocks conditions still prevailed. Leukemia could be eradicated. BMT with haploidentical donor BM for SAA after conditioning with ATG yielded spectacular results (Speck et al. 1977). Today, we know that ATG, rather than the cells, was responsible for the outcome. The introduction of intensive induction regimens for AML enabled stable phases of complete first remission (CR1) (Crowther et al. 1970). The discovery of CSA, as the first of its kind of novel IS agents, opened new dimensions in BMT and other organ transplantation (Kay et al. 1980). It became acceptable to transplant patients in the early phase of their disease, e.g., CR1 or first chronic phase (CP1) (Thomas et al. 1975). The boom of BMT began (Thomas 2007; Gratwohl et al. 2015a). The first patient in the EBMT database dates back to 1965. In 1973, at the first informal gathering in St. Moritz, the database comprised 13 patients, 4 transplanted in that year. In 1980, a total of 285 HCT were performed, increasing to 4025 10 years later.

HCT rapidly diversified in terms of donor type, by including autologous and allogeneic stem cells from related and unrelated donors, and of stem cell source, from BM and peripheral blood to cord blood. Indications expanded from the early congenital immunodeficiency, leukemia, and aplastic anemia to a full variety of severe congenital disorders of the hematopoietic system, to other hematological malignancies such as myeloma and lymphoma, and to non-hematological malignancies, e.g., germ cell tumors. The HCT technology improved to encompass a variety of in vivo and ex vivo GvHD prevention methods and conditioning regimens of varying intensities with or without TBI. HCT became open to centers with no links to radiobiology institutes and was no longer bound to “sterile units” and to selected countries (Gratwohl et al. 2015a; Copelan 2006).

The previously informal gatherings and the database no longer sufficed to share the urgently needed information exchange. EBMT became a formal structure, with elections for presidents and working party chairs. It was listed in PubMed

for the first time in 1985 (EBMT 1985). The meetings were no longer confined to ski resorts and became open to all involved in patient care and scientific analyses (Table 2.1). Obviously,

organization of the annual meeting is today a major undertaking and only possible with the support of corporate sponsors. Still, the initial spirit remains.

**Table 2.1** List of EBMT meetings and presidents

Year	Location of annual meeting	Participating groups	EBMT president
1974			Informal gathering
1975	St. Moritz, Switzerland	1st P	
1976	St. Moritz, Switzerland	2nd P	B. Speck <sup>a</sup>
1977	Courchevel, France	3rd P	B. Speck <sup>a</sup>
1978	Courchevel, France	4th P	B. Speck <sup>a</sup>
1979	St. Moritz, Switzerland	5th P	E. Gluckman
1980	Sils-Maria, Switzerland	6th P	E. Gluckman
1981	Courchevel, France	7th P	E. Kubanek
1982	Courmayeur, Italy	8th P	E. Gordon-Smith
1983	Oberstdorf, Germany	9th P	E. Gordon-Smith
1984	Granada, Spain	10th P	J. Barrett
1985	Bad Hofgastein, Austria	11th P, 1st N	J. Barrett
1986	Courmayeur, Italy	12th P, 2nd N	A. Marmont <sup>a</sup>
1987	Interlaken, Switzerland	13th P, 3rd N	A. Marmont <sup>a</sup>
1988	Chamonix, France	14th P, 4th N	G. Gharion
1989	Bad Hofgastein, Austria	15th P, 5th N	G. Gharion
1990	The Hague, Netherlands	16th P, 6th N	J. Goldman <sup>a</sup>
1991	Cortina d'Ampezzo, Italy	17th P, 7th N	J. Goldman <sup>a</sup>
1992	Stockholm, Sweden	18th P, 8th N	J. Goldman <sup>a</sup>
1993	Garmisch-Partenkirchen, Germany	19th P, 9th N	J. Goldman <sup>a</sup>
1994	Harrogate, UK	20th P, 10th N	A. Gratwohl
1995	Davos, Switzerland	21st P, 11th N	A. Gratwohl
1996	Vienna, Austria	22nd P, 12th N	A. Gratwohl
1997	Aix-les-bains, France	23rd P, 13th N	A. Gratwohl
1998	Courmayeur, Italy	24th P, 14th N	A. Bacigalupo
1999	Hamburg, Germany	25th P, 15th N	A. Bacigalupo
2000	Innsbruck, Germany	26th P, 16th N	A. Bacigalupo
2001	Maastricht, Netherlands	27th P, 17th N	A. Bacigalupo
2002	Montreux, Switzerland	28th P, 18th N, 1st DM	J. Apperley
2003	Istanbul, Turkey	29th P, 19th N, 2nd DM	J. Apperley
2004	Barcelona, Spain	30th P, 20th N, 3d DM	J. Apperley
2005	Prague, Czech Republic	31st P, 21st N, 4th DM	J. Apperley
2006	Hamburg, Germany	32nd P, 22nd N, 5th DM	D. Niederwieser
2007	Lyon, France	33rd P, 23d N, 6th DM, 1st P&F	D. Niederwieser
2008	Florence, Italy	34th P, 24th N, 7th DM, 2nd P&F	D. Niederwieser
2009	Goteborg, Sweden	35th P, 25th N, 8th DM, 3rd P&F	D. Niederwieser
2010	Vienna, Austria	36th P, 26th N, 9th DM, 4th P&F	A. Madrigal
2011	Paris, France	37th P, 27th N, 10th DM, 5th P&F	A. Madrigal
2012	Geneva, Switzerland	38th P, 28th N, 11th DM, 6th P&F, 1st QM, 1st Ped	A. Madrigal
2013	London, UK	39th P, 29th N, 12th DM, 7th P&F, 2nd QM, 2nd Ped	A. Madrigal
2014	Milan, Italy	40th P, 30th N, 13th DM, 8th P&F, 3d QM, 3d Ped	A. Madrigal

(continued)

**Table 2.1** (continued)

Year	Location of annual meeting	Participating groups	EBMT president
2015	Istanbul, Turkey	41st P, 31st N, 14th DM, 9th P&F, 4th QM, 4th Ped	M. Mohty
2016	Valencia, Spain	42nd P, 32nd N, 15th DM, 10th P&F, 5th QM, 5thPed, 1st Pha	M. Mohty
2017	Marseille, France	43rd P, 33rd N, 16th DM, 11th P&F, 6th QM, 6th Ped, 2nd Pha, 1st Psy	M. Mohty
2018	Lisbon, Portugal	44th P, 34th N, 17th DM, 12th P&F, 7th QM, 7th Ped, 3d Pha, 2nd Psy	M. Mohty
2019	Frankfurt, Germany	45th P, 35th N, 18th DM, 13th P&F, 8th QM, 8th Ped, 4th Pha, 3rd Psy, 1st TC	N. Kröger
2020	Madrid, Spain <sup>b</sup>	46th P, 36th N, 19th DM, 14th P&F, 9th QM, 9th Ped, 5th Pha, 4th Psy, 2nd TC, 1st MSH-IC	N. Kröger
2021	Madrid, Spain <sup>b</sup>	47th P, 37th N, 20th DM, 15th P&F, 10th QM, 10th Ped, 6th Pha, 5th Psy, 3rd TC, 2nd MSH-IC, 1st LT	N. Kröger
2022	Prague, Czech Republic <sup>b</sup>	48th P, 38th N, 21st DM, 16th P&F, 14th QM, 11th CT, 11th Ped, 7th Pha, 6th Psy, 4th TC, 3rd MSH-IC, 2nd LT, 1st Trainee	N. Kröger
2023	Paris, France	49th P, 39th N, 22nd DM, 17th P&F, 15th QM, 12th CT, 12th Ped, 8th Pha, 7th Psy, 5th TC, 4th MSH-IC, 3rd LT, 2nd Trainee	A. Sureda

Participating groups: *P* physicians, *N* nurses, *DM* data manager, *P&F* patient and family day, *QM* quality manager, *Ped* pediatricians, *Pha* pharmacists, *Psy* psychologists, *TC* transplant and search coordinators, *MSH-IC* multi-stakeholder forum and innovative cellular therapies, *LT* laboratory technicians

<sup>a</sup> Deceased

<sup>b</sup> Virtual meetings

## 2.3 The Present

Today, EBMT ([www.ebmt.org](http://www.ebmt.org)) is a nonprofit organization with a clear mission statement: “To save the lives of patients with blood cancers and other life-threatening diseases by advancing the fields of blood and marrow transplantation and cell therapy worldwide through science, education and advocacy” (<https://www.ebmt.org/ebmt/what-we-do>). It is formally a professional society with legal residence in the Netherlands and an administrative office in Barcelona, Spain. EBMT is chaired by the president, who is elected by the members for 4 years with a prior year of president elect. The Board of Association is the administrative body responsible for defining the strategic direction of the EBMT and running operations and decisions that are not due to be taken by the General Assembly (GA) and it is constituted by the Executive Committee as well

as by the chair and co-chair of the Scientific Council, the education and registry representative of the Scientific Council, and the president of the Nurses Group. The Scientific Council defines the scientific and education policy of the EBMT to be approved by the General Assembly. Its members consist of the chairs of each of the 12 Working Parties (WP) and the Nurses Group President. The EBMT Committees have been established over the years in response to various needs identified by the EBMT community that span different functions of the EBMT. Their objectives are to support and advise the EBMT Board and other committees and management, as well as the Working Parties, as they carry out their activities. The strong commitment of EBMT on training and education as well as on the equality, diversity, and inclusion (ED&I) approach led to the development of two new successful committees: the trainee and the EDI committee

(Table 2.2). The operational structure of EBMT has significantly increased its complexity over time; EBMT has more than 100 employees that are physically located in four different offices: Barcelona, Paris, Leiden, and Shanghai, the latter one being opened with the development of an increasingly strong collaboration between EBMT and transplantation centers in China.

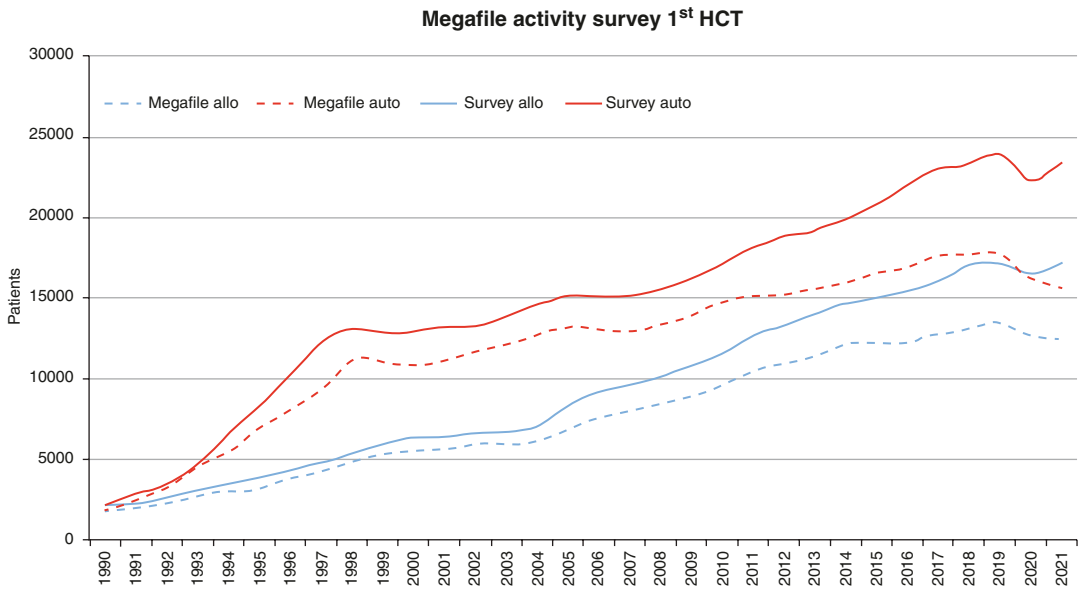
The EBMT is devoted to the promotion of all knowledge associated with the transplantation of hematopoietic cells or immunomodulatory cells from all donor sources and donor types including basic and clinical research, education, standardization, quality control, and accreditation

for transplant procedures. The EBMT registry is one of EBMT's most precious jewels. It currently contains information on more than 700,000 patients treated with HCT and more than 40,000 new patients are reported each year (Fig. 2.1). Most of the scientific production of the EBMT is based on information extracted from the registry data. At the present time and after the incorporation of other cell therapy strategies—CART cells—the EBMT registry contains information on more than 5000 patients treated with this new therapeutic option and our aim is that the information in the registry reflects the reality of the EBMT member centers in this field as much as possible. The annual congress is the most important educational activity; Paris 2023—the first face-to-face congress after 3 years of virtual life—brought together more than 4700 face-to-face delegates and more than 1100 virtual delegates. However, EBMT's educational offer has grown significantly over the years with a multitude of medium and small educational events, mostly organized by the WPs and an e-learning platform, developed already before the COVID-19 pandemic, which has allowed to keep education in our virtual life and increase the participation of groups of professionals for whom it was unreachable. Finally, the concepts of accreditation, quality, benchmarking, and advocacy constitute the third fundamental pillar of EBMT's life. JACIE has been and is one of the great triumphs of EBMT; JACIE accreditation has evolved over time and is now of paramount importance for the accreditation and qualification of CART centers. The benchmarking exercise is an ongoing project, which has already resulted in the publication of two manuscripts. EBMT's participation as a scientific society within the Common Representation of Substances of Human Origin (CoRe SoHO) ensures our representation in this specific EU area.

Members of the EBMT are mainly centers active in transplantation of hematopoietic cells or any other organization involved in the care of donors and recipients of HCT. Currently and

**Table 2.2** EBMT working parties and committees

<i>Working parties</i>	
ADWP	Autoimmune Diseases Working Party
ALWP	Acute Leukemia Working Party
CMWP	Chronic Malignancies Working Party
CTIWP	Cellular Therapy and Immunobiology Working Party
HWP	Haemoglobinopathies Working Party
IDWP	Infectious Diseases Working Party
IEWP	Inborn Errors Working Party
LWP	Lymphoma Working Party
PDWP	Paediatric Diseases Working Party
SAAWP	Severe Aplastic Anaemia Working Party
TCWP	Transplant Complications Working Party
<i>Committees</i>	
	Nuclear Accident Committee
	Donor Outcomes Committee
	Statistical Committee
	Registry Committee
	JACIE Committee
	Global Committee
	Legal and Regulatory Affairs Committee
	Pharmacist Committee
	Patient Advocacy Committee
	Trainee Committee
	Practice Harmonization Committee
	Equality, Diversity and Inclusion Committee
	Accreditation Committee
	Quality Managers Committee
	Inspectors Committee
	Nurses Group Research Committee
	Nurses Group Scientific Committee
	Nurses Group Pediatric Committee
	Nurses Group Global Committee



**Fig. 2.1** Numbers of patients with a first HCT by main donor type and year of transplant. The lines reflect the difference in patient numbers with and without information

in the database (megafile). (Courtesy: Annelot van Amerongen, EBMT Registry Unit, Leiden; Helen Baldomero, EBMT activity survey office, Basel)

after the GA of the Annual Meeting 2023, EBMT holds 6031 members in 582 full center members and 334 members in 50 associate center members, 176 members in 28 provisional center members, 256 individual members, and 35 honorary members from 58 different countries. EBMT is supported in its activities through the membership fees and the revenue of the annual meetings and by its corporate sponsors (<https://www2.ebmt.org/Contents/Members-Sponsors/Sponsors/Listofcorporatesponsors/Pages/List-of-corporate-sponsors.aspx>). EBMT is part of the global network of organizations involved in HCT, the Worldwide Network for Blood and Marrow Transplantation (WBMT), and in close link with national and other international professional organizations involved in HCT, such as AFBMT, APBMT, CIBMTR, EMBMT, LABMT, or WMDA. The GOCART coalition, with the slogan “If you want to go fast, go alone; if you want to go far, go together,” has EBMT and the European Society of Haematology (EHA) as founding partners and aims to become the umbrella that brings together the concerns and

needs of the different actors involved in cell therapy strategies. In March/2019 EBMT received the positive qualification opinion from the European Medical Association (EMA) on the use of its patient registry to support novel CART cell therapies; this was the beginning of the development of the post-authorization studies (PASS) which will enable marketing authorization holders (MAH) to provide information on the long-term and real-life efficacy and safety of the different CART constructs to European authorities.

## 2.4 The Future

Again, according to the big history concept, predicting the future is a difficult task: “There are no data about the future; from an empirical scientific point of view, it is impossible to say what lies ahead of us” (Spier 2015). But we can project scenarios; we know the past, and we see the today. We live in the rapidly evolving world of the industrial revolution IV, dominated by global-



ization, digitization, and personalized medicine. Targeted therapies promise cures; gene-modified cells destroy hitherto untreatable cancers; immunomodulation with checkpoint inhibitors has become a reality (Hochhaus et al. 2017; Tran et al. 2017; Le et al. 2015). If HCT is to remain a valuable treatment, mentalities and methods of the past no longer suffice. The idea of beliefs, hence physicians creating their own Goldilocks conditions, will lead to the end of HCT. It has to be replaced by a stringent scientific approach. The sad story of HCT for breast cancer, with more than 40,000 transplants but no clear answer, must not to be repeated (Gratwohl et al. 2010).

Hence, prediction number one: The idea of “a donor for everybody” will be abandoned. HCT has to provide for the individual patient the best outcome regarding overall survival, quality of life, and costs. The outcome after HCT must be superior, in these three aspects, to any of the modern drugs or treatments, including “watch and wait” strategies or palliation. Assessment of risks needs to integrate risk factors relating to the patient, his or her disease, the donor, the stem cell source, the transplant technology, and micro- and macroeconomic risk factors (Gratwohl et al. 2015b, 2017). For some patients, early transplant will be the optimal approach; for others, HCT may need to be delayed. For others, HCT will never be the preferred option. Obviously, the transplant physician is no longer in a position to adequately assess risk in comparison to the multiple alternative strategies, as it was possible in the old times of the simple EBMT risk score. Machine-learning algorithms will replace risk assessment; the competent physician will still be needed to discuss the results with his or her patients and their families and to conduct the transplant (Verghese et al. 2018). The introduction of CART cells as a new cell therapy strategy has represented significant changes in the treatment of some hematological malignancies; the number of both autologous and allogeneic transplants has decreased significantly in lymphoma patients (Snowden et al. 2022). New gene therapy strategies will undoubtedly also change the cur-

rent perception of transplantation in benign hematological pathology.

Hence, prediction number two: The WHO guiding principles for cell, organ, and tissue transplants, “data collection and data analysis are integral parts of the therapy,” need to become a mandatory reality for all transplant teams (WHO 2010). The gap between transplant numbers and reports (Fig. 2.1) has to be closed. Reporting has to become real time and lifelong. The EBMT and transplant centers have to adapt. Data and quality management will become a “condition sine qua non” for all, with close interactions between local, national, and international organizations. Machine learning will end the individualistic center unique transplant techniques. It will no longer be possible to apply hundreds of different GvHD prevention methods and a multitude of conditioning regimens, just by the argument “I have good experience with my method.” Standardization will permit correct personalized medicine, as outlined above. Obviously, assessment of outcome can no longer be restricted to transplanted patients; it will need the correct comparison with nontransplant strategies on a routine basis.

Hence, prediction number three: HCT centers and the EBMT will no longer be isolated in the treatment landscape. HCT will need to be integrated into the treatment chain, from diagnosis to early treatment, transplant decisions, and secondary treatment, up to lifelong follow-up. Not all of these steps have to occur at the transplant center, but they need to be coordinated by the expert team. Data have clearly shown that transplant experience, as measured in patient numbers and years, is associated with outcome (Gratwohl et al. 2015b). No center will have sufficient expertise for all diseases amenable to HCT or for all transplant techniques, e.g., BM harvest. HCT centers will have to decide on their priorities, jointly with their referral and their after-care chain, within their city, their country, or with neighboring countries for coordination.

Hence, final prediction: EBMT can take the science-based lead for coordination and stan-

standardization, guide in reorganization of networks with non-transplant treatment chains, and prioritize comparative studies, independent of pressure groups. This is the focus of EBMT's strategic plan for 2023–2026: (a) to transform science on BMT and advanced CT through collaborative platforms and translational evidence and innovation, to become the EU reference in the field with global impact; (b) transform to leverage the community's awareness and people's knowledge, skills, and attitudes with non-bias and reliable educational resources to improve standards of BMT and CT and patient outcomes across borders; and (c) as a global community, to continually improve the cell therapy delivery for all involved. Then, history will tell, whether the proverb from a contemporary of von Humboldt, Georg Wilhelm Friedrich Hegel (1770–1831) “History teaches us that man learns nothing from history” (Spier 2015), can be overcome. The potential is here.

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# Registries: A Bridge Between Donor and Recipient

# 3

Irina Evseeva, Lydia Foeken, and Juliana Villa

## 3.1 Introduction

### 3.1.1 What Is a Registry?

A registry plays a critical role in the recruitment, donation, collection, testing and distribution of cells for use in cellular therapy. As a bridge between donation and transplant, registries can serve either as donor registries, providing cell products from their donors/cord blood banks (CBBs) to national and international transplant centres, or patient registries, partnering with local transplant centres, making the search of the best cell source and requesting cell products. In most cases, registries serve in both roles.

Registries have different structures, some are governmental organizations, others are foundations, and a third category is part of a larger organization such as a hospital, blood bank or university. Some have affiliated donor or patient

management entities located in different countries. WMDA provides operational and regulatory information on each operating registry and CBB on WMDA Share (<https://share.wmda.info/x/4gdcAQ>).

### 3.1.2 What Is WMDA?

WMDA was initiated by three pioneers, Professors John M. Goldman (United Kingdom), E. Donnell Thomas (United States) and Jon J. van Rood (the Netherlands) and founded in 1994. WMDA is a merger organization of BMDW, NetCord Foundation, EMDIS and WMDA. Over 100 registries and CBBs collaborate by sharing both donor and cord blood (CB) data for international search through WMDA's Search & Match Service (WSMS) (<https://share.wmda.info/x/4gdcAQ> and <https://statistics.wmda.info/>) (Fig. 3.1).

WMDA operates a certification programme ensuring that cells are provided according to internationally agreed standards and can be imported without administrative burden into a country. In 2023, over 90% of the donors registered are assessed through WMDA's Certification Programme (<https://www.wmda.info/professionals/quality-and-accreditation/wmda-standards/>).

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## Unrelated donors and cord blood units registered in 2022 per continent



**Fig. 3.1** WMDA Global Trends Report 2022

### 3.2 The Role of the Registry: Patient Perspective

#### 3.2.1 Find the Best Suitable Graft: Search and Match Service

The choice of the best suitable cell source depends on patient's medical condition and clinical situation and is determined by patient HLA type, sex, weight, CMV status, alloantibody profile, ABO blood group and other factors that the transplant physician may find important. Transplant and search coordinators can utilize Search & Match Service, available at <https://search.wmda.info/login>, to register patient data. This can be done manually through a web form or automatically through an API connection that electronically transfers the data. Within seconds, a list of potentially matched URD/CBUs is available. Registries are encouraged to upload their complete URD/UCB data on a daily base.

Nearly 95% of the registered donors have been DNA typed for HLA-A,-B,-DRB1 loci, with more than half having additional information on HLA-

C,-DQB1,-DPB1. The chance of finding a suitable cell source varies, depending on patient race and ethnicity. The likelihood of a patient of White (Caucasian) ancestry identifying an 8/8 HLA-matched URD is 79%, the rate is much lower for patients of non-White or multi-ethnic heritage—47% for Asian, 48% Hispanic/Latino and 29% for Black or African Americans. High genetic diversity, increased barriers to donor availability and underrepresentation in the global inventory are contributing factors to lower match likelihoods experienced by patients of non-Caucasian ancestry (Gragert et al. 2023) (<https://bethematch.org/transplant-basics/how-blood-stem-cell-transplants-work/how-does-a-patients-ethnic-background-affect-matching/>). To facilitate these complex searches multiple mismatched unrelated donors are selected, as described in Chap. 12. WMDA allows to search for multiple mismatches.

WMDA offers a set of filters that can be used to reduce the number of potential cell sources for your patient. Examples of filters are age, gender and certification status. WMDA provides accurate information at initial search for quick identi-

fication of the most suitable cell source and is working towards service development, e.g. implementing a flag on a CBU with all necessary releasing tests completed, listing of adult cryopreserved donor units and an algorithm on assessing donor’s commitment to donate.

WMDA lists over 800,000 UCBs and provides information to shortlist the best suitable CBUs—HLA match to the patient, cellularity parameters, frozen volume and CBB information to follow published guidelines (Politikos et al. 2020; Little et al. 2021). To further facilitate the selection process, WMDA is working to standardize the presentation through a report that includes information about potency and quality. A critical factor for assessing the quality is whether the CB is stored in an AABB or NetCord-FACT-accredited CBB. If this is not the case, it is recommended to seek information through the registry, Eurocord survival data and WMDA information sources.

### 3.2.2 Back-Up Donor Strategy

While availability of URD at workup is a WMDA key performance indicator, registries cannot

always meet the timeframe requirements as individual and unexpected medical or personal issues can lead to unavailability of a donor. It is recommended that at least one backup donor who is prepared and willing to donate and/or a reserved UCB be chosen at workup with a primary donor, to ensure that impact to patient care is minimized should the primary donor become unavailable.

### 3.2.3 Registries as Partners of Transplant Centres

Once a cell source has been identified, the transplant centre will notify their affiliated registry to facilitate the delivery of the cells. Annually, over 20,000 cell products are transported across borders to meet the needs of patients requiring a HCT (Fig. 3.2). Registries work closely with transplant physicians to assist with URD and CBU selection, evaluation of product quality and education and training.

If there is no national registry in your country, WMDA can assist with the identification of the most suitable cell source and provide a match list (Fig. 3.3).

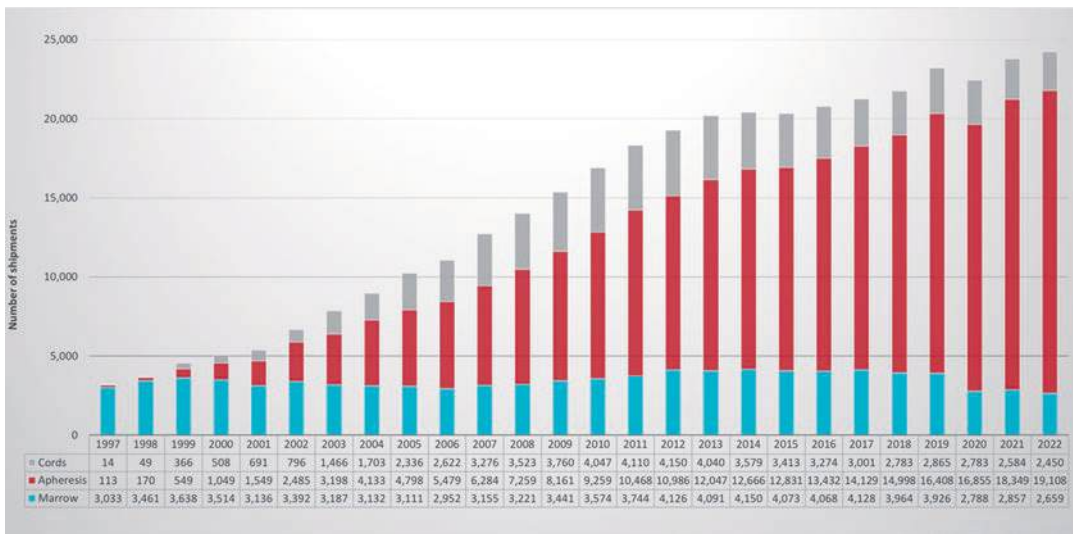


Fig. 3.2 URD and CBUs shipped annually (WMDA Global Trends 2022 Report)



Patient list Add patient

Romée Brouwer

Patient list / Results

Patient [2022\_J\_Janssen]



10/10 search (at HLA-A, B, C, DRB1, DQB1) | 2 mismatche alloud | A, B, DR donor search | algorithm HAP-E

Edit search

Filters: Donor status CMV Registry Accreditation Bloodgroup Sex All filters

1,994 donor results (Bold) = antigen mismatches Underlined = allele mismatches Italics = indicate uncertainty

	A	B	C	DRB1	DQB1	DPB1	Registry	Sex	Age	Bloodgroup
Patient details										
Standard	29:02:01	38:01:01	12:03:01	04:03	03:02:01	02:01P		Female	29	O+
	32:01:01	35:01:01	04:01:01	11:01	03:01:01	05:RGPW				
10/10 (potential) allele matches										
# 1	GRID: 498700000054629434		Status: available	CMV: N 2003-10-02	Last contact: 2019-11-19	Ethnicity: CAEU	ADCU	Full report		
0: 2%	<b>M</b>	<b>A</b>	<b>A</b>	<b>L</b>	<b>P</b>	<b>P</b>	7450 IT-Milano	Female	25	O+
1: 10%	0%	100%	100%	0%	10%	11:01:01G				
2: 7%	01:ABGEP	01:NRAR	01:NRAR	04:03	03:02:01	04:03:01G				
	01:ABGEP	35:01:01	35:01:01	11:01	03:01:01					
# 2	GRID: 498700000054629434		Status: available	CMV: N 2003-10-02	Last contact: 2019-11-19	Ethnicity: CAEU	Full report			
0: 2%	<b>M</b>	<b>A</b>	<b>A</b>	<b>L</b>	<b>P</b>	<b>P</b>	7450 IT-Milano	Female	25	O+
1: 10%	0%	100%	100%	0%	10%	11:01:01G				
2: 7%	01:ABGEP	01:NRAR	01:NRAR	04:03	03:02:01	04:03:01G				
	01:ABGEP	35:01:01	35:01:01	11:01	03:01:01					
# 3	GRID: 498700000054629434		Status: available	CMV: N 2003-10-02	Last contact: 2019-11-19	Ethnicity: CAEU	Full report			
0: 2%	<b>M</b>	<b>A</b>	<b>A</b>	<b>L</b>	<b>P</b>	<b>P</b>	7450 IT-Milano	Female	25	O+
1: 10%	0%	100%	100%	0%	10%	11:01:01G				
2: 7%	01:ABGEP	01:NRAR	01:NRAR	04:03	03:02:01	04:03:01G				
	01:ABGEP	35:01:01	35:01:01	11:01	03:01:01					
# 4	GRID: 498700000054629434		Status: available	CMV: N 2003-10-02	Last contact: 2019-11-19	Ethnicity: CAEU	Full report			
0: 2%	<b>M</b>	<b>A</b>	<b>A</b>	<b>L</b>	<b>P</b>	<b>P</b>	7450 IT-Milano	Female	25	O+
1: 10%	0%	100%	100%	0%	10%	11:01:01G				
2: 7%	01:ABGEP	01:NRAR	01:NRAR	04:03	03:02:01	04:03:01G				
	01:ABGEP	35:01:01	35:01:01	11:01	03:01:01					
# 5	GRID: 498700000054629434		Status: available	CMV: N 2003-10-02	Last contact: 2019-11-19	Ethnicity: CAEU	Full report			
0: 2%	<b>M</b>	<b>A</b>	<b>A</b>	<b>L</b>	<b>P</b>	<b>P</b>	7450 IT-Milano	Female	25	O+
1: 10%	0%	100%	100%	0%	10%	11:01:01G				
2: 7%	01:ABGEP	01:NRAR	01:NRAR	04:03	03:02:01	04:03:01G				
	01:ABGEP	35:01:01	35:01:01	11:01	03:01:01					

Fig. 3.3 Search & Match Service

### 3.3 The Role of a Registry: Donor Perspective

#### 3.3.1 Donor Recruitment

Annually, registries add two million new volunteer URDs. Donor recruitment is challenging as registries must target young donors and ensure they are well-informed. Methods for recruitment include patient-related drives, targeting specific groups, engaging blood donors, social media campaigns and online recruitment. When donors sign up as volunteers, they agree to register in the global database. They provide biological material and personal information for testing and potential matching. Confidentiality and traceability are crucial. To ensure this registries follow European General Data Protection Regulation and have implemented Global Registration Identifier for Donors (GRID) (Neller et al. 2017). Annually, WMDA is honouring the donors on the third Saturday in September during World Marrow Donor Day (<https://worldmarrowdonoraday.org/>).

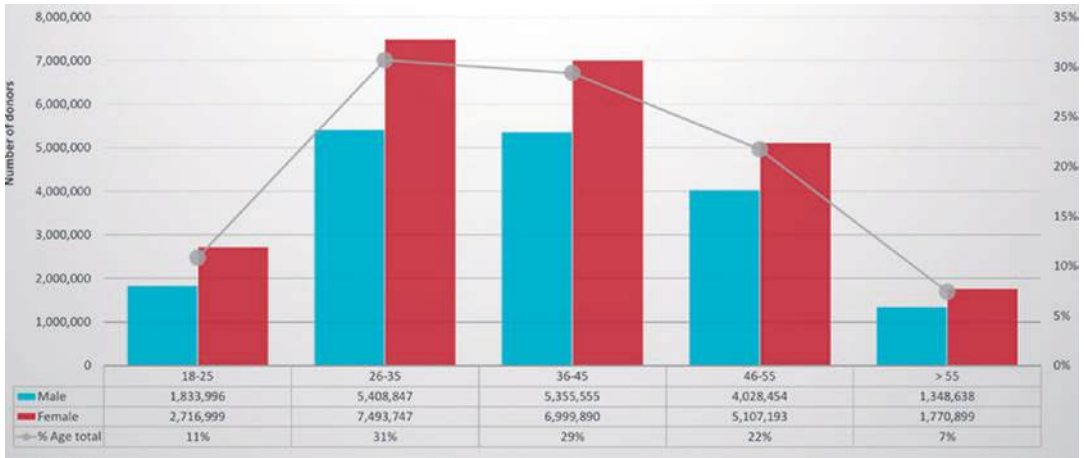
#### 3.3.2 Donor Pool HLA Diversity

New conditioning regimens allow an increase in the selection of mismatched unrelated donors (Nagler et al. 2022), and the disparities between patients from different ethnicities being transplanted are considerable (Auletta et al. 2021). For 30% of the patients that are registered, the number of options to find a suitable cell source is limited (Fig. 3.4). The main obstacles are rare alleles or haplotype combinations (e.g. multi-race ancestry). Strategies are explored to focus on targeted recruitment within non-Caucasian populations and encouraging the establishment of new and emerging registries. WMDA has published a handbook on how to start a registry to facilitate this process (available on request, [mail@wmda.info](mailto:mail@wmda.info)). Another strategy is training of search coordinators in exploring alternative options (e.g. mismatched URD, CB). In collaboration with EBMT, WMDA has developed an online education programme on donor and cord blood selection.



Fig. 3.4 Search results (WMDA Global Trends 2022 Report)





**Fig. 3.5** Age and gender of unrelated donors

### 3.3.3 Donor Profile: Young, Healthy and High-Resolution Typed

Registries target young and healthy volunteers (Fig. 3.5). The age range of the donor pool is between 16 and 60 years old, and younger donors are preferred due to better transplant outcomes (Kollman et al. 2016; Shaw et al. 2018). In addition, other testing such as CMV status and blood group can be done. WMDA’s donor medical suitability guidelines are publicly available (Lown et al. 2014 and <https://share.wmda.info/x/FABtEQ>; Worel et al. 2022).

Donor/patient HLA matching is an aspect in selecting a cell source. The classic approach is to consider the best match on a minimum of four HLA loci-HLA-A,-B,-C,-DRB1 for adult and CB donors, where additional HLA loci (-DQB1,-DPB1) may be included (Fleischhauer et al. 2012; Mayor et al. 2018). The number of mismatches allowed depends on the type of the graft and clinical situation. WMDA provides flexibility in search tools to identify matched and mismatched donors in line with the patient needs (Shaw et al. 2017; Eapen et al. 2017).

### 3.3.4 Donor Availability: Focus of Registries Worldwide

Donor retention is influenced by cultural and traditional factors. Donor availability is a key focus, as time to transplant is a significant factor in overall survival (Craddock et al. 2011). While donors have the right to withdraw at any stage, registries aim to keep in touch with donors to ensure up-to-date information and increase the number of collection centres to meet desirable turnaround times. WMDA has defined key performance indicators (KPI) on donor availability (<https://share.wmda.info/x/1YC1EQ>).

Improvements in HLA typing methodologies have resulted in donors with high HLA-resolution typing, allowing for unambiguous determination of compatibility with patients in need of HCT. The health and availability check (HAC) is an emerging strategy that can expedite a patient’s timeline to transplant, as well as improving both donor experience and potentially patient outcomes.

### 3.4 Working Together to Deliver Best Care to Patient and Donor

To deliver the best care for donor and patient, transplant centres work closely with local registries providing necessary information and helping to maintain donor commitment and satisfaction.

#### 3.4.1 Backup Donor

Transplant centres must consider the cost, unnecessary testing and workload of managing backup donors while ensuring care for their patients. Having prepared backup donors is crucial to avoid delays if the primary donor is unavailable. The transplant centre should collaborate with the registry to identify and prepare backup donors promptly, following ethical and regulatory guidelines.

#### 3.4.2 Serious (Product) Events and Adverse Reactions (SPEAR)

WMDA operates a service that collects and analyses information on serious adverse events (SAE) and reactions (SAR) related to stem cell donation (Shaw et al. 2013; Jöris et al. 2021). This service is known as SPEAR (serious [product] adverse events and reactions). The aim of SPEAR is to gather information and to provide a rapid alert system for disseminating information to the international community that affect donors and/or products and/or recipients. By reporting these incidents (Sorensen et al. 2016), WMDA understands the potential risks and improves the safety of the donation process. Transplant centres are welcome to report on related donors. Info is available at [sear-spear@wmda.info](mailto:sear-spear@wmda.info) and at <https://share.wmda.info/x/YotOGg>.

#### 3.4.3 Donor Follow-Up

The donation process in general has proven to be safe, but in rare cases severe and even fatal events have been reported. Better supportive care and the administration of reduced-intensity conditioning regimens have contributed to an increase in HCT in older patients, whose related donors are usually also older. As a consequence, the median age of related donors has increased and is approximately 10 years higher than that of unrelated donors (Halter et al. 2009) leading to potentially more donors with occult or manifest comorbidities at the time of donation. Donor follow-up is organized in different ways (Sánchez Ibáñez et al. 2023) and registries can assist transplant centres to organize donor follow-up for related donors (Ruesch et al. 2022).

#### 3.4.4 Patient Follow-Up

To ensure safety of HCT, registries would like to share patient follow-up information with both the collection centre and the donor. Both external parties are important to be informed: the collection centre from a quality perspective (as stated in JACIE standards) and, if a donor is interested, they can be informed about the status of the patient. Registries send forms ([https://wmda.info/wp-content/uploads/2022/04/WMDA-FORM-TF1\\_Group\\_v1.1.pdf](https://wmda.info/wp-content/uploads/2022/04/WMDA-FORM-TF1_Group_v1.1.pdf)) to receive this information from transplant centres, in case they do not have access to the EBMT database.

#### 3.4.5 Addressing Crisis Situations

Several times the provision of cells has been challenged by situations such as volcano eruptions or political conflicts, and none of them have been so disruptive as the COVID-19 pandemic. For the first time, a negative development in the



number of donations was observed due to logistical reasons such as travel and lockdown restrictions or limited capacity at collection centres. Registries should be able to respond to crisis situations and to collaborate internationally (Joris et al. 2022).

One of the EBMT/WMDA recommendations was to withhold the conditioning regimen of the patient until the cells had safely arrived at the transplant centre. This was possible by cryopreserving the products at origin or destination (<https://www.ebmt.org/covid-19-and-bmt>). This methodology had as a negative consequence a high number of non-transfused donations, some registries reporting as many as 5–10% of their donations going unused (Schmidt et al. 2021). Although the latter waves of the pandemic showed fewer logistic problems, cryopreservation needs to be reviewed to assess the impact on donor burden and patient outcome.

### 3.4.6 Donor-Patient Contact

Donor-patient contact after donation is generally not recommended. There may be ethical and psychological implications for donors and recipients if contact is made. Therefore, it is important to follow the guidance provided by their medical professionals and the ethical guidelines established by regulatory bodies. However, there are circumstances where contact can be facilitated in a controlled manner, such as anonymous correspondence or facilitated meetings with medical professionals present to monitor and guide the interaction.

### 3.4.7 Related Donor

In recent years, registries have expanded their services to include support for related donors

who live in different countries or who are unable to collect cells at their local transplant centre. This includes providing logistical and administrative assistance to ensure the safe and timely transport of cells from the donor to the patient's transplant centre. Some registries also offer pre- and post-collection support, as well as assistance with travel and accommodation arrangements.

### 3.4.8 Research

Registries want to make sure that donors are fully informed of the risks and benefits of participating in clinical trials and that their decision to participate is entirely voluntary. By participating in clinical trials, donors can help to further medical knowledge and potentially benefit patients in the future. Donors should be made aware of their right to withdraw from the study at any time, without any negative consequences for their care or the donation process. Registries have a responsibility to ensure that donors are properly informed about any research studies and that their participation is strictly voluntary (King et al. 2012).

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## 3.5 Future of Registries

WMDA and its registries are a community of healthcare professionals focused on innovation and striving for equal access for all patients. The connection between donors and patients is going beyond the traditional unrelated donor. Areas that registries can assist transplant centres are:

- Care, administrative and logistical support for related donors
- Logistics of products for cellular therapy
- Complex logistical procedures

### Key Points

- WMDA and its members strive for a world where access to life-saving cellular therapies for all patients is assured and donors' rights and safety are protected.
- International collaboration resulted in more than 41 million URD and CBUs being potentially available for patients requiring HCT. A potential match can quickly be identified by performing a search in WMDA's Search & Match Service.
- Registries are important partners for transplant centres in requesting URD/CBUs, to ensure quality and legal compliance. Registries are exploring other products and services to support the clinical community, e.g. care for related donors and supporting cellular therapy.
- Efforts are being made to reduce time to transplant by increasing the HLA diversity, by performing high-quality HLA typing and by educating donors on their role in the donation process to avoid the donor not being available.

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## The HCT Unit

# 4

Walid Rasheed, Dietger W. Niederwieser,  
and Mahmoud Aljurf

### 4.1 Introduction

Hematopoietic cell transplantation (HCT) is an advanced therapeutic intervention that is required for a number of malignant and nonmalignant medical conditions, often for critically ill patients. The establishment of an HCT program requires the efforts of experienced and appropriately trained personnel to lead the program. Clearly, this also requires financial, legal, ethical, and other institutional support. Without the commitment of the hospital director, allocation of resources, support of the national health authorities, and politicians, an HCT program will not be successful. For newly starting programs, it would be essential to identify minimal requirements for establishing an HCT unit in order to optimize resource utilization as well as maintain safe patient care. While these minimal requirements also apply to well-established units, its structure helps to understand and implement additional steps for larger units which plan to offer addi-

tional transplant services and have access to more resources. The recent advent of more cellular therapy types, including immune effector cell therapy, has added another layer of complexity necessitating additional requirements by HCT programs to ensure patient safety.

More than 20 years ago the EBMT and the ISCT (International Society of Cellular Therapy) formed the Joint Accreditation Committee—ISCT and EBMT (JACIE) based on the FACT (Foundation for the Accreditation of Cellular Therapy) program. Efforts of these bodies have culminated in the establishment of standards related to HCT and cellular therapies to assure quality and safety in the practice of HCT. Although program accreditation with JACIE is not mandatory worldwide, these standards are very helpful as guidelines to understand requirements to establish an HCT unit.

Table 4.1 summarizes basic minimal requirements of an HCT unit, which are discussed in more details in the following sections.

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**Table 4.1** HCT unit minimal requirements

Inpatient unit	Clean single bedded rooms with isolation capability
Ancillary medical services	<ul style="list-style-type: none"> <li>• Intensive care unit</li> <li>• Emergency room service</li> <li>• Gastroenterology and pulmonary service<sup>a</sup></li> </ul>
Outpatient clinic	Single-patient examination rooms
Blood bank	<ul style="list-style-type: none"> <li>• 24 h on-site blood bank service: ABO typing and crossmatch, RBC and platelets for transfusion</li> <li>• Irradiation and leukocyte—depletion of blood products</li> </ul>
Laboratory	<ul style="list-style-type: none"> <li>• Hematology cell count and chemistry lab</li> <li>• Serology for viral screen</li> <li>• Microbiology for basic bacterial and fungal cultures</li> <li>• CMV PCR or antigenemia<sup>a</sup></li> <li>• Access to CSA/tacrolimus levels<sup>a</sup></li> </ul>
HLA typing lab <sup>a</sup>	Access to ASHI or similarly accredited HLA typing lab
Stem cell collection	<ul style="list-style-type: none"> <li>• PBSC apheresis capability</li> <li>• Bone marrow harvesting facility and expertise for matched sibling donor<sup>a</sup></li> </ul>
Stem cell processing facility	<ul style="list-style-type: none"> <li>• FACS CD34 enumeration</li> <li>• Refrigerator for blood and bone marrow</li> <li>• Controlled cryopreservation capability for freezing of autologous stem cell product</li> <li>• Equipment and expertise to process ABO-mismatched cellular product<sup>a</sup></li> </ul>
Radiology	<ul style="list-style-type: none"> <li>• Routine X-ray radiology, ultrasound, CT scanner</li> <li>• Placement of central venous catheters</li> </ul>
Pharmacy	<ul style="list-style-type: none"> <li>• Availability of conditioning chemotherapy drugs</li> <li>• Availability of antimicrobial agents (broad-spectrum antibiotics, antiviral and antifungal drugs)</li> <li>• Availability of immunosuppressive agents for GVHD prophylaxis and treatment<sup>a</sup></li> </ul>
Human resources	<ul style="list-style-type: none"> <li>• Medical director: licensed physician with adequate training and experience in HCT</li> <li>• Nursing staff with training in chemotherapy administration, infection control, and handling of stem cell products</li> <li>• Clinical laboratory director—clinical pathology trained</li> <li>• Appropriately trained lab scientist and technicians</li> <li>• Clinical pharmacist trained in chemotherapy and conditioning regimen administration</li> <li>• Multidisciplinary medical staff (radiology, pathology, ICU, surgery, gastroenterology<sup>a</sup>, pulmonary<sup>a</sup>)</li> </ul>
Outcome database	Monitor patient demographics, treatment, and outcomes (Level I data reporting)
Quality management	<ul style="list-style-type: none"> <li>• Written institutional protocols/guidelines</li> <li>• Regular audits of various HCT procedures and patient treatment outcomes</li> <li>• System to detect errors or adverse events for corrective or preventative actions</li> </ul>

<sup>a</sup> Requirements for allogeneic HCT programs

## 4.2 Inpatient Unit

The inpatient HCT unit should have a minimum number of single bedded rooms with isolation capability. The number and space of rooms should be adequate for the type and volume of transplant activity performed at the transplant center. These rooms must adhere to the standards of safety and comfort of patients in a tertiary care hospital facility. Every location or room should have a sink and tap for handwashing.

There needs to be a working station or room for nurses involved in patient care. A similar working space for physicians is required. Medical and nursing staff coverage should be available 24 h a day, including public holidays. The ratio of nurses to patient beds depends on the type and intensity of transplants being performed, e.g., autologous versus allogeneic, but generally a ratio of one nurse to three patients is reasonable. Emergency cart with drugs for resuscitation should be available in the inpatient unit.

Infections, including bacterial, viral, or fungal infections, are potential significant complications in transplant recipient and may lead to significant morbidity and mortality. Therefore, HCT units should have established measures for infection control. Guidelines for infection prevention and prophylaxis in HCT patients, endorsed by several scientific organizations, are available and highly recommended to follow. HCT recipients should be placed in single-patient rooms. Furthermore, at a minimum, standard precautions should be followed in all patients including hand hygiene, wearing of appropriate protective equipment (gloves, surgical masks or eye/face protection, gowns) during procedures/activities that are likely to generate splashes or spray of blood, body fluids, or secretions. Hand hygiene is essential, using alcohol-based hand rubs or washing with soap and water. In patients with suspected or proven of having an infection, additional precautions are required accordingly, e.g., airborne, droplet, or contact isolation. HCT units should be cleaned at least daily with special attention to dust control. During building construction intensified mold control measures are required and a multidisciplinary team (including infection control, engineers, infectious disease staff, and transplant clinicians) should be involved. Testing patients for multiresistant bacteria is important in order to isolate positive carriers and minimize transmission risk.

Other important infection control measures include well-sealed rooms, positive pressure differential between patient rooms and the hallway, self-closing doors, more than 12 air exchanges per hour, and continuous pressure monitoring. HEPA (high-efficiency particulate air) filters have shown efficacy in providing protection against acquisition of fungal infections in immune-compromised hematology patients, including HCT patients, and during hospital construction or renovation works. While HEPA filters are not absolutely required as a minimal requirement in newly established centers with less complicated transplant activities, they are certainly preferred and highly recommended as newly established centers expand their activities

to include more complicated (especially allogeneic) transplant activities. HEPA filters are especially indicated in countries with high fungal burden, and therefore, HEPA filters are always preferred. Flowers and plants are not allowed in HCT units!

There is no agreed upon minimum number of transplants to be performed in a program. However, to ensure continuing proficiency in a transplant program, the ASBMT recommends for programs performing only one type of HCT (autologous or allogeneic), at least ten transplants of that type are to be performed per annum; programs performing both allogeneic and autologous transplantations should perform a minimum of ten transplants of each kind per annum.

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### 4.3 Ancillary Medical Services

HCT patients often require other medical specialties' involvement in their complicated care. This include the risk of developing life-threatening infections or other posttransplant complications, hence the importance of having access to emergency room as well as intensive care services at the same tertiary care hospital facility where the transplant program is being established. Intensive care units should have the ability of providing inotropic support, respiratory support (including mechanical ventilation), and renal replacement (hemodialysis) if required.

Input from infectious disease physicians can be valuable in HCT patients who are at risk of a multitude of opportunistic and potentially life-threatening infections; this is especially important for programs that perform allogeneic transplants. Availability of gastroenterology specialist with endoscopy services is critical for allogeneic programs, as often diagnostic endoscopy is required to histologically differentiate GVHD from other etiologies of gastrointestinal complications. Similarly, pulmonary medicine service with access to diagnostic bronchoscopies is required for such patients with pulmonary abnormalities. Other ancillary medical services may include dermatology, ophthalmology, and gynecology.

cology which can be valuable in the diagnosis and treatment of organ-specific GVHD complications in centers performing allogeneic HCT.

HCT programs that perform transplants using radiotherapy as part of a conditioning regimen (total body irradiation) should have available radiation oncology service on-site. The radiation oncology team, including the radiation oncologist and physicist, should have adequate training in the technique of TBI and appropriate equipment and procedures must be in place to deliver successful and safe radiation component of these conditioning regimen.

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#### **4.4 Outpatient Unit**

HCT patients attend to the outpatient unit, both for pretransplant assessment and workup as well as posttransplant follow-up and management. Single-patient examination rooms are a minimal requirement for the outpatient service of the program. These rooms should be adequately equipped to allow clinical assessment of patients. It is important to implement infection control measures to minimize risk of transmitting infections, including hand hygiene measures and availability of appropriate room to isolate patients who are identified to be potentially infectious to others, e.g., due to herpes zoster infection. A dedicated infusion area would be ideal as transplant recipients often require IV fluid and electrolyte replacement or blood product administration.

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#### **4.5 Blood Bank**

Availability of blood banking services is a critical component of a successful transplant program. A 24-h on-site blood banking service is required for ABO typing, crossmatch, and urgent supply of red blood cells and platelets for transfusion. Meeting minimal standard criteria according to recognized international blood bank societies such as the American Association for Blood Banking (AABB) or equivalent are important.

Blood bank staff, including blood bank director, scientists, and technicians, should be adequately qualified and trained in blood banking procedures.

Transplant recipients are severely immune-compromised and are at risk of transfusion-associated GVHD, caused by unrestricted proliferation of donor lymphocytes in the immune-compromised host. Hence, it is critical that transplant recipients receive irradiated blood products to prevent this complication. The use of leukocyte-depleted blood products is recommended to reduce the risk of HLA alloimmunization in the multiply transfused hematology patients, as well as to reduce the incidence of transfusion reactions. In allogeneic programs, clear documented pathways for transfusion support in cases of ABO mismatch should be available for both blood bank and clinical staff as guidance.

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#### **4.6 Laboratory**

A 24-h on-site hematology cell count and basic chemistry lab is required. Furthermore, microbiology laboratory service is essential in the clinical management of transplant recipients, including routine bacterial and fungal cultures of various patient specimens. Serology screening for relevant viral and bacterial infections is also required for pretransplant workup of recipients as well as donor screening, including testing for HIV, viral hepatitis, CMV, HSV, HTLV1, toxoplasma, and syphilis among others. For allogeneic transplant recipients, monitoring for cytomegalovirus (CMV) reactivation is essential and results must be available in a timely manner to allow therapeutic intervention; both molecular technique by quantitative PCR (preferable) and antigenemia method are acceptable. In the allogeneic setting, monitoring drug levels, e.g., cyclosporine or tacrolimus, is required, and same-day service is recommended to allow interventions aiming at keeping levels of these important drugs within the target therapeutic range.



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## 4.7 HLA Typing Lab

Access to an HLA typing laboratory is mandatory for allogeneic programs. Such service can be available on-site or alternatively provided in reference laboratory. JACIE standards state that clinical programs performing allogeneic transplantation shall use HLA testing laboratories that are capable of carrying out DNA-based intermediate- and high-resolution HLA typing and are appropriately accredited by the American Society for Histocompatibility and Immunogenetics (ASHI), European Federation for Immunogenetics (EFI), or other accrediting organizations providing histocompatibility services appropriate for hematopoietic cellular therapy transplant patients.

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## 4.8 Stem Cell Collection

Access to peripheral blood stem cell apheresis service on-site is a minimal requirement in each program. This is often part of the blood bank service or alternatively under the administration of the clinical program. Having at least two cell separators would be beneficial, as the second cell separator would be a backup in situations of unexpected machine faults and for routine servicing. Daily operation of apheresis facility requires appropriately trained and experienced nursing staff and a medical director with adequate qualification and experience in clinical and laboratory aspects of the apheresis procedure. Institutional written protocols and policies covering all aspects of apheresis procedure should be available for guidance. JACIE standards require a minimum average of ten cellular therapy products collected by apheresis per year for program accreditation.

A BM stem cell source is sometimes recommended for better patient outcome, e.g., patients with BM failure. Programs performing allogeneic HCT for such indication should have a bone marrow harvest facility on-site. This requires convenient and easy access to surgical operating room with anesthesia service. Appropriate equip-

ment for the BM harvest procedure are required. Physicians with adequate training and experience in BM harvesting are crucial to perform the procedure successfully.

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## 4.9 Stem Cell Processing Facility

The stem cell processing facility requires a designated area, usually within the laboratory. It should be appropriately equipped for the processing of various stem cell products depending on the types of transplants performed and the size of the program. Availability of flow cytometry for the enumeration of CD34 cell count is mandatory. Controlled cryopreservation capability, using liquid nitrogen, for freezing of autologous stem cell product is essential. This may also be used in allogeneic sibling products. Standard quality control measures, including systems to closely monitor and record the temperature in all freezers and refrigerators, are critical. Allogeneic programs should have appropriate equipment and expertise on-site for the timely and safe processing of ABO-mismatched stem cell products as required, including the need to perform red cell or plasma depletion procedures when indicated. The processing facility should be operated by adequately trained staff, including scientist, technicians, and a medical director. Written standard operating procedures explaining all aspects of stem cell processing performed at the facility are required.

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## 4.10 Radiology

Standard routine (X-ray), ultrasound, and computed tomography (CT scan) imaging services are the minimal requirements and should be available on-site for the routine diagnostic imaging. Availability of magnetic resonance imaging (MRI) is preferred, as it is often useful in the diagnosis of specific clinical conditions relevant to stem cell transplant recipients, such as iron overload, CNS infections, and posterior reversible encephalopathy syndrome (PRES) related to



calcineurin inhibitor toxicity. Placement of central venous catheters in transplant recipients is obviously required in each program. Depending on the institutional setting, this service may be provided by various hospital services; often this is done by the radiology service under ultrasound guidance. Having well-trained and experienced interventional radiologist to perform this procedure is crucial for the safety of patients.

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#### 4.11 Pharmacy

Pharmacy services are essential in each HCT program. Availability of conditioning chemotherapy agents is clearly required; specific drugs depend on the type and complexity of transplant procedures performed in each program. Commonly used agents in conditioning regimens include busulfan, cyclophosphamide, fludarabine, and melphalan. Antithymocyte globulin (ATG) may also be required in the allogeneic setting (e.g., in aplastic anemia) and requires special attention and training by nursing, pharmaceutical, and medical staff in relation to its administration.

Broad-spectrum antibiotics should be available for urgent use as required in transplant recipient. Likewise, access to antiviral and antifungal agents is important for both prophylaxis and treatment. Allogeneic programs should also have access to immunosuppressive drugs used for GVHD prophylaxis such as cyclosporine, methotrexate, and tacrolimus, as well as medications required for GVHD treatment.

A trained pharmacist is crucial for the HCT program. The pharmacist should review all conditioning chemotherapy protocols and ensure appropriate dispensing and administration of cytotoxic agents.

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#### 4.12 Staffing and Human Resources

Appropriately trained and experienced medical and nursing staffs are crucial for the HCT program. The clinical medical director of the pro-

gram should be a licensed physician (specialty certification in hematology, oncology, or immunology) with adequate training at a BMT program. A minimal BMT training duration of 6–12 months is suggested. JAICIE standards indicate that the clinical program director shall have 2 years of experience as an attending physician responsible for the direct clinical management of HCT patients in the inpatient and outpatient settings. A minimum of one (1) additional attending transplant physician is required in the program. Modern technologies like supervisory telemedicine may be useful to cope with lack of experience.

The success of a transplant program relies heavily on the presence of appropriately trained and experienced nursing staff. This includes training in chemotherapy administration, infection control, management of neutropenic patients, and handling of stem cell products.

Other important staff includes appropriately trained and experienced personnel in the laboratory (including laboratory director, scientist, and technicians), trained pharmacist, and medical professionals of ancillary medical services. Continuous education activities are required for all healthcare professionals involved in the management of HCT patients. Written HCT protocols are essential and should be signed by the physician and the pharmacist.

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#### 4.13 Chimeric Antigen Receptor T-Cell (CAR-T Cell) Unit

The use of this type of immune effector cell therapy is gradually expanding and is typically implemented in well-established HCT centers. CAR-T therapy represents a novel technology, not just in the production and cellular process that underpin the mechanism of action, but also in the processes, clinical pathways, and complications. Institutions, therefore, need to develop clear guidance for all healthcare providers who are involved in the CAR-T therapy process. All relevant staff involved in the prescribing, dispensing, or administering of CAR-T cell thera-

pies should be aware of how to manage the risks of cytokine release syndrome (CRS) and neurological complications and should be trained and certified in the respective manufacturers' risk evaluation and mitigation strategy (REMS) programs. The complexity and toxicity profile necessitate that CAR-T cell therapy is delivered in a center and a program that is experienced in the management of patients undergoing cellular therapy and its complications with close liaison and access to intensive care. Hence, the start of CAR-T cell therapy in HCT programs should be preceded by adequate preparation addressing multiple elements that include, but are not limited to, materials, equipment, personnel, documents (standard operating procedures and institutional guidelines), staff training, and facilities. On-site availability of relevant ancillary medical services (intensive care, neurology, and infectious disease), as well as medications required for the treatment of CRS and immune cell-associated neurotoxicity syndrome (ICANS), is paramount.

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#### **4.14 Institutional Database and Data Manager**

Monitoring patient demographics, treatment details, and outcomes is an essential minimal requirement. Each program should keep complete and accurate patient records using unique patient numbers (UPN), and a database containing relevant patient data should be established and regularly maintained. Appropriate patient consent needs to be obtained for such database. An example of the minimal data required to be obtained on each transplant patient is the information required in the CIBMTR or EBMT MED-A forms. Having a data manager in a transplant program to initiate and maintain this institutional transplant database is highly recommended. Often data managers have a nursing background with experience in HCT. Attending training data management courses during international meetings or through links with other experienced and well-established programs would be valuable.

#### **4.15 Quality Control**

The JACIE standards require that all essential clinical, collection, and processing facilities in the transplant center evaluate and report patient outcomes. Regular audits of various HCT procedures and patient treatment outcomes are required. Essentially, a system is required to be in place to detect errors/adverse events, so that these can be evaluated in order to implement preventative measures to minimize the risk of recurrence of these incidents. Furthermore, each program should have written institutional clinical protocols in relation to the various aspects of the transplant patient care to standardize practice. Likewise, stem cell collection and processing facilities should have standard operating procedures that serve as a guidance for all staff to follow to enhance patient's safety. Access to or relationship with experienced HCT program is often very helpful and highly recommended via shared protocols/telemedicine and/or web-based conferencing.

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#### **4.16 Transplant Coordinator**

HCT is a complex therapeutic intervention and coordination of the pretransplant, transplant, and posttransplant patient care is important. A transplant coordinator can play a pivotal role in this context, acting as a facilitator and educator as well as a point of contact for the patients and their families. Transplant coordinators ensure the smooth and safe running of the HCT service starting from scheduling and arranging pretransplant workup of patients and planning the roadmap for the transplant recipient with continued involvement and education of the patients and their families until the time of admission. Furthermore, the transplant coordinator would play a significant role in the coordination of post-HCT follow-up and care in clinics. For allogeneic HCT, the transplant coordinator would be very valuable in arranging donor search starting from HLA typing of the recipient and his/her family members, in addition to initiating and following a search for unrelated donor in national or interna-

tional registries. The transplant coordinator involvement may extend to organizing the logistics of getting the stem cells from the donor from the donor center where the recipient may be in another health facility (national or international). Moreover, transplant coordinators will often lead the HCT team's weekly planning meetings and discussions with the arrangement of the HCT waiting list. Typically, transplant coordinators have a nursing background with significant experience in stem cell transplantation.

### Key Points

- The inpatient unit should have single bedded rooms with isolation capabilities. Single outpatient examination rooms are also required.
- Laboratory, blood bank, and pharmacy services are critical to the success of HCT programs.
- Stem cell collection and processing capabilities are minimal requirements for any HCT program; the level of such capabilities depends on the type and complexity of HCT performed in each center.
- Ancillary medical services are essential components of successful HCT programs, including intensive care, emergency, and radiology services. Additional medical services are required in allogeneic programs.
- Appropriately trained and experienced staff (medical, nursing, laboratory, pharmacy) are crucial for the HCT program.
- The complexity and toxicity profile necessitate that CAR-T therapy is delivered in a center and a program that is experienced in the management of patients undergoing cellular therapy and its complications with close liaison and access to intensive care. Hence, the start of CAR-T cell therapy in HCT programs should be preceded by adequate preparation addressing multiple elements, to

mitigate risks associated with immune effector cell therapy.

- Monitoring patient characteristics and transplant outcomes is essential.
- A local quality control system is required in all aspects involved in the HCT procedure.
- Having a data manager for the HCT program, to initiate and maintain institutional minimal transplant database, is highly recommended.
- Transplant coordinators play a pivotal role in the management of HCT patients, starting from pre-SCT workup, right through posttransplant care.

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# JACIE Accreditation of HCT Programs

# 5

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## 5.1 Introduction

The complexity of HCT as a medical technology and the frequent need for close interaction and interdependence between different services, teams, and external providers (donor registries, typing laboratories, etc.) distinguish it from many other medical fields. This complexity led to efforts by transplantation professionals to standardize processes based on consensus as a way to better manage inherent risks of this treatment. HCT was, and continues to be, a pioneer in the area of quality and standards.

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## 5.2 Background

In 1998, EBMT and the International Society for Cell and Gene Therapy (ISCT) established the Joint Accreditation Committee, ISCT and EBMT (JACIE). JACIE aimed to offer an inspection-based accreditation process in HCT against established international standards. JACIE is a committee of the EBMT, its members are appointed by and are accountable to the EBMT board, and ISCT is represented through two members of the committee. JACIE collaborates with the US-based Foundation for the Accreditation of Cellular Therapy (FACT) to develop and maintain global standards for the provision of quality medical and laboratory practice in cellular therapy.

The JACIE and FACT accreditation systems stand out as examples of profession-driven initiatives to improve quality in transplantation and which have subsequently been incorporated by third parties, such as healthcare payers (health insurers, social security) and competent authorities (treatment authorization). The JACIE Accreditation Program was supported in 2004 by the European Commission under the public health program 2003–2008 and was acknowledged as an exemplary project in a 2011 review of spending under the public health program.

### 5.3 Impact of Accreditation in Clinical Practice

Much literature indicating a better clinical outcome in teaching hospitals and centers of excellence has been available since the 1990s (Hartz et al. 1989; Birkmeyer et al. 2005; Loberiza et al. 2005). Initial evidence of a positive relationship between the implementation of a quality management system and outcome of HCT in Europe was published in 2011 (Gratwohl et al. 2011). In this study, patients' outcome was systematically better when the transplantation center was at a more advanced phase of JACIE accreditation, independent of year of transplantation and other risk factors.

Another analysis (Gratwohl et al. 2014) was performed on a large cohort of patients who received either an allogeneic or an autologous transplantation between 1996 and 2006 and reported to the EBMT database. The authors showed that the decrease of overall mortality in allogeneic procedures over the 14-year observation period was significantly faster in JACIE-accredited centers, thus resulting in a higher relapse-free survival and overall survival at 72 months from transplant. Such improvement was not shown in autologous transplantation. Further confirmation with an updated health economic analysis was provided in Gratwohl et al. (2015).

Similar results published by Marmor et al. (2015) in an American study showed that centers accredited by both FACT and Clinical Trial Network (CTN) demonstrated significantly better results for more complex HCT such as HLA-mismatched transplants. Further, Anthias et al. (2016) suggested that the introduction of FACT-JACIE standards has improved the related donor care.

These data reinforce the concept that clinical improvement is driven by the implementation of a quality management system embedded in external accreditation standards, especially in the context of more complex procedures. This process also results in a wider standardization of procedures across different countries and geographic

areas, therefore contributing to providing patients with similar treatment expectations even when accessing different health management systems. Comprehensive reviews of this area have been recently published (Snowden et al. 2017, 2021).

### 5.4 JACIE-FACT Accreditation System

JACIE and FACT accreditation systems are based on the development and continuous update of standards covering the entire transplantation process, from selection of the donor/patient to follow-up, including collection, processing, and storage of the graft. Considering the different competences included in the process, the standards are divided in four parts:

- Clinical program
- Bone marrow collection
- Apheresis collection
- Processing facility

A quality management (QM) section is embedded in each section, aimed at providing a tool for both the applicants to develop a comprehensive system of quality assessment and for the inspectors to check the compliance of the program to the standard. Stand-alone processing laboratories can apply; however, the target of the accreditation is the transplantation program, intended as a process in its entirety, thus requiring a full integration of units, laboratories, services, and professionals. Each section focusses on the competence of personnel, listing topics for which the evidence of specific training is required which also includes the minimum experience requirements for positions of responsibility. Maintaining these competencies is required for all professionals.

The standards are revised on a 3-year basis by a commission formed of experts appointed by JACIE and FACT, including HCT administration, cell processing and storage, blood apheresis, transplant registries, and QM specialists. The standards are based on published



evidence and, when this is not available, on expert consensus. A legal review and comparison with current regulations are carried out for each version. When the developmental phase is finalized, the standards are published for public review and comment and finally approved by JACIE and FACT. The standards incorporate sound principles of quality medical and laboratory practice in cellular therapy, but do not cover legal requirements of local competent authorities.

The compliance to the standards is ensured through an inspection system, carried out by voluntary inspectors, trained and coordinated by the JACIE office. The JACIE inspection is a multi-step procedure: the applicant center is provided with all the application documents and is then required to submit a set of documentation to the JACIE accreditation coordinators. The on-site inspection is then planned in agreement with the applicant.

JACIE inspections are carried out in most cases in the language of the applicant. The inspectors' report is then assessed by the JACIE accreditation committee, which may request supplementary information, modifications, or another on-site visit. Once all aspects are shown to be compliant, accreditation is awarded. An accreditation cycle is 4 years for JACIE, and facilities must complete an interim, documentation-based audit at 2 years post-accreditation. Accredited facilities must reapply for reaccreditation and may also be reinspected in response to complaints or information that a facility may be noncompliant with the standards, in response to significant changes in the program and/or facility.

Many tools are made available to prepare the accreditation through the JACIE website, including a quality management guide, the welcome guide, e-learning materials and webinars. JACIE runs training courses throughout the year, and the JACIE staff are available to support the applicants. An accreditation manual provides detailed explanations and examples for each single item of the standards. JACIE and FACT also collaborate on an initiative for transplant programs in

low- and middle-income countries (LMICs), where full accreditation might not be feasible due to resources and/or cultural issues. This “step-wise” option encourages the programs to connect with an international network of professionals and may also stimulate local authorities to support further progress toward full accreditation in the interests of patients, donors, and the professional community.

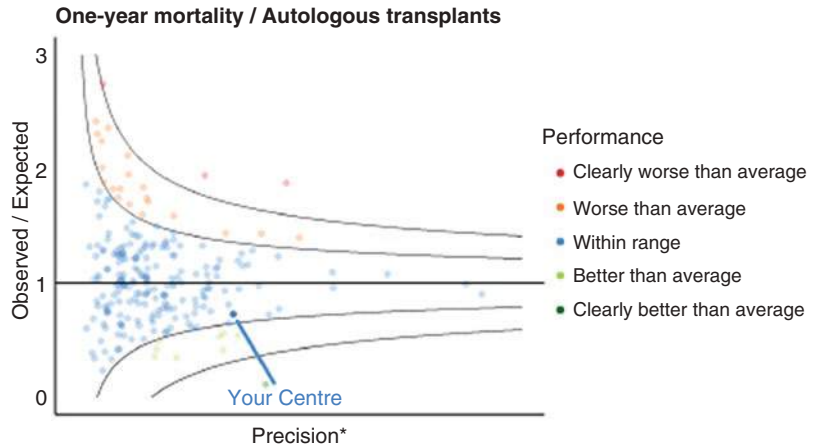
The standards cover the use of different sources of hematopoietic stem cells and nucleated cells from any hematopoietic tissue source administered in the context of the transplant process, such as DLI. The term “hematopoietic” in the title is to define the scope of these standards, due to an increasing number of accredited facilities that also support non-hematopoietic cellular therapies. Starting with version 6.1, the standards include new items specifically developed for other cellular therapy products, with special reference to immune effector cells (IECs). This reflects the rapidly evolving field of cellular therapy through mainly, but not exclusively, genetically modified cells, such as CAR-T cells. The standards do not cover the manufacturing of such cells but include the chain of responsibilities where the product is provided by a third party and ensure the competence of the personnel in the management of adverse events related to the infusion.

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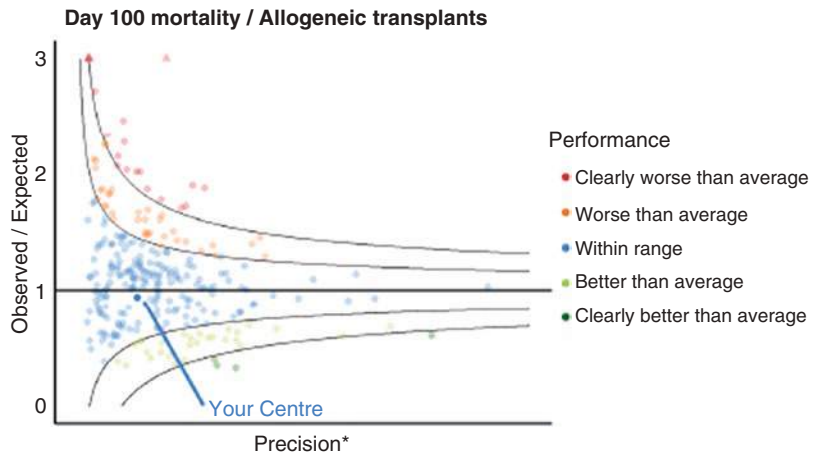
## 5.5 Benchmarking

Introduction of “benchmarking” standards related to 1-year survival/mortality, 1 year event-free survival, 100-day survival mortality, and other patient outcomes has developed into a collaboration between JACIE and the EBMT registry which will enable centers to address these new JACIE standards within their own BMT community and across the international boundaries. The “benchmarking” initiative at the EBMT is comprehensively covered in Snowden et al. (2020) and Saccardi et al. (2023). Based on individual center EBMT registry returns and a minimum level of data completeness over a 5-year

**Fig. 5.1** Example of funnel plot for 1-year mortality following autologous transplantation comparing observed over expected mortality adjusted for case mix and center follow-up. \*Precision reflects increasing number of transplants per centre



**Fig. 5.2** Example funnel plot for 100-day mortality following allogeneic transplantation comparing observed over expected mortality adjusted for case mix and center follow-up. \*Precision reflects increasing number of transplants per centre



period, annual reports are issued reflecting center performance “benchmarked” against the broader EBMT returns with adjustment for case mix and follow-up.

Only centers reporting at least 80% of follow-up in transplants performed in the 5-year observed interval are included in the benchmarking analysis. Results are presented as “funnel plots” and other data outputs for autologous and allogeneic transplants. Results are highly affected by data completeness and quality of follow-up over the period. Examples of funnel plots are provided

below comparing “observed over expected” 1-year and 100-day survival/mortality (Figs. 5.1 and 5.2).

## 5.6 Activity of JACIE, Including Impact of Pandemic

JACIE is run on a nonprofit basis, resourced almost entirely on application fees. Fees depend on the configuration of the program and supplementary fees for additional sites and discounts



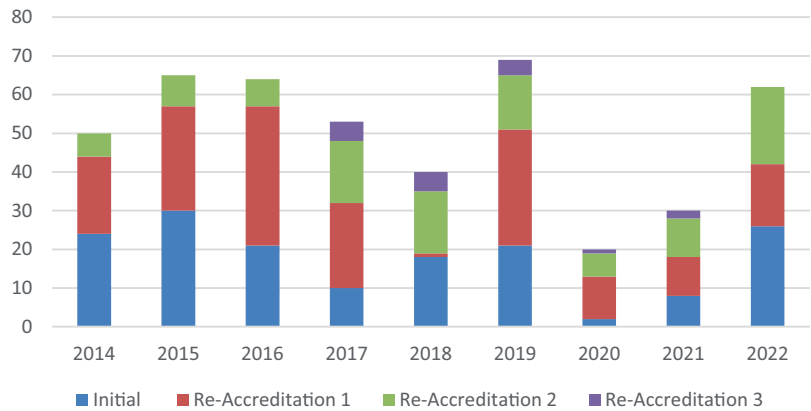
for active inspectors based at the applicant center are also applied (*see* JACIE website for details).

Over the last 25 years JACIE has carried out over 800 inspections and accredited over 700 centers in 30 countries across Europe and further afield with increasing numbers of centers going through the second and third reaccreditation cycle (Fig. 5.4). The Covid-19 pandemic brought the inspection activity to a halt in early 2020, and

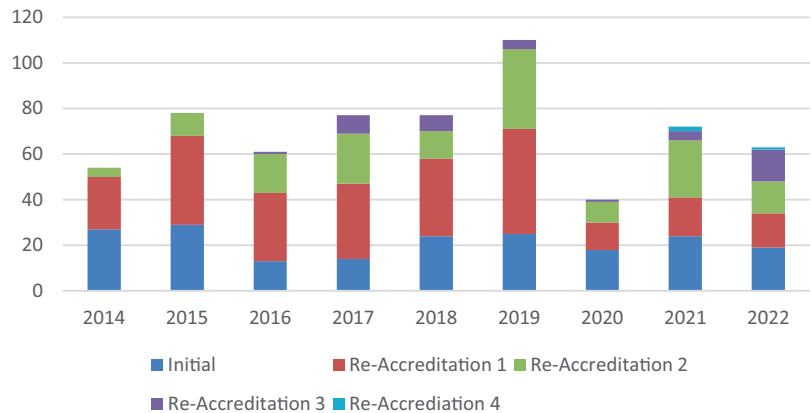
despite developing a virtual inspection model, the inspections did not restart until late 2021. The pandemic was also reflected in the number of applications and the accreditations during this period (Figs. 5.3 and 5.5).

JACIE accreditation is now mandatory in several European countries to apply for reimbursement of the procedure and/or to be authorized to perform HCT.

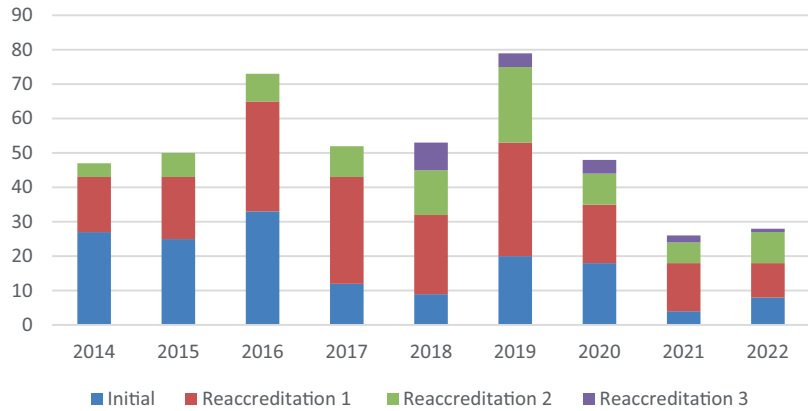
**Fig. 5.3** JACIE applications 2014–2022



**Fig. 5.4** JACIE Inspections 2014–2022



**Fig. 5.5** JACIE accreditations 2014–2022



### Key Points

- JACIE accreditation is based on an internationally-agreed quality standard system led and delivered by the professionals in the field of HCT and cell therapy.
- The standards are regularly updated, incorporating advances in the evidence base while reflecting the practical view of experienced experts on clinical and laboratory practice of HCT and cell therapy.
- Published data support a positive improvement in the clinical outcome related to the accreditation process, also promoting a progressive standardization of HCT practice across different countries.
- **JACIE continues to develop the standards to meet the changing needs of the evolving field in CAR-T and immune effector cells (IEC), “benchmarking” of patient survival and extending the access in LMIC countries through “stepwise” initiative.**

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# Statistical Methods in HCT and Cellular Therapies

# 6

Simona Iacobelli and Liesbeth C. de Wreede

## 6.1 Introduction

The analysis of data describing the outcomes of patients who have received an HCT is not only fundamental to assessing the effectiveness of the treatment but can provide invaluable information on the prognostic role of disease and patient factors. Thus, the appropriate analysis and understanding of such data are of paramount importance. This document provides an overview of the main and well-established statistical methods, as well as a brief introduction of more novel techniques. More insight is provided in the *EBMT Statistical Guidelines* (Iacobelli 2013). Additionally, the paper by de Wreede et al. (2022) explains the most important concepts and related pitfalls in the analysis of HCT studies.

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## 6.2 Endpoints

The outcomes most commonly studied in HCT analyses are the key events occurring at varying times post-HCT, e.g. engraftment, GVHD, relapse/progression and death. Besides the clinical definition of the event of interest, it is important to define the corresponding statistical endpoint and to use a proper method of measuring the occurrence of the event (Guidelines 2.1).

The main distinction is between events that occur with certainty during a sufficiently long observation period (follow-up), death in particular and events which are precluded from occurring once another event occurs, e.g. not all patients will experience a relapse of their disease because some die before. We define death without prior relapse (usually called NRM; see Guidelines 2.1.2) as the ‘competing event’ of relapse. The name ‘NRM’ is preferable to TRM, the proper analysis of which requires individual adjudication of causes of death.

**Standard survival endpoints:** In addition to death, other examples of events of the first type are the combinations of (negative) events of interest, which in total have 100% probability of occurrence, for example, PFS which considers as failure the event ‘either relapse/progression or death, whatever occurs first’. The components of PFS are the two competing events mentioned above, relapse/progression and NRM.

**Competing risks endpoints:** In addition to relapse/progression and NRM, other examples

are death of a specific cause and all intermediate events during an HCT history (engraftment, GVHD, achievement of CR, CMV infection) including the long term (diagnosis of secondary malignancy). Notice that the definition of an endpoint requires specifying which are the competing events. Usually, this will be death without prior event of interest, but depending on the disease and the aims of the analysis, other competing events might be included in the analysis, e.g. a second transplantation or other treatment can be considered as competing event for achievement of response.

### 6.3 Analysis of Time-to-Event Outcomes

Each event of interest may occur at variable times posttransplant, so in statistical terms, it has two components—whether it occurs at all and, if it does, when. However, at the end of the follow-up, there can be patients who have not yet had the event of interest but are still at risk for it: their observation times are called ‘censored’. Censoring occurs at different timepoints for different patients. The inclusion of censored data precludes the use of simple statistical methods such as the chi-square or T-test and requires the methods of survival (or competing risks) analysis. The crucial assumption of most methods in survival analysis is that the patients censored at a timepoint are ‘represented’ by those who remain under follow-up beyond that timepoint. In other words, the fact that a patient is censored should not indicate that their prognosis is worse or better than the prognosis of a similar patient who remains under observation. This assumption is called ‘independent and uninformative’ censoring.

#### 6.3.1 Kaplan-Meier Curves

The main method to summarize survival endpoints is the Kaplan-Meier curve (Kaplan and Meier 1958), estimating for each point in time  $t$

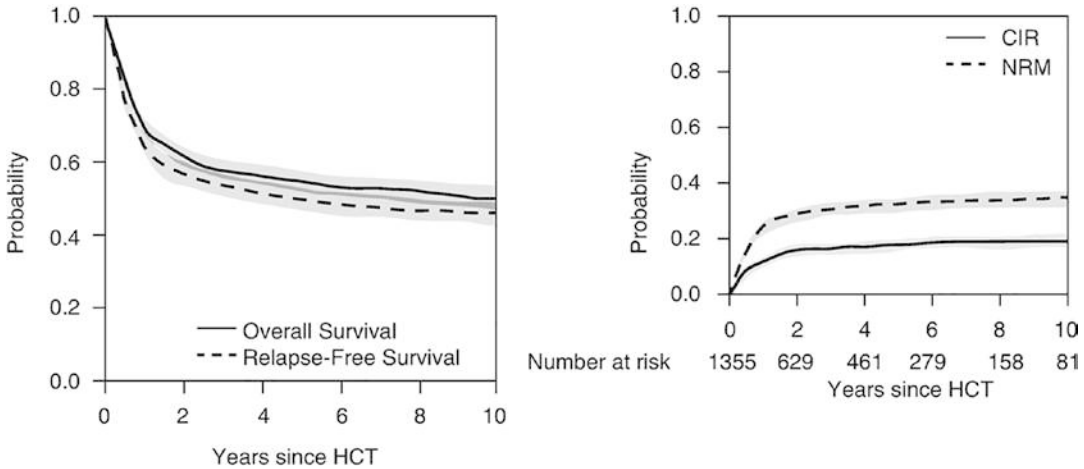
after HCT the probability  $S(t)$  of surviving beyond that time. This curve is decreasing from 100% and will reach 0% with complete follow-up. A long flat tail of the curve (often called ‘plateau’) is often based on a few censored observations at late times, corresponding to very unreliable estimates of the long-term survival. It is useful to report each  $S(t)$  with its 95%CI (confidence interval at 95% level, best obtained using the Greenwood formula) or at least the number of patients still at risk at different timepoints. The median survival time is the minimum time when  $S(t)$  is equal to 50% (Fig. 6.1).

#### 6.3.2 Cumulative Incidence Curves

The appropriate method to summarize endpoints with competing risks is the cumulative incidence curve (Gooley et al. 1999), estimating for each point in time  $t$  the probability  $F(t)$  of having had the event of interest before that time. This curve is increasing from 0% and will not reach 100% even with complete follow-up if the competing event was observed for some patients. It is always useful to interpret cumulative incidence curves of competing events together, to understand, e.g. when a category of patients has a small risk of relapse, if this means that they have a good prognosis or that they died too early from complications to experience a relapse (shown by a high NRM curve) (Fig. 6.1).

#### 6.3.3 Comparison of Groups

The main method to compare survival curves for two or more independent groups is the log-rank test. This test is based on the comparison of the underlying hazard functions, which express the instantaneous probability of the event at a time  $t$  among patients currently at risk. It has good properties in the situation of proportional hazards (PH, described in the next section), but it should be avoided (or considered carefully) when the survival curves cross; with converging curve



**Fig. 6.1** Probability curves of the four main outcomes after HCT. CIR cumulative incidence of relapse. CIR and NRM add up to 1-RFS. Number at risk indicates the num-

ber of patients in follow-up who have not yet experienced an event. The grey zones indicate 95% confidence intervals

alternatives like the Wilcoxon signed-rank test should be preferred.

In the comparison of cumulative incidence curves, the main method is the Gray test. Also the log-rank test can be applied to compare groups in the case of competing risks, when the object of interest is not the cumulative probability of occurrence of the event but its instantaneous probability among the cases at risk at each time, which is called ‘cause-specific hazard’. For the interesting difference of the two approaches to the analysis of competing risks endpoints, see Dignam and Kocherginsky (2008).

We refer to Sects. 1.3 and 1.4 of the guidelines for remarks on statistical testing and about proper settings for comparisons of groups. Importantly, the simple methods described in this chapter can be applied only to groups defined at or before the time origin (e.g. transplantation); assessing differences between groups defined during the follow-up requires other approaches, as those described in Sect. 6.4.1 (Guidelines page 14).

### 6.3.4 Proportional Hazard Regression Analysis

The above tests do not give a summary measure of the difference in outcomes between

groups, nor can they be used when the impact of a continuous risk factor (e.g. age) has to be assessed. Furthermore, any comparison could be affected by confounding. These limitations are typically overcome by applying a (multi-variable) regression model. The one most commonly used for survival endpoints is the proportional hazard (PH) Cox model (Cox 1972). Results are provided in terms of hazard ratios (HR), which are assumed to be constant during the whole follow-up (Guidelines 4.3.1). The Cox model in its simplest form is thus not appropriate when a factor has an effect that strongly decreases (or increases) over time, but time-varying effects can be accommodated for in more complex models. Effects of characteristics which change during follow-up can be assessed by including them as time-dependent covariates.

For endpoints with competing risks, two methods can be used, which have a different focus: the Cox model can be used to analyse cause-specific hazards, whereas a regression model for cumulative incidence curves was proposed by Fine and Gray (1999).

The use of these regression models requires a sound statistical knowledge, as there are many potential difficulties with the methods both in application and interpretation of results.

## 6.4 Advanced Methods

Many more advanced methods than the ones described above exist that help to get more insights from the available data. A good application of these needs expert statistical knowledge. The brief introductions given below are primarily meant to help understanding papers where these methods have been applied. For a more in-depth discussion, see, e.g. Therneau and Grambsch (2000).

### 6.4.1 Multi-State Models

The methodology of multi-state models (Putter et al. 2007) has been developed to understand the interplay between different clinical events and interventions after HCT and their impact on subsequent prognosis. Their primary advantage is that sequences of events, such as HCT, DLI, GVHD and death, and competing events, such as relapse and NRM, can be modelled simultaneously (see Fig. 6.2 for an example). This is in contrast to analysing composite survival outcomes such as GVHD-free survival where all failures are combined and resolution of GVHD is not considered. Some studies applying this method that offer new insights into the outcomes after HCT are Klein et al. (2000) about current leukaemia-free survival, Iacobelli et al. (2015) about the role of second HCT and CR for MM patients and Eefting et al. (2016) about the evalu-

ation of a TCD-based strategy incorporating DLI for AML patients.

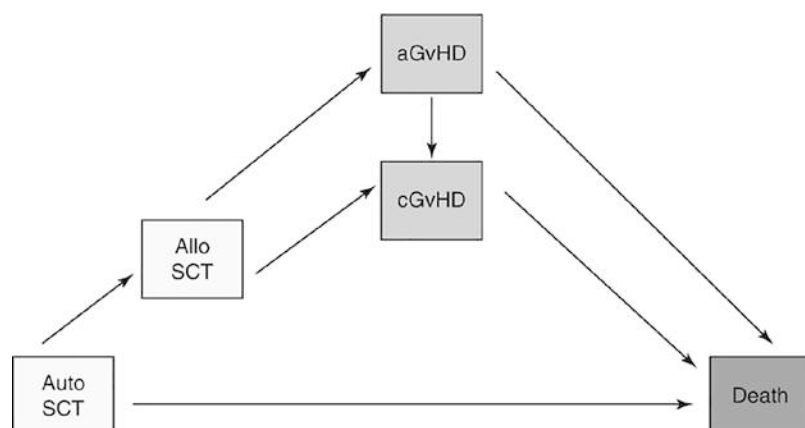
### 6.4.2 Random Effect Models

In standard methods, all patients are considered as independent, and each patient only contributes one observation for each endpoint. There are, however, situations when this does not hold, for instance, when patients within the same centre tend to have more similar outcomes than those from another centre or when one patient can experience more than one outcome of the same kind, e.g. infections. In these cases, the outcomes within one ‘cluster’ (a centre or a patient) are more correlated than the outcomes between clusters, which has to be accounted for in the analysis. This is usually done by random effect models, which assume that each cluster shares an unobserved random effect. In survival analysis, these are called frailty models (Therneau and Grambsch 2000, Chap. 9). If the outcome is not an event but a value measured over time, e.g. CD8 counts, the appropriate regression models are mixed models and joint models (Baart et al. 2021).

### 6.4.3 Long-Term Outcomes: Relative Survival/Cure Models

With improved long-term outcomes and increasing numbers of older patients, a substantial num-

**Fig. 6.2** Example of a multi-state model. All patients start in state 1 (event-free after HCT). They can then proceed through the states by different routes. Each arrow indicates a possible transition



ber of patients will die from other causes than the disease for which they have been transplanted and the direct and indirect consequences of its treatment. This so-called population mortality can be quantified by methods from relative survival, using population tables describing mortality of the general population (Pohar Perme et al. 2016).

Especially for transplanted children, a period with a high risk of mortality can be followed by a very long and stable period where the death risk is (almost) zero. When the focus of an analysis is on the probability of long-term cure, cure models can be used that assess the impact of risk factors on this but only if follow-up is sufficiently long (Sposto 2002).

#### 6.4.4 Propensity Scores

Propensity scores (PS) are useful to compare the outcomes of two treatments in the absence of randomization, to control confounding due to the fact that usually the choice of the treatment depends on patient's characteristics (confounding by indication) (Rosenbaum and Rubin 1983). First, the PS, defined as the probability of receiving one treatment instead of the other, is estimated for each patient. Then PS can be used in various ways (mainly stratification or pair matching), allowing comparison of treatment outcomes among cases with a similar risk profile.

#### 6.4.5 Methods for Missing Values

Missing values in risk predictors are a common problem in clinical studies. The simplest solution is to exclude the patients with missing values from the analysis (CC [complete case analysis]). This solution is not optimal, however: firstly, not all information is used (an excluded patient might have other characteristics known), and secondly, this approach can lead to bias if patients with missing values have on average a different outcome from the patients with observed values.

If values can be imputed on the basis of observed values in the dataset, these patients can be retained in the analysis to increase precision of estimates and (under certain conditions) avoid bias. The replacement of all missing values of a variable by a single value, e.g. its mean or mode if categorical, should be avoided; instead multiple imputation (MI) can be used. MICE (White et al. 2011) is the most common approach for MI; another method to consider more suitable for Cox models is called SMC-FCS (Bartlett et al. 2015). A major advantage of MI is that it properly takes into account the uncertainty caused by the imputation in the estimates. For practical advice on when and how to use MI, see Carpenter and Smuk (2021). If data are missing not at random—meaning their values cannot be predicted from the observed variables—multiple imputation can at most decrease the bias but not fully remove it.

##### Key Points

- Survival and competing risk endpoints need specific methods.
- Survival analysis methods: Kaplan-Meier, log-rank test and Cox model.
- Competing risks methods: cumulative incidence curve, Gray test, Cox model and fine and Gray model.
- Including events/changes of status occurring during follow-up in an analysis requires specific (advanced) methods, like multi-state models.

**Acknowledgements** We thank Myriam Labopin, Richard Szydlo and Hein Putter for their contributions to this chapter.

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## **Part II**

### **Biological Aspects**

**Topic Leaders: Chiara Bonini, Raffaella  
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# Biological Properties of Hematopoietic Stem Cells: Scientific Basis for Hematopoietic Cell Transplantation

Alessandro Aiuti, Serena Scala, and Christian Chabannon

## 7.1 Introduction

Hematopoiesis—from the Greek term for “blood making”—is the adaptive process by which mature and functional blood cells are continuously replaced over the entire lifetime of an individual. Erythrocytes, platelets, and the various subsets of leukocytes all have finite although different life spans. As a consequence, the daily production of red blood cells, platelets, and neutrophils under homeostatic conditions amounts to more than 300 billion cells.

In mammals, after the emergence of the first hematopoietic progenitors in extraembryonic structures such as the yolk sac in mice, cells of hematopoietic nature are first detected in the

aorto–gonado–mesonephric (AGM) region of the developing embryo, where they derive from the endothelium (Costa et al. 2012; Yvernogeu et al. 2020; Zhu et al. 2020). The site of hematopoiesis then moves to the fetal liver and next to the bone marrow (BM) where it remains established until the death of the individual. In humans, extramedullary hematopoiesis denotes a myeloproliferative syndrome.

The considerable knowledge accumulated over more than a century of experimental hematology has led to the early understanding that all hematopoietic lineages derive from a small subpopulation of undifferentiated and self-renewing stem cells. Hematopoietic stem cells (HSCs) represent the most accurately explored model of somatic stem cells that are present in most if not all tissues and organs, contributing to tissue homeostasis and repair. The existence of a population of HSCs also has practical implications in terms of developing innovative therapies aiming at the definitive replacement or enhancement of a function in cells from one or several hematopoietic lineages, including the possibility of establishing durable hematopoietic chimerism in recipients of allogeneic hematopoietic cell transplantation (HCT).

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## 7.2 Self-Renewal

A general property of stem cells is self-renewal, assuming that when these cells divide, at least one of the “daughter cells” fully recapitulates the

biological properties of the “mother stem cell.” Self-renewal of the HSC population prevents exhaustion while the hematopoietic tissue proliferates and differentiates extensively under steady-state conditions to repair various damages. Demonstration of self-renewal at the clonal level remains an arduous task, even though high-throughput analytical tools have been developed. There is a growing body of evidence suggesting aging of the HSC population and decline of stem cell function with age (de Haan and Lazare 2018; Goodell and Rando 2015; Hammond et al. 2023). Appearance of “passenger mutations” in clonal hematopoiesis is one hallmark of aging (Cooper and Young 2017; Xie et al. 2014). Recent lines of evidence have suggested links between lifestyle and aging through direct or indirect mechanisms as well as opportunities for therapeutic interventions in order to slow the aging process (Kaastrup and Gronbaek 2021). The significance of such observations remains to be fully elucidated but obviously raises questions when it comes to soliciting elderly individuals to donate HSCs for the benefit of a related patient. Since increasing pieces of evidence suggest that younger age of the donors is associated with better overall survival, donor age has become an important variable in the selection of matched unrelated and haploidentical donors for allogeneic transplantation. However, it remains difficult to dissect the role of donor age in HSC functionality (i.e., hematopoietic and lymphoid reconstitution) vs. other graft components and variables influenced by age, which may impact engraftment, non-relapse mortality, and disease relapse (DeZern et al. 2021; Ciurea et al. 2020; Pruitt et al. 2023).

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### 7.3 Commitment and Differentiation: New Data Challenge the Historical View of Hematopoietic Hierarchy

The traditional view of HSC differentiation is a hierarchical representation of an inverted tree, where discrete and homogeneous populations

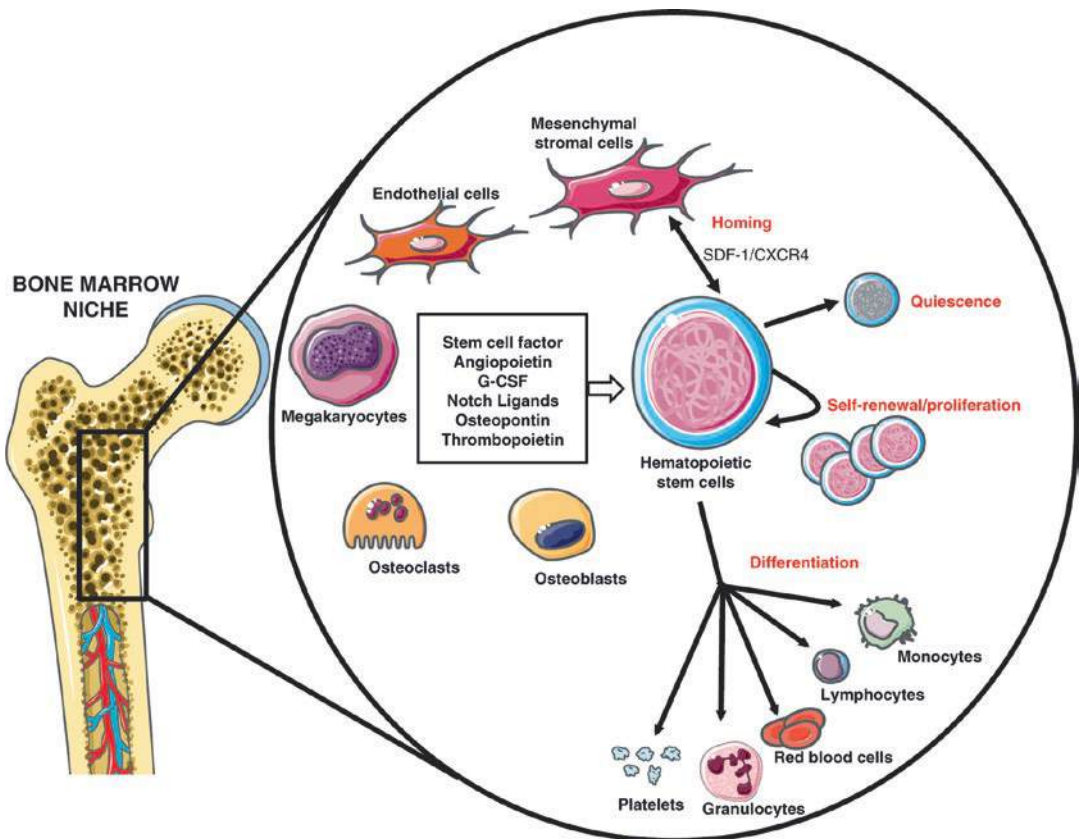
branch from one another, with successive restrictions in differentiation potentials. This oversimplifying view has been increasingly challenged by recent studies reporting on non-invasive genetic experiments and clonal analyses in mice (Busch and Rodewald 2016; Laurenti and Gottgens 2018). These studies suggest that hematopoietic differentiation uses different mechanisms under steady-state and stress conditions (Goyal and Zandstra 2015); however, both under steady-state conditions and in transplantation models, only a small fraction of HSCs contributes to long-term and stable reconstitution without compromising on the reestablishment of hematopoiesis (Hofer and Rodewald 2016; Schoedel et al. 2016), whereas most stem cells remain quiescent or proliferate infrequently. Single-cell transcriptional landscapes also suggest that differentiation occurs as a continuous rather than a discrete physiological process and that restriction of differentiation is not the result of a “symmetric split” between the myeloid and lymphoid compartments, as long believed by the phenotypic identification of “common myeloid progenitors” (CMPs) and “common lymphoid progenitors” (CLPs).

Commitment to one or several lineages, or conversely restriction in differentiation abilities, results from the expression of a controlled genetic and epigenetic program (Antoniani et al. 2017; Gottgens 2015; Pouzolles et al. 2016); these mechanisms remain partially understood and can thus only be partially harnessed for in vitro engineering of HSCs and their progeny (Rowe et al. 2016). Pathways vary during ontogeny, thus likely reflecting changes in these genetic and epigenetic programs (Keita et al. 2023). The fate of HSCs and their progeny is additionally regulated by extrinsic signals, among which hematopoietic growth factors and cytokines play an important role in survival, proliferation, and amplification (Kaushansky 2006). Experimental pieces of evidence also suggest that murine HSCs may directly sense external signals, such as from pathogens (Hysenaj et al. 2023).

## 7.4 The Bone Marrow Niches and Maintenance of Stemness

Recent years have witnessed considerable progress in our understanding of the organization and function of the bone marrow microenvironment (Fig. 7.1). HSCs establish interactions in the context of microanatomical organizations termed “niches.” Progress has been made both in understanding the heterogeneity of niches within successive hematopoietic sites and in identifying various categories of cells—either of non-hematopoietic or of hematopoietic origin—that interact with HSCs (Christodoulou et al. 2020). The various types of bone marrow niches closely associate with the neurovascular network that

infiltrates the central bone marrow and the endosteal region. The nature of the signaling between these different cell populations is also increasingly deciphered and involves many pathways, with some of them unexpected at first (Calvi and Link 2015; Crane et al. 2017). Replicating some of these interactions in vitro is key to successful expansion or genetic engineering of isolated HSCs. Among the many molecular actors that govern interactions between HSCs and the various cells present in niches, the C-X-C motif chemokine 12 (CXCL12) and its most important receptor CXCR4 are of particular interest: direct or indirect modulation of this axis is clinically used to amplify the compartment of circulating stem cells that exist in low numbers under steady-state conditions (Crees et al. 2023).



**Fig. 7.1** HSC properties and BM niche components

## 7.5 Preclinical Models of HCT

Most of the current knowledge on the biology of HSCs and on the therapeutic mechanisms of HCT derives from studies in animal models (Sykes and Scadden 2013; Eaves 2015). Classical murine transplantation studies have shown that single or few engrafting HSCs are sufficient and necessary to sustain long-term hematopoiesis in a reconstituted mouse. Human-in-mouse xenografts have become a fundamental tool to study hematopoietic dynamics upon HCT. The generation of immune-deficient mice bearing a deletion of the interleukin-2 receptor gamma chain on the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) background (NSG mice) was instrumental for studying HSC homing, engraftment, lineage differentiation, and serial transplantation capacity. This model has been further modified by introducing human myeloid cytokine genes to increase myeloid differentiation (Doulatov et al. 2012) or loss-of-function mutation in the KIT receptor to efficiently support engraftment of human HSCs without the need for conditioning therapy (Cosgun et al. 2014). To overcome the lack of human components in the murine BM, humanized BM niche systems, which are based on human stromal cells implanted on a specific scaffold or directly injected with the extracellular matrix to generate BM micro-ossicles, have been recently developed (Di Maggio et al. 2011; Reinisch et al. 2016). These strategies provide novel tools to study the behavior of human HSCs in their physiological context and to dissect the role of the niche upon transplantation. However, homing and engraftment parameters in xenografts may be different from the natural setting and most HCT models follow recipient mice for few months after transplantations, thus making long-term outcome difficult to assess.

Dogs provide an ideal preclinical modeling system for HCT studies due to their large body size, life span, and high genetic diversity, which more appropriately recapitulate the human scenario. Preclinical canine modeling has been fundamental for the clinical translation of conditioning regimens and the importance of

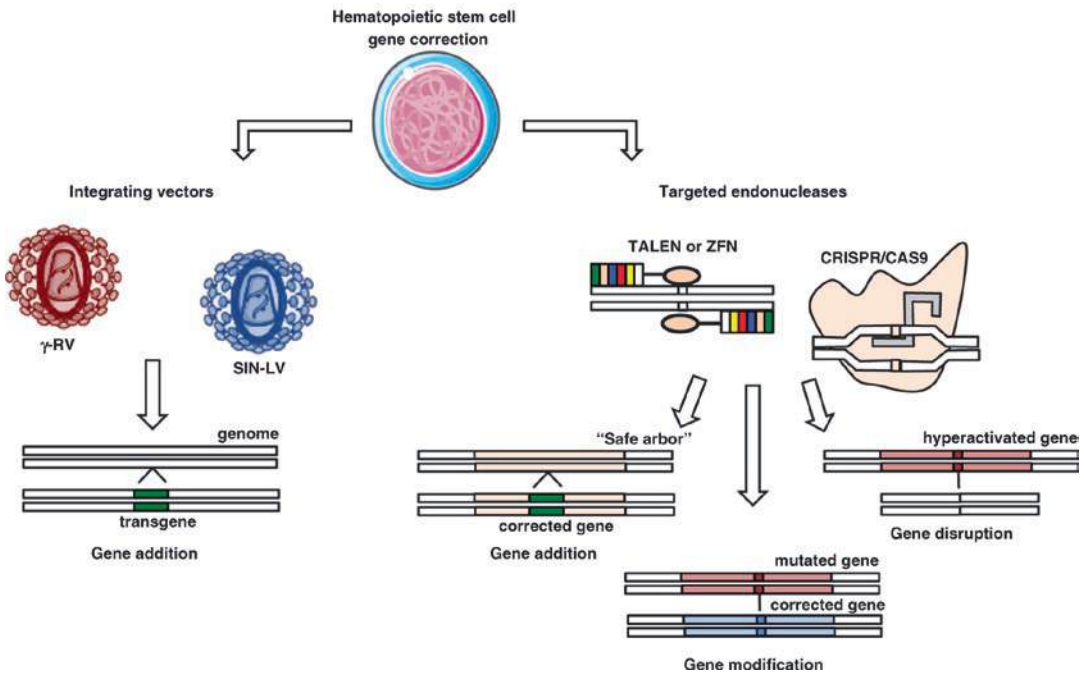
major histocompatibility complex (MHC) donor/recipient matching. However, the lack of canine reagents and the logistic difficulties of working with large animal models have precluded widespread availability (Stolfi et al. 2016). Autologous HCT in nonhuman primates (NHPs) is arguably the experimental model most closely resembling humans; their treatment conditions—including the use of CD34<sup>+</sup> cells, mobilization, and conditioning regimens—all parallel those commonly used in human transplantation. Although ethical issues and costs are increasingly limiting their availability and use to selected centers, these animals are able to maintain long-term hematopoiesis up to several years after transplantation, thus allowing the study of HCT dynamics in a close-to-human manner (Koelle et al. 2017).

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## 7.6 Gene Transfer/Gene Editing/ Gene Therapy (GT) Targeting HSCs

Ex vivo HSC gene therapy (GT) is based on the genetic modification of autologous HSCs to correct monogenic disorders or to provide novel features to hematopoietic cells for treating infectious diseases or cancers (Naldini 2011) (Fig. 7.2). It is now well-established that HSCs can be efficiently gene modified to continuously produce a cell progeny expressing the therapeutic gene while maintaining the ability to engraft in the long term, for at least 15 years (Cicalese et al. 2016). According to a recent meta-analysis, more than 400 patients have received hematopoietic stem and progenitor cell (HSPC) GT for the treatment of 14 genetic diseases, in the context of 55 clinical trials (Tucci et al. 2022). The potential advantages of this approach over allogeneic HCT include the lack of graft-versus-host disease (GVHD) or rejection and the possibility of engineering HSCs in order to achieve a supraphysiological level of the corrected protein (Naldini 2011; Cicalese et al. 2016; Tucci et al. 2022; Aiuti and Naldini 2016). Despite robust and consistent results in terms of long-term durability, efficacy, and safety of the treatment, there are differences in terms of gene correction in the





**Fig. 7.2** Gene correction of HSCs for cell-based therapies

intended populations and BM-transduced cell chimerism. The gene engineering platform, the stem cell source, the disease background, and the conditioning regimen might account for some of these distinctions.

Currently, integrating vectors derived from retroviruses represent the most efficient platform for engineering HSCs and providing permanent and heritable gene correction.  $\gamma$ -Retroviral vectors (RVs) have been used in many clinical applications, including GT of inherited immunodeficiencies and cancer therapy, but the use of  $\gamma$ -RVs is associated with risks of insertional mutagenesis due to activation of proto-oncogenes, also depending on the disease type (Cicalese et al. 2016; Tucci et al. 2022). Self-inactivating (SIN) lentiviral vectors (LVs) are currently the tools of choice for most of the HSC GT applications, given their ability to transduce nondividing cells at higher efficiency, to carry larger and more complex gene cassettes, and to display a safer insertion site (IS) pattern in human HSCs (Naldini 2011; Locatelli et al. 2022; Scala et al. 2023). The recent development of designer endonucleases has led to the advent of gene tar-

geting approaches. In contrast to viral vectors, which can mediate only one type of gene modification (gene addition), genome editing technologies can mediate gene addition, gene disruption, gene correction, and other targeted genome modifications (Dunbar et al. 2018; Ferrari et al. 2023). These strategies have the potential to overcome vector IS genotoxicity and to handle diseases due to dominant negative mutations and have started to enter clinical trials and practices (Frangoul et al. 2021). Despite the great promises, several challenges need to be addressed, including long-term clinical safety. Primitive, slow-cycling, human BM-derived HSCs are highly resistant to ex vivo manipulations required for gene targeting, and the current efficiency of gene editing by homology-directed repair into repopulating HSCs may not be suitable for clinical applications requiring high levels of correction (Dunbar et al. 2018; Kohn 2017).

In most cases, mobilized peripheral blood (MPB) has become the preferred HSC source for patients undergoing HCT and HSPC GT, also owing to the higher numbers of stem cells collected. Recent findings have shown that the

use of MPB HSCs is associated not only with faster neutrophil and platelet reconstitution but also with an overall increased clonality of the engrafted HSCs (Scala et al. 2023) and that a better characterization of the HSPC compartments has been more informative than the total CD34<sup>+</sup> cell dose.

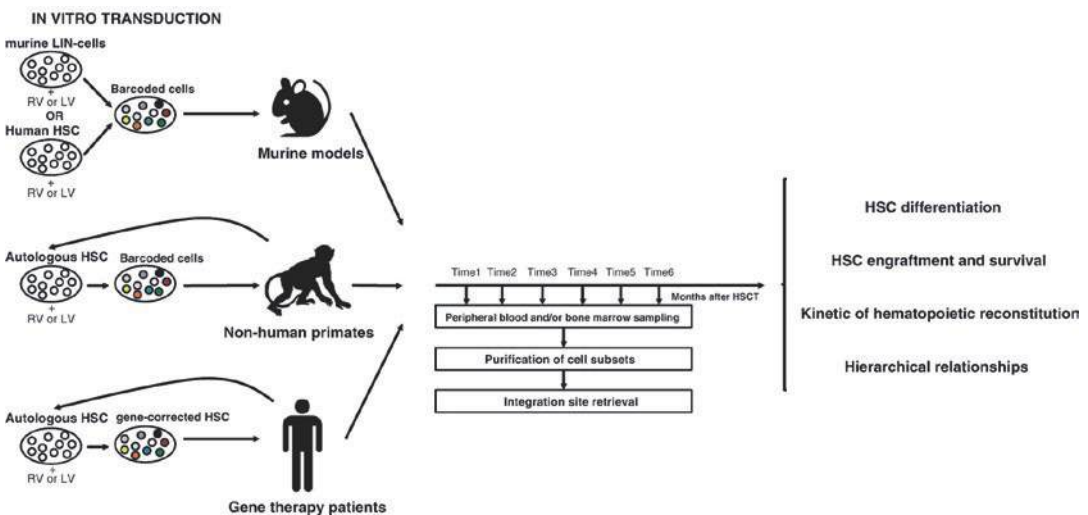
Despite these progress, a collection of HSCs still present challenges under certain pathological conditions with low HSC content, heavily treated patients, or low-body-weight pediatric subjects. In vitro expansion protocols are an attractive strategy to increase the quality and/or the amount of transplantable HSCs. High-throughput screening of chemical compounds has resulted in the identification of at least two promising molecules, namely, StemRegenin1 and SR1 (Wagner Jr. et al. 2016), and the pyrimidoindole derivative UM171 (Dumont-Lagace et al. 2021; Fares et al. 2014), which are able to achieve great expansion of long-term repopulating HSCs. The ideal drug or combination has yet to be reported, although encouraging results paving the way for non-conditioned or non-genotoxic cell transplants or cellular therapies have been reported in mice (Wilkinson et al. 2019; Srikanthan et al. 2020; Omer-Javed et al. 2022).

## 7.7 Studying the Dynamics of Hematopoietic Reconstitution Upon HCT

Upon gene correction, each transduced cell and its progeny becomes univocally marked by a specific IS. The analysis of RV or LV IS has emerged as one of the most effective strategies for tracing the activity of genetically engineered hematopoietic cells directly in vivo in animal models and in GT-treated patients. Retrieving the IS from mature blood cells after HCT allowed studying the kinetics of blood cell production from individual stem cells within a heterogeneous population (Scala et al. 2023) (Fig. 7.3).

In the murine setting, the finding that the vast majority of the ISs after transplantation were present in either lymphoid or myeloid cells with few ISs shared by both lineages led to the concept that murine HSCs are heterogeneous and already biased for their fate. The possibility of directly translating these models in human beings is currently under investigation (Lu et al. 2011; Yamamoto et al. 2013).

Clonal tracking studies in nonhuman primates have been pivotal in studying HCT dynamics in an experimental setting close to humans. The



**Fig. 7.3** Clonal tracking for studying the hematopoietic reconstitution dynamics upon HCT



results of these works showed a common pattern of hematopoietic reconstitution upon transplantation: clonal fluctuation in the early phases post-HCT, potentially due to the initial contribution to the hematopoiesis of short-term unilineage progenitors, followed by a recovery of a stable hematopoietic output, likely related to the takeover of long-term multipotent HSC contribution. Thus, differently from murine studies, long-term HSCs are able to provide multilineage engraftment and there is no evidence of a predetermined lineage choice at the stem cell level in primates (Koelle et al. 2017; Kim et al. 2014). Additionally, NHPs have been useful in interrogating the effect of older HSCs in HCT, showing reduced multilineage output and clonality of the graft in aged vs. young animals (Yu et al. 2018).

To date, few cutting-edge studies have used IS retrieval from GT-treated patients, allowing, for the first time, to study the complexity of the hematopoietic system and hematopoietic reconstitution upon HCT in humans (Scala et al. 2023; Biasco et al. 2016; Scala et al. 2018; Wang et al. 2010). These studies showed that transplanted gene-repaired HSCs are able to engraft and generate a polyclonal multilineage output overtime. Longitudinal analyses allowed unveiling that unilineage clones active during the first 6 months after GT tend to be replaced by multilineage long-term clones, indicating HSC-derived activity. Finally, based on the number of ISs recaptured overtime, it has been estimated that about 1 in  $10^5$ – $10^6$  infused gene-corrected cells have the potential to engraft in the long term. These approaches represent a prototypical example of the power of translational studies, providing information relevant to the human hematopoietic system, complementing and expanding the data derived from animal models.

Alternative approaches based on single-cell high-throughput analyses have also allowed to explore the dynamics of hematopoietic posttransplant reconstitution in human recipients and its relation to posttransplant clinical events (Huo et al. 2023).

## 7.8 From Experimental Hematology to Medical Practices and Hematopoietic Cellular Therapies

As already stressed in this brief review, a considerable amount of knowledge has been accumulated over years, thus allowing us to understand part of the mechanisms that control HSC behavior and take advantage of this knowledge; many of these observations have cross-fertilized other disciplines. However, a large gap persists between the technological sophistication of research tools and the rudimentary nature of clinical-grade reagents, devices, and laboratory tests. In clinical transplantation or even in the most modern forms of hematopoietic cellular therapies, stem cells remain identified as “CD34<sup>+</sup> cells,” which can at best be considered as a gross approach to stemness; functional assays are limited to clonogenic cultures in routine practice; flow cytometry-activated cell sorting has barely entered the clinical field, and most cell selection procedures rely on immune selection with magnetic beads. Despite these limitations, and as can be seen from the content of the other chapters in this book, HCT remains the only example of a worldwide and widely used cell transplant procedure, with many of its underlying conceptual aspects and techniques being used to design innovative and highly personalized somatic cell therapy or gene therapy medicinal products (Chabannon et al. 2018).

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# Biological Properties of Cells Other Than HSCs

# 8

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## 8.1 Introduction

The array of cellular players involved in the biology of hematopoietic cell transplantation (HCT) clearly extends beyond hematopoietic stem cells (HSCs) themselves and, in the case of transplantation from allogeneic sources, importantly includes cells of the innate and adaptive immune systems. Historically, the discovery of the human leukocyte antigen (HLA) system and the functional characterization of the different immune cell types had a transformational impact on our current understanding of the pathobiological “sequelae” of allo-HCT (rejection, graft-versus-host disease (GVHD), graft-versus-leukemia

(GVL) effect). This body of knowledge coupled to the most recent “exploitation” of biotechnology has allowed us to design strategies for in vivo stimulation or adoptive transfer of specific immune cell types with the potential to dramatically improve transplantation outcome.

In this chapter, we will review the biological properties of cells other than HSCs, which have so far been therapeutically investigated in human allo-HCT. Since, apart from vaccination, antigen-presenting cells and myeloid cells at large have seldom been therapeutically investigated in human allo-HCT, they will not be discussed here. Conversely, we will briefly touch on mesenchymal stromal cells (MSCs), which, although not classifiable as immune cells “stricto sensu,” have been widely employed in allo-HCT.

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## 8.2 Conventional or Alpha Beta T Cells

The majority of mature T cells is characterized by the expression of the  $\alpha\beta$  T-cell receptor (TCR), which endows major histocompatibility class (MHC)-restricted recognition of peptides derived from non-self-proteins. The mutually exclusive co-expression of CD8 or CD4 further conveys specificity toward MHC class I/MHC class II/peptide complexes, respectively. CD8<sup>+</sup> T cells recognize intracellular peptides, mainly derived from viruses or mutated genes, mediating the cytotoxicity of infected or transformed cells, and

thence the name cytotoxic T lymphocytes (CTLs). Conversely, CD4<sup>+</sup> T cells recognize extracellular pathogen-derived peptides, providing antigen-specific “help” to bystander immune cells, such as B cells in antibody production and phagocytes in the killing of engulfed pathogens. Alloreactivity occurs because of  $\alpha\beta$  TCR-mediated recognition of mismatched HLAs or of non-HLA polymorphic peptides presented in the context of matched HLAs, e.g., those derived from H-Y (male-specific histocompatibility antigen). The latter are known as minor histocompatibility antigens (mHags) and play a major role in GVHD and the GVL effect after HLA-matched transplantation.

The adoptive transfer of CTLs specific toward important opportunistic viruses in allo-HCT (cytomegalovirus (CMV), Epstein–Barr virus (EBV), adenovirus (ADV)) has been one of the first manipulated cellular immunotherapies to be tested in humans (Bollard and Heslop 2016) and in some European Union (EU) countries is now available as an off-the-shelf therapy from HLA-matched donors. Conversely, it has been proposed that naïve T cells, i.e., cells that have never encountered their cognate antigen, may be more alloreactive than memory T cells, i.e., antigen-experienced cells that have persisted even after clearing the infection. This concept is at the basis of protocols for the depletion of naïve T cells from the graft as a way to prevent GVHD while retaining a strong GVL effect (Bleakley et al. 2015). Promising are also attempts at translating this approach against hematological tumor antigens for treating overt leukemia relapse after allo-HCT (Chapuis et al. 2013). On a different page, given the overall complexity of immune responses, it is not surprising that during evolution, some immune cell types have evolved with the specific task of immune regulation. T regulatory cells (Tregs) are thymus-derived cells characterized by constitutive expression of the transcription factor FoxP3. Tregs are potent

suppressors of alloreactivity and are now being investigated for GVHD management after their ex vivo expansion (Brunstein et al. 2016).

### 8.3 Unconventional T Cells

Unconventional T cells are subsets of T cells, which often reside at mucosal sites and sense a wide range of non-polymorphic ligands, especially of bacterial origin. They include (but are not limited to) TCR $\gamma\delta$  T cells, invariant natural killer T cells (iNKT) cells, and mucosal-associated invariant T (MAIT) cells. They have a limited TCR repertoire diversity and get activated quickly, bridging innate to adaptive immunity.

1. High  $\gamma\delta$  T cells after HCT are associated with a favorable outcome (Arruda et al. 2019). In TCR $\alpha\beta$ /CD19-cell depleted haplo-HCT,  $\gamma\delta$  T cells are a dominant subset, accounting in part for a GVL effect (Airoldi et al. 2015). A subset of  $\gamma\delta$  T cells (V $\gamma$ 2V $\delta$ 9) is activated by phosphoantigens and can be safely expanded in vivo by the bisphosphonate zoledronate (Merli et al. 2020). In addition, while the V $\gamma$ 9V $\gamma$ 2 subset usually predominates, patients reactivating CMV showed an expansion of the V $\gamma$ 1 subset (Ravens et al. 2017).
2. Type I invariant NKT is a distinct population of semi-invariant  $\alpha\beta$  T cells that recognize lipids presented in the context of broadly distributed CD1d. An early iNKT reconstitution has been linked to a reduced GVHD incidence (Rubio et al. 2012; Chaidos et al. 2012). A GVL potential has been reported in pediatric leukemia patients given haplo-HCT (de Lalla et al. 2011).
3. MAIT cells (CD3<sup>+</sup>CD4<sup>-</sup>CD161<sup>high</sup>) are abundant in mucosal tissues, display a repertoire of limited diversity, and recognize bacterial metabolites. Their reconstitution positively correlated with the diversity of the gut microbiota. Several studies have



reported an association between low circulating MAIT cell counts and GVHD (Bhattacharyya et al. 2018; Ben Youssef et al. 2018).

## 8.4 NK Cells

Natural killer (NK) cells belong to the innate immune system and provide immediate reactivity against virally infected tumor targets. NK cytotoxicity is controlled by a balance between several germ line-encoded inhibitory and activating receptors, such as killer immunoglobulin-like receptors (KIRs) and natural cytotoxicity receptors (Vivier et al. 2011). The importance of NK cells in allo-HCT has surfaced after the demonstration of their pivotal role in preventing leukemia relapse and decreasing GVHD risk after grafting from HLA-haploidentical donors (Ruggeri et al. 2002). Since then, there has been a growing interest in using both autologous and allogeneic NK cells in patients with leukemia or other high-risk hematological tumors also in the non-transplant setting (Koehl et al. 2016). These trials have uniformly shown the safety and potential efficacy of infused NK cells. Nevertheless, they have also documented the emergence of powerful immune escape mechanisms, raising the question on how to improve NK cell-based therapies (Koehl et al. 2018). Various trials are under way in order to investigate ways to achieve better NK cell cytotoxicity and overcome the immunosuppressive tumor microenvironment, including:

1. Combination of novel checkpoint inhibitors with activated NK cells
2. Bi- or tri-specific antibodies for directly binding NK cells to cancer cells
3. Chimeric antigen receptor (CAR)-modified NK cells for direct targeting of cancer cells

The latter strategy is particularly interesting since CAR-modified NK cells are expected to retain their natural antitumor reactivity, thus exerting potentially synergistic effects. The first clinical CAR-modified NK cell studies targeting

CD19 and NKG2D ligands have been initiated (ClinGov Nos NCT03056339, NCT01974479, NCT00995137, NCT03415100) with promising initial results (Liu et al. 2020) and will likely be instrumental in demonstrating proof of concept.

## 8.5 Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) are multipotent cells. In the musculoskeletal system, MSCs are responsible for generating bone cells, cartilage cells, and other cell types. Since many of these cells are derived from the embryonic mesenchyme, the name “mesenchymal stromal cells” was coined. Additional investigations revealed complex modes of action beyond the formation of individual cell types: secretory, anti-inflammatory, hematopoietic stem cell niche-supporting and immunomodulatory properties of MSCs and their ability to migrate to sites of damage and inflammation (Wilson et al. 2019). Based on these activities, graft-versus-host and autoimmune diseases, neurological conditions, cancers, or other diseases are addressed by MSC-based therapies. Many organ systems are targeted, and the potential clinical use of MSCs seems enormous. Therefore, subsequent to hematopoietic stem cells, MSCs are the second-most frequently used cell source for cell therapeutic applications. Notwithstanding their widespread use, MSCs are currently the stem cell population with the least defined identity and properties (Hoffmann et al. 2017). Despite the many promising reports, a multitude of clinical trials with MSCs have failed and there is a rising perception that MSCs might present “doubtful drugs” (Sipp et al. 2018).

In the majority of studies, mononuclear cells, including the rare MSCs, are isolated from bone marrow by a density gradient or from solid tissues by enzymatic digestion and explant cultures. A small fraction of the isolated cells is able to adhere to cell culture polystyrene: a retrospective and nonspecific isolation resulting in heterogeneous cell populations. The adherent cells are expanded in two-dimensional static cultures and characterized by their morphology, proliferation (interpreted as self-renewal), a pattern of cell sur-

face antigens, and the forced differentiation *in vitro* into mesenchymal cell types (taken as evidence for multipotent differentiation). These features do not withstand rigorous assessments of stem cell features.

Contrasting with this, pioneering studies have prospectively isolated stromal cells from the bone or bone marrow based on the specific presence (positive selection) or absence (negative selection) of selected cell surface antigens and clearly demonstrated their central stem cell features of self-renewal and differentiation *in vivo* (Tikhonova et al. 2019; Leimkühler et al. 2021; Crisan et al. 2008; Sacchetti et al. 2007; Chan et al. 2018), without preceding cell culture. From their data, the notion emerges that different populations of stem cells may exist in different compartments of the bone, bone marrow, or—if at all—in other tissues. For example, these studies point at cell surface antigens that have not yet been considered as “typical” MSC surface molecules, like CD146, PDPN, and CD164. Based on such improved cell isolation strategies, single-cell RNA sequencing revealed different subpopulations of MSCs (Tikhonova et al. 2019; Leimkühler et al. 2021; Ruoss et al. 2021; Xie et al. 2022). A critical reappraisal of these different cell populations, harmonization of the methods for their isolation and expansion, including novel strategies to mimic the *in vivo* stem cell niche by three-dimensional dynamic and hypoxic *in vitro* culture systems, and a clear description of the anticipated mode of action, including the development of validated potency assays, is therefore necessary for harnessing the full therapeutic potential of MSCs in the future (Lavrentieva et al. 2020).

### Key Points

- HCT rather than a solo play is an orchestral concert, where different cellular players contribute to the overall final result of the symphony.
- Besides obviously HSCs, the key contributors are cells of the innate and adaptive immune systems. Both have evolved for the key task of self/non-self-

discrimination, with each however focusing on the recognition of different classes of molecules, from proteins to glycolipids.

- The tremendous knowledge in immunobiology acquired in the last few decades has enabled the utilization of the properties of these cells or the amelioration of the outcome of HCT.

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# Histocompatibility

# 9

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## 9.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) has revolutionized the treatment of many hematological and oncological disorders, offering a potential cure for patients with otherwise fatal diseases. However, successful transplantation depends on the compatibility of polymorphic human leukocyte antigens (HLAs) between the donor and the recipient. HLA molecules are critical components of the immune system, responsible for presenting antigenic peptides to specific immune receptors, thereby triggering both pathogen-specific and alloreactive responses. The latter mediates not only graft-versus-host disease (GVHD), a major complica-

tion occurring after allo-HCT, but also the beneficial graft-versus-leukemia (GVL) effect.

The first indications for a major histocompatibility complex (MHC) were provided by the work of Peter Gorer in 1936, as a result of his studies in mice (reviewed by Thorsby (2009)). Subsequently, immune-mediated rejection of tissue allografts was described in 1945 by the British immunologist Peter Medawar, followed by the discovery of the MHC carrying the histocompatibility genes by George Snell in 1948 and of the human leukocyte antigen (HLA) molecules by Jean Dausset, Jon van Rood, and Rose Payne a decade later (reviewed by Thorsby (2009)). The importance of these discoveries was recognized by the Nobel Prize awarded in physiology and medicine to Medawar in 1960 and Benacerrat, Snell, and Dausset in 1980, respectively. Since then, the MHC has emerged as the single most polymorphic gene locus in eukaryotes, with 36,263 HLA alleles reported to date in the international ImMunoGeneTics information system/HLA (IMGT/HLA) database (release 3.52, (<https://www.ebi.ac.uk/ipd/imgt/hla/about/statistics/>). Accessed 9 May 2023; Barker et al. (2023)).

The concept of HLA matching in transplantation was first proposed in the 1960s, when it became apparent that HLA antigens played a critical role in the recognition and rejection of foreign tissues. The early attempts at HLA matching were based on serological methods, which relied on the detection of antibodies that bind to HLA molecules. However, this approach was

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limited by the low resolution of serological typing and the high frequency of HLA alleles in the human population.

Over the past few decades, advances in molecular biology and genomics have revolutionized HLA typing, allowing for a more accurate and comprehensive matching of donors and recipients, thereby greatly contributing to increased safety and feasibility of allo-HCT in recent years (Gooley et al. 2010; Penack et al. 2020). Today, HLA typing is typically performed by high-throughput next-generation sequencing (NGS), allowing for unambiguous high-resolution typing in most cases (Cornaby et al. 2021).

HLA matching has become a critical component of allo-HCT, with studies showing that better HLA matching leads to improved outcomes and lower rates of GVHD. However, achieving perfect HLA matching is not always possible, particularly for patients from ethnic or racial minority groups with limited donor options. In these cases, partially HLA-mismatched donors, in particular haploidentical relatives or mismatched unrelated donors (UDs), are used with increasing clinical success (Kanakry et al. 2016; Slade et al. 2017, Shaw et al. 2021), in addition to umbilical cord blood (UCB) transplantation with frequent HLA mismatches (Ballen et al. 2013). Moreover, non-HLA polymorphisms have also been recognized as important players, in particular minor histocompatibility antigens (mHAgs), killer immunoglobulin-like receptors (KIRs), and other polymorphic gene systems (Dickinson and Holler 2008; Gam et al. 2017; Heidenreich and Kröger 2017; Spierings 2014).

In this chapter, we will provide an overview of HLA matching in the context of allo-HCT, including the history of HLA typing, the clinical importance of HLA matching, and the current methods for HLA typing and matching. We will also discuss the challenges and limitations of HLA matching and the alternatives available for patients who cannot find a perfectly matched donor.

## 9.2 The Biology of Histocompatibility

### 9.2.1 Major Histocompatibility Antigens

The human MHC spans about 4 million base pairs of DNA on the short arm of chromosome 6 (6p21.3) and contains approximately 260 genes, many of which with immune-related functions (Trowsdale and Knight 2013). The MHC can be divided into three main regions—classes I, II, and III—which contain HLA-A, HLA-B, and HLA-C as well as HLA-DR (HLA-DRA1, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5), HLA-DQ (HLA-DQA1 and HLA-DQB1), and HLA-DP (HLA-DPA1 and HLA-DPB1), respectively, and complement factor as well as tumor necrosis factor (TNF) genes. MHC genes are codominantly expressed and follow Mendelian inheritance patterns, resulting in a 25% probability for two siblings to be HLA-identical or to have inherited the same MHC from both parents. An additional feature of the MHC is the nonrandom association of alleles at different HLA loci, known as linkage disequilibrium (LD), and relatively high recombination rates exceeding 1%, also referred to as “crossing over” (Martin et al. 1995).

### 9.2.2 Structure and Function of HLA Class I and II Molecules

HLA class I and II molecules are immunoglobulins (Igs) found on the surface of cells that present peptides in their highly polymorphic antigen-binding groove (Madden 1995). HLA class I proteins, encoded in the classical HLA-A, HLA-B, and HLA-C loci, consist of heterodimers of a polymorphic  $\alpha$ -chain and a monomorphic  $\beta$ 2 microglobulin, with molecular weights of 45 kDa and 12 kDa, respectively. The  $\alpha$ -chain has three hypervariable Ig-like domains, with the  $\alpha$ 1 and  $\alpha$ 2 domains forming the antigen-binding

groove, the  $\alpha 3$  domain contacting the CD8 coreceptor on T cells, and a transmembrane region anchoring the molecule to the cell membrane. HLA class I is expressed on all healthy nucleated cells and presents peptides, which are protein fragments of mostly intracellular origin generated through proteasomal cleavage and transported to the endoplasmic reticulum via the transporter associated with antigen processing (TAP; Vyas et al. (2008)). Cell surface HLA class I peptide complexes can be recognized by the T-cell receptor (TCR) of CD8<sup>+</sup> T cells, leading to the activation of cytotoxic and/or cytokine effector functions, or by KIRs on natural killer (NK) cells, leading to the inhibition or activation of effector functions (Heidenreich and Kröger 2017). HLA class II proteins, encoded by the classical HLA-DR, HLA-DQ, and HLA-DP loci, consist of heterodimers of an  $\alpha$ - and a  $\beta$ -chain of approximately 30 kDa each. Despite the different composition, the overall structure of the heterodimeric HLA class II proteins is highly similar to that of the HLA class I proteins. While the peptide-binding groove involves the membrane-distant  $\alpha 1$  and  $\beta 1$  domains, the region contacting the CD4 coreceptor on T cells is located in the membrane-close domains. Both chains anchor to the cell membrane with their respective transmembrane parts. For HLA-DR, the polymorphisms are mostly clustered in the  $\beta$ -chain Ig-like domain forming the antigen-binding groove, i.e., the  $\beta 1$  domain. For HLA-DQ and HLA-DP, both the  $\alpha$ - and  $\beta$ -chains are polymorphic, with an increased level of polymorphism in the  $\alpha 1$  and  $\beta 1$  domains. HLA class II proteins are constitutively expressed on professional antigen-presenting cells, such as B cells, macrophages, and dendritic cells, and can be upregulated on various cell types by pro-inflammatory cytokines, such as interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$ . HLA class II molecules present peptides generally of extracellular origin generated through protein degradation in the phagolysosome (Vyas et al. 2008). Peptide loading onto HLA class II molecules occurs in the dedicated MHC (MHC class II) compartment and is cata-

lyzed by two nonclassical HLA molecules that are also encoded in the MHC, i.e., HLA-DM and HLA-DO. HLA class II peptide complexes on the cell surface can be recognized by CD4<sup>+</sup> T cells, leading to the activation of cytokine-mediated helper or regulatory functions. To date, only a single receptor on NK cells with binding activity to HLA class II, the activating NKp44 engaged by a subset of HLA-DP allotypes, has been described (Niehrs et al. 2019).

### 9.2.3 HLA Polymorphism and Tissue Typing

HLA molecules were first detected by serological methods, through the ability of the sera from sensitized individuals to agglutinate some but not all leukocytes (hence the term “human leukocyte antigen”) (Thorsby 2009). Until the mid-1990s, serological typing was the main method of tissue typing. With the advent of polymerase chain reaction (PCR) techniques, molecular tissue typing took over and unraveled a far greater degree of HLA allelic polymorphism than previously appreciated (Erllich 2012). HLA nucleotide polymorphism is clustered in the so-called hypervariable regions (HVRs) mainly in exons 2, 3, and 4 of HLA class I and exons 2 and 3 of HLA class II, encoding the functional antigen-binding groove and CD4/CD8 coreceptor-binding regions. Therefore, PCR-based molecular typing focused on these exons, leading to different levels of typing resolution (Table 9.1). With the advent of next-generation sequencing (NGS) for tissue typing purposes (Gabriel et al. 2014, Cornaby et al. 2021), allelic or at least high-resolution typing can be achieved in most cases. Moreover, NGS enables high-throughput sequencing of the entire HLA coding and noncoding regions, unraveling an additional layer of polymorphism, with more than 50% of all submissions resulting from NGS-based typing techniques (Barker et al. 2023). Due to the ability to sequence large numbers of samples in a single run and multiple loci per individual, the NGS technology allows for highly

**Table 9.1** HLA typing resolution and appropriate typing methods

HLA typing resolution <sup>a</sup>	Appropriate typing methods <sup>b</sup>
Low (first field)	Serology, SSP, SSOP, others
High (second field)	NGS, SBT
Allelic (all fields)	NGS, SBT
Intermediate	SSP, SSOP, SBT

<sup>a</sup> As defined in Nunes et al. (2011). *Low*: A serological typing result or DNA-based typing at the first field in the DNA-based nomenclature. *High*: A set of alleles that encode the same protein sequence in the antigen-binding groove and that exclude alleles not expressed on the cell surface. High resolution thus includes alleles reported with the suffix P (a set of alleles encoding the same amino acid sequence at the antigen-binding groove). *Allelic*: Unique nucleotide sequence for a gene as defined using all the digits in a current allele name. *Intermediate*: A level of resolution that falls between high and low resolution, as agreed with the entity requesting the testing. Examples are restriction to common and well-documented (CWD) alleles (Sanchez-Mazas et al. 2017), common intermediate and well-documented (CIWD) alleles (Hurley et al. 2020), or reporting by the National Marrow Donor Program (NMDP) codes (<https://bioinformatics.bethematchclinical.org/hla-resources/allele-codes/allele-code-lists/>)

<sup>b</sup> *Serology* complement-dependent cytotoxicity of specific antisera, *SSP* sequence-specific priming, *SSOP* sequence-specific oligonucleotide probing, *Others* additional molecular typing approaches, including quantitative PCR and restriction fragment length polymorphism (RFLP), *SBT* sequencing-based typing (Sanger sequencing), *NGS* next-generation sequencing

accurate and reliable HLA typing, which is essential for selecting the best possible donor and minimizing the risk of GVHD. As a result, NGS has become an indispensable tool in the field of transplantation and has greatly improved the success rates of allo-HCT (Penack et al. 2020).

Next-generation sequencing has also become an increasingly important tool for HLA typing in registry donors. The high-throughput sequencing capabilities of NGS make it possible to efficiently process large numbers of donor samples. It enabled registries to rapidly expand their donor pools, increase the likelihood of finding a suitable match for any given patient, and extend the basic typing for each donor to the HLA-DPB1, HLA-DPA1, HLA-DQA1, and HLA-DRB345 loci. The introduction of NGS has significantly increased the quality of the donor registry typing per individual registry donor. When comparing

the data from 2016 and 2022, the percentage of registry donors with at least an HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1 has almost doubled to 43% worldwide in the past years (source: WMDA Global Trends Report 2022; <https://wmda.info/publications>. Accessed 26 June 2023). Moreover, qualitatively, the introduction of NGS into registry typing has catalyzed faster donor selection and workup procedures in recent years. Overall, NGS has transformed the landscape of HLA typing for registry donors, providing a more comprehensive and accurate means of identifying donors, thus improving the likelihood and speed of selecting a well-matched donor upfront, associated with the lowest possible clinical risks.

## 9.2.4 T-Cell Alloreactivity

The ability of T cells to specifically recognize non-self, allogeneic tissues is called T-cell alloreactivity. T-cell alloreactivity is the main mediator of both the major benefit and the major toxicity of allogeneic HCT i.e. GVL and GVHD, respectively. T-cell allorecognition can be either direct or indirect. Direct T-cell alloreactivity is targeted to intact mismatched HLA-peptide complexes expressed on the cell surface of allogeneic cells and can be mediated by both naïve and memory T cells (Archbold et al. 2008). Alloreactive T-cell receptors (TCRs) cross-recognize foreign HLA molecules associated with the so-called allopeptides, i.e., different peptides of a largely unknown sequence and origin within the global array of peptides displayed in the antigen-binding groove, the immunopeptidome (Meurer et al. 2021; van Balen et al. 2020; Crivello et al. 2023). Instead, indirect T-cell alloreactivity refers to the recognition of peptides derived from the mismatched HLA proteins and presented in the antigen-binding groove of self-HLA molecules (Gokmen et al. 2008). These peptides are also referred to as Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE, see Sect. 3.5) (Geneugelijk and Spierings 2018; Geneugelijk et al. 2019). A special form of indirect T-cell alloreactivity is the recognition of foreign peptides not deriving from



mismatched HLA but from any other expressed polymorphic gene and presented by self-HLA molecules. These peptides are referred to as minor histocompatibility antigens (mHAg) (Spierings 2014); mHAg are the only targets of T-cell alloreactivity in HLA-matched allo-HCT and are mainly recognized by naïve T cells.

#### Key Points

- HLA molecules are encoded by highly polymorphic genes in the human MHC and play a crucial role in peptide antigen recognition by T cells.
- HLA tissue typing can be performed at different levels of resolution, with the highest being attainable only by NGS-based methods, which are unraveling an unprecedented degree of polymorphism in the MHC.
- Alloreactive T cells can recognize non-self HLA molecules on healthy and malignant cells after allo-HCT, mediating both toxic GVHD and beneficial GVL.

are HLA-compatible, meaning that they carry the same HLA alleles at least at the 4 loci, namely, HLA-A, HLA-B, HLA-C, HLA-DRB1, and are therefore 8 out of 8 allele-identical. In most cases, 8 out of 8 matched UD are also 10 out of 10 matched i.e. they are also matched for the 2 HLA-DQB1 alleles, due to the strong LD between HLA-DR and HLA-DQ. In contrast, the LD is much weaker for HLA-DP, and, therefore, most UD are mismatched for one or both HLA-DP alleles. This should be taken into consideration by discriminating between related donors and UD in HLA-matched allo-HCT. International registries collectively contain the HLA typing information of more than 41 million potential UD (<https://wmda.info/>. Accessed 6 June 2023). The probability of finding a volunteer UD matched for 8/8 HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles varies between 30% and more than 90%, according to the ethnic group of the patient (Gragert et al. 2014, Gragert et al. 2023). If a fully matched UD cannot be found, then a mismatched UD can be used with increasing success, due to new platforms of GVHD prophylaxis based on post-transplant cyclophosphamide (PTCy) (Kanakry et al. 2016; Shaw et al. 2021). For UD HCT, HLA identity should be confirmed at the highest resolution level possible (allelic, high, or intermediate resolution, Table 9.1), to be agreed between the transplant center and the tissue typing laboratory. Another increasingly used donor type is represented by haploidentical relatives, who share one but not the other HLA haplotype with the recipient (Luznik 2008). These donors are available for more than 90% of patients and can be found in parents or offspring (100% likelihood), siblings (50% likelihood), and the extended family. Moreover, HLA haploidentity should be confirmed by family studies wherever possible. As an alternative, unrelated UCB units are used by many centers as a stem cell source (Ballen et al. 2013). Several hundred thousand UCB units, collected from the umbilical cord and placenta after childbirth, are stored in cord blood banks around the world and are readily available. Given the immature immune system transplanted with

## 9.3 HLA Matching in Allo-HCT

### 9.3.1 Donor Types

By inheritance, HLA-identical sibling donors share with the patient the same parental HLA haplotypes, i.e., the HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ, and HLA-DP alleles, in the MHC located on the short arms of each of the two parental chromosomes 6. Siblings have a 25% probability of being HLA-identical according to Mendelian rules. Genotypic HLA identity should be confirmed by family studies for the HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, and HLA-DPB1 loci, to exclude recombination. If this is confirmed, then HLA-identical sibling donors are matched for both HLA alleles at each of the 6 loci, meaning that they are 12 out of 12 allele-identical. In contrast, fully matched UD

these grafts, HLA mismatches are better tolerated, and fully matched UCB allo-HCT is the exception. On the downside, protective immunity is slower to develop post-transplant.

### 9.3.2 HLA Matching in Unrelated HCT

#### 9.3.2.1 Matched UD

The key loci to include in the typing and matching process for UDs are minimally HLA-A, HLA-B, HLA-C, and HLA-DRB1 at a high resolution and optionally HLA-DQB1 and HLA-DPB1. Each mismatch at HLA-A, HLA-B, HLA-C, and HLA-DRB1 increases the risk of mortality by approximately 10% (Lee et al. 2007, Fürst et al. 2013). HLA-DQ mismatches seem to be better tolerated (Tie et al. 2017) and are therefore the mismatch of choice if a 10 out of 10 matched UD is not available. HLA-DP disparity is protective of relapse (Shaw et al. 2010) and several models for permissive, clinically tolerated HLA-DP mismatches have been developed in the last two decades (Zino et al. 2004; Fleischhauer et al. 2012; Thus et al. 2014b; Petersdorf et al. 2015). These are discussed below (see Sect. 3.5).

#### 9.3.2.2 Mismatched UD

The availability of a 10 out of 10 matched UD varies starkly amongst ethnic groups, from more than 75% in European Whites to less than 30% in African Americans (Gragert et al. 2023). In these cases, a single mismatched donor (9 out of 10) is a valid alternative. Apart from HLA-DQ mismatches, which are tolerated best, no specific hierarchy could be detected between HLA-A, HLA-B, HLA-C, and HLA-DRB1 regarding clinical tolerability. For HLA-DQ, taking both  $\alpha$ - and  $\beta$ -chain polymorphism into account might be helpful for the identification of low-risk combinations (Petersdorf et al. 2022). With the introduction of PTCy as a new platform of GVHD prophylaxis, the clinical risks associated with single or even multiple HLA mismatches at any locus have dramatically decreased, and, nowadays, mismatched UDs represent a promising

option to improve access to transplant for patients without a suitable fully matched UD (Kanakry et al. 2016; Shaw et al. 2021; Auletta et al. 2023).

### 9.3.3 HLA Matching in Haploidentical HCT

Biological parents of the recipients and the recipient's biological children are by definition haploidentical for their genomic content, including their HLA. Therefore, guidelines by the European Federation for Immunogenetics require to confirm the presence of a shared haplotype by descent or, if not proven by descent, via high-resolution HLA typing, possibly for all six HLA loci to exclude recombination (European Federation for Immunogenetics, Standards for histocompatibility & immunogenetics testing, Version 8.0. <https://efi-web.org/committees/standards-committee> – accessed 19 May 2023). The introduction of PTCy as GVHD prophylaxis has allowed for successful transplantation across an entire mismatched HLA haplotype, even with the so-called T-cell-replete grafts i.e. grafts that are not depleted from donor T cells. Haploidentical family donors are generally mismatched for 6 out of 12 HLA alleles; however, accidentally, 1 or more alleles can also be identical on the unshared haplotype. No advantage of these “less-than-haploidentical” donors could be found so far (Lorentino et al. 2017). In contrast, certain types of mismatches, including those involving a B-leader match, an HLA-DPB1 nonpermissive mismatch (see Sect. 3.5), and an HLA-DRB1 mismatch in the graft-versus-host direction, were associated with better outcomes compared to others and might be taken into consideration when selecting a haploidentical family donor (Fuchs et al. 2022). Moreover, in 25% of cases, leukemia relapse after haploidentical allo-HCT displays a specific form of immune evasion, termed “HLA loss,” by which the unshared haplotype is selectively lost and replaced by a duplicated shared haplotype (Vago et al. 2012; Crucitti et al. 2015). This aspect must be taken into consideration, especially in the case of post-transplant relapse, where

diagnosis of HLA loss by HLA typing of the recurrent leukemia is recommended.

### 9.3.4 HLA Matching in Unrelated Cord Blood HCT

Cord blood serves as a valuable and readily available source for allo-HCT. The advantage of cord blood is that it can be stored in cord blood banks, allowing for quicker access compared to searching for a matched adult donor. HLA matching in umbilical cord blood transplantation (UCBT) follows a similar process as in other transplantation methods. However, due to the unique properties of cord blood stem cells, a less stringent HLA match can still be considered acceptable in certain circumstances. The flexibility of cord blood stem cells allows for a greater degree of HLA mismatch, making it possible to identify suitable cord blood units even when a perfect match is not available. As such, minimal matching procedures address HLA matching at a serological split antigen level for HLA-A and HLA-B and at a high-resolution level for HLA-DRB1 (Politikos et al. 2020). Various studies have, however, shown that inclusion of HLA-C (Eapen et al. 2011) and matching at a high-resolution (Eapen et al. 2017) improve the outcome. As such, the following criteria involving HLA matching are being advised for cord blood units that meet the minimal cell number requirements (Fatobene et al. 2020):

- (a) Execute high-resolution typing for HLA-A, HLA-B, HLA-C, and HLA-DRB1 of patients and UCB units.
- (b) The first choice is  $\geq 5/6$  HLA-matched units considering HLA-A, HLA-B, and DRB1 and preferably also considering allele-level typing.
- (c) Potentially include HLA-C and steer toward  $\geq 6/8$  HLA-matched units.
- (d) If no  $\geq 5/6$  is available, then  $4/6$  HLA-matched units are acceptable (HLA-A and HLA-B at the antigenic split level and HLA-DRB1 at the allelic level).
- (e) In double UCBT, a unit-to-unit HLA match is not required.

### 9.3.5 Models of High-Risk/Nonpermissive HLA Mismatches

HLA mismatches that are clinically less well-tolerated than others are referred to as high-risk or nonpermissive. This classification is based on the observation that limited T-cell alloreactivity is generally sufficient for the beneficial effect of GVL without inducing clinically uncontrollable GVHD, whereas intolerable toxicity can be induced by excessive T-cell alloreactivity, leading to severe treatment refractory GVHD. Therefore, high-risk or nonpermissive HLA mismatches are those associated with excessive T-cell alloreactivity compared to their low-risk or permissive counterparts. The number and TCR diversity of alloreactive T-cell responses have been shown to be dependent on the degree of immunopeptidome overlaps between the mismatched HLA alleles, which, in turn, is reflective of the genetic polymorphism in the antigen-binding groove (Meurer et al. 2021, van Balen et al. 2020, Crivello et al. 2023). As a result, families of related HLA-DP molecules, classified into the so-called T-cell epitope (TCE) groups, also based on alloreactive T-cell cross-reactivity, define core-permissive, non-core-permissive, and nonpermissive mismatches at this locus (Fleischhauer and Shaw 2017, Arrieta-Bolanos et al. 2022). TCE groups are in LD with genetically controlled expression levels of mismatched HLA-DPB1, which are associated with GVHD risks after UD-HCT, a concept that has also been explored for HLA-C mismatches (Petersdorf et al. 2014, 2015). Structural similarity and hence immunopeptidome overlaps are also at the basis of the specific high-risk HLA-C and HLA-DPB1 allele mismatch combinations proposed in the past (Fernandez-Vina et al. 2014; Kawase et al. 2009). Finally, the total number of PIRCHE-I (presented by HLA class I) and PIRCHE-II (presented by HLA class II), as a measure of the potential level of indirect alloreactivity after transplantation, has also been proposed to be predictive of outcome (Geneugelijk and Spierings 2018; Geneugelijk et al. 2019). The PIRCHE model is attractive since it is potentially applicable



to any HLA-mismatched donor transplantation, including <8/8 matched UD and haploidentical HCT. Comparative evaluation of three models for high-risk/nonpermissive HLA-DP mismatches (i.e., TCE expression and PIRCHE-II) has recently shown similar associations with clinical outcome (Buhler et al. 2021), suggesting that they might be at least partly surrogates of each other, possibly reflecting LD in the HLA-DP region. An overview of different models for high-risk/nonpermissive HLA mismatches can be found in Table 9.2.

### 9.3.6 Factors Influencing the Role of Histocompatibility

Next to donor–recipient HLA matching status, donor age has been shown as the single most important factor associated with post-transplant survival (Kollman et al. 2016; Shaw et al. 2018). Instead, other clinical factors, including donor sex, blood group, and cytomegalovirus serosta-

**Table 9.2** Models of high-risk/nonpermissive HLA mismatches

Model	HLA locus, donor type, and clinical association
T-cell epitope (TCE) groups <sup>a</sup>	HLA-DPB1; 8/8 UD; mortality and acute GVHD
Expression levels <sup>b</sup>	HLA C and DPB1; 7–8/8 UD; acute GVHD
Mismatch combinations <sup>c</sup>	HLA C and DPB1; 7–8/8 UD; mortality, acute GVHD and relapse
PIRCHE <sup>d</sup>	HLA C and DPB1; 8/8 UD; 9/10 UD, CBU, acute GVHD

<sup>a</sup> *TCE groups*: HLA-DPB1 mismatches involving alleles from the same (permissive) or different (nonpermissive) TCE groups (Fleischhauer and Shaw 2017, Meurer et al. 2021)

<sup>b</sup> *Expression levels*: HLA-C or HLA-DPB1 mismatches involving a high-expression allele in the patient, as predicted by noncoding, single nucleotide expression polymorphisms (Petersdorf et al. 2014, 2015)

<sup>c</sup> *Mismatch combinations*: High-risk allele mismatches defined by statistical associations (Fernandez-Vina et al. 2014; Kawase et al. 2009)

<sup>d</sup> *PIRCHE*: Predicted, indirectly recognizable HLA epitope numbers as predicted by online tools (Thus et al. 2014a; Thus et al. 2014b; Thus et al. 2016; Geneugelijck and Spierings 2018; Geneugelijck et al. 2019)

tus, have not been conclusively associated with patient outcomes. As mentioned above, the GVHD prophylaxis used, in particular PTCy, has an important impact on the role of HLA. Further work is needed to redefine the rules of HLA mismatching in this particular context. For HLA-mismatched allo-HCT, donor-specific antibodies (DSAs) should be searched according to the guidelines of the European Federation for Immunogenetics (European Federation for Immunogenetics, Standards for histocompatibility & immunogenetics testing, Version 8.0. <https://efi-web.org/committees/standards-committee> – accessed 19 May 2023) and avoided.

### 9.3.7 Guidelines for UD Selection by Histocompatibility

Consensus guidelines for donor selection have been established in many countries both in Europe (Spierings and Fleischhauer 2019) and overseas (Dehn et al. 2019), through the collaboration between donor registries and national immunogenetic societies. The general recommendation is the selection of an 8/8 HLA-A, HLA-B, HLA-C, and HLA-DRB1 (in Europe often 10/10 i.e. including the HLA-DQB1 locus) matched UD if an HLA-identical sibling is not available, followed by a 7/8 (or 9/10) UD or a haploidentical donor. Avoidance of high-risk or nonpermissive HLA mismatches according to any of the models outlined in Table 9.2 is usually regarded as optional.

#### Key Points

- Allo-HCT donor types (in parenthesis the percentage probability of their identification for a given patient) include genotypically HLA-identical siblings (25%), HLA-haploidentical family donors (>90%), UDs (30–90%), and UCB units (>80%).
- When considering allo-HCT from HLA-identical donors, related and unrelated donors should be regarded separately

because most of the latter carry HLA-DP mismatches, which impact T-cell alloreactivity and GVHD as well as relapse risks.

- HLA typing strategies, including family studies for related donors and typing resolution level for UDs, should be agreed upon between the transplant center and the tissue typing laboratory.
- In UD-HCT, the survival probability decreases by 10% with every mismatch at HLA-A, HLA-B, HLA-C, and HLA-DRB1, in patients transplanted under GVHD prophylaxis not based on PTCy.
- After HLA, the most relevant factor influencing patient survival is donor age.
- Models for high-risk/nonpermissive HLA mismatches eliciting excessive T-cell alloreactivity and toxicity include structural mismatches leading to high immunopeptidome divergence, expression levels, and PIRCHE.
- The introduction of PTCy as GVHD prophylaxis has allowed successful transplantation across multiple HLA mismatches. The role of histocompatibility in this setting, in particular of high-risk/nonpermissive HLA mismatches, will have to be redefined.
- Consensus guidelines established at the national level between donor registries and immunogenetic societies aid in the selection of HCT donors.

## 9.4 Non-HLA Immunogenetic Factors

### 9.4.1 An Overview

HLA alleles are the most but not the only polymorphic genes in humans. Overall, interindividual gene variability by single nucleotide polymorphism (SNP) or copy number variation (CNV) affects 0.5% of the  $3 \times 10^9$  bp in the human genome. Although most of these polymorphisms

are probably nonfunctional, some of them can give rise to polymorphic proteins that can be mHAGs, as described in Sect. 2.2, affect the expression of different genes, including those encoding immunologically active cytokines, or themselves act as immune ligands or receptors relevant to transplantation biology. Among the latter, the KIR gene locus on the long arm of human chromosome 19 displays considerable polymorphism, with 1617 alleles reported to the Immuno Polymorphism Database/KIR (IPD/KIR) database, release 2.12, December 2022 (<https://www.ebi.ac.uk/ipd/kir/about/statistics/>. Accessed 9 May 2023; Barker et al. 2023). Similar to high-risk or nonpermissive HLA mismatches, the role of non-HLA polymorphism in allo-HCT is still incompletely defined. It is impossible to provide a comprehensive overview of all non-HLA factors under study, and the list of factors listed in Table 9.3 and discussed in Sect. 4.2 is only a selection based on the existing evidence for their clinical impact in certain transplant settings.

### 9.4.2 Clinical Impact of Non-HLA Immunogenetic Factors

*mHAGs* are the only targets of T-cell alloreactivity in HLA-identical HCT (see Sect. 2.2) and, as such, play an important role in both GVHD and

**Table 9.3** Non-HLA immunogenetic factors and HCT outcome

Non-HLA factor	Clinical outcome association
mHAG <sup>a</sup>	GVHD and relapse
KIR <sup>b</sup>	Relapse and mortality
MIC <sup>c</sup>	GVHD, relapse, and transplant-related mortality
Others <sup>d</sup>	GVHD and transplant-related mortality

<sup>a</sup> Minor histocompatibility antigens (Spierings 2014)

<sup>b</sup> Killer Ig-like receptors (Heidenreich and Kröger 2017; Shaffer and Hsu 2016)

<sup>c</sup> MHC class I-related chain (Isernhagen et al. 2016)

<sup>d</sup> Cytokine, chemokine, and immune response gene polymorphisms, including tumor necrosis factor, interleukin (IL)-10, the IL-1 gene family, IL-2, IL-6, interferon- $\gamma$ , tumor growth factor  $\beta$  and their receptors, NOD-like receptors (NOD2/CARD15), toll-like receptors, and micro-RNAs (Dickinson and Holler 2008; Gam et al. 2017; Chen and Zeiser 2018)

GVL (Spierings 2014). This dual function is related to their different modes of tissue and cell expression, i.e. hematopoietic system-restricted or broad. Broadly expressed mHAgS can cause both GVHD and GVL, and donor–recipient matching for these mHAgS is therefore desirable yet virtually impossible due to their large number, with many of them probably currently undefined. In contrast, mHAgS restricted to hematopoietic cells are more prone to induce selective GVL. The latter is being explored as a target for HCT-based immunotherapy of hematological malignancies, in which mHAg-specific responses are specifically enhanced to promote GVL.

*KIRs* are predominantly expressed by NK cells and recognize certain HLA class I specificities on target cells. *KIRs* have either long-inhibitory or short-activating cytoplasmic domains and are stochastically co-expressed on NK cells. The eventual outcome of *KIR* interaction (or lack thereof) with its HLA class I ligand (inhibition or activation) is a complex process that depends on the relative number of inhibitory or activating *KIRs* and on the state of education of the NK cells. Educated NK cells from individuals expressing the cognate HLA ligand are strongly reactive against cells missing that ligand. This “missing-self” reactivity is at the basis for the potent GVL effect attributed to NK cells in the setting of HLA-mismatched transplantation, in particular haploidentical HCT (Heidenreich and Kröger 2017). Depending on the donor *KIR* gene asset, a role of NK cell-mediated GVL has also been postulated in the HLA-matched setting (Shaffer and Hsu 2016). Based on all this evidence, *KIR* typing is increasingly being adopted as an additional criterion for donor selection.

*MHC class I chain-related (MIC) A* and *B* are nonclassical MHC class I genes. *MICA* encodes a ligand for *NKG2D*, an activating NK receptor. The SNP Val/Met at position 129 of the *MICA* protein results in isoforms with high (Met) and low affinities (Val) for *NKG2D*. Consequently, various studies suggest a role for this SNP in the HCT outcome, including GVHD, relapse, and survival (Isernhagen et al. 2016).

*Immune response gene* polymorphisms have also been reported to contribute to the risks associated with HCT (Dickinson and Holler 2008; Gam

et al. 2017; Chen and Zeiser 2018). They often comprise SNPs in cytokine- or chemokine-coding genes or their regulatory elements such as microRNAs (miRNAs). These variations in both the donor and the recipient can have a significant impact on transplant outcome and the development of GVHD; however, their relative role in different transplant settings is not yet fully elucidated.

### Key Points

- Non-HLA immunogenetic factors that have been associated with clinical outcome of HCT include polymorphic mHAg, *KIR*, *MIC*, and immune response genes.
- Hematopoietic tissue-specific mHAgS are used for specific cellular immunotherapy of hematological malignancies.
- Polymorphic *KIRs* are responsible for “missing-self” recognition by alloreactive NK cells mediating selective GVL after HCT, and *KIR* genotyping is therefore increasingly included into donor selection algorithms.

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# Clinical and Biological Concepts for Mastering Immune Reconstitution After Hematopoietic Cell Transplantation: Toward Practical Guidelines and Greater Harmonization

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and Jaap Jan Boelens

## 10.1 Introduction/Background

Not only the underlying mechanisms driving a long-term cure but also life-threatening side effects after hematopoietic cell transplantation (HCT) are primarily mediated by reconstitution of the immune repertoire. The composition and

dynamics of reconstitution are influenced by the conditioning regimen, cell dose, graft composition, and age and type of immune suppression. However, our understanding of these mechanisms is limited due to many variations in clinical programs, including the specific type of transplantation procedure, and the absence of standardized immune monitoring after HCT. While the process of donor selection has seen significant advancements based on new biological insights, little attention has been given to optimizing cell product design in terms of numbers and composition to minimize inter-patient variability. In addition, the high inter-patient disparities in the clearance of agents used during the conditioning are rarely investigated. The lack of prospective clinical studies addressing these concepts, coupled with limited pharmaceutical company interest, fosters a consensus discussion. Our goal is to harmonize HCT interventions by exploring how individual patient differences and overall transplantation strategies impact the final effector mechanisms of HCT, specifically aiming for timely and well-balanced immune reconstitution.

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### 10.1.1 Impact of Conditioning Regimens on Immune Reconstitution and Outcomes: Pharmacokinetics–Pharmacodynamics (PK–PD) and Individualized Dosing

Over the last decade, it has become evident that various agents, such as busulfan, fludarabine, anti-thymocyte globulin (ATG), and anti-T-lymphocyte globulin (ATLG), administered as part of the conditioning regimen and post-HCT, have a substantial impact on both relapse and non-relapse mortality due to graft-versus-host disease (GvHD) and viral reactivation. Consequently, these agents significantly influence survival chances (Soiffer et al. 2017; Lakkaraja et al. 2022; van Roessel et al. 2020; Admiraal et al. 2017). Comprehensive pharmacokinetic (PK) and pharmacodynamic (PD) modeling has provided evidence that exposure to most of these agents can affect both short- and long-term immune reconstitution.

An important example is the development and validation of a population pharmacokinetics model for ATG (Thymoglobuline). It was found that clearance of ATG mainly depends on weight (when patients weigh <40 kg) and the receptor load (represented by absolute lymphocyte count; ALC) before the first dosing (Haanen et al. 2020). Using population PK modeling, a new dosing nomogram was developed, which has been recently validated in a prospective trial (Admiraal

et al. 2022). Patients who received individualized dosing were more likely to attain CD4<sup>+</sup> immune reconstitution, defined as CD4<sup>+</sup> >50/μl at two consecutive time points before day 100. Importantly, it was confirmed that this definition of CD4<sup>+</sup> immune reconstitution is a reliable predictor of outcomes in multiple transplantation settings (adults, pediatrics, T-replete, T-deplete, cord blood (CB), bone marrow (BM), and peripheral blood stem cells (PBSCs)) (Admiraal et al. 2022) and is easy to use at all transplant centers. In Table 10.1, optimal exposures of ATG after transplant associated with optimal outcomes are presented (Soiffer et al. 2017; Lakkaraja et al. 2022; Admiraal et al. 2017; Haanen et al. 2020; Admiraal et al. 2022) (Table 10.1). Although no validated population PK model for ATLG has yet been published, data from a post hoc analysis of a randomized controlled trial allowing three different types of regimens showed that ATLG had opposite effects on the outcome parameters of chronic GvHD and leukemia-free survival, resulting in overlapping curves for these primary end points (Soiffer et al. 2017). This study showed that agents used for conditioning had a significant impact on the ALC prior to dosing of ATLG and thus influence immune reconstitution and clinical outcomes, i.e., a similar impact as shown for ATG.

More recently, when using body surface area (BSA)-based dosing, it has been found that fludarabine exposure is highly variable (range 10–66 mg\*h/L, median exposure 26 mg\*h/L)

**Table 10.1** Suggested novel ATG (Thymoglobuline) dosing nomograms based on PK–PD modeling for (non-)myeloablative settings in pediatrics and adults

Setting	Dosing on	Target AUC after HCT (AU <sup>a</sup> d/mL) and donor source	Starting day	References
Pediatrics; myeloablative	Weight ALC Cell source	<20 for cord blood <50 for bone marrow	–9 <sup>b</sup>	Admiraal et al. (2015)
Pediatrics/adults ex vivo T cell depletion (TCD)		<20	–12 <sup>b</sup>	Lakkaradja et al. (2022)
Adults: Non-myeloablative	ALC	60–90 for peripherally mobilized stem cells	–9	Admiraal et al. (2017)

Level C evidence (retrospective studies). ALC, absolute lymphocyte count; AUC, area under the curve

<sup>a</sup> Prospective validation trial (PRAISE IR) almost closed to accrual

<sup>b</sup> Confirmed in a prospective validation trial (PARACHUTE) (Admiraal et al. 2022)



(Langenhorst et al. 2019). Immune reconstitution was found delayed in patients with an exposure  $>25$  mg\*h/L, which was associated with more viral reactivations and higher probability of non relapse mortality (NRM). Using a validated population PK model, both glomerular filtration rate (GFR) and weight were identified as predictors of clearance of fludarabine. An association between fludarabine exposure and outcomes was also shown in CD19 chimeric antigen receptor T-cell (CAR T) recipients (Fabrizio et al. 2022; Dekker et al. 2022), suggesting that individualized fludarabine dosing to improve outcomes is a viable option beyond the HCT setting. Prospective validation trials in bone marrow transplantation and in immune effector cell transplant strategies (e.g., CAR T) are underway.

Posttransplant cyclophosphamide (PT-Cy) has emerged as an elegant and effective pharmacological strategy to overcome human leukocyte antigen (HLA) barriers in the setting of allogeneic HCT from haploidentical donors and more recently in matched donor transplants (Battipaglia et al. 2021). Several biological mechanisms are responsible for PT-Cy effectiveness in terms of GvHD reduction (Radojicic and Luznik 2019), and new understandings are currently emerging (i.e., reduction in the proliferation of alloreactive CD4<sup>+</sup> effector T cells and the preferential recovery of CD4<sup>+</sup> regulatory T cells (Tregs); functional impairment of surviving alloreactive CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells) (Nunes and Kanakry 2019). Moreover, PT-Cy has an indirect effect on Tregs (Fletcher et al. 2023) due to the expansion of functional myeloid-derived suppressor cells. A retrospective study has recently compared immune reconstitution across ATG and PT-Cy strategies (Massoud et al. 2022). ATG resulted in faster reconstitution of CD8<sup>+</sup> T, natural killer (NK), natural killer T (NKT), and  $\gamma\delta$ T cells, whereas CD4<sup>+</sup> T cells and B cells reconstituted faster after PT-Cy. Similar reconstitution was observed for Tregs and B cells. Even though differences in immune reconstitution (IR) were associated with a decreased incidence of infections and moderate/severe chronic GvHD in the ATG

group, they had no impact on any of the other long-term outcomes.

Collectively, these studies present compelling evidence that achieving “predictable” immune reconstitution is paramount when investigating the efficacy of maintenance therapies involving novel drugs, donor lymphocyte infusions, and advanced cell therapy interventions. Such predictability serves as a standardized predictor, enabling meaningful comparisons across studies and accounting for the numerous variables inherent to the HCT setting.

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## 10.2 Graft Composition as an Additional Predictor of Immune Reconstitution and Clinical Outcomes

Although transplant physicians carefully monitor the levels of many drugs, such as cyclosporine or antibiotics, an additional opportunity to further harmonize the transplantation procedure arises from the surprising clinical observation that substantial cell dose variations are currently accepted across patients. The hesitation to monitor cell numbers in the graft or after HCT, and to act on them, is, of course, partially driven by the confusing magnitude of immunological subsets, the narrow nature of many immunological programs with a lack of consensus on immune monitoring, and also the rather limited immunological education across the majority of transplant physicians. However, currently available retrospective and prospective studies can provide guidance. A retrospective EBMT study indicated that graft T-cell numbers in matched unrelated donors frequently vary between 50 and 885  $\times 10^6$ /kg and that the highest quartile in CD34<sup>+</sup> cells as well as T cells associate with an inferior clinical outcome (Czerw et al. 2016). As we cannot expect randomized trials to address in the future the impact of different graft compositions in T cell-replete transplantations on clinical outcomes, avoiding higher numbers of CD34 and T cells within the highest quartile might be reasonable, as high T-cell numbers have been associated with the risk of developing chronic GvHD (Czerw et al. 2016). For

haploidentical donors, even lower T-cell numbers might be advised (Mussetti et al. 2018), as, in this context also, higher numbers of T cells are associated with increased incidences of chronic GvHD. However, different cohort analyses are desirable to confirm these intriguing studies. Higher numbers of NKT cells (Malard et al. 2016) and  $\gamma\delta$ T cells (Perko et al. 2015) in the graft have been reported to associate with favorable immune reconstitution, and a positive clinical outcome, most likely due to their impact in controlling GvHD (Du et al. 2017) and acting on cytomegalovirus (CMV) as well as on leukemia (Scheper et al. 2013; de Witte et al. 2018). However, these variables are more difficult to control in daily clinical practice. Direct ex vivo graft engineering provides an elegant solution to further control immune subsets in the graft and the consecutive immune reconstitution. It also allows for the standardization of cell numbers, as well as subsets per patient, e.g., selecting CD34-positive cells alone has been reported to associate with less chronic GvHD, whereas the graft-versus-leukemia effect is maintained (Pasquini et al. 2012). Increased activity of the next generation of graft engineering through depletion of  $\alpha\beta$ T cells has been reported over the last decade (de Witte et al. 2023), emphasizing the better awareness of an opportunity to define graft compositions more precisely before transplantation. Depletion of  $\alpha\beta$ T cells is associated with not only lower frequencies of infection and extremely low GvHD rates but also a different immune repertoire (de Witte et al. 2021a) and with a good efficacy/safety profile used during the pandemic (Nijssen et al. 2023). Thus, each transplantation platform needs to be carefully evaluated for immune reconstitution as it might substantially differ and, consequently, differently impact later interventions (de Witte et al. 2021b; Schmid et al. 2021).

### 10.2.1 Monitoring: Immune Cell Phenotyping

Variables that may impact immune reconstitution are (A) the immune status before the immune intervention, (B) the immune composition of the

graft, (C) the dynamics of the reconstituting immune subsets and their function, and (D) the exposure to drugs administered in the conditioning regimen prior to intervention (as discussed above; Table 10.1). The most important questions that arise when monitoring the immune cells after transplant using clinical flow cytometry are what markers should be followed and how to use these markers in a meaningful way? These questions are particularly important in an era when post-HCT pharmaceutical maintenance interventions and donor lymphocyte infusion (DLI) or the administration of other Advanced therapy medicinal products (ATMPs) have become more common over the last decade (Soiffer and Chen 2017).

Flow cytometry is broadly available to monitor immune cell reconstitution in accredited laboratories within transplant centers. Markers identifying the most common leukocyte subsets are broadly used and can therefore be considered as a “standard” panel: CD45 (lymphocytes), CD3 (T cells), CD19 (B cells),  $\alpha\beta$  T-cell receptor (TCR),  $\gamma\delta$ TCR, and CD16/CD56 (NK) cells. For  $\gamma\delta$ T cells, it is important to note that  $\delta 2$ -positive and  $\delta 2$ -negative  $\gamma\delta$ T cells always need to be distinguished as they are biologically two completely different populations (Sebestyen et al. 2020). In some laboratories, this panel is extended to identify the differentiation and activation state of subsets of T (T-helper, cytotoxic, regulatory T cells, naive, effector/memory or recent thymic emigrants), B (switched and non-switched) and NK(T) cells, and cells from the myeloid lineage (monocytes, dendritic cell subsets). This knowledge is important because the success of cell-based immunotherapies, as well as agents modulating the immune system after transplantation, will significantly depend on the presence or absence of different immune subsets. As described above, from all markers, CD4<sup>+</sup> T cells >50/uL (at two consecutive time points < 100 days) have shown to be the best early immune cell markers to predict outcomes in many different transplant settings. More recently, in a large (>500 pt) pediatric and young adult cohort with B cells >25 cells/uL <100 days, the combination of CD4<sup>+</sup> T cells (>50 cells/uL) and B cells (>25 cells/uL) in particular has been

found to be a predictor of outcomes (e.g., NRM, GvHD, and Overall Survival (OS)) (van Roessel et al. 2020). This new combination of B and CD4<sup>+</sup> T cells as potential biomarkers of outcomes needs confirmation in separate cohorts. Interestingly, the relationship between CD4<sup>+</sup> T-cell immune reconstitution and exposure to ATG and fludarabine was not found between the conditioning drugs and B-cell immune reconstitution. In the near future, mastering the diversity might allow for the definition of patient subpopulations who would benefit from certain adjuvant therapies as maintenance after HCT (e.g., checkpoint inhibitor treatment and tyrosine kinase inhibitors (TKIs)) (Davids et al. 2016; Mathew et al. 2018). Moreover, certain myeloid subsets are suggested to have an impact on outcomes (Mussetti et al. 2018), but more studies are needed to confirm this. Therefore, on top of clinical flow panels, discovery panels (in a research setting) can potentially provide more insight into what the optimal immune milieu is for disease and toxicity control. To be able to compare results from different trials and individual centers, it is important to develop standardized operational protocols for sample handling and staining protocols for both fresh and biobanked samples.

### 10.2.2 Immune Monitoring: Secretome Analyses

Measuring the production of cytokines, chemokines, and growth factors in the serum or plasma represents an integral part of immunomonitoring during immunotherapeutic treatments. Proteomic biomarkers may distinguish diverse diseases/response patterns, identify surrogate markers of efficacy, and provide additional insights into the therapeutic mode of action. Over the last decade, advances in highly multiplexed technologies have allowed for the discovery and validation of several blood biomarkers of acute and chronic GvHD and graft-versus-tumor reactivity.

As examples, proteins, such as interleukin (IL)-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), ST2 (suppressor of tumorigenicity

2), and soluble IL-2a, have shown to be biomarkers of GvHD, whereas increased levels of tumor necrosis factor-alpha (TNF- $\alpha$ ) and IL-6 are associated with robust immune responses to viral reactivations (de Koning et al. 2016). It is noteworthy that these biomarkers show diagnostic and prognostic potential (Milosevic et al. 2022), can be informative in predicting more severe GvHD and NRM (McDonald et al. 2015; Srinagesh et al. 2019), and may be informative to categorize patients based on their likelihood to respond to therapy (Hess et al. 2021). The main challenge, however, remains to identify predictors very early after or even before cell infusion.

Peripheral blood is often the only source for protein analysis, which may lack the sensitivity to reflect local responses in affected tissues. The most common methods to identify these markers include antibody-based enzyme-linked immunosorbent assays (ELISAs) or multiplex platforms, such as protein microarrays, liquid chromatography–mass spectrometry (LC-MS), electrochemiluminescence, and bead-based or proximity extension multiplex immunoassays (MIAs). Again, different technologies and reagents (e.g., antibodies and recombinants for standard curves) may lead to different concentrations, and dramatic variability in results, depending on how the pre-analytical samples are handled (e.g., differences in processing and storage, including duration of storage). Cytokine levels may differ considerably between serum and plasma samples obtained from the same donor, due to release of platelet-associated molecules into the serum. Moreover, the type of anticoagulant used in plasma isolation and time- and/or temperature-sensitive changes need to be considered (Keustermans et al. 2013). These phenomena underscore the need for extensive documentation with respect to all biomarker analyses before any conclusions can be made when comparing patient cohorts treated at multiple sites.

While the detection of specific cell subsets and proteins offers valuable insights, functional assays can provide additional information to enhance our understanding of the biological mechanisms and assess the effectiveness of a patient's immune system. For example, the func-

tionality of natural killer (NK) cells and regulatory T cells (Tregs) can be evaluated through assays that measure their ability to induce target cell killing by degranulation as well as by their capacity for proliferation and suppression, respectively. Moreover, recent advancements in single-cell proteomic technologies have enabled the combination of both approaches, wherein the analysis of secreted proteins at the single-cell level generates an immune fitness score. This score has demonstrated its potential in predicting the responsiveness to checkpoint inhibitor therapy (Haanen et al. 2020), but its value in assessing immune fitness post-HCT has still to be assessed.

### 10.2.3 Immune Monitoring of Virus-Specific T-Cell Responses

Virus-specific immune responses are mainly assessed for cytomegalovirus (CMV) (Tassi et al. 2023; Krawczyk et al. 2018; Wagner-Drouet et al. 2021), human herpesvirus 6 (HHV6) (Noviello et al. 2023), adenovirus (AdV) (Cesaro and Porta 2022), Epstein–Barr

virus (EBV), BK virus (Annaloro et al. 2020), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Anon 2022). Different assays have been adopted to assess virus-specific T cells (Table 10.2): flow cytometry-based tests (e.g., intracellular cytokines, MHC multimer binding), interferon- $\gamma$  (IFN- $\gamma$ ) enzyme-linked immunospot (ELISpot), QuantiFERON-CMV, other home-made tests (e.g., proliferation assays or different CMV-specific T-cell subsets). In a recent EBMT survey, only 13.8% centers have reported to perform at least one type of virus-specific immune monitoring, whereas 31% additional centers are planning to start to do so in the future (Greco et al. 2023; Cordonnier et al. 2021). The quantitative and functional assessment of virus-specific T-cell responses may be more relevant to patient's risk stratification and clinical decision-making, thereby encouraging immune monitoring of patients. While still experimental and often limited to research studies, adoptive immunotherapy with virus-specific lymphocytes could benefit from more data on virus-specific IR to extend its applicability on a broader scale.

**Table 10.2** Example of platforms for virus-specific immunological monitoring (i.e., CMV)

Characteristics	IFN- $\gamma$ QuantiFERON	IFN- $\gamma$ ELISpot	Dextramer
Sample	Whole blood	PBMCs	Whole blood
Turnaround time	24 h	24–48 h	24 h
Functional analysis	Yes	Yes	No
Phenotypic characterization	No	No	Yes
Differentiation CD8 <sup>+</sup> /CD4 <sup>+</sup>	No (only CD8 <sup>+</sup> )	Yes	No (only CD8 <sup>+</sup> )
HLA restriction	Yes	No	Yes
Cost	+	++	++
Advantages	Simple to perform and highly standardized (routine)	Highly sensitive	Increased detectable fluorescence intensity and improved avidity
Limitations	<ul style="list-style-type: none"> <li>• Only measures CD8<sup>+</sup> cells</li> <li>• Sensitive to lymphopenia</li> <li>• HLA-restricted</li> </ul>	<ul style="list-style-type: none"> <li>• Lack of standardization</li> <li>• Operator-dependent</li> <li>• Requires PBMC isolation (hardworking)</li> </ul>	<ul style="list-style-type: none"> <li>• Only measures CD8<sup>+</sup> cells</li> <li>• HLA-restricted</li> <li>• Standardized kit</li> </ul>

*PBMC* peripheral blood mononuclear cell

### 10.3 From Transplantation to Immune Monitoring of CAR T Cells, Harmonization Is Needed

Adoptive transfer of CAR T cells has revolutionized the treatment of several hematological malignancies by overcoming chemotherapy refractory and/or relapsed disease. CAR T therapy shares many similarities with hematopoietic cell transplantation. First detailed immunological analyses of long-term responders become available (Cappell and Kochenderfer 2023; Melenhorst et al. 2022). In addition to clinical trial data, a large body of real-world evidence (RWE) has been compiled in different registries, with the EBMT CAR T registry being the largest European registry to be successfully used for post-authorization safety studies (PASSs) for most approved CAR T products (McGrath et al. 2020). Notably, only a minimal core set of accepted clinical end points are identical across trials and registries, leaving important additional clinical parameters not comparable between trials and RWE. The ongoing GoCART Coalition initiative (<https://thegocartcoalition.com>) aims to harmonize not only clinical data collection as needed for PASSs but also exploratory clinical data for earlier clinical trials and biomarker analyses. Optimal time points and, e.g., flow cytometry panels for associated exploratory biomarker programs are not harmonized across centers, trials, or products. This lack of harmonization in clinical and biomarker programs hampers scientific advances, quality control efforts, and/or benchmarking and urgently calls for a coordinated effort to harmonize parameter sets, data structures, and time points for the assessment of clinical and biomarker data enabling health-care professionals, health-care providers and payers, and, of course, patients, to optimize their decision-making. Therefore, under the umbrella of the GoCART Coalition, EBMT, European Hematology Association (EHA), and T2Evolve

started a new initiative in 2023 (CART-CD) to generate a harmonized European parameter set via the Delphi process (Webbe et al. 2023), a structured process, to involve the broader stakeholder community. This will allow to harmonize, over the next years, the data structure with common time points for clinical end points and a set of biological parameters. This harmonization, including harmonization of collecting samples for immune monitoring, will improve and facilitate cross-study comparability and generate real-world data for CAR T cell therapies and beyond.

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### 10.4 In Summary

The failure or success of HCT is significantly impacted by the patient's immune status. However, only a minority of HCT programs systematically consider individualized drug monitoring during conditioning, graft design, and immune monitoring as key for patient surveillance, in order to maximally control and capture essential details of the intervention HCT. Therefore, guidelines are needed to further harmonize the HCT procedure and standardized immune monitoring to allow for distillation of the key features for success and failure. First, careful recommendations for individualized drug dosing and graft compositions can be made based on available data sets. However, within the new cellular therapy registry of EBMT, it will be key to register additional details of drug dosages, graft compositions, and immune reconstitution, to capture clinical variations in programs, as well as defined immune reconstitutions. This will enable a retrospective increase in insight into daily clinical practice, and its impact on immune reconstitution, as well as clinical outcome. Moreover, clinical trials should adopt such consensus measurements. Nevertheless, the markers and phenotypes studied in one setting may not be considered relevant in another, supporting the definition of a set of general recommended protocols and a set of add-on

**Table 10.3** Panels under consideration in the panel discussion of the CTIWP (Greco et al. 2018). General parameters that could be included in harmonized immune monitoring protocols across most studies/centers and advanced parameters that may be of great value in specific studies and that can only be performed in specialized immunology laboratories or analyzed in a central laboratory

	General		Advanced
Graft composition	$\alpha\beta$ T	$\alpha\beta$ TCR, CD45RO/RA, CD3, CD4, CD8, CD27	Intracellular cytokines after phorbol myristate acetate (PMA)/ionomycin stimulation
	$\gamma\delta$ T	$\gamma\delta$ TCR, CD45RO/RA, CD3, CD27	Specific TCR by multimer approach
	Treg	CD45, CD4, CD25, CD127, FoxP3	
	B	CD45, CD19, CD38, CD27, IgM/G/D, CD21	
	NK/NKT	CD45, CD3, CD56, TCR24/11)	
Cell phenotyping pre- and posttransplantation	$\alpha\beta$ T	$\alpha\beta$ TCR, CD45RO/RA, CD3, CD4, CD8, CD27	Intracellular cytokines after PMA/ionomycin stimulation
	$\gamma\delta$ T	$\gamma\delta$ TCR, $\delta$ 2TCR, $\delta$ 1TCR	Specific TCR by multimer approach
	Treg	CD45RO/RA, CD3, CD27	$\alpha\beta$ TCR and $\gamma\delta$ TCR repertoire
	B	CD45, CD4, CD25, CD127, FoxP3	
	NK/NKT	CD45, CD19, CD38, CD27, IgM/G/D, CD21	
	DC/mono	CD45, CD3, CD56, TCR24/11) CD11c, HLA-DR, CD14, CD16, CD1c, CD141, CD303	
Secretome	–	Multiplex panel (e.g., IL-7, ST2, TNF- $\alpha$ , IL-6, HGF, IL-2R, IL-8, GM-CSF, etc.)	
Cell function	–	NK cell lyses T-cell proliferation upon antigens and mitogens B-cell maturation	
PK	Busulfan, fludarabine, ATG, Campath (if part of conditioning)	Trial drug	
Minimal Residual Disease (MRD)	qPCR (targets expressed, flow cytometry)	Next-generation sequencing	
Viral load	CMV, EBV, HV6, adenovirus	–	

*qPCR* quantitative PCR

trial-specific parameters (Table 10.3). A new survey is currently being prepared by the Cellular Therapy and Immunobiology Working Party (CTIWP) of EBMT and GoCART Coalition for both hematopoietic cell transplantation and CAR T therapies. A harmonization procedure to achieve

a more balanced immune reconstitution might have a more profound impact on patient survival than any other novel maintenance therapy (Admiraal et al. 2017; Boelens et al. 2018) and allow for a better success rate for novel drugs tested as maintenance therapy.



### Key Points

- The failure or success of HCT is significantly impacted by the patient's immune status.
- Harmonizing individualized drug monitoring during conditioning, graft design, and immune monitoring is key for patient surveillance.
- A harmonization procedure to achieve a more balanced immune reconstitution might have a more profound impact on patient survival (and quality of life) than any other novel maintenance therapy and allow for a better success rate for novel drugs tested as maintenance therapy.

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## **Part III**

### **Methodology and Clinical Aspects**

**Topic Leaders: Nicolaus Kröger, Arnon Nagler,  
Enric Carreras and Selim Corbacioglu**



# Evaluation and Counseling of Candidates

# 11

Pere Barba and Alessandro Rambaldi

## 11.1 Evaluation of Candidates and Risk Factors for HCT

Pere Barba

### 11.1.1 Introduction

The evaluation of candidates and the analysis of individual risk factors for hematopoietic cell transplantation (HCT) allow establishing four fundamental aspects of the procedure:

1. The indication for HCT
2. Providing proper information to the patient
3. Choosing the best donor, conditioning, and post-HCT immunosuppression
4. Conducting benchmarking and comparative studies between centers

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## 11.1.2 Candidates' Evaluation Workflow

### 11.1.2.1 The First Visit

The most relevant aspects to include in the first visit are as follows:

- Evaluating medical history (both past and present) and conducting a physical examination (see Sect. 11.1.2.4)
- Reviewing diagnostic tests (in referred patients)
- Reevaluating human leukocyte antigen (HLA) typing of the patient and potential donors (in the case of allo-HCT)
- Providing preliminary information on:
  - Therapeutic options and results
  - The HCT procedure
  - Possible complications and side effects (see specific chapters in Part V)
- Scheduling reevaluation of the current status of the disease (see Sect. 11.1.3)
- Scheduling visits with a radiation therapist (in the case of total body irradiation (TBI)), a dentist, a gynecologist, a blood bank (for a list of blood/platelet donors), an HCT unit supervisor nurse, etc.
- Obtaining the patient's signature on the informed consent form for HCT and for procurement of hematopoietic stem cells (HSCs) (in the case of auto-HCT)

### 11.1.2.2 Pre-Apheresis Visit

The important aspects involved in the pre-apheresis visit include the following:

- Assessing the results of pretransplant tests
- Providing complete information on the procedure
- In the case of peripheral blood stem cells (PBSCs), assessing the status of venous accesses. Programming central venous catheter (CVC) (if necessary) and the mobilization schedule
- Scheduling a preanesthetic visit in the case of bone marrow (BM)
- Program manipulation of HCT (if applicable) and/or cryopreservation

### 11.1.2.3 The Last Visit Before Admission

This involves the following aspects:

- Obtaining the final and complete patient information (see Sect. 11.1.2.5)
- Reviewing pretransplant tests (see Sect. 11.1.3)
- Scheduling admission and conditioning therapy
- If necessary, then scheduling CVC placement
- In the case of allo-HCT, confirming that the donor's evaluation is correct and that there are no contraindications for donation (see Chap. 12)
- In the case of auto-HCT, confirming that the cryopreserved cellularity is correct
- Submitting donor and recipient information to the blood bank (group, cytomegalovirus (CMV) serology, previous transfusions, etc.)
- In the case of TBI, confirming that the dosimetry has been carried out and that the radiotherapy (RT) has been programmed
- Confirming storage of patient and donor samples for serotheque and cellular library

### 11.1.2.4 Medical History

The following patient information should be collected:

Medical background; childhood illnesses and vaccines; allergies and adverse drug reactions;

surgical interventions (previous anesthesia); medications not related to the basic process; previous transfusion history, family tree, and relevant family history; and, in women, menarche/menopause, pregnancy and childbirth, contraceptive methods used, last menstrual period, and gynecological checkups

Travel to malaria, trypanosomiasis, and human T-lymphotropic virus type I/II (HTLV-I/II) endemic areas

Previous relevant infections, including coronavirus disease 2019 (COVID-19) and vaccination status

All data about the current illness, such as:

- The date of disease onset and initial symptomatology
- The methodology used (staging)
- Radiotherapy treatments (doses and dates) conducted
- Other treatments undertaken
- Disease status

Social aspects, such as:

- Alcoholism and other drug use
- Habits
- Place of accommodation (whether close to the center) and means of transport
- Role of family members
- Cultural, economic, and intellectual aspects

### 11.1.2.5 Information to Provide (See Detailed Information in the Counseling Section)

Ask the patient (privately) if he or she wishes someone else to be present for the session. For adolescents, follow the rules of each country, thus respecting the right of information. Transmit as much information as possible in writing. She/he must be informed about:

- The most frequent early and late complications (see specific chapters in Parts V and VI), including graft failure, gastrointestinal (GI) complications, alopecia, sinusoidal obstruction syndrome/ veno-occlusive disease (SOS/VOD), acute graft-versus-host disease

(GvHD), early infections, chronic GvHD, late infections, relapse of the disease, infertility, endocrine complications, neoplasms, and other adverse events

- Serious potential complications (intensive care unit (ICU) admissions) and possibility of death, the availability of an advanced health-care directive registry, and the need to appoint a person to make decisions in case the patient is unable to do so.
- Estimated duration of admission
- Most frequent complications on discharge, outpatient follow-up, likelihood of readmission, and need for caregivers at discharge

### 11.1.3 Pretransplant Evaluation

All the following studies must be performed within 30 days prior to the HCT, except the assessment of the baseline disease status (7–15 days) and the pregnancy test (7 days):

- Complete blood count (CBC) and basic coagulation; complete biochemistry (including ferritin); blood type and Rh/irregular antibodies; dosage of immunoglobulins (Igs); serology CMV, Epstein–Barr virus (EBV), Herpes simplex virus (HSV), varicella zoster virus (VZV), toxoplasma, syphilis, hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), and HBsAb (Hepatitis B surface antibody) (HTLV-I/II and Chagas disease according to the patient's origin); nucleic acid test (NAT) for hepatitis B virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV); pregnancy test
- Chest X-ray; respiratory function tests (including forced expiratory volume in 1 s (FEV1) and diffusing capacity of the lung for carbon monoxide (DLCO)); electrocardiogram; echocardiogram or isotopic ventriculography (depending on the previous treatment)
- Reevaluation of the minimal residual disease (MRD) (see specific chapters in Part IX).
- Dental evaluation; gynecological evaluation; psychological/psychiatric evaluation

- Nutritional assessment
- HLA typing (recheck) (see Chap. 9).

### 11.1.4 Risk Assessment

#### 11.1.4.1 Individual Risk Factors

There are a group of variables that have a prognostic value in all predictive models.

Variables	High risk
Age	Older. Do not use as a single criterion. Relative importance
General condition	Karnofsky index <80%
Disease	Not in remission. See specific chapters
Type of donor	Others rather than HLA-identical siblings
HLA compatibility	Any HLA-A, HLA-B, HLA-C, and DRB1 difference
CMV serology	Different serology than the donor
Donor	Age > 35–40 years For a male recipient, a female donor (especially if multiparous)
Interval diagnosis of HCT	Prolonged (relevant in chronic myeloid leukemia (CML) and Severe aplastic anemia (SAA))
Comorbidities	See HCT-CI model
Iron overload	Present
Experience of the center	Non-JACIE/FACT-accredited centers

*HCT-CI* HCT-specific Comorbidity Index, *JACIE* Joint Accreditation Committee of International Society for Cell and Gene Therapy-European Society for Blood and Marrow Transplantation (ISCT-European Society for Blood and Marrow Transplantation (EBMT)), *FACT* Foundation for the Accreditation of Cellular Therapy

#### 11.1.4.2 Predictive Models

##### The Disease Risk Index (DRI)

The Disease Risk Index categorizes people according to disease type and its status at the time of HCT. It does not take into account factors such as age or comorbidities. This score index classifies the disease into four prognostic groups and anticipates the overall survival, progression-free survival, cumulative incidence of relapse, and cumulative incidence of non-relapse mortality (see Table 11.1) (Armand et al. 2012, 2014).



**Table 11.1** The disease risk index (Armand et al. 2012, 2014)

Risk	Disease
Low	AML with favorable cyt., CLL, CML, indolent B-cell NHL
Intermediate	AML intermediate cyt., MDS intermediate cyt., myeloproliferative neoplasms, MM, HL, DLBCL/transformed indolent B-NHL, MCL, T-cell lymphoma nodal
High	AML adverse cyt., MDS adverse cyt., T-cell lymphoma extranodal
Risk	Stage
Low	CR1, CR ≥ 2, PR1, untreated, CML CP, PR ≥ 2 (if RIC)
High	PR ≥ 2 (if MAC), induction failure, active relapse, CML AP or BP

Disease risk	Stage risk	Overall risk	OS at 4 years
Low	Low	Low	64% (56–70%)
Low	High	Intermediate	46% (42–50%)
Intermediate	Low	High	26% (21–31%)
Intermediate	High		
High	Low	Very high	6 (0–21%)
High	High		

Adapted from Armand et al. (2012)

AML acute myeloid leukemia, MM multiple leukemia, cyt. cytogenetics, CLL chronic lymphocytic leukemia, NHL Non-Hodgkin’s lymphoma, MDS myelodysplastic syndrome, HL Hodgkin’s lymphoma, DLBCL diffuse large B-cell lymphoma, MCL mantle cell lymphoma, CR1 first complete remission, CR complete remission, PR1 first partial remission, RIC reduced-intensity conditioning, PR partial response, MAC myeloablative conditioning, AP accelerated phase, BP blast phase

**The EBMT Risk Score (Gratwohl et al. 1998, 2009)**

This predictive score, validated with 56,505 patients, allows estimating the 5-year probability of OS and the treatment related mortality (TRM) for the main diseases (see Tables 11.2, 11.3, and 11.4).

The EBMT risk score is also useful for predicting OS and TRM in patients receiving a second HCT (Rezvani et al. 2012) and in those receiving a T-cell depletion (TCD) HCT (Lodewyck et al. 2011).

Some authors have introduced modifications in this risk score (including the concept of disease stage) to improve its predictivity (Terwey et al. 2010; Hemmati et al. 2011). Similarly, it has been combined with the HCT-CI (Barba et al. 2014).

**Table 11.2** EBMT risk score (Gratwohl et al. 2009)

Variables	Value of variables	Points
Age	<20 Years	0
	20–40 Years	1
	>40 Years	2
Disease status <sup>a</sup>	Early	0
	Intermediate	1
	Advanced	2
Interval diagnosis of HCT <sup>b</sup>	<12 Months	0
	≥12 Months	1
Donor	HLA-identical sibling	0
	Unrelated donor	1
Gender of donor–recipient	Female to male	1
	Other combinations	0

Adapted from Gratwohl et al. (2009)

<sup>a</sup>Does not apply in patients with SAA. Early = AL in CR1; MDS in CR1 or untreated; CML in the first chronic phase; NHL/MM untreated or in CR1. Intermediate = AL in CR2; CML with another status than accelerated phase or blastic phase; MDS in CR2 or in PR; NHL/MM in CR2, PR, or stable disease. Late = AL in other stages; CML in blastic crisis; MDS in all other stages; NHL/MM in all other stages

<sup>b</sup>Does not apply to patients in CR1

**Table 11.3** Probability (%) of TRM at 5 years by applying the EBMT risk score

Points	0	1	2	3	4	5	6–7
AML	14	20	25	30	36	40	41
ALL	15	23	24	30	40	47	53
CML	15	22	30	38	45	52	55
AA	18	26	40	49	52		
MDS	25	28	30	35	38	46	50
MM			29	35	40	42	52
NHL	15	24	28	30	34	36	38

Extracted from Gratwohl et al. (2009)

**Table 11.4** Probability (%) of OS at 5 years by applying the EBMT risk score

Points	0	1	2	3	4	5	6–7
AML	68	59	52	38	30	23	18
ALL	66	52	43	38	22	16	14
CML	76	72	60	51	39	26	14
AA	81	72	60	49	45		
MDS	56	52	46	40	35	28	25
MM			48	40	36	22	17
NHL	75	59	50	48	43	40	38

Extracted from Gratwohl et al. (2009)

This score has been validated by many groups and for many diseases (AML, ALL, myelofibrosis (MF), CLL, and CML, among others).

**HCT-Specific Comorbidity Index (HCT-CI) (Sorrer et al. 2005)**

This was developed in Seattle in 2005. It is an adaptation of the classical Charlson Comorbidity Index (CCI). It has been validated in several cohorts and is widely used. The score analyzes 17 comorbidities and their degree (see Table 11.5).

**Table 11.5** HCT-specific Comorbidity Index, including age variable (Sorrer et al. 2005, 2014)

Comorbidity/definition	Points
<i>Age</i> ≥ 40 years	1
<i>Arrhythmia</i> Atrial fibrillation, flutter, sick sinus node syndrome, or ventricular arrhythmias	1
<i>Cardiac</i> Coronary heart disease, congestive heart failure, IAM, FEVE ≤50%	1
<i>Inflammatory bowel disease</i> Crohn’s disease or ulcerative colitis that has required treatment	1
<i>Diabetes</i> Requiring insulin or oral antidiabetic medication in the 4 weeks prior to HCT	1
<i>Cerebrovascular</i> CVA or TIA or cerebral thrombosis	1
<i>Psychiatric</i> Depression, anxiety, or others requiring ongoing treatment (not on demand)	1
<i>Mild liver</i> Chronic hepatitis, elevated bilirubin <1.5 × UNL or AST/ALT <2.5 × UNL Previous HBV or HCV infection	1
<i>Obesity</i> BMI >35 kg/m <sup>2</sup>	1
<i>Previous infection</i> Infection in admission requiring continuation of treatment beyond day 0	1
<i>Moderate lung</i> DLCO and/or FEV1 66–80% or minimal stress dyspnea	2
<i>Rheumatology</i> Systemic lupus, rheumatoid arthritis, polymyositis, polymyalgia rheumatica, connective tissue disease	2
<i>Peptic ulcer</i> Endoscopic or radiological diagnosis (does not score if only reflux or gastritis)	2
<i>Renal</i> Creatinine >176 memol/L, dialysis, or previous kidney transplant	2

**Table 11.5** (continued)

Comorbidity/definition	Points
<i>Previous tumor</i> <sup>a</sup> Neoplasia at some point (excludes non-melanoma skin tumor)	3
<i>Heart valve</i> Diagnosed (except mitral prolapse)	3
<i>Severe pulmonary</i> DLCO and/or FEV1 ≤ %, dyspnea at rest or oxygen at home	3
<i>Severe liver disease</i> Bilirubin ≥0.5 for VN or AST or ALT ≥0.5 for VN or cirrhosis	3

<sup>a</sup>The most recent version has also included in this category hematological tumors of a different lineage, which motivates transplantation (e.g., lymphoma in an AML patient but not previous MDS in an AML patient)  
CVT cerebral vein thrombosis, TVA transient ischemic attack, AST aspartate transaminase, ALT alanine transaminase, BMI body mass index, FEVE left ventricular ejection fraction, IAM myocardial infarction, UNL upper normal value

Given the impact of age on outcomes, the authors modified the model (Sorrer et al. 2014), including a 1-point score for patients aged 40. This modification significantly improved the predictive capacity of the model. Consequently, the patients could be classified into three different risk groups (0 points: low risk; 1–2 points: intermediate risk; and 3 or more points: high risk) that clearly correlated with 2-year non-relapsed mortality (NRM).

Other authors re-stratified the HCT-CI index (flexible HCT-CI) as low risk: 0–3 points; intermediate risk: 4–5 points; and high risk: >5 points, with this classification being a better predictor of NRM. In an RIC setting, the 100-day and 2-year NRM incidence in these risk categories was 4%, 16%, and 29% and 19%, 33%, and 40%, respectively. The authors found this predictive NRM value using neither the original HCT-CI nor the pre-transplant assessment of mortality (PAM) or Charlson Comorbidity Index (CCI) models. Regarding the 2-year OS, this flexible HCT-CI score was also associated with the highest predictive hazard ratio (Barba et al. 2010).

HCT-CI has also been validated in CD34<sup>+</sup>-selected HCT (Barba et al. 2017) and combined with the EBMT risk score, thus allowing a higher discrimination (Barba et al. 2014; Versluis et al. 2015).

### The EBMT Machine Learning Algorithm (Shouval et al. 2015)

This algorithm is based on an alternating decision tree that is able to detect variables associated with the primary outcome, assigning weights and ignoring redundancies. This score was developed to not only analyze the NRM at day +100 in patients with acute leukemia but also predict NRM, Disease Free-Survival (DFS), and OS at 2 years.

The variables included in the model are age, Karnofsky index ( $\geq 80$ ;  $< 80$ ), diagnosis (AML; ALL), disease stage (CR1; CR2; all other stages), interval diagnosis of HCT ( $< 142$  days;  $\geq 142$  days), donor–recipient CMV status (both sero+; both sero-; any other combination), donor type (matched sibling donor (MSD); matched unrelated donor (MUD)), conditioning (MAC; RIC), and annual allo-HCT performed at the center ( $< 20$ ;  $\geq 21$ ). The total + 100 NRM and 2-year NRM, DFS, and OS could be obtained through the web page <http://bioinfo.lnx.biu.ac.il/~bondi/web1.html>.

Recently, this algorithm has also been validated by an independent set of data from GITMO (Gruppo Italiano Trapianto di Midollo Osseo) (Shouval et al. 2017).

### The Myelofibrosis Transplant Scoring System (MTSS) Score for Myelofibrosis

A clinical molecular myelofibrosis transplant scoring system (MTSS) has been proposed for patients for whom an allogeneic transplant is advised. This score is applicable to primary and post-essential thrombocythemia/polycythemia vera (ET/PV) myelofibrosis and seems promising in predicting posttransplant outcome better than disease-specific systems. Risk factors were incorporated into a four-level MTSS with a 5-year survival rate of 83% for low-risk patients (score, 0–2), 64% for intermediate-risk (score, 3–4), 37% for high-risk (score, 5), and 22% for very high-risk (score,  $> 5$ ) (Gagelmann et al. 2019).

#### 11.1.4.3 The Predictive Capacity of these Models

Unfortunately, all these models have a limited predictive capacity, and none of them stand out more than the rest.

Authors	Predictive/s model/s	Predictive capacity
Sorror et al. (2005)	HCT-CI	0.65
Xhaard et al. (2008)	rHCT-CT, PAM	0.49, 0.57
Gratwohl et al. (2009)	EBMT	0.63
Barba et al. (2010)	rHCT-CI, PAM	0.67, 0.63
Barba et al. (2014)	HCT-CI, EBMT	0.60, 0.54
Versluis et al. (2015)	(HCT-CI-EBMT)	0.58, 0.58 (0.64)

rHCT-CI reduced model, without PFTs, HCT-CI flexible model (modified scale)

### 11.1.5 Practical Applications of Risk Assessment

Election of the conditioning	In patients with a high risk of NRM following one of the mentioned risk scores, an RIC should be considered
Relative contraindications	Uncontrolled infection, severe or chronic liver disease (excluding cirrhosis), severe disturbances in heart function (FEV $< 40\%$ ), respiratory (DLCO $< 40\%$ ), or renal (creatinine clearance $< 30$ mL/min). <i>Cirrhosis</i> : Even compensated cirrhosis receiving RIC has a high likelihood of hepatic decompensation (Hogan et al. 2004)
Absolute contraindications	Pregnancy

#### Key Points

- Evaluation of a candidate must be carried out according to a preestablished work plan designed by each institution. The use of standardized procedures reduces the risk of errors or omissions.
- Pretransplant variables (such as age) have a clear impact on the results of the procedure but, when assessed in isolation, are highly insufficient to predict the results.
- Models (DRI, EBMT risk score, HCT-CI, PAM) allow a much more realistic approach to the real possibilities of a given candidate and adapt the procedure to their needs.

## 11.2 Counseling of Candidates

Alessandro Rambaldi

### 11.2.1 Introduction

Allo-HCT is a potentially curative treatment modality for otherwise incurable diseases. Unfortunately, after transplantation, patients may experience not only the persistence or recurrence of their own disease but also some dramatic clinical complications and toxicities, including death. The clinical indications for transplant have been addressed in the section “Indications” of this book, but, in general, when allo-HCT is advised, the strength of the indication (the likelihood to be cured by transplant), the patient’s fitness, and his/her personal commitment to transplantation must be carefully evaluated for each candidate.

Obviously, in a patient, the first distinction must be made between neoplastic and non-neoplastic disease, and the transplant option should be progressively discussed with the patient during the course of the disease, particularly in the case of hematological malignancies. Many professionals should concur to illustrate to the patients the curative potential of allo-HCT and help them understand the severe complications that can eventually develop. It is clear that different indications remarkably affect the way in which a patient is advised. However, there is a time when the transplant option must be formally presented and advised. Therefore, evaluation of each transplant candidate must be based on well-predefined formal standard operating procedures to collect exhaustive clinical, instrumental, and laboratory data that may lead to a robust definition of the risks and benefits related to allo-HCT. All in all, counseling is tailored to conduct such evaluation of the individual patients (Shouval et al. 2015), according to both objective data and subjective data such as patient propensity and fear of side effects. At the end of this process, the patient should be aware of the rationale, the benefit and toxicity associated with each step, and the component of the transplantation procedure. In this chapter, I will hereby summa-

rize the main topics that I cover with my patients when they come to my office to discuss the option of allo-HCT.

### 11.2.2 Understanding the Benefits and Risks of Allogeneic Transplantations

Patients must be informed that allo-HCT is a therapeutic option that is always proposed with the intent to achieve a permanent cure of the underlying disease, but, despite this premise, disease progression or relapse may eventually happen. The indication for allo-HCT depends not only on the disease characteristics but also on patient-related factors such as age and comorbidities (Sorrer et al. 2007) so that the transplant proposal is the result of an accurate and wise evaluation of both these factors (Sorrer 2013; Wang et al. 2014). In addition, the source of the stem cell graft and the donor type (sibling matched, matched or mismatched unrelated, haploidentical or cord blood) are associated with different transplant modalities for the graft procurement, the conditioning regimen, and the GvHD prophylaxis. All these details require time that the physicians must dedicate to their transplantation candidates.

The patient should understand the specific risk/benefit balance associated with a conventional versus a transplant-based proposal, and this may be remarkably different if he/she has been diagnosed with a non-neoplastic disease such as thalassemia or sickle cell anemia, a bone marrow failure syndrome like aplastic anemia, or a blood cancer, such as an acute leukemia. Even when allo-HCT may, in theory, represent the most efficacious treatment modality to get a permanent cure of a specific disease, an accurate description of the available alternatives must be presented. This is particularly important when the non-transplant options, albeit not curative, may have the chance to keep the patient alive for a long time (Samuelson Bannow et al. 2018) or, even more importantly, when conventional treatment may lead to a definitive cure such as in the case of some patients with acute leukemia with

intermediate-risk genetic factors or those achieving deep molecular remission after conventional chemotherapy (Cornelissen and Blaise 2016).

### **11.2.3 Understanding the Transplant Procedure: The Donor, the Conditioning Regimen, and the Clinical Complications**

Once the indication for transplantation has been confirmed, patients and their relatives must be informed on how the transplant is performed. Patients should understand that identifying a stem cell donor is an absolute prerequisite to perform a transplant. Accordingly, patients should be informed about the human leukocyte antigen (HLA) genetic system, its specificity toward each individual, how it is inherited by parents according to the Mendelian laws, and what is the probability of finding a compatible donor in the family group. Understanding the HLA system is crucial to explain why the use of an HLA family-matched sibling donor is considered standard and when such a sibling is not available, an international search has to be performed to identify an HLA-compatible unrelated donor. It is important to underline that more than 40 million potentially available donors are registered by the World Marrow Donor Association (WMDA) and that the probability of finding a compatible donor is between 50 and 80% according to the ethnical origin of each patient.

Once such a matched unrelated donor is identified, this type of transplant is considered a standard of care, and its clinical outcome is fully comparable to that observed when using an HLA-identical sibling. In patients for whom an MSD or an MUD is not available, the patient should be informed that two additional options are available, namely, the use of HSCs obtained by a family mismatched donor (commonly defined as haploidentical because sharing only one of the patient's HLA haplotypes) or banked cord blood units (CBUs). Patients should understand how HLA diversity between the patient and donor has been overcome by specific programs of

in vitro or in vivo manipulation of the graft. In recent years, the number of transplantations performed using an haploidentical donor has markedly increased since the clinical outcomes of this transplant modality are comparable to those achieved using matched related donors. Prospective clinical trials are ongoing to define if a MUD or an haplo donor can offer a clinical benefit, particularly in terms of leukemia-free survival. For this reason, dedicated counseling about the choice between an haplo and a MUD is frequently requested. Based on ethical considerations and the related probability of finding a donor within the international registries and also financial aspects, each transplant center must have standard operating procedures detailing the reasons why an haplo or a MUD donor is the preferred choice. The transplant candidate should be advised accordingly. In the current clinical practice, the use of cord blood units is declining and it is usually restricted to a minority of patients for whom a MUD or an haplo graft is not available. Still, the potent anti-leukemic activity of this transplant modality should be kept in mind, particularly for patients with resistant hematological malignancies (Horgan et al. 2023). Specific counseling to illustrate the benefits and risks (particularly the delayed engraftment and immune reconstitution) of this transplant modality should be undertaken.

Patients should be reassured that the incidence and severity of GvHD, the most important side effect of allo-HCT, does not seem to be higher than observed with a MUD and also an haplo donor. In addition, patients should know that many studies reported that transplants performed with these alternative stem cell sources proved to be effective and safe even when offered to patients of advanced age and/or with existing accompanying illnesses or when the disease was refractory to conventional treatment. All in all, at the present time, the clinical outcome of these alternative types of transplants compares reasonably well with those achieved with a MUD. Therefore, the decision to use this type of stem cell source only when an HLA-matched donor is not available is mostly related to the lack



of randomized clinical trials that are planned to be performed in the near future.

The goal of allo-HCT is to eradicate the patient's hematopoiesis that is either neoplastic or normal. This is achieved by delivery of the conditioning regimen and by the lifelong *in vivo* role played by the donor's immune system. Most often, high doses of chemotherapy and/or radiation are included in the preparations, although remarkable differences exist depending on the disease needing transplant and patient tolerance. The patient should understand that the intensity of the conditioning regimen may be particularly important in the case of hematological malignancies when the aim to remove most of the neoplastic cells present in the patient's body is the first goal. However, to avoid at least part of the treatment toxicity, the intensity of the preparative regimen can be down-modulated, leading to the definition of this preparative regimen as non-myeloablative or reduced intensity. The depletion of the patient bone marrow stem cells induces prolonged pancytopenia and the need for donor-derived healthy stem cells to grow and establish a new blood cell production system.

Allogeneic HSCs, collected from the donor's BM or PB or a frozen CBU, are infused through the central venous catheter into the bloodstream: HCT is not a surgical procedure, and it is highly similar to receiving a blood transfusion. The stem cells find their way into the bone marrow and begin reproducing and growing new, healthy blood cells. It is extremely important to explain how the donor immune system will develop progressively after transplantation and will either play a crucial beneficial role against residual neoplastic cells or restore the immune competence against infections, but it could mediate the most harmful GvHD effect against the patient.

After the transplant, supportive care is provided to prevent and treat infections, side effects of treatments, and complications. Prolonged anemia, thrombocytopenia, and leukopenia can be dangerous and even life-threatening. A low platelet count can be potentially associated with bleeding in the lungs, GI tract, and brain. Leukopenia, including either a defect of neutro-

phils and lymphocytes, leads to the development of frequent infections, the most common clinical complications after transplantation. Infections can include not only bacterial, most likely when the patient has severe bone marrow suppression, but also viral and fungal pathogens. Infections can require an extended hospital stay, prevent or delay engraftment, and/or cause permanent organ damage. On average, the time to hematological engraftment (recovery of the neutrophil and platelet function) is about 2–3 weeks, but a protective recovery of the immune system can take months and sometimes years. High doses of chemotherapy and radiation can cause remarkable toxicities that include but are not limited to severe mucositis (inflammation of the mouth and GI tract) that favors bacterial translocation with related infections and GvHD and multi-organ failure of mainly the lungs, heart, liver, and kidneys.

Particular attention should be paid to the risk of graft failure that can occur early or late after transplantation. Graft failure is more frequent in some diseases such as myelofibrosis or as a result of infection or when the stem cell content of the graft is insufficient to guarantee a durable engraftment. Graft rejection can also happen after a reduced intensity conditioning regimen (when the immune system of the host is not completely eradicated and can actively reject the donor stem cells).

Finally, and most importantly, patients must be aware of what GvHD is, when and how it may develop, and why it represents the most serious complication of HCT, being not only life-threatening but also the principal reason of a long-lasting poor quality of life. Transplantation candidates should be aware that GvHD is the negative counterpart of the deep interaction of the donor immune system within the patient's body that at the same time may lead to a definitive cure of an otherwise incurable disease. In other words, when transplant is advised, patients must realize that they are accepting the possible onset of a chronic, often invalidating, autoimmune disease. GvHD can appear at any time after transplant.

Patients must be reassured that specific and validated protocols to prevent GvHD have been implemented. These programs to prevent GvHD prophylaxis may differ substantially based on the patient's age, the conditioning regimen, the type of disease, and, most importantly, the stem cell graft and HLA compatibility. Patients must understand that despite GvHD prophylaxis, unfortunately, GvHD may eventually occur, and, in this case, specific treatment programs exist. It is important to teach the patient that GvHD can be distinguished into an acute form, which usually develops within the first 100 days after transplantation, and a chronic form, which occurs later in the transplant course. Patients who develop acute GvHD are also more likely to develop the chronic form of GvHD. Patients must understand the importance of their compliance to all the treatments given posttransplant to prevent GvHD and how this is instrumental for a successful transplant. GvHD occurs when the donor's immune system reacts against the recipient's tissue. At variance to what happens after a solid organ transplant where the patient's immune system is driven to reject only the transplanted organ, in GvHD, the donor immune system can react against many different organs of the recipient. This is because the new cells do not recognize the tissues and organs of the recipient's body as self. Over time, owing to the effect of immunosuppressive drugs, a progressive tolerance can develop. The most common sites for GvHD are the GI tract, liver, skin, and lungs.

#### 11.2.4 Posttransplant Treatment Options

An important new development in transplantation is represented by the opportunity to deliver additional posttransplant antitumor treatments. The patients should be aware that transplantation may represent an immunotherapy platform, which does not impede the administration of additional antineoplastic treatments. The latter can give time to the donor immune system to mount an effective graft-versus-leukemia activity.

#### 11.2.5 Alternative Treatment Options to Autologous and Allogeneic Transplantation

Recent development of innovative cellular therapies can represent an effective treatment alternative to both autologous and allogeneic transplant. Autologous chimeric antigen receptor T cells (CAR-T) cells as third- or even second-line treatment for patients with relapsed or refractory large B-cell lymphoma resulted in significantly longer overall survival than standard care (Westin et al. 2023). The results achieved in clinical trials have been confirmed in the real-world setting (Jacobson et al. 2020).

The benefit of this treatment option must be discussed with each potential patient, taking into consideration the most commonly reported side effects (cytokine release syndrome and neurotoxicity) as well the high costs of this treatment not always fully covered by national health systems or private insurances.

CAR-T cells have also been shown to be effective in relapsed and refractory ALL, with impressive results achieved in clinical trials conducted in pediatric and adult patients (Maude et al. 2018; Shah et al. 2021) and confirmed in the real-life setting (Pasquini et al. 2020). Despite the unprecedented rate of Complete Remission (CR) rate achieved in advanced ALL, many patients can still relapse. Therefore, a subsequent, allogeneic transplantation remains a treatment option to be discussed with the patient and his/her relatives, particularly in the pediatric setting. The high costs of a sequential CAR-T cell treatment and allogeneic transplant may represent a financial burden that is not always affordable.

#### 11.2.6 Logistics

After discharge from the transplant ward, patients are followed up in the outpatient clinic two to three times per week until day +100. Patients should be helped to realize how complex the transplant procedure is and that the time spent in the hospital represents only the first part of the treatment pro-



gram. All allo-HCT patients should ideally stay within 1 h of the hospital until it is about 3 months from the day of the transplant. Patients and their families should also realize that the overall recovery time varies from person to person and that, in general, this process takes about 1 year to be considered satisfactory. Allogeneic transplantation is therefore a long-lasting form of immunotherapy, and the interaction between the donor immune system and the patient requires careful and prolonged medical assistance, quite often lifelong.

### Key Points

Counseling of patients should be carefully performed to inform candidates that:

- The specific characteristics of both the disease and patient are equally important to advise transplant.
- Allo-HCT is performed to cure otherwise incurable diseases.
- Despite transplantation, the disease may persist or relapse may occur.
- Transplantation can severely compromise the quality of life of patients.
- Transplantation is a form of immunotherapy requiring long-term follow-up care.
- CAR-T cells are an alternative to autologous or allogeneic transplantation.
- Transplantation is an immunotherapy platform that does not impede additional posttransplant options.
- Logistics are important to ensure adequate care and assistance.

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# Donor Selection for Adults and Pediatrics

# 12

Francis Ayuk, Adriana Balduzzi, and Nina Worel

## 12.1 Introduction

The availability of a suitable stem cell graft is an absolute prerequisite for the performance of allo-hematopoietic cell transplantation (HCT). Beyond donor–recipient histocompatibility, other factors such as stem cell source, donor age and gender, donor–recipient cytomegalovirus (CMV) status, and ABO compatibility, besides the stem cell dose contained in the graft, may play a role in transplant outcome.

In this chapter, we discuss the results of studies investigating these factors and conclude with an algorithm for donor selection. Issues peculiar to pediatric recipients are also analyzed and discussed.

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## 12.2 Donor Human Leukocyte Antigen (HLA) Compatibility (See Chap. 9)

The outcome of HCT depends in part on the matching between the donor and the recipient for the human leukocyte antigens (HLAs), encoded by a group of genes on chromosome 6; genes and products are labeled the major histocompatibility complex (MHC). The HLA system is the most polymorphic genetic region known in the human genome. A set of HLA gene alleles, called haplotypes, is inherited from each parent; therefore, the probability that a child inherits and shares both parental haplotypes with a full sibling is 25%. Such an HLA-identical sibling is still considered an optimal donor.

The most relevant genes for transplantation belong to class I (HLA-A, HLA-B, and HLA-Cw) and class II (HLA-DR, HLA-DQ, and HLA-DP). HLA compatibility with the donor is usually defined by high-resolution typing (4 digits) for 10 alleles, HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ (Petersdorf 2013), even though there is increasing evidence supporting the relevance of DPB1 matching (reviewed by Fleischhauer and Shaw (2017)).

The concept of “compatibility” between cord blood (CB) donor–recipient pairs is still under debate. In the past, any CB unit, which was 6/6 or 5/6 matched was considered HLA-compatible (matched donor (MD)), as defined by low-resolution typing at the A and B loci and

high-resolution typing at the DRB1 locus; more recently, a high resolution for at least A, B, C, and DRB1 loci has been requested and, progressively, the same criteria used for volunteer donors are considered to define CB HLA matching (Eapen et al. 2017).

## 12.3 Donor Selection for Adult Patients

### 12.3.1 Donor Type

#### 12.3.1.1 Matched Related Siblings and Unrelated Donors (URDs)

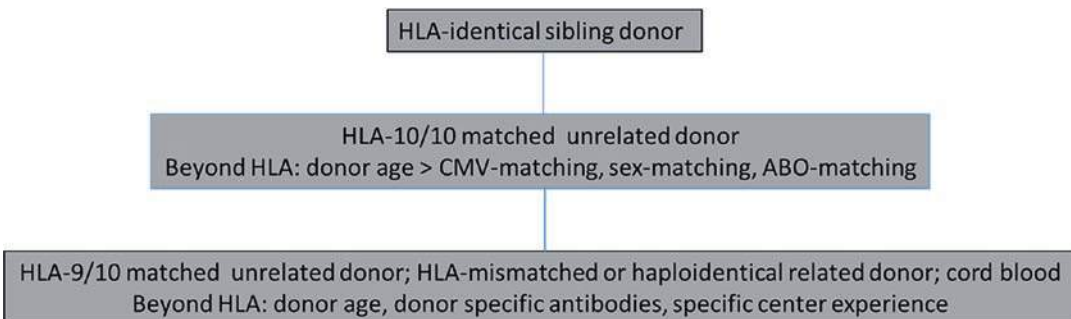
Donor–recipient histocompatibility is one of the key variables in allo-HCT. An HLA-identical sibling donor is generally considered the best donor for allo-HCT; however, less than a third of patients will have one available, with the proportion varying mainly according to family size. The algorithm for donor selection is described in Fig. 12.1.

Unrelated donor registries worldwide now include more than about 41 million volunteer donors, with most of them in North America and Europe ( [www.bmdw.org](http://www.bmdw.org)). The probability of finding a fully matched unrelated donor (MUD) (8/8 or 10/10) varies on average between 16% and 75% (Gragert et al. 2014; Buck et al. 2016) depending on ethnicity, with the lowest and highest probabilities in patients of African and

European descent, respectively. Over time, increasing ethnic diversity will further limit the chances of finding a fully matched unrelated donor.

Till date, no randomized trial has compared the outcome of transplants from different donors. However, one prospective analysis (Yakoub-Agha et al. 2006) and several retrospective analyses indicate that outcomes after matched sibling donor (MSD) and fully MUD (8/8 or 10/10) HCT are comparable (Schetelig et al. 2008; Szydlo et al. 1997; Arora et al. 2009; Gupta et al. 2010; Woolfrey et al. 2010; Saber et al. 2012). Increase in donor–recipient HLA disparity in HLA-A, HLA-B, HLA-C, or HLA-DRB1 is associated with poorer outcome after unrelated donor transplantation (Lee et al. 2007; Shaw et al. 2010; Woolfrey et al. 2011; Horan et al. 2012; Fürst et al. 2013; Pidala et al. 2014; Verneris et al. 2015). The overall decrease in survival can be explained by the increase in non-relapse mortality (NRM) with no positive effect on relapse. Disparities in HLA-DQB1 and C-allele disparities in C\*03:03 vs. 03:04 have been reported to be permissive with no negative effects on outcome (Lee et al. 2007; Fürst et al. 2013; Morishima et al. 2015; Pidala et al. 2014; Crivello et al. 2016). Disparities in HLA-DPB1 are observed in the majority of HLA-A, HLA-B, HLA-C, and HLA-DQB1 (10/10) MUD transplants. Nonpermissive mismatches in DPB1 defined according to T-cell epitope matching (Zino et al. 2004; Crocchiolo et al. 2009; Fleischhauer

### Algorithm for donor selection for adult patients with hematological malignancies



**Fig. 12.1** The algorithm for donor selection

et al. 2012; Pidala et al. 2014; Oran et al. 2018), allele cell surface expression levels (Petersdorf et al. 2015), or a combination of both (Ruggeri et al. 2023) are associated with poorer outcome compared to full matches or permissive mismatches. Associations of permissive DPB1 mismatches with a lower relapse incidence are currently being explored (Fleischhauer and Beelen 2016; Fleischhauer and Shaw 2017) and have been demonstrated in a recent large retrospective study (Ruggeri et al. 2023).

In HLA-mismatched unrelated donor transplantations, recent data have shown that outcome depends on the HLA-mismatched locus and HLA-B leader dimorphism, indicating that the success of HLA-mismatched unrelated transplantation might be enhanced through the judicious selection of mismatched donors (Petersdorf et al. 2020). Similar findings have subsequently been reported for haploidentical related donor and cord blood transplantations (Fuchs et al. 2022; Petersdorf et al. 2021).

The impact of the killer immunoglobulin-like receptor (KIR) ligand on the outcome, especially disease relapse, has been mostly reported for HLA-mismatched transplantations (Ruggeri et al. 2002; Hsu et al. 2006; Cooley et al. 2014). More recent data have also shown that natural killer (NK) cell reactivity may still affect the outcome in the era of posttransplant cyclophosphamide (Solomon et al. 2018; Wanquet et al. 2018). The NKG2D (natural killer group 2, member D) axis has also been explored and holds promise for further improvement of patient selection (Petersdorf et al. 2023).

### 12.3.1.2 Haploidentical Related Donors

Improvements in transplant technology, including pretransplant anti-T-cell globulin (ATG) (Huang et al. 2006), posttransplant cyclophosphamide (PT-CY) (Luznik et al. 2008), and  $\alpha/\beta$  T-cell depletion (TCD) (Bertaina et al. 2014), have led to improved outcome and rapidly increasing use of haploidentical related donor transplantation (Passweg et al. 2014). Several retrospective comparison studies have reported a

similar outcome for haploidentical and MUD transplants (summarized by Fuchs 2017). The results of prospective comparative trials are eagerly awaited. Selection of an optimal haploidentical donor also takes into account HLA-mismatched loci, including B-leader, NK reactivity, and the other non-HLA factors described below.

### 12.3.2 The Role of Non-HLA Donor Characteristics

Besides donor–recipient histocompatibility, donor age is now considered the most relevant non-HLA donor characteristic in unrelated donor HCT (Kollman et al. 2001, 2016; Wang et al. 2018) with a 2-year survival being 3% better when a donor 10 years younger is selected (Shaw et al. 2018). These findings have impacted daily practice to the extent that the percentage of selected donors under 30 years of age has increased from 36% in the period 1988–2006 to 51% in 1999–2011 up to 69% in 2012–2014 (Kollman et al. 2016). There is accumulating evidence that transplantation with grafts from younger matched unrelated donors may even lead to improved outcomes compared to older matched related donors (Kröger et al. 2013; Guru Murthy et al. 2022). This also seems to hold true for haploidentical HCT, at least for patients over 40 years of age (Canaani et al. 2018).

Matching for patient/recipient CMV serostatus also seems to be a determinant of transplant outcome, with the best outcome seen in seronegative patients receiving seronegative grafts (Ljungman 2014; Kalra et al. 2016; Shaw et al. 2017). How recent improvements in CMV prophylaxis and treatment will impact the relevance of CMV in donor selection remains to be defined.

The impact of sex mismatch on outcome is more controversial, possibly reflecting different definitions of sex mismatch, which has been considered for only male recipients (Gratwohl et al. 2009, 2017; Nakasone et al. 2015) or for both male and female in other reports (Kollman et al. 2016). Interestingly, all three studies confining sex mismatch to male recipients reported a



significant impact for this variable, albeit possibly dependent on the conditioning regimen.

The impact of ABO (blood group) compatibility on outcome has been reported to be modest and seems to have further diminished in recent years probably due to changes in transplant practice, including the less frequent use of bone marrow (BM) grafts (Seebach et al. 2005; Kollman et al. 2016; Shaw et al. 2018). Nevertheless, the use of peripheral blood (PB) instead of BM eliminates ABO incompatibility infusion-related issues but does not prevent possible ABO-incompatibility related-issues in the posttransplant course, with the occurrence of delayed hemolysis, delayed erythropoiesis, and pure red cell aplasia (Balduzzi et al. 2021).

The impact of non-HLA donor characteristics may be less conspicuous in matched and mismatched related donor transplantations using PT-CY. It must, however, be taken into consideration that the close association between donor age and donor-patient relation, on the one hand, with patient age, on the other hand, makes these analyses more complex (McCurdy et al. 2018; Robinson et al. 2018). Larger patient cohorts and prospective studies are required for more definite conclusions.

### 12.3.3 Donor Choice According to Stem Cell Source

The three graft sources for allo-HCT are BM, PB stem cells (PBSCs), and CB. In matched related donor and unrelated donor HCT, survival outcome is similar for both BM and PBSCs. However, hematological recovery is more rapid and graft rejection less frequent after PB compared to BM HCT, whereas the incidence of chronic graft-versus-host disease (cGvHD) and, to a lesser extent, acute GvHD tends to be higher after PB HCT (Bensinger et al. 2001; Couban et al. 2002; Schmitz et al. 2002; Couban et al. 2016; Anasetti et al. 2012). In allo-HCT for non-malignant diseases, in particular for severe aplastic anemia (SAA), BM is still the preferred stem cell source in high-income countries, despite improvements in outcome after PB HCT (Schrezenmeier et al. 2007; Chu et al. 2011; Bacigalupo et al. 2012; Kumar et al. 2016).

Traditionally, BM has been used as a stem cell source for haploidentical HCT with PT-CY (Luznik et al. 2008), whereas granulocyte colony-stimulating factor (G-CSF)-stimulated BM has been used for haploidentical HCT with ATG (Huang et al. 2006) and PBSCs for haploidentical HCT with  $\alpha/\beta$  T-cell depletion (Bertaina et al. 2014). There are no prospective studies comparing different stem cell sources within these strategies. When PT-CY is used, PBSCs seem to be associated with a higher risk of acute and chronic GvHD and a lower risk of relapse in patients with leukemia (Bashey et al. 2017).

The use of umbilical CB grafts continues to decrease with the rise in the numbers of haploidentical transplants performed (Passweg et al. 2023). Due to the limited number of stem cells per unit, CB grafts have been more frequently used in pediatric HCT and will be discussed in the specific CB chapter.

### 12.3.4 Anti-HLA Antibodies

The abovementioned improvements in transplant technology have led to an increased use of grafts from HLA-mismatched donors. Detection of donor-specific anti-HLA antibodies in the patients' serum has been associated with an increased risk of graft failure and also poorer survival of those patients with graft failure (Ciurea et al. 2015) after haploidentical HCT. The risk of graft failure and overall mortality may however also depend on the type and intensity of TCD used. The European Society for Blood and Marrow Transplantation (EBMT) has recently published a consensus guideline on detection and treatment of donor-specific antibodies in haploidentical HCT (Ciurea et al. 2018).

## 12.4 Donor Selection for Pediatric Patients

Donor selection criteria may vary between adult and pediatric recipients. According to the "motto" of the Pediatric Disease Working Party, "children are not small adults;" besides the size, what makes HCT in children different is mainly related

to indications and the biology of a growing individual.

The most frequent indication for transplantation in children is acute leukemia, but a growing proportion of transplanted children is affected with nonmalignant disorders (NMDs), mainly inherited diseases.

### 12.4.1 Pediatric Recipient Size

In terms of size, the recipient weight may vary from few kilograms in most patients transplanted for immunodeficiencies to a full adult size in some adolescents. The recommended cell dose in the graft is shown in Table 12.1 (Gluckmann 2012). The lower the recipient weight, the smaller is the amount of the requested absolute total nucleated cell and stem cell count in the graft, which makes the harvest easier, often matching the transplant center requests. An appropriate cell dose in the graft yields a lower risk of rejection, which is actually the lowest in pediatrics. On the other hand, the lower amount of cells requested to ensure engraftment in children makes CB a more valuable source than in adults.

### 12.4.2 Indications

In terms of indications, according to the most recent EBMT survey, out of the 2920 children and young adults younger than 18 years transplanted in 2021, the main indications for allogeneic HCT were acute leukemias (48%), followed by NMDs (46%), with primary immunodeficiencies representing 37% of them (Passweg et al. 2023).

As NMDs, mainly inherited disorders, namely, immunodeficiencies, hemoglobinopathies, inborn errors of metabolism, and congenital bone marrow failures, do not benefit from any allo-

activity, the closest HLA matching (possibly “10 out of 10” HLA alleles) is recommended. On the contrary, a small degree of HLA incompatibility is tolerated in malignancies, as the detrimental effect of HLA disparity, triggering a higher risk of GvHD and consequent higher risks of toxicity and mortality, might be counterbalanced by the so-called “graft-versus-leukemia (GVL)” or “graft-versus-tumor” effect, which is the alloreactivity of immunocompetent donor cells potentially eradicating residual malignant cells in the patient and playing a role in the prevention of malignant disease recurrence.

### 12.4.3 Donor Type

Due to the decreasing size of modern families in the so-called Western countries, HLA-identical siblings are available for less than 25% of the children in need of a transplant, as shown by the few studies performing a “randomization by genetic chance,” based on the availability of an HLA-identical sibling or not (Balduzzi et al. 2005). As a consequence, 75% of the patients may need to run a search for an unrelated donor.

In the Acute Lymphoblastic Leukemia-Stem Cell Transplantation-Berlin–Frankfurt–Muenster 2003 (ALL-SCT- BFM 2003) and ALL-SCT-International BFM 2007 (ALL-SCT-I-BFM 2007) studies, out of 569 very-high-risk ALL patients, eligible for HCT from any donor, a total of 106 patients (26%) were transplanted from a mismatched donor (MMD), mainly haploidentical. The 4-year NRM was higher for patients transplanted from MMD ( $23 \pm 4\%$  versus  $9 \pm 1\%$ ,  $p < 0.001$ ). In multivariate analysis, MMD grafts were detrimental in terms of OS, event-free survival (EFS), and NRM.

More recently, in the aforementioned EBMT survey (Passweg et al. 2023), donors for pediatric

**Table 12.1** Number of cells according to stem cell source

	Volume collected	Med CD34 content	Med CD3 content	Target cell dose
Bone marrow	10–20 mL/kg	$2\text{--}3 \times 10^6/\text{kg}^a$	$25 \times 10^6/\text{kg}$	$>2 \times 10^8$ TNC/kg
Peripheral blood	150–400 mL	$8 \times 10^6/\text{kg}$	$250 \times 10^6/\text{kg}$	$5\text{--}10 \times 10^6$ CD34 <sup>+</sup> /kg
Umbilical cord blood	80–160 mL	$0.2 \times 10^6/\text{kg}$	$2.5 \times 10^6/\text{kg}$	$>3 \times 10^7$ TNC/kg

<sup>a</sup>Per kilogram recipient body weight



recipients transplanted in 2021 were unrelated in 54% and related in 46% of the cases, with haplo-identical grafts being 42% of the latter ones. In terms of stem cell source, BM was used as the stem cell source in 48% of the patients, whereas PBSCs were used in 47% and CB was used in 140 pediatric patients, 84% of which were unrelated.

The eligibility criteria for HCT in malignant diseases vary overtime, resulting from the balance between the outcome of frontline and relapse chemotherapy protocols and the outcome of transplantation, which partially depends on the degree of compatibility within each donor–recipient pair. Similarly, the eligibility for transplantation in NMD increased as the safety profile of the procedure improved. Some patients are considered eligible for transplantation only in case an HLA-identical sibling is available; as the risk profile of the patient worsens, a broader degree of HLA mismatching is considered acceptable.

Within the International BFM (I-BFM) Study Group, focusing on pediatric malignancies, regardless of their relationship with their recipient, donors are defined as HLA-matched (MD) if the donor–recipient pairs are fully matched (10/10) or have a single allelic or antigenic disparity (9/10) or are defined as a mismatched donor (MMD) if the donor–recipient pairs have two (8/10) or more allelic or antigenic disparities, up to a different haplotype (Peters et al. 2015). Any donor who is not an HLA-identical sibling or an MD, as defined above, is considered an MMD. Both MD and MMD could be either related or unrelated to their recipient. A related donor who is not an HLA-identical sibling is actually regarded as an MD, and GvHD prophylaxis is planned accordingly (Peters et al. 2015).

Results from a BFM study and an I-BFM prospective study showed that transplantation from a “10 or 9 out of 10” matched donor, either related or unrelated, was not inferior to transplantation from an HLA-identical sibling in terms of EFS, OS, and cumulative incidence of relapse (CIR) in pediatric patients with ALL (Peters et al. 2015; Balduzzi et al. 2019). As a consequence, the eligibility criteria for HCT might be reviewed and extended to those for MSD HCT, at least in ALL,

and, possibly, considered for other malignant diseases. Therefore, an unrelated donor search activation and transplantation might be recommended in the future virtually for every child for whom an allo-HCT is indicated and disparities within donor–recipient pairs can be progressively accepted as the risk profile of the patient increases.

Unfortunately, some inherited disorders, in particular sickle cell disease (Gluckman et al. 2017) or other recessively inherited disease, the incidence of which is highly increased by a parental blood relation, have higher incidences in non-Caucasian ethnicities, which are less represented within stem cell donor banks. The consequence is that well-matched donors often lack when a perfect matching is crucial; progresses in haploidentical HCT progressively broadened its indications and may overcome this issue (de la Fuente et al. 2020).

Depending on each transplant center experience, MMD might be preferred, carrying the advantage of prompt donor availability and flexible schedules and bringing about higher degrees of alloreactivity, potentially associated with a lower relapse risk. HCT from MMD is widely recommended when timing adjustment is crucial, as in an advanced disease phase in malignancies and in case of posttransplant relapse.

#### 12.4.4 Haploidentical Donors in Pediatrics

Successful haploidentical HCT has mainly evolved in pediatrics over the last two decades from ex vivo T-cell depletion by CD34<sup>+</sup>-positive selection, to CD34<sup>+</sup>-negative selection, up to selective CD3  $\alpha\beta$  depletion, to allow other cells in the graft, potentially protecting from viral infections (Handgretinger et al. 2001; Klingebiel et al. 2010). In pediatrics, an improved immune recovery after T-cell receptor (TCR)  $\alpha\beta$ -depleted haploidentical HCT (Lang et al. 2015), a similar outcome between TCR  $\alpha\beta$ -depleted and matched sibling and matched unrelated donor HCT in children with acute leukemia (Locatelli et al. 2017) and in NMD, (Bertaina et al. 2014), has been recently reported and confirmed by a multi-

center phase I/II study (Lang et al. 2017). Moreover, some reports of PT-CY in pediatric patients show promising results (Jaiswal et al. 2016; Sawada et al. 2014; Wiebking et al. 2017; Fierro-Pineda et al. 2023).

One of the parents mostly serves as a donor in haploidentical donors for pediatric recipients. The choice between the mother and the father is still debated. Better survival was shown in patients transplanted from the mother than from the father (51% vs. 11%;  $P < 0.001$ ), due to both reduced incidence of relapse and transplant-related mortality (TRM), with a protective effect on the risk of failure (hazards ratio (HR) 0.42;  $P = 0.003$ ), possibly explained by transplacental leukocyte trafficking during pregnancy, inducing long-term, stable, reciprocal microchimerism in both the mother and child (Stern et al. 2008).

As donor-derived alloreactive NK cells have been shown to play a key role in the eradication of leukemic cells, favorable NK matching should guide donor selection (Stringaris and Barrett 2017; Mavers and Bertaina 2018). Moreover, anti-HLA antibodies should be checked and accounted for to guide donor selection.

#### 12.4.5 Stem Cell Source

BM is usually recommended as a stem cell source. A donor with a body weight allowing for a graft containing at least  $3 \times 10^8$  nucleated cells/kg recipient body weight and  $3 \times 10^6$  CD34<sup>+</sup> cells/kg body weight should be selected, in order to yield more than 95% neutrophil engraftment chances at a median of 21 days in the setting of hematological malignant diseases (Simonin et al. 2017).

It is rare in pediatrics to require PB just in order to obtain an adequate amount of cells to ensure engraftment, as the absolute cell dose needed rarely overcomes the maximum amount, which could be harvested from a donor. As higher numbers of CD3 cells are obtained in PB grafts, it is recommended not to exceed an amount of  $10 \times 10^8$  CD3<sup>+</sup> cells/kg recipient body weight.

The increased risk of chronic, and possibly acute, GvHD after PBSC transplantation, as

compared to BM, is commonly reported. In a recent European retrospective study, including 2584 pediatric patients transplanted from 2003 to 2012 for ALL, both TRM and chronic GvHD have appeared to be significantly higher after PBSCs, as compared with other stem cell (SC) sources, despite the similarity of the overall survival for both stem cell sources (Simonin et al. 2017). In the prospective ALL-SCT-BFM 2003 study, the same OS was reported, and no difference could be demonstrated in TRM, acute GvHD, and relapse, irrespective of the stem cell source in the two cohorts of patients transplanted from HLA-identical siblings and other matched donors. Nevertheless, within patients transplanted from HLA-identical siblings, the cumulative incidence of chronic GvHD was higher in PB than in BM recipients (Peters et al. 2015).

Reinforced GvHD prophylaxis may be recommended when PBSCs are used, mainly when no serotherapy is included as for GvHD prophylaxis, as in most protocols in the HLA-identical sibling setting in malignancies (Simonin et al. 2017).

Nowadays, in the ongoing prospective FORUM trial, the algorithm for choosing the stem cell source recommends BM as the first choice. To date, there is no demonstration for a better GVL effect after PB HCT in the pediatric population (Peters et al. 2021).

Due to the increased risk of cGvHD after PB transplant, which is almost consistent among investigators, it is definitely recommended to avoid PB in nonmalignant disorders.

From the first CB transplantation performed for a Fanconi anemia patient in 1987, CB appeared as a useful and an efficient stem cell source, due to two major features: high proliferative capacity, allowing engraftment despite 1-log fewer cells, and immune plasticity, allowing a wider HLA disparity within each donor–recipient pair (Gluckman et al. 1989).

The possibility of adopting less stringent HLA-matching criteria enlarged the availability of grafts to at least 90% of the pediatric patients in need of an allogeneic transplant (Eapen et al. 2017). According to Eurocord consortium recommendations, unrelated CB with two or less

HLA disparities typed in low resolution (i.e., two digits) for class I (A and B loci) and high resolution (i.e., four digits) for class II (DRB1 locus) and with more than  $2.5 \times 10^7$  nucleated cell dose/kg or  $2 \times 10^5$  CD34<sup>+</sup> cells/kg are suitable for engraftment (Gratwohl et al. 2009). Recent studies from Eurocord, NetCord, EBMT, and Center for International Blood and Marrow Transplant Research (CIBMTR) have recommended high-resolution HLA typing for A, B, C, and DRB1 and a maximum of one or two mismatched loci with a cellularity of  $3 \times 10^7$  total nucleated cells (TNC)/kg or higher (Eapen et al. 2014).

In the EBMT Survey reporting 2021 data, BM was used as the stem cell source in 1402 patients of which 62% were family donors. PBSCs were used in 1378 patients with similar proportions seen in both family ( $n = 699$ ) and unrelated donors ( $n = 679$ ). Cord blood stem cells were used in 140 pediatric patients of which 118 (84%) were from unrelated cord blood donors.

Two prospective studies could demonstrate no benefit of double CB in pediatric patients transplanted for malignant diseases (Wagner et al. 2014; Michel et al. 2016).

#### 12.4.6 Other Donor–Recipient-Related Factors

Besides HLA compatibility and stem cell source, donor age, gender, female parity, weight, ABO blood group, and viral serological status should also be considered in the decision-making process for donor selection, whenever more than one donor is available, which may not often be the case (Wang et al. 2018).

Most studies report that a young donor is better than an older one. Few studies also report that a male donor is better for a male recipient and better than a multiparous woman for any recipient, even though this finding is not consistent through the literature. The donor gender effect may be mild and may need a larger series of patients to be demonstrated (Friedrich et al. 2018). Unfavorable weight disparity, with donors weighing less than their recipient, should be avoided, when possible (Styczynski et al. 2012).

CMV-immunoglobulin G (IgG) and Epstein–Barr virus (EBV)-positive patients should be grafted from CMV- and EBV-positive donors, respectively (Jeljeli et al. 2014; Bontant et al. 2014). ABO matching is usually preferred, especially instead of a major or even minor incompatibility (Booth et al. 2013). Donor location might also be considered, as overseas deliveries increase the time elapsing between collection and infusion, thus reducing cell viability and potentially jeopardizing engraftment. More recently, KIR genotyping has allowed identification of alloreactive donors who may contribute to preventing relapse in the non-haploidentical setting as well (Mavers and Bertaina 2018).

Even though it is mainly clear which variant should be preferred within each variable, there is no consensus regarding the hierarchical order by which the above factors should be combined. In a recent survey within the Pediatric Diseases Working Party of the EBMT, the above features have been listed in the following order of importance, on an average, but evaluations widely differed among the responders (Balduzzi et al. 2021):

- HLA compatibility, with 10/10 better than 9/10
- CMV serological status of positive donors in case of positive recipients
- BM as a stem cell source
- Donor age, with a younger donor being preferable over an older one
- Donor gender, with a male donor preferred, particularly for a male recipient
- ABO major compatibility
- Donor center location
- ABO minor compatibility

The recent use of letermovir as per CMV prophylaxis might limit the role of donor CMV serological status in the donor selection process in pediatrics also and in the adult setting. Experiences in pediatrics are relatively scarce, but the prevention of CMV reactivation seems as successful as in adults (Körholz et al. 2023), therefore CMV serostatus might become less and less relevant in the donor selection process.

Moreover, the presence of anti-HLA antibodies directed to any mismatched HLA alleles should be ruled out, mainly in heavily transfused nonmalignant diseases, such as hemoglobinopathies or bone marrow failures (Ciurea et al. 2018).

### Key Points

- An HLA-identical sibling is considered a donor of first choice.
- For patients with hematological malignancies, transplantation from a fully HLA MUD (8/8 or 10/10) is not inferior to transplantation from HLA-identical siblings in terms of EFS. Recent data have indicated that outcome after transplant from a young matched unrelated donor may be better than that from older (>10 years) related donors.
- The choice of alternative donors (haploidentical related donors, cord blood, mismatched unrelated donors) depends on center experience, urgency of transplant procedure, donor age, and detection of donor-specific anti-HLA antibodies.
- For pediatric patients and patients with nonmalignant disorders, BM is the preferred stem cell source.
- For adult patients with hematological malignancies, survival outcome after HCT with PBSCs and BM is comparable.
- In URD transplantation, donor age is probably the most relevant non-HLA donor factor.

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## 13.1 An Overview

Hematopoietic cell transplantation (HCT) is a potentially curative therapeutic procedure in a broad range of malignant and nonmalignant hematological disorders. Conditioning is the preparative regimen that is administered to patients undergoing HCT before the infusion of stem cell (SC) grafts. Historically, pre-HCT conditioning was aimed at eradicating the hematological malignancy in case of malignant indication for HCT. It was also used for providing sufficient immune suppression (IS), to ensure engraftment and to prevent both rejection and graft-versus-host disease (GVHD), and for providing stem cell niches in the host bone marrow (BM) for the incoming stem cells. The third purpose is now controversial as it was demonstrated in animal models that with mega doses of hematopoietic stem cells (HSCs) and repeated administrations, engraftment can be achieved without conditioning. In addition, the donor immune system can pave the way for engraftment.

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A conditioning regimen consists of two components: myeloablation, which targets the host stem cells, and lymphodepletion, which targets the host lymphoid system. The relative intensities of myelosuppression and immune suppression differ between the different regimens. Some of the compounds used in the conditioning are more myeloablative (MA) in nature, for example, melphalan (MEL) or busulfan (BU), whereas some are more lymphodepleting like fludarabine (FLU) or cyclophosphamide (CY). The pretransplant conditioning may include total body irradiation (TBI) (that provides both myelosuppression and immune suppression) or, in rare and specific instances, other types of irradiation like total lymphoid irradiation (TLI). Alternatively, the pre-HCT conditioning can be radiation-free, including only chemotherapy. In recent years, serotherapy, specific targeted novel compounds, and monoclonal antibody (MoAb) and radiolabeled Ab have started getting incorporated into specific disease-oriented conditioning regimens.

Conditioning regimens can be grouped by dose intensity. Historically, the conditioning protocols were myeloablative conditioning (MAC) in nature, and the two most popular ones were the CY/TBI (intravenous (IV) CY 60 mg/kg × 2 days followed by TBI 12 Gy) and the BU/CY protocol (oral BU 4 mg/kg × 4 days and CY 60 mg/kg × 2 days). However, MAC is associated with significant organ- and transplant-related toxicity (TRT), limiting allo-HCT to younger patients in good medical conditioning, typically up to the

age of 55 years. During the past two decades, non-MA (NMA), RIC, and reduced toxicity conditioning (RTC) regimens have been developed, aiming at reducing organ toxicity and transplant related mortality (TRM) while keeping the anti-malignant effect and allowing allo-HCT in elderly and medically infirm patients. These are relatively nontoxic and more tolerable regimens designed not to maximally eradicate the malignancy but rather to provide sufficient IS to achieve engraftment and to allow induction of graft versus leukemia (GVL) as the primary treatment. A group of experts attempted to define and dissect the intensity of the conditioning regimen based on the expected duration and reversibility of cytopenia after HCT and the theoretical need for stem cell support (Bacigalupo et al. 2009). MAC was defined as a conditioning regimen that results in irreversible cytopenia in most patients, and stem cell support after HCT is required. Truly NMA regimens cause minimal cytopenia and can theoretically be administered without stem cell support. RIC regimens cause profound cytopenia and should be administered with stem cells, but cytopenia may not be irreversible. Mixed chimerism may occur more often following the less intensive regimens. RTC regimens were later defined as new versions of MAC that

cause less toxicity such as with the substitution of CY with FLU.

Several new regimens and approaches have been introduced over the last few years. These comprise newly included chemotherapy agents such as treosulfan and thiotepa and new immunosuppressive agents like clofarabine. In addition, there are new doses and schedules, such as different doses of BU, or new schedules, such as sequentially administering novel chemotherapy combination (FLAMSA (fludarabine, Ara-C, and amsacrine)), to be followed by RIC conditioning. The old RIC/MAC classification may not accurately classify these regimens and there may be significant overlapping. The European Society for Blood and Marrow Transplantation (EBMT) started an effort to redefine and measure transplant conditioning intensity (TCI) (Spyridonidis et al. 2020). They assigned intensity weight scores for the most often used components of conditioning regimens. The sum of these scores results in grouping TCI into low, intermediate, or high TCI. This score better predicted non-relapse mortality (NRM) than did the original RIC/MAC classification. An intermediate TCI score overlaps with what was previously defined as RTC. Further validation and refinement of these scores is underway.

**Table 13.1** Summary of randomized studies comparing various conditioning regimens with different dose intensities

	Author	Disease/age	Conditioning regimens	Outcome
MAC vs. RTC	Lee et al. (2013)	AML Age 17–59 years	BuCy 64 FluBu4 62	BuCy better RI and OS, similar NRM
	Rambaldi et al. (2015)	AML Age 40–65 years	BuCy 124 FluBu4 127	FluBu4 less NRM, similar RI and OS
	Bornhäuser et al. (2012)	AML Age 18–60 years	Cy/TBI 99 Flu/TBI8 99	Similar outcomes
	Peters et al. (2021)	ALL Age 4–21 years	TBI/VP16 212 Flu/TT/Bu 201 Or flu/TT/Treo	TBI less RI and better OS
MAC vs. RIC	Kröger et al. (2017)	MDS Age 18–60 years	BuCy 64 FluBu4 65	Similar outcomes
	Scott et al. (2017)	AML/MDS	MAC 135 RIC 137	MAC lower RI, higher NRM, better OS
Various RIC	Blaise et al. (2013)	Hematological malignancies MAC-ineligible	Flu/Bu2 69 Flu/TBI2 70	FluBu2 lower RI, higher NRM, similar NRM
	Beelen et al. (2020)	AML/MDS MAC-ineligible	Flu/Treo10 221 Flu/Bu2 240	Flu/Treo lower NRM, better OS
	Craddock et al. (2021)	AML/MDS MAC-ineligible	RIC 122 FLAMSA 122 /RIC	Similar outcomes

Table 13.1 summarizes the major randomized studies that have been published in recent years, which compare various regimens with different dose intensities. The optimal condition regimen can be selected based on patient age and comorbidity scoring (including organ-specific toxicity risk), disease status at the time of transplantation, and donor type (sibling, matched unrelated or alternative donors such as umbilical cord blood, or haploidentical donors). Disease type may also direct conditioning components by adding, for example, disease-targeted agents. Different regimens are used in pediatrics considering the aspects of growth and puberty, and different regimens may be needed for nonmalignant disorders where more emphasis is placed on engraftment and prevention of GVHD rather than GVL. The overall dose intensity as well as the relative myeloablative and immunosuppressive components of the regimen can be selected. The selection of the regimen for the prevention of GVHD has also been undergoing significant change in recent years and will be discussed in another chapter. This selection may have an impact on conditioning components such as when selecting post-transplant CY.

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### 13.2 Total Body Irradiation

TBI is one of the major constituents of MAC regimens. In HCT, TBI serves a dual purpose. It allows cell killing, which contributes to the eradication of malignant cells, potentially complemented by additional high-dose systemic chemotherapy. It provides homogeneous dose distribution in the whole body, including sanctuaries not easily reached by systemic chemotherapy such as the central nervous system (CNS) and testicles. It provides a different mechanism of tumor cell killing against chemotherapy-resistant cell clones. TBI also provides immunosuppression to facilitate engraftment. Most studies have shown the equivalence of chemotherapy-based MAC, mostly BU/CY and CY/TBI conditioning for AML (Nagler et al. 2013). In contrast, despite the absence of consensus, TBI remains the first choice in many centers for adult ALL (Giebel et al. 2023a, 2023b).

There is high variability in TBI scheduling among transplantation centers in terms of dose, fractionation, dose rate, and administration of additional chemotherapy (Giebel et al. 2014). Historically, TBI was included in MAC platforms, but, in recent years, to reduce associated toxicities, there have been efforts to reduce the dose and fractionation by including TBI in RIC and RTC regimens. TBI  $\geq 5$  Gy in a single dose or  $\geq 8$  Gy in fractionated doses is considered MAC (Bacigalupo et al. 2009).

Historically, TBI combined with CY has been the standard regimen used for conditioning in acute leukemia. TBI is typically administered at a dose of 12 Gy in six fractions delivered twice a day over 3 days (Thomas et al. 1982). Higher doses of TBI up to 14.25 Gy resulted in improved anti-leukemic effects, but this was counterbalanced by increased toxicity and TRM (Clift et al. 1990). A randomized study comparing standard CY/TBI with fludarabine and a lower dose of TBI (total 8 Gy) in patients with AML in CR1 showed similar survival rates (Bornhäuser et al. 2012). Recently, a registry-based study of the Acute Leukemia Working Party (ALWP) has defined that 8-Gray TBI is sufficient for adult patients with acute lymphoblastic leukemia (ALL) transplanted in CR1 with no additional benefit of augmenting the conditioning intensity to 12-Gray (Spyridonidis et al. 2023).

TBI has evolved since its introduction in the late 50s, but acute toxicities and long-term morbidity remain, especially in younger pediatric patients. The acute toxicities include nausea, vomiting, diarrhea, stomatitis, temporary loss of taste, hemorrhagic cystitis, parotitis, and rash. The late toxicities include interstitial pneumonitis, sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD), cataracts, infertility, hormone-related disorders, osteoporosis, growth retardation, and secondary malignancies (Gruen et al. 2022). These long-term known side effects of TBI can significantly impair the quality of life of patients still during childhood and/or when they reach adulthood.

Although the benefit of a TBI-based conditioning regimen has been shown in children in a large randomized study (Peters et al. 2021), concerns remain about whether TBI should remain

the standard conditioning regimen for all children with ALL. The introduction of sensitive methods for the detection of measurable residual disease (MRD) and new immunotherapies using bispecific antibodies or chimeric antigen receptor (CAR) T-cells in combination with non-TBI-based conditioning regimens have already shown promising results comparable with the outcome after TBI-based standard transplants or even better (Handgretinger and Lang 2022). Additional efforts should be made besides optimization of conditioning regimens to prevent relapses post-transplant and reduce toxicities.

New strategies of radiotherapy, such as helical tomotherapy, are being explored, which is widely used for some solid tumors and is a path for the improvement of outcomes and toxicities in TBI. It has a sparing capacity to reduce the dose to critical organs such as the eyes, thyroid, liver, and lungs (Paix et al. 2018). Several research groups are evaluating the clinical outcomes of this novel hypo-fractionation strategy for patients receiving total marrow irradiation (TMI) and total marrow and involved lymphoid irradiation (TMLI) as part of the conditioning regimen before HCT with interesting results (Shi et al. 2020; Paix et al. 2018). Recently, preclinical models have shown that antibody drug conjugates (ADCs) targeting hematopoietic cells can specifically deplete host stem and immune cells and enable engraftment, using an anti-mouse CD45-targeted ADC combined with TBI (Saha et al. 2022).

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### 13.3 Myeloablative Non-TBI-Containing Conditioning

MAC consists of high-dose chemotherapy, mostly alkylating agent-based regimens used in HCT. These preparative regimens may or may not include a radiation component (see the previous section). MAC causes, by definition, profound and prolonged cytopenia that may last more than 21 days and necessitates stem cell graft in order to recover (Bacigalupo et al. 2009). This high-intensity conditioning can be adminis-

tered only to fit patients with low comorbidities as they are associated with unacceptable toxicity in patients with a low performance status and high co-morbidity scores (Shouval et al. 2022). Historically, BU/CY has been the prototype of chemotherapy-based MAC. It was developed by the Johns Hopkins group in the early 80s as an alternative to TBI in an effort to reduce the incidence of long-term radiation-induced toxicities and improve the planning of HCT in institutions lacking easy availability of linear accelerators (Tutschka et al. 1987). The original studies used oral BU that has an erratic and unpredictable absorption with wide inter- and also intra-patient variability with the risk of increased toxicity, mainly SOS/VOD in patients with a high area under the curve of BU plasma concentration versus time, whereas low BU concentrations may be associated with a higher risk of graft rejection and relapse (Hassan 1999). The common solution was monitoring BU levels and dose adjustments that allowed for better control of the dose administered and reduction of the abovementioned risks (Deeg et al. 2002). The development of IV BU with more predictable pharmacokinetics, achieving tight control of plasma levels, and less need for plasma level testing and dose adjustments significantly reduced BU-mediated SOS/VOD and TRM (Nagler et al. 2014).

Subsequently, in an attempt to further reduce regimen-related toxicity, CY was replaced with FLU, a nucleoside analogue with considerable IS properties that also has a synergizing effect with alkylators by inhibiting DNA repair as well as a highly favorable toxicity profile. A well-designed two-arm study compared BU/CY to BU/FLU, demonstrating a significant reduction of TRM in the FLU/BU arm with no difference in relapse incidence (Rambaldi et al. 2015). FLU is replacing CY in many of the current conditioning protocols, including in combination with TBI (Bug et al. 2023; Giebel et al. 2023b). Other alkylators like thiotepe (Eder et al. 2017) and other nucleoside analogues like clofarabine (Chevallier et al. 2012) have been incorporated into MAC protocols for both acute myeloid leukemia (AML) and ALL in an attempt to reduce the risk of relapse



with equivalent results to TBI-containing conditioning protocols. Other MAC regimens include MEL in combination with BU or replacing BU (Vey et al. 1996; Duque-Afonso et al. 2022).

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### 13.4 Non-Myeloablative, Reduced Intensity and Reduced Toxicity Conditioning

NMA and RIC have been widely introduced over the past 20 years in an attempt to reduce organ toxicity and TRM, allowing HCT in elderly and medically infirm patients who are not eligible for standard MAC. In addition, RTC, based on FLU and MA doses of an alkylating agent, was designed to allow safer administration of dose-intensive therapy. Multiple such protocols have been reported over the years with somewhat overlapping dose intensities and, to a certain extent, unclear categorization.

NMA regimens cause minimal myelosuppression and can theoretically be administered with no stem cell support. They usually result, at least initially, in mixed chimerism. The original NMA conditioning protocols consisted of FLU with a low-dose TBI of only 2 Gy (the Seattle protocol). Other examples include the FLU/CY and the fludarabine, cytarabine, and granulocyte colony-stimulating factor (G-CSF) with idarubicin (FLAG-IDA) conditioning protocol pioneered at the MD Anderson Cancer Center.

RIC regimens are more dose-intensive. They cause profound myelosuppression that may be reversible after a prolonged period of time if there is no stem cell support. They usually associate with earlier achievement of complete chimerism. The most popular regimen in this subgroup is the FLU/BU2 combination (including half of the myeloablative BU dose). The other most popular regimen is the combination of FLU and MEL.

RTC regimens such as the FLU/BU4 regimen are now more often categorized with MAC regimens as discussed in the previous section.

There are several relatively new novel conditioning protocols with a dose intensity that is not

easily categorized. Treosulfan (TREO) is a pro-drug of a bifunctional alkylating agent with strong myeloablative and immunosuppressive characteristics and is associated with a low pro-inflammatory cytokine release and a favorable toxicity profile (Danylesko et al. 2012). FLU/TREO, with a total treosulfan dose of 36–42 g/m<sup>2</sup>, can be categorized as an RTC regimen (Shimoni et al. 2018). It allows safer administration of dose-intensive conditioning in less fit or older patients. A lower dose of TREO of 30 g/m<sup>2</sup> can be grouped with RIC (Beelen et al. 2020). FLU/TREO may have a specific advantage in patients transplanted for myelodysplastic syndrome (MDS) (Shimoni et al. 2021). The thiotepa–busulfan–fludarabine (TBF) regimen consisting of TT, BU, and FLU has RIC and MAC versions and has gained a lot of popularity in alternative donor transplants (Saraceni et al. 2017). A sequential approach was initially developed to treat high-risk leukemia with encouraging results. This approach includes an AML salvage chemotherapy followed in a few days by RIC. The most known type is the FLAMSA conditioning, which comprised of sequential chemotherapy, including FLU, Ara-C, and amsacrine, followed by RIC (Schmid et al. 2005). These regimens had a promising outcome in refractory leukemia.

Multiple retrospective studies compared the various regimens. As a general rule, more intensive regimens are associated with a lower relapse rate, a higher TRM rate, and similar overall survival (Aoudjhane et al. 2005; Luger et al. 2012). The less intensive regimens may have a disadvantage in patients with active disease at transplantation (Shimoni et al. 2006). In particular, a truly NMA approach may be less effective in acute leukemia (Luger et al. 2012).

However, these comparisons are biased by the selection of better-fit patients to MAC, whereas older patients or those with comorbidities were most often administered RIC. Several randomized studies compared MAC and RIC but produced in conflicting results. The Blood and Marrow Transplantation Clinical Trial Network (BMT CTN) conducted a phase III randomized trial comparing MAC (BU/CY, FLU/BU4, or

CY/TBI) with RIC (FLU/BU or FLU/MEL) in patients with AML and MDS who were MAC-eligible (Scott et al. 2017). RIC resulted in lower TRM but much higher relapse incidence compared with MAC, resulting in improved leukemia-free survival (LFS) and a trend for an advantage in overall survival compared with MAC. However, the advantage was limited to patients with a positive MRD at the time of transplantation. A randomized study comparing RIC and MAC in patients with MDS demonstrated similar 2-year RFS and OS with no difference between the two conditioning regimens (Kröger et al. 2017). Randomized studies comparing MAC and RTC did not show a survival difference as discussed in the previous subchapters (Bornhäuser et al. 2012; Rambaldi et al. 2015).

Despite being included in the same dose-intensity category, not all RIC regimens are similar in terms of toxicity profile and expected transplantation outcomes. Several randomized and retrospective studies compared various types of RIC. A randomized French study, which compared FLU/BU to FLU/low-dose TBI, demonstrated less relapse with the FLU/BU regimen but higher TRM, resulting in similar LFS and OS (Blaise et al. 2013). FLU/BU and FLU/MEL were retrospectively compared. FLU/MEL is usually associated with better anti-leukemic effects and a lower relapse rate but with higher NRM, in particular due to GVHD (Shimoni et al. 2007; Eapen et al. 2018). Augmenting a RIC regimen (FLU/BU) by FLAMSA pre-conditioning sequential therapy did not improve survival compared to standard RIC in a randomized UK study in patients with AML and MDS (Craddock et al. 2021). Finally, a randomized study comparing FLU/TREO (with a low dose of treosulfan) and FLU/BU2 showed better survival with the FLU/TREO regimen due to lower rates of NRM (Beelen et al. 2020).

Patients with MDS and myeloproliferative disease (MPD) are often older and are administered RIC or RTC regimens rather than MAC. There is no evidence for an advantage of any regimen over the others in this setting. A large European study used FLU/BU/ATG RIC for myelofibrosis with promising outcome. More

recently, a TBF double alkylator regimen has gained more popularity (Battipaglia et al. 2021). Regimens that are similar to AML are used in MDS. The numbers of allogeneic transplantations for lymphomas is reducing. Most patients are administered RIC such as fludarabine with a combination of an alkylator agent such as melphalan, busulfan, thiotepa or cyclophosphamide.

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### 13.5 Conditioning Regimens for Allo-HCT from Alternative Donors: Mismatched Unrelated Donor (MMUD), CB, and Haploidentical

Historically, these types of allo-HCT were the most challenging ones with a relatively high incidence of non-engraftment and high TRM, on one hand, and increased GVHD rate, on the other. Recent development in the field of transplantation, including novel conditioning regimens and better supportive care, has resulted in major improvement in the results of HCT from alternative donors and marked increase, in particular with the haplo-HCT (Nagler and Mohty 2022). The increase in haplo-HCT is mostly related to the switch from extensive T-cell depletion to non-T-cell depletion techniques (Lee et al. 2017; Kanakry et al. 2016). There are two major approaches for non-T-cell-depleted transplantation: one is posttransplant CY (PTCY)-based (the Baltimore approach) and the other is ATG-based (the Chinese approach). PTCY allows the elimination of alloreactive T cells and enhancement of regulatory T cell (Treg) activity. The original protocol was non-myeloablative using fludarabine and low-dose CY with low-dose TBI pretransplant and PTCY with tacrolimus and MMF posttransplant with BM as an haplo donor stem cell source. This regimen allows haploidentical donor HCT in elderly and medically infirm patients with promising results. Other approaches followed using more intensive and even MAC regimes, with the TBF regimen used most often, and also using peripheral blood stem cells (PBSCs) rather than BM. The ATG approach is



based on intensive pretransplant conditioning and intensive posttransplant immune suppression and the use of G-CSF-mobilized BM and PBSCs as SC sources. This regimen has promising results in younger patients, mostly in China.

Following the improvement of haplo-HCT, umbilical cord blood HCT is currently used less often in adults. There are MAC and RIC regimens. The most popular MAC regimens are based on TBI or, more recently, on the TBF regimen. The RIC regimen is often based on low-dose TBI with FLU and CY. A more detailed discussion of alternative donor transplants will be provided in other chapters.

### 13.6 Preparative Conditioning for Autologous HCT

Auto-HCT is mainly performed for malignant lymphoma and multiple myeloma (MM). The most popular conditioning protocol for auto-HCT in lymphoma is BEAM (BCNU (bischloroethylnitrosourea, carmustine), VP16, Ara-C, and MEL), but other BCNU-based regimens have also been used such as BEAC (BCNU, VP16, Ara-C and CY instead of MEL) or CBV (cyclophosphamide, BCNU, VP16 (etoposide)). Some centers use thiotepa-based regimens, substituting thiotepa for BCNU (such as TEAM (thiotepa, etoposide, cytarabine, and melphalan) or TECAM (thiotepa, etoposide, cyclophosphamide, cytarabine, and melphalan) protocols). Others tried to replace BCNU with bendamustine (the so-called BeEAM (bendamustine, etoposide, cytarabine, and melphalan) protocol). Thiotepa, BCNU, and etoposide pass the blood–brain barrier and are included in the treatment of lymphoma that involves the CNS.

High-dose MEL remains the most used regimen in multiple myeloma with some centers adding bortezomib or BU to the conditioning. The numbers of auto-HCT in acute leukemia went down in the last two decades in parallel to the increase in the numbers of allo-HCT with RIC and from alternative donors with BuCY or BU/MEL been the most popular preparative regimen (Gorin et al. 2017). Some disease-specific regimens are used in solid tumors and are discussed in other chapters.

#### Key Points

- Conditioning regimens are an integral part of HCT, enabling engraftment and providing an antitumor effect.
- The conditioning regimen pretransplantation should take into consideration patient and disease characteristics, including age, comorbidities, and disease status, including measurable residual disease.
- Conditioning regimens may include TBI, chemotherapy, serotherapy, monoclonal antibodies, and targeted therapy, which vary in terms of different malignancies and types of donors.
- The dose intensity of the pre-HCT conditioning ranges between MAC, RTC, RIC, and NMA in decreasing order of dose intensity.
- Both NMA and RIC significantly reduce transplant-related organ toxicity and mortality, enabling transplants in elderly and medically infirm patients. More intensive regimens may have an advantage in acute leukemia, particularly with positive MRD at the time of transplant.
- Specific conditioning regimens are used for allo-HCT from cord blood and haploidentical donors.

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# Selection of Stem Cell Source

# 14

Claire Horgan and David Valcárcel

## 14.1 Introduction

Selection of stem cell source is an important consideration for any physician planning an allogeneic haematopoietic cell transplant (HCT) and has evolved considerably since bone marrow (BM) was used as the stem cell source in the first successful allogeneic HCT in 1968 (Gatti et al. 1968). BM remained the only source of stem cells for the two decades that followed until experimental work demonstrating that peripheral blood (PB) stem cells can be enriched by pre-treatment with certain chemotherapy agents and haematopoietic growth factors (Richman et al. 1976; Socinski et al. 1988; Duhrsen et al. 1988) resulted in the first peripheral blood stem cell transplant in 1986 (Korbling and Freireich 2011). Alongside this, the recognition of cord blood (CB) as a rich source of stem cells (Prindull et al. 1978) led to the successful use of cord blood as a third stem cell source in allogeneic HCT in the late 80s (Gluckman et al. 1989).

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Following the introduction of PB as a stem cell source, its use evolved significantly, and, by the early 2000s, it had overtaken BM as the predominant stem cell source in adult HCT (Giebel et al. 2019). This trend has continued to increase, with PB being by far the most commonly employed stem cell source, now used in more than 80% of adult allogeneic HCT across the globe (CIBMTR n.d.; Passweg et al. 2023). In contrast, BM remains the stem cell source of choice in the paediatric setting (CIBMTR n.d.), and, despite its appeal as an intuitively attractive alternate donor source in adults and paediatrics (Wynn et al. 2022), cord blood use is declining (Passweg et al. 2021).

## 14.2 Factors Influencing the Selection of the Source

There are a number of factors that must be considered when selecting the most appropriate stem cell source for HCT and the advantages and disadvantages for both the patient and donor must be considered as well as specific disease-related considerations.

### 14.2.1 Bone Marrow

The advantages with using BM as a stem cell source include the fact that it can be harvested from a paediatric donor, which is particularly

relevant in the paediatric matched-related donor setting, and there is no need for donor mobilization using granulocyte colony-stimulating factor (G-CSF). There are also fewer T cells in bone marrow grafts compared with peripheral blood, which results in less graft-versus-host disease (GVHD).

The reduced donor T-cell contamination of the graft may be disadvantageous in the malignant disease setting since T cells are involved in the graft-versus-leukaemia (GVL) effect of allogeneic HCT (Sweeney and Vyas 2019). In addition to this, the cell dose is limited by the volume of the donor marrow and is therefore generally lower than the stem cell dose obtained with mobilized peripheral blood stem cells. Red blood cell (RBC) contamination may be significant, and grafts may therefore require RBC depletion when the donor and recipient are ABO-incompatible, which may further reduce the stem cell dose.

Furthermore, BM harvesting is an invasive procedure requiring a general anaesthetic operation for the donor and, in some circumstances, red blood cell transfusion in those cases in which high cellularity is needed.

### 14.2.2 Peripheral Blood

The advantages of peripheral blood stem cells include the large donor CD34 stem cell dose, which is generally collected. This helps in more rapid engraftment and immune reconstitution, leading to lower rates of graft failure, reduced risk of infectious complications and faster hospital discharge. Furthermore, the higher number of T cells in the graft may drive engraftment, particularly in reduced intensity conditioning, and may enhance the GVL (graft-versus-leukaemia) effect in malignant diseases. Apheresis of peripheral blood normally includes only very few red blood cells, so depletion is not necessary in most cases.

The disadvantages of peripheral blood stem cells include the increased risk of chronic GVHD due to the higher amount of T-cell content of the product. This may be associated with a prolonged need for immunosuppression therapy in the long-term, post-transplant period and may adversely affect the quality of life. Allogeneic peripheral blood stem cell donation is avoided in the paediatric setting due to ethical considerations with the use of paediatric donor G-CSF mobilization. Additionally, the procedure requires adequate venous access, which can be highly challenging in children.

For donors, PB stem cell harvests are also much less invasive and more tolerable, as no operation is required. However, they need to receive G-CSF to mobilize progenitors (although the safety profile has been demonstrated in large series with long-term follow-up) in some cases, a central vein catheter may be needed, or a second day of collection may be required for some poor mobilizers.

### 14.2.3 Cord Blood

The advantages of using cord blood include the fact that it is readily available since it is already tissue-typed and cryopreserved. Additionally, the less stringent human leukocyte antigen (HLA) matching required makes it easier to find a donor across HLA barriers (Gragert et al. 2014), which is particularly important for ethnic minorities. Cord blood transplant is associated with rapid immune reconstitution where no serotherapy is used (Chiesa et al. 2012) and higher rates of chimerism in some diseases (Wynn et al. 2022). Recent evidence has also demonstrated that cord blood transplant is associated with reduced relapse and therefore a greater GVL effect (Horgan et al. 2023; Milano et al. 2016).

Disadvantages of cord blood use include a higher transplant-related mortality (Eapen et al. 2007) compared with other donor sources and a



high cost, which makes it challenging in resource-limited settings. The cell dose is also limited, especially in adults or larger patients. This can be overcome using a double cord, although this adds to the complexity of the HCT and further increases the cost and transplant-related mortality (TRM). In addition to this, engraftment may be slower. Recent advances have shown the possibility of expanding progenitor cells from CB, achieving an amount of CD34 near to that obtained in PB transplants (Horwitz et al. 2019). Another disadvantage is the impossibility of obtaining additional cells that can be used for donor lymphocyte infusion (DLI) in the event of disease relapse or the need to improve immune reconstitution.

#### 14.2.3.1 Disease-Related Considerations

Disease-related factors must also be considered when deciding the most appropriate stem cell source for an individual patient. In non-malignant diseases, particularly severe aplastic anaemia, BM is still the preferred stem cell choice due to the lower risk of GVHD (Schrezenmeier et al. 2007). BM is also generally considered to be the stem cell source of choice in the paediatric setting, particularly when paediatric sibling donors are used for the reasons outlined previously. The higher cell dose and more rapid engraftment result in PB being generally preferred as a stem cell source in the adult malignancy setting, particularly where reduced intensity conditioning is used.

### 14.3 Differences in the Composition of BM vs. PB

BM and PB differ in terms of cellular content, with a higher number 2 to 3 times more CD34<sup>+</sup> cells in PB than in BM and a higher number of lymphocytes (in the range of 1 log higher) than in

BM. The next table shows the differences in the composition of the different sources.

	Peripheral blood ( $\times 10^6/\text{kg}$ )	Bone marrow ( $\times 10^6/\text{kg}$ )	Ratio of PB to BM
CD34	7.3	2.4	3
CD3	701	49	14
TCR $\alpha/\beta$	663	42	14
TCR $\gamma/\delta$	26	2	13
CD3 <sup>+</sup> CD4 <sup>+</sup>	393	26	15
CD4 <sup>+</sup> CD45RA <sup>+</sup>	188	11	17
CD4 <sup>+</sup> CD45RO <sup>+</sup>	169	10	17
CD3 <sup>+</sup> CD8 <sup>+</sup>	236	18	13
CD3 <sup>+</sup> CD4 <sup>-</sup> CD8 <sup>-</sup>	45	5	9
CD19 <sup>+</sup>	93	15	6
CD16 <sup>+</sup> CD56 <sup>+</sup> CD3 <sup>-</sup>	77	6	13
CD45 <sup>+</sup> CD14 <sup>+</sup>	599	25	24

Adapted from Körbling and Ottinger (Körbling and Anderlini 2001)

### 14.4 Main Results of Studies Comparing BM and PB

There are several randomized trials, retrospective studies and meta-analyses comparing PB and BM as a source of cells in the allogeneic stem cell transplant setting.

Globally, the comparisons suggest that PB transplantation results in faster engraftment and lower graft failure compared to bone marrow (BM) transplantation. However, there is a higher incidence of acute and chronic GVHD associated with PB transplantation.

The risk of relapse appears to be slightly lower when using PB, possibly due to the higher incidence of GVHD. This effect is particularly evident in cases involving HLA identical donors and advanced stage diseases, resulting in a benefit in disease-free survival without significant differences in the overall survival across most studies. The main results of some of these studies are presented in the following table.



	Peripheral blood	Bone marrow	P-value
<b>SCTCG meta-an, 1:1. HLA id sib (S.C.T.C Group 2005)</b>	N = 538	N = 554	
Neutrophil engraftment (days)	14	21	<0.001
Platelet engraftment (days)	14	22	<0.001
Acute GVHD (%)	41	38	0.5
Grade III/IV acute GVHD (%)	26	21	0.03
Chronic GVHD (%)	73	56	0.01
Extensive GVHD (%)	51	35	0.01
3-Year NRM (%)	16	25	0.04
3-Year relapse (%)	21	27	0.01
3-Year DFS (%)	59	53	0.02
3-Year OS (%)	62	59	0.17
<b>Holtick meta-an, Rel and URD (Holtick et al. 2015)</b>	N = 653	N = 677	
Neutrophil engraftment (days)	16	20	<0.001
Platelet engraftment (days)	13	19	<0.001
Acute GVHD (HR)	Reference	1.03	0.67
Grade III/IV acute GVHD (HR)	Reference	0.75	0.07
Chronic GVHD (HR)	Reference	0.72	<0.001
Extensive GVHD (HR)	Reference	0.69	0.006
NRM (HR)	Reference	0.98	0.91
Relapse (HR)	Reference	1.3	0.07
DFS (HR)	Reference	1.04	0.6
OS (HR)	Reference	1.07	0.43
<b>Anasetti. RCT. MA. UD (Anasetti et al. 2012)</b>	N = 273	N = 278	
Neutrophil engraftment (days)	Reference	5 days later	0.001
Platelet engraftment (days)	Reference	7 days later	0.001
Engraftment failure (%)	3	9	0.002
Acute GVHD (%)	≈50	≈50	NS
Chronic GVHD (%)	53	41	0.01
Extensive GVHD (%)	48	32	0.001
2-Year NRM (%)	≈25	≈25	0.66
2-Year relapse (%)	≈30	≈30	0.74
2-Year DFS (%)	≈45	≈45	0.38
2-Year OS (%)	≈50	≈50	0.33
<b>Savani. Registry. RIC (Savani et al. 2016)</b>	N = 9011	N = 837	
Neutrophil engraftment (days)	16	20	<0.001
Acute GVHD (%)	24	17	0.005
Chronic GVHD (%)	36	29	<0.001
2-Year NRM (%)	20	18	0.19
2-Year relapse (%)	35	43	<0.001
2-Year DFS (%)	44	39	0.009
2-Year OS (%)	50	47	0.05
<b>Ruggeri. Registry. Haplo donor (Ruggeri et al. 2018)</b>	N = 191	N = 260	
Neutrophil engraftment (days)	17	18	0.001
Acute GVHD (%)	38	21	0.01
Grade III/IV acute GVHD (HR)	14	4	0.01
Chronic GVHD (%)	32	36	0.28
2-Year NRM (%)	23	23	0.61
2-Year relapse (%)	22	26	0.38
2-Year DFS (%)	54	49	0.39
2-Year OS (%)	56	55	0.57
2-Year GRFS	43	44	0.82

	Peripheral blood	Bone marrow	P-value
<b>Simonin. Registry. Paediatrics.</b> (Simonin et al. 2017)	N = 719	N = 1743	
Neutrophil engraftment (days)	16	19	0.001
Acute GVHD (odd ratio)	Reference	1.07	0.55
Chronic GVHD (%)	44	21	<0.001
3-Year NRM (%)	20	12	0.002
3-Year relapse (%)	26	29	0.29
3-Year DFS (%)	54	59	<0.001
3-Year OS (%)	62	67	<0.001

*Meta-an* Meta-analysis, *Sib* Sibling, *Rel* Related, *UR* Unrelated, *NS* Not significant, *MA* Myeloablative, *RIC* Reduced intensity conditioning

## 14.5 The Role of Cord Blood

Cord blood is an intuitively attractive stem cell source owing to its greater permissiveness for HLA mismatch and subsequent increased donor pool (Schrezenmeier et al. 2007), low rates of chronic GVHD (Keating et al. 2019; Sharma et al. 2020; Barker et al. 2020) and ready availability, yet its recent decline has been mirrored by a rise in haploidentical transplantation (Passweg et al. 2021). This is largely due to the fact that cord blood transplants are more challenging, with a higher TRM (Eapen et al. 2007) that may in part be attributed to lower stem cell doses and T-replete strategies. Despite this, cord remains an important stem cell source and has specific utility in both malignant and non-malignant disease settings. Indeed, T-replete cord blood transplant is associated with reduced relapse and improved event-free survival in paediatric patients with high-risk myeloid malignancy (Horgan et al. 2023), and a similar reduction in relapse with cord has been observed in adults (Milano et al. 2016; Sharma et al. 2020). In non-malignant disease, the superior chimerism associated with cord blood (Church et al. 2007) results in improved outcomes in patients with metabolic disease (Lum et al. 2017; Boelens et al. 2009). Strategies to reduce the high TRM associated with cord blood transplant may serve to broaden its applicability as a stem cell source and safeguard the future of cord blood transplant, particularly in settings where the use of alternate strategies may be associated with an adverse outcome. In this line, a recent randomized study has shown that ex vivo expansion of CB is associated with faster

engraftment and less early transplant complications in comparison with standard CB transplantation.

### Key Points

- The use of PB compared to BM is associated with earlier haematological recovery, a higher risk of GVHD and, possibly, a greater GVL effect. Overall, there are no significant differences observed in terms of overall survival or disease-free survival.
- In patients at a high risk of relapse, the use of PB may be associated with a GVL effect, especially in the context of reduced-intensity transplantation.
- In general, for patients with non-neoplastic diseases, particularly aplastic anaemia, the use of BM is preferred.
- Since the overall results are similar and there is a lower incidence of chronic GVHD, in patients with standard-risk diseases, the consideration of BM transplantation is important as it may be associated with better long-term quality of life.

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# Bone Marrow Harvesting for HCT

# 15

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## 15.1 Introduction

Historically, bone marrow (BM) has been the first source of stem cells considered since the early 1960s for hematopoietic cell transplantation (HCT) (Gorin et al. 1977). In 1986, the first success of an unrelated cord blood (UCB) transplantation in a child promoted UCB (Gluckman et al. 1997) as an alternative source in certain settings. Since 1994 and the initial demonstration that peripheral blood (PB) mobilized by cytokines (granulocyte colony-stimulating factor (G-CSF) first and, more recently, when needed, plerixafor) could be used as well as BM, the proportion of PB transplants has considerably increased to

reach about 65% (haplo), 90% (allo), and 100% (auto) (Passweg et al. 2021) so that nowadays bone marrow transplantation (BMT) accounts for a minority of transplants.

This chapter schematically compares the advantages of PB with those of BM and details the technique of BM harvesting. It is not the purpose of this chapter to review the benefit/risk ratio of BM versus PB stem cells (PBSCs). However, the use of BM should always be considered in non-neoplastic diseases, such as Severe Aplastic Anemia (SAA), in which no graft-versus-tumor effect is needed and graft-versus-host disease (GVHD) should be avoided.

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## 15.2 Advantages and Disadvantages of BM

BM	For the patients	For the donors
Disadvantages	Lower hematological and immunological recovery and consequently: <ul style="list-style-type: none"> <li>– A higher risk of infectious complications</li> <li>– More days of hospitalization</li> </ul>	They need: <ul style="list-style-type: none"> <li>– To be anesthetized</li> <li>– Hospital admission</li> <li>– Autotransfusion</li> </ul>
Advantages	Lower incidence of chronic GVHD and consequently: <ul style="list-style-type: none"> <li>– A lower risk of late infections</li> <li>– Less late complications due to prolonged IST (e.g., osteoporosis, aseptic necrosis, etc.)</li> <li>– A better quality of life</li> <li>– A higher risk of relapse in neoplastic diseases</li> </ul>	<ul style="list-style-type: none"> <li>– No need to administer G-CSF, avoiding possible short- and long-term secondary effects [1] (see this chapter)</li> </ul>

*IST* Immunosuppressive treatment [1]. This is a much-debated topic, but in both donors, receiving the drug for a few days, and patients, receiving it for prolonged periods, no greater risk of long-term hemopathies than in those who do not receive it has been demonstrated

## 15.3 The Technique of BM Collection

- **Admittance:** Confirm that the posterior pelvic area has been shaved, the autotransfusion obtained before (if needed), the preanesthetic visit done, and the informed consent form signed.
- **Preparation:** In a sterile operating room, as currently only posterior iliac crests are punctured, the donor should be positioned in a prone position. In the case of a pediatric donor, completing the volume to be extracted by puncturing the anterior crests by turning the donor during the procedure can be considered. Monitor and avoid compression of the breasts and genitalia.
- **Anesthesia:** Usually, general anesthesia is performed, and the use of a laryngeal mask to avoid postintubation throat discomfort is advised. In exceptional cases where general anesthesia is contraindicated, subarachnoid anesthesia (a spinal block) can be used.
- **Preparation of the area:** Disinfect the skin of the posterior pelvic region with povidone iodine, and, then, dry with a sterile gauze. Cover the donor with sterile drapes, leaving the upper part of the posterior pelvis free. Fix the carvings and cover the field with a Steri-Drape.
- **Aspiration:** A physician will be placed on each side of the operating table. Using puncture needles, through 1–2 cutaneous points in the posterosuperior area of both iliac crests, perform 100–200 punctures by varying the direction and depth of the needle. Once the needle is inserted, the clamp is removed, the syringe is connected, the first aspiration is performed, the clamp is reinserted, and so on. The needle is advanced a few millimeters, and the same process is repeated several times at each new puncture site. Each time, 4–5 mL of marrow blood is aspirated to avoid blood contamination, and the contents are emptied into the collection bag. Needles with multiple holes at different levels simplify the process but increase the risk of blood contamination. Once the syringe has been emptied, it is washed with saline solution with sodium heparin, from a container prepared for this purpose. Periodically and gently shake the collection bag with the volume to be aspirated. The maximum volume to aspirate is 15–20 mL/kg donor weight.

Some registries, such as the National Marrow Donor Program (NMDP), use the following formula to **predict the cellularity** that can be obtained from a BM donor considering that the

maximum volume of BM to be obtained is 15–20 mL/kg donor weight and that the “expected Total Nucleated Cells (TNC)” is  $0.22 \times 10^8$  TNC per mL aspirated.

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Then, total TNC expected = maximum volume (in milliliters)  $\times$  “expected TNC.”

Total TNC  $\times$  kilogram receptor weight = Total TNC expected / receptor weight in kilograms

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- Objective:** The aim of aspiration is to obtain  $2\text{--}4 \times 10^8$  nucleated cells/kilogram of recipient weight. In case of Unrelated Donors (UNR), it is advisable to request and attempt to collect  $>3 \times 10^8$  TNC/kg. To ensure this goal, it is advisable to perform a cell count after having collected about 500–600 mL to assess the efficacy of the aspiration. Unlike PBSCs, CD34<sup>+</sup> cell counts, despite being quantified, are not standard with BM.
- A recent study has shown that the cellularity of BM collections has been decreasing over the last few years, despite a better selection of donors (younger and more males with a larger body volume). This decrease is attributed to the lack of experience of the collection centers (worse results in centers with  $<6$  collections/year) and to the reduction of the collection time, which, although beneficial to the donor from an anesthetic point of view, implies a greater contamination of the product by medullary blood, either by aspirations with a greater volume or by the use of new aspiration equipment that facilitates the aspiration of medullary blood (Prokopishyn et al. 2019). This multicenter observation has recently been replicated in a large single-center series (Spitzer et al. 2021). Recently, as a result of the coronavirus disease 2019 (COVID-19) pandemic, this observation has acquired special relevance due to the loss of cellularity that can be caused by delays in the delivery of products and their cryopreservation, although the latter factor does not seem to have a relevant clinical impact (Fernandez-Sojo et al. 2021), except in some cases of bone marrow aplasia (Eapen et al. 2020).
- Filtering:** Classically, once the desired cellularity is reached, the collection bag (that contains a prefilter of 850  $\mu\text{m}$ ) is connected to two additional filters of 500 and 200  $\mu\text{m}$  (included in the collection kit), which, in turn, are connected to the final collection bags. By gravity, the collected volume passes through the filters, reaching the final bags. It is preferable to distribute the aspirated Bone Marrow (BM) in two to three bags as a precaution against possible breakage during transport or handling. This procedure is under revision due to the destocking of the classic BM collection kits, which are being replaced by in-house kits (see below).
- Labeling:** All products, especially if they are to be cryopreserved, should be labeled for an adequate traceability. ISBT 128 (proposed by the International Society of Blood Transfusion) is a labeling method that is voluntary in some countries and mandatory in others, supported by scientific and professional societies and required by the Joint Accreditation Committee of International Society for Cell and Gene Therapy-European Society for Blood and Marrow Transplantation (ISCT-EBMT) (JACIE)-accredited cell therapy facilities. In 2017, the use of the Single European Code (SEC) on tissues and cells was enforced within the European Union (EU) or exported from the EU. SEC intended standardization within the EU and the integration of the two existing codes (Regulation EU 2017/745). As ISBT 128 provides all the information required in SEC, except the country identifier and the tissue establishment code, which are constants, most centers use ISBT 128 labeling.



Due to the increasing numbers of exported hematopoietic stem cell products, it was necessary to generate a distinct identification code linking donors and products to maintain adequate traceability. As a result, a Global Registration Identifier for Donors (GRID) of Hematopoietic Cells was implemented in 2014 by the World Marrow Donor Association (WMDA). Over the years, the initial concept “GRID for life” changed, the GRID was abandoned by cord blood products, and the number could be changed if the issue organization of the donor changed. Despite that, nowadays, GRID has become a crucial code for unrelated donors and has been included in the ISBT 128/SEC (see Fig. 15.1).

- **Autotransfusion:** Depending on the volume collected, two attitudes regarding transfusion during BM collection may be followed: no transfusion and liquid replacement or autotransfusion collected 2–3 weeks preceding marrow collection. Allogeneic transfusions should be avoided by all means in healthy donors. Depending on hemoglobin and ferritin pre-donation and volume obtained, a dose of intravenous Fe could be administered.
- **Post-aspiration:** After disinfecting the wounds, apply dressing and a compressive bandage. Administer analgesia. The morning after aspiration, remove the compressive bandage, assess the puncture site aspect, and apply dressing on each side. Continue with the prescribed analgesia for approximately 1 week and oral Fe. Avoid violent exercise or heavy lifting for 7 days.
- **Sodium (Na) heparin or anticoagulant citrate dextrose solution (ACD) solution a (ACD-A)?:** Many collection centers only use Na heparin as an anticoagulant for BM collection, but international standards (JACIE and the Association for the Advancement of Blood and Biotherapies (AABB) clearly recommend:
  - **JACIE** (Standards eighth edition, CM5.1.12.2): Use of additives for a long shelf life. Explanation: ACD should be used as an anticoagulant (may be combined with heparin) when shipping long-acting cell therapy products for centralized manufacturing.
  - **AABB** (Standards 32 edition): Heparin is used to prevent clotting in BM. However, given the potential for marrow “clumping” during longer storage (as might be the case when marrow is transferred to another center for transplantation), additional anticoagulation may be used. ACD-A at a 10% concentration appears to decrease the likelihood of marrow clotting and clumping. The AABB also recommends it for those marrows to be processed.

While many products continue to be cryopreserved (i.e., in a pandemic situation), it seems reasonable to employ this additional measure. Since it is not a matter of changing the form of collection for those who only use heparin, simply add ACD-A at 10% at the time of preparing the product for transport, especially if it is to be cryopreserved.

 A9996 15 987654 	 4700 <b>O</b> RhD Positive
Collection Date/Time 22 JAN 2015 13:59	For Use by Intended Recipient Only
Do Not Irradiate Do Not Use Leukoreduction Filters	GRID: 9999 0000 1234 5678 930 
 S1125400 DESIGNATED	 0150241359 Expiration Date/Time: 24 JAN 2015 13:59
<b>HPC, MARROW</b> 3rd Party Blood Component Present See Accompanying Documentation	Intended Recipient: PATRICK, ANNE Recipient ID: 123456721 Date of Birth 07 JAN 1964
Total Volume _____ mL containing approx _____ mL Heparin (_____ U/mL) Store at 1 to 10 C	Processing Facility 2nd Line of Name City, State, Zip Code
SEC: XX001234A999615987654 A0S112500020150124	

**Fig. 15.1** An example of an ISBT 128 label, including two-dimensional (2D) GRID and SEC code (adapted from Neller et al. (2017))

## 15.4 In-House Collection Kits

After the introduction of a new European legislation on medical devices (2017/745), the commercial BM extraction kits lost the European Conformity (EC) mark and were no longer permitted for use in Europe. Some member states asked for a derogation of the EC mark for the kits, but commercial BM manufacturers stated that they were unable to resume the supply in Europe. This

has led to the production of different in-house protocols, varying mainly in the filtration process of the cells, depending on the filters and other materials available, the site of filtering (in the operating (OP) room or the processing laboratory), and the regulations set by the regional authorities of each country. Some examples of this protocols have been shared by different groups as the “New Method for Bone Marrow Collection and Filtration” presented as a poster by E. Berger et al.

EBMT 2023 and the recently published “In-house Bone Marrow Collection kit” by J. Fernandez-Sojo, which has compared the results of the harvest performed with a commercial kit and found no differences (Fernandez-Sojo et al. 2023).

## 15.5 Major and Minor Complications According to the NMDP (Pulsipher et al. 2014)

**Major:** They are extremely rare (0.25%), and most of them have a rapid resolution.

- Severe hypotension with electrocardiogram (ECG) changes, hypo K
- Abdominal thrombosis, sepsis due to *Escherichia coli*
- Severe laryngospasm after extubating
- Pulmonary edema
- Asystole, arrhythmia, desaturation
- Significant pain, severe anemia

Another publication analyzes **serious complications** of BM donation and describes 12 cases in 27,770 donations analyzed (0.04%), with the most notable being 1 death due to massive Pulmonary thromboembolism (PTE) 15 days after donation; among the major nonfatal complications are cardiac arrest (4), severe hypotension (2), pulmonary edema (1), cerebral vascular accident (1), and subdural hematoma due to heparin-induced thrombocytopenia (1) (Halter 2009).

**Expected minor that prolongs admission:** Similarly, infrequent, 1.4%. They resolve in 1–2 days.

- Nausea, vomiting
- Pain, fever
- Syncope
- Anesthesia-related problems: Hypotension, urinary retention, bradycardia, bronchospasm

**Unexpected minors, prolong admission:** Also infrequent, 0.2%, few days duration

- Infection, unusual pain
- Pulmonary edema, chest pain

**Chronic or disabling complications:** Referred in 5%, some cases >3 months duration

- Prolonged hip, back, or joint pain

modulatory and anti-inflammatory effect (treat GVHD). MSCs can be isolated from 30–150 mL BM harvesting of healthy consent allogeneic donors (individually or in pool (Kuci et al. 2016)). Donor selection must be in accordance with the national legislation and international (Foundation for the Accreditation of Cellular Therapy (FACT)/JACIE and WMDA) regulations. A considerable variability regarding in vivo effects of BM MSCs for bone formation and hematopoiesis support exists between manufacturer’s centers (Liu et al. 2017).

### Key Points

- BM, when compared with PBSCs, results in less TRM, less GVHD (in particular chronic extensive GVHD), but a less GVL/lymphoma/tumor effect.
- Harvest with small (2–5 mL) aspirate volumes to avoid dilution with blood.
- The goal should be at least  $3 \times 10^8$  nucleated cells per kilogram, but the more the better. The maximum volume collected should not exceed 20 mL/kg donor body weight. Cryopreservation should be avoided unless under specific conditions.
- A correct filtering of the product and labeling of the bags is mandatory. Validate all the in-house procedures.
- The decision for no transfusion with liquid replacement (recommended) or autotransfusion (second-best option) relies on the judgment of the local medical team. Allo-transfusion must be avoided in healthy donors.

## 15.6 Other Therapeutic Applications of BM Harvesting

### 15.6.1 Culture-Adapted Mesenchymal Stromal Cells (MSCs)

These cells have the potential to stimulate the tissue repairing process and exercise an immune

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Nina Worel, Yavuz M. Bilgin, and Patrick Wuchter

## 16.1 Introduction

The intravenous infusion of HSC to restore BM damage is the basic principle of high-dose chemotherapy, since otherwise the patient would expect long-lasting aplasia with life-threatening infections. Therefore, a sufficient collection of HSC before application of high-dose therapy is mandatory. Since HSC expresses CD34 on their surface, the number of CD34+ cells in the transplant material is considered the major indicator of the HSC content.

In principle, there are two ways how to collect stem cells: under anesthesia by repeated aspiration of BM from the pelvic crest or by leukapheresis after mobilization of HSC into the PB. The latter one is favored and considered as standard because it is less stressful for the patient or donor and leads to faster engraftment and hematologic reconstitution which may improve patient outcomes (Gertz 2010).

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Usually, HSC circulates in a very small number in the PB (<0.05% of the leukocytes). For HSC mobilization from the BM to the PB agents which stimulate CD34+ cells are needed. For this purpose the cytokine granulocyte colony-stimulating factor (G-CSF) represents the “golden-standard” since several decades. G-CSF induces myeloid hyperplasia and the release of CD34+ cells into the circulation through proteolytic cleavage of adhesion molecules (Lapidot and Petit 2002). Currently, the two G-CSF cytokines filgrastim and lenograstim have market approval for mobilization of HSC in Europe. In case of sufficient mobilization, HSCs are collected by leukapheresis, which is preferentially performed by peripheral venous access or the use of central venous catheters where necessary. Finally, in case of autologous transplantation, the autograft will be cryopreserved using dimethyl sulfoxide (DMSO) until transfusion.

The aim of infusion of HSC from a donor for allogeneic SCT is to restore BM damage and to treat the patient’s disease. It represents a permanent cellular immunotherapy by adding a graft versus tumor effect in malignant diseases.

## 16.2 Strategies of Autologous Stem Cell Mobilization

There are two different strategies to mobilize autologous HSC from the BM to the PB: the so-called steady-state mobilization and the mobili-

zation by chemotherapy. Both approaches have specific advantages and disadvantages, but the relapse rate is comparable, as documented in several clinical trials (Tuchman et al. 2015).

### 16.2.1 Mobilization Without Chemotherapy (“Steady State”)

Using this approach, HSC will be mobilized by the use of cytokines only. The only approved cytokine for mobilization is G-CSF. The approved doses for steady-state mobilization are filgrastim 10 µg (1.0 Mio. I.E.)/kg/day SC for 5–7 consecutive days or lenograstim 10 µg (1.28 Mio. I.E.)/kg/day SC for 4–6 consecutive days. The use of biosimilar G-CSF has equivalent efficacy (Schmitt et al. 2016; Lisenko et al. 2017).

Leukapheresis usually is performed on day 5 independent whether filgrastim or lenograstim was used for mobilization. If the number of cells collected is inadequate, mobilization with G-CSF may be continued for 1–2 days. However, if the collection goal is not reached after the third leukapheresis, a successful mobilization is unlikely.

The major advantages of steady-state mobilization are the relatively low toxicity including reduced in-hospital patient days, the predictable time of leukapheresis, the outpatient administration, and the reduced costs compared to chemo-mobilization. The major disadvantages are variable mobilization failure rates, and the lower CD34+ cell yields compared to chemo-mobilization. Mobilization with G-CSF only may be used in patients without further need of chemotherapy, e.g., in patients with a stable remission of the underlying disease or patients with multiple myeloma.

### 16.2.2 Mobilization with Chemotherapy

The use of chemotherapy in combination with G-CSF is the preferred way of mobilization for patients who will need further decrease of tumor burden. CY in a dose of 2 g/m<sup>2</sup> is widely used for HSC mobilization. However, a higher dosage of

4 g/m<sup>2</sup> CY leads to an increased toxicity (Baertsch et al. 2017). In multiple myeloma patients, chemo-mobilization with low-dose cyclophosphamide (2 g/m<sup>2</sup>) is a safe mobilization regimen with stem cell collection rates comparable to that of high-dose cyclophosphamide (4 g/m<sup>2</sup>) (Zannetti et al. 2021).

It is also an option to mobilize HSC not by a separate mobilization chemotherapy but as part of the disease-specific chemotherapy, e.g., to mobilize HSC following salvage treatment in lymphoma patients. The choice of a specific chemo-mobilization approach is based on patient’s disease characteristics and local clinical practice guidelines.

The officially approved doses of G-CSF for HSC mobilization after myelosuppressive therapy are filgrastim 5/µg (0.5 Mio. I.E.)/kg/day SC and lenograstim 150/µg (19.2 Mio. I.E.)/m<sup>2</sup>/day SC. In clinical practice, the dosage is often rounded up to 5–10/µg/kg/day filgrastim or 150–300 µg/m<sup>2</sup>/day lenograstim, respectively. Mobilization with G-CSF should start after completion of chemotherapy at the earliest and at the leukocyte nadir at the latest and should continue until the last leukapheresis session. Most protocols recommend the initiation of G-CSF within 1–5 days after the end of chemotherapy.

The major advantage of adding chemotherapy to cytokines, besides the effect on the tumor, is the expected improvement of the collection yield with fewer apheresis sessions (Sung et al. 2013). The major disadvantages of chemo-mobilization are the therapy-related toxicity, the requirement of in-hospital treatment in most cases, the bone marrow damage by the chemotherapy which may impair future mobilizations, and higher mobilization costs. Therefore chemo-mobilization may not be the approach of first choice in situations where chemotherapy is not required for the underlying disease.

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## 16.3 CD34+ Cell Count and Timing of Leukapheresis

Up to date, CD34+ cell count in mobilized peripheral blood products is the most important parameter of graft quality, as it is the only



**Table 16.1** Recommended start of G-CSF and start of CD34+ monitoring for most used mobilization chemotherapy regimens

Chemotherapy	Start G-CSF	Start CD34+ monitoring
CY 2 g/m <sup>2</sup>	Day 5	Day 10
CAD	Day 9	Day 13
(R)CHOP/CHOEP	Day 6	Day 11
(R)DHAP	Day 9	Day 14
(R)ICE	Day 6	Day 12
(R)AraC/TT	Day 5	Day 10

Day 1: First day of chemotherapy application (without rituximab). Adapted from (Kriegsmann et al. 2018)

recognized predictor of stable hematopoietic engraftment after auto-HCT (Saraceni et al. 2015). Following chemotherapy, an exact prognosis of the CD34+ cell peak in the PB and the optimal start of leukapheresis are difficult and require daily monitoring of CD34+ cells in the PB. Table 16.1 summarizes a recommendation of timing of G-CSF following most of the currently used chemotherapy regimens and start of monitoring of CD34+ cells in the PB.

If not otherwise specified by the protocol, CD34 monitoring should be initiated at the latest if leukocytes increase up to 1000  $\mu$ L during recovering from aplasia or at day 4–5 of G-CSF in steady-state mobilization. A start of leukapheresis is indicated when a CD34+ cell count of >20/ $\mu$ L is reached (Mohty et al. 2014).

## 16.4 Target HSC Collection Count

The target number of HSC to be collected is dependent among others on the kind of transplant (e.g., autologous or allogeneic), the stem cell source (BM or PB), and the underlying disease (Table 16.2).

### 16.4.1 Cell Target for Autologous Transplantation

The generally accepted minimum CD34+ cell yield to proceed to transplantation is  $2 \times 10^6$  cells/kg bw (Mohty et al. 2014). However, higher

**Table 16.2** Overview on CD34+ dosage

	Autologous transplantation	Allogeneic transplantation
Minimum dose of mobilized CD34+ cells for one transplant	$2 \times 10^6$ CD34+ cells/kg bw	$4 \times 10^6$ CD34+ cells/kg bw
Optimal dose of mobilized CD34+ cells for one transplant	$2.5\text{--}5 \times 10^6$ CD34+ cells/kg bw	$5\text{--}8 \times 10^6$ CD34+ cells/kg bw <sup>a</sup>

<sup>a</sup>Or higher when further processing is intended

yields of  $2.5\text{--}5 \times 10^6$  CD34+ cells/kg bw have been associated with faster hematopoietic recovery, reduced hospitalization, blood transfusions, and antibiotic therapy (Stiff et al. 2011; Giralt et al. 2014). It may be advantageous to collect cells in this range, if the mobilization status of the patient allows it without additional leukapheresis session.

Most patients with NHL or HL will need one autograft. Depending on their risk stratification and the therapeutic protocol, patients may have the need of two or even more transplantations (mainly patients with MM). In these cases, it is essential to collect the required number of HSC before the first high-dose therapy since mobilization after high-dose therapy has an increased risk of failure. For tandem transplantation, the required cell dose for one transplantation is also at least  $2 \times 10^6$  CD34+ cells/kg bw. With new therapies for certain hematological malignancies, such as chimeric antigen receptor (CAR) T cells, the need for tandem transplantation in MM patients may decrease as CAR T cells become widely available.

### 16.4.2 Cell Target for Allogeneic Transplantation

In allogeneic transplantation, the target quantity of HSC to be collected depends among others on the graft source, the HLA-compatibility between donor and recipient (HLA identical, HLA-haploidentical), the underlying disease (malignant, non-malignant), and the planned graft processing (T-cell depletion, cryopreservation). The generally accepted minimum CD34+



cell dose is  $\geq 4 \times 10^6$  cells/kg bw of the recipient, which is associated with optimal engraftment kinetics (Mifflin et al. 1997). If further processing is planned, doses of up to  $8.0 \times 10^6$  CD34+ cells/kg bw or even more could be necessary.

However, higher doses of CD34+ peripheral blood stem cells are also associated with increased mortality from cGVHD after allogeneic HLA-identical sibling transplantation (Mohty et al. 2003). The probability of extensive cGVHD at 4 years was 34% in patients receiving a CD34+ cell dose  $< 8.3 \times 10^6$ /kg bw, as compared to 62% in patients receiving  $> 8.3 \times 10^6$ /kg bw CD34+ ( $P = 0.01$ ).

## 16.5 Leukapheresis

Collection of peripheral HSC by leukapheresis is a well-established process. In the allogeneic setting, the duration of one leukapheresis session should not exceed 5 h and two consecutive sessions.

In the autologous setting, some centers perform large volume apheresis with more than 4 times total blood volume to be processed. CD34+ cell collection has been shown to be more effective with larger apheresis volume (4.0–5.3 times the patient's total blood volume), and no difference in CD34+ cell viability was observed compared with normal-volume apheresis (2.7–3.5 times the patient's total blood volume) (Abrahamsen et al. 2005). The duration of one leukapheresis session should still be within the range of 5 h (or within the maximum recommended run time by the manufacturer, i.e., 480 min. for the Spectra Optia), and the total number of leukapheresis sessions should not exceed three consecutive procedures since more sessions are futile in most cases.

To estimate the necessary duration of the leukapheresis session, it may be helpful to use an algorithm based on the CD34+ cell number in PB of the donor and the collection efficiency (CE2) (Wuchter et al. 2017; Rajsp et al. 2022; Kayser et al. 2023).

## 16.6 Management of Poor Mobilizers

### 16.6.1 Mobilization Failure Among Patients (Autologous SCT)

Despite widespread and established practice, current mobilization strategies vary between centers and differ in terms of feasibility and outcome. The majority of patients are able to mobilize sufficient CD34+ cells for at least a single auto-HCT. Although the incidence of mobilization failure among patients is not fully documented, approximately 15% of the patients fall in this category (Wuchter et al. 2010). A second attempt to remobilize with G-CSF is usually less effective and has a lower success rate.

Poor mobilizers are defined as patients with less than  $2 \times 10^6$  CD34+ cells/kg collected or patients mobilizing less than 20 CD34+ cells/ $\mu$ L into the PB. In general, there are two groups of poor mobilizers: predicted poor mobilizers and proven poor mobilizers (Olivieri et al. 2012).

Plerixafor (AMD3100) is a bicyclam molecule, which reversibly blocks chemokine receptor-4 (CXCR-4), thereby inhibiting binding with its ligand stroma-cell-derived factor-1 (SDF-1). This mechanism results in the release of hematopoietic progenitor cells in the blood circulation (Uy et al. 2008) and makes plerixafor an important tool to overcome poor mobilization. The addition of plerixafor (recommended dose 0.24 mg/kg bw/day SC or 20 mg abs. in adult patients up to 83 kg bw) to the mobilization scheme should be considered in case of inadequate mobilization (Worel et al. 2017).

If a patient has below 20 CD34+ cells/ $\mu$ L, plerixafor application should be considered. In the “grey area” between 10 and 20 CD34+ cells/ $\mu$ L, the decision to use plerixafor is based on disease characteristics and treatment history. Furthermore, if it is not possible to collect at least one-third of the collection goal with the first apheresis session, plerixafor should be applied as a rescue strategy because of high risk of mobilization failure (Mohty et al. 2014; Cheng et al. 2015). Below 10 CD34+ cells/ $\mu$ L, the use of plerixafor is clearly indicated to avoid mobiliza-

tion failure. Plerixafor is recommended when the CD34+ is  $<10/\mu\text{L}$  on days 4–5 of mobilization with G-CSF alone. If the CD34+ is still  $<10/\mu\text{L}$  in patients with chemo-mobilization after 12–14 days, plerixafor is recommended if the leukocyte count is increasing (Bilgin 2021).

With the use of plerixafor, patients spend less time on apheresis with less blood volume to be processed and higher CD34+ cell yields with the first apheresis, leading to a decreased number of apheresis sessions needed. This has a direct effect on reducing mobilization costs (Hundemer et al. 2014; Mohty et al. 2018). In case of a failed first mobilization attempt, the use of plerixafor for remobilization is possible and may well be effective (Hubel et al. 2011; Yuan et al. 2013).

Predicted poor mobilizers are defined by baseline patient or disease characteristics which are associated with poor mobilization. These factors are listed in Table 16.3. In patients with one or more of these risk factors, the preemptive use of plerixafor should be considered (Worel et al. 2017). It is generally accepted that the most robust predictive factor for poor mobilization is the CD34+ cell count in PB before apheresis. Patients with low premobilization platelet count ( $<140 \times 10^9/\text{L}$ ), age  $> 65$  years and previous radiotherapy were significant predictors of mobilization failure with plerixafor. Also patients who received fludarabine- and lenalidomide-based induction treatment may require plerixafor more often (Bilgin et al. 2015). Nowadays daratumumab, a monoclonal CD38 antibody, is administered to transplant-eligible newly diagnosed myeloma patients and these patients required plerixafor significantly more often (Chhabra et al. 2023).

### 16.6.2 Mobilization Failure in Allogeneic HSC Donors

In healthy donors, mobilization failure with G-CSF is uncommon, with an estimated incidence rate between 5% and 10% (Ings et al. 2006).

**Table 16.3** Factors described as predictive of poor mobilization or mobilization failure

Risk factors for poor mobilization
Age $> 60$ years
Refractory or advanced stage of underlying disease
High number of prior treatment lines
Therapy with fludarabine, melphalan, lenalidomide and daratumumab
Low CD34+ cell count before apheresis
Low platelet count before mobilization

Modified from (Mohty et al. 2014)

The use of plerixafor is not approved for allogeneic SCT. However, since 2011 several reports mentioned that donors had a successful mobilization using plerixafor after poor mobilization and failure to mobilize adequate CD34+ cell numbers with G-CSF. In several studies, plerixafor was added to the mobilization scheme, with approximately three-fold increase in CD34+ cells (Cid et al. 2021; Hölig et al. 2021).

## 16.7 Future Directions

At this time, the number of CD34+ cells in the graft is the most important indicator for graft quality. A sufficient number of CD34+ cells are essential to overcome the toxicity of high-dose chemotherapy and to facilitate hematopoietic recovery. However, there is an increasing understanding that other graft subsets, e.g., CD34+ subpopulations or immune cell subsets (B cells, T cells, NK cells, dendritic cells), influence immune recovery. There are also reports that the mobilization regimen has a major impact on graft immune composition and patient's outcome (Saraceni et al. 2015). Therefore, stem cell mobilization could not only be an important part of high-dose therapies but could also be part of an effective immunotherapy. The delineation of this approach has just been started.

More recently, in 2023, the first biosimilar of plerixafor has been approved by the EMA. The impact of this decision is yet unclear regarding marketing price and availability.

### Key Points

- Mobilization with chemotherapy plus G-CSF is the preferred method for patients who will need decrease of tumor burden or who have to collect a high number of HSC.
- Steady-state mobilization should be considered for patients who are not in need for chemotherapy due to the underlying disease (e.g., MM) or disease status (i.e., stable remission).
- Up to date, CD34+ cell count in the PB is the most important parameter of graft quality.
- The required dose for one autologous transplant is at least  $2 \times 10^6$  CD34+ cells/kg bw.
- The required dose for one allogeneic transplant is at least  $4 \times 10^6$  CD34+ cells/kg bw.
- The indication for the use of plerixafor depends on the CD34+ cell count in the PB, the collection goal, the collection yield with the first apheresis, and the presence of risk factors.

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# Mobilization and Collection of HSCs in Children

# 17

Volker Witt, Herbert Pichler, and Norbert Ahrens

## 17.1 Introduction

Collecting or harvesting hematopoietic stem cells (HSCs) from children is a challenge, not only because children have physiological and anatomical differences but also because the psychological, legal, and ethical concerns in minors differ from those in adult donors. In addition, parents and/or legal guardians have to be addressed in all issues and can only assent and not consent to the procedure. The main difference to the adult setting is the small body weight (bw), the difficulties in venous access, especially in the leukapheresis setting, and the need for red blood cell (RBC) substitution.

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## 17.2 Patient Selection and Pre-Procedure Preparation

### 17.2.1 Patient Selection and Testing

In children, the indications for autologous HSC harvesting is well-established (Passweg et al. 2014). Using children in the allogeneic setting as donors is a completely different issue (Bitan et al. 2016). Children should not donate HSCs if a comparable adult volunteer HSC donor is available, if the indication for the stem cell therapy is not first line, or if the therapy is experimental (Sheldon 2004; Zinner 2004).

Patient pre-procedure analyses follow the same local legal requirements as in adults for blood counts, blood group, clinical chemistry, and infectious disease testing. It may extend in allogeneic sibling donors for parameters that are specific to the transplantation indication, e.g., hemoglobin electrophoresis.

To perform HSC harvesting in children, physicians and nurses must be experienced in the care of children and knowledgeable about the normal age-dependent physiological parameters, like vital signs, growth, and psychological and motor development, and should be trained in the communication with children, parents, and/or their legal guardians (Anthias et al. 2016).



### 17.2.2 Bone Marrow (BM) vs. Peripheral Blood Stem Cells (PBSCs) and Risk Analysis

The main graft resources are BM and PBSCs. The basic techniques are quite similar to those used in adults. For BM collection, the iliac crests or, in extremely small children, the tibia is used. For harvesting HSCs from the PB, leukapheresis is used with the same apheresis systems as in adults.

A study from the European Group for Blood and Marrow Transplantation (EBMT) Pediatric Diseases Working Party describes which factors influence the safety of HSC collection. In this prospective evaluation, 453 pediatric donors were included. The children donated either BM or PBSCs according to center policy. A large variability in approach to donor issues was observed between the participating centers. Significant differences were observed between BM and PBSC donors regarding pain, need for RBC support, duration of hospitalization, and iron supplement; however, differences between the groups undergoing BM vs. PBSC donation preclude direct risk comparisons between the two procedures. The most common adverse event was pain, mainly reported by older children after BM harvesting but also observed after central venous catheter (CVC) placement for PBSC collection. With regard to severe adverse events, one patient developed pneumothorax with hydrothorax after CVC placement for PBSC collection. The risk of allo-transfusion after BM harvesting was associated with a donor age of <4 years and a BM harvesting volume of >20 mL/kg. Children <4 years were at a higher risk than were older children for RBC support after BM harvesting, and there was a higher risk of complications from CVC placement before apheresis. It was concluded that PBSC and BM collection are both safe procedures in children (Styczynski et al. 2012).

### 17.2.3 Children as Allogeneic Donors

Pediatric-aged donors vary widely in their ability to assent or consent to the risks of a donation pro-

cedure. There are key regulations and ethical imperatives, which must be addressed in deciding which donation procedure is appropriate for minors (van Walraven et al. 2013). In order to have general guidance, in 2010, the American Academy of Pediatrics published a recommendation on this issue. The authors strongly recommend the inclusion of the pediatric donor in all decision-making processes to the extent that they are capable. An independent chaperon should stand as the minor's advocate to not only protect the rights of the donor but also help prevent any delay of the donation procedure (Chan and Tipoe 2013).

The decision to consider a minor family donor, especially in inherited diseases, is complicated due to the fact that phenotypically healthy or minor symptomatic siblings with a mild carrier status might be eligible to donate to the severely ill recipient. One simple example is a sibling with thalassemia minor for a recipient with a thalassemia major (Biral et al. 2008). There are many other major diseases, including primary immunodeficiencies, chronic granulomatous disease, or sickle cell disease, where carriers are used as HSC donors. Potential family sibling donors with medical or psychological problems should not be considered as donors and therefore should not be HLA-typed (Bitan et al. 2016).

## 17.3 Bone Marrow Harvesting

Extending from Chap. 15, the collection of HSCs from the BM is historically the oldest technique. Multiple punctures of the iliac crest are performed under general anesthesia by experienced physicians. The bone marrow is harvested by aspirations through adequately dimensioned needles. In extremely small children, and if the iliac crest is anatomically not suitable for punctures, then the aspirations could also be performed by punctures of the proximal tibia.

For successful HCT, it is necessary to obtain enough progenitor cells during the BM harvesting procedure. Most centers use multiple aspirations of maximum 2 mL BM, whereas others use

few larger amounts of aspirations for BM harvesting (20–100–250 mL). It could be shown that the latter methods result in comparable grafts for transplantation (Witt et al. 2016). For some young donors with anatomical limitations or in diseases where a suitable donor should be used for more than one recipient, a minimally harming procedure is warranted for bone marrow harvesting (Biral et al. 2008).

More recently, adult donors have received granulocyte colony-stimulating factor (G-CSF) because stimulated BM is richer in HSCs and therefore results in quicker engraftment (Ji et al. 2002). However, data for pediatric donors are ambiguous (Frangoul et al. 2007; Furey et al. 2018). A recent study has shown that a dose of  $3\text{--}5 \times 10^6$  CD34<sup>+</sup> hematopoietic progenitor cells (HPCs)/kilogram of recipient body weight was the optimal CD34<sup>+</sup> cell dose infused to attain graft-versus-host disease (GVHD)/relapse-free survival in children with an matched sibling donor (MSD) while constraining donor side effects.

## 17.4 Peripheral Blood Stem Cell Harvesting

### 17.4.1 Mobilization and Preparation

For mobilization of HPCs into the PB, the longest experience exists with G-CSF in combination with chemotherapy in the autologous setting, but plerixafor has also been reported in case series and in the MOZAIC (Multicenter International Study of Oxaliplatin/Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer) study, a two-arm phase I/II study, as being safe for the use in children in combination with standard G-CSF mobilization (Chambon et al. 2013; Morland et al. 2020). The dose proposed in this study is 240  $\mu\text{g}/\text{kg}$  bw 8–12 h before apheresis (Karres et al. 2020). In two case series, plerixafor is reported as feasible and safe in haploidentical and allogenic donors with an unfavorable donor/recipient bw ratio (Kurnakova et al. 2021; Zubicaray et al. 2021).

As in adults, leukapheresis should be performed if meaningful numbers of CD34<sup>+</sup> HPCs

are mobilized in the peripheral blood, to achieve the minimal threshold of  $2\text{--}5 \times 10^6/\text{kg}$  recipient with a minimum number of procedures (Fritsch et al. 2010).

### 17.4.2 Vascular Access

Vascular access can be frequently achieved with only peripheral venous access lines (Witt et al. 2008). For central access, Hickman catheters are usually sufficient, and temporary Shaldon catheter placement is only required in a minority of patients (Doberschuetz et al. 2019). Alternative line management with arterial access is also possible (Goldstein 2012; Even-Or et al. 2013; Hunt et al. 2013).

### 17.4.3 Apheresis Techniques

PBSCs are harvested by leukapheresis in extremely small children even below 6 kg body weight and have been described since the 1990s of the last century (Kanold et al. 1994; Klingebiel et al. 1995; Diaz et al. 1996). Special experience and techniques are required to perform safe leukapheresis procedures in pediatric patients using apheresis systems, which are constructed for use in adults. Priming of these systems with saline and citrate as for adults may cause anemia and possibly dilution coagulopathy in children below, e.g., 15–30 kg and 10 kg body weight, respectively, due to the large extracorporeal volume of the apheresis systems (ca. 160–220 mL) (Moog 2010). Blood warmers, if used, take an additional 50 mL (Pasko et al. 2023). The expected blood loss for the procedure should therefore be calculated (Witt et al. 2007). This has to be done individually to decide whether a priming of the set is needed. In most of the newest versions of the apheresis systems, an algorithm guides the user through this pediatric priming procedure. For priming only irradiated and leukodepleted packed RBCs should be used. This can be supplemented by plasma products as applied for intrauterine exchange transfusion, if coagulopathy is anticipated.



After completion of a primed apheresis, tube rinsing with saline is usually omitted to prevent circulatory overload from the priming red cell unit in the patient.

Priming for low-body-weight children includes the adjustment of apheresis machine pump rates, if less than 10 mL/min inlet flow is required. A possible workaround for apheresis machine setting limitations is by priming with an artificial body weight setting, e.g., 50 kg and by reducing this to the patient's body weight immediately before connecting the patient. Caution should be exercised for continuous pump operations, as this may be technically limited to 5 mL/min. In addition, blood flow speed can be too slow to achieve timely anticoagulation.

For anticoagulation, citrate is mostly used in extremely small children with an initial anticoagulant citrate dextrose solution A (ACD-A) rate of 1:12 and increases in case of clumping and low blood flow with a high risk of hypocalcemic side effects (Pasko et al. 2023). Alternatively, ACD-A may be supplemented by heparin, e.g., 5000 IE per 500 mL ACD-A that allows rates of up to 1:22 (Salazar-Riojas et al. 2015).

#### 17.4.4 Apheresis Monitoring

To avoid hypocalcemia-related adverse effects, meticulous, ionized calcium monitoring by blood gas analysis is recommended (Kreuzer et al. 2011; Maitta et al. 2014). Calcium substitution is frequently required and recommended to be applied by a separate line, as low flow rates considerably increase the risk of intravenous coagulation by calcium excess compared to adults.

Volume management is a crucial aspect of pediatric stem cell apheresis to ensure patient safety and optimize the collection of HSCs. Adequate volume management involves careful consideration of blood flow rates, processed volumes, replacement fluids, and monitoring of patient hemodynamics.

Patient compliance to the procedure is frequently sufficiently high to avoid sedation, especially if parents are present. However, if children display discomfort, this is typically tied to central

venous pressure variations that impair collection efficacy. In these instances, sedation as required is recommended, e.g., using propofol (Devasia et al. 2021).

#### Key Points

- Pediatric donors can safely donate HSCs if an experienced team is performing the harvesting procedure.
- Donors below 4 years of age have a higher risk of harvesting-associated complications. With BM harvesting, they have a higher need for RBC support and there is a higher risk of complications from CVC placement before apheresis.
- Minors should only be recruited as HSC donors if no medically equivalent histocompatible adult is available for donation and if there is a reasonable likelihood that the recipient will benefit from the procedure.
- An informed consent (child assent) for HSC donation has to be obtained by the legal guardians and from the pediatric donor. A donor advocate with expertise in pediatric development should be appointed for all minors who are considered as potential HSC donors.
- Long-term follow-up data should be collected to help determine the actual medical and psychological benefits and risks of child donors.

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# Procurement and Management of Cord Blood Unit for Allogeneic Transplantation

# 18

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## 18.1 Introduction

Umbilical cord blood (UCB) cells for allogeneic use are collected and frozen in more than 160 public CB banks worldwide. More than 800,000 CB units (CBUs) are available for transplantation. In this chapter, we will describe some procedures for cord blood collection, processing, banking, and recommendations on how to choose a single or double UCB unit for transplantation (Querol et al. 2021).

## 18.2 Collection

During the antenatal period, objective information on the opportunity to donate UCB is given by trained health care providers. After consent, qualified personnel determines donor eligibility to ensure that donation is safe for future recipients. To assess donor eligibility, a donor questionnaire and a medical history review are conducted for identifying risk factors for trans-

missible and genetic diseases. In addition, infectious disease markers (IDMs) are tested on maternal blood samples obtained within 7 days before or after the collection of the UCB unit. According to local regulations, IDMs are assessed for evidence of infection by HIV-1, HIV-2, hepatitis B, hepatitis C, HTLV-I, HTLV-II, syphilis, and any additional markers required.

Collection technique must not interfere with maternity hospital regular delivery care. Successful collection aims having a high volume that correlates with higher cell contain, avoiding microbial contamination and ensuring traceability. High-quality CBUs are compatible with delayed cord clamping usually up to 1–2 min (Frändberg et al. 2016). After cord clamping, thorough disinfection, venipuncture of umbilical vein to drain by gravity into a primary container containing anticoagulant to avoid clotting. Collection bag and accompanying maternal samples shall be appropriately labeled.

There are two main techniques to collect UCB: before the placenta is delivered (in utero) or after the placenta is delivered (ex utero). Both collection techniques are equivalent regarding quality, providing that collectors are adequately trained and the procedure is validated in each collection center.

After collection, collector will complete a report describing labor characteristics and informing on incidences like the presence of fever or other complications, type of delivery, drugs administered, etc. In case of unexpected adverse reactions during collection, they need to

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be communicated to the competent authority. After collection, it is advisable to follow-up the donor including health questionnaires. Additionally, if any abnormal result is detected during testing, a counseling process should be in place.

Once collection is finished, samples are locally stored in validated conditions before transportation to the processing center. As this facility may be far away from the collection sites, a validated procedure for transportation between these two facilities is needed to demonstrate a controlled environment. Standard operating procedures shall be in place to describe time and temperature of storage and transportation methods. All transportation records shall allow tracking back from the collection site to the UCB bank, and any deviation should be recorded.

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## 18.3 Processing and Banking

### 18.3.1 UCB Cell Processing

Unrelated UCB unit must arrive at the processing laboratory in time to allow initiation of cryopreservation within 48 h of collection (this time is extended to 72 h for related or directed UCB donations). The decision as to whether a collected UCB unit will be acceptable for processing and banking will be made based on the acceptance criteria specified by the UCB bank. Many banks have further refined their acceptance criteria based on economics and the desire to build an international inventory of high-quality UCB units with very high TNC or shifted toward ethnic minorities. Many UCB banks are now committed to processing and storing only those UCB units with high TNC (e.g.,  $>150 \times 10^7$  TNC or higher), based on the greater likelihood of these units being used (Saccardi et al. 2016).

Once accepted for further processing, volume reduction of UCB is considered essential to the provision of a high-quality product and cost-effective UCB banking. Reducing the volume of the final product depleting of plasma and RBC allows for storage efficiency in terms of space and cost and, most importantly, reduces

the risk of ABO incompatibility and DMSO toxicity to the potential recipient. Despite some cell losses, volume reduction has additional practical and clinical benefits; the process yields RBC and plasma components as waste products that can be used for immediate or future testing, thereby minimizing the loss of the actual stem cell product for testing purposes. Volume reduction is mainly performed by centrifugation using automatic devices and adding some macromolecules to improve buffy coat like hydroxyethyl starch.

The selection of a suitable protocol for cryopreservation of UCB for use in transplantation is critical to optimize the recovery of functionally viable progenitor cells, most of which lie within the CD34<sup>+</sup> compartment. Some important considerations that are potential sources of cell damage include the type and concentration of cryoprotectant, the cell concentration, and the cooling and warming rates. UCB units must be stored in freezing bags designed and approved for the cryopreservation of human cells and placed into metal canisters to afford protection during freezing, storage, transportation, and shipping. It is important that after filling, each freezing bag is visually examined for possible leaking and breakage of seals.

UCB units should be cryopreserved using a controlled rate freezer with a validated freezing program. Liquid nitrogen-based tanks have been used to ensure long-term maintenance. Minimizing transient-warming events is very important to maintain viable cells. Stability programs should be designed in order to establish the expiration time of the UCB stored.

### 18.3.2 Testing and Quality Assessment

Table 18.1 shows release specification for UCB units. Quality assessment is written below:

#### 18.3.2.1 Safety

It is essential that UCB is screened for those infectious diseases which can be transmitted via blood (as described above). In addition, product

**Table 18.1** Lists the specification requirements for CBU stored for clinical application, according to appendix V of the seventh edition NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration ([www.factwebsite.org](http://www.factwebsite.org))

Specification requirements for cord blood units stored for clinical administration				
Test	Unrelated specification		Related specification	
	Fresh post-processing sample	Post-thaw attached segment or representative sample prior to release	Fresh post-processing sample	Post-thaw attached segment or representative sample prior to release
Total nucleated cell count	$\geq 5.0 \times 10^8$		Enumerated	
Total nucleated cell recovery	Should be $\geq 60\%$		Should be $\geq 60\%$	
Total viability	$\geq 85\%$		$\geq 70\%$	
Viable CD34 count	$\geq 1.25 \times 10^6$			
Viability of CD34 cells		$\geq 70\%$		
CFU (or other validated potency assay) <sup>a</sup>		Growth (or positive result for potency)		Growth (or positive result for potency)
Sterility	Negative for aerobes, anaerobes, and fungus		Negative for aerobic and anaerobic bacteria and fungi—OR—Identify and provide results of antibiotic sensitivities	
Donor screening and testing	Acceptable as defined by applicable law and NetCord-FACT standards		Acceptable as defined by applicable law and NetCord-FACT standards	
Identity		Verified		Verified

<sup>a</sup>There should be evidence of potency by CFU or other validated potency assay on a fresh post-processing sample

should be free of microbial contamination (or with an appropriate antibiogram for related uses). Prior to release for administration, each UCB unit must have undergone hemoglobinopathy screening.

### 18.3.2.2 Identity

At least, HLA-A, HLA-B, HLA-C, and DRB1 loci must be determined using DNA-based methods and result included when listing a UCB unit on the search registries. It is recommended that HLA typing is performed in an accredited laboratory. ABO blood group and Rh type must be reported prior to listing a UCB unit for search. Prior to the release of a UCB unit for administration, it is imperative that HLA identity is con-

firmed. Ideally, confirmatory typing will be performed on a sample taken from a contiguous segment. HLA typing on maternal blood may also be performed prior to release of a UCB unit. Haplotype matching between maternal donor and infant donor confirms linkage between the two and serves as a secondary confirmation of identity.

### 18.3.2.3 Purity

UCB unit specifications report total nucleated cells, total nucleated RBC count, and CD34<sup>+</sup> cells, and a cell blood count with differential should be performed, with parameters for neutrophils, lymphocytes, monocytes, and platelets defined.



### 18.3.2.4 Potency

Potency testing to determine the growth potential and viability of progenitor cells in a UCB unit should be performed post-processing (prior to cryopreservation), in addition to being performed on a representative thawed sample prior to release for administration.

## 18.4 Selecting CBU for Transplantation

The success of the UCB transplantation (UCBT) will depend on the characteristics of the CBU. Tables 18.2 and 18.3 list the recommendation for selection of single and double cord blood units, respectively, for allogeneic transplantation.

**Table 18.2** Recommendations for unrelated CBU selection and transplantation<sup>a</sup>

<i>Initial selection of single CBU should be based upon</i>	
(a)	HLA matching of the recipient and CBU
(b)	CBU cell dose (TNC $\pm$ CD34 <sup>+</sup> ) at cryopreservation
(c)	Patient's diagnosis (malignant versus nonmalignant)
(d)	Avoiding CBU containing Ag that matches the specificity of any pretransplant donor-specific anti-HLA Ab in the recipient
<i>HLA matching</i>	
• <i>Malignant disorders</i> (Eapen et al. 2014)	
HLA matching should be based upon allelic typing for HLA-A, HLA-B, HLA-C, and HLA-DRB1 for single CBT	
1.	Select an HLA-matched (8/8) CBU. TNC dose should be $>3 \times 10^7/\text{kg}$
2.	If an HLA-matched (8/8) CBU is unavailable, select a CBU matched at 7/8 HLA loci. HLA-A or HLA-B mismatches are preferable to HLA-DRB1 mismatches. TNC dose should be $>3 \times 10^7/\text{kg}$ for 5–7/8 matched units
3.	If a CBU matched at 8/8 or 7/8 HLA loci is unavailable, consider a CBU matched at 5 or 6/8 HLA loci. Avoid mismatches in HLA-DRB1
4.	If CBU 4/8 matched, CBU may rarely be considered as a single CB graft if no other option is available. TNC dose should be $>5 \times 10^7/\text{kg}$ for 4/8 matched units
5.	CBU 3/8 HLA-matched CBU is not recommended
• <i>Nonmalignant disorders</i> (Eapen et al. 2017)	
1.	CBU with HLA 8/8 or 7/8 gives same survival results
2.	CBU with HLA 6/8 and 5/8 gives inferior survival rates and is alternative options
3.	We do not recommend selecting cord blood units with more HLA disparities
<i>TNC and CD34<sup>+</sup> cell dose</i>	
• <i>Malignant disorders</i>	
Nucleated cell dose <sup>b</sup>	At freezing, minimum TNC dose $3.0 \times 10^7/\text{kg}$ , or After thawing, minimum TNC of $2.0\text{--}2.5 \times 10^7/\text{kg}$
CD34 <sup>+</sup> cell dose <sup>c</sup>	At freezing, $1.0\text{--}1.7 \times 10^5/\text{kg}$ , or After thawing, around $1.0\text{--}1.2 \times 10^5/\text{kg}$
• <i>Nonmalignant disorders</i> <sup>d</sup>	
Nucleated cell dose	At freezing, minimum cell dose $3.5 \times 10^7/\text{kg}$ , or After thawing, minimum cell dose $3.0 \times 10^7/\text{kg}$
CD34 <sup>+</sup> cell dose	At freezing or after thawing, $>1.7 \times 10^5/\text{kg}$
Colony-forming unit assay: This assay is important to evaluate the functional capacity of HPCs after thawing an aliquot or after thawing the product; however, it is difficult to establish a generalized CFU-GM dose due to variations of colony setup and counting between centers	
<i>Other considerations when selecting single CB units</i>	
If many CBUs meeting the criteria above are available, the following factors should also be considered	
• <i>Use accredited cord blood banks.</i> For safety, only accredited banks recognized by national and international organizations should be used	
• <i>ABO compatibility:</i> ABO compatibility may be associated with improved outcomes, although the data are conflicting	

**Table 18.2** (continued)

<i>Initial selection of single CBU should be based upon</i>
• <i>NIMA</i> : If the cord banks have the mother's HLA typing, the potential effect of NIMA should be noted in context of clinical trials
• <i>KIR ligand</i> : Due to conflicting data, KIR ligand matching should not be used in the selection of CBUs
• <i>Sex matching</i> : Sex matching between CBUs and patients in single or double UCBT is not necessary

<sup>a</sup>Based on Eurocord recommendations (Ruggeri 2019, modified)

<sup>b</sup>If the infused TNC dose is  $1.0\text{--}2.0 \times 10^7/\text{kg}$ , the number of CD34<sup>+</sup> cells or CFU-GM should be taken into consideration to predict the probability of neutrophil recovery and to discuss the possibility of a second transplant. If both cell doses are lower than recommended, a BM aspirate and chimerism analysis should be performed between days +20 and 28. The absence of engraftment indicates the need for a second transplant; preliminary data shows that haploidentical or double CBT should be considered

<sup>c</sup>Due to variation in counting CD34<sup>+</sup> cells, this recommendation should be taken with caution. However, if colonies are not growing, the transplant physicians should consider a second transplant after day +30

<sup>d</sup>For patients with BMF syndromes (aplastic anemia or congenital bone marrow failure states) or hemoglobinopathies, the number of TNC at freezing should be greater than  $5 \times 10^7/\text{kg}$

**Table 18.3** Additional criteria for double CBU selection

– When a single CBU contains insufficient cells (as specified above), double UCBT is recommended for the treatment of malignant disorders	
– There are currently insufficient data to make recommendations for double UCBT in the treatment of nonmalignant disorders	
<i>HLA matching</i>	
• The recommended indications for HLA matching for single CBU with the recipient for double UCBTs should be used, i.e., the minimum acceptable HLA matching between either CBU and the recipient is 4/6 using low/intermediate typing (antigen) for HLA-A and HLA-B and high-resolution typing (allelic) for HLA-DRB1	
• There is no requirement for intercord HLA matching	
<i>Cell dose</i>	
Nucleated cell dose	At freezing, the sum of both CBUs $>3.5 \times 10^7/\text{kg}$ The minimum cell dose of each unit should be $>1.5 \times 10^7/\text{kg}$
CD34 <sup>+</sup> cell dose	At freezing or after thawing, the sum of both CBUs $>1.8 \times 10^5/\text{kg}$
<i>ABO matching</i>	
Two prospective trials (Wagner et al. 2014; Michel et al. 2016) did not demonstrate an advantage of double UCBT in children and young adults with hematological malignancies using a MAC regimen, when a single CBU with an adequate cell dose is available.	

### Key Points

- Cord blood donation comprises the following steps: informative and consent process, revision of eligibility criteria, cord blood collection, and finally fresh storage before a standardized transportation to the processing cell lab.
- Cell processing labs require coordination of production and quality control labs to transform the altruistically donated raw material in a medicinal product with pre-defined specifications that ensure its safety, identity, purity, and potency.

- A public cord blood bank is a stem cell registry that provides ready-to-use banked medicinal products for any patient in need through international networking of stem cell donor organizations.
- Cord blood selection is based on sorting CB units using primary criteria (cell content at cryopreservation and HLA matching, based on allele level matching and including HLA C match) followed by ranking based on secondary criteria depending on the type of disease, disease status, conditioning regimen, patients age, and weight.

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# Graft Manipulation

# 19

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## 19.1 Introduction

Graft manipulation is performed to define and to optimize the volume and cellular composition of stem cell sources like apheresis products, bone marrow, or umbilical cord blood.

Basic manipulations comprise centrifugation procedures for depletion of erythrocytes and volume reduction and are required to cryopreserve grafts in the presence of cryoprotectants like DMSO (dimethyl sulfoxide) (Rowley 1992). These are standard procedures for BM and CB, while apheresis products can usually be cryopreserved without further manipulation.

More complex manipulations are used to optimize the cellular composition and to meet requirements of the individual transplant regimen. Selection of CD34<sup>+</sup> or AC133<sup>+</sup> progenitors from apheresis or BM has been used to produce concentrated stem cell grafts. In recent years, the selective depletion of unwanted cells like CD3<sup>+</sup>, TcR $\alpha\beta$ <sup>+</sup>, or CD45RA<sup>+</sup> T-cells and others provides a custom-tailored graft. For both, positive selection and

depletion, immunomagnetic cell sorting using monoclonal antibodies and paramagnetic microbeads in combination with semi- or fully automated devices has become the standard technique in most laboratories and increasing the activity of graft engineering has been observed in a recent EBMT surveil across Europe (Witte et al. 2023).

## 19.2 Graft Manipulation

### 19.2.1 Physical Manipulations

#### 19.2.1.1 Volume Reduction

Volume reduction might be necessary in small children and is done by a simple centrifugation process and removal of the supernatant.

#### 19.2.1.2 Washing to Reduce Plasma Antibodies or Anticoagulants

Washing might be necessary in case of unwanted isoagglutinins or to lower the heparin concentration and is also done by centrifugation in a bag or dedicated devices and by exchange of plasma with a suitable solution like 0.9% NaCl. Addition of anticoagulant is not necessary as coagulating agents are washed out by the treatment.

#### 19.2.1.3 Depletion of Erythrocytes

Depletion of erythrocytes is necessary in case of blood group incompatibilities and usually confined to bone marrow. Several procedures are

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employed including centrifugation with an apheresis device (Kim-Wanner et al. 2017) or centrifugation in bags or tubes and subsequent harvest of the buffy coat. Especially in small grafts (<125 mL), a separation using density gradient centrifugation (e.g., Ficoll) can be useful with an even stronger depletion of erythrocytes.

## 19.2.2 Immunomagnetic Procedures

### 19.2.2.1 CD34 Enrichment

Enrichment of CD34<sup>+</sup> stem cells was the first method which provided grafts with a very low number of T-cells and therefore allowed to avoid GvHD highly effective even in haploidentical HCT (Ringhoffer et al. 2004; Handgretinger et al. 2001).

The method has also been successfully used in MSD and MUD HCT to minimize the rate of GvHD (Pasquini et al. 2012; Lang et al. 2003a) and showed a clear advantage regarding combined cGVHD-free and relapse-free survival compared to unmanipulated grafts in myeloid diseases (Tamari et al. 2018).

Moreover, CD34<sup>+</sup> selection is used as a graft backbone to which other cell types (unmanipulated DLI, CD45RA depleted DLI, regulatory and conventional T-cells, and others) can be added.

Enrichment can be performed with the Miltenyi Biotec CD34 reagent system which uses a mAb for the CD34 class 2 epitope and therefore has to be detected by an Ab to a different epitope (normally class 3). Stem cells after separation normally show high purity with extremely low amounts of other contaminating cell types. In some cases, various amounts of monocytes are found without detrimental effect on the graft. Due to the small size of the graft, absolute numbers of contaminating T-cells remain low even if a significant percentage might persist. B-cells are passively depleted as well, whereas CD34<sup>+</sup>CD19<sup>+</sup> B-cell precursors are retained: 1–3% in PB, up to 30% in BM preparations.

Recovery of CD34<sup>+</sup> cells is in the range of 50–90% (Schumm et al. 1999).

### 19.2.2.2 CD133 Enrichment

CD133 detects a slightly smaller subpopulation of CD34<sup>+</sup> cells and can also be used for enrichment of stem cells with similar results (Koehl et al. 2002; Lang et al. 2004). This method is currently only rarely used.

### 19.2.2.3 T-Cell Depletion

Immunomagnetic TCD is technically more demanding than CD34<sup>+</sup> enrichment as the processed grafts contain a much higher overall number of cells and even extremely low percentages of contaminating T-cells can endanger the success of the manipulation. Moreover, the correct enumeration of T-cells in a depleted graft is challenging and needs special protocols.

### CD3 Depletion

Depletion of CD3<sup>+</sup> T-cells provides almost untouched grafts with potential antileukemic effectors (e.g., NK cells) enabling fast engraftment and reliable prevention of GvHD. Prospective phase I/II trials showed low TRM rates after haplo-HCT in combination with toxicity- and intensity-reduced conditioning regimens in children and adults with various diagnoses (Federmann et al. 2012; Lang et al. 2014; Flaadt et al. 2023).

The depletion efficacy can be 0.5 log lower than in CD34<sup>+</sup> selection. Since in haplo-HCT residual T-cells should not exceed  $50 \times 10^3/\text{kg}$ , it might be occasionally necessary to perform a CD34<sup>+</sup> selection with parts of the apheresis to remain below the requested thresholds and to guarantee a sufficient number of progenitor cells (Lang et al. 2014; Federmann et al. 2012; Huenecke et al. 2016).

It should be ensured that during the incubation process, all cells come into contact with the CD3 reagent to avoid unstained T-cells which can impair the result of the depletion significantly. This may happen when transferring stained cells into a second bag system leaving unstained cells in the tubing ends and crinkles of the bag behind. Even smallest amounts of 20–50  $\mu\text{L}$  can contain more T-cells than the whole graft should have.

Analysis of CD3 depleted grafts needs special protocols and has to take into account the rare

number of T-cells among the huge overall number of cells. Therefore, a multigating strategy should be implemented and validated, and T-cells should be determined using several parameters. Exclusion of myeloid cells by CD33 could be helpful as well as the use of CD3 in a bright fluorochrome like APC. Gating can be facilitated by using a “spiked” probe with cells of the negative fraction, and a small percentage of cells from the positive fraction was added to set the gate for subsequent analysis of the negative fraction. For statistical reasons, a minimum of  $1 \times 10^6$  events should be acquired. To prevent takeover of cells from a previous tube, special care should be taken like flushing the cannula with water before the actual acquisition or to clean the cannula on the outside (Schumm et al. 2013).

### TcR $\alpha\beta$ Depletion

This procedure removes  $\alpha\beta$  + T lymphocytes via a biotinylated anti-TcR $\alpha\beta$  Ab followed by an anti-biotin Ab conjugated to magnetic microbeads while retaining both  $\gamma\delta$  + T lymphocytes and natural killer cells in the graft.

Depletion with the TcRab reagent has been shown to be associated with a high depletion efficacy (4.7 log), better than after CD3 depletion (4.0 log) and similar to CD34<sup>+</sup> enrichment (4.6 log). Moreover, the results differ less than those after CD3 depletion, resulting in  $<50 \times 10^3$ /kg infused residual TCR $\alpha\beta$ + T-cells, even in small children (Schumm et al. 2013).

Compared to CD34 selected grafts, a faster expansion was seen for CD3<sup>+</sup> and for CD56<sup>+</sup> in the early phase after haplo-HCT, probably caused by expansion of co-transfused  $\gamma\delta$  T-cells and NK cells (Lang et al. 2015). Moreover, clinical trials in children and adults demonstrated a very low incidence of acute and chronic GvHD as well as favorable engraftment and TRM rates in malignant and nonmalignant diseases (Locatelli et al. 2017; Kaynar et al. 2017; Bethge et al. 2022). Therefore, this method is currently the most widely used. Moreover, the approach was successfully used to avoid GvHD also in MSD/MUD HCT (Maschan et al. 2016; Sperl et al. 2022).

Detection of TcR $\alpha\beta$ + T-cells should be done with the same precaution used for CD3 depleted

cells, with a minimum of  $1 \times 10^6$  events and several parameters for the identification of the TcR $\alpha\beta$ + cells. Pregating on CD3-PE vs 7-AAD has been shown to be very helpful as well as gating on TcR $\alpha\beta$  and TcR $\gamma\delta$  cells in the consecutive dot plot (Schumm et al. 2013).

Graft engineering is a time-consuming process, and a recent retrospective analyses showed that 48 hours is an acceptable time frame. Also freezing and thawing of engineered graft are an option in particular in the time of a pandemic (Nijssen et al. 2023).

### CD19 Depletion

Depletion of CD19<sup>+</sup> B-cells can be done together with CD3 or TcR $\alpha\beta$  depletion and prevents effectively the occurrence of EBV-associated PTL. Although the threshold dose of contaminating B-cells is still not defined, no cases of PTL were observed in two multicenter trials with 104 children and adults after infusion of median numbers of 28 and  $7 \times 10^3$  CD20<sup>+</sup> cells/kg BW, respectively (Lang et al. 2014; Federmann et al. 2012).

Alternatively, B-cell depletion can be done *in vivo* by infusion of therapeutic anti-CD20 mAbs (Locatelli et al. 2017).

Detection of CD19<sup>+</sup> B-cells needs special attention as the binding of fluorescence-labeled antibody is impaired when cells were preincubated with the CD19 reagent. Therefore, the detection has to be done with an antibody for CD20 which is co-expressed on B cells (Schumm et al. 2006).

### Stem Cell Boosts

Poor graft function after HCT is a relevant complication and is defined as at least bilinear severe cytopenia and/or transfusion requirement, which occurs in a situation of full donor chimerism.

Administration of stem cell boosts from the original donor offers a therapeutic option (Remberger et al. 1998).

To reduce the risk of GvHD, *ex vivo* TCD procedures as mentioned above are recommended (Olsson et al. 2013). Most experience exists with CD34 selected boosts. Response rates of 80% and a low risk of *de novo* GvHD between 6% and



22% were observed, even in the case of mismatched donors. Moreover, not only hematological recovery but also recovery of lymphocyte subsets could be improved (Askaa et al. 2014; Mainardi et al. 2018). Add back of donor T-cells can be considered to avoid non-engraftment and enhance immune reconstitution (see Sect. 19.2.3).

### 19.2.3 Add Back of Donor T-Cells

#### 19.2.3.1 Unmanipulated T-Cells

Unmanipulated T-cells can be added to a backbone of CD34<sup>+</sup> selected or T-cell depleted grafts to provide T-cell immunity in various situations. For example, engraftment and immune recovery could be significantly improved without increasing the rate of GvHD by adding a defined number of T-cells to positive selected grafts (Seitz et al., 2018). In the post-transplant phase, unmanipulated T-cells can be used to exert alloreactive effects. The tolerable dose of T-cells varies strongly depending on the HLA disparity, the T-cell chimerism in the patient, and the time after transplantation. In MUD HCT or in haploidentical HCT, it can be helpful to cryopreserve a number of vials with a defined number of T-cells (i.e.,  $100 \times 10^3$  CD3<sup>+</sup>/kg and  $25 \times 10^3$  CD3<sup>+</sup>/kg, respectively). Dosage can vary for MUD and family donors between  $1 \times 10^5$  to  $1 \times 10^6$  cells/kg depending on the time from transplant (3–6 months). For haploidentical donors, lower dosages are recommended ( $1$ – $2$ ,  $5 \times 10^4$ /kg).

#### 19.2.3.2 CD45RA Depletion

DLI with CD45RA<sup>+</sup>-depleted T-cells takes advantage of the CD45RO<sup>+</sup> T-cells which obviously exert little graft-versus-host reaction but can provide antileukemic and antiviral activity. Depletion can be done using the same equipment and reagents for depletion. Depletion is highly effective, and contaminating CD45RA<sup>+</sup> cells cannot be found at all (Teschner et al. 2014).

#### 19.2.3.3 Regulatory and Conventional T-Cells

Adoptive transfer of regulatory T-cells has been employed by several groups to reduce GvHD

incidence. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cells can be selected from a donor leukapheresis product by a 2-step magnetic selection process: a negative selection of CD19<sup>+</sup> and CD8<sup>+</sup> cells followed by a positive selection of CD25<sup>+</sup> cells. In this case, the final product is highly enriched in regulatory T-cells (Di Ianni et al. 2011). Donor regulatory T-cells can also be obtained with FACS sorting and ex vivo expanded through different approaches. Purity of the regulatory T-cell product and the modalities of its infusion vary depending on the different strategies used (Edinger and Hoffmann 2011; Mancusi et al. 2019). Overall, regulatory T-cell adoptive transfer for GvHD prevention showed promising efficacy in key studies (Brunstein et al. 2011; Brunstein et al. 2016; Meyer et al. 2019). Also, CD4<sup>+</sup>CD25<sup>+</sup> donor regulatory T-cells can be infused 2–3 days before conventional T-cells and at the time of transplant together with CD34<sup>+</sup> cells. The adoptive transfer of donor regulatory T-cells allows for the infusion of a large number of donor conventional T-cells with no or limited use of post-transplant pharmacologic immune suppression. This strategy helped to reduce leukemia relapse and chronic GvHD rates with an acceptable incidence of acute GvHD in pilot trials (Martelli et al. 2014; Pierini et al. 2021). Larger studies are needed to validate such promising approach.

#### 19.2.3.4 Virus-Specific T-Cells

Virus-specific T-cells can be enriched from peripheral blood or an unstimulated apheresis of the original (seropositive) stem cell donor or—if not possible—alternatively from a partially matched third-party donor.

Donor-derived-specific T-cells against ADV-, CMV-, or EBV-associated antigens have been already used in many patients suffering from life-threatening infections post-transplant, and clinical or virological response rates between 70% and 86% in clinical trials and in real world data were observed (Icheva et al. 2013; Feucht et al. 2015; Feuchtinger et al. 2010; Heinz et al. 2023). Thus, antigen-specific T-cells have become a widely used therapeutic option for refractory viral diseases. A large phase III trial is currently ongoing.

The most common technique in the field of graft manipulation is the cytokine capture system which employs the secretion of IFN $\gamma$  after stimulation with appropriate Ag or peptide mixtures for immunomagnetic selection of specific T cells. Simultaneous stimulation with several Ag is possible and generates multispecific T-cells.

The selection procedure can be done with a CliniMACS Prodigy® from a maximum of  $1 \times 10^9$  cells from a nonmobilized or a mobilized apheresis and yields  $0.1\text{--}2 \times 10^6$  CD3<sup>+</sup> IFN $\gamma$ <sup>+</sup> target cells.

Accompanying debris and dead cells require an accurate analysis. Moreover, the small amount of target cells limits the sample size available for analysis, and therefore a single platform procedure including cell count and viability in one measurement is recommended. The first step should be done without washing and includes a cell gate to exclude debris. CD45 and 7-AAD can be used for proper determination of cell viability. A second sample can be analyzed after washing for CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> numbers and the percentage of IFN $\gamma$ <sup>+</sup> cells in these subsets. Bystander cells like B cells, monocytes, and granulocytes can be found in low numbers (Feuchtlinger et al. 2006).

### 19.3 Regulatory Requirements

Graft manipulation is regarded as drug manufacturing in most countries and has to follow the requirements of the EU GMP guidelines, the European Pharmacopoeia, and several EU directives. Therefore, clean room areas are required for the manufacturing and a manufacturing license, and a marketing authorization is mandatory for the distribution of the product. A quality assurance system has to be implemented, and specifications have to be in place for both raw material and drug product. In most cases, volume, cell number, cell dose, viability, and composition are minimum parameters. Sterility in the form of microbiological examination of cell-based preparations according to Pharm. Eu. 2.6.27 has to be shown either before release of the product or, in the case of limited

stability, after release. A supply chain must be established to ensure that all agents are available in a timely manner. The equipment used must be qualified regularly and comply with the current state of the art in order to meet all regulatory requirements.

Peripheral blood stem cells from both blood and bone marrow for hematopoietic reconstitution are regarded as non-ATMP.

#### Key Points

- CD34 enrichment yields stem cell preparations with low contaminating T- and B-cells.
- CD3/CD19 depletion preserves large numbers of NK cells in the grafts.
- TcR  $\alpha\beta$ /CD19 depletion provides large numbers of NK cells and  $\gamma\delta$  T cells with very low amounts of TcR $\alpha\beta$  T-cells.
- DLI with CD45RA-depleted T-cells might reduce the risk of GvHD.
- Donor regulatory and conventional T-cell adoptive immunotherapy with no post-transplant immune suppression provide low incidence of leukemia relapse and chronic GvHD when employed together with a CD34<sup>+</sup> selected graft.
- Virus antigen-specific donor- or third-party-derived T-cells can be utilized post-transplant in patients with therapy-refractory viral infections.

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# Processing, Cryopreserving, and Controlling the Quality of HSC

# 20

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## 20.1 Assessment of HSC by Measuring CD34 and the Presence of Other Cell Subsets

The efficiency of an autologous, as well as an allogeneic, HSC graft is mainly determined by the number of CD34<sup>+</sup> cells present. The dose of transplanted CD34<sup>+</sup> cells per kg body weight (bw) determines the kinetics of the neutrophil and platelet engraftment after auto-HCT (Weaver et al. 1995). The measurement of CD34<sup>+</sup> cells by flow cytometry is, therefore, an important method to assess the graft quantity.

The minimal number of CD34<sup>+</sup> cells for an autologous transplant is  $\geq 2.0 \times 10^6$  CD34<sup>+</sup> cells/kg bw (Mohty et al. 2014). Transplants below this threshold should only be used in cases where no additional stem cell collection is feasible, and there is a vital indication for the autologous stem cell transplantation. Most transplant centres regard a cell dose of  $2.5\text{--}5 \times 10^6$  CD34<sup>+</sup> cells/kg bw as optimal, based on the published clinical data (Duong et al. 2014; Perez-Simon et al. 1999; Giralt et al. 2014). For an allo-HCT, a cell dose of  $\geq 4.0 \times 10^6$  CD34<sup>+</sup> cells/kg bw is regarded as ade-

quate in most cases. If further processing (i.e., cryopreservation, cell depletion, or purging) is planned, higher doses of  $5\text{--}8 \times 10^6$  CD34<sup>+</sup> cells/kg bw could be necessary.

In the autologous setting, it has been speculated that the quality of CD34<sup>+</sup> cells from poor mobilizers may be inferior. However, studies have found that the proportions of primitive and quiescent CD34<sup>+</sup> subsets were comparable across mobilization groups (Jiang et al. 2012), and leukocyte and platelet recovery after transplantation was not different (Wuchter et al. 2010). The application of plerixafor in order to overcome insufficient HSC mobilization not only increases the number of CD34<sup>+</sup> cells but also the proportion of more primitive HSC subsets, the absolute lymphocyte count, and the numbers of lymphocytes in various subsets (CD19<sup>+</sup> cells, CD3<sup>+</sup> cells, T-cells, and NK-cells) in the autograft (Fruehauf et al. 2009; Taubert et al. 2011; Varmavuo et al. 2013). However, these variances do not translate into relevant clinical differences regarding hematopoietic recovery. Taken together, the graft quality from poor mobilizers can be regarded equivalent compared to that from good mobilizers, regardless of the use of plerixafor.

Since CD34 is a marker for heterogeneous subgroups of cells, the composition of hematopoietic subpopulations as progenitor cells of the hematopoietic system might contribute to a better understanding of the graft quality (Roug et al. 2014; Saraceni et al. 2015; Dmytrus et al. 2016; Heuer et al. 2023). However, based on the cur-

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rently published data, no final conclusion can be drawn and further investigations are warranted to determine the potential effects of autograft cell subsets on the patients' clinical outcomes. As delineated in an EBMT position statement, determination of cell subsets other than CD34<sup>+</sup> cells is not routinely performed in clinical practice but only in clinical trials (Mohty et al. 2014). Accordingly, assessment of tumour cell contamination is usually not part of the clinical routine but can be of interest in clinical trials.

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## 20.2 HSC Cryopreservation

HSC should be processed and stored in accordance with the respective Medical Council, responsible local and overarching authorities as well as scientific society's guidelines (e.g. EU: Guideline 2004/23/EG 2004; Guideline 2006/17/EG 2006; EU-GMP-Guideline 2012; Heuer et al. 2023).

If necessary, collected cells can be stored for a maximum of up to 72 h at 2–6 °C before cryopreservation. However, cryopreservation within 48 h or less is recommended to maintain an optimal viability of the cells. In the case of storage for >24 h prior to cryopreservation, the maximum nucleated cell (NC) concentration should not exceed  $2 \times 10^8/\text{mL}$ .

For cryopreservation, a number of different protocols are used worldwide. Usually, the maximum accepted NC concentration is  $\leq 4 \times 10^8/\text{mL}$ . If necessary, PBSC products can be diluted with autologous plasma, human albumin, or commercial resuspension medium. Increasing the cell concentration by volume depletion minimizes the number of cryostored bags needed, but the upper limit of the NC concentration needs to be considered. The final product includes 5–10% dimethyl sulfoxide (DMSO) as a cryoprotectant and 0.05–0.25 mL of ACD-A stabilizer solution per ml of transplant. Freezing at a controlled rate of 1–2 °C per minute is recommended. Cells need to be stored in the vapour phase nitrogen at a temperature of  $\leq -140$  °C. Cross-contamination while preparing and storing the cells must be prevented by taking appropriate measures.

At the time of auto-HCT, cryopreserved bags must be thawed at the site of transplantation and PBSCs should be reinfused within a maximum time span of 20 min of thawing using standard transfusion filters in order to minimize the detrimental effect of DMSO upon HSC. Previous washing for purposes of DMSO depletion is not routinely performed, as the loss and damage of HSC are regarded as too high.

Several studies demonstrated that under these storage conditions, CD34<sup>+</sup> HSC remained viable for up to 19 years (Ferynhough et al. 2013; McCullough et al. 2010; Spurr et al. 2002). In addition, it could be demonstrated that the duration of cryostorage of the transplant had no impact on the haematologic reconstitution after transplantation (Lisenko et al. 2017).

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## 20.3 HSC Quality Assessment

HSC product quality assessment needs to be performed at several time points during cell processing and storage. Volume measurement, enumeration of NC and red blood cells, and flow cytometry-based CD34<sup>+</sup> cell quantification should be performed directly after PBSC collection in accordance with the Stem Cell Enumeration Committee Guidelines of the International Society of Hematotherapy and Graft Engineering (ISHAGE) (Sutherland et al. 1996). A validated protocol and external quality control (e.g. the round robin test) is strongly recommended (Whitby et al. 2012).

Shortly before freezing, microbiological culture samples must be obtained. NC enumeration and NC viability measurement (e.g. by staining with trypan blue, 7-aminoactinomycin D [7-AAD] or propidium iodide) should be performed from aliquots of the final cell product after freezing and thawing. This viability testing is only valid for a defined and limited time span, often 2–5 years based on local guidelines, before it needs to be repeated prior to transplantation. Therefore, a sufficient number of reference samples should be prepared for each HSC product (the recommended minimum number is 3).



Target values need to be defined for the final product, mostly in accordance with local authorities. In most transplant centres in Europe, the following criteria are mandatory (together with additional criteria) for the release of an autologous transplant: NC concentration  $\leq 4 \times 10^8/\text{mL}$ , CD34<sup>+</sup> cell number  $\geq 2 \times 10^6/\text{kg}$  bw, red blood concentration  $\leq 0.1$  mL per mL of transplant, no microbial growth and minimum NC viability of  $>70\%$  after freezing and thawing.

## 20.4 Collection of Reference Samples for Quality Control

Reference (or retention) samples for quality control must be taken and stored from the cell product. These samples allow the proof of quality and potency of the transplant in terms of sterility, purity, and viability of the cells. In the case of an allo-HCT, reference samples may also need to be collected from the donor, depending on the respective local legal requirements, to allow for a retrospective analysis in terms of serological testing.

Reference samples are prepared in parallel with the cell product and should be stored under the same cryoconditions until they are analysed. As a release criterion for an autologous stem cell transplant, a reference sample should be cryopreserved for  $>24$  h under the identical conditions as the cell product before the viability of nucleated cells is analysed. Performing a clonogenic assay (e.g. colony-forming assay) from the reference samples can assess the haematopoietic potency of the cells. However, this is not regarded as a release criterion but should be performed for process validation or in the case of prolonged cryostorage of a transplant ( $>5$  years).

The final cell product must be labelled in accordance with respective legal requirements. In order to transport a cryopreserved HSC product, a validated shipping container is required, preferably with continuous temperature monitoring. The treating physician is responsible for the application of the HSC transplant after evaluating its integrity and the accompanying documents.

### Key Points

- Minimal number of CD34<sup>+</sup> cells is  $\geq 2.0 \times 10^6/\text{kg}$  bw for an auto-HCT and  $\geq 4.0 \times 10^6/\text{kg}$  bw for an Allo-HCT.
- Determination of HSC subsets is not routinely required, but may be part of clinical studies.
- Cryopreservation needs to be performed within 72 h, preferably  $<48$  h.
- The maximum NC concentration in the cryostored transplant should be  $\leq 4 \times 10^8/\text{mL}$ .
- The final product includes 5–10% DMSO as a cryoprotectant and 0.05–0.25 mL of ACD-A stabilizer solution per ml of transplant.
- Freezing at a controlled rate of 1–2 °C per minute is recommended, and cells need to be stored in vapour phase nitrogen at a temperature of  $\leq -140$  °C.
- NC viability should be  $>70\%$  after freezing and thawing.
- At the time of ABSCT, cryopreserved bags must be thawed and reinfused within a maximum of 20 min. of thawing.
- Reference samples for quality control must be prepared and cryostored in parallel and under identical conditions as the cellular product.

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# Documentation of Engraftment and Chimerism After HCT

# 21

Peter Bader, Hermann Kreyenberg,  
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## 21.1 Definition

Chimerism defines the proportion of donor and recipient cells after allogeneic stem cell transplantation. Complete or full donor chimerism is defined as the presence of >95% donor cells in the myeloid and in the lymphoid compartment. Mixed donor chimerism is defined as a proportion of 5–95% donor cells in the myeloid and in the lymphoid compartment. Absent donor chimerism is defined as <5% donor cells. Split donor chimerism defines patients with different degrees of donor chimerism in the myeloid or lymphoid compartment.

## 21.2 Introduction

For a long time, it was believed that complete donor hematopoiesis is necessary to maintain engraftment after allo-HCT. A few decades ago,

it became apparent that donor and recipient hematopoiesis may coexist. This state of coexistence of hematopoietic cells is called mixed chimerism (MC). If all cells are of donor origin, the patient is referred to as “complete chimera” and he shows a “complete chimerism.”

It is important to note that the state of hematopoietic chimerism may underlay a certain dynamic. Patients with a complete chimerism may develop a “mixed chimerism” at a later time point or vice versa. In a later period after transplant, the amount of autologous cells may “increase” or “decrease.” The patients then develop an “increasing mixed chimerism” or a decreasing mixed chimerism. To avoid misunderstandings as to whether donor or recipient hematopoiesis changes, it is recommended to report “increasing mixed donor chimerism” or “increasing mixed recipient chimerism.”

Nowadays, it has become possible to analyze hematopoietic chimerism also in single cell subpopulations. If a patient’s hematopoiesis is mixed only in different cell lines, these patients are referred to have a “split chimerism.” Finally, the applied method for chimerism analysis has also an impact on the degree of chimerism. A patient could be completely chimera with a method detecting about 1% autologous cells, whereas recipient cells could have been detected with a more sensitive technique (Bader et al. 2005).

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### 21.3 Methods for Chimerism Analysis

Different methods have been developed for the assessment of hematopoietic chimerism. All these methods followed the same principle using differences in polymorphic genetic markers and their products. Historically, restriction fragment length polymorphism (RFLP), cytogenetics, red cell phenotyping, and fluorescence in situ hybridization techniques were used for the assessment of hematopoietic chimerism. All of these techniques have been very time-consuming and did not always offer the possibility to be used in every patient–donor constellation.

Widespread and timely clinical applicability has become possible after polymerase chain reaction (PCR) techniques were developed. During the 1990s, these analyses were mainly performed by amplification of variable number of tandem repeats (VNTRs). Later in the decade, short tandem repeats (STRs) were used. Fluorescent labeling of the primers and resolution of PCR products with capillary electrophoresis allowed immediate and accurate quantification of the degree of chimerism. Semiautomated PCR analysis using the appropriate hardware allowed moreover high sample throughput. This made it possible to study chimerism in all patients and in short time intervals already early after transplantation. Accurate monitoring of engraftment as well as surveillance of impending graft rejection in patients transplanted for nonmalignant disease has become possible (McCann and Lawler 1993; Thiede et al. 2001).

To increase the sensitivity of chimerism analysis below the detection limit of STR-PCR, various techniques such as quantitative real-time PCR (qPCR) (Alizadeh et al. 2002), digital PCR (dPCR) (Stahl et al. 2015), and next-generation sequencing (NGS) (Aloisio et al. 2016) have become available and can be employed to identify low degree of chimerism termed microchimerism. These approaches commonly employ the targeting of biallelic markers, such as single nucleotide polymorphisms (SNPs) or deletion insertion polymorphisms (DIPs), to achieve discrimination. While the achievable sensitivity is

comparable for all approaches, the accuracy of dPCR and NGS is superior to qPCR. The drawback arises when utilizing these highly sensitive methods, as most samples tend to exhibit detectable recipient portions (Willasch et al. 2014). Therefore, it becomes crucial to establish the biological background within the individual's clinical context (Vynck et al. 2021). Continuous observation combined with mathematical description of chimerism dynamic could improve outcome prediction (Sellmann et al. 2018).

Based on these issues, the STR-PCR with fluorescent-labeled primers and resolution of the fragments with capillary electrophoresis is currently still considered to be the gold standard for the assessment of post-transplant chimerism (Blouin and Askar 2022). It is important to stress that whatever method is employed to study chimerism, it is important that the procedure is standardized and the chimerism laboratory is accredited and is participating in quality control rounds (Lion et al. 2012).

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### 21.4 Chimerism Investigation in the Clinical Setting

#### 21.4.1 Chimerism in Nonmalignant Diseases

Allo-HCT is the only curative treatment option for many patients with inherited or acquired nonmalignant diseases such as immunodeficiency, storage diseases, osteopetrosis, thalassemia, sickle cell disease, severe aplastic anemia, bone marrow failure syndromes, and many others.

The aim of the transplant procedure in these diseases is to achieve stable and durable engraftment to (1) improve the hematopoietic function, (2) correct the immune competence, and/or (3) increase or normalize the respective enzyme shortage. As a consequence, it is not always necessary to replace the recipient hematopoiesis completely. For many diseases, it is sufficient to implement a state of mixed hematopoietic chimerism to improve the patients' well-being. To minimize toxic side effects intensity of conditioning regimens in these diseases is often reduced and

therefore less myeloablative. MC is more likely, and graft rejection or non-engraftment remained the major causes of treatment failures in these patients (Bader et al. 2005; Thiede et al. 2001).

It could be shown that rapid donor cell presence and maintenance of early complete donor chimerism in NK and T-cells may play an important role in achieving sustained engraftment especially in patients who were treated with reduced intensity conditioning regimens. Analysis of chimerism in disease characterizing cell subpopulations in patients with nonmalignant disease, e.g., in patients with severe combined immune deficiencies (SCIDs) or in patients with storage disease, enables the documentation of success of the transplant procedures (Preuner et al. 2016).

#### 21.4.1.1 Intervention to Influence the Evolution of Chimerism: Transfusion of DLI

In patients with nonmalignant diseases, MC occurs frequently. The question whether individual patients with MC are at risk to reject their graft depends on the diagnosis and on the conditioning regimens. Studies have clearly shown that MC can be influenced by DLI. MC can be stabilized or even converted to complete donor chimerism by DLI. However, in treating patients with MC and DLI, physicians have to bear in mind the potential risk to induce GVHD which should be avoided in patients with nonmalignant disease with all efforts.

#### Hemoglobinopathies

In *thalassemia patients*, large studies have been published already from the Pesaro group of Guido Lucarelli, evaluating the influence of MC and disease recurrence. In general, it was found that patients whose recipient MC increased to >30% autologous cells were by far more likely to ultimately reject and be transfusion-dependent. However, there are patients with persisting high-level MC who remained transfusion independent. Retrospective studies have been performed evaluating the possibility of influencing MC by DLI. It could be shown that a state of MC may be sufficient to remain transfusion independent. It

was also shown that DLI is capable to convert MC to CC. However, no general recommendation could be given at the time being (Fitzhugh et al. 2014; Karasu et al. 2012; Abraham et al. 2017).

In *sickle cell disease (SCD)*, the impact of MC has been studied intensively as more and more patients with SCD were transplanted from matched but also from mismatched donors. In the late 1990s, first studies concluded that 10% of donor cell engraftment and persistence were needed for the effective treatment of SCD in patients who were treated with a homozygous healthy donor; however, if the patient was grafted with the stem cells of a heterozygous HbAS donor, 30–40% donor cells are required. The presence of MC in patients transplanted for sickle cell disease does not warrant DLI per se. In patients with less than 30% of donor chimerism, DLI might be considered. In a most recent study, Fitzhugh and colleagues developed a mathematical model by which they could show that a donor chimerism in the myeloid compartment of 20% is necessary to reverse the sickle cell phenotype and to prevent patients from disease recurrence (Fitzhugh et al. 2017).

#### 21.4.2 Chimerism in Malignant Diseases

Chimerism detected by molecular methods allows the assessment of persisting or reappearing recipient cells after allo-HCT. These cells might be a reflection of either survival of malignant cells or of survival or recurrence of recipient hematopoietic cells or a combination of both. It could be shown by prospective studies already in the early 1990s that a MC frequently occurs in the mononuclear cell fraction, weakens thereby the GvL effect, and facilitates recurrence of the underlying leukemia.

Chimerism analysis does provide information about the alloreactivity and/tolerance induction of the graft and thereby serves more likely a “prognostic factor” than as an indirect marker for MRD. It has become evident that the development of post-transplant chimerism is a dynamic



process. Hence, if chimerism analyses are performed in the intention to detect impending relapse, investigations need to be performed in short time intervals (Bader et al. 2004b; Thiede et al. 2001; Kröger et al. 2010a, b).

Initially, many pediatric studies using serial analysis of chimerism could clearly demonstrate that patients who develop a MC post-transplant have an increased risk for future relapse of their leukemia. This could later also be confirmed by studies in adult patients. Moreover, these and subsequent studies undoubtedly showed that by immunotherapeutic interventions, e.g., withdrawal of IS or transfusion of DLI, MC could be converted to complete chimerism, GvL effect restored, and many patients prevented from developing overt hematological relapse (Platzbecker et al. 2012; Bader et al. 2004a).

Based on its limited sensitivity to detect minor cell population of about 1%, chimerism analysis by STR-PCR in the whole blood is not suitable to serve as a MRD marker. For the assessment of MRD, other techniques should be used, if possible. In patients and diseases lacking a disease-specific marker, for example, regularly in patients with MDS and often in patients with AML, chimerism analysis could be performed in cell subpopulations. Thiede et al. could clearly demonstrate that by the characterization of chimerism in the CD34-positive cell fraction, leukemia relapse could be anticipated in advance in many patients with AML and MDS. In ALL patients, several studies have been performed investigating the impact of MC after enrichment of entity specific subpopulation, e.g., CD 10, CD19, and CD 34 for precursor B ALL and CD3, CD4, CD5, and CD8 for T-lineages. Remarkable correlation between MRD and chimerism in different subsets could be proven (Platzbecker et al. 2012; Bornhäuser et al. 2009; Rettinger et al. 2011; Gambacorta et al. 2020).

Advances in testing methods and increased sensitivity have shown that MC occurs more frequently than previously thought. The occurrence of increasing MC that is not associated with impending relapse highlights the dynamic nature of chimerism state. It further emphasizes that serial and quantitative analysis of chimerism is

necessary to allow for the identification of patients at highest risk for relapse (Sellmann et al. 2018).

Not all patients can be identified, and time interval between the onset of MS and relapse is often short. It is essential to perform the analysis frequently and ideally: chimerism should be combined with MRD analysis to optimize the predictive value. These investigations can form the basis for individual preemptive immunotherapy strategies to prevent recurrence of the underlying disease.

### Key Points

- Documentation of engraftment is the important step on the way to successful HCT.
- Post-transplant patients are carrying two different genetic profiles and are called chimera.
- Analysis of hematopoietic chimerism offers the possibility to realize impending graft rejection and may also serve as an indicator for the recurrence of the underlying disease.
- Since several years, these investigations have become the basis for intervention strategies to:
  - Avoid graft rejection.
  - Maintain engraftment.
  - To treat imminent relapse by preemptive immunotherapy.

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# Short- and Long-Term Controls After HCT

# 22

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## 22.1 Introduction

Patients undergoing HCT (mainly allo-HCT) have a risk of developing complications related to pre-, peri-, and post-HCT. The resulting morbidity of the HCT process makes it necessary for patients to adopt a healthy lifestyle that promotes health and contemplate preventive measures for the detection and treatment of possible complications.

The short- and long-term controls allow for regular and systematic screening and at the same time are an opportunity to give advice on healthy lifestyle habits. Monitoring should be multidisciplinary with involvement of hematology, other medical specialties, physicians of primary care, nursing, and mental health professionals.

Early and late complications, as well as psychological problems, are discussed in Parts IV, V, and VI of the Handbook.

After discharge, a long-term follow-up plan for each patient is needed which takes the given treatment and individual risk factors into account.

The recommendations related to screening and prevention post-HCT can be consulted in several web pages (see References).

## 22.2 Monitoring Depending on the Type of HCT

### 22.2.1 Autologous HCT

Timing	Monitoring
From discharge to day +100	Until full hematologic recovery, it is recommended to live near the hospital Recommended controls <sup>a</sup> : <ul style="list-style-type: none"><li>– Clinical evaluation and transfusions when necessary</li><li>– Basic hematological and biochemical tests</li><li>– Specific markers for different diseases</li></ul>

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Timing	Monitoring
At +3 months	Evaluate the status of the primary disease Recommended controls <sup>a</sup> : <ul style="list-style-type: none"> <li>– Hematological and biochemical tests, specific tumoral markers</li> <li>– MRD evaluation: Immunophenotype and molecular specific adapted to each disease</li> <li>– BM biopsy in case of NHL, HL, MPS, and solid neoplasms with previous marrow affectionation, in the remaining disease BM smears (see specific chapters)</li> <li>– Imaging tests depending on primary disease</li> </ul>
Long term	Visits every 6 months up to 2 years and then annually Recommended controls <sup>a</sup> : <ul style="list-style-type: none"> <li>– Analytical and complementary explorations: See Table 22.1</li> <li>– Baseline disease: Control of possible progression or relapse during at least 5 years</li> <li>– In patients treated with chemotherapy + radiotherapy, assess the risk of second malignancies or MDS after HCT</li> </ul>

<sup>a</sup>Variable frequency depending on the patient's condition

### 22.2.2 Allogeneic HCT

Timing	Monitoring
From discharge to day +100	It is recommended that the patient resides near the transplant center during the first 3–6 months after HCT Recommended controls <sup>a</sup> : <ul style="list-style-type: none"> <li>– Weekly clinical evaluation, during the first month, every other week until second month, every 2 weeks until third month, and then monthly up to 6–12 m, unless problems arise. It must include complete physical examination, with special emphasis on data of acute GvHD, infections, and pulmonary complications</li> <li>– Blood samples: Complete blood count, liver and kidney function, Mg, levels of IS agents, quantify CMV by PCR (and EBV if ATG); chimerism evaluation at 1 and 2 month</li> <li>– BM aspirate (or biopsy) in diseases with previous marrow affectionation (usually within 1 month of HCT)</li> </ul>
At 3 months	Usually, this moment marks the turning point so that, if the patient does not have major problems, he/she can be monitored by the referring doctor. However, the patient should be periodically reevaluated at the transplant center (every 3–4 months during the first year, every 4–6 months during the second year, and annually after the third year) Recommended controls <sup>a</sup> : <ul style="list-style-type: none"> <li>– Visit and complete physical exploration with special emphasis on the signs of acute and chronic GvHD (assessment by organs as indicated in Chaps. 43 and 44 and paragraph 22.3)</li> <li>– Blood test: Complete blood count, kidney function, liver function, clearance creatinine, IS levels; chimerism and sample for MRD follow-up. In patients aged &lt;17 years, weight and height every 3 months</li> </ul>
Long term	It depends on the complications that arise during follow-up. If there are no complications, it is recommended that a patient visits to the center every 6 months up to 3 years and annually thereafter Recommended controls: <ul style="list-style-type: none"> <li>– Visit and complete physical examination including gynecological evaluation and endocrinological, if appropriate</li> <li>– Analytical and complementary explorations: See sect. 22.3</li> <li>– Specific controls: Specific MRD studies on diseases with markers (see corresponding chapters)</li> <li>– In patients treated with chemotherapy + radiotherapy, the risk of secondary neoplasms</li> </ul>

<sup>a</sup>Variable frequency depending on the patient's condition (every 4–6 weeks)

## 22.3 Organ-Specific Long-Term Monitoring

Table 22.1 analyzes organ by organ the long-term follow-up recommendations.

**Table 22.1** Organ-specific monitoring<sup>a</sup>

Recommended screening <sup>b</sup>	6 months	1 year	An.	Comments
<i>Ocular</i> (see Chap. 48)				
– Clinical symptom evaluation	1	1	1	– Immediate examination if visual symptoms
– Visual acuity and fundus exam	+	1	+	– Special attention to <i>sicca</i> syndrome
<i>Oral</i> (Chap. 50)				
– Preventive oral health and dental maintenance	1	1	1	– Avoid smoking, sugar beverages, or oral piercing
– Clinical assessment	1	1	1	– If oral cGvHD, high-risk squamous cell cancer; evaluation every 6 months
– Dental assessment (+children)	+	1	1	
<i>Respiratory</i> (Chap. 52)				
– Clinical pulmonary assessment	1	1	1	* Active or passive
– Smoking tobacco avoidance*	1	1	1	– If cGVHD, spirometry test in each control (recommended for many authors)
– PFT (+chest Rx if symptoms)	+	+	+	
<i>Cardiac and vascular<sup>c</sup></i> (Chap. 55)				
– CV risk factor assessment	+	1	1	– Counseling on heart healthy lifestyle – Active treatment of risk factors
<i>Liver</i> (Chaps. 38 and 49)				
– Liver function testing	1	1	1	– Monitor viral load by PCR if HCV or HBV
– Serum ferritin testing		1	+	– Additional testing if high ferritin levels (MRI/FerriScan®)
<i>Kidney</i> (Chap. 51)				
– Blood pressure screening	1	1	1	– Hypertension should be investigated and treated appropriately
– Urine protein screening	1	1	1	
– BUN/creatinine testing	1	1	1	– Avoid nephrotoxins
<i>Muscle and connective</i> (Chap. 54)				
– Physical activity counseling	1	1	1	– If risk of cGvHD, test joint mobility and touch skin to detect sclerotic changes
– Evaluation muscle weakness	2	2	2	– Treat cramps symptomatically
<i>Skeletal</i> (Chap. 54)				
– Bone density testing <sup>d</sup>		1	+	– Prevent bone loss and fractures with exercise, vitamin D, and calcium
<i>Nervous system</i> (Chap. 53)				
– Neurologic clinical evaluation	+	1	1	* Special attention of cognitive development in pediatric patients
– Cognitive development*		1	1	
<i>Endocrine</i> (Chap. 56)				
– Thyroid function testing		1	1	– Annual gynecological evaluation in women
– Growth speed in children		1	1	– Hormonal replacement if necessary
– Gonadal function assessment <sup>e</sup>	1	1	1	
– Gonadal function assessment <sup>f</sup>		1	+	
– Gonadal function assessment <sup>g</sup>		+	+	
<i>Mucocutaneous</i> (Chap. 54)				
– Skin self-exam, sun counseling	1	1	1	– Avoid sunlight without adequate protection
– Gynecological exam in women		1	1	
<i>Immunity</i>				
– Encapsulated microorg. Prophylaxis*	2	2	2	* If chronic GvHD and IS therapy, consider endocarditis prophylaxis in high-risk patients
– PJP prophylaxis (see Chap. 39)	1	2	2	
– Immunizations (see Chap. 29)	1	1	1	

(continued)

**Table 22.1** (continued)

Recommended screening <sup>b</sup>	6 months	1 year	An.	Comments
<i>Secondary neoplasia</i> (Chap. 47)				
– Counseling and autoexamination		1	1	– Reduce UV skin exposure – Special attention to high-risk organs
– Same population screening		1	1	– If TBI, increase frequency mammography – Consider personal and familiar history of malignancy
<i>Psychosocial and sexual</i>				
– Psychosocial assessment (see Chap. 30)	1	1	1	– Add spousal/caregiver psychological adjustment and family functioning
– QOL assessment (see Chap. 34)	1	1	1	
– Evaluation of sexual function	1	1	1	

An. annually, 1 recommended for all transplant recipients, 2 recommended for patients with ongoing chronic GvHD or IS, + reassessment recommended for abnormal testing in a previous time period or for new signs/symptoms

<sup>a</sup>Adapted from Majhail et al. (2012). Similar recommendations but focused in children have been elaborated by the Children's Oncology Group <http://www.survivorshipguidelines.org>

<sup>b</sup>In patients with chronic GVHD, these controls should be tightened and their frequency increased

<sup>c</sup>Follow the American Heart Association for endocarditis prophylaxis in high-risk HCT recipients

<sup>d</sup>Adult women, all allo-HCT, and patients at high risk for bone loss

<sup>e</sup>Prepubertal men and women

<sup>f</sup>Postpubertal women

<sup>g</sup>Postpubertal men

## 22.4 Fertility (See Chap. 56)

## 22.5 Quality of Life (See Chap. 34)

### Key Points

- Patients auto- and mainly allo-HCT have a risk of developing complications related to pre-, peri-, and post-HCT.
- The resulting morbidity of the HCT process makes it necessary for patients to adopt a healthy lifestyle that promotes health and contemplate preventive measures for the detection and treatment of possible complications.
- The short- and long-term controls allow for regular and systematic screening and at the same time are an opportunity to give advice on healthy lifestyle habits. These recommendations can be based on comorbidity and individual risk factors.

- Monitoring should be multidisciplinary with involvement of hematology, other medical specialties, physicians of primary care, nursing, and mental health professionals.

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## **Part IV**

### **General Management of the Patient**

**Topic Leaders: Selim Corbacioglu,  
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Simone Cesaro and Giulia Caddeo

## 23.1 Introduction

The execution of the complex procedure of hematopoietic cell transplantation (HCT) requires a stable and safe venous access. This aim is usually achieved by the insertion of a central venous catheter (CVC). CVC is a key tool to ensure a safe central venous access for multiple purposes (chemotherapy, parenteral nutrition, supportive therapy, hydration, and blood sampling), to reduce the need for repeated, painful venipunctures, and to preserve the peripheral venous asset. The use of CVC is associated with the occurrence of complications (malfunctioning, occlusion, dislodgement, kinking, rupture, thrombosis, and catheter-related bloodstream infections-CRBSI) that can cause its premature removal or be life-threatening for the patient. For this reason, education and continuous training of health personnel are fundamental to preserve the CVC life span from complications. Table 23.1 shows the essential steps of CVC maintenance (Cesaro et al. 2016; Cellini et al. 2022).

CVCs are being designated by:

- Duration (e.g., temporary or short-term versus permanent or long-term).
- Site of insertion (e.g., subclavian vein, femoral vein, jugular vein, and basilic vein).
- Number of lumens (single, double, or triple lumen).
- Characteristic of tip (open tip or closed tip).
- Materials to reduce complications (e.g., impregnation with heparin, antibiotics, or silver).

## 23.2 Type of CVC Materials

Catheter materials should be biocompatible, kink resistant, inherently chemically resistant, and neutral, biostable, soft, and deformable and should have a high tensile strength (Lim et al. 2018; Crocoli et al. 2022). The most commonly used materials are polyurethane and silicone. Silicone catheters are flexible, chemically stable, and well tolerated. Polyurethane has a superior tensile strength. Non-tunneled, semirigid catheters are usually made of polyurethane, while tunneled catheters are usually made of both silicone and polyurethane (Lim et al. 2018). The choice of a polyurethane or a silicone catheter is debated. Silicone catheters are more prone to material failure as a result of the development of surface irregularities due to loss of barium sulfate molecules and thrombotic occlusion. The introduction

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**Table 23.1** CVC maintenance: recommended rules

1. Assessment of line functionality and dressing site daily for inpatients or every 2–3 days for outpatients
2. CVC care and maintenance as dictated by local policy or standard operating procedure <sup>a</sup>
3. Vigorous mechanical scrub for manual disinfection prior to each CVC access and allow it to dry. Acceptable disinfecting agents include 70% isopropyl alcohol, iodophors (i.e., povidone-iodine), or >0.5% chlorhexidine in alcohol solution
4. Change gauze dressing every 7 days or before in case of soiled, dampened, and loosened dressing
5. Change the transfusion administration set and filter after the completion of each unit or every 4 h. If more than 1 unit can be infused in 4 h, the transfusion set can be used for a 4-hour period
6. Change intermittent administration sets every 24 h
7. Replace administration sets for parenteral nutrition solutions at least every 24 h
8. Replace administration sets used for intravenous fat emulsions infused separately every 12 h
9. Change caps every 72 h (or 7 days if pressure-positive or neutral needle free connector is used)

<sup>a</sup>There may be a variability among EBMT centers regarding the practice of CVC care and maintenance such as the use of sterile gloves and mask by provider/assistant, the adoption of aseptic technique for all catheter entries, the use of prepackaged dressing change kit, the frequency of flushing, and the type of solution used for flushing CVC

of third-generation polyurethane catheters with power-injectable technology, that allows flows up to 5 mL/s and contrast medium injection, made this type of CVC a favorite with many operators (Crocoli et al. 2022).

### 23.3 Type of CVC

CVCs are classified in two main categories: *tunneled and non-tunneled*, according to whether or not they follow a subcutaneous route before accessing the central vein. Non-tunneled catheters are directly inserted into a peripheral or large central vein. Both tunneled and non-tunneled CVCs may have a single or multiple lumen. Tunnelling of CVCs was introduced to reduce the risk of infectious and mechanical (dislodgement) complications, and this type of CVC is ideal for long-term care (Cellini et al. 2022). Non-tunneled

CVCs are usually inserted for a short to medium period (from 2–4 weeks to 1–3 months) (Lee and Ramaswamy 2018; Padmanabhan 2018). Tunneled CVCs are in turn classifiable in two subgroups: partially implanted and totally implanted. Partially implanted CVCs are characterized by an external part outside the patient's body whose extremity (hub) is used to draw blood sampling or to connect the infusion lines, a tunneled subcutaneous part with a Dacron cuff at a few centimeters from the skin entry point, and a final intravenous part with the tip positioned at the border between the superior vena cava and the right atrium. The Dacron cuff stimulates a fibrotic reaction of the subcutaneous tissues over 2–4 weeks ensuring stability and CVC securement. Both cuff and subcutaneous course are fundamental to prevent the CVC from becoming infected due to the migration of external microbes along the CVC.

The tip of CVC can be open or closed: open tip requires the clamping of the external part of CVC when it is not in use to avoid blood backflow with breath or body movements, while closed tip has lateral valves that open as fluid is withdrawn or infused and remain closed when the CVC is not used. The clamping of CVC with open tip is not needed if a neutral pressure needle-free connector is used to prevent blood backflow.

*Totally implanted* catheters consist of a reservoir (port) placed in a pocket in the subcutaneous tissue, anteriorly on the chest wall, below the clavicle, that is connected to the catheter. This type of CVC preserves the patient's body image and ensuring almost no limitations on sports activities, and body hygiene. Accessing to this type of CVC needs skin puncture with a special "non-coring" needle (Huber needle or gripper system). In case of frequently accesses, the procedure can be painful or discomforting for the patient, requiring the application of topical skin anesthetic for its prevention. "Non coring" needle does not permit the infusion or the extraction of high volumes making it less suitable for patients requiring high infusion or blood extrac-

tion rates. The recent introduction of port models with a modified reservoir chamber (vortex, tidal, power port) has allowed to obtain a higher flow rate suitable for leukapheresis, red blood cell exchange, extracorporeal photopheresis, and therapeutic plasma exchange (Blanco-Guzman 2018; Lim et al. 2018).

The *peripherally inserted central catheter (PICC)* is a CVC inserted into a vein of the arm, usually the basilic or cephalic veins; its tip is advanced through the axillary and subclavian veins up to the cavo-atrial junction (Cornillon et al. 2017). For more information on PICCs, see Chap 32.

### 23.4 Venous Access

Central lines are usually inserted through the subclavian, the jugular, or, less frequently, the femoral vein. This last venous access is associated with a higher risk of infectious complications (O’Leary 2016), and it is more commonly used in critically ill patients admitted to intensive care units who require a non-tunneled CVC. It is recommended that the ratio of catheter caliber to vein diameter should not exceed 1/3. Using the subclavian or jugular access, the tip of the catheter has to lie in the superior vena cava, just before the entrance of the right atrium, about 29–55 mm below the level of trachea carina (in adults). The incidence of pneumothorax after CVC insertion is about 1.5–3.1%, and it is higher with subclavian vein catheterization, whereas the risk of hemorrhage and bruise is slightly more common with the jugular venous line access.

In the positioning of a PICC, the incannulation of the basilic vein is preferred to that of the cephalic vein as it has low risk of complications. To minimize the risk of complications due to venous catheterization, the routine use of ultrasound guidance to cannulate the vein is recommended instead of the classical (blind) technique (Crocoli et al. 2015; Crocoli et al. 2022).

A chest X-ray must be performed at the end of the CVC insertion procedure to confirm that the line is positioned inside the superior vena

cava, and for the cannulation of subclavian or jugular veins, no pneumothorax was inadvertently caused. Alternatively, the use of intracavitary ECG (electrocardiographic method) is a noninvasive method to evaluate the correct position of the catheter tip.

## 23.5 CVC Complications

Catheter-related complications may be classified into *infectious* (local or systemic) and *mechanical* (occlusion, rupture, dislodgement, accidental self-removal, and thrombosis) (Cesaro et al. 2009). As the catheter is itself a risk for developing complications, it should be removed as there is no further need for it. Premature CVC removal is indicated in case of mechanical complication with persistent malfunction, CRBSI by *Candida* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus aureus*, persistent bacteria colonization, recurrent CRBSI, or CVC thrombosis.

### 23.5.1 Special Measures to Prevent Catheter-Related Infections

The key rules to prevent infections are proper handwashing by the performing provider, the use of aseptic technique over the patient at insertion time, thorough cleaning of the insertion site, and periodic review of the CVC exit site (Cesaro et al. 2016; Cellini et al. 2022). To prevent CRBSI and tunnel or exit-site infection, medication-impregnated dressings with different antimicrobial materials were developed to decrease the production of the biofilm by microorganisms and decrease the adhesion of them to the catheter walls. Impregnating medications may contain chlorhexidine gluconate, silver sulfadiazine, rifampin, and minocycline. Chlorhexidine gluconate is among the most used, by impregnating the whole dressing or applying an impregnated sponge (e.g., Biopatch®) and covered by a transparent polyurethane semipermeable transparent dressing. The efficacy of flushing or locking CVC with an antibiotic solution to prevent infec-

tion is debated, and it is associated with the risk of increasing microbial antibiotic resistance; therefore, it is not recommended and it should be reserved to selected cases (van den Bosch et al. 2021).

### 23.6 Catheters for Leukapheresis

The procedure of stem cell collection by apheresis is performed both for auto- and allo-HCT to obtain stem cells (O’Leary 2016). As the procedure requires sustained high blood flow rates (50–100 mL/min), an adequate venous access is needed. Peripheral access placed in the basilic, cephalic, brachial, median cubital, and radial veins is recommended. Considering that the placement of a central CVC is associated with potentially life-threatening complications such as pneumothorax, bleeding, and embolism, its use is not recommended for PBSC collection of a healthy volunteer donor. Conversely, in the case of auto-PBSC, if the patient has no adequate peripheral or central venous access, a temporary non-tunneled CVC may be placed in the internal jugular, subclavian, or femoral veins. Catheter removal is performed on donor laboratory values (platelets  $>50 \times 10^9/L$ ) and after the assessment of an adequate CD34<sup>+</sup> dose collection and cryopreservation.

Partially implanted silicone CVCs are often used by pediatric oncologists-hematologists because they are most suitable for long-term complex treatment. In the case of leukapheresis procedure, silicone CVCs are not ideal because they are more prone to collapse during automatic apheresis (Ridyard et al. 2017). On the other hand, the harvesting procedure of peripheral stem cell collection, which requires high blood flow rates and a large needle, may be difficult in children below 10 kg using a temporary peripheral venous access due to the small size of veins (Padmanabhan 2018). In this case, the placement of a short-term CVC made of polyurethane may be needed (Cooling 2017). In younger children, the rigidity of such material and the narrower lumens of the veins may represent a potential risk for thrombosis and infection.

#### Key Points

CVC indications and insertion		
1. Type of CVC	Tunneled CVCs/port/PICCs	Long-term therapy (months, years) Port for intermittent use, tunneled CVC for continuous use Suitable for inpatient and outpatient
	Non-tunneled CVCs	Short-term therapy (2–4 weeks, 1–3 months) Suitable for inpatient
2. Number of lumens	Single lumen vs double lumen	Double lumen in patients undergoing HCT, critically ill patients, intensive intravenous therapy
3. Insertion	Percutaneous/minimally invasive	Ultrasound guidance recommended Adequate training required
	Cutdown approach	Very limited indication (premature infants) Experienced operator
4. Material	Silicone	Tunneled CVC
	Polyurethane	Tunneled and non-tunneled CVC

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### 24.1 General Aspects

Many recommendations in this chapter are based on evidence from studies including a broad variety of diseases. Only a few studies addressed transfusion strategy specifically in patients undergoing HCT (see review Christou et al. 2016). Many recommendations are derived from patients with cytopenia in nontransplant settings. There are both need and opportunity to address issues regarding transfusion of HCT patients in clinical trials. So far, there is a paucity of studies on the impact of transfusion on HCT-specific outcomes.

Red blood cell (RBC), platelet concentrate (PC), and fresh frozen plasma (FFP) for patients who are candidates for HCT should be leukocyte-reduced, i.e. should contain  $<1 \times 10^6$  leukocytes/unit. Leukocyte reduction reduces febrile non-hemolytic transfusion reactions and decreases the incidence of alloimmunization to leukocyte antigens and the risk of CMV transmission. All cel-

lular blood components (RBC, PC, and granulocyte transfusions) must be irradiated (see below).

### 24.2 Irradiation for Prevention of Transfusion-Associated GvHD (ta-GvHD)

Ta-GvHD is a rare complication of transfusion wherein viable donor T lymphocytes in cellular blood products mount an immune response against the recipient (Kopolovic et al. 2015). Some of the clinical presentations of ta-GvHD resemble that of GvHD (fever, cutaneous eruption, diarrhea, and liver function abnormalities). Also, many patients develop pancytopenia. Since mortality is high (>90%), prevention of ta-GvHD is critical (Kopolovic et al. 2015; Foukaneli et al. 2020; Wiersum-Osselton et al. 2021). HCT recipients are at a risk of ta-GvHD and should receive irradiated cellular blood products (Kopolovic et al. 2015). It is recommended that no part of the component receives a dose  $<25$  Gy and  $>50$  Gy (European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS) 2023). Some pathogen-reduction technologies have been shown to inactivate lymphocytes, and additional irradiation is not required (Kleinman and Stassinopoulos 2018).

There is no consensus on the duration of the use of irradiated blood products in HCT recipients (Foukaneli et al. 2020; Wiersum-Osselton et al. 2021). Standard practice is (1) auto-HCT, at

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least 2 weeks prior to stem cell collection until at least 3 months after HCT and (2) allo-HCT, at the latest starting with conditioning until at least 6 months after HCT or until immune reconstitution. However, some centers recommend lifetime use of irradiated products since it is difficult to confirm complete and sustained immunological reconstitution.

Allogeneic cellular blood components that transfused hematopoietic stem cell donors within 7 days prior to or during the harvest should also be irradiated (Foukaneli et al. 2020).

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### 24.3 Prevention of CMV Transmission

The highest risk of transfusion-transmitted CMV (TT-CMV) remains in CMV-seronegative recipients of matched CMV-negative HCT (Ljungman 2014). Risk of TT-CMV can be reduced by transfusion of leukocyte-reduced blood products (i.e.,  $<1$  to  $5 \times 10^6$  residual leukocytes per unit) or by transfusion of blood components from CMV-seronegative donors (Ziemann and Thiele 2017). The UK Advisory Committee for the Safety of Tissues and Organs recommended that CMV-unselected components could be safely transfused without increased risk of CMV transmission. It is unclear whether the “belt and suspender approach,” i.e., the use of both leukocyte-reduced and seronegative products, further reduces the risk of TT-CMV. Donations from newly CMV-IgG-positive donors bear the highest risk for transmitting CMV infections (Ziemann and Thiele 2017). Currently, no international consensus on risk mitigation for CMV transmission exists, and surveys revealed substantial heterogeneity of clinical practice (Lieberman et al. 2014; Morton et al. 2017). Also, there is no consensus how long CMV-

seronegative products should be given to transplant recipients: the current practice ranges from 100 days after transplant till lifelong (or until CMV seroconversion) (Lieberman et al. 2014).

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### 24.4 Red Blood Cell Concentrates (RBCs)

A restrictive RBC transfusion threshold of 7–8 g/dL hemoglobin is recommended for adult patients who are hemodynamically stable. A restrictive RBC transfusion threshold of 8 g/dL is recommended for patients with existing cardiovascular disease (Carson et al. 2021; Carson et al. 2016). These cut-offs are derived from studies on a broad range of indications. Only one randomized clinical trial is available specifically for patients undergoing HCT (TRIST trial, NCT01237639). It randomly assigned 300 patients to either a liberal strategy (Hb threshold  $<90$  g/L) or a restrictive strategy (Hb threshold  $<70$  g/L). Health-related quality of life was similar between groups, and there were no significant differences in clinical outcomes between the transfusion strategies (Tay et al. 2020). The median number of RBC units transfused was lower in the restrictive strategy compared to the liberal strategy group, but this did not reach statistical significance (Tay et al. 2020). One randomized feasibility trial is ongoing in children undergoing allogeneic hematopoietic cell transplantation. It compares restrictive versus liberal red cell transfusion strategies using hemoglobin concentrations of  $<6.5$  g/dL and  $<8.0$  g/dL (ISCRTN17438123).

In adult recipients, one unit of RBC increases the hemoglobin concentration by about 1 g/dL. In children, the dose should be calculated by the formula:

$$\text{Volume (mL RBC)} : \text{Target Hb after transfusion (g/dL)} - \text{pretransfusion Hb (g/dL)} \times 4 \times \text{weight (kg)}$$

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A randomized trial in patients with hematological malignancies indicated that transfusion of a single is not inferior to double RBC transfusion

for patients receiving a bone marrow transplant or intensive chemotherapy in a hematological intensive care unit (Chantepie et al. 2023). The

single RBC transfusion policy did not reduce the number of RBC units transfused per stay (Chantepie et al. 2023).

Several randomized trials showed no evidence that transfusion of fresh RBC reduced morbidity or mortality compared to standard issue RBCs. Thus, the AABB recommends that patients should receive RBC selected at any point within their licensed dating period (Carson et al. 2016).

Chronic RBC transfusions result in iron overload. The impact of pretransplant iron overload on outcome after HCT remains controversial. A meta-analysis (Armand et al. 2014) and a prospective cohort study suggest that iron overload, as assessed by liver iron content, is not a strong prognostic factor for overall survival in a general adult HCT population. Heparin or labile plasma iron levels may be more specific for iron overload and complications in HCT patients.

## 24.5 Platelet Concentrates (PCs)

PC should be transfused prophylactically to non-bleeding, nonfebrile patients when platelet counts are  $\leq 10 \times 10^9/L$  (Schiffer et al. 2018). Prophylactic platelet transfusions may be administered at higher counts based on clinical judgment (Schiffer et al. 2018). Patients with active bleeding, febrile conditions, or active infections should receive prophylactic PC transfusions at a threshold of  $20 \times 10^9/L$ . Also, in case of specific transplant-related toxicity which might increase the risk of bleeding (acute GvHD, mucositis, hemorrhagic cystitis, or diffuse alveolar hemorrhage), a threshold of  $20 \times 10^9/L$  or even higher, based on careful clinical observation, might be justified.

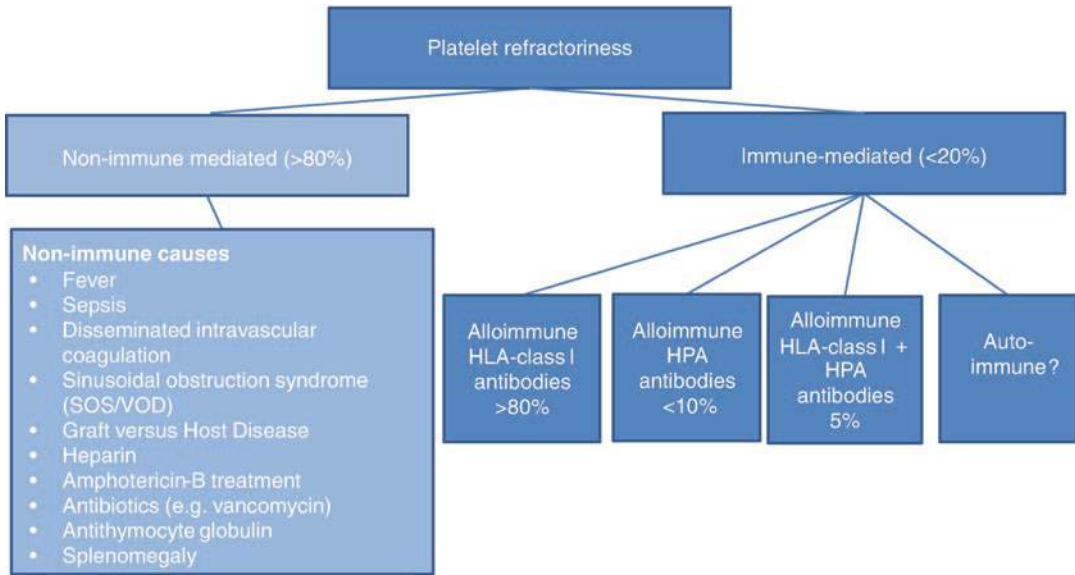
Two prospective randomized control trials comparing prophylactic versus therapeutic PC transfusion in adult patients ( $\geq 16$  years) suggest that a therapeutic transfusion strategy might be feasible in patients after auto-PB HCT but cannot be easily transferred to other indications (AML, allo-HCT) for whom special attention to the increased risk of bleeding, in particular, CNS bleeding, is needed (Stanworth et al. 2013; Wandt et al. 2012). The results may not be generalizable

to children since a subset analysis of the PLADO trial demonstrated that bleeding rates were significantly increased among children, particularly among those undergoing autologous HCT (Josephson et al. 2012).

The randomized PLADO trial compared different doses of PC transfusions (“low dose,” “medium dose,” and “high dose” defined as  $1.1 \times 10^{11}$ ,  $2.2 \times 10^{11}$ , and  $4.4 \times 10^{11}$  platelets per  $m^2$  BSA, respectively) (Slichter et al. 2010). While a strategy of “low-dose” transfusion significantly reduces the overall quantity of platelets transfused, patients required more frequent PC transfusion events (Slichter et al. 2010). At doses between  $1.1 \times 10^{11}$  and  $4.4 \times 10^{11}$  platelets/ $m^2$ , the number of platelets in the prophylactic transfusions had no effect on the incidence of bleeding.

Both apheresis PC and pooled PC from whole blood donations are safe and effective. Available data suggest equivalence of the products in non-allosensitized recipients (Schrenzenmeier and Seifried 2010). A clear advantage of apheresis PCs can only be demonstrated in allosensitized patients with HLA and/or HPA antibodies who receive antigen-compatible apheresis PCs.

Some patients experience inadequate increment after PC transfusions, i.e., a corrected count increment (CCI) below  $5000/\mu L$  at 1 h after transfusion of fresh, ABO-identical PCs on at least two subsequent transfusions. Refractoriness can be caused by non-immunological factors ( $>80\%$ ) or immunological factors ( $<20\%$ ) (Fig. 24.1). If platelet refractoriness is suspected and no apparent nonimmune causes can be identified, screening for the presence of HLA-Ab is recommended. If HLA-Ab is present, the patient should receive apheresis PCs from matched donors (Juskewitch et al. 2017; Stanworth et al. 2015): ideally all four antigens (HLA-A, HLA-B) of donor and recipient are identical. Also PCs from donors expressing only antigens which are present in the recipient can be used. If PCs from such donors are not available, donors with “permissive” mismatches in HLA-A or HLA-B shall be selected (e.g., based on cross-reactive groups or computer algorithms that determine HLA compatibility at the epitope level). A noninferior-



**Fig. 24.1** Etiology of platelet transfusion refractoriness (modified according to Pavenski et al. 2012)

ity, crossover, randomized trial compared HLA epitope-matched platelets with HLA standard antigen-matched platelet transfusion. It supported a role for epitope-matched platelets for HLA alloimmunized, platelet refractory patients (Marsh et al. 2021). If no better-matched donor is available, antigen-negative platelets, i.e., not expressing the target antigen(s) of the recipients’ HLA allo-Ab, can be transfused. Screening for antibodies against human platelet antigens (HPAs) should be performed if refractoriness persists also after transfusion of HLA-matched PCs and nonimmune causes are unlikely. Approaches for patients without compatible platelet donors are autologous cryopreserved platelets (e.g., collected in remission prior to allogeneic HCT), IS (e.g., rituximab), and high-dose IVIg and plasmapheresis.

### 24.6 Immunohematological Consequences of ABO-Mismatched Transplantation

About 40–50% of allo-HCT are ABO-mismatched. While transplantation across the ABO barrier is possible, immunohematological problems have to be taken into account. There is

a risk that ABO incompatibility between donor and recipient causes hemolytic transfusion reactions. In case of major ABO mismatch and a recipient anti-donor isoagglutinin titer  $\geq 32$ , the red cell contamination in PBSC graft should be kept  $< 20$  mL, and RBC depletion of BM grafts must be performed. If recipient anti-donor isoagglutinin titers are low ( $\leq 16$ ), no manipulation of the PBSC graft is required and RBC depletion of a BM graft might be considered in this situation but is not mandatory. In case of minor ABO incompatibility and a high donor anti-recipient isoagglutinin titer ( $\geq 1:256$ ), plasma depletion of both PBSC and BM grafts should be performed. If the donor anti-recipient isoagglutinin titer is low ( $\leq 128$ ), no manipulation of the PBSC graft is required and plasma depletion of a BM graft might be considered but is not mandatory. In case of bidirectional ABO incompatibility and high titers of anti-recipient isoagglutinins, both RBC and plasma depletion are required.

Delayed hemolysis can occur in minor ABO-mismatched HCT, in particular after RIC, due to hemolysis of remaining recipient red cells by isoagglutinins produced by donor B lymphocytes.

Major or bidirectional ABO-incompatible HCT can cause pure red cell aplasia (PRCA), delayed engraftment, and increased RBC transfu-

sion requirement (Griffith et al. 2019). The risk is higher if a group O recipient with high-titer anti-A isoagglutinins receives a group A graft. If no spontaneous remission of PRCA occurs and anti-donor isoagglutinins persist, various treatments to remove isoagglutinins, to reduce their production, or to stimulate erythropoiesis can be used (see review Worel 2016; Marco-Ayala et al. 2021).

### 24.7 Transfusion in ABO- or RhD-Incompatible HCT

The change of blood group and the persistence of recipient isoagglutinins require a special approach for transfusion support in ABO-incompatible HCT considering several aspects: isoagglutinins might target engrafting progenitors and transfused platelets to which variable amounts of ABO antigens can be bound. ABO blood group antigens are expressed in many non-hematopoietic tissues which continue to express the recipients' ABO antigens also after engraftment. ABO antigens can be secreted into body fluids. If possible, exposure of HSC recipients to isoagglutinins should be avoided. RBCs which

are ABO compatible with both HSC donor and recipient are mandatory. Plasma and PCs which are compatible with both the donor and the recipient should be preferred. Table 24.1 summarizes the recommendation for ABO preference of transfusions in ABO-incompatible HCT.

For PCs, some choices of blood groups might not always be available. To reduce the risk of adverse events due to isoagglutinins, apheresis PC donors with high-titer ABO antibodies should be excluded. However, a preferred strategy is the use of plasma-reduced PC (both for apheresis PC and pooled PC from whole blood donations). These are suspended in platelet additive solution with only about 30% plasma volume remaining.

HSC recipients should receive RhD-negative RBC and also RhD-negative PC except when both HSC donor and recipient are RhD-positive. If the HSC donor is RhD-positive and the recipient is RhD-negative, platelet transfusion can be switched to RhD-positive products after erythroid engraftment, i.e., appearance of RhD-positive red cells.

Whenever possible, RBC should be compatible both with HCT donor and recipient for CcEe antigens. If Rh antigens of HCT donor and recipient differ in a way that compatibility with both is

**Table 24.1** RBC, platelet, and plasma transfusion support for patients undergoing ABO-incompatible HCT

			Phase I <sup>a</sup>	Phase II and phase III <sup>a</sup>				
ABO incompatibility	Recipient	Donor	All products	RBC	Platelets		Plasma	
				Choice <sup>b</sup>	First choice	Second choice <sup>b</sup>	First choice	Second choice
Major	O	A	Recipient	O	A	AB, B, O	A	AB
	O	B	Recipient	O	B	AB, A, O	B	AB
	O	AB	Recipient	O	AB	A, B, O	AB	–
	A	AB	Recipient	A, O	AB	A, B, O	AB	–
	B	AB	Recipient	B, O	AB	B, A, O	AB	–
Minor	A	O	Recipient	O	A <sup>c</sup>	AB, B, O	A	AB
	B	O	Recipient	O	B <sup>c</sup>	AB, A, O	B	AB
	AB	O	Recipient	O	AB <sup>c</sup>	A, B, O	AB	–
	AB	A	Recipient	A, O	AB <sup>c</sup>	A, B, O	AB	–
	AB	B	Recipient	B, O	AB <sup>c</sup>	B, A, O	AB	–
Bidirectional	A	B	Recipient	O	AB	B, A, O	AB	–
	B	A	Recipient	O	AB	A, B, O	AB	–

– Not applicable

<sup>a</sup>Phase I until preparative regimen, phase II until complete engraftment, phase III after complete engraftment

<sup>b</sup>Choices are listed in the order of preference

<sup>c</sup>For practical reasons, the use of donor type platelets might be defined as first choice, in phase III, i.e., after complete engraftment



not possible (e.g., recipient CCD.ee, donor cCD.EE), then RBC compatible with the recipient shall be chosen in the period until engraftment. After the appearance of donor-derived red cells, RBC supply should switch to compatibility with the graft. Patients should receive K-negative RBC except when both recipient and donor are K positive.

## 24.8 Granulocyte Concentrates

In life-threatening non-viral infections during neutropenia, the use of irradiated granulocyte transfusions should be considered. Response and survival after granulocyte transfusion correlate strongly with hematopoietic recovery. Thus, granulocyte transfusions may mainly bridge the gap between specific treatment and neutrophil recovery (Nguyen et al. 2020). Granulocyte transfusions can help to control active fungal infections in a very high-risk population of patients who otherwise are denied by transplant program. A retrospective study suggested that granulocyte transfusion might maintain the mucosal integrity and thus reduces bacterial translocation and triggers for GvHD. In the randomized RING trial, success rates for granulocyte and control arms did not differ within any infection type. The overall success rates for the control and granulocyte transfusion group were 41% and 49% (n.s.) (Price et al. 2015). However, patients who received high dose ( $\geq 0.6 \times 10^9$  granulocytes/kg per transfusion) fared better than patients who received lower doses. The collection center should ensure to provide a high-dose concentrate by appropriate donor selection, pre-collection stimulation, and apheresis techniques. The optimal number of granulocyte transfusions is unclear. Adverse events of granulocyte infusions are fever, chills, pulmonary reactions, and alloimmunization. Studies demonstrated that overall risk of alloimmunizations was low and there was no effect of alloimmunization on the primary outcome (survival, microbial response), the occurrence of transfusion

reactions, or post transfusion neutrophil increments. Alloimmunization remains a problem because of its negative impact on increments after platelet transfusion and potential increase of graft failure after HCT. Donor-specific HLA-Ab might be implicated in early graft failure. If granulocyte transfusions are used prior to a planned unrelated HCT, recipients should be monitored for the development of HLA-Ab and the search algorithm for the UD should take into account donor-specific antibodies. Granulocyte concentrates must be irradiated and should be obtained from CMV-seronegative donors, ideally also confirmed by CMV-PCR to avoid donations in the serological window period.

### Key Points

- Patients undergoing HCT must be transfused with irradiated blood products (at least 2 weeks prior to stem cell collection in auto- and starting with the conditioning in allo-HCT).
- A restrictive RBC transfusion threshold of 7–8 g/dL hemoglobin is recommended for adult patients who are hemodynamically stable.
- RBC must be compatible with both the HSC donor and the recipients.
- Platelet concentrates should be transfused to non-bleeding, nonfebrile patients when platelet counts are  $\leq 10 \times 10^9/L$ .
- Prophylactic platelet transfusion remains the standard of care for thrombocytopenic patients undergoing allogeneic HCT.

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Annic Baumgartner and Philipp Schuetz

### 25.1 Introduction

Patients undergoing HCT, particularly allo-HCT, are at risk for malnutrition (Fuji et al. 2012). Malnutrition is associated with poor clinical outcome, decreased OS, higher risk of infectious and immunologic complications, delayed neutrophil engraftment and prolonged hospital stay (Baumgartner et al. 2016, 2017). Importantly, most patients are well-nourished or even overweight upon admission to HCT but experience rapid deterioration of nutritional status during treatment (Fuji et al. 2014). Weight loss results from a complex interplay of toxic, inflammatory and immunological mechanisms leading to caloric deficits by anorexia as well as a catabolism of the metabolism.

Nutritional support is meant to reduce caloric deficit and reduce the risks for negative metabolic effects. However, there is a lack of large-scale trials proving benefit of nutritional interventions in this setting (Baumgartner et al. 2017). The cur-

rent nutritional approach is thus based on physiological considerations and results of observational and some smaller interventional trials and needs to be adapted to an individual patient's situation.

### 25.2 Screening for Malnutrition

Pre-existing malnutrition is an important additional risk factor in patients undergoing HCT. International guidelines such as the European Society of Enteral and Parenteral Nutrition (ESPEN) recommend screening for malnutrition at admission for transplantation (Bozzetti et al. 2009). There is no international consensus on how to assess malnutrition in this patient population. For reasons of practicability, the use of the NRS 2002 is generally recommended (Bozzetti et al. 2009). In the acute setting, weight assessment may be inaccurate because of inflammatory fluid retention.

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### 25.3 Nutritional Recommendations (See General Recommendations in Table 25.1 and Fig. 25.1; Monitoring in Table 25.2 and Nutritional Strategies in Fig. 25.1)

#### 25.3.1 Nutrition in Allo-HCT

##### 25.3.1.1 Route of Administration

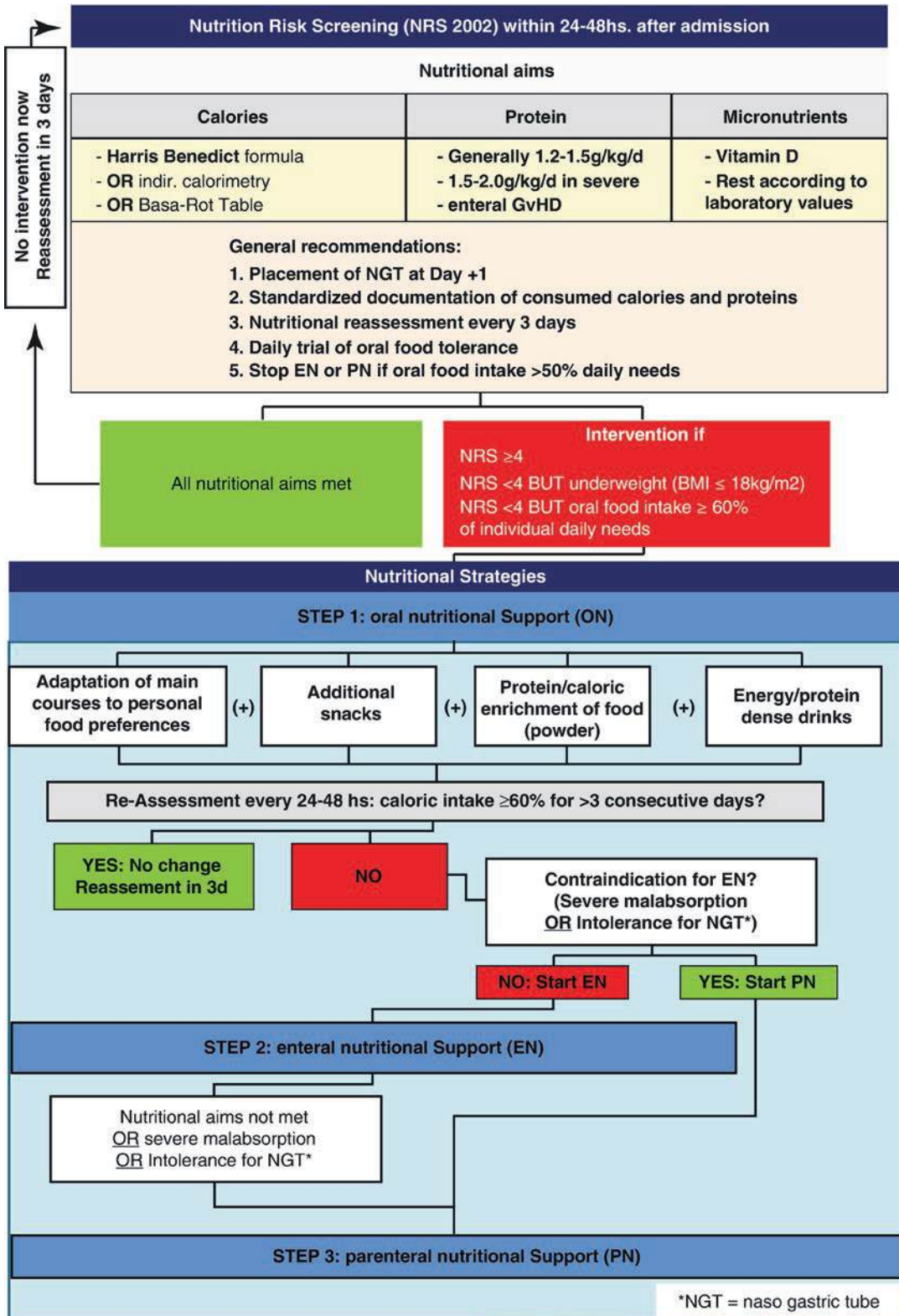
Due to its positive effects on GI integrity and microbiome, enteral nutritional (EN) support is generally preferred over parenteral nutrition (PN) in case of a functioning GI tract.

During allo-HCT, patients often experience GI failure so PN is used instead. Yet, higher risk of central line infections as well as hyperglycaemia associated with PN demand restricted use (Seguy et al. 2012).

Small, prospective, nonrandomized trials on EN found satisfying results on feasibility and safety with lower infection rates as well as beneficial effects such as earlier neutrophil engraftment and lower rates of severe GI GvHD (Seguy et al. 2012; Guièze et al. 2014). Some studies even report higher OS (Seguy et al. 2012). Results of a large prospective trial are expected (Lemal et al. 2015).

**Table 25.1** Summary of general recommendations for nutritional support

Screening for malnutrition	
Indication	All patients to estimate risk for pre-existing malnutrition
Tools	NRS 2002
Nutritional support	
General management	<ol style="list-style-type: none"> <li>1. Early involvement of dietitians</li> <li>2. Consider placement of nasogastric tube on day +1</li> <li>3. Standardized monitoring of nutritional intake</li> <li>4. Nutritional reassessment every 3 days using the NRS 2002</li> </ol>
Indication of intervention	<ol style="list-style-type: none"> <li>1. Oral intake &lt;60% for 3 days consecutively</li> <li>2. Consider nutritional support in all patients with preexisting malnutrition and/or BMI &lt;18</li> </ol>
Discontinuation	Oral intake >50% for 3 days consecutively
Estimation of caloric needs	According to Harris–Benedict formula (ideal body weight) OR BASA-ROT table (25–30 kcal/kg ideal body weight)
Route of nutritional support	<ol style="list-style-type: none"> <li>1. Intensification of oral nutrition</li> <li>2. Enteral nutrition</li> <li>3. Parenteral nutrition</li> </ol>
Forms of nutritional support	
Intensified oral nutrition	<i>Indication:</i> Malnutrition or underweight (BMI <18 kg/m <sup>2</sup> ) and preserved oral intake <i>Options:</i> Additional snacks rich in proteins and energy, protein or calorie enrichment of main courses, additional protein and energy drinks (ONS) <i>Standardized supplementation:</i> None
Enteral nutrition	<i>Indication:</i> If nutritional goals cannot be reached by oral support alone <i>Standardized supplementation:</i> Vitamin K once weekly
Parenteral nutrition	<i>Indication:</i> If nutritional goals cannot be reached in patients with gastrointestinal failure and/or intolerance for NGT <i>Standardized supplementation:</i> Lipid-soluble vitamins (ADEK) Water-soluble vitamins Trace elements
Vitamin and trace elements	Multivitamin generally recommended Vitamin D: Supplementation recommended (bolus of 40,000E at admission, maintenance therapy with 1500E orally per day) Other vitamins or trace elements if overt deficiency
Immunonutrition	Generally not recommended



**Fig. 25.1** Algorithm for guided nutritional support



**Table 25.2** Monitoring of nutritional parameters

Parameter	Frequency of assessment	Significance and implications
Anthropometry		
Weight	Daily	Correlation with fluid balance Evaluation of diuretics and albumin supplementation
Bioimpedance assessment	Individually	Uncontrolled, unexplained weight loss Severe, prolonged inflammation
Nutritional assessment		
Oral food consumption	3× daily	Evaluation of nutritional support
Laboratory parameter		
Albumin	Weekly	Evaluation of supplementation in anasarca
Sodium, potassium	Daily	Adaptation of potassium supplementation
Calcium, magnesium, phosphate	Twice weekly	Adaptation of supplementation CAVEAT refeeding, gastrointestinal loss
INR, quick	Twice weekly	Evaluation of supplementation CAVEAT low content in certain products for EN/PN
Glucose	3–6× daily if PN or preexisting diabetes mellitus otherwise twice weekly	Adaptation of insulin dose
Creatinine	Daily	Correction of fluid balance CAVEAT toxic damage
Liver function tests	Twice weekly	Evaluation of toxic damage, infection, hepatic GvHD, VOD, or relapse
Triglycerides	Twice weekly if PN	Adaptation of PN
Vitamin D	At admission	Begin routine supplementation
Vitamin B12	At admission	Supplementation pretransplantational individually

We encourage the use of EN as a first-line measure. Indication for PN should be limited to intolerance for nasogastric tube and GI failure including severe malabsorption or limited gastro-intestinal passage.

### 25.3.1.2 Indications and Timing

There are few study data regarding optimal timing of nutrition. The ESPEN guidelines recommend implementation of nutritional support if oral caloric intake falls below 60–70% of basic requirements for 3 days consecutively (Bozzetti et al. 2009).

Discontinuation of EN or PN should be considered, if >50% of daily requirements are met by oral intake (Bozzetti et al. 2009). To enhance early return to oral food intake patients should be encouraged to maintain minimal oral intake throughout therapy.

### 25.3.1.3 Estimation of Caloric Needs

Most studies investigating energy expenditure by indirect calorimetry have been performed in small paediatric populations. Validity of the data for adults therefore is limited, and results are controversial (Sharma et al. 2012; Duro et al. 2008).

Determination of energy requirements based on calculations, e.g., by the BASA-ROT table or Harris–Benedict Formula, does not differ significantly from results by indirect calorimetry (Sharma et al. 2012; Valentini 2012; Harris and Benedict 1918). Therefore, we recommend estimation of energy requirements according to an adjusted Harris–Benedict formula.

## 25.3.2 Nutrition in Auto-HCT

In general, effects of auto-HCT on nutritional status are less pronounced. Nutritional support is not generally recommended and has to be evaluated individually in patients experiencing severe complications or in patients with pre-existing malnutrition.

### 25.3.3 Nutrition in Acute Gastrointestinal GvHD

GvHD of the digestive tract leads to excessive diarrhoea, abdominal pain, nausea, vomiting, gastrointestinal bleeding, dysphagia and malabsorption. Patients experience malnutrition to a higher extent and show significantly more additional complications (van der Meij et al. 2013).

Caloric demands are mainly driven by energy loss through diarrhoea. Enteral solutions should be low in fibre and fat and not contain lactose. Maintaining a minimal amount of oral or enteral nutrition facilitates early dietary recovery (Imataki et al. 2006; Andermann et al. 2016). Complete bowel rest and total PN are indicated in severe GvHD grade IV and stool volume >1500 mL in 24 h (Bozzetti et al. 2009; Imataki et al. 2006).

Protein requirements are elevated. Recommendations range from 1.2 to 2.5 g/kg/day. We recommend aiming for 1.5–2 g/kg/day in the absence of severe renal impairment (Bozzetti et al. 2009; Muscaritoli et al. 2002).

Vitamin and trace elements are often deficient and need to be measured regularly to evaluate need of supplementation.

### 25.3.4 Low Bacterial Diet/Low Microbial Diet/Neutropenic Diet

A low microbial diet has been installed in the 1980s to prevent potential threat of food-borne infections from organisms colonizing the gastrointestinal tract.

There is no standardized protocol, and variations amongst centres, contradictions even, are high. Yet, there is no proof of efficacy in preventing infections or death.

In line with most current publications, we recommend safe food handling and strict hand

hygiene as proposed by the FDA or the EC over a neutropenic diet.

## 25.4 Immunonutrition

A meta-analysis on glutamine found reduced severity and duration of mucositis and GvHD (Kota and Chamberlain 2017). To date, no randomized controlled trial showed a benefit on overall survival or reduction of infection rates (Crowther et al. 2009).

Pre- and probiotics may enhance diversity of the GI microbiome. So far, no study has evaluated their effects compared to placebo. Again, there might be a benefit on severity of GvHD (Ladas et al. 2016). Safety has been evaluated in a pilot study in children and adolescents and proved satisfying.

There are no randomized controlled trials assessing the benefits of omega-3 fatty acids or trace elements. Except for vitamin D, there is no proven benefit of a routine supplementation (Hall and Juckett 2013). Based on this data, we do not recommend routine use of immunonutrients.

## 25.5 Long-Term Follow-Up

Follow-up should include regular nutritional screening and documentation of weight, BMI, appetite, and functional status based on patients' history. A balanced, Mediterranean diet can be recommended along with regular physical training to regain muscle mass. An increase in weight should be addressed early to avoid full development of a metabolic syndrome because of high baseline cardiovascular risk in transplanted patients.

Persisting malnutrition, especially in chronic GvHD, should be handled by an interdisciplinary team. Caloric needs seem to be elevated and often require in- and out-hospital nutritional support.

### Key Points

- There is high risk for malnutrition upon HCT treatment
- Malnutrition is an independent risk factor in these patients
- The potential benefit of all nutritional interventions remains largely unproven
- All dietary recommendations are based on physiological considerations and results of mainly observational trials
- Adherence to a systematic approach to nutritional support improves transparency, comparability, and generally reduces use of unnecessary PN
- Oral and enteral nutritional support is recommended over parenteral support in case of functioning gastrointestinal tract
- A minimal oral or enteral food intake is beneficial for recovery of mucosa and microbiome
- Immunonutrients did not show significant beneficial effects and therefore are not recommended for routine use
- Neutropenic diets did not show a benefit over safe food handling approaches

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### 26.1 Introduction

A potentially life-threatening complication of allogeneic hematopoietic cell transplantation (allo-HCT) is graft-versus-host disease (GVHD), which occurs when T-cells from the recipient recognize host antigens on healthy tissues. Despite 50 years of history and over 750,000 procedures performed worldwide, GVHD remains a challenging issue that physicians are facing on a daily basis.

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Overall, 30–50% of patients undergoing allo-HCT will develop acute GVHD, and around 10% will have severe acute GVHD (grades III–IV). The main risk factor for developing chronic GVHD is the previous development of the acute form of the disease.

The pathophysiology, diagnosis, and management of both acute and chronic GVHD will be covered by other chapters in this Handbook (Chaps. 43 and 44). This chapter will summarize the use of immunosuppression (IS) to prevent the development of acute GVHD because attempts to prevent chronic GVHD basically rely on the ability to prevent the acute disease. Readers with interest in a more detailed overview of the biological process, prevention, and therapy of acute GVHD can refer to two excellent recent reviews (Zeiser and Blazar 2018; Hill et al. 2021).

### 26.2 GVHD Prophylaxis After MAC: The “Gold” Standard Combination of CNI and MTX

In the mid-1980s, Storb and colleagues found that the combination of cyclosporin-A and methotrexate (CSA/MTX) (Table 26.1) was superior to CSA alone in a series of randomized phase 3 trials (Storb et al. 1986). This gold standard regimen remains the most widely used prophylaxis regimen in Europe today, especially after MAC.

In the late 1990s, another CNI-based prophylactic regimen using tacrolimus (TAC) in

**Table 26.1** CSA/MTX for GVHD prophylaxis

	Cyclosporine	Methotrexate
Dosage	3 mg/kg/day IV till engraftment then orally	15 mg/m <sup>2</sup> day +1 10 mg/m <sup>2</sup> day +3, +6, +11
Adjusting dose	Target dose to reach a plasma level of 150–200 ng/mL; adjust to renal function	Day +11 dose may be omitted if grade III/IV mucositis
Interaction	Numerous; ++ with azoles	
Adverse effects	Numerous Renal insufficiency, CNS, and endothelial toxicities	Mucositis

conjunction with MTX was developed and two randomized phase 3 trials were published after MAC in HLA-identical sibling donors and matched/mismatched unrelated donors (Ratanatharathorn et al. 1998; Nash et al. 2000). Although both reported a significant decrease in the incidence of grade II–IV acute GVHD, none could demonstrate an improved survival with TAC/MTX compared to CSA/MTX. The reasons for this lack of improvement may be that (1) in the trial performed from HLA-identical sibling, there was an imbalance of disease risk among the two groups with higher risk patients with leukemia among patients receiving TAC/MTX, and (2) for the trial in URD, the HLA-typing methodology at that time was serologically based and thus included a very high proportion of patients with almost certainly a high degree of mismatching. Nevertheless, the TAC/MTX regimen is currently considered as the American gold standard, whereas it never reached broad popularity in Europe. More recently, a BMT CTN phase 3 trial aimed at comparing the standard prophylaxis TAC/MTX with CNI-free prophylaxis based on CD34+ selection or PTCy alone after bone marrow allo-HCT with MUD (Luznik et al. 2022). While CNI-free prophylaxis yielded lower rates of moderate/severe chronic GVHD, this did not improve OS or TRM.

CSA and TAC inhibit GVHD by preventing the activation of the nuclear factor of activated T-cell (NFAT) family of transcription factors, thereby reducing the transcription of interleukin-

2 and the activation of effector T-cells, albeit with a concurrent reduction in levels of interleukin-2-dependent anti-inflammatory Tregs.

### 26.3 GVHD Prophylaxis After RIC: Is CNI Plus MMF Standard?

From the early development of the RIC, two regimens have been used: CSA (or TAC) alone or in combination with MMF (reviewed in Zeiser and Blazar 2018). Surprisingly, the combination of CSA/MMF—while largely used worldwide—has never been tested in a large randomized clinical trial. In 2014, a meta-analysis of 33 studies representing 3440 patients failed to demonstrate benefits of the combination of CNI and MMF on acute GVHD incidence (Ziakas et al. 2014). In a retrospective study from CIBMTR of 1564 patients who underwent MRD or MUD/MMUD allo-HCT after RIC, CNI and MMF were associated with a higher risk of acute GVHD in unrelated donor transplants and did not improve survival (Hamilton et al. 2019). CNIs in this setting are usually used at the same dose (and share the same toxicity profile) as after MAC. MMF's toxicity mainly consists of hematological toxicity. Attention must be paid to the use of ganciclovir or valganciclovir (for CMV reactivation) in addition to MMF because of the risk of severe pancytopenia. MMF is usually delivered at the dose of 30 mg/kg/day split into two or three doses.

### 26.4 New Immunosuppressive Regimens for GVHD Prophylaxis

With the current prophylactic treatment strategies summarized above, the rate of grades II–IV acute GVHD remains of concern in the range of 20–30% (Shouval et al. 2019). As reviewed elsewhere in the Handbook, the treatment of acute and of chronic GVHD with high-dose steroids remains unsatisfactory with 30–50% of patients being steroid resistant or dependent. There is thus still an unmet clinical need in GVHD prophylaxis.



After years of no new agent in this setting, improved knowledge of basic T-cell immunology and improved knowledge of the pathophysiology of the disease, some new agents have been tested, mostly in phase 2 trials. This section summarizes the drugs with the most advanced development that reported an acute GVHD incidence in the 20% range (i.e., a range that may warrant development of subsequent phase 3 trials).

- *Sirolimus (SIR)*, an mTOR inhibitor, is a more potent suppressor of the expansion of conventional T-cells than Tregs, owing to the greater dependence of conventional T-cells on the mTOR-protein kinase B pathway. This was the basis of the development by the Dana-Farber Cancer Institute (DFCI) group of a regimen that leads to a cumulative incidence of grades II–IV acute GVHD of 20% and less than 5% of grades III–IV acute GVHD. This prompted a large randomized trial of the BMTCTN comparing TAC/SIR to TAC/MTX. There was no difference in the risk of grade II–IV acute GVHD-free survival (67% vs. 62%,  $P = 0.38$ ), and the risk of grade II–IV GVHD was similar (26% vs. 34%,  $P = 0.48$ ) (Cutler et al. 2014). A smaller randomized single-center phase 2 study found however a reduced risk of grade II–IV acute GVHD (43% after TAC/SIR vs. an unexpectedly high rate of 89% after TAC/MTX) (Pidala et al. 2012). In the setting of nonmyeloablative allo-HCT with matched unrelated donors, the addition of SIR to CSA + MMF reduced the risk of grade II–IV acute GVHD and translated into an improved survival in a randomized phase 3 trial (Sandmaier et al. 2019). Likewise, the addition of SIR to CSA + MMF reduced the risk of acute GVHD and resulted in an improved survival—when compared to a historical cohort—in a trial in patients receiving nonmyeloablative allo-HCT with mismatched unrelated donors (Kornblit et al. 2020).
- *Bortezomib (BOR)*, in combination with TAC/MTX, yielded encouraging rates of acute GVHD after mismatched RIC allo-HCT in a phase 1/2 study (Koreth et al. 2012). This combination was tested together with a combination of TAC/MTX/maraviroc and TAC/MMF/PTCy, respectively, against the reference combination of TAC/MTX in a randomized phase 2 trial (the BMTCTN 1203 trial) after RIC. The trial revealed that the combination of TAC/MMF/PTCy was the most promising regimen in comparison to TAC/MTX (Bolaños-Meade et al. 2019). This trial prompted the design of the randomized BMTCTN 1703 trial that directly compared TAC/MTX to TAC/MMF/PTCy (see *infra*) (Holtan et al. 2022). Finally, in an open-label three-arm phase 2 randomized controlled trial, investigators at the DFCI compared conventional TAC/MTX (A) vs. BOR/TAC/MTX (B) and vs. BOR/SIR/TAC (C) in 138 URD RIC allo-HCT recipients. Grade II–IV acute GVHD rates were similar (A: 33%, B: 31%, C: 21%) as was the 2-year NRM. Overall, the BOR-based regimens did not seem to improve outcomes compared with TAC/MTX therapy (Koreth et al. 2018).
- *Vorinostat*, a histone deacetylase inhibitor, has anti-inflammatory and immunoregulatory effects. Pavan Reddy's group in Michigan provided compelling evidence from preclinical models that vorinostat reduced the risk of GVHD through suppressed proinflammatory cytokines, regulated APCs, and enhanced Treg functions. In two separate clinical trials (Choi et al. 2014, 2017), authors translated their findings in the clinical setting. In one trial where vorinostat was added to standard prophylaxis after RIC in HLA-identical siblings, acute GVHD grade II–IV rate was 22% and that of grades III–IV is 6%. In another trial after MAC in URD, the acute GVHD rates were similar.
- *Tocilizumab*, a humanized anti-IL-6 receptor monoclonal antibody, added to CNI/MTX standard prophylaxis has been tested by two different groups (Kennedy et al. 2014; Drobyski et al. 2018). IL-6 levels are increased early during GVHD and are present in all target tissues. Blockade of the IL-6 signaling pathway has been shown to reduce the severity of GVHD and to prolong survival in experimental models. Investigators in Milwaukee

and in Brisbane conducted two separate phase 2 trials using tocilizumab, and both found very low rates of grade II–IV acute GVHD (less than 15%). However, a phase 3 randomized double-blind trial using Tocilizumab showed nonsignificant reduced incidence of grade 2–4 aGVHD in recipients from HLA-matched VUDs and no improvements in long-term survival (Kennedy et al. 2021).

- *Vedolizumab*, an anti- $\alpha 4\beta 7$  monoclonal antibody that inhibits the migration of lymphocytes across the gut endothelium, was evaluated in a phase 1b trial for the prevention of gut GVHD in combination with the standard prophylaxis TAC/MTX and leads to very promising results regarding the incidence of acute gut GVHD (19% of patients in the 300 mg dose cohort) (Chen et al. 2019). More recently, a phase 3 randomized and placebo-controlled trial confirmed the benefits of adding vedolizumab to standard prophylaxis: The improvement of acute GI GVHD-free survival by 180 days following HCT was 85.5% (95% CI, 79.2–90.0%) vs 70.9% (95% CI, 61.6–77.2%) for those assigned to placebo (HR, 0.45; 95% CI, 0.27–0.73;  $P < 0.001$ ) (presented orally in the plenary session of the 2023 EBMT meeting).
- *Abatacept*: A phase 2 trial assessed safety, efficacy, and immunologic effects of adding T-cell costimulation blockade with abatacept to CNI/MTX-based GVHD prophylaxis. The primary end point was day +100 grade 3–4 AGVHD, with day +180 severe-AGVHD-free survival (SGFS) a key secondary end point. Adding abatacept to URD HCT was safe, reduced AGVHD, and improved SGFS and was approved by the FDA. This promising drug warrants further evaluation in phase 3 randomized trial (Watkins et al. 2021).

## 26.5 ATG

Rabbit anti-thymoglobulin (ATG) and anti-T lymphoglobulin (ATLG) are polyclonal sera obtained immunizing rabbits against either human thymocytes or Jurkat cell line, respec-

tively. The mechanism of action is only partially known and includes T- and B-cell depletion, inhibition of migration of inflammatory cells and dendritic cells, sparing Treg compartment. ATG and ATLG contain different antibody specificities, are produced from different pulsed antigens and different manufacturing processes, and no pharmacological comparison of the doses can be done, since no head-to-head comparison on the choice of the brand is left to center policy. Several randomized studies comparing weight-based dosed ATG/ATLG with standard CNI and anti-metabolites strategy demonstrated the efficacy about GVHD prevention in both unrelated (Bacigalupo et al. 2001; Walker et al. 2016; Finke et al. 2017; Soiffer et al. 2017) and related transplants (Kröger et al. 2016; Chang et al. 2020; Cho et al. 2021) (Table 26.2), although no survival advantage was demonstrated (which may be due to offsetting GvHD-related mortality for infection or relapse related mortality), even with long-term follow-up (Bacigalupo et al. 2001; Walker et al. 2016; Finke et al. 2017; Bonifazi et al. 2019). GRFS and quality of life were significantly better in ATG/ATLG-treated patients (Bonifazi et al. 2019). There is no definitive agreement about the doses in all the transplant settings (Bonifazi et al. 2020). Recently, a population pharmacokinetic model showed that ATG exposure after transplant was highly variable, due to highly variable clearance. It was found that weight (<40 kg) and ALC prior to dosing were the only predictors for clearance, suggesting that ALC-based dosing could improve outcomes. This was subsequently reinforced in a phase 2 prospective clinical trial (Admiraal et al. 2022), where a simple weight and ALC-based dosing nomogram was used. Improved outcome was mainly due to better immune reconstitution resulting in a fivefold lower non-relapse mortality in those with immune reconstitution ( $CD4+ > 50/\mu L$  at 2 consecutive time points <100 days), while GvHD rates were similar. Also, in the RCT using ATLG by Soiffer et al. the post hoc analyses suggested that ALC prior to dosing impacts survival; those with low ALC (mainly TBI pt) and ATLG had lower survival probability, while those with higher ALC (mainly chemo pt) had

**Table 26.2** Randomized trials using ATG for GVHD prophylaxis

	Walker	Finke	Soiffer	Kroger	Cho	Chang
Settings	URD/MSD PBSC (prevalent) MAC/RIC	URD 100% PBSC (prevalent) MAC	URD PBSC (prevalent) MAC	MSD PBSC MAC	MSD PBSC MAC (prevalent)	MSD PBSC (prevalent) MAC
Doses of ATG	T—4.5 mg/kg	G—60 mg/kg	G—60 mg/kg	G—30 mg/kg	T—2.5 mg/kg	T—4.5 mg/kg
Primary end point	IS withdrawal at 12 months	Treatment failure: aGvHD 3–4 and/or death <100 days	Mod-severe cGvHD free survival	2 years cGVHD	2 years cGVHD	aGVHD 2–4
Years of enrollment	2010–2013	2003–2007	2011–2015	205–2014	2017–2019	2013–2018
aGvHD 3–4	Lower for ATG	Lower for ATG	Lower for ATLG (2–4)	Not different	Not different	Lower for ATG (2–4)
cGvHD	Lower for ATG	Lower for ATG	Lower for ATLG	Lower for ATLG	Lower for ATG	Lower for ATG
Extensive cGvHD	Lower for ATLG	Lower for ATLG	Lower for ATLG	Lower for ATLG	Lower for ATG	Lower for ATG
NRM	Not different	Not different	Not different	Not different	Not different	Not different
Relapse	Not different	Not different	Not different	Not different	Not different	Not different
OS	Higher for ATG (long-term)	Not different	Lower for ATLG	Not different	Not different	Not different

better survival when they received ATLG (Soiffer et al. 2017). Although relapse is a major concern, the majority of the randomized studies failed to confirm these data. RCT however do not fully cover the area of conditions with higher risk of relapse (advanced phases and RIC) where the use and the doses of ATG/ATLG should be evaluated in the context of a risk/benefit evaluation. There is an overall tendency to use lower doses in the real-world experience and in clinical trials too. However, randomized controlled trials using individualized ATG (or ATLG) dosing may be the more individualized answer.

## 26.6 Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody directed against the 21–28 kD cell surface glycoprotein, CD52. It is marketed for multiple sclerosis and for B-cell chronic lymphocytic leukemia (B-CLL). Alemtuzumab induces a rapid but long-lasting depletion of B- and T-cells in the peripheral blood and secondary lymphoid organs.

Although no randomized study has been run to establish the efficacy of alemtuzumab as GVHD prevention, retrospective analyses and prospective non-controlled studies showed Alemtuzumab able to significantly reduce both acute and chronic GVHD both in nonmalignant and in malignant diseases, especially for transplant in SAA (Kanda et al. 2011; Locatelli et al. 2017; Marsh et al. 2019); however due to unpredictable, mostly too high, exposure, immune reconstitution is significantly delayed (compared to ATG) (Willemsen et al. 2015). Consequently, infections and relapse are important limitations using Alemtuzumab.

## 26.7 Naïve T-Cell Depletion

In a large phase 2 trial, 138 patients with acute leukemia received T<sub>N</sub>-depleted PBSC from HLA-matched related or unrelated donors. GVHD prophylaxis was with TAC +/-MTX or MMF. Subjects received CD34-selected PBSC and a defined dose of memory T-cells depleted of

$T_N$ . Depletion of  $T_N$  from PBSC allografts results in very low incidences of severe acute and any cGVHD, without apparent excess risks of relapse or nonrelapse mortality (Bleakley et al. 2022).

## 26.8 PTCy

Nowadays, the non-T-cell-depleted haploidentical transplant represents a feasible treatment option for patients lacking matched donor. The classical Baltimore's PTCY prophylaxis includes CY 50 mg/kg on days +3 and +4 followed by CNI/MMF given from day +5 has led to increasing numbers of haploidentical transplant in the recent years. Another scheme used in some centers includes PTCY given on days +3 and +5 with early introduction of CsA on day -1 or 0 followed by MMF from day +1. According to a retrospective study, this last regimen was more frequently used with MAC and with BM as the source of stem cells and revealed a lower incidence of grade II–IV aGVHD; however, when both regimens mentioned above were compared, there were no differences in the cumulative incidence of cGVHD and extensive GVHD (Ruggeri et al. 2020).

The CY does target and deplete proliferating alloreactive T-cell while preserving Tregs. The use of high doses of PTCY can be associated with hemorrhagic cystitis and cardiac injury that can lead to congestive heart failure.

PTCY has been increasingly used in the setting of URD and MSD and was assumed to lead to decreased rates of GVHD, especially in cGVHD. Recently, these results were supported by two randomized phase 3 studies from the BMT CTN. In the first one (BMT CTN 1301), PTCY was administered as a single agent after a MAC regimen, using BM as a stem cell source. Similar rates of severe cGVHD and OS were observed after PTCy when compared to TAC + MTX. However, PTCY alone was associated with higher aGVHD, and a reduced relapse rate and RFS (Bolaños-Meade et al. 2019). The BMT CTN 1703 randomized phase 3 trial com-

pared PTCY + MMF + TAC to TAC + MTX after in allogeneic HCT using PBSC and RIC conditioning regimen. The results showed a significant reduction in acute and chronic GVHD with PTCY + TAC + MMF without increased risk of relapse or death. There was no difference neither in the relapse/progression rate at 1 year or in the 1-year OS rate. These results are promising and may lead to the development of a new standard of care (Holtan et al. 2022).

PTCY has also been associated with ATG as GVHD prophylaxis in the haploidentical transplantation setting. A retrospective study of patients with AML who underwent haploidentical transplantation and received PBSC and PTCY alone vs ATG + PTCY (associated with MMF + CsA in both groups) as GVHD prophylaxis showed a lower 2-year incidence of cGVHD of all grades in the ATG + PTCY group, with no statistical difference in the cumulative incidence of aGVHD nor extensive cGVHD (Battipaglia et al. 2022).

Currently, there is no consensus on the best conditioning regimen and/or its intensity in the context of haploidentical HCT. Similarly, it is currently unknown if other combinations like sirolimus (SIR) + MMF can be substituted to CNI/MMF in addition to PT-CY in the haploidentical setting.

## 26.9 Conclusion and Perspective

Despite decades of experience with allo-HCT and several trials of prophylactic regimens, GVHD remains a common complication of allo-HCT. When acute GVHD develops, the main treatment is high-dose steroids. However, around one-third of the patients will be steroid-resistant/dependent. Steroid resistance remains associated with a dismal prognosis (30–40% 1-year survival). These data urge for developing new strategies to prevent GVHD. Fortunately, based on preclinical findings and improved knowledge of the immune biology of HCT, recent drug combination opens the gate for future improvements.

### Key Points

- Standard GVHD prophylaxis relies on CNI + short-term MTX after MAC and of CSA ± MMF after RIC.
- ATG significantly reduce rate and severity of acute GVHD in most randomized clinical trials.
- PTCy (+CNI/MMF) is currently the standard GVHD regimen after haplo-identical HCT, although this regimen has never been formally tested in randomized trial. In non-haploidentical transplant, phase 3 randomized clinical trials demonstrated the superiority of this regimen (as compared to CNI/MTX) after RIC, while PTCy alone failed to demonstrate superiority after MAC.
- Randomized clinical trials testing new prophylactic regimens are still deeply warranted since GVHD remains a common, and potentially life-threatening, complication after allo-HCT.

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# Infection Control and Isolation Procedures

# 27

Malgorzata Mikulska

## 27.1 Introduction

*Infection prevention and control practices (IPC)* are defined as a set of measures aimed at preventing or stopping the spread of infections in healthcare settings. All HCT recipients should follow general guidelines (e.g. provided by CDC) for preventing healthcare-associated infections through hand hygiene, disinfection and sterilisation, environmental infection control, isolation precautions and prevention of intravascular catheter-related infection [(Sehulster et al. 2004; Centers for Disease Control and Prevention 2002; Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007; Guidelines for Environmental Infection Control in Health-Care Facilities 2003), all available at <https://www.cdc.gov/infection-control/guidelines/>].

Dedicated and detailed international recommendations for HCT recipients on preventing infectious complications have been published in 2009 (Tomblin et al. 2009; Yokoe et al. 2009). As there were no well-executed randomised or controlled trials and little evidence from cohort or multiple time-series studies, most data come from descriptive studies, reports of expert com-

mittees and the opinions of respected authorities. Hence, recommendations on infection control could only be graded as level III.

*Isolation procedures* in HCT recipient comprise precautions universal for all healthcare settings (Standard Precautions and Transmission-Based Precautions) and those specific for HCT and employed to prevent transmission of spores of filamentous fungi, mainly *Aspergillus*, with unfiltered air.

There is no consensus on specific *protective environment*, also called reverse isolation, for neutropenic patients. HCT recipients should be placed in a single-patient room, with adequate ventilation system (see below), if possible. However, no clear benefit of routine footwear exchange or use of disposable gloves and gowns on the rate of infections has been demonstrated, and procedures vary significantly between institutions, with routine use of masks and disposable gloves and gowns in some but not others. On the contrary, the negative effect of strict protective isolation on patient's quality of life and well-being should be acknowledged and weighted against the evidence of benefits of single protective measures (Abad et al. 2010).

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## 27.2 Standard Precautions

**Should be used universally for all patients and they include**

1. Proper hand hygiene

2. Use of standard personal protective equipment (PPE)
3. Appropriate cleaning and disinfection protocols (including those for shared equipment or toys and play areas in paediatric units)
4. Safe injection practices
5. Infection control practices for special procedures (e.g. surgical masks for lumbar puncture)

### 27.2.1 Hand Hygiene

It is by far the most effective means of prevention of pathogen transmission (Freifeld et al. 2011; Tomblyn et al. 2009). The preferred method of hand decontamination is with an alcohol-based hand rub, due to its superior convenience and reduced drying of the skin. Handwashing with soap and water is recommended if hands are visibly soiled, for example, with blood or body fluids, and after potential contact with spores of *Clostridioides difficile* or with *Norovirus*. Of note, 15–30 s is the minimum necessary hand-washing time.

### 27.2.2 Other Standard Precautions

PPE used routinely by healthcare workers during patient care and procedures are gloves, gowns (used if direct contact with patient's fluids is expected) and mouth, nose and eye protection (used during procedures which are likely to generate splashes or sprays of blood, body fluids, secretions and excretions). Routine donning of gowns upon entrance into a high-risk unit, including HCT unit, is not indicated.

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## 27.3 Transmission-Based Precautions

These are the measures used in addition to standard precautions for patients with documented or suspected infection or colonisation with highly transmissible or epidemiologically important pathogens for which additional specific precautions are neces-

sary to prevent transmission. The main types of transmission-based precautions are *contact* precautions, *airborne* precautions and *droplet* precautions. The specific PPE and the examples of pathogens which require each type of transmission-based precautions are outlined in Table 27.1.

*Transmission-based precautions* should also be applied in a *pre-emptive* way, e.g. in case of patients transferred from high-risk facilities, pending the results of surveillance cultures or during diagnostic workup, e.g. for suspected infectious diarrhoea.

There are no clear criteria for appropriate discontinuation of contact precautions (usually multiple negative swabs from a site known to be colonised), but there are data suggesting that colonisation with multidrug-resistant (MDR) pathogens might persist longer and reappear after several negative swabs, and *C. difficile* shedding might be present even after the resolution of diarrhoea (Banach et al. 2018). Therefore, in HCT setting, continuing contact precautions until discharged home and reculturing patients to document clearance of MDR carriage only after an interval free of hospitalisations, antimicrobial therapy and invasive devices might be more appropriate (Sehulster et al. 2004; Banach et al. 2018). The information on contact precautions in place should be clearly stated on the discharge information form for the centres which will care for such patient subsequently. In case of MDR Gram-negative pathogens, full antibiotic susceptibility results should be provided to allow appropriate empirical therapy in case of severe subsequent infection. In case of *Candida auris* colonisation, no routine reassessment of colonisation is recommended since it may persist for many months and discontinuation of contact precautions in healthcare facility is not recommended (updated recommendations available at <https://www.cdc.gov/fungal/candida-auris/c-auris-infection-control.html>).

*Cough etiquette* should be promoted. Additionally, transplant recipients, particularly those with respiratory symptoms, should use surgical masks and maintain special separation from others in common waiting areas, ideally a distance of at least 1 m.

**Table 27.1** Transmission-based precautions to be applied in addition to standard precautions

Type of precaution	Patients' placement and PPE to be used by patients	PPE for healthcare personnel	Example of pathogens and comments
Contact	<ul style="list-style-type: none"> <li>– Single room; if not available, cohorting of those colonised/infected by the same pathogen</li> <li>– During transport, cover patient's colonised/infected areas</li> </ul>	<ul style="list-style-type: none"> <li>– Disposable gloves and gowns</li> <li>– Use patient-dedicated or disposable equipment; if not feasible, clean and disinfect thoroughly</li> </ul>	<ul style="list-style-type: none"> <li>– Infection with <i>Clostridioides difficile</i></li> <li>– Colonisation or infection with MDR pathogens</li> <li>– Infectious diarrhoea due to pathogens such as <i>Salmonella</i>, <i>Norovirus</i>, <i>Rotavirus</i>, etc.</li> </ul> <p>All the units and other hospitals involved in patient's care should be notified about all the isolated pathogens requiring contact precautions</p>
Droplet	<ul style="list-style-type: none"> <li>– Single room; if not available, cohorting of those infected by the same pathogen</li> <li>– Surgical mask</li> <li>– Follow CDC's respiratory hygiene/cough etiquette in healthcare setting</li> </ul>	<ul style="list-style-type: none"> <li>– Mask (surgical)</li> <li>– In many cases in HCT also disposable gloves and gowns, since both droplet and contact precautions are necessary e.g. in case of respiratory viruses</li> </ul>	<ul style="list-style-type: none"> <li>– Pathogens transmitted by respiratory droplets (i.e. large-particle droplets &gt;5 µm in size) that are generated by a patient who is coughing, sneezing or talking, e.g. influenza or other respiratory viruses</li> </ul> <p>In case of transplant recipients, the duration of precautions should be extended due to the possibility of prolonged shedding caused by immunodeficiency</p>
Airborne	<ul style="list-style-type: none"> <li>– Preferably airborne infection isolation room, i.e. with at least 6 (existing facility) or 12 (new construction/renovation) air changes per hour and direct exhaust of air to the outside (if not possible, the air may be returned to the air-handling system or adjacent spaces if all air is directed through HEPA filters)</li> <li>– Surgical mask</li> <li>– Follow CDC's respiratory hygiene/cough etiquette in healthcare setting</li> </ul>	<ul style="list-style-type: none"> <li>– N95 or higher-level respirator for respiratory protection</li> </ul>	<ul style="list-style-type: none"> <li>– <i>Mycobacterium tuberculosis</i> (patients with respiratory tuberculosis and sputum with direct evidence of mycobacteria)</li> <li>– Measles, chickenpox and disseminated herpes zoster</li> </ul>

HEPA high-efficiency particulate air, MDR multidrug resistant, PPE personal protective equipment

Current WHO guidance (from June 2020) recommends for healthcare workers caring for suspected or confirmed SARS-CoV-2 positive the use of droplet and contact precautions, and airborne and contact precautions in case of aerosol generated procedures, although there are data supporting the universal use of airborne + contact precautions. Recent local and international guidelines should be followed for updated procedures on prevention of SARS-CoV-2 infection.

Upon entering HCT unit, visitors should be screened for the presence of symptoms of easily transmissible diseases such as viral respiratory

tract infections, gastroenteritis, etc. and, if present, advised to postpone their visit until no longer symptomatic. Also, healthcare workers with respiratory symptoms should refrain from direct patient care until the symptoms resolve. Non-immune persons who were exposed to communicable diseases such as measles or chickenpox should refrain from contact with HCT recipients or transplant candidates until the incubation period passes without developing the disease.

Instructional materials for patients and visitors on recommended hand hygiene, respiratory hygiene/cough etiquette practices and the appli-

cation of transmission-based precautions should be provided. Vaccination of healthcare workers and household contacts is paramount and discussed in the dedicated chapter.

## 27.4 Management of the Threat of MDR Bacteria

In the era of increasing bacterial resistance, an important part of infection control deals with *prevention of colonisation and infection with MDR bacteria* (Siegel et al. 2007). Active surveillance, for example, with rectal swabs for detecting colonisation with carbapenem-resistant *Enterobacteriaceae* (CRE) or vancomycin-resistant enterococci (VRE) or nasal swabs for methicillin-resistant *Staphylococcus aureus* (MRSA) should be performed in institutions where these pathogens are encountered or in patients coming from such institutions.

The need for screening for different pathogens may vary according to local epidemiology and is performed in some settings (Girmenia et al. 2015), while recent ASTCT guidelines recommend that when CRE are uncommon, limiting screening to patients referred from CRE-endemic areas is another reasonable strategy (Satlin et al. 2021). The screening for MDR pathogens allows to choose the appropriate empirical therapy in case of neutropenic fever and to implement contact precautions to prevent nosocomial transmission (Girmenia et al. 2015). Careful evaluation of the possibility and risk/benefit ratio of decolonisation in selected cases through oral administration of non-absorbable molecules or faecal microbiota transplantation is warranted (Girmenia et al. 2015; Bilinski et al. 2017), although evidence is insufficient to provide a recommendation (Tacconelli et al. 2019).

In order to counteract the threat of MDR pathogens and the shortage of agents active against Gram-negative MDR bacteria, *antimicrobial stewardship program* should be implemented in every centre (Gyssens et al. 2013). Additionally, national systems for surveillance, with obligation of notification and recommendations for contain-

ment and infection control measures, should be put in place (Tacconelli et al. 2014).

The aim of *antimicrobial stewardship* is to limit the negative impact of MDR pathogens on patients' outcome, and its *main elements* are detailed in Table 27.2.

Successful implementation of antimicrobial stewardship is based on a multidisciplinary approach and close collaboration between the treating haematologists, microbiology laboratory and infectious diseases consultation service, and includes also infection control unit, hospital pharmacy and hospital authorities who should recognize that this is an important step in high-quality management of infectious complications after HCT.

*Surveillance of effectiveness of infection control practices* should be put in place, with regular

**Table 27.2** Main elements of antimicrobial stewardship program

1. Regularly updated (e.g. every 6–12 months) surveillance of local epidemiology of infections in HCT recipients, through reports on:
  - (a) Resistance rates to main antibiotics in top 10 most frequent pathogens
  - (b) Data on antibiotic consumption
  - (c) Data on patient outcomes in case of most frequent/difficult infections, particularly due to resistant pathogens
2. Implementation of updated diagnostic methods and prompt reporting of microbiologic results by the laboratory in order to provide clinicians with
  - (a) Correct and timely diagnosis (e.g. of viral or fungal infections or *Clostridium difficile*, which may allow to avoid unnecessary antibiotic therapy)
  - (b) Rapid results of antimicrobial susceptibility testing to allow choosing the best targeted antibiotic therapy
3. Promoting appropriate antibiotic use, for example
  - (a) Implementing timely de-escalation or discontinuation of antibiotic treatment, particularly during neutropenia
  - (b) Appropriate dosing for different indications
  - (c) Optimised infusion strategies for time- and dose-dependent molecules, e.g. use of extended or continuous infusion of time-dependent molecules such as beta-lactams
4. Establishing and regularly updating protocols for prevention and treatment of infections, e.g. identifying antibiotic and antifungal regimens for empirical therapy in accordance with local epidemiology (e.g. prevalence of extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae*, methicillin-resistant staphylococci, azole-resistant aspergilli, etc.)

monitoring of adherence. In case of contact-transmission pathogens, such as *Clostridium difficile* or MDR bacteria, laboratory data should be regularly analysed to detect any trends indicating possible increase in transmission.

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## 27.5 HCT Environment

Flowers, fountains, water leaks and water-retaining bath toys carry the risk of *water-associated infections* with Gram-negative bacilli such as *Pseudomonas aeruginosa* or *Legionella* and thus should be avoided in the areas where severely immunocompromised patients are being cared for (Yokoe et al. 2009). In addition, there are issues specific for HCT recipients, such as room ventilation, intensified protective measures applied during hospital construction and renovations, avoidance of contact with soil (including potted plants) and avoidance of dust, both permanently (e.g. non-carpeting and no porous surfaces) and while cleaning, all aimed at decreasing the risk of invasive aspergillosis (Yokoe et al. 2009).

CIBMTR/ASBMT/EBMT global recommendations on protective environment concerning hospital *room design and ventilation* are available (Yokoe et al. 2009). Briefly, allo-HCT recipients should ideally be placed in protective environment rooms that incorporate several features including central or point-of-use HEPA (high-efficiency particulate air) filters with 99.97% efficiency for removing particles  $\leq 0.3 \mu\text{m}$  in diameter and  $\geq 12$  air exchanges/hours, with directed airflow and consistent positive air pressure differential between the patient's room and the hallway  $\geq 2.5$  Pa. All these measures remove airborne fungal spores and are aimed at preventing airborne infections with filamentous fungi such as aspergilli. The efficacy of protective isolation measures in case of auto-HCT recipients is less well established.

Currently, *HEPA-filtered rooms* are available in almost all centres, while few centres fulfilled all the CIBMTR/ASBMT/EBMT requirements. However, the knowledge on the details and maintenance of protective environments in the HCT

setting was recently found inadequate, requiring education efforts and cooperation with hospital infection control and the hospital maintenance services (Styczynski et al. 2018).

During *construction and renovations*, due to high density of fungal spores, protective environmental measures are particularly important, and mould-control measures should be intensified and filtration efficiency should be monitored frequently to best determine appropriate time for replacement. Specific recommendations are available and should be followed (Schulster et al. 2004). For example, construction and renovation areas should have negative air pressure relative to HCT patient care areas to ensure that air flows from patient care areas towards construction areas, and a portable, industrial-grade HEPA filter should be used between a construction zone and the HCT unit if a large area is under construction and negative pressure differential cannot be guaranteed. In addition, HCT recipients may benefit from wearing N95 respirators outside HEPA-filtered areas, particularly during ongoing constructions, since unlike surgical masks, higher efficiency masks offer protection against *Aspergillus* spores. Active monitoring of cases of invasive mould infections should be performed in order to detect any possible outbreak.

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## 27.6 Food Safety in Transplant Recipients

Drinking *water* should be safe; thus boiled or bottled water is preferred. Tap water in highly populated areas is usually regarded as safe from bacterial contamination because tested regularly. However, it may still contain *Cryptosporidium*. Water from private wells should be avoided.

The use of *low-microbial diet*, which prohibits fresh fruits and vegetables and unprocessed food, did not result in a decreased incidence of infections in neutropenic patients (Sonbol et al. 2015; van Dalen et al. 2016). Standard food safety practices that emphasize safe handling and washing or thoroughly cooking food were found to be just as safe and produced no increase in infection rates or incidence of neutropenic fever. Similarly,



to other immunocompromised patients, HCT recipients should avoid foods possibly contaminated by *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella enteritidis*, *Toxoplasma gondii*, etc.

Main high-risk foods to avoid include:

- Raw or undercooked meat, poultry, fish or shellfish
- Refrigerated smoked fish
- Unpasteurised milk
- Foods with raw or undercooked eggs
- Unwashed fruits and vegetables
- Raw sprouts
- Soft cheeses made from unpasteurised milk like brie, camembert and blue-veined and fresh cheese (can be eaten if cooked)
- Hot dogs, deli meats and luncheon meats that have not been reheated to steaming hot or to 75 °C
- Unsafe water and ice made of it

Food safety practices for food handling should be followed, and specific information for cancer patients is available online (<https://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM312793.pdf>). It should be kept in mind that too restrictive diet recommendations, in the absence of the clear benefit of avoiding foods other than above-mentioned, may have negative impact on patient's nutritional status and/or quality of life.

#### Key Points

- General guidelines for preventing healthcare-associated infections should be followed, and hand hygiene is the single most effective measure.
- Mandatory isolation procedures comprise Standard Precautions and Transmission-Based Precautions if appropriate: airborne, contact or droplets.
- Specific recommendations on ventilation, room design and protective environment during construction/renovation

are provided to protect HCT from transmission of spores of filamentous fungi, mainly *Aspergillus*.

- Protocols for the prevention of colonisation and infection with multidrug-resistant bacteria should be put in place, particularly in centres where these bacteria are already present.
- Antimicrobial stewardship program should be implemented in every centre to promote optimal use of antibiotics.
- Standard food safety practices should be applied, and only selected foods should be avoided (e.g. raw/undercook/underheated meat, fish or eggs, unpasteurised milk, unwashed fruits and vegetables, unsafe water).

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## General Management of the Patient: Specific Aspects of Infectious Disease Supportive Care in Children

Elio Castagnola and Andreas H. Groll

Many of the conditions requiring allogeneic HCT and related complications are similar in adults and children and are covered in other chapters of this handbook. However, there are a few exceptions where approaches to management can be different. Infections are frequent causes of post-transplant morbidity and generally life-threatening if not appropriately controlled. Most of the available data on their epidemiology and treatment are derived from studies in adults, and not all aspects can be directly transferred to children.

In the following paragraphs, we will analyze specific aspects of the management of bacterial and fungal infections and address selected issues on viral infections in children.

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### 28.1 Management of Bacterial Infections

*Antibacterial prophylaxis* for febrile neutropenia has been frequently administered to children undergoing allogeneic HCT but has never been specifically analyzed in an appropriately designed randomized clinical trial, and its effectiveness has been questioned in a real-life experience (Ricci et al. 2020). In the context of pediatric-specific safety issues associated with the fluoroquinolones and increasing concerns about the selection of antibiotic-resistant bacteria, the 2020 guidelines of the European Conference on Infections in Leukemia (ECIL) for the use of antibiotics in pediatric patients with cancer or post-HCT made a strong recommendation against the routine use of antibacterial prophylaxis of febrile neutropenia during the pre-engraftment period in children (Lehrnbecher et al. 2021). This recommendation is based on carefully analyzed data from randomized trials and meta-analyses, European Medicines Agency recommendations, and information from long-term observational studies on resistance. Indeed, in a multicenter, multinational retrospective analysis of 1291 bloodstream infections reported in 1031 patients, occurrence of bloodstream infection due to antibiotic resistant pathogens was significantly associated with previous exposure to antibiotics for prophylaxis and treatment (Castagnola et al. 2021). Of note, the recommendation against its routine use does not exclude prophylaxis in

individual patients after careful risk–benefit analysis, depending on the clinical situation (Lehrnbecher et al. 2021).

Independently of age, administration of *empirical antibacterial therapy* is a standard in granulocytopenic children with new onset of fever or any signs or symptoms of a new infection. Important considerations regarding the initial choice of antibacterial agents include the clinical status of the patient, previous infections, colonization of the patient with resistant bacteria, and the local epidemiology of resistant bacteria at the referring or the transplanting center. In the standard situation of a clinically stable patient at low risk of resistant infections, monotherapy with an antipseudomonal non-carbapenem  $\beta$ -lactam plus  $\beta$ -lactamase inhibitor combination or a fourth-generation cephalosporin is strongly recommended as initial therapy by the ECIL guidelines (Lehrnbecher et al. 2021). For clinically unstable patients, an anti-pseudomonal carbapenem with or without a second anti-Gram-negative agent, with or without a glycopeptide, is recommended with similar strength, even when there appears to be a low risk of resistant infections; for patients who are colonized or were previously infected with resistant Gram-negative bacteria, or in centers with a high rate of resistant pathogens, empirical treatment should be adjusted on the basis of the results of resistance testing (Lehrnbecher et al. 2021).

Apart from the initiation and choice of antibacterial therapy, the current ECIL guidelines also provide considerations for de-escalation of antibacterial therapy (Lehrnbecher et al. 2021), but most of these considerations do not apply to the pre-engraftment granulocytopenic phase that last approximately 20 days on average in a standard pediatric transplant setting (Linke et al. 2020) and are associated with mucositis and other complications in many patients. Thus, in most instances, empirical antibacterial therapy is administered until recovery from neutropenia and defervescence, and adequate treatment of an infection, if documented.

Empirical antibacterial therapy should also be considered post engraftment particularly in the presence of a central venous line and/or GVHD because of the significant risk of Intensive Care

Unit admission and consequent mortality in the presence of bacteremia (Castagnola et al. 2014b, 2021).

An important issue that has become more relevant in recent times is the use of *pharmacokinetic and pharmacodynamic* information to optimize the administration of antibacterial agents to improve outcome in severe infections and reduce the risk of resistant strain selection. For example, it has been demonstrated that the intermittent dosing of piperacillin–tazobactam at 100 mg/kg (of piperacillin) every 8 h over 5 min frequently does not result in optimal exposure in critically ill or febrile granulocytopenic children. Differently, the use of a loading dose followed by continuous infusion or at least a prolonged infusion over 3 h administered every 6 h may be used to maintain the therapeutic target concentration of 4 times the minimum inhibitory concentration of the infecting organisms during continuous administration, or at pre-infusion time (trough) in case of intermittent schedule (De Cock et al. 2017; Saffioti et al. 2019; Thorsted et al. 2019). Similar observations and considerations have been made for optimal dosing of vancomycin (Shimamoto et al. 2021), just to name two of the most essential agents in pediatric transplantation. In addition to optimizing the pharmacokinetics and pharmacodynamics, dosing of antibacterial agents in critically ill children and those with febrile granulocytopenia or GvHD, the possible presence of one or more of the following situations must also be considered: augmented renal clearance (creatinine clearance  $>130$ – $160$  mL/min/ $1.73$  m<sup>2</sup>) that may lead to diminished plasma and tissue concentrations; hypalbuminemia that may increase the amount of free drug of highly protein-bound agents, increasing their elimination particularly in the presence of augmented renal clearance; and acute kidney injury that is sometimes observed at the onset of severe infections and may cause drug accumulation and toxicity, but frequently resolves within 48 h. This observation provides a strong rationale for administration of full dosages of antibiotics with a high therapeutic index and renal elimination, such as the  $\beta$ -lactams, for the first 48 h to prevent suboptimal concentrations in the first hours of

**Table 28.1** Prophylaxis and therapy of invasive bacterial infections in children undergoing allogeneic HCT

<i>Antibacterial prophylaxis for febrile neutropenia</i>
Routine antibacterial prophylaxis of febrile neutropenia during the pre-engraftment period of HCT is not recommended
<i>Empirical therapy for febrile neutropenia, or fever after engraftment, especially in the presence of GvHD</i>
The choice of antibacterial agents to be administered for empirical therapy should be based on the clinical status of the patient, previous infection or colonization of the patient with resistant bacteria, and the local epidemiology of resistant bacteria at the referring or the transplanting center. For initial therapy in a clinically stable patient at low risk of resistant infections, monotherapy with an antipseudomonal noncarbapenem $\beta$ -lactam plus $\beta$ -lactamase inhibitor or a fourth generation cephalosporin is recommended
<i>Antibacterial agents recommended for initial empirical therapy</i>
Piperacillin–tazobactam 100 mg/kg (max 4000 mg) of piperacillin q6h intravenously, preferentially over 3 h. The daily dose can also be administered as continuous infusion, fractionated every 6 h because of drug stability. In this case, a loading dose (100 mg/kg max 4000 mg of piperacillin in 1 h) should be administered
Cefepime 50 mg/kg (max 2000 mg) q8h intravenously over 2 h
<i>Antibacterial combinations recommended for clinically unstable patients</i>
Meropenem 20 mg/kg (max. 2000 mg) q8h intravenously, preferentially over 3 h. The daily dose can also be administered as continuous infusion, fractionated every 6 h because of drug stability. In this case, a loading dose (20 mg/kg max. 2000 mg of meropenem in 1 h) should be administered. Higher doses could be needed in patients colonized by Gram-negatives with reduced susceptibility to carbapenems
+/- glycopeptide (vancomycin 20 mg q8h or teicoplanin 10 mg q24h (max. 800 mg; day 1 10 mg q12h) plus therapeutic drug monitoring)
+/- second Gram-negative agent (amikacin 20 mg/kg (max 1500 mg) in 30–60' q24h, ciprofloxacin 20 mg/kg (max. 750 g) q12h)
<i>Antibacterial combinations recommended for patients colonized/previously infected with resistant bacteria, or in centers with a high rate of resistant bacteria</i>
Adjustment of empirical treatment on the basis of the results of resistance testing of these bacteria
<i>Treatment of documented infections</i>
Treat under consideration of localization and results of susceptibility testing. For treatment of infections by multiresistant bacteria, consultation of an infectious diseases specialist is recommended

treatment of a severe infection with optional dose adaptation if renal impairment persists beyond the first 2 days (Castagnola et al. 2022). Recommendations for use and dosing of antibacterial agents in pediatric allogeneic HCT recipients are summarized in Table 28.1.

*Clostridioides difficile* may become a cause of severe, and sometimes recurrent, diarrheal disease, especially in allogeneic HCT recipients (Haeusler et al. 2022). However, children below the age of 2 years may harbor this pathogen in their intestinal tract without developing disease (Lees et al. 2016; Enoch et al. 2011), and other intestinal pathogens, including but not limited to enteric viruses, may be the cause of diarrhea and gastroenteritis in the posttransplant situation (Castagnola et al. 2016). Preferred options for treatment of *Clostridioides difficile* infection include oral vancomycin and oral fidaxomicin; intravenous metronidazole is an option for patients who are unable to take oral medication,

and the monoclonal antibody bezlotoxumab is reserved for patients with recurrent infection but is not yet approved in patients below 18 years of age. Of note, no solid data exist for fecal transplantation in immuno-compromised children (Diorio et al. 2018).

## 28.2 Management of Invasive Fungal Diseases

Invasive fungal diseases (IFDs) are an important cause of posttransplant morbidity in pediatric allogeneic HCT and are associated with high mortality and significantly decreased overall survival probability (Cesaro et al. 2017; Castagnola et al. 2018; Linke et al. 2020). Apart from the pivotal impact of prolonged granulocytopenia, the use of glucocorticosteroids in therapeutic doses, and limited to candidemia the use of central venous catheters, increasing age have been

additional risk factors for the development of IFD (Fisher et al. 2018). However, multivariable analyses showed that age is no longer significant in the presence of severe acute or chronic extensive GvHD or in cases of primary graft failure or rejection (Castagnola et al. 2014a).

Based on natural incidence rates that are estimated to be well above 10%, both the initial (Groll et al. 2014) and the updated 2020 ECIL recommendations for the diagnosis, prevention, and treatment of invasive fungal diseases in pediatric patients with cancer or post-HCT (Groll et al. 2021) strongly recommend primary antifungal prophylaxis pre- and post-engraftment until discontinuation of immunosuppression and immune recovery, and in situations of augmented immunosuppressive treatment in the context of acute or extensive chronic GvHD. Based on large randomized clinical trials performed in adults and regulatory approval in pediatric patients, antifungal prophylaxis in the transplant setting is largely azole-based with echinocandins and polyenes as secondary options (Groll et al. 2021). While the local epidemiology is an important additional consideration for selecting an institutional prophylaxis strategy, mold-active prophylaxis is strongly recommended by ECIL and other international consortia in the context of acute and chronic GvHD because of the predominance of invasive mold infections in these settings (Groll et al. 2021; Lehrnbecher et al. 2020).

According to the ECIL guideline, *empirical antifungal therapy* may be initiated during the pre-engraftment phase in pediatric patients with granulocytopenia after 96 h of fever of unclear cause that is unresponsive to broad-spectrum antibacterial agents. Primarily recommended agents for empirical therapy include liposomal amphotericin B or caspofungin with discontinuation of antifungal prophylaxis (Groll et al. 2021). The alternative is a *pre-emptive, diagnostic-driven approach* that requires rapid availability of pulmonary CT imaging and of galactomannan-assay results, and, ideally, the ability to perform diagnostic bronchoscopies in the case of pulmonary findings (Santolaya et al. 2018; Lehrnbecher and Groll 2019; Groll et al. 2021). If all studies including blood cultures are negative for the pres-

ence of an IFD, mold-active antifungal prophylaxis may be continued and the patient further monitored. Conversely, if the diagnosis of a probable or proven IFD is made, this IFD is treated according to the respective treatment recommendations (Groll et al. 2021).

*Diagnosis of IFDs* is based on isolation of fungal pathogens from cultures of sterile sites or tissue invasion demonstrated by histology or by the presence of fungal biomarkers in blood or cerebrospinal fluid or bronchoalveolar lavage, associated with suggestive imaging in children with a compatible clinical picture (Donnelly et al. 2020). Since the lung is the most important site of invasive mold infections, a chest CT is strongly recommended in granulocytopenic patients with fever for  $\geq 96$  h and in those with clinical findings suggestive of pneumonia. As non-typical findings are frequent in pediatric pulmonary mold infection, detection of any pulmonary infiltrates may be indicative of an IFD and should prompt a diagnostic work-up and initiation of mold-active antifungal therapy. Furthermore, appropriate cranial imaging should be considered after diagnosis of a probable or proven pulmonary mold infection because central nervous system involvement may occur in up to 30% and will require specific treatment considerations (i.e., use of voriconazole) (Groll et al. 2021; Pana et al. 2023).

Detection of galactomannan antigen in serum is widely used for the diagnosis of IFD and has similar diagnostic performance in children and adults (Lehrnbecher et al. 2016; Ferreras-Antolin et al. 2022). While an option in high-risk situations in children not on mold-active prophylaxis, prospective monitoring in serum is discouraged by the current ECIL guidelines if anti-mold prophylaxis is administered because of poor sensitivity in this setting (Lehrnbecher et al. 2016; Groll et al. 2021). In contrast, assessment of galactomannan in serum is strongly recommended for diagnostic use in prolonged febrile neutropenia and in those with abnormalities on chest CT, and the galactomannan assay may also be a useful diagnostic adjunct in bronchoalveolar lavage and cerebrospinal fluid (Groll et al. 2021). Of note, no adequate data exist for beta-D-Glucan



and polymerase chain reaction-based monitoring in serum (Lehrnbecher et al. 2016; Groll et al. 2021). However, the use of PCR and other molecular methods on bronchoalveolar lavage fluid, diagnostic aspirates, or tissue specimen is strongly recommended whenever such specimens are obtained (Groll et al. 2021).

The options for *treatment of probable or proven IFDs* in pediatric patients are similar to those in adults: for invasive candidiasis, echinocandin-based fungicidal regimens are preferred, and for invasive aspergillosis, azole-based regimens with liposomal amphotericin B being an alternative option due to its better tolerance (Fisher et al. 2021; Groll et al. 2021). In case of mucormycosis, rare molds, rare yeasts, cryptococcal meningitis and infections by dimorphic fungi, transplant physicians are referred to existing international European Confederation of Medical Mycology guidelines that include pediatric-specific considerations (Hoenigl et al. 2021).

There are some notable caveats for the use of antifungal drugs. *Voriconazole* frequently needs to be administered at higher dosages in subjects below 5 years of age to achieve and maintain effective plasma concentrations (Soler-Palacin et al. 2012; Neely et al. 2015; Castagnola and Mesini 2018). Inflammation, steroid administration, or obesity can further modify its concentrations (Castagnola and Mesini 2018; Natale et al. 2017) and so do genetic factors (Teusink et al. 2016). Finally, severe cutaneous adverse events can also be observed in children when voriconazole is administered for prolonged periods in conjunction with immunosuppression and sun exposure (Goyal et al. 2015; Bernhard et al. 2012).

*Posaconazole* was first approved in adults in 2006 in the form of oral suspension. This formulation had variable absorption resulting in subtherapeutic concentrations in a large proportion of patients (Jancel et al. 2017), especially those with intestinal acute GvHD (Heinz et al. 2016). Administration with a fatty meal and/or other “bundle” measures or using doses based on body surface area were recommended to overcome this limitation (Castagnola and Mesini 2018).

Nevertheless, the pediatric development of the oral solution was discontinued after a formal dose-ranging study failed to demonstrate a consistent dose-exposure relationship (Arrieta et al. 2019). Following introduction of an intravenous solution and gastro-resistant tablets in adults, a novel pediatric gastro-resistant/delayed-release powder for oral suspension has been developed and is now approved together with the intravenous solution in pediatric patients 2 years and older for prophylaxis of IFD (Groll et al. 2020; European Medicines Agency 2022). For children not tolerating a suspension, various approaches exist and may be used for appropriate dosing of the gastro-resistant tablet (Castagnola and Mesini 2018; Mesini et al. 2018; Tragiannidis et al. 2019).

*Isavuconazole*, administered as the water-soluble prodrug isavuconazonium sulfate, is an intravenous and oral triazole approved in 2015 for first-line treatment of invasive aspergillosis and treatment of mucormycosis (Groll et al. 2022a). After identification of an adequate pediatric pharmacokinetic profile (Arrieta et al. 2021), approval of the compound for patients >2 years of age is to be expected shortly (Groll et al. 2022a).

All mold-active antifungal triazoles interfere with the cytochrome P450 system which may lead to many relevant drug interactions that must be considered during their use, particularly during immunosuppression (Groll et al. 2017). Inter- and intraindividual variability of exposure is a problem that is predominantly inherent to the use of voriconazole. The current ECIL guidelines strongly recommend therapeutic drug monitoring for use of itraconazole and voriconazole, and with lesser emphasis, for posaconazole and isavuconazole, both for prophylactic and therapeutic use (Lewis et al. 2015; Groll et al. 2021). Recommendations for use and dosing of antifungal agents in pediatric allogeneic HCT recipients are summarized in Table 28.2.

*Pneumocystis jirovecii* pneumonia is a severe, life-threatening fungal infection in allogeneic-HCT recipients. Primary prophylaxis is highly recommended in children undergoing allogeneic-HCT from the time of engraftment onward until

**Table 28.2** Agents used for prophylaxis and therapy of invasive fungal diseases in children undergoing allogeneic HCT

Antifungal agent	Summary of EMA approved indications <sup>a</sup>	Pediatric dosage range	Specific comments
Amphotericin B deoxycholate	Treatment of IFDs	0.7–1.5 mg/kg/day IV in single daily dose	PK similar to adults; clinical usefulness limited by infusion-related reactions and nephrotoxicity
Amphotericin B lipid complex	2nd line treatment of invasive <i>Candida</i> or <i>Aspergillus</i> infections	5 mg/kg/day IV in single daily dose	PK similar to adults; similar rate/extent of infusion-related reactions, but less nephrotoxicity relative to amphotericin B deoxycholate
Liposomal amphotericin B	Treatment of IFDs and empirical therapy in granulocytopenic patients	1–>5 mg/kg/day IV in single dose	PK similar to adults; infusion-related reactions and nephrotoxicity less frequent relative to amphotericin B deoxycholate or lipid complex Dosages used for prophylaxis (non-approved indication): 1 mg/kg IV in one single dose or 2.5 mg/kg IV twice weekly
Flucytosine	Treatment of invasive candidiasis and cryptococcosis in combination with amphotericin B	100–150 mg/kg/day IV in 3–4 divided doses + TDM	No published PK and safety data for pediatric patients; robust phase III efficacy data for cryptococcal meningoenzephalitis
Fluconazole	Treatment and prevention of superficial and invasive <i>Candida</i> infections; treatment of cryptococcosis and coccidioidomycosis	6–12 mg/kg/day IV/PO in single daily dose	Maximum dose of 12 mg/kg strongly recommended for invasive infections, favorable safety profile, but potential for drug–drug interactions Prophylaxis: no activity against molds
Itraconazole	Treatment of superficial <i>Candida</i> infections; second line treatment of invasive candidiasis, aspergillosis and cryptococcosis; prophylaxis in granulocytopenic patients	5 mg/kg/day PO of the suspension in two divided doses + TDM	Limited pediatric PK data, but generally similar to adults. Similar problems with oral bioavailability. High potential for relevant drug–drug interactions. Not licensed in the EU in pediatric patients, no PK data for children <2 years
Isavuconazole	Treatment of invasive. Aspergillosis and treatment of mucormycosis in patients for whom amphotericin B is inappropriate	10 mg/kg IV/PO once daily with a maximum. Dose of 372 mg isavuconazolium sulfate (day 1 and 2: three times daily)	Not licensed in the EU in pediatric patients; pediatric investigation plan for ≥2 years of age completed, regulatory approval expected
Posaconazole	1st line treatment of invasive aspergillosis second line treatment of fusariosis, chromoblastomycosis, and coccidioidomycosis; treatment of oropharyngeal candidiasis; antifungal prophylaxis in AML/MDS and allogeneic HCT patients	<i>Intravenous solution:</i> 6 mg/kg once daily (max. 300 mg; day 1: 6 mg/kg twice daily) <i>Delayed release tablets:</i> Patients >40 kg 300 mg/day in one single dose (day 1: 300 mg BID) <i>Powder for delayed release oral suspension:</i> ≤40 kg: weight-based once daily dosing (day 1: twice daily). For details, see SPC <i>Oral suspension:</i> not approved	Approved for antifungal prophylaxis in pediatric patients ≥2 years of age, not yet for primary treatment of invasive aspergillosis. Potential for relevant drug–drug interactions. TDM suggested

**Table 28.2** (continued)

Antifungal agent	Summary of EMA approved indications <sup>a</sup>	Pediatric dosage range	Specific comments
Voriconazole	Treatment of invasive aspergillosis, fusariosis, scedosporiosis; treatment of candidemia in non-granulocytopenic patients; antifungal prophylaxis in allogeneic HCT patients	2–<12 years/12–14 years and <50 kg: 8 mg/kg BID (day 1: 9 mg/kg BID) IV and 9 mg/kg BID PO; ≥15 years and 12–14 years and ≥50 kg: 4 mg/kg BID (day 1: 6 mg/kg BID) IV; 200 mg BID PO +TDM (all)	Optimal dose uncertain, age-dependent; high intra- and inter-individual PK variability requires TDM. Relevant potential for drug-induced adverse events, and relevant drug–drug interactions. Not licensed in subjects <2 years of age
Anidulafungin	Treatment of invasive candidiasis	1.5 mg/kg/day (day 1: 3 mg/kg) IV in one single dose	PK similar to adults; favorable safety profile, virtually no interactions; approved by the EMA for pediatric patients ≥1 month
Caspofungin	Treatment of invasive candidiasis, invasive aspergillosis (second line), and for empirical antifungal therapy in granulocytopenic patients	≥3 months–17 years: 50 (d1:70) mg/m <sup>2</sup> /day IV in one single dose; Max. dose 70 mg/day; ≥18 years: 50 mg/day IV (d1: 70 mg) Infants <3 months and neonates: 25 mg/m <sup>2</sup> /day IV	Robust pediatric PK data sets/models; favorable safety profile, virtually no interactions; approved for pediatric patients of all age groups Dosage used for prophylaxis (non-approved indication): 50 (d1:70) mg/m <sup>2</sup> /day IV in one single dose Data from an adequate randomized trial suggest prophylactic activity of echinocandins against <i>Candida</i> and <i>Aspergillus</i>
Micafungin	Treatment of esophageal and invasive candidiasis, prophylaxis of invasive <i>Candida</i> infections in granulocytopenic patients	1–4 mg/kg/day IV (≥50 kg: 50–200 mg) in one single dose Neonatal dose: 10 mg/kg/day IV	Robust pediatric PK dataset/models; favorable safety profile, virtually no interactions; approved for pediatric patients of all age groups Approved dosage for prophylaxis: 1 mg/kg IV once daily (max. 50 mg); non-approved dosage for prophylaxis: 2.5 mg IV twice weekly Data from an adequate randomized trial suggest prophylactic activity of echinocandins against <i>Candida</i> and <i>Aspergillus</i>

Table adapted from reference Groll et al. (2021)

IV intravenously, PO orally, DAMB amphotericin B deoxycholate, TDM therapeutic drug monitoring, PK pharmacokinetics

<sup>a</sup>Summarized tabulation; for specific wording, please refer to the respective summary of product characteristics (SPCs)

immune reconstitution and in general for up to 1 year posttransplant. Prophylaxis is highly effective, and in case of documented failure, especially in adolescents, patient compliance needs to be questioned (Maertens et al. 2016; Castagnola and Mesini 2018), as well as intestinal absorption.

### 28.3 Management of Viral Infection and Disease

No principal differences are notable between children and adults regarding the clinical presentation, diagnosis, prevention, and management of systemic viral infections. Apart from Epstein–Barr virus (EBV) and adenovirus infections, cytomegalovirus (CMV) infection is frequent post pediatric allogeneic HCT and associated with considerable morbidity and potential to progress to fatal end-organ disease. Letemovir is a new antiviral agent with a novel mechanism of action and has become standard prophylaxis in seropositive adult HCT recipients (Jakharia et al. 2021). Pediatric development of the compound is in advanced stages (Groll et al. 2022b) and will hopefully lead to approval in the future (Körholz et al. 2023). Control of EBV, adenovirus, and CMV may become challenging, and the option of treatment with virus-specific T-cells may be explored early in foreseeable complicated patient courses (Zajac-Spychala et al. 2022).

Primary systemic and respiratory viral infections may be seen more frequently in pediatrics, and in this setting, household contacts and health-care workers may represent important sources, with possible in-hospital spreading. In the last years, the SARS-CoV-2 pandemics provided an important challenge worldwide and in particular to immunocompromised patients. Notable and different from observations in adults, the incidence of COVID-19, the disease caused by the virus, was lower in immunocompromised children than in adults even if severe cases and deaths were also observed in pediatrics (Haeusler et al. 2021; Mukkada et al. 2021). This provides a stringent rationale to vaccinate these patients,

their household contacts, and health care workers (Cesaro et al. 2022).

### 28.4 Nonpharmacological Prevention

Application of bundle procedures for screening of colonization by resistant bacteria and isolation of positive patients (Castagnola et al. 2019) together with correct hand hygiene, appropriate isolation procedures, correct vascular access handling, and the use of HEPA filters can all be of great utility in the prevention of difficult to treat infections post allogeneic HCT (Castagnola et al. 2019; Ifversen et al. 2021). Interestingly, multi-variable analyses of data collected in the aforementioned survey on bloodstream infections (Castagnola et al. 2021) did not identify colonization by resistant Gram-negatives as a cause of mortality or ICU admission; indeed, these two outcomes were associated with methicillin-resistant *S. aureus* colonization. This observation is important considering the effectiveness of decolonization procedures against this pathogen (Fueller et al. 2022).

Vaccines also represent an important tool for the prevention of viral and certain bacterial (e.g., *S. pneumoniae*) infections in the posttransplant setting, and re-immunization with inactivated vaccines may be started as early as 6 months post-transplant, while vaccines with live attenuated viruses must be administered later and in the absence of severe immunosuppression (Cordonnier et al. 2019; Sticchi et al. 2019; Zajac-Spychala et al. 2022).

#### Key Points

##### In children undergoing allogeneic HCT

- Effectiveness of antibacterial prophylaxis of febrile neutropenia in the pre-engraftment period has not been specifically studied in randomized clinical trials, and there is the risk of selecting resistance, so this practice is not recommended.

- Empirical antibacterial therapy of febrile neutropenia with an antipseudomonal non-carbapenem  $\beta$ -lactam plus  $\beta$ -lactamase inhibitor combination or a fourth-generation cephalosporin is recommended in a clinically stable patient at low risk of resistant infections, while in clinically unstable patients, an antipseudomonal carbapenem with or without a second anti-Gram-negative agent, with or without a glycopeptide, is recommended, even when there appears to be a low risk of resistant infections. However, more in general, the choice should be based on epidemiological data from the referring or the transplanting center.
- A similar approach could be used in non-neutropenic patients with GvHD, based on the high frequency of bacteremia observed in this patients population.
- Pharmacokinetic/pharmacodynamics data should be used to optimize effectiveness and reducing the risk of resistance selection.
- Primary mold-active antifungal prophylaxis should be administered both in the pre- and in the post-engraftment phases. Triazoles represent the first choice, but liposomal amphotericin B could represent a good alternative.
- Diagnosis of invasive fungal disease can be a challenge and requires a multimodal approach with combined use of clinical, radiological (CT scan), and biomarkers (galactomannan, polymerase chain reaction) and sometimes aggressive diagnostic procedures (bronchoalveolar lavage, biopsies).
- Empirical antifungal therapy (i.e., administration of antifungals in the presence of persistent febrile neutropenia in the absence of an etiological diagnosis) is a possible option when a diagnostic driven approach (biomarkers and imaging) is not available, with simi-

lar results in terms of efficiency. Therapeutic options are like those recommended for adults, even if pediatric registration of the newest drugs is not always promptly available.

- Management of viral infections, vaccination schedules, and infection prevention procedures is similar to those recommended in adults, even if pediatric registration of the newest drugs is not always promptly available.

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## 29.1 General Concepts

Vaccination should be considered a *routine practice for all HCT recipients*, either autologous or allogeneic, adults or children. It should be implemented in all HCT programs. Adult cover is particularly important as they represent 90% of HCTs. To obtain this objective, the following are necessary:

- To have in place a standardized program specific for HCT patients.
- The collaboration of the Preventive Department of the hospital and primary care physicians.
- The program must be simple, with a clear chronology, and convenient for the patient and physician (no increase in the number of visits).
- FACT-JACIE Standards (version 8.1, December 2021) require that policies/SOP are in place for posttransplant vaccination schedules and indications.

*The vaccination program should include not only the patient but also those who live with the patient and the healthcare workers (HCWs).*

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*There is not a unique vaccine schedule for all HCT patients.* Each center should discuss and adapt a specific vaccine program.

- The practical application of the immunization programs shows important variations across centers (Miller et al. 2017).
- Auto-HCT recipients are generally vaccinated with the schedule used for allogeneic recipients with small differences (see Table 29.1).

### *Reasons for universal vaccination of HCT patients*

**General interest: as a general healthcare principle, all the population should be correctly vaccinated, including adults and of course HCT recipients. If an increasing collective of patients, like HCT, is not well vaccinated, which can generate holes of immunity that can be a risk for the health of the general population.**

- *Individual interest for each HCT patient: vaccination protects the patient against infections that can cause important morbi-mortality. There are frequent infections in HCT that have safe vaccines (pneumococcus, influenza, and HBV) and other rare infections associated with high mortality that have an unsatisfactory prevention/treatment but can be prevented by immunization (tetanus, diphtheria, measles, and polio).*

**Table 29.1** ECIL recommendations for allogeneic-HCT recipients (Cordonnier et al. 2019)

Vaccine	No. of doses <sup>a</sup>	Time post-HCT to initiate vaccine	Grading
<b>Influenza</b> (inactivated)	1 (or 2, special cases) <sup>b</sup>	<ul style="list-style-type: none"> <li>&gt;6 months</li> <li>As long as patient is judged to be IS</li> <li>Yearly, lifelong</li> <li>From 3 months in case of a community outbreak</li> </ul>	AIIr BIIr BIIr
<b>Measles–mumps–rubella</b> <ul style="list-style-type: none"> <li>Measles</li> </ul> In sero(–) patients, with no GVHD, no IS, no REL of underlying disease, and no IGIV during the previous months, at least 3 months, ideally between 8 and 11 months <ul style="list-style-type: none"> <li>Rubella</li> </ul> In sero(–) women and of childbearing potential, with same precautions as for measles vaccine	1 (2 in children) MMR 1 MMR	<ul style="list-style-type: none"> <li>≥24 months</li> </ul> ≥12 months in case of measles outbreak in patients with low-grade IS <ul style="list-style-type: none"> <li>≥24 months</li> </ul>	BIIu CIII CIIu
<b>Virus hepatitis B<sup>c</sup></b> <ul style="list-style-type: none"> <li>Sero(–) patients before HCT and patients vaccinated pre-HCT but lost their immunity at 6 months)</li> <li>Previously infected and anti-HBs &lt;10 IU/L</li> <li>Sero(–) patients with a donor with positive anti-HBc</li> </ul>	3 <sup>d</sup>	6–12 months 6–12 months Vaccine before transplant	BIIr BIII BIII
<b>Human papilloma virus (HPV)</b> Follow recommendations for general population in each country	According to official label	From 6 to 12 months	BIIu
<b>Inactivated polio</b>	3 <sup>e</sup>	6–12 months	BIIu
<b>Live-attenuated varicella vaccine</b>	1	Can be considered in sero(–) patients, with ALL the following: >24 m from HCT, no GVHD, no IS, no REL of the underlying disease, and no IGIV in the previous months, at least 3 months, ideally between 8 and 11 months	BIIr
	2	The addition of a second dose in adults may be considered in patients who were sero(–) before HCT or had no history of VZ infect	
<b>Live-attenuated zoster vaccine</b>	Not recommended		DIII
<b>Pneumococcal conjugate (PCV)</b>	3	3 months	AI
<b>Polysaccharidic vaccine</b> In case of GVHD, use PCV instead of PPS for this fourth dose (BIIr)	1	12 months (no earlier than 8 weeks after last PCV)	BI
<b>Meningococcal conjugate</b> (in accordance with country recommendations and local prevalence)	2	From 6 months <ul style="list-style-type: none"> <li>For men-C or tetravalent vaccine</li> <li>For men-B vaccine</li> </ul>	BIIu BIII
<b>Haemophilus influenzae conjugate</b>	3	3 months or 6 months	BIIr
<b>Diphtheria-tetanus</b> (DT is preferred to Td CIII)	3 <sup>e</sup>	From 6 months	BIu
<b>Pertussis (acellular)</b> (DTaP is preferred over Tdap CIII)	3 <sup>e</sup>	From 6 to 12 months	CIII

Note for auto-HCT: same recommendations but grading changes for some vaccines

<sup>a</sup>If not specified otherwise, the interval between dose is 1 month

<sup>b</sup>Influenza: a second dose of influenza vaccine, after 3–4 weeks from the first, may have a marginal benefit and should preferably be considered in patients with severe GVHD or low lymphocyte count (B II r) and also for the patients vaccinated early (from 3 months after transplant) (B II r). Children ≥6 months through 8 years, receiving influenza for the first time after transplant, should receive a second dose at least 4 weeks after the first dose

**Table 29.1** (continued)

<sup>c</sup>HBV. After posttransplant vaccination, if anti-HBs are <10 mIU/mL 1–2 months after the initial series of three vaccine doses, an additional three doses should be considered, but the benefit of this second series of vaccination is uncertain. IDSA guidelines (Rubin et al. 2014): give the same recommendations (strong, low); for adolescents and adults, a high dose of vaccine (40 µg) is recommended for these booster doses (strong, low)

<sup>d</sup>Three doses: interval 0, 1, and 6 months

<sup>e</sup>At 1–2 months interval

## 29.2 General Principles of Vaccination in HCT Patients

### 29.2.1 The Pretransplant Vaccination

The pretransplant vaccination is not effective to maintain a prolonged posttransplant immunity. In order to protect the HCT recipient, a complete series of posttransplant vaccinations is required. This is different from what is recommended for solid organ transplant (SOT) recipients for whom pretransplant vaccination is an essential part of the vaccination program. Post-HCT recipients should be viewed as “never vaccinated” regardless of the pre-HCT vaccination history of the recipient or the donor (Rubin et al. 2014).

### 29.2.2 The Pre-HCT Immunity

The pre-HCT immunity for a specific pathogen is not a reason to withhold vaccination after transplant. The majority of patients will lose their immunity after HCT.

As a general rule, *live vaccines should be considered contraindicated* (there are exceptions, see later). The inactivated, subunit, or protein/polysaccharide vaccines can be safely administered.

There are few randomized trials in HCT recipients, and many of the studies have been done in patients transplanted with BM/PB, using MAC. The experience with other sources (CBU), conditioning regimens (RIC), and donors (haplo) is scarce.

Many vaccines are administered by *intramuscular route*, which can be a problem for severe

thrombocytopenic patients (less than  $50 \times 10^9$  platelets/L). For severe thrombocytopenic patients, some vaccines can be safely administered SC (inactivated poliomyelitis, conjugate pneumococcal vaccine) or even intradermic route (for influenza vaccine). Clinical experience suggests that intramuscular injections are safe if the platelet count is  $\geq 30\text{--}50 \times 10^9/L$ , a  $\leq 23$ -gauge needle is used, and constant pressure is maintained at the injection site for 2 min (Rubin et al. 2014).

### 29.2.3 The Dose of Vaccine

The dose of vaccine used is the same for general population, with some exceptions (see Table 29.1). A uniform specific interval between doses cannot be recommended, as various intervals have been used in studies. As a general guideline, a minimum of 1 month between doses may be reasonable.

### 29.2.4 Several Patient and Vaccine Characteristics Impact on the Vaccine Response

#### 29.2.4.1 Time from Transplantation

As a general rule, the later time a vaccine is administered, the better response is obtained (there are exceptions; see pneumococcal vaccine section). Usually >12 months from transplant is associated with better responses.

Postponing vaccination with a non-live vaccine should be the exception. Reasonable situations to postpone vaccination with a non-live vaccine are: recent rituximab administration

(<6 months); profound hypogammaglobulinemia (<3 g/L); unstable condition (due to uncontrolled grade 3–4 acute GVHD, uncontrolled infection, and admission to intensive care unit); and current treatment with ATG. The presence of GVHD under control with treatment should not delay vaccination. Some centers delay vaccination until the patient shows a minimum level of lymphocytes (for example, >200 CD4/ $\mu$ L). However, there are no data to support any specific lymphocyte level for starting vaccines, and delaying the vaccination increases the at-risk period for the patient.

#### 29.2.4.2 Type of Vaccine

T-cell-dependent vaccines obtains better response than T-cell-independent vaccines because it triggers memory response that leads to a longer protection compared with T-cell-independent vaccine.

#### 29.2.4.3 Other Factors

*The presence of GVHD or ongoing IS treatment* has been associated with a decrease in vaccine response, particularly for polysaccharide-based vaccines.

- Some vaccine responses seem to be not impaired by the presence of GVHD/IS treatment. This is the case of conjugated *Haemophilus* vaccine, conjugated pneumococcal vaccine, conjugated meningococcal vaccine, inactivated polio vaccine, and diphtheria-tetanus vaccine.
- International guidelines recommend different attitudes in patients with GVHD for the moment of vaccine administration.
- Although GVHD may impair response to vaccines, these patients have higher risk of infection and are likely to benefit from vaccination.

*The use of rituximab decreases serological vaccine response at least to tetanus, influenza, and SARS-CoV-2.*

- ECIL 2019 guidelines (Cordonnier et al. 2019): patients who have received rituximab from transplant should have their vaccine pro-

gram delayed at least more than 6 months after the last dose. As the antibody response is uncertain, specific antibody assessment after vaccination can be helpful.

### 29.2.5 Types of Vaccines in HCT Recipients

*Generally recommended for all HCT (auto and allogeneic)*

- Influenza (inactivated/subunit), SARS-CoV-2, poliomyelitis (inactivated), human papillomavirus, pneumococcus, *Haemophilus influenzae*, hepatitis B, meningococcus, tetanus, diphtheria, pertussis, and measles–mumps–rubella (special conditions, see Sects. 29.4 and 29.5).

*Optional/special situations, to cover situations such as after disease exposure or before travel to areas endemic for infections*

- Hepatitis A, tick-borne encephalitis (see Chap. 38, Sect. 38.7.2), Japanese B encephalitis, rabies, yellow fever (live), varicella (Varivax<sup>®</sup>, live).

*Contraindicated: As a general rule, all live vaccines*

- Oral polio vaccine, bacillus Calmette–Guérin, oral typhoid, zoster vaccine (Zostavax<sup>®</sup>), intranasal influenza vaccine, and oral rotavirus vaccine.
- The exceptions for this rule are live vaccines for measles–mumps–rubella that are recommended following strict safety rules (see Sect. 29.4), yellow fever (live) (see specific section), and varicella (Varivax<sup>®</sup>, live); all these vaccines are contraindicated (DIII) before 24 months post-HCT or in case of active GVHD or IS.

#### 29.2.5.1 Use of IVIG and Vaccines

For inactivated vaccines, Ig do not inhibit immune responses. For live virus vaccines, vaccination should not be administered *at least 3 months and ideally 8 months after the last immunoglobulin infusion.*



## 29.3 Benefits and Risks of Vaccination in HCT Recipients

### 29.3.1 Benefits

#### 29.3.1.1 Direct Benefits

The prevention of the specific infectious disease targeted by the vaccine, as shown by pneumococcus, influenza, SARS-CoV-2, and varicella-zoster (subunit vaccine) vaccination. Nonetheless, the majority of the efficacy studies in HCT recipients are based on surrogate markers (serology response) and not on the demonstration of a reduced risk of the infectious disease.

#### 29.3.1.2 Indirect Benefits

The benefits of vaccination can go beyond the prevention of a particular infection, as shown by influenza vaccine. Influenza immunization with inactivated vaccine is recommended by cardiologists as part of comprehensive secondary prevention with the same enthusiasm as the control of cholesterol, blood pressure, and other modifiable risk factors (Davis et al. 2006). In patients with cardiovascular disease, influenza vaccination is associated with a lower risk of all-cause, cardiovascular mortality, and major adverse cardiovascular events compared with control (Yedlapati et al. 2021). Although all these studies were performed in general population, it is logical to assume a similar trend in HCT recipients.

### 29.3.2 Risks

Evidence indicates that *inactivated vaccines* have the same safety profile in immunocompromised patients as in immunocompetent individuals (Beck et al. 2012; Rubin et al. 2014; Cordonnier et al. 2019), and there is no evidence that they induce or aggravate GVHD (Cordonnier et al. 2019). The exception is the SARS-CoV-2 vaccine. There is a risk of worsening/eliciting GVHD in allogeneic HCT recipients with SARS-CoV-2

vaccines. This risk needs to be considered when deciding about time for vaccination (Cesaro et al. 2022). It is possible that the risk for GVHD using the protein-subunit vaccine might be lower and could be considered in individual patients after careful risk assessment (Cesaro et al. 2023).

*Live vaccines* represent a real risk for HCT and should not be used except in special situations with strict requirements (see section of varicella vaccine and ECIL vaccination guidelines table). Fatal disseminated VZV infections due to vaccine strain have been reported in HCT recipients after varicella vaccine and zoster vaccine, even when vaccine was administered several years after transplant (Cordonnier et al. 2019).

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## 29.4 Vaccination Recommendations

There are several international recommendations focused on HCT recipients. The best known are those by the Infectious Disease Working Party (IDWP) of the EBMT, ECIL, CDC, NCCN, and Infectious Diseases Society of America (IDSA). Here, we follow the ECIL recommendations (Cordonnier et al. 2019). *A specific recommendation for vaccination of children after HCT generated and endorsed by the Pediatric Diseases Working Party of EBMT is provided in Sect. 29.6 and Fig. 29.1.*

The IDWP of the EBMT was one of the first cooperative groups that published recommendations specific for HCT recipients. The first ones were published in 1995, with updates in 1999 and 2005. In 2017, guidelines were reviewed and updated under the umbrella of the ECIL group, (Cordonnier et al. 2019) (Table 29.1).

For SARS-CoV2, there are specific guidelines for HCT recipients. IDWP-EBMT had published several updates (<https://www.ebmt.org/covid-19-and-bmt>). ECIL recently updates vaccine recommendations in the ECIL-9 meeting (Cesaro et al. 2022, 2023). For a summary of SARS-CoV-2 vaccination in HCT recipients, see Chap. 38.

## 29.5 Specific Vaccines

### 29.5.1 Influenza

#### 29.5.1.1 Clinical Manifestations

Twenty percent of HCT with confirmed influenza are afebrile.

It is a serious disease in HCT: one-third develops pneumonia, 10% requires mechanical ventilation, and 6% died (Ljungman et al. 2011) (i.e., 100–300 times higher the mortality of influenza in general population). Other complications include encephalitis, which can be lethal, and myocarditis.

#### 29.5.1.2 Influenza and Cardiovascular Disease (CVD)

The majority of influenza deaths are related to lung complications. Nonetheless, in general population up to a third of deaths related to influenza are CV deaths (Loomba et al. 2012).

The risk of acute myocardial infarction is significantly increased after laboratory-confirmed influenza infection (Kwong et al. 2018).

HCT recipients are at high risk of developing CVD. At 10 years, 8% will develop CVD (Armenian et al. 2012).

#### 29.5.1.3 Vaccine

##### Evidence of Vaccine Efficacy

- A retrospective study showed a protection rate of 80% in the rates of virologically confirmed influenza (Machado et al. 2005).
- A systematic review and meta-analysis showed significantly lower odds of influenza-like illness after vaccination in transplant recipients (HCT and SOT) compared with patients receiving placebo or no vaccination (Beck et al. 2012). Seroconversion and seroprotection were lower in transplant recipients compared with immunocompetent controls.
- Given the suboptimal immunogenicity in HCT recipients, family members and health-care professionals involved in the care of these populations should be vaccinated.

##### Vaccine Response (Engelhard et al. 2013; Cordonnier et al. 2019)

- Longer interval from transplant is associated with better serology response. Vaccination

within the first 6 months after transplant produces poor serology responses. Nonetheless, seasonal vaccination against influenza can boost the cellular immune response in HCT recipients as early as 3 months after HCT, but the protective effect is lower compared with healthy controls (Engelhard et al. 2013).

- Conflicting data exist on the benefit of a second dose of vaccine, and marginal benefit was seen with the use of GM-CSF.
- In a recent randomized phase II trial in pediatric allogeneic-HCT, high-dose (60 µg) antigen trivalent IIV was more immunogenic but induced more injection-site reactions than a standard dose (15 µg) (Schuster et al. 2023).
- Rituximab administration during the year before vaccination was associated with a lack of seroprotective titer.
- Active GVHD and low lymphocyte counts at vaccination are associated with poor immune response.

*Live, attenuated influenza vaccine* is contraindicated in HCT recipients.

### 29.5.2 Respiratory Syncytial Virus (RSV)

RSV is a frequent and serious infection in HCT recipients and occur in 1–12% of adult patients with hematological malignancy and HCT. Progression to LRTID is observed in 38% of leukemia and HCT recipients, with an average mortality of 32% (range, 0–70%) (Hirsch et al. 2013).

Recently, 2 RSV vaccines have been approved by the FDA in May 2023 [Abrysvo (Pfizer; unadjuvanted) (Walsh et al. 2023) and Arexvy (GSK; uses same adjuvant as Shingrix) (Papi et al. 2023)], and one by EMA in June 2023 (Arexvy). Both are indicated for active immunization for the prevention of lower respiratory tract disease (LRTD) caused by respiratory syncytial virus in adults 60 years of age and older. Both use a recombinant glycoprotein F stabilized in the pre-fusion conformation. Both vaccines demonstrated significant vaccine effectiveness against RSV-induced lower respiratory tract infection (85.7–

82.6%) among older adults that lasted over at least 2 consecutive seasons. Co-administration with influenza vaccine appears safe without a statistically significant effect on vaccine effectiveness for either vaccine. Availability of both vaccines is anticipated for the 2023–2024 winter RSV season (mid-September through mid-May; peaks late December to mid-February).

Neither has been studied in immunocompromised patients, including HCT. Studies have to be done, but as with other protein recombinant vaccines, no serious problems are anticipated.

### 29.5.3 Measles, Mumps, and Rubella

**The clinical impact and the reasons for immunization in HCT recipients differ among these viruses**

- *Measles*: Severe and also fatal measles infections (pneumonia, encephalitis) have been reported in HCT recipients. The aim of vaccination is to protect the patient of severe consequences of infection.
- *Rubella*: There are no reports of severe rubella disease occurring in HCT recipients. The main indication for rubella vaccination is the prevention of congenital rubella in fertile women.
- *Mumps*: There are no reports of severe mumps occurring in HCT recipients. The indication for mumps vaccination is therefore weak. There is no indication for routine mumps vaccination after HCT. However, mumps is included in combination vaccines with measles and rubella.

#### 29.5.3.1 Vaccines

Only live-attenuated vaccines are available. Presentations: measles alone, combined measles–mumps–rubella, combined measles–mumps–rubella–varicella (live).

### 29.5.4 Hepatitis B Virus (HBV)

*Prevention of infection and reverse seroconversion*

- Approximately 40–70% of HCT recipients obtain a titer of anti-HBs of >10 mIU/mL after post-HCT vaccination, a rather low response

compared with healthy controls. Even those who fail to obtain a response may benefit from vaccination as it can prevent reverse seroconversion.

- Patients who have evidence of a previously resolved hepatitis B infection prior to the transplant (i.e., HBsAg negative but anti-HBs and/or anti-HBc) are at risk of reverse seroconversion.
- Immunization for HBV can prevent HBV reverse seroconversion even in non-responders to hepatitis B vaccine after allo-HCT (Takahata et al. 2014). Probably, antigen-specific memory T-cells and cytotoxic T-cells induced by hepatitis B vaccine are largely responsible for the prevention of reverse seroconversion in non-responders to the vaccine. This reinforces the need of HBV vaccination.

### 29.5.5 Human Papilloma Virus (HPV)

In HCT, women nearly 40% will have genital HPV infection in long-term follow-up (Shanish et al. 2018). HPV is associated with cervical, vulvar, and vaginal cancer in females, penile cancer in males, and anal cancer and oropharyngeal cancer in both females and males.

In long-term survivors, second neoplasias are a significant complication after allo-HCT. Cervix cancer is one of the most frequent. Squamous cell cancers, the commonest posttransplant solid tumors, are associated with HPV infection. Genital HPV disease is a significant late complication of allo-HCT, occurring in one-third of women. Prolonged systemic IS treatment for cGVHD is associated with a higher risk of developing HPV-related squamous intraepithelial lesions.

Regular gynecologic examination, cervical cytology, and HPV testing after HCT are recommended for all women (Majhail et al. 2012) as preventing measure for HPV-related cancer and as a tool for early diagnose and treatment of genital GVHD.

#### 29.5.5.1 Vaccine

- HPV vaccine is a noninfectious, virus-like particle (VLP) vaccine. There are three formu-

lations of HPV vaccines that differ in the number of HPV covered: a 9-valent HPV vaccine (6, 11, 16, 18, 31, 33, 45, 52, and 58 VLPs) (Gardasil 9<sup>®</sup>), quadrivalent HPV vaccine (6, 11, 16, and 18 VLPs) (Gardasil<sup>®</sup>), and bivalent vaccine (16, 18 VLPs) (Cervarix<sup>®</sup>).

- The experience with HPV vaccine in HCT is limited, 20 children (MacIntyre et al. 2016) and 64 adults (Stratton et al. 2020), but shows a good immune response, similar to health women, with no specific safety issue.
- HPV vaccine is recommended in all guidelines (Ljungman et al. 2009; Hilgendorf et al. 2011; Rubin et al. 2014; Cordonnier et al. 2019) but with a low grade of recommendation (B II u to C III) due to the limited experience in HCT recipients. The recommended number of doses is three (Hilgendorf et al. 2011; Rubin et al. 2014).

## 29.5.6 Poliovirus

The WHO European Region was declared polio-free in 2002. Imported wild-type and vaccine-type polioviruses still remain a threat to unvaccinated people in the EU/EEA. Maintaining high vaccination coverage in all population groups remains an essential tool for keeping Europe polio-free.

Only inactivated poliovirus vaccines are used in all EU/EEA countries.

Oral polio vaccine (OPV) is contraindicated for HCT recipients due to the risk of paralytic poliomyelitis. This complication has occurred after vaccination of patients with severe combined immune deficiency and acute lymphoblastic leukemia but has not been described in HCT recipients.

## 29.5.7 Varicella Zoster Virus (VZV)

### 29.5.7.1 Prevention of VZV After HCT

Antiviral prophylaxis (acyclovir/valacyclovir) is the primary mode of prevention. It should be given for at least 1 year after allo-HCT and for 3–6 months after auto-HCT (Cordonnier et al. 2019).

### 29.5.7.2 Types of Vaccines

There are three available vaccines.

- Live-attenuated varicella vaccine, a low-titer VZV vaccine (Varivax1<sup>®</sup>, Varilix1<sup>®</sup>). It is also available in combination in the same vaccine with measles, mumps, and rubella.
- Varicella vaccine can be used in HCT following strict requirements (see ECIL and IDSA vaccination guidelines) (Cordonnier et al. 2019; Rubin et al. 2014). Although vaccination with varicella-attenuated vaccine is indicated/considered in guidelines, in practice it is rarely used due to concerns of safety, particularly in adults (Miller et al. 2017). The commercial availability of the VZ subunit vaccine makes the use of attenuated vaccines even lower.
- Live-attenuated zoster vaccine, a high-titer vaccine (Zostavax1<sup>®</sup>). It contains more than 14 times more virus than varicella vaccine. In all guidelines, this vaccine is contraindicated in HCT patients.
- New adjuvanted VZV subunit vaccine (Shingrix1<sup>®</sup>)

*Adjuvanted VZV subunit vaccine* (Shingrix<sup>®</sup>) consists of recombinant VZV gE antigen mixed with AS01B adjuvant. It was approved by the FDA (October 2017) and EMA (March 2018) for the prevention of herpes zoster (HZ) and post-herpetic neuralgia in adults 50 years of age or older, and in adults 18 years of age or older at increased risk of HZ (in this group, HCT recipients are included). This vaccine is not indicated for the prevention of primary varicella infection (chickenpox). It is administered IM in two doses separated by 60 days.

In a randomized phase 3, double-blind trial in auto-HCT (Bastidas et al. 2019), 2 doses of adjuvanted VZV subunit vaccine (or placebo) were given: the first at 50–70 days after HCT and the second dose 1–2 months thereafter. The vaccine showed a 68.2% efficacy in preventing post-transplant zoster and 89% in preventing post-herpetic neuralgia. The vaccine was well-tolerated, and most symptoms were mild and transient. At the time of ECIL-7 (Cordonnier et al. 2019) guidelines publication, this study

was not published and therefore this vaccine was not commented on the guidelines. Nonetheless, this vaccine has been incorporated to the routine vaccination calendar in many HCT centers. It is also recommended in the last NCCN guidelines (National Comprehensive Cancer Network 2023) for auto-HCT and can be considered in allogeneic.

No randomized study has been done in allogeneic HCT with adjuvanted VZV subunit vaccine, and only limited experience is available. Therefore, the efficacy and safety of this vaccine in allogeneic-HCT have not been established.

### 29.5.8 Pneumococcus

Pneumococcus is a frequent and *serious complication in HCT*. The incidence of invasive pneumococcal disease (IPD) in HCT is 50 times higher compared to the general population (Shigayeva et al. 2016). In spite of this high incidence of IPD, less than one in five HCT recipients with IPD had received pneumococcal vaccine.

#### 29.5.8.1 Types of Vaccine

- Polysaccharidic (PS) vaccine.
  - 23-valent polysaccharidic (PS) vaccine (Pneumo 23<sup>®</sup>, Pneumovax23<sup>®</sup>): poor immunogenic, T-cell-independent response, no boost benefit
  - Poor responses, particularly in patients with GVHD.
  - PS after PCV vaccine increases and expands the response obtained with PCV. Some non-responders to PCV will achieve a response with PS vaccine.
- Conjugate vaccine (PCV): highly immunogenic, T-cell-dependent response, with boost benefit.
  - 13-valent in the majority of countries (Prevenar 13r<sup>®</sup>) (that replace the previous 7-valent vaccine) or 10-valent available in some countries (Synflorix<sup>®</sup>).
  - Five trials have shown a good response to PCV after three doses (range 54–98%). Four trials used 7-valent conjugated vac-

cine and one the 13-valent vaccine (Cordonnier et al. 2019). These responses are much better compared with what is obtained with PS vaccine.

- Early vaccination at 3 months is not inferior to late vaccination (9 months) after allo-HCT.
- PCV should always be administered before PS vaccine.

Conjugate vaccine (PCV) has shown higher efficacy in preventing invasive pneumococcal disease (IPD) compared to polysaccharidic (PS), both in autologous and allogeneic HCT recipients (Roberts et al. 2020).

### 29.5.9 Diphtheria–Tetanus–Pertussis

The exposure to *tetanus* in the environment is a real risk for HCT recipients, so the aim of vaccination after transplant is to protect the patient.

*Diphtheria* has essentially been eradicated, but ongoing vaccination is critical for immunity. Diphtheria cases are still happening in Europe with an increase of 280% from 2009 to 2014. The reappearance of diphtheria cases in countries like Spain diphtheria-free for more than 30 years (Jane et al. 2018) is alarming and another reason to vaccinate all our HCT recipients.

There are very limited published data of *per-tussis* in HCT and no reported case of severe or fatal pertussis infection after HCT in adults. Therefore, the objective of vaccination in these patients is avoiding pertussis transmission by HCT recipients.

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## 29.6 Vaccinations in Children Post-allogeneic Hematopoietic Cell Transplantation

Early and comprehensive re-immunization is important post-HCT and should recognize step-wise recovery of the immune system after HCT and potential impact of immunosuppressive therapy (IST) for prophylaxis/treatment of GvHD that impacts immune recovery.

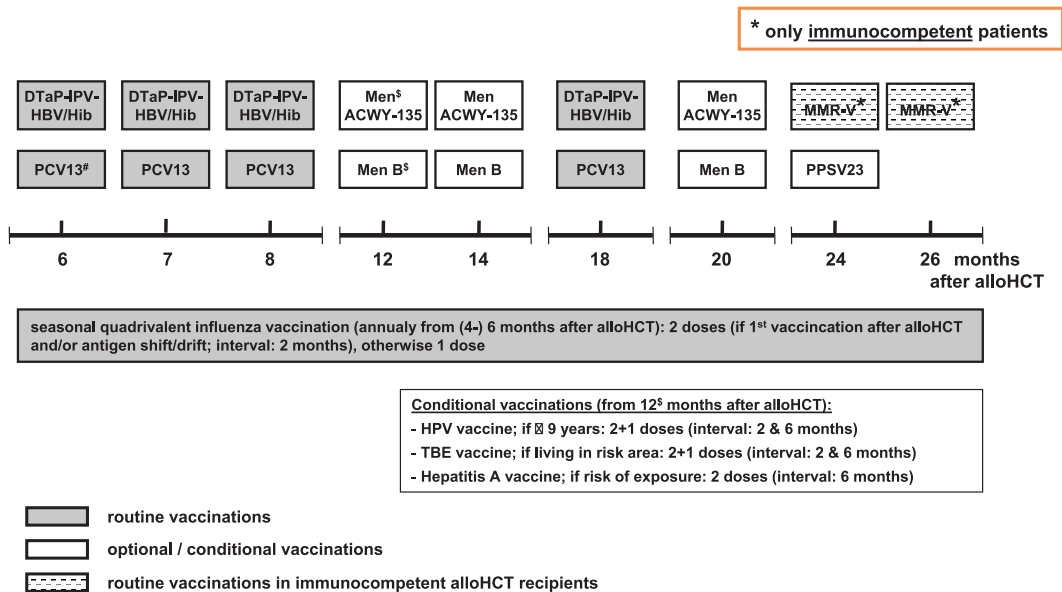
An expert group of pediatric infectious disease and transplant physicians within the Pediatric Diseases Working Party of the EBMT identified the specific clinical demand, reviewed currently available evidence *focusing on the pediatric age group* and in 2021, generated the current consensus recommendation detailed in Fig. 29.1 and publication (Ifversen et al. 2021). Major aspects are summarized in the following paragraphs.

### 29.6.1 General Principles for Immunization Post-HCT in Children

Based on data from a retrospective analysis of revaccination of pediatric HCT recipients from Great-Britain (Patel et al. 2007), the prospective IKAST trial on vaccination of children after HCT (Meisel et al. 2007), and a trial on 13-valent pneumococcal conjugate vac-



## Consensus recommendation for vaccination of children after alloHCT



**Fig. 29.1** Recommended and optional/conditional vaccinations after allogeneic hematopoietic cell transplantation in childhood.

DTaP-IPV-HBV/Hib, hexavalent diphtheria, tetanus, acellular pertussis, inactivated poliovirus, hepatitis B, Haemophilus influenzae type B vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; Men ACWY-135, quadrivalent meningococcal conjugate vaccine; Men B, recombinant meningococcal type B vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; MMR-

V, live attenuated measles, mumps, rubella, varicella-zoster virus vaccine; HPV, human-papilloma-virus vaccine; TBE, tick-borne-encephalitis vaccine; HAV, hepatitis A vaccine. #Start of vaccination at 3 months post-HCT possible after individual risk-benefit analysis (please refer to text). §Start of vaccination at 6 months post-HCT possible after individual risk-benefit analysis (please refer to text). \*Only immunocompetent patients post-HCT, if ≥3 months without immunosuppressive therapy and ≥3 months without active cGvHD



nation in HCT recipients (Cordonnier et al. 2015), the following recommendations are made:

- Use a fixed starting time point for re-vaccination with the newborn DTaP/IPV/HBV/Hib combination vaccine and the 13-valent pneumococcal conjugate (PCV13) vaccine 6 months post-HCT [if leukocyte engraftment and platelets  $\geq 50 \times 10^9/L$ ] and immunize irrespective of donor/graft type, GvHD, IST, and/or measures of immune recovery.
- Use combination vaccine DTaP/IPV/HBV/Hib irrespective of chronologic age.
- Optional/conditional vaccinations should not interfere with evidence-based immunizations (DTaP/IPV/HBV/Hib, PCV13) starting at 6 months. Optional/conditional vaccinations preferably start at 12 months post-HCT.
- Immunization with *non-live vaccines* is safe during IVIG replacement as there is no specific risk besides non-response. Check titers 3 months after stopping IVIG.
- Start vaccination with live vaccines (MMR-V) not earlier than 24 months post-HCT and restrict to immunocompetent patients without GvHD and IST  $\geq 3$  months and off IVIG substitution.
- Consider checking antibody concentrations prior and 1 month after primary series in patients with GvHD, IST, IVIG treatment, and/or delayed immune reconstitution.

Additional note: Single-center experience indicates that providing non-live vaccines earlier than 6 months post-HCT may be feasible in children with very swift immune recovery. However, there are no published data on this policy and limited induction of immunologic memory and duration of protection must carefully be weighed against potential earlier protection.

## 29.6.2 Specific Recommendation for Selected Vaccines

### 29.6.2.1 Influenza

High risk for life-threatening influenza-virus infection post-HCT mandates annual immunization with inactivated influenza vaccines comprising quadrivalent strain coverage. Two doses should be given for first influenza vaccination post-HCT and after antigenic shift/drift.

### 29.6.2.2 Pneumococcus

PCV13 comprises the majority of pneumococcal serotypes detected in invasive pneumococcal disease post-HCT. Administration of the 23-valent pneumococcal polysaccharide vaccine (PPSV23) at 24 months may broaden protection. However, sparse data on the immunogenicity of PPSV23 with regard to serotypes reaching beyond PCV13 result in an optional recommendation for PPSV23 post-HCT.

### 29.6.2.3 Meningococcus

No data exist on the specific risk for invasive meningococcal disease post-HCT, but immunocompromised patients represent candidates for meningococcal vaccination. Clinical relevant protection requires vaccination with both A/C/W/Y135 conjugate and recombinant MenB vaccines. Only few disappointing data are available for A/C/W/Y135 conjugate and no data with MenB vaccination post-HCT resulting in an optional recommendation for meningococcal vaccination starting at 12 months post-HCT.

### 29.6.2.4 Human Papilloma Virus

All adolescent transplant recipients should receive HPV vaccination starting from 12 months post-HCT.

### 29.6.2.5 Varicella-Zoster Virus

High incidence of VZV reactivations with substantial morbidity exists in the first 2 years post-HCT. Only few case series report on the use of the live-attenuated VZV vaccine in pediatric

HCT recipients. Immunization can only be instituted at 2 years after HCT coming too late to prevent the major burden of VZV reactivation. These considerations lead to an optional recommendation for immunization with live-attenuated VZV vaccine in immunocompetent children at least 24 months post-HCT. Vaccination of family members and household contacts is urgently recommended. If a post-vaccination rash develops, the vaccinated should avoid contact with HCT recipients who may receive aciclovir prophylaxis. Non-live VZV vaccines have recently been investigated in immunocompromised hosts. An inactivated VZV vaccine as well as an adjuvanted VZV subunit vaccine prevented zoster reactivation in adult autologous HCT recipients. No data are available for either of these vaccines in the post-HCT setting. Thus, no recommendation can be made for their use in children post-HCT.

### 29.6.2.6 COVID-19

During the COVID-19 pandemic and with COVID-19 now being endemic, it seems prudent to administer COVID-19 vaccines—under the condition that they are non-live and non-replicating—to all recipients of allogeneic HCT, as far as they are available and labeled for use in children and adolescents. In analogy with the recommendation for the influenza vaccination, in the current active pandemic situation, the immunization may be started earlier than 6 months after alloHCT e.g., at 3 months. It is recommended to do antibody assessments whenever available prior to and 4 weeks after (last) vaccination of the primary series in order to assess immunogenicity as information on this is lacking, in particular in the setting of pediatric alloHCT. This recommendation is in accordance with the EBMT guideline for COVID-19 vaccination in allogeneic HCT recipients (<https://www.ebmt.org/covid-19-and-bmt>). In the current endemic situation, it appears logic to re-vaccinate annually as with influenza vaccines.

**Table 29.2** Vaccinations before travel to areas endemic for infections (Ljungman et al. 2009)

If contraindications for the vaccine exist, the patient should be advised not to travel to endemic areas (CIII) Vaccination is one of the precautions that the HCT patients should observe. There are other equal important measures that should be followed: chemoprophylaxis against malaria; mosquito-oriented precautions; food safety to prevent traveler's diarrhea; avoiding sun exposure, particularly for those under treatments associated with photosensitivity (like voriconazole)	
Tick-borne and Japanese B encephalitis	<ul style="list-style-type: none"> <li>• According to local policy in endemic areas (CIII), no data exist regarding the time after HCT when vaccination can be expected to induce an immune response</li> </ul>
Rabies	<ul style="list-style-type: none"> <li>• Rabies vaccine is made from killed virus and cannot cause rabies. Nonetheless, there are no data regarding safety, immunogenicity, or efficacy among HCT recipients</li> <li>• Preexposure rabies vaccination should probably be delayed until 12–24 months after HCT</li> <li>• Postexposure administration of rabies vaccine with human rabies Ig can be administered any time after HCT, as indicated</li> </ul>
Yellow fever (live)	<ul style="list-style-type: none"> <li>• Limited data regarding safety and efficacy (C III). Yellow fever vaccine has been safely administered to a limited number of post-HCT patients (Rubin et al. 2014)</li> <li>• The risk–benefit balance may favor the use of the vaccine in patients residing in or traveling to endemic areas</li> </ul>

## 29.7 Vaccinations Before Travel to Areas Endemic for Infections (See Table 29.2) (Ljungman et al. 2009)

**Table 29.2** (continued)

Hepatitis A	<ul style="list-style-type: none"> <li>• Follow recommendations for general population in each country (CIII)</li> <li>• Ig should be administered to hepatitis A-susceptible HCT recipients who anticipate hepatitis A exposure (for example, during travel to endemic areas) and for postexposure prophylaxis</li> </ul>
Typhoid (IM), inactivated vaccine	<ul style="list-style-type: none"> <li>• No data were found regarding safety, immunogenicity, or efficacy among HCT recipients. DIII. Remember that typhoid oral vaccine is live attenuated and is contraindicated in HCT patients (DIII)</li> </ul>
Cholera	<ul style="list-style-type: none"> <li>• No data were found regarding safety and immunogenicity among HCT recipients. Vaccine is not recommended (DIII)</li> </ul>

## 29.8 Serological Testing

For the majority of vaccines, no pre- or postvaccination serology is recommended. Nonetheless, there are exceptions for this rule (Ljungman et al. 2009).

Antibody titer assessment can be useful to evaluate the need for some vaccines (e.g., HBV 6 months after transplant, measles, mumps, rubella, and LAVV 24 months after transplant),

to decide the need for vaccination or for a second dose or series in the presence of predictors of poor response (Cordonnier et al. 2019).

### 29.8.1 Prevaccination

Testing for Abs to measles is recommended in adults, with vaccination performed if the patient is seronegative (BIIfu).

If vaccination against varicella is contemplated, testing of immunity should be carried out and vaccination should be administered to seronegative patients only (BIIfr).

### 29.8.2 Postvaccination

Pneumococcal vaccine: Pneumococcal antibodies can be assessed at 24 months, although the practical consequences of such assessments—boost or full revaccination program—are to be prospectively evaluated.

Hepatitis B: Testing should be carried out 1 month or later after the third vaccine dose. A second three-dose vaccination schedule is recommended in nonresponders:

Testing should be conducted approximately every 4–5 years to assess for immunity to HBV, measles, tetanus, diphtheria, and polio (BIII).

## 29.9 Vaccinations for Donors, Close Contacts/Family, and HCWs of HCT Recipients (See Table 29.3) (Ljungman et al. 2009; Cordonnier et al. 2019; Rubin et al. 2014)

**Table 29.3** Vaccinations for donors, close contacts/family, and HCWs of HCT recipients

<i>General comments</i>		
Inactivated vaccines can be safely given for donors, close contacts, and HCWs of HCT patients		
For live vaccines, a careful evaluation should be done (see below). Some have no safety issues for HCT recipients, but other can cause severe damage		
<i>Donors</i>		
Guidelines do not recommend donor vaccination for the benefit of the recipient <sup>a,b</sup>		
<ul style="list-style-type: none"> <li>• Only vaccines that are indicated and recommended based on the donor's age, vaccination history, and exposure history should be administered</li> <li>• Nonetheless, vaccination of the donor has been shown to improve the posttransplant immunity of the patient in the case of tetanus, diphtheria, 7-valent pneumococcal conjugate vaccine (PCV), and <i>Haemophilus influenzae</i> type b-conjugate vaccines. Donation is an opportunity to update the donor vaccination calendar. If the donor has to receive any of these vaccines in his/her own interest, the administration of at least one dose pre-collection of stem cells could also benefit the receptor</li> </ul>		
Administration of MMR, MMRV, varicella, and zoster vaccines should be avoided within 4 weeks of stem cell harvest <sup>b</sup> . By extension, all live vaccines should be avoided before stem cell collection due to the risk of transmission of the pathogen with the graft <sup>c</sup>		
<i>Vaccines recommended for close contacts and HCWs of HCT recipients</i>		
<i>Who?</i>	<i>Vaccine</i>	<i>Dose/notes</i>
All	Influenza, inactivated	<ul style="list-style-type: none"> <li>• Annually, as long as there is in contact with an IS recipient<sup>a</sup>: close contacts: AII<sup>a</sup>–AIII<sup>c</sup>; HCWs: AI<sup>a</sup>–AIII<sup>c</sup></li> </ul>
All sero(–) VZ	Varicella: AIII <sup>a</sup>	<ul style="list-style-type: none"> <li>• 2 doses, separated by at least 28 days</li> </ul>
HCWs Sero(–)	Measles	<ul style="list-style-type: none"> <li>• AIII<sup>a</sup>; recommended, not graded<sup>b,c</sup></li> </ul>
<i>Live vaccines given for close contacts or HCWs of HCST patients: precautions</i>		
<i>Intranasal influenza vaccine</i>		<ul style="list-style-type: none"> <li>• If live influenza vaccine is administered to a close contact/HCWs, contact between the IS patient and household member should be avoided for 7 days (weak, very low)<sup>b</sup></li> </ul>
Measles-mumps-rubella		<ul style="list-style-type: none"> <li>• No risk for the HCT patient</li> </ul>
Varicella		<ul style="list-style-type: none"> <li>• The vaccination dose or doses should be completed &gt;4 weeks before the conditioning regimen begins or &gt;6 weeks (42 days) before contact with the HCT recipient is planned (BIII)<sup>a</sup></li> <li>• If a varicella vaccine develops a postvaccination rash within 42 days of vaccination, the vaccinee should avoid contact with HCT recipients until all rash lesions are crusted or the rash has resolved<sup>a</sup></li> </ul>
<i>Oral polio vaccine (OPV)</i>		<ul style="list-style-type: none"> <li>• Oral polio vaccine (OPV) should not be administered to individuals who live in a household with IS patients (strong, moderate)<sup>b</sup>. These vaccinated contacts shed the live-attenuated poliovirus strains of the vaccine in the stools that can induce paralytic poliomyelitis in immunocompromised patients like HCT</li> <li>• If live-attenuated oral polio vaccine, that is still available in some non-US/non-European countries, is given to a household contact, a 4–6-week furlough is advised</li> </ul>
Rotavirus		<ul style="list-style-type: none"> <li>• Rotavirus vaccine is included in the children vaccine calendar of many countries, so it will be frequent that a HCT patient has a child candidate for the vaccine</li> <li>• Virus is shed in stools for 2–4 weeks after vaccination. Transmission from vaccinated to IS person has been confirmed, but there are no reported cases of symptomatic infection in contacts</li> <li>• Highly IS patients should avoid handling diapers of infants who have been vaccinated with rotavirus vaccine for 4 weeks after vaccination (strong, very low)</li> <li>• HCT recipients should have no contact with the stools or diapers of vaccinated children for 4 weeks following vaccination<sup>c</sup></li> </ul>
<i>Vaccines for travel: Yellow fever vaccine; oral typhoid vaccine</i>		<ul style="list-style-type: none"> <li>• Can safely be administered<sup>b</sup></li> </ul>

HCWs: healthcare workers

<sup>a</sup>Ljungman et al. (2009)

<sup>b</sup>Rubin et al. (2014)

<sup>c</sup>Cordonnier et al. (2017)

### Key Points

- Vaccination should be considered a routine practice for all HCT recipients, either autologous or allogeneic, adults or children. It should be implemented in all HCT programs.
- There is no unique vaccine schedule for all HCT recipients. Each center should discuss and adapt a specific vaccine program.
- To obtain this objective, it is necessary to have in place a standardized program specific for HCT recipients with a simple and clear chronology and the collaboration of the Preventive Department of the hospital and primary care physicians.
- Postponing vaccination with a non-live vaccine should be the exception.
- The vaccination program should include not only the HCT recipients but also those who live with the patient and the healthcare workers (HCWs).
- There are two main reasons for universal vaccination of HCT recipients: (a) the general interest as all the population should be correctly vaccinated to avoid holes of immunity that can be a risk for the health of the general population and (b) individual interest for each HCT recipient.

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Alice Polomeni and Angela Scherwath

## 30.1 Introduction

The growing scientific knowledge in the field of allogeneic hematopoietic cell transplantation (alloHCT) has enabled a greater number of patients to access this curative therapy. However, advances in alloHCT such as the expansion of treatment indications and the age of eligible patients, new preemptive protocols and the development of haploidentical transplantation raise new ethical and clinical questions.

AlloHCT remains associated with significant physical and psychological morbidity that may have a negative impact on patients' and their relatives' health-related QOL (Majhail and Rizzo 2013). Psychosocial difficulties occur throughout the alloHCT process, from pretransplant to recovery phase and even in long-term survivors. Insofar, psychological support of alloHCT recipients and caregivers should be based on a preventive and sustainable approach, comprising a broad range of QOL aspects. Psychooncological interventions are regularly planned and con-

ducted in an interdisciplinary approach taking medical and social issues into account.

## 30.2 The Period Preceding HCT

Since alloHCT often appears to be the only therapeutic cure, this can cause high expectations in patients and their families, who may overestimate alloHCT's benefits and underestimate the procedure's morbidity and mortality risks (El-Jawahri et al. 2015b). Information about alloHCT including prognosis, posttransplant effects and its impact on QOL could not only promote 'understood consent' (D'Souza et al. 2015) but also help patients and their close relatives to face the persistent side effects post-HCT (Jim et al. 2014). This suggests a thorough medical as well as psychosocial preparation regarding risks and challenges with concomitant offering of possible coping resources as understanding of prognosis can be associated with depression and worsening QOL over time (El-Jawahri et al. 2015a).

Pre-alloHCT, anxious-depressive symptoms and sleep disruption are frequently described and linked to the burden of uncertainty about treatment outcomes. Baseline anxiety and depression predict worsening QOL during hospitalisation and posttreatment adjustment and are risk factors for survival (Artherholt et al. 2014). Besides, a significant correlation among QOL, fatigue and anhedonia in HCT recipients has been demonstrated (Amonoo et al. 2020). Therefore, a

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thorough survey of the psychosocial anamnesis and a brief screening of psychosocial issues in the course of treatment and survivorship are essential, but screening tools are still underutilized (Barata et al. 2016). To avoid evitable strain, short instruments such as the Distress Thermometer, the Patient Health Questionnaire, the Cancer Treatment-Related Distress Scale and the EORTC QLQ-C30 should be used to measure distress, anxiety, depression and health-related QOL. Moreover, alloHCT teams should screen patients' and caregivers' needs, including psychosocial support to identify and address unmet needs. Tay et al. (2019) consider screening of other psychosocial aspects such as substance abuse, non-compliance and coping styles pre-HCT, not to contraindicate the procedure, but to suggest target interventions. This is especially important as psychosocial factors are intercorrelated with OS (Solh et al. 2020) and NRM (Hong et al. 2022). Pretransplant psychosocial assessment tools such as the Stanford Integrated Psychosocial Assessment for Transplant (SIPAT), the Transplant Evaluation Rating Scale (TERS) or the Psychosocial Assessment of Candidates for Transplant (PACT) may be used (Randall et al. 2022).

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### 30.3 Hospitalisation for HCT

During hospitalisation, patients struggle with considerable changes, including loss of physical abilities and autonomy. HCT hospitalisation constraints, combined with poor physical condition, may increase patients' feelings of isolation and dependence, negatively affecting psychological well-being (Tecchio et al. 2013). Symptoms of depression, anxiety, sleep disruption and adjustment disorders are frequently reported (El-Jawahri et al. 2015b). While anxiety does not change over time, depression levels increase more than twofold after 2 weeks of isolation (Tecchio et al. 2013). These symptoms can go unrecognised but interfere with HCT treatment. Depression during hospitalisation, for example, is associated with longer hospital stay, increased mortality risk, posttransplant anxious-depressive

symptoms and posttraumatic stress syndrome (PTSS) (El-Jawahri et al. 2016).

HCT survival is associated with the presence of a family caregiver (FC) during hospitalisation (Foster et al. 2013). The support provided by the HCT team can also help patients to better cope with hospitalisation and facilitate psychological adjustment after discharge, reducing difficulties in the transition towards outpatient care.

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### 30.4 Post-HCT

Data show that patients in remission for 2–5 years post-HCT have a high probability of long-term survival. Nevertheless, HCT-related morbidity is substantial, negatively affecting physical well-being, psychological functioning and social integration. HCT's late effects have been well described, notably for cGVHD, the severity of which is significantly related to significant anxiety and depression symptoms, impairing psychosocial functioning and diminishing QOL (Majhail and Rizzo 2013; Jacobs et al. 2019).

The evaluation of haploidentical HCT has also raised questions regarding QOL. Most of the retrospective studies indicate that patients with haplo-HCT have an equivalent or even higher QOL (including emotional well-being) compared to patients with other graft sources (Zhang et al. 2022).

Regarding psychopathology post-HCT, several studies reported high rates of anxiety and depression in patients, even several years after transplantation (Jim et al. 2016). An unsettling fact is that depression post-HCT has been associated with higher mortality and increased risk of suicide (Tichelli et al. 2013). Importantly, between 5 and 19% of the patients fulfil a diagnosis of post-traumatic stress disorder (PTSD) (Esser et al. 2017). Since medical complications predicted severity of PTSD symptomatology 1 year after HCT, healthcare professionals should be aware of psychological strain among patients suffering from long-term medical complications.

Another important side effect is cognitive dysfunction. Owing to neurotoxic pretreatments, cognitive impairment occurs in 47% of patients

prior to allo HCT and is found in 41% of patients 1 year after transplant (Scherwath et al. 2013). Depressive symptoms and sleep disorders may increase cognitive dysfunctions. Sleep disruption remains an issue for 43% of HCT patients after transplant (Jim et al. 2016), whereby the association between fatigue, depression and neurocognitive dysfunction needs more clarification (Kelly et al. 2018). Poor neurocognitive functioning leads to lax medication management and adherence to recommended monitoring guidelines, which in turn may increase posttreatment morbidity risks (Mayo et al. 2016).

Psychosocial issues have also been explored in QOL research. Fatigue, sleep disorders, neurocognitive impairment, neurobehavioural problems and sexual dysfunction may persist (Esser et al. 2017). Fear of relapse, feelings of disability and barriers to social rehabilitation are frequent concerns, even several years after the procedure, with only a minority of disease-free transplant survivors consider themselves having ‘returned to normal’ (Syrjala et al. 2012).

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### 30.5 Family Caregivers and Related Donors

Family caregivers (FCs) can contribute to patients’ recovery and to better survival following HCT (Ehrlich et al. 2016). Current research shows that FC experience a significant burden across the treatment trajectory. Before HCT, FCs present higher levels of anxiety and depression symptoms than patients (Posluszny et al. 2019). At the time of transplant, FCs report high levels of fatigue, sleep disorders, depression and anxiety, as well as poorer QOL compared to general population norms (Jamani et al. 2018). FCs may have more emotional difficulties than patients, and their well-being can be impaired well past posttransplant: they face obstacles in their own professional and social lives and express marital dissatisfaction after alloHCT (Langer et al. 2017).

Qualitative data indicate that the main FC difficulties are related to long-term HCT consequences and the unpredictable, uncertain

character of their evolution. Assuming not only daily tasks but also the patients’ psychological support, FCs may feel overwhelmed by the complex demands of the caregiving role and the social impact of a lengthy rehabilitation (Applebaum et al. 2016). In spite of the difficulties met during this posttransplant period, FCs rarely benefit from regular psychosocial support. Like for patients, sufficient information, preparation and guidance should be available for FC to help them manage symptoms of distress and promote adaptive coping (Langer et al. 2020).

Related donors (RDs) deserve particular attention. RDs’ experience is influenced by family dynamics, the quality of recipient–donor interpersonal relationships, the emotional support received and the (often disproportionate) expectations about the benefits of the treatment. Although positive effects of related donation have been demonstrated (e.g. deep personal satisfaction and higher degree of self-esteem), there is also a negative impact, notably pain, anxiety, depression and guilt related to the recipient’s medical condition and HCT outcomes (Garcia et al. 2013). The complexity of related donation is increased in the context of haploidentical transplants. Worel et al. (2022) propose new recommendations regarding donors’ management. The authors stress the importance of considering psychosocial aspects in the initial assessment of the donation. In sum, data suggest that psychological support and follow-up should also be offered to RD.

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### 30.6 Adolescents and Young Adults (AYA)

The adolescent and young adult (AYA) group represents a particular group that significantly varies from non-AYA patients, especially in psychosocial aspects (Mathanda et al. 2020). HCT appears to be a risk factor for poor health-related QOL and social functioning in AYA cancer survivors. In their review, Mehta et al. (2018) identify unmet needs in this population who presents with specific psychosocial issues, notably social reintegration (school, work, peer relationships)

posttreatment. Compared to the healthy general population, these patients show more difficulties in physical, emotional and social functioning. An important issue concerns fertility preservation and providing counselling on this subject prior to HCT is imperative.

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### 30.7 Paediatric Patients

Paediatric alloHCT has been shown to induce disruptions in family life, with an increased incidence of anxious-depressive disorders and post-traumatic stress syndromes in patients, parents and siblings. Paediatric patients also experience declines in cognitive abilities, social functioning and self-esteem. Multiple chronic health conditions, cGVHD, fatigue and pain are related to worse mental health in these patients (Di Giuseppe et al. 2020).

Despite high rates of psychological symptoms, which may persist over time, psychosocial issues may be underestimated by HCT teams. Pai et al. (2019) propose that a systematic screening of these issues using a standard tool (PAT-HCT) may promote the development of targeted interventions for patients and families.

As follow-up of childhood HCT survivors including surveillance of physical and psychological late effects of allo-HCT is fundamental, special attention should be paid to the risk of withdrawal as they journey towards adulthood (Chow et al. 2016).

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### 30.8 Psychological Interventions

Despite their incidence, anxious-depressive symptoms and psychosocial difficulties are not currently reported in HCT settings. Barriers to approach psychosocial services are, for example, patients' fear of being stigmatised and doctors who tend to prioritise strictly medical aspects. Healthcare professionals often poorly evaluate psychological symptoms: anxiety is overrated, depression is underestimated and the consistency between the patients' and the medical teams'

evaluation seems insufficient. However, even when psychosocial preHCT evaluation takes place and patients were subsequently encouraged to follow-up with psychosocial services, only 14% followed-up with psychotherapy. Importantly, high-need patients underutilised this offer, while a subset of patients with low levels of distress, depression and anxiety made use of it (Penalba et al. 2018). Another study shows that of alloHCT recipients reporting distress, only 39% were taking antidepressant or anxiolytic medications and 22% were receiving psychotherapy (Hefner et al. 2014).

Psychological support should be offered during all stages of HCT, from before the procedure to after care, and should cover different approaches like psychodynamic interviews, psychoeducation, biobehavioural methods and communication skills. Cioce et al. (2020) show that patient education, conducted by a nurse, a dietician and a psychologist about a week before HCT, reduces anxiety and depression, ameliorating patients' QOL. Specific techniques to ameliorate anxiety as well as side effects like pain, sleeplessness, nausea or restlessness comprise relaxation, imagery and hypnotherapeutic approaches (Syrjala et al. 2012). After alloHCT, manualised psychooncological therapies combining psychoeducational elements with group-format psychological therapy are well-trying. A telephone-based cognitive-behavioural approach showed to decrease general distress as well as depressive and PTSD symptoms (DuHamel et al. 2010), while internet-based interventions on coping had not improved recipients' psychological functioning (David et al. 2013). A personalised online program focused on cancer-related distress, depression, fatigue and healthcare needs proposed to alloHCT survivors (INSPIRE) has been reached notably by patients with cGVHD and moderate levels of cancer-related distress, while engagement in this program was not influenced by social factors (Syrjala et al. 2018).

Target interventions have also been developed to support FC, like problem-solving skills, cognitive-behavioural interventions and expressive talking (Applebaum et al. 2016) as well as

couple-based communication intervention for HCT survivors and their partners (Langer et al. 2018).

Regarding AYA, psychosocial interventions should include specific problems such as family relationships and social integration (school and work). The role of peer support has not been sufficiently explored, but Rini et al. (2014) show that a peer support intervention reduced general distress and improved QOL in alloHCT survivors experiencing high survivorship problems. In the paediatric setting, interventions to reduce caregiver distress have proven to be effective (Manne et al. 2016).

### Key Points

- The rates of psychological morbidity in HCT patients emphasise the need for clinical assessment throughout the procedure and at regular intervals.
- Given their vital role in the patients' recovery process, HCT teams should also assess FC for psychological adjustment and family functioning.
- Particular attention should be given to RDs, who do not benefit systematically from a medical and psychological follow-up. Haploidentical transplant raises new ethical and clinical issues regarding related donation.
- Paediatric and AYA patients are especially vulnerable groups with a high psychosocial burden needing specific survivorship care to support them on their way of transition to adulthood.
- Regardless of the overwhelming evidence of psychological morbidity in HCT patients and in FC, barriers still exist in discussing psychosocial issues in routine care.
- Systematic screening may contribute to stimulate discussion of psychological symptoms, but quality psychosocial care requires team training and an effective multidisciplinary approach.

- Effectiveness of psychooncological interventions is widely proven and should be adapted to patients and FCs all along the course of alloHCT.

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# Clinically Relevant Drug Interactions in HCT

# 31

Tiene Bauters and Adrin Dadkhah

## 31.1 Introduction

Patients undergoing HCT receive polymedication which carries the potential to result in drug interactions. To avoid unexpected outcomes, attention to drug interactions is crucial, especially when drugs with a narrow therapeutic index or inherent toxicity profile are involved (Leather 2004; Glotzbecker et al. 2012; Gholaminezhad et al. 2014).

Drug interactions can be defined as changes in a drug's effect due to recent or concurrent use of another drug, food, or environmental agent. The net effect of the combination can result in enhanced activity of the affected drug, possibly leading to toxicity, or reduced activity leading to therapeutic failure (Thanacoody 2012).

In general, drug interactions can be categorized as being pharmacodynamic, pharmacokinetic, or pharmaceutical in nature.

### 31.1.1 Pharmacodynamic Interactions

Pharmacodynamic interactions occur when the effect of one drug is changed by the presence of another drug at its site of action. They compete for specific receptor sites or interfere indirectly with physiological systems.

The effect can be additive/synergistic or antagonistic. An example of an additive interaction is the concurrent use of QT-prolongating drugs (e.g., ciprofloxacin and fluconazole) which substantially increases the risk of torsades de pointes or other ventricular tachyarrhythmias.

Specific antagonists can be used to reverse the effect of another drug at the receptor site (e.g., naloxone, an opioid receptor antagonist which reverses signs of opioid intoxication) (Lexicomp drug interactions 2023).

### 31.1.2 Pharmacokinetic Interactions

Pharmacokinetic (PK) interactions occur when one drug alters the rate or extent of absorption, distribution, metabolism, or elimination of another drug resulting in diminished effects or drug potentiation (Palleria et al. 2013). An example of decreased absorption is observed for quinolones in combination with polyvalent cations or several tyrosine kinase inhibitors (e.g., acalabrutinib and dasatinib) with proton pump inhibitors. However, the most frequent and significant

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drug interactions relate to drug metabolism. These will be further discussed here.

### 31.1.2.1 Cytochrome P450 Enzyme System

Several enzyme families are involved in drug metabolism, cytochrome P450 (CYP450) being the most important one. CYP450 consists of an unique group of isoenzymes grouped into families (1–3) and divided into subfamilies (A–E). They are primarily found in the liver and are genetically encoded (Ingelman-Sundberg and Rodriguez-Antona 2005; Lynch and Price 2007).

The effect of a CYP450 isoenzyme on a particular substrate can be altered by interaction with other drugs. Drugs can be substrates for a CYP450 isoenzyme and/or may inhibit or induce the isoenzyme (Larson 2018; Glotzbecker et al. 2012; Leather 2004):

*Inhibition:* Leads to reduced metabolism of the substrate with an increase in the steady-state concentration. It potentiates the effect and might lead to enhanced or toxic effects, especially in drugs with a narrow therapeutic index like cyclosporine and tacrolimus. Its onset occurs within 1–3 days for drugs with a short half-life, while the maximal effect may be delayed for drugs with a long half-life.

*Induction:* Increases the activity of CYP450 enzymes and usually results in decreased concentration/effect of the affected drug with the risk of therapeutic failure. Since the process of enzyme induction requires new protein synthesis, the effect usually occurs over days to weeks after starting an inducer.

Prodrugs rely on enzymes for conversion to their active form(s). The combination of a pro-drug (e.g., thiotepa or cyclophosphamide) with a CYP450 inhibitor may result in therapeutic failure because of little or no production of the active drug. Conversely, an exaggerated therapeutic effect or adverse effect can be expected when a CYP450 inducer is added (Lynch and Price 2007).

In general, any drug metabolized by one of the CYP450 enzymes has the potential for

PK-interaction, and concurrent use should be done with caution. For instance, the inhibition of CYP1A2 by ciprofloxacin may result in a ten-fold increase of tizanidine exposition and therefore leads to sudden hypotension. As CYP3A4 is responsible for the metabolism of more than 50% of clinically administered drugs (Ingelman-Sundberg and Rodriguez-Antona 2005; Larson 2018), examples of CYP3A4 substrates, inhibitors, and inducers used in HCT are presented in Table 31.1.

Mutations in CYP genes give rise to four major phenotypes: poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultrarapid metabolizers (Ingelman-Sundberg and Rodriguez-Antona 2005; Ahmed et al. 2016). Polymorphisms in CYP450 are of concern in the study of interindividual altered drug metabolisms and/or adverse drug reactions.

### 31.1.2.2 Drug Transportation

P-glycoprotein (PgP) is a plasma membrane transporter involved in the excretion of drugs. Its activity is inhibited or induced by most drugs that inhibit/induce CYP3A4, resulting in increased or decreased bioavailability/clearance of PgP substrates (Ingelman-Sundberg and Rodriguez-Antona 2005; Glaeser 2011; Thanacoody 2012).

### Monoclonal Antibodies

Metabolism of monoclonal antibodies (MABs) does not involve CYP450 enzymes or drug transporters; therefore, PK interactions between MABs and conventional drugs are very limited. However, current information in this area is not abundant, and more research is needed (Ferri et al. 2016).

## 31.1.3 Pharmaceutical Interactions

Pharmaceutical interactions manifest when two or more drugs and their diluents are mixed in the same infusion bag/syringe or when infusion lines (including parenteral nutrition) meet at a Y-site junction. They are the result of incompatibilities and can cause visible changes including color, turbidimetry, and precipitation or non-visible

**Table 31.1** CYP3A4 substrates, inhibitors, and inducers commonly used in HCT (non-limitative list) (Lexicomp drug interactions 2023, MedicinesComplete 2023, Stockley's Interactions Checker)

Substrates	Inhibitors	Inducers
Acalabrutinib	Amiodarone	<b>Barbiturates (phenobarbital)</b>
Apixaban	Aprepitant	<b>Carbamazepine</b>
Benzodiazepines <sup>a</sup>	Cimetidine	Corticosteroids
Budesonide	Ciprofloxacin	<b>Phenytoin</b>
Calcium Channel Blockers <sup>b</sup>	<b>Clarithromycin</b>	Rifabutin
Carbamazepine	Diltiazem	<b>Rifampicin</b>
Corticosteroids	Erythromycin	St John's wort
Cyclosporine	Fedratinib	
Etoposide	Fluconazole	
Gilteritinib	Grapefruit juice	
Macrolide antibiotics <sup>c</sup>	<b>Itraconazole</b>	
Rivaroxaban	<b>Ketoconazole</b>	
Sirolimus	Letermovir	
Statins <sup>d</sup>	Nirmatrelvir- <b>Ritonavir</b>	
Steroids <sup>e</sup>	<b>Posaconazole</b>	
Tacrolimus	<b>Voriconazole</b>	
Thiotepa	Verapamil	
Quetiapine		
Venetoclax		
Miscellaneous <sup>f</sup>		

Bold font indicates strong inhibitors/inducers

<sup>a</sup> Alprazolam, diazepam, midazolam

<sup>b</sup> Amlodipine, diltiazem, verapamil

<sup>c</sup> Clarithromycin, erythromycin, NOT azithromycin

<sup>d</sup> Atorvastatin, simvastatin NOT pravastatin, fluvastatin

<sup>e</sup> Estradiol, progesterone, testosterone

<sup>f</sup> Aprepitant, fentanyl, ondansetron, zolpidem

changes that may also lead to a significant drug degradation. To assess the compatibility and stability of intravenous drug mixtures, multiple factors need to be taken into consideration. Therefore, establishing compatibility charts or consulting the hospital pharmacy is advisable.

### 31.2 Drug Interactions in HCT Practice

Drug interactions can occur as early as during the conditioning regimen. Drugs as etoposide and thiotepa rely on CYP450 enzymes for metabolism, while cyclophosphamide needs to be converted to become functional. Also, there

are several clinically relevant drug interactions for busulfan, which is known to have a high interindividual variability considering the ratio of dose and drug exposure. Therefore, to maintain the narrow therapeutic range, it is recommended to conduct therapeutic drug monitoring for busulfan in myeloablative conditioning regimens (McCune and Holmberg 2009). A non-limitative list of PK interactions with busulfan and recommendations for management are summarized in Table 31.2.

Many clinically relevant interactions have been reported with calcineurin inhibitors (cyclosporine and tacrolimus) and sirolimus. A non-limitative overview of PK interactions with these drugs is presented in Table 31.3.

**Table 31.2** Drug interactions with busulfan (BU) (non-limitative list)<sup>a</sup>

Interacting drug	Proposed mechanism	Effect	Recommended action
Blinatumomab	Unclear (probably cytokine-mediated CYP3A4-inhibition)	▲ BU levels	
Deferasirox	Unclear (probably inhibition of CYP2C8 and CYP1A2)		– Concurrent administration possible, however TDM necessary
Paracetamol	Competition for glutathione		– Avoid paracetamol within 72 h prior to or concurrently with BU
Metronidazole	CYP3A4 inhibition Competition for glutathione		– Monitor for increased BU concentrations/toxicity when used concurrently
Itraconazole Posaconazole Voriconazole	Unclear (probably CYP3A4 inhibition)		– Monitor for increased BU concentrations/toxicity when used concurrently
Phenytoin	CYP3A4/glutathione-S-transferase induction	▼ BU levels	– Use alternative antiepileptic (levetiracetam)

<sup>a</sup> Lexicomp drug interactions (2023), Glotzbecker et al. (2012), Myers et al. (2017), Sweiss et al. (2019) and Essmann et al. (2021)

**Table 31.3** Pharmacokinetic interactions with cyclosporine (C), tacrolimus (T) and sirolimus (S) (non-limitative list)<sup>a</sup>

Interacting drug	Proposed mechanism	Effect	Recommended action
<i>Anticoagulants</i>			
Dabigatran Edoxaban	P-gP/ABCB1 inhibition	– ▲ dabigatran/edoxaban level	• Monitor closely for excessive clinical response to dabigatran/edoxaban
<i>Antivirals</i>			
Letermovir	CYP3A4 inhibition	– ▲ C/T/S levels	• Monitor C/S/T levels (decrease letermovir dose when combined with C)
<i>Antiepileptics</i>			
Carbamazepine Phenobarbital Phenytoin	CYP3A4 induction	– ▼ C/T/S level	• Monitor C/T/S levels • Increased C/T/S doses will likely be needed • Consider therapy modification (levetiracetam)
<i>Antifungals</i>			
Caspofungin	Unknown	– C: ▲ adverse/toxic effect of caspofungin – ▼ T/S levels	• Monitor liver function/hepatotoxicity in combination with C • Monitor T/S levels and adjust as necessary
Fluconazole Itraconazole Posaconazole Voriconazole	CYP3A4 and/or P-gP inhibition	– ▲ C/T/S levels	• Monitor clinical response of C/T/S closely • Monitor C/T/S levels closely • Decreased C/T/S doses will likely be needed • Itraconazole: consider therapy modification (C/T/S) • Posaconazole/voriconazole: consider therapy modification (C/T), avoid combination (S)

**Table 31.3** (continued)

Interacting drug	Proposed mechanism	Effect	Recommended action
<i>Calcium channel blockers</i>			
Diltiazem Verapamil	CYP3A4 inhibition	– ▲ C/T/S levels	<ul style="list-style-type: none"> <li>• Monitor C/T/S levels</li> <li>• Decreased doses of C/T/S might be needed</li> <li>• Monitor for decreases in blood pressure (C/T)</li> <li>• Consider therapy modification (C/S)</li> </ul>
<i>Calcineurin inhibitors</i>			
Cyclosporine	CYP3A4 competition	<ul style="list-style-type: none"> <li>– T: ▲ levels/nephrotoxicity of C/T</li> <li>– S: ▲ levels of S (of specific concern with modified C)</li> </ul>	<ul style="list-style-type: none"> <li>• Discontinue C/T therapy at least 24 h prior to initiating therapy with the other agent</li> <li>• C/T: avoid combination</li> <li>• Monitor for toxic effects of S</li> <li>• S: ▲ risk of C-induced HUS/TTP/TMA</li> <li>• Administer oral doses of S 4 h after doses of C</li> <li>• C/S: consider therapy modification</li> </ul>
Tacrolimus		<ul style="list-style-type: none"> <li>– C: ▲ levels/nephrotoxicity of C/T</li> <li>– S: ▲ adverse/toxic effect of T/S, ▼ level of T</li> </ul>	<ul style="list-style-type: none"> <li>• Avoid combination with C/S (enhanced toxicity of C/T/S)</li> </ul>
<i>Corticosteroids</i>	CYP3A4/PgP induction CYP3A4 substrate	<ul style="list-style-type: none"> <li>– ▼ C/S/T levels</li> <li>– ▲ effect of corticosteroid</li> </ul>	<ul style="list-style-type: none"> <li>• Monitor for changes in C/T levels and toxic effects of T/C when tapering corticosteroids</li> </ul>
<i>Macrolide antibiotics (not azithromycin)</i>			
Clarithromycin Erythromycin	CYP3A4/PgP inhibition	<ul style="list-style-type: none"> <li>– ▲ C/T/S levels</li> <li>– S: ▲ level of erythromycin</li> </ul>	<ul style="list-style-type: none"> <li>• Monitor C/T/S levels and adjust dose accordingly</li> <li>• S/T: consider therapy modification</li> </ul>
<i>Proton pump inhibitors (PPI, not pantoprazole)</i>			
Omeprazole Lansoprazole	C: Unclear T: CYP3A4/ CYP2C19 inhibition	– ▲ C/T level	<ul style="list-style-type: none"> <li>• Monitor C/T levels closely when starting or stopping therapy with PPI and adjust dosage if necessary</li> <li>• Inconsistent data (omeprazole), pantoprazole may be less likely to significantly interact</li> </ul>
<i>Statins</i>			
Atorvastatin Simvastatin	CYP3A4 inhibition and inhibition of OATP1B1-mediated hepatic uptake	<ul style="list-style-type: none"> <li>– C: ▲ level of atorvastatin/simvastatin</li> <li>– T: Limited effect</li> </ul>	<ul style="list-style-type: none"> <li>• Monitor for increased risk for statin-related toxicities (myopathy and rhabdomyolysis)</li> <li>• C: Avoid concurrent use atorvastatin/simvastatin</li> <li>• T: No action needed</li> </ul>
<i>Miscellaneous</i>			
Grapefruit juice	CYP3A4 inhibition (intestinal)	– ▲ C/T/S levels (C/T: Primarily limited to orally administered C/T)	<ul style="list-style-type: none"> <li>• Monitor C/T/S levels</li> <li>• Avoid combination with orally administered C/S/T</li> </ul>
Metronidazole	CYP3A4 inhibition	– ▲ C/T levels	<ul style="list-style-type: none"> <li>• Monitor C/T/S levels (standard measures)</li> </ul>

(continued)



**Table 31.3** (continued)

Interacting drug	Proposed mechanism	Effect	Recommended action
Mycophenolate mofetil (MMF)	Decreased enterohepatic recirculation	<ul style="list-style-type: none"> <li>– C: ▼ active metabolite mycophenolic acid ▲ glucuronide metabolite concentrations (associated with mycophenolate adverse effects)</li> <li>– MMF: ▼ C exposure in children</li> <li>– T: does not affect PK of mycophenolic acid (one study suggests ▲ T exposure)</li> </ul>	<ul style="list-style-type: none"> <li>• Monitor MMF dosing and response to therapy particularly closely when adjusting concurrent C (starting, stopping, or changing dose)</li> </ul>
Rifampicin	CYP3A4/PgP induction	<ul style="list-style-type: none"> <li>– ▼ C/T/S levels</li> </ul>	<ul style="list-style-type: none"> <li>• Monitor levels, increase dose C/T/S accordingly</li> <li>• Consider therapy modification</li> </ul>
St John's wort (SJW)	CYP3A4/PgP induction	<ul style="list-style-type: none"> <li>– ▼ C/T/S levels</li> </ul>	<ul style="list-style-type: none"> <li>• Consider alternatives to SJW</li> <li>• If it cannot be avoided, monitor C/T/S levels</li> </ul>

▼ = decreased; ▲ = increased

<sup>a</sup> Lexicomp drug interactions (2023) and Glotzbecker et al. (2012)

## 31.3 Interactions with Herbal Drugs and Food

### 31.3.1 Herbal Drugs

The use of herbal drugs is growing worldwide, and a number of serious interactions with conventional drugs have been reported (Enioutina et al. 2017). Patients often do not perceive herbal supplements as drugs and prescribers are not always aware that patients are taking these products. A thorough drug history anamnesis is important and should be performed by asking very specific questions about herbal drug use.

An example of an herbal drug frequently involved in major drug interactions is St John's wort (SJW) (*Hypericum perforatum*). SJW is an over-the-counter product commonly used in HCT patients for the treatment of mild depression. SJW can reduce the serum concentration of CYP3A4 substrates as cyclosporine and tacrolimus by induction of CYP3A4 or by increasing PgP expression, resulting in lack of response. Concomitant use of SJW with drugs metabolized by CYP3A4 should be avoided or monitored if no alternative for SJW is available (Enioutina et al. 2017; Lexicomp drug interactions 2023).

### 31.3.2 Food

Drug interactions with food and drinks are known to occur. Grapefruit juice is a potent inhibitor of intestinal CYP3A4, and many clinically relevant interactions have been reported (e.g., with simvastatin and calcineurin inhibitors). Cruciferous vegetables (Brussels sprouts, cabbage, and broccoli) contain substances that are inducers of CYP1A2 but do not appear to cause clinically important drug interactions (Thanacoody 2012).

## 31.4 Resources for Drug Interactions

Drug interactions in HCT can be numerous. Whenever a potential clinically relevant drug interaction is recognized, a management plan should be recommended (modification in drug therapy or closer monitoring of efficacy and adverse reactions) (Tannenbaum and Sheehan 2014). A number of resources are available to help identifying and managing drug interactions (e.g., Lexicomp drug interactions 2023; ClinicalKey 2023; MedicinesComplete 2023). Interpretation of interactions must be performed

carefully to avoid the risk of over-alerting. The patient's clinical status, comorbidities, and severity of the drug interactions presented should always be taken into account.

### 31.5 Conclusion

Drug interactions can occur at all levels during HCT. Attention to and management of interactions are crucial to prevent severe clinical consequences. Due to the complexity of the therapy and the risk of drug interactions, an active collaboration in a HCT multidisciplinary team, including physicians, pharmacists, and nurses, is of paramount importance.

#### Key Points

- Drug interactions in HCT are common and can occur at all levels.
- Knowledge of mechanisms involved in drug metabolism might help in anticipating interactions.
- A multidisciplinary approach is important to reduce the risk of drug interactions.

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Michelle Kenyon and John Murray

### 32.1 Introduction: HCT Nursing

Haematopoietic cell transplantation (HCT) is undoubtedly one of the most challenging and complex forms of treatment for malignant and non-malignant blood disorders as well as autoimmune disease. As a result, nursing in the field of HCT and cellular therapy offers a wealth of opportunities to make a positive difference to patient experience through high-quality specialist nursing care and the unique role of nursing within the transplant MDT. In this section, we will describe the nursing roles that surround the patient pathway and offer an overview the particular aspects of patient care that they encompass.

More than 82,000 transplants are performed globally each year (Niederwieser et al., 2021), and this is increasing, leading to ongoing workforce demands on the background of a largescale shortage of qualified nurses. While global regions face their own specific challenges, poor pay and working conditions for many nurses globally means sustained commitment by governments

and policymakers are needed to urgently address these issues (Jester 2023). Our transplant units, where it takes time to train and develop new staff, are vulnerable to the current workforce deficit and while recruitment is challenging in the face of a global shortage, we can do a lot to retain the staff that we do have. Well-defined career pathways together with a professional development strategy and specialist training opportunities can be a very effective investment.

There is great potential to improving the care that we provide to patients and opportunities for nursing research and innovation should be optimised through local and national research networks. In a recent review of cancer nursing research priorities (Dowling et al., 2023), the role of technology in improving patient and caregiver symptoms and health outcomes was top. Other priorities included those focused on culturally sensitive psychosocial care, financial toxicity, social determinants of health and scope of practice; all highly relevant to the transplant and cell therapy sphere.

In this continually evolving speciality, where nurses are pivotal to patient outcomes, the importance of a well-equipped, professionally educated, knowledgeable and competent workforce is critical. This is reinforced in the FACT-JACIE International Standards where accreditation requires that the clinical programme has access to personnel who are formally trained, experienced and competent in the management of patients receiving cellular therapy (JACIE 8th

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edition standards 2021). Therefore, transplant centres must commit to ongoing nurse education, professional development and research in all aspects spanning novice to expert to ensure sustainability and capability to deliver complex, state-of-the-art treatments in the years ahead.

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## 32.2 The Roles of Nursing Throughout the Patient Transplant and Cell Therapy Pathway

Nursing is recognised as both an emerging profession and an academic discipline. The diverse and continually evolving field of transplantation and cell therapy creates many opportunities for innovation in nursing roles and as such these have been developed to support the patient pre-, peri-, and post-treatment. Nurses and in particular the Clinical Nurse Specialist (CNS) are evaluated as having a positive impact on service delivery (Kerr et al., 2021) in relation to continuity of care, patient advocacy and increased access to services such as the medical consultant (Beaver et al. 2010, Ream et al. 2009). Regardless of the stage of pathway that nurses deliver care to patients, they should be aware of the complications in order to play a role in prevention or early detection of illness such as sepsis, dehydration, electrolyte imbalance and organ dysfunction, taking appropriate measures to minimise adverse effects and deliver prescribed treatment. This care is complex and requires a high level of skill (Walhult et al. 2023).

### 32.2.1 (Central) Venous Access Devices (cVADs)

All nurses, regardless of role, require education and training on the care and maintenance of cVADs, and this should include patient wellbeing and safety. There are a range of devices, and device selection should be based on considerations such as diagnosis, proposed treatment and vein condition.

Within the available devices, a PICC (peripherally inserted central catheter) is frequently used, especially in autologous transplant and CAR-T, with a tunneled catheter such as a Hickman often reserved for allogeneic transplant particularly those receiving myeloablative conditioning therapy.

Nurses are responsible for the care of cVADs and are the main users of these devices for infusions of chemotherapy, medication, parenteral nutrition and transfusions. Safe handling and care of the cVAD and infusions are vital in this process due to the risks associated with catheter-related infections. Aseptic Non-Touch Technique (ANTT) (Pratt et al. 2007, Rowley et al. 2010) has led to a decrease in catheter-related infections.

### 32.2.2 GvHD

Graft-versus-host disease (GvHD) is the most recognised complication post-HCT and was first observed in 1956 (Ghimire et al. 2017) and remains a leading cause of non-relapse mortality and is associated with a high morbidity that increasingly affects quality of life (Lee et al. 2002). Nursing care of patients with GvHD is highly complex and extremely stressful especially in the acute setting in patients with grades 3–4 skin and GI involvement. Supportive nursing care to complement medical interventions aims to offer symptomatic comfort and relief. There are many manifestations of GvHD, and nurses are able to advise patients with respect to many of these including eye, mouth and genital care. For further readings refer to the GvHD chapter in *The EBMT Textbook for Nurses* (Kenyon & Babic, 2023).

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## 32.3 The Transplant Coordinator

The transplant coordinator (TC) is responsible for the preparation of patients and donors in a complex process that requires an expert level of communication and planning. Each patient and their families need to be physically and psycho-

logically prepared to enable them to manage the treatment; the TC is at the centre of this process. Many transplant coordinators are nurse specialists who focus their role on the individual needs of the patient and families; however, some centres have medical staff that occupy this role. Transplant coordinators provide a high level of care and management, with explanations of complicated and complex tests and many have a wide area of latitude to make clinical decisions within departmental SOP's. The TC will participate in specific or advanced nursing practices, e.g. bone marrow sampling, HLA typing, and transplant recipient care.

The TC ensures that a suitable source of cells (autologous or allogeneic) is available. This entails requests to donor search panels and ordering cells once an ideal match has been identified by the transplant physician. The TC supports the patient with verbal and written information and educates them about the whole process. The TC will coordinate all of the care and embodies a clinical nursing function where emphasis is placed on specialisation in a clearly defined area of care.

The TC may also in some institutions take care of the donor, arranging tissue typing and will liaise with the apheresis or bone marrow harvesting team to ensure a smooth process. Sibling donors may often have additional anxieties to that of an unrelated donor, and support from an experienced TC is vital. The TC is involved in the creation of information tools for the patient and the donor which are evaluated in order to have an accurate knowledge of patients' needs. The TC actively participates in the JACIE process of accreditation of transplant centres by writing and evaluating SOPs and being a valued member of the MDT and ward team offering teaching and advice (Kenyon, Babic 2023).

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### 32.4 The Apheresis Nurse

The apheresis nurse is a highly skilled practitioner, who is able to handle the technicalities of harvesting as well as the social, emotional and

ethical aspects (Neyrink & Vrielink, 2019) and be adept at managing an often-fluid starting date and time. Apheresis nurses are an integral part of the transplant process, and the work-up begins as soon as a patient or donor is identified by the team as suitable for harvest. The process for patients and donors (siblings or unrelated) is similar. There are routine blood tests and screening to be performed that are driven by the specific protocol which must be in place prior to the harvest commencing. Once a proposed harvest date is set, for those undergoing an autologous procedure the conditioning therapy can be commenced. For donors, a date for the first dose of G-CSF is given. The day of harvest may change in real time as blood counts may not be optimal on the first day of attendance. This requires excellent administrative and communication skills to rearrange the procedure and inform all concerned parties at short notice of the delay. Often more than 1 day of apheresis is required which again may lead to logistical challenges. Especially if an unrelated donor and cells are to be collected to be taken elsewhere.

The apheresis nurse spends a significant amount of time with the patient or donor during the day(s) of harvest and is able to provide information and answer questions about the transplant process. This can often be a nervous time for the patient who is aiming to provide enough cells for a transplant (HCT or CAR-T) in the future or for the donor who is hoping to give enough cells for a successful transplant for a relative or unrelated recipient. An apheresis nurse is not just a technician.

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### 32.5 The HCT Ward Nurse

Nurses, in particular those caring for patients during the peri-transplant period and the early months after engraftment, are pivotal in implementing practices to prevent and manage infections and other serious effects following HCT (Kenyon & Babic, 2023) such as:

- Bleeding caused by thrombocytopenia



- Fatigue caused by decreased haemoglobin levels and the effects of chemo/radiotherapy and associated medication
- Oral hygiene and pain management due to mucositis
- Gastrointestinal toxicity including nausea, vomiting, diarrhoea and constipation
- Sepsis
- Impaired nutritional intake, weight loss and malnutrition
- Psychosocial concerns
- Effects of protective isolation

The ward nurse, working at the bedside with the patient, is best placed to monitor for early and acute complications. Early complications are generally considered to be those that occur during the first 3 months following transplantation when the patient has reduced tolerance due to neutropenia and/or increased intestinal permeability and in the allogeneic setting, high dose immunosuppression. In neutropenia, the number of white blood cells decreases significantly, resulting in increased risk of infection. An increased permeability of the intestinal wall caused by intensive chemotherapy damages the gastrointestinal mucosa. As a result, pathogenic bacteria (bodily bacteria or bacteria from the diet) can enter the bloodstream and cause sepsis.

In the early phase of HCT, the main risk factors for infections are neutropenia-barrier breakdown due to mucositis, indwelling catheters, depressed T-cell and B-cell function and aGvHD. Two of the most common early complications, **oral mucositis and sepsis**, will be discussed below. Other complications such as haemorrhagic cystitis (HC), eosinophilic syndrome (ES) and diffuse alveolar haemorrhage (DAH) occur less often but can be serious when they do arise. Transplant-associated thrombotic microangiopathy (TA-TMA) and veno-occlusive disease (VOD) are analysed in Chaps. 42 and 49. For all complications, there are locally agreed SOPs often supported by national guidelines, recommendations for prevention and principles for nursing care, with monitoring and prompt intervention that can influence patients' morbidity and mortality.

### 32.5.1 Oral Mucositis (OM)

Oral mucositis (OM) is the inflammation of the mucosal membrane, characterised by ulceration, which may result in pain, swallowing difficulties and impairment of the ability to talk (Al-Dasoogi et al., 2013). The mucosal injury offers a ground for infection which can potentially lead to sepsis and septicaemia (Quinn et al., 2020). However, OM is not the only oral complication seen within the transplant setting but most patients undergoing autologous and allogeneic HCT will experience mucosal changes within their oral cavity leading to difficulties in eating, sleeping and talking and a reduction in quality of life.

Care strategies are aimed at optimising care of the oral cavity, preventing oral damage, infection prevention and treatment of oral complications when they arise. More information on oral mucositis, refer to the early and acute complications chapter in *The EBMT Textbook for Nurses* (2023).

### 32.5.2 Sepsis

Patients undergoing HCT are at increased risk of infection, and this is a leading cause of morbidity and mortality. The signs and symptoms of sepsis can be subtle and sometimes difficult to recognise in the presence of neutropenia and other transplant complications. Preventive measures are important but increased monitoring and the use of early warning scores alongside team collaboration and immediate action can be life-saving, allowing for prompt and appropriate sepsis management.

In the early phase of transplant, the main risk factors for infection are (Rovira et al., 2012)

- Neutropenia
- Barrier breakdown
- Depressed T- and B-cell function
- Presence of aGvHD

Responsibility for the implementation of strategies for infection prevention and control extends to the whole HCT MDT and includes:

- Hand hygiene
- Respiratory hygiene
- PPE
- Safe management and care of equipment
- Safe management of the environment
- Management of laundry
- Management of blood and body fluid spills
- Waste management
- Management of exposure

Early recognition and treatment are vital for a successful outcome of sepsis. Temperature, pulse, blood pressure, respirations and saturation (vital signs) should be frequently monitored. Signs of infection are not always obvious, but if the patient has a temperature  $\geq 38.0$  °C, cultures should be taken, IV antibiotics and IV fluids started or increased and oxygen therapy initiated. The goal is always to *start antibiotic treatment within 1 h* from detection of fever (Swedish “Pro Sepsis” Programme Group Sepsis 2015). This is sometimes referred to as “the golden hour” (or “door-to-needle time” for patients admitted from outside the hospital) and is the most critical period in the patient’s survival from sepsis.

The concept of the *Sepsis Six* has been developed as a guide to prioritise interventions and offer a resuscitation bundle in patients where sepsis is suspected (Daniels et al. 2011).

1. Oxygen therapy
2. Blood cultures
3. IV antibiotics
4. Fluid resuscitation
5. Serum lactate
6. Assess urine output (may require catheterisation)

Patients with sepsis are likely needed additional nursing care such as assistance with oral care and personal hygiene. It is important to ensure that the patient’s and caregivers’ information, education and support needs are met.

On discharge from the hospital, we need to ensure that the patient and their caregiver are aware of when, why and how to contact the clinic

or hospital that they have a fever thermometer at home, know when to take their temperature and are aware of the level that constitutes a fever. For further information on the nursing care of the septic patient refer to the EBMT Textbook for Nurses (2023).

Since transplantation is a complex treatment with significant risk for some patients, a previously curative intent may evolve to end of life care. The nurse is a key advocate for their patients and should have the opportunity to work with the MDT to ensure that the patient’s wishes and best interests are taken into consideration when a positive treatment outcome no longer be possible.

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## 32.6 Advanced Clinical Practice Nursing in HCT

The advanced clinical practice (ACP) nurse role in HCT has rapidly developed over the past 20 years in Europe. The ACP may be ward- or outpatient-based, will usually have prescribing, admission and radiology rights and will diagnose and manage patients alongside medical colleagues. The aim is to improve patient care through medication management, patient and staff education, implementation of protocols and guidelines and developing quality improvement initiatives to improve outcomes (Mahmoudjafari et al. 2023). This position is embedded in a multi-professional framework for advanced clinical practice developed in 2017 in England (Multi-professional framework for advanced clinical practice in England—Advanced Practice (hee.nhs.uk) 2017), and similar documentation exists across Europe that allows for new, dynamic and flexible ways of working and delivering high quality care. There are capabilities that underpin ACP such as clinical practice, leadership and management, education and research that if applied to this post ensure a quality service is provided. The ACP as an experienced member of the transplant team bridges nursing and medical care to enhance patient experience.

### 32.7 Post-HCT Nursing

Embedding SOPs and protocols within transplant care has enabled nurses to develop important roles in the follow-up period after HCT. Nurses are an important source of support in the immediate period after patients go home. Even though patients are given information during their transplant and prior to discharge to prepare them for this stage of recovery, this is a time of great uncertainty and change. In addition, patients remain clinically vulnerable to the development of early complications and regular HCP contact and support are critical to outcomes. The nurse involved in post-HCT care requires specific training on monitoring protocols, signs and symptoms of early and intermediate complications such as infection, GvHD and late onset VOD as well as others.

The nurse plays an important part in navigating follow-up visits, is often involved in arranging transfusion support and offers medication advice, information and replenishment. They reinforce the safety messages given to the patient at discharge particularly with regard to infections, signs and symptoms to report and the risks of delays in this.

The nurse working in the area of posttransplant care is also well placed to offer practical guidance on food and nutrition, exercise, relationships and sex. These topics among many others are not always easily broached in the transplant clinic and the nurse–patient relationship supports this dialogue. Conversations around the emotional toil of transplant recovery are important to identify those for whom more formal psychological support may be beneficial and the use of standardised assessment tools validate a range of concerns, triggering conversations that enable referrals to other services as needed.

Nurses have become increasingly involved in late effects care. The clear guidelines for late effects surveillance and screening have been incorporated within local SOPs, facilitating the development of nurse roles in this area. Support with health behaviour and lifestyle modifications such as smoking cessation, exercise and nutri-

tion; and also, immunisation, sexual function and vocational rehabilitation are components of late effects care amenable to the attention of nurses working in the long-term follow-up clinic.

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### 32.8 Ambulatory and Day Unit Nursing

Ambulatory and day unit nursing is an evolving area. Treatments that were previously given in the in-patient setting are now transferring to ambulatory care and day units, such as complex cytotoxic and supportive medications. The result is that the nursing staff in these areas now require skills on par to colleagues working on in-patient departments. Many hospitals now operate a rotation of staff through all patient areas. Nurses in ambulatory and day care require additional skills such as management of a computerised ambulatory delivery device or CADD pumps and how to infuse DLI that are different from ward-based teams.

These areas are usually small requiring fewer staff with the expectation that the patient population is pre-selected and not in need of routine medical review. Nurses will need to be competent in managing acutely unwell patients and be aware of how to access hot beds and medical team assessments out of hours. There should be clear pathways for rapid hospital assessment and treatment of potential sepsis. This exciting area offers nurses opportunities to develop their roles and ultimately improve patient care.

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### 32.9 Summary

It is clear that nurses working in HCT have a wealth of opportunities to make a difference to patients with each role described here offering a vital component of care. Nurses are a vital component of the transplant team complementing the other roles in the MDT. Continuous modifications and developments in treatment undoubtedly influence this highly complex but highly rewarding area of nursing care.

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### 33.1 Introduction

Ethics is a branch of philosophy, and, like mathematics, moral philosophy does not give ready-made answers to questions but teaches how one could systematically analyse and resolve a problem. Philosophy's main tool, to achieve this, is logic, where accurate premises are linked together to support a conclusion within a sound and valid ethical argument (West 2009). This chapter aims to explain this process using examples from blood and marrow transplantation practices.

Ethical discourse requires a theory of ethics (Thompson 2005). One requires a landmark to understand their ethical position. One needs to know on what basis one can decide if an action is wrong or right, bad or good; a theory of ethics should help this. It will also allow better understanding of common threats to ethics such as appealing to religion, using relativism to justify accepting different truths to different situations

or explaining that ethical stands are unreasonably demanding (Blackburn 2001).

The most known ethical theories are Kant's deontological theory and Bentham and Mill's utilitarianism (Vardy and Grosch 1999). Kant argued for our duty to pursue a set of intrinsically ethical rules that can be universally applied. Ethics is the search for such rules. On the other hand, utilitarianism argues that an action or a rule is moral if their outcomes bring the greatest pleasure and happiness to the greatest numbers of people. No doubt, these theories would ignite an interesting discussion on transplant ethics but may not provide clear enough guidance to healthcare practitioners to help tackle the dilemmas that they regularly encounter.

During the last four decades, Beauchamp and Childress (2013) defended, and significantly developed, the four principles ethical theory for healthcare profession. These principles include:

1. Respect for autonomy: respecting the decision-making capacity of autonomous persons.
2. Nonmaleficence: avoiding the causation of harm.
3. Beneficence: providing benefits as well as balancing such benefits against risks and cost.
4. Justice: distributing benefits, risks and costs fairly.

According to Beauchamp and Childress (2013), beneficence is the primary goal of

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medicine and healthcare, whereas respect for autonomy, along with nonmaleficence and justice, sets the moral limits on the professional's actions in pursuit of this goal.

Ethical obligations towards patients (and sometimes their relatives) are well known to healthcare professionals. In the field of transplantation, management of donors adds another dimension to the ethical complexity. Ethical practice requires one to apply the above four principles to all field of work, every time an ethical issue is raised. Transplantation practice is full with issues that can raise serious and sometimes disturbing ethical concerns. The following is a discussion of some aspects of the ethical implications of high-risk treatment, lack of enough funding for healthcare and issues with donor care.

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### 33.2 Ethical Challenges of High-Risk Treatment

Blood and marrow transplantation is mostly used to treat life-threatening illnesses but also it carries serious complications that are themselves life-threatening. Resistance disease or a recipient with significant comorbidities can make transplant risks too high and brings risks of futility to the equation. Although guidelines and outcomes data are available in the literature, the application of such evidence may require the support of colleagues or other experts within a multidisciplinary team. This should help in striking the desirable balance between expected benefits and possible harm (the beneficence and the nonmaleficence principles). Although risks may be too high, one ought to ask 'is it the best option available for that particular patient with that particular disease?' (Snyder 2016). Moreover, the implications of undertaking a transplant procedure with limited benefits on resources and other patients ought to be considered. The limitation of transplant rooms, for example, may explain how a decision to transplant a particular patient could affect another.

A transplant procedure that carries only 10–20% chance of success can be a source of worry to staff as it brings the beneficence/nonma-

leficence balance to a critical point. However, the other two ethical principles may help. What the patient wants to do? And will such a transplant jeopardise other patients care or face funding rejection? Obviously for a keen patient and supportive healthcare payers, the decision is less problematic. The balance of forces may be different in another situation with the same clinical ground. This brings uncomfortable variations into practice which can only be minimised by the development of constructive ethical discourse.

An unbiased list of options ought to be discussed with the patient (and possibly with their relatives). To obtain an autonomous consent, staffs have to ensure that the patient has fully understood all options and has made a choice that is not influenced by any coercive factors. Obtaining such a valid consent requires arrangements, and it will take some time and effort (Cusatis et al. 2023). This, however, not only meets our moral obligations but also has practical benefits, as a well-consented patient is likely to cooperate with the demand of treatment and work with staff to fight complications. Respect of autonomy dictates that patients are well informed about decisions that they make, and it also dictates that staffs accept such decisions even if decisions sound counterintuitive. A self-funding patient who refuses life-saving transplant to save the money for their young children may pose difficult and very uncomfortable challenges to staff. This patient can be helped through exploring charitable funds for their treatment, but ignoring their autonomous decisions is not an ethical option.

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### 33.3 Engagement with Funding Issues as a Professional Moral Obligation

Establishing funding rules for transplantation treatment has been, on many occasions, considered the job of healthcare payers or insurers. Medical staffs are involved in setting up guidelines, publishing data on outcomes, and advising in some complex cases. However, an ethical assessment of the issue will put medical staff in

the centre of decision-making. After all, healthcare payers and insurers will base all their decisions not only on medical information but also on the interpretation of such information as provided by medical staff. It is prudent to think that it is unethical that medical staffs do not engage actively in this process. The same ethical desire that drives staffs to treat illness and complications ought to drive their engagement in mending funding practices that do not meet patients' needs, as both issues are detrimental to patients' outcomes.

The respect to autonomy principle dictates involvement of patients' representatives in funding decisions. Most healthcare services have such an arrangement, and the job of the medical staff is to educate representatives to be able to make valid and informed decisions. The principle of beneficent, in this setting, can be applied by gathering, analysing and publishing good data to support funding decisions. Whilst publishing papers may have been considered as an option for academic progression, it seems that it has become an ethical obligation. Nonmaleficence means that delays in introducing new development in the field must be avoided. Transplant field is rapidly changing (for the better), and such delays could devote patients from a helpful treatment modality that could make a difference to them. The principle of justice is in the heart of healthcare funding. However, this ought to not mean 'sticking to the rule.' Most rules have legitimate exceptions and the job of the transplant physician to fight the corner of the patients in this regard. Some healthcare services support cord blood transplantation but not the use of double cord because of cost implications. The recent dramatic development of haplo-identical HCT appears as a more cost-effective option and seems to provide the same overall results. The desire to establish an ethical process of funding may have led the English National Healthcare Service to establish Clinical Reference Groups, including one for transplantation. This group is composed of a medical chair, eight other transplant physicians and three members to represent patient and public voice (NHS England 2018). Medical ethics is mainly seen as a direct issue between a professional and a

patient. This discussion showed the ethical obligations of professionals outside the clinic and the hospital ward. This is obviously demanding but also more helpful to patients.

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### 33.4 The Ethical Issues in Donor Management

Transplant donation is a fertile subject for ethical debate as all types of donation carry some moral concerns. These are mainly around respect of donor autonomy, risk of exploitation or possible harm to donor. According to national and international guidance including JACIE quality management, both related and unrelated donors have to be supported and managed by professionals other than staff who look after the recipient. Unrelated donations have some financial and reputational benefits to the donor registries. However, given existing professionalism and code of practice, this has rarely raised concerns.

Whilst the balance of risks and benefits of most types of treatment offered to a particular patient can be established, a major dilemma in donor ethics is the fact that assessing harm and inconvenience to one person (the donor) in relation to expected benefits to another (the recipient) is highly problematic. Staffs occasionally make the decision themselves and argue that some temporary aches and pains and minimal risks of ruptured spleen (G-CSF side effects) are acceptable risks to justify a life-saving donation, particularly to a family member. Staff position makes 'some sense,' but it does not respect donor autonomy, and so it cannot be accepted as a universal rule that could be practiced widely, i.e. it lacks ethical grounds. It should be noted that even haplo-identical donations from a parent to a child may cause some psychological stress (Aguilera et al. 2022).

Child donors, pregnancies conceived for HCT and donation from a family member who lack capacity have been debated. Minor sibling donors require particular consideration as their autonomy is harder to prove. There is evidence that a child donor is subjected to both physical and psychological implications. This prompted (the)

American Academy of Pediatrics Committee on Bioethics to recommend that five conditions are met to ensure morally justified donations from children (AAP 2010). These include lack of suitable adult donor, the expected benefit to recipient is reasonably high, strong relationship between donor and recipient, potential physical and psychological harms to donor must be minimised and, finally, obtaining parents' consent and child assent. Child assent and agreement are hard to confirm, and the availability of independent committee or assessor to look after such donors has been recommended and is enforced by rules in some countries.

Moreover, a family donation from an adult with full capacity can be morally challenging for two reasons. Firstly, not all family members want to donate. Some of them find the process too demanding, and if they were 'given the choice,' they will rather not. The story of one such donor was in the news. A newspaper (the Daily Mail, UK) reported the situation using the following headline: 'Sentenced to die by my sister, leukemia victim refused her only chance of transplant' (Oldfield 1997). The sister refused to donate bone marrow because of the phobia of hospitals. The subsequent media debate led the donor to reconsider her position. This is a moral position that is hard to defend. Secondly, the health risks to family donors are not minimum or negligible. They are more likely to encounter significant complications than unrelated donors (Halter et al. 2009). Documented experience from unrelated donations cannot be used to advise family donors, and the comparison between harm to donor and benefit to recipient is even harder in the family donor situation. Many authors (van Walraven et al. 2010; Brand et al. 2011) attempted to raise awareness of these issues, and many argued that a system that is separate to and not influenced by patient care ought to be in place to manage family donors. This led to the introduction of changes to JACIE management quality manual (version 7) to demand two different teams and network for donors and recipients medical evaluation.

Transplantation, like other healthcare practices, requires an accurate balance between expected benefits and possible harm as well as

valid patient consent. Given limited resources, the implication of one transplant on another ought to be considered. Given the life-saving and life-threatening nature of this modality of treatment, ethical issues with transplantation are likely to be challenging. Staffs are expected to let patients decide for themselves. Moreover, staffs ought to escalate complex issues to the legal system or more commonly to the ethics committee within their institution. In the European Union, Directive 2001/20/EC established ethics committees as an independent body to agree complex ethical challenges.

### Key Points

- Clinical ethics teaches skills to tackle moral dilemmas but does not provide ready-made answers.
- Clinical ethics now extends, beyond patient–clinician relationship, to donor care as well as engagement with fund holders and insurers.
- The four-principles ethical theory (autonomy, beneficent, nonmaleficence and justices) provides reasonable basis for moral assessment of ethical issues in most fields of practice.
- The donation process requires ethical vigilance. Family donors have high health risks and, given the potential social pressure, are not always autonomous.

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# Quality of Life Assessment After HCT for Pediatric and Adults

# 34

Anna Barata and Heather Jim

## 34.1 Introduction

Methodological advances in the HCT field have increased the population of survivors worldwide. However, HCT is associated with significant morbidity that impairs survivors' recovery and adversely affects their QoL. A significant body of literature has addressed QoL after HCT and highlights significant deficiencies in physical, psychological, social, and role functioning both in adult and pediatric survivors (Pidala et al. 2010). These data are clinically relevant as they help to understand the impact of HCT on patient's lives. Clinically, assessment of QoL can inform patient education and be used to evaluate the benefit of supportive care interventions.

## 34.2 QoL Assessment

QoL can be considered as a patient-reported outcome (PRO). PROs are defined by the US Food and Drug Administration (FDA) as the "measure-

ment of any aspect of a patient's health status that comes directly from the patient, without the interpretation of the patient's response by a clinician or anyone else" (US Food and Drug Administration 2009). Thus, PROs specifically describe the impact that HCT has on patients' lives and provide information unavailable from other sources. PROs are also used in pediatric populations, although parents or other proxies might be used as a source of information when children are unable to report their own QoL. However, the use of patients' own reports is clearly recommended because significant discrepancies are found when comparing patients' self-reported QoL to reports of physicians, parents, or other proxies (Kurosawa et al. 2017; Russell et al. 2006). In general, measures to assess patient- and proxy-reported QoL are questionnaires.

These instruments can be broadly categorized as general or disease- or procedure-specific. General measures assess QoL of the general population and can also be administered to specific populations, such as HCT recipients. These questionnaires allow comparisons of QoL across populations, such as between HCT survivors and individuals without cancer. In contrast, disease- and procedure-specific instruments examine specific aspects of the health conditions assessed. These measures capture specific PROs that are likely to be important to patients.

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### 34.3 Measures to Assess QoL in Adults and Pediatric Patients Undergoing HCT

There are numerous measures assessing QoL on adults and pediatric HCT recipients. Measures used have been both general and disease-specific. The following sections list some of the most commonly used questionnaires in the field of HCT.

#### 34.3.1 Adults

Interest in assessing QoL in adult HCT recipients is reflected in the variety of measures used to assess this outcome. However, there is a need for the scientific community to reach consensus about which questionnaires to use in order to

facilitate comparison across studies (Shaw et al. 2016). Table 34.1 summarizes alphabetically some of the most common questionnaires to assess QoL in adults.

#### 34.3.2 Pediatrics

There is less research on QoL on pediatric patients than adult patients. Initial pediatric studies focused on a single aspect of functioning, such as psychosocial and physical limitations. It was not until the early 1990s that pediatric QoL began to be addressed as a multidimensional construct. Most of the measures used in pediatric studies were originally developed to be used in the general population or in children with specific illnesses. Table 34.2 lists alphabetically the most common measures used to assess QoL in pediatric population.

**Table 34.1** Questionnaires assessing QoL in adult HCT survivors

(a) General	
<i>European Quality of Life-5 Dimensions (EQ-5D-5L)</i> (van Reenen and Jansen 2015)	
Aim	Health status
Items	6
Domains/subscales	Mobility, self-care, usual activities, pain, anxiety, depression
Results	Profile of each of the domains assessed, and an index of the health status. Higher scores indicate better health status
Translations	Available in more than 130 languages
<i>Medical Outcomes Study-Short Form (MOS SF-36)</i> (Ware et al. 1994)	
Aim	QoL
Items	36; shorter versions feature 12 items (SF-12) or 8 items (SF-8)
Domains/subscales	General health, physical, role, emotional and social functioning, mental health, pain, vitality
Results	Physical Component Score; Mental Component Score and Global Score. Higher scores indicate better QoL
Translations	Available in more than 170 languages
<i>Patient-Reported Outcomes Measurement Information System (PROMIS)</i> (Cella et al. 2010)	
Aim	Mental, physical, and social health and QoL in healthy populations as well as those with chronic conditions
Items	Multi-item measures varying in length and complexity; for example, PROMIS-29 has 29 items, PROMIS-43 has 43 items, and PROMIS-57 has 57 items
Domains/subscales	Each subscale measures a single domain; PROMIS profile measures assess multiple domain
Results	Higher scores indicate more of the concept being measured. Measures use standardized T-score metric against normative data for the US population
Translations	Available in Spanish and several other languages



**Table 34.1** (continued)

(b) Cancer and HCT-specific	
<i>European Organization for Research and Treatment of Cancer QoL Questionnaire Core 30 (EORTC QLQ-C30) version 3.0</i> (Aronson et al. 1993)	
Aim	QoL in cancer
Items	30 items
Domains/subscales	Functional scales, symptom scale, and a QoL scale
Results	Higher scores in functional and QoL scales indicate better well-being. Higher scores in the symptom scale indicate worse symptomatology
Translations	Available in more than 100 languages
<i>Functional Assessment of Cancer Therapy—Bone Marrow Transplant (FACT-BMT)</i> (McQuellon et al. 1997)	
Aim	QoL in HCT
Items	47
Domains/subscales	Consists of the FACT-G (Cella et al. 1993) and the BMT concerns subscale
Results	Higher scores indicate better QoL
Translations	Available in more than 38 languages
<i>Functional Assessment of Cancer Therapy—General Scale (FACT-G)</i> (Cella et al. 1993)	
Aim	QoL in cancer
Items	33
Domains/subscales	Physical, functional, social and emotional well-being
Results	Higher scores indicate better well-being and global QoL
Translations	Available in more than 60 languages

**Table 34.2** Questionnaires assessing QoL in pediatric HCT survivors

(a) General	
<i>Child Health Questionnaire (CHQ)</i> (Landgraf et al. 1996)	
Aim	QoL
Versions	Parent-reported versions feature 50 items (CHQ-PF50) or 28 items (CHQ-PF28) and are intended for parents of children aged 5–18 years. The child-report version (CHQ-87) has 87 items and is appropriate for children aged 10–18 years
Domains/subscales	Global health, physical functioning, role/social-physical functioning, bodily pain/discomfort, role/social-emotional functioning, role/social behavior, parental impact time, parental impact emotional, self-esteem, mental health, global behavior, family activities, family cohesion, and changes in health
Results	Higher scores indicate higher physical and psychosocial well-being
Translations	The CHQ-PF50 and CHQ-PF28 are available in more than 80 languages, and the CHQ-87 in 34
<i>Patient-Reported Outcomes Measurement Information System (PROMIS)</i> (Hinds et al. 2013)	
Aim	Health and QoL in healthy populations as well as those with chronic conditions
Versions	Multi-item measures varying in length and complexity: PROMIS-25 has 25 items, PROMIS-37 37 items, and PROMIS-49 49 items. PROMIS measures are child- and parent-reported. Child report measures are intended for children aged 8–17 years, and parent report for children 5–17 years
Domains/subscales	Physical, mental and social health, and a global QoL score
Results	Higher scores indicate more of the concept being measured. PROMIS use standardized T-score metric against normative data for the US population
Translations	Children and proxy measures are available in Spanish and in several other languages
<i>Pediatric Quality of Life Inventory (PedsQL™) 4.0 Generic Score Scales</i> (Varni et al. 2001)	
Aim	QoL in healthy children or those diagnosed with an acute or chronic disease
Versions	Parent-report form for children aged 2–4 has 21 items, and child and parent reports for children aged 5–18 have 23 items
Domains/subscales	Physical, emotional, social, and school functioning
Results	Physical health summary score; psychosocial health summary score; total score. Higher scores indicate better QoL
Translations	Available in more than 70 languages

(continued)

**Table 34.2** (continued)

(b) Cancer and HCT-specific	
<i>Child Health Rating Inventories (CHRIs)- and Disease-Specific Impairment Inventory-Hematopoietic Stem Cell Transplantation (DSII-HCT)</i> (Parsons et al. 2005)	
Aim	The disease-specific (DSII-HCT) module assesses QoL of childhood HCT survivors
Versions	10-item module intended to child report (aged 5–12), adolescent report (13–18), and parents report (5–18)
Domains/subscales	Items are grouped in three domains reported by parents and patients to be most salient to the HCT experience: worry, hassles, and body image
Results	Higher scores indicate better QoL
Translations	The questionnaire is available in English
<i>Peds Quality of Life Cancer Module 3.0 (PedsQL CM™)</i> (Varni et al. 2002)	
Aim	QoL in children with cancer
Versions	Parent report form for children aged 2–4 has 25 items, child and parent reports for children aged 5–7 has 26 items, and child and parents reports for children more than 8 years has 27 items
Domains/subscales	Pain and hurt, nausea, procedural anxiety, treatment anxiety, worry, cognitive problems, perceived physical appearance, and communication
Results	Higher scores indicate better QoL
Translations	Available in more than 100 languages
<i>The Behavioral, Affective and Somatic Experiences Scales (BASES)</i> (Phipps et al. 1994)	
Aim	QoL during the acute phase of HCT
Versions	There are separate versions to be completed by nurses (BASES-N), parents (BASES-P), and children (BASES-C). The BASES-N and BASES-P have 38 items, and the BASES-C has 14 items. The questionnaire is intended to be used in child aged 5–17
Domains/subscales	Somatic distress, mood disturbance, compliance, quality of interactions, and activities
Results	Higher scores indicate more distress/impairment
Translations	Available in English

### 34.4 Challenges when Implementing QoL Assessment

Improvement in patients' QoL is included among the strategic goals of major cancer organizations such as the American Society of Clinical Oncology and regulatory agencies such as the FDA and the European Medicines Agency. Recognition of the importance of the patient experience is reflected in the increasing incorporation of patient-reported QoL measures in observational research and clinical trials. However, some aspects should be considered when implementing patient-reported QoL measures.

Historically, studies and clinical trials have used diverse patient-reported QoL measures which make results difficult to compare (Shaw et al. 2016), although there are available tools to map common

PRO QoL measures to one another, such as the PROMIS with the SF-36 (Choi et al. 2012). Second, the mode of administration should also be considered. PRO measures have traditionally been administered by paper and pencil, but new technologies offer the potential to use electronic measures. Electronic measures administered before or during a clinic visit allow results to be available at the time of consultation and may facilitate symptom monitoring to guide supportive treatment. One example is the PROMIS instrument, which is available using computer adaptive testing or through REDCap software. Computer adaptive testing selects questions based on the previous responses that patients have provided to approximate the construct being measured in the fewest number of questions. The implementation of routine assessment of patients' QoL on clinical care and clinical trials has the potential to improve patients' well-being.

### Key Points

- Assessing HCT survivors' QoL is essential in order to know the impact that the HCT, its morbidity, its treatments, and related interventions have on survivors' well-being.
- Enhanced efforts should be made in order to include QoL assessment in routine clinical practice. Engaging clinicians in using QoL assessments, potentially by means of electronic administration, as well as broadening the interpretation of their scores into the clinical field, might facilitate incorporation.
- Further efforts should elucidate to what extent QoL results are incorporated into management decisions, treatment recommendations, and patients' education.
- Additional efforts should also be made to include QoL outcomes in clinical trials.
- The incorporation of QoL assessment into clinical and research practice has the potential to improve HCT outcomes.

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## **Part V**

### **HCT Complications and Management**

**Topic Leaders: Enric Carreras, Jan Styczynski  
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Malgorzata Mikulska

## 35.1 Introduction

Fever during neutropenia is almost universal after an HCT. In neutropenic HCT recipients, clinicians are faced with a unique combination of issues: (1) high incidence of bacterial bloodstream infections, (2) high mortality in case of infections due to Gram-negative bacteria unless effective antibiotic treatment is provided promptly, and (3) numerous other causes of fever.

Additionally, in the absence of neutrophils which are responsible for most of clinical signs or symptoms during a localised bacterial infection (abscess formation, prominent lung infiltrates, pyuria, etc.), fever is frequently the only symptom present also in these cases. On the other hand, fever is a highly unspecific sign, and there are numerous causes of fever during neutropenia other than bacterial infections, including (a) viral infections, (b) fungal infections, (c) drug reactions (e.g. ATG), (d) transfusion reactions, (e) cytokine release syndrome (CRS), (f) mucositis (g) engraftment syndrome, (h) GvHD, (i) rejection, (j) haemophagocytosis, and (k) underlying disease.

However, since infection due to Gram-negative bacteria, particularly *Pseudomonas*

*aeruginosa*, can result in rapid deterioration of clinical conditions and death, this possibility should always be considered and appropriate empirical antibiotic therapy started while awaiting the results pointing to the actual cause of fever or absence of bloodstream infection. The issue of prevention of fever and infections during neutropenia through antibiotic prophylaxis with fluoroquinolones has been seriously challenged by a worldwide increase in antibiotic resistance (Mikulska et al. 2018).

## 35.2 Initial Management of Fever During Neutropenia

Initial management of fever during neutropenia should include all the following (Freifeld et al. 2011; Averbuch et al. 2013; Lehrnbecher et al. 2023).

### 35.2.1 Diagnostic Procedures

- |  |
|--|
| (a) At least two sets (1 set = 1 aerobic and 1 anaerobic bottle) of <b>blood cultures</b>  |
| 1. Including at least one set from the central venous catheter (CVC), if present   |
| 2. Using an aseptic methodology to reduce the risk of contamination  |
| 3. Providing adequate blood volume (10 ml in each bottle), since the volume of blood is essential to ensure optimal detection of bacteraemia or candidemia |

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- (b) **Clinical exam** with particular attention to subtle signs of a localised infection
4. Signs of infection of exit/entry of CVC
  5. Perineal pain suggestive of an abscess
  6. Skin or nail lesions suggestive of fungal infection
  7. Abdominal defence or diarrhoea suggestive of neutropenic enterocolitis, *C. difficile* infection, etc.
  8. Upper respiratory tract symptoms such as rhinorrhoea suggestive of viral respiratory infection
  9. Mucosal lesions
  10. CNS signs or symptoms (focal lesions, e.g. with fungal infection or bacteria abscess vs. being confused in severe systemic infection or viral encephalitis)
  11. Pleuritic chest pain suggestive of invasive mould infection
- (c) Any **other microbiological tests** based on the clinical presentation (e.g. sputum culture, nasal swab for respiratory viruses, urinary antigen for *legionella*, viral DNA (CMV, ADV, etc.), *Clostridioides difficile* toxin, etc)
- (d) **Radiological tests** based on the clinical presentation (for suspected lung involvement, lung CT should be used since chest X-ray has too low sensitivity for detecting pneumonia in neutropenic patients) and the previous episodes of fever

### 35.2.2 Evaluation of the Risk of Clinically Severe Infection

Such an evaluation, based on comorbidities and clinical presentation, leads to the decision on additional diagnostic tests, the spectrum of antibiotic coverage, the need for close monitoring for signs of further clinical deterioration, and, in case of outpatients, on hospital admission.

### 35.2.3 Evaluation of the Risk of Infection Due to Resistant Bacteria (Particularly Gram-Negative)

This risk is considered high in case of:

- (a) Colonisation with a resistant bacterial strain.
- (b) Previous infection caused by a resistant bacterial strain.

- (c) Local epidemiology with high incidence of infections caused by resistant pathogens.

### 35.2.4 Choice of the Appropriate Empirical Antibiotic Therapy

In order to provide immediate active treatment and prevent overexposure to broad spectrum agents/combinations, the choice of escalation and de-escalation strategy (see Table 35.1) and the most appropriate antibiotic must be made.

### 35.2.5 In High-Risk Patient's Assessment of the Need for Antifungal Therapy

- (a) Assess the risk of candidemia in patients not receiving antifungal prophylaxis and presenting with septic shock.
- (b) Assess the probability of invasive aspergillosis (IA) based on the incidence of IA (taking into account risk factors, mould-active prophylaxis, etc.) and the results of galactomannan (GM) or *Aspergillus* PCR screening or targeted testing.

Empirical antifungal therapy (adding antifungal agent in patients persistently febrile despite broad-spectrum antibiotics) could be replaced by diagnostic-driven strategy based on the use of diagnostics, such as lung CT, fungal serum markers (GM, PCR,  $\beta$ -D-glucan) and targeted treatment following diagnosis (see Chap. 37).

## 35.3 Main Changes in the Management of Neutropenic Fever

The main change in the management of febrile neutropenia occurred due to an increasing rate of *multidrug-resistant (MDR) bacteria* in certain countries or centres, in particular Gram-negative rods resistant to almost all antibiotics routinely

**Table 35.1** The main characteristics of escalation and de-escalation strategy

Strategy	Escalation	De-escalation
Definition	Empirical treatment active against susceptible <i>Enterobacteriaceae</i> and <i>P. aeruginosa</i>	Starting upfront an empirical coverage of <b>MDR</b> bacteria, particularly <b>Gram-negative</b> , which is <b>later (72–96 h) reduced (de-escalated)</b> if a MDR pathogen is <i>not</i> isolated, i.e.: <ul style="list-style-type: none"> <li>• Susceptible strain isolated</li> <li>• No microbiological results</li> </ul>
Antibiotics usually used	Monotherapy with anti-pseudomonal cephalosporin (cefepime, ceftazidime) or piperacillin-tazobactam	<ul style="list-style-type: none"> <li>• Carbapenem or a new combination of <math>\beta</math>-lactam/<math>\beta</math>-lactam-inhibitor (e.g. ceftolozane/tazobactam or ceftazidime/avibactam), to cover ESBL producers and some resistant <i>P. aeruginosa</i> strains</li> </ul> Combinations, examples <ul style="list-style-type: none"> <li>• <math>\beta</math>-lactam + aminoglycoside</li> <li>• <math>\beta</math>-lactam + coverage of resistant Gram-positives</li> </ul> Optimal choice will depend on colonising MDR strain or local epidemiology
Main advantages	Less induction or selection of resistant strains (carbapenem sparing) Less toxicity	Appropriate therapy before culture results are available > <b>lower mortality</b>
Main limitations	In case of empirical therapy inactive against isolated Gram-negative, prognosis is significantly worsened	Overuse of broad-spectrum antibiotics/combinations leading to high antibiotic pressure, particularly in case of failure to de-escalate
Who	All patients, unless criteria for de-escalation approach are present	Patients at risk for infections due to resistant bacteria, such as those with: <ul style="list-style-type: none"> <li>• Colonisation with a resistant pathogen</li> <li>• Previous infection with a resistant pathogen</li> <li>• Cared for in centres in which resistant pathogens are frequently isolated</li> </ul> Particularly if presenting in severe clinical conditions

MDR multidrug resistant

used in febrile neutropenia (e.g. *Enterobacteriaceae* resistant to third-generation cephalosporins  $\pm$  piperacillin-tazobactam, i.e. producers of extended-spectrum  $\beta$ -lactamases [ESBLs]; *Enterobacteriaceae* or *Pseudomonas aeruginosa* or *Acinetobacter baumannii* resistant to carbapenems). Therefore, traditional protocols consisting of starting a standard  $\beta$ -lactam in all febrile patients and changing treatment in case of persistent (48–72 h) fever (called escalation strategy) might not be applicable to all patients, and individualised approach might be required.

### 35.3.1 De-escalation Strategy

Patients who are at high risk of infections due to resistant bacteria (particularly Gram-negative)

should immediately receive treatment with antibiotics active against such strain since the delay in starting effective antimicrobial therapy has been associated with an increased mortality (Averbuch et al. 2017). Therefore, a de-escalation strategy, typically used in critically ill patients in intensive care units (ICU), has also been proposed for neutropenic haematology patients (Averbuch et al. 2013).

Traditional *escalation empirical therapy* (Table 35.1) is still appropriate in most of cases, especially in countries or centres when resistance rates are low among pathogens commonly causing infections in neutropenia. With this approach, we avoid routine use of carbapenems or combinations of a  $\beta$ -lactam with aminoglycoside (which have been shown associated with more toxicity and no clinical advantage) (Averbuch et al. 2013; Drgona

et al. 2007). The empirical use of an antibiotic active against resistant Gram-positive bacteria (such as vancomycin or agents active against vancomycin-resistant enterococci) is not recommended neither as initial therapy nor in persistently febrile patients, unless the patient has signs or symptoms suggesting a Gram-positive aetiology (e.g. skin or CVC involvement or pneumonia) or a documented Gram-positive infection (Freifeld et al. 2011; Beyar-Katz et al. 2017; Kamboj et al. 2019).

*De-escalation strategy* consists of starting with a broad initial empirical regimen, chosen due to on the severity of the patient's clinical presentation and the risk of infection due to resistant (mainly Gram-negative) bacteria based on individual factors for harbouring MDR bacteria and the local bacterial epidemiology. The key issues of de-escalation approach are (1) providing immediate effective treatment of a potentially life-threatening MDR pathogen and (2) reducing as much as possible the unnecessary use of broad-spectrum drugs (to avoid selection or induction of resistant strains). Data from neutropenic cancer patients in ICU and HCT recipients showed that de-escalation approach is safe and feasible (Mokart et al. 2014; Snyder et al. 2017; Gustinetti et al. 2018), but its implementation is not universal HCT centres (Verlinden et al. 2020). Main characteristics of escalation and de-escalation approach are reported in Table 35.1. The choice of antibiotics for de-escalation strategy will depend on susceptibility results in case of previous infection or colonisation with MDR Gram-negative (in that case empirical treatment can be decided as soon as these results are available, i.e. before the development of fever and indicated in the clinical chart to be started in case of fever) or local epidemiology, e.g. high prevalence of extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in bloodstream isolates.

### 35.3.2 Antibiotic Discontinuation

Another issue of management of febrile neutropenia is the *length of antibiotic therapy*, particu-

larly in the absence of clinically or microbiologically documented infection. Traditionally, antibiotic treatment was continued until neutrophil recovery, with the aim of avoiding infection relapse. This approach has been challenged by IDSA and ECIL guidelines, with the latter stating that antibiotics can be safely discontinued after  $\geq 72$  h of IV therapy in patients that are and have been haemodynamically stable since the onset of fever and are afebrile for  $\geq 48$  h, irrespective of the granulocyte count and the expected duration of neutropenia (Averbuch et al. 2013; Lehrnbecher et al. 2023). The rationale for this recommendation was the fact that alteration of patient's microbiota leads to an increased risk of colonisation/selection of resistant pathogens, which might subsequently cause life-threatening infections.

The safety of *discontinuation of empirical antibiotic* therapy after few days of treatment, provided the antibiotic treatment is restarted immediately in case of fever reappearance, has been reported and demonstrated in several studies (Orasch et al. 2015). A randomised open-label trial performed in 157 high-risk febrile neutropenic haematology patients without clinically or microbiologically documented infection showed that antibiotics can be safely discontinued after 72 h of apyrexia and clinical recovery, irrespective of the neutrophils count, saving days of antibiotic exposure (Aguilar-Guisado et al. 2017). Another randomised trial aimed to validate a clinical practice of discontinuing empirical carbapenem therapy of febrile neutropenia after 3 days, irrespective of fever resolution, as long as no clinically or microbiologically documented infection was present (de Jonge et al. 2022). In that study, most (>60%) of 281 included patients were autologous transplant recipients. There was no difference in treatment failure and no deaths due to carbapenem-susceptible bacteria, but serious adverse events were more frequent in short treatment group (mainly readmissions and mainly in those persistently febrile), suggesting that obtaining clinical improvement before discontinuation might be required.

### 35.4 Fever Persistent despite Empirical Antibiotic Therapy

Fever persistent despite empirical antibiotic therapy is not an infrequent event. Patient's general clinical conditions are the most important factor to consider.

*If no signs or symptoms of clinical deterioration* (e.g. septic shock, confusion, worsening respiratory function) are present, slow response to antibiotic treatment should be considered, particularly if accompanied by improvement in inflammatory markers such as C-reactive protein or procalcitonin (the latter particularly for Gram-negative bloodstream infections). In alternative, nonbacterial infections (e.g. viral or fungal) or noninfectious causes, such as mucositis, CRS or engraftment syndrome, should be considered. Usually, changes in antibiotic regimen are not necessary if clinical conditions are stable. Routine addition of antibiotics against resistant Gram-positives (glycopeptides) has not been shown effective (Beyar-Katz et al. 2017).

Results of serum GM, PCR or other fungal markers, performed either in screening or at the onset of fever, should be available by day 2–3 of fever and should guide antifungal treatment. In patients at high risk of IA, lung CT scan should be performed. *Empirical antifungal treatment* has been introduced when noninvasive diagnostic tests were not available and CT availability was extremely limited. When these diagnostic measures became available, *pre-emptive (called also diagnostic-driven) approach* has been shown able to provide earlier treatment than empirical approach (Maertens et al. 2005) (see Chap. 37). Empirical antifungals might be provided while awaiting the results of diagnostic tests or, in case of mould-active prophylaxis, the confirmation of adequate blood levels, but appropriate diagnostics should be performed to confirm or exclude

the presence of invasive fungal disease. In a randomised trial, pre-emptive antifungal strategy was safe in high-risk neutropenic patients on fluconazole prophylaxis, and reduced by half the number of patients receiving antifungals without excess mortality or increase in invasive fungal infections (Maertens et al. 2023).

*If clinical conditions deteriorate*, usual management steps are:

1. *Aggressive diagnostic workup* (repeated blood cultures, additional testing for viruses and fungi, CT scan, BAL lavage in case of pneumonia, lumbar puncture in case of CNS symptoms, etc.), and while awaiting the results:
2. *Escalation* of antibacterial treatment
3. In some cases starting an *antifungal therapy*.

There is no universal scheme for antibiotic escalation therapy, but it usually covers resistant Gram-negatives (including those producing ESBLs, e.g. with a carbapenem or an addition of aminoglycoside) and methicillin-resistant staphylococci or ampicillin-resistant enterococci (e.g. with a vancomycin or novel agents). Coverage of other resistant bacteria should be based on the local epidemiology, the epidemiology of a centre where the patient was cared for before transplant and on patient's past history of infections and colonisation. In case of diarrhoea, diagnosis of *Clostridioides difficile* infection is mandatory since antibiotic escalation without appropriate therapy for *C. difficile* might worsen the infection. Nonbacterial infections (viral, fungal, toxoplasma, etc) and less frequent agents (e.g. Legionella, mycobacteria, or *Nocardia*) should be considered in differential diagnosis and tested for based on clinical presentation and patient's past exposure. Empirical antifungal treatment in this setting might be warranted while awaiting the results of the whole diagnostic workup.

### Key Points

- Numerous causes of fever during neutropenia exist, but usually neutropenic fever should be managed as suspected bloodstream infection due to Gram-negatives until proven otherwise.
- The initial management includes diagnostics (two sets of blood cultures) and the assessment of the risk of (1) clinically severe infection and (2) infection due to resistant bacteria.
- In patients with severe presentation and the risk of resistant bacteria, de-escalation approach should be used in order to cover the most probable resistant strain(s).
- In other cases, escalation approach is appropriate and the choice of the first-line empirical antibiotic therapy should be based on antibiotic susceptibility of Gram-negative bacteria most frequently isolated in one's centre.
- Empirical antifungal therapy could be replaced in most cases by diagnostic-driven (pre-emptive) strategy.
- In the absence of clinically or microbiologically documented infection, empirical antibiotic can be safely discontinued after 72 h of afebrile and clinical recovery, irrespective of the neutrophils count, and it saves exposure to antimicrobials.
- In case of clinical worsening and persistence of fever, extensive diagnostic workup is mandatory.

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Dina Averbuch

## 36.1 Introduction (Tomblin et al. 2009; Averbuch et al. 2017, 2020; Misch and Andes 2019; Dandoy et al. 2020; Martinez-Nadal et al. 2020; Vu et al. 2020)

The most frequent bacterial infections in HCT patients are bloodstream infections (BSI). They occur in 5–10% of auto-HCT and 20–50% of allo-HCT patients, with higher rates before engraftment, and are associated with increased morbidity and mortality. BSI can be divided into primary and secondary. Primary BSIs are mainly central line catheter-related (CRBSI) or mucosal barrier injury-associated (MBI-BSI). The latter occurs when the pathogen originates from the oral cavity or GI (e.g., Enterobacteriales) in patients with neutropenia and/or MBI (GI GVHD grade 3/4, severe diarrhea). Secondary BSI accompanies site-specific infection (e.g., *Pseudomonas aeruginosa* pneumonia and BSI). Other frequently clinically documented bacterial infections include pneumonia, skin and soft tissue infection, and typhlitis. Patient-related risk factors for bacterial infections include older age,

comorbidities, low functional capacity, high-risk hematological disease (active malignancy, aplastic anemia in allo-HCT, lymphoma, or leukemia in auto-HCT), intestinal domination of specific bacteria, and decreased microbiome diversity. Transplant-related risk factors are specific to the post-HCT period. During the early pre-engraftment phase, neutropenia and disruption of anatomical barriers (mucosal damage and vascular devices) predispose to infection resulting from Gram-positive cocci (GPC) and GNB. Haploidentical HCT and CBT are associated with slower engraftment, delayed immune reconstitution, and higher infection risk. During the intermediate phase, starting at engraftment (+30 to +100 days), the main risk factors are CVC, lack of immune reconstitution, and GVHD-related factors, including its severity and treatment (e.g., etanercept, steroid-resistant disease). Later, humoral and cellular immunodeficiency predisposes to infections due to encapsulated pathogens (*Streptococcus pneumoniae* and *Haemophilus influenzae*), and, rarely, infections due to *Mycobacteria*, *Nocardia*, *Listeria*, and others. The main risk factors for BSI-associated mortality include older age, high comorbidity score, steroid use, persistent neutropenia, ICU-acquired BSI, shock at presentation, multi-drug resistant (MDR) bacteria (non-susceptible to  $\geq 1$  agent in  $\geq 3$  therapeutically relevant antimicrobial categories), and inadequate empirical treatment.

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### 36.2 Epidemiology and Treatment of Bacterial Infections (Averbuch et al. 2017; Misch and Andes 2019; Dandoy et al. 2020; Martinez-Nadal et al. 2020; Vu et al. 2020; Treçarichi et al. 2023)

GNB has become an increasingly common cause of BSI, although sizable intercenter variability in epidemiology is reported. Attributable mortality is usually higher in patients with GNB-BSI than GPC-BSI. Increase in MDR GNB infections limits treatment options, leads to inappropriate empirical therapy, and increases mortality. The prevalence of resistance is influenced by local antibiotic use policies (prophylaxis and treatment), infection control measures, and resistance patterns throughout the specific hospital and countrywide. In the multinational prospective EBMT study, half GNBs were resistant to non-carbapenem  $\beta$ -lactams, the first-line treatment for febrile neutropenia; 18.5% was carbapenem-resistant (CR); and 35% was MDR. Higher resistance rates were reported in allo- versus auto-HCT patients, and in southeastern as compared with northwestern Europe. The main risk factors for carbapenem-resistant GNB are prior colonization or infection with the same bacteria, breakthrough on carbapenems, and ICU hospitalization. In this session, we address infections caused by the most frequent GPC and GNB and the main treatment options for the severe infections they cause. There are differences in recommendations and levels between American and European guidelines.

### 36.3 Gram-Positive Infections

*Coagulase-negative Staphylococcus* (CoNS) causes 7–63% of BSI. True CoNS BSI, defined as at least two consecutive positive blood cultures, is usually CVC-related. Methicillin resistance is frequent, prompting treatment with glycopeptides. The prognosis is usually good.

*Staphylococcus aureus* causes 3–8% of BSI, and its attribute mortality is high (12–40%).

Cefazolin and oxacillin are the therapeutic mainstays against methicillin-susceptible *Staphylococci*. Methicillin-resistant *Staphylococcus aureus* (MRSA) is frequent in some countries but not all. The main treatment options for MRSA BSI include vancomycin and daptomycin, with a higher daptomycin dose (8–12 mg/kg/day) considered for BSI treatment. As daptomycin is inactivated by surfactant, it should not be used to treat pneumonia. Reduced vancomycin susceptibility has been reported (VISA), with some strains daptomycin nonsusceptible. Other active agents for specific indications (pneumonia and/or skin and soft tissue infections) include ceftaroline, lipoglycopeptides, delafloxacin, and linezolid (linezolid is myelosuppressive).

*Enterococci* cause 4–24% of BSI, usually occurring later after HCT. *E. faecium* is more common than *E. faecalis*. Risk factors for the vancomycin-resistant Enterococci (VRE) BSI include previous colonization, mucositis, and broad-spectrum antimicrobial exposure. It typically occurs in patients in poor clinical condition and in association with GVHD, perhaps explaining its high associated mortality and the failure of VRE-active empirical therapy to reduce that mortality. The main treatment option for ampicillin-susceptible enterococcal infection is ampicillin; for ampicillin-resistant infection, vancomycin; and for VRE, linezolid or daptomycin or a combination of daptomycin with either ampicillin, ceftriaxone, or ceftaroline. Reduced daptomycin susceptibility has been reported among VRE; increased dosage is thus recommended.

*Streptococcus viridans* (VS) causes 7–24% of BSI, usually occurring soon after HCT. VS BSI can be accompanied by ARDS and septic shock associated with high mortality. Mucositis, especially following cytarabine, exposure to fluoroquinolones (FQ) or ceftazidime, antiacids, MAC, and haploidentical HCT, predispose to VS BSI. VS is susceptible to most  $\beta$ -lactams used empirically for febrile neutropenia except for ceftazidime. The possibility of  $\beta$ -lactam-resistant VS infections, mainly observed after exposure to  $\beta$ -lactams or in nosocomial BSI, justifies the vancomycin addition in neutropenic patients with septic shock.

HCT patients are at risk for *invasive pneumococcal disease* (IPD). The main presentations are BSI, pneumonia, and meningitis, occurring late (sometimes years) after HCT, with a mortality of 13–30%. Predisposing factors include allo- versus auto-HCT, hypogammaglobulinemia, and cGVHD. Vaccination against IPD is important. Antibiotic prophylaxis, preferably with oral penicillin (if local penicillin resistance rates are low), shall be considered in patients with cGVHD on immune suppressive therapy and those with hypogammaglobulinemia who do not receive replacement therapy, regardless of prior vaccinations.

### 36.4 Gram-Negative Infections (Paul et al. 2022; Tamma et al. 2022)

GNB infecting HCT patients include mainly *Enterobacterales* (~70%) and non-fermentative GNB (NFGNB, ~24%). *E. coli* is the most frequent GNB prior to engraftment; after engraftment, increase is seen in the proportion of non-*E. coli* Enterobacteriaceae (with higher resistance rates) and NFGNB. GNB infections may present with BSI, sepsis/septic shock, pneumonia, enterocolitis, and soft tissue infections (e.g., ecthyma gangrenosum, typically *Pseudomonas aeruginosa*-associated). Without appropriate supportive and antibiotic treatment, death may occur within hours. The main GNB, their resistance pattern, and treatment recommendations are addressed below.

#### 36.4.1 Broad-Spectrum $\beta$ -Lactamase-Producing *Enterobacterales*

The main resistance mechanism to empirical therapy in *Enterobacterales* is broad-spectrum  $\beta$ -lactamase production, due to:

1. Extended-spectrum  $\beta$ -lactamase production (ESBL-E; 2–44% of *Enterobacterales* in HCT patients). They are typically resistant to ceftriaxone, but in vitro can appear susceptible to

other non-carbapenem  $\beta$ -lactams. The treatment of choice is carbapenem. Piperacillin-tazobactam, ceftazidime, or cefepime are not recommended for treating severe ESBL-E infections, even if susceptibility is demonstrated.

2. AmpC production, that can be inducible (*Enterobacter cloacae*, *Klebsiella aerogenes*, *Citrobacter freundii*), meaning resistance can develop on treatment with non-carbapenem  $\beta$ -lactam. Carbapenem is a treatment of choice. Cefepime can be used if the bacteria are fully susceptible and ESBL production is excluded.

European guidelines recommend carbapenem as a treatment of choice for patients with severe infection resulting from third-generation cephalosporines-resistant Enterobacterales.

#### 36.4.2 Carbapenemase-Producing *Enterobacterales* (CPE)

The main carbapenem resistance mechanism in *Enterobacterales* is carbapenemases production, including *Klebsiella pneumoniae* carbapenemase (KPC), metallo- $\beta$ -lactamases (New Delhi metallo- $\beta$ -lactamase (NDM), VIM, etc.), and OXA-48-like enzymes. Among Enterobacterales, carbapenem resistance is more frequent in *Klebsiella pneumoniae*. Several new  $\beta$ -lactams are active against carbapenemase-producing Enterobacterales; clinical data on treatment with some of them and specifically data in immunocompromised patients with BSI is, however, very limited, and resistance has been reported on treatment or regardless of previous exposure.

Severe infections due to KPC-producing Enterobacterales can be treated with meropenem-vaborbactam or ceftazidime-avibactam. Other options are imipenem-relebactam and cefiderocol, but clinical data on their use is very limited. OXA-48-like-producing *Enterobacterales* can be treated with ceftazidime-avibactam or alternatively with cefiderocol. Metallo- $\beta$ -lactamases-producing Enterobacterales can be treated with aztreonam plus a ceftazidime-avibactam combination, or

with cefiderocol. Treatment with meropenem-vaborbactam or ceftazidime-avibactam has been associated with better outcomes than the older (mainly colistin-based) regimens. Polymyxins are not suggested for the treatment of CRE infections susceptible to  $\beta$ -lactams.  $\beta$ -lactams nephrotoxicity rates were significantly lower than that of polymyxin-based or aminoglycoside-based regimens. A routine combination of  $\beta$ -lactams with aminoglycosides, polymyxins, or FQ is not recommended. For patients with severe infections caused by CRE, susceptible in vitro only to polymyxins, aminoglycosides, tigecycline, or fosfomycin, or in the case of non-availability of new antibiotics, treatment with more than one active drug is suggested.

### 36.4.3 *Pseudomonas aeruginosa* (PA)

PA causes 4–16% of BSI with a 39–79% mortality, especially in ICU-acquired and resistant PA infections. PA in HCT patients is frequently resistant to  $\beta$ -lactams, FQ, and aminoglycosides; 25–71% are MDR, and resistance can develop on treatment. “Difficult-to-treat” resistant (DTR) PA is not susceptible to any of the following: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin. Non-carbapenem  $\beta$ -lactams treatment (i.e., piperacillin-tazobactam, ceftazidime, cefepime, and aztreonam) is preferred for infections caused by susceptible PA. To treat carbapenem-resistant but non-carbapenem  $\beta$ -lactam-susceptible PA infection, high-dose extended infusion of non-carbapenem  $\beta$ -lactam can be used. Ceftolozane-tazobactam treatment is preferred for patients with DTR PA, as outcomes are better and nephrotoxicity rates lower than on other regimens (mainly polymyxin- and aminoglycoside-based), according to observational studies. Ceftolozane-tazobactam resistance, with ceftazidime-avibactam cross-resistance, can develop on treatment. Other treatment options for DTR PA include imipenem-relebactam and ceftazidime-avibactam. An alternative is cefiderocol, but clinical data on its use are limited.  $\beta$ -lactam combination with aminoglycosides, FQ, or polymyxins is not routinely recommended for

$\beta$ -lactam-susceptible PA infections. Severe PA infections, especially in neutropenic patients, are, however, frequently treated with combination therapy, at least until the patient stabilizes, as prognosis is poor. Nephrotoxicity remains a concern. For DTR PA infections resistant to all  $\beta$ -lactams, a combination of less in-vitro resistant  $\beta$ -lactam with an aminoglycoside or polymyxin B can be considered. If severe CRPA infections are treated with polymyxins, aminoglycosides, or fosfomycin, two in-vitro active drugs should be considered.

### 36.4.4 Other NFGNBs

Other NFGNBs are rarely responsible for infection in HCT patients. *Stenotrophomonas maltophilia* can cause severe hemorrhagic pneumonia or BSI. It is resistant to multiple antibiotics, with intrinsic resistance to carbapenems and aminoglycosides. TMP/SMX is a treatment of choice, but resistance has been reported, and the sulfonamide can be poorly tolerated. For severe infections, a combination with one of minocycline (preferred)/tigecycline/levofloxacin/cefiderocol is an option. Theoretically, a combination of ceftazidime-avibactam with aztreonam can overcome resistance mechanisms.

Infections resulting from carbapenem-resistant (CRAB) and MDR *Acinetobacter baumannii* are typically ICU-associated, with mortality in HCT patients of 49–95%. Recommended therapy in patients with severe CRAB infections is a combination of at least two in-vitro active agents. High-dose extended/continuous ampicillin-sulbactam infusion is a preferred therapy, to be considered even when susceptibility is not demonstrated. Agents generally used in combination include minocycline (preferred), tigecycline (alternative, high dose), and polymyxins. Some consider the addition of high-dose, extended-infusion meropenem as a part of a combination for infections caused by non-highly resistant CRAB, with a step-down to single agent after improvement. Cefiderocol treatment has been associated with higher mortality than other therapies and should not be used routinely. Sulbactam-durlobactam is a novel combination with promising activity against CRAB.

### 36.5 Bacterial Infection Syndromes (Freifeld et al. 2011; Misch and Andes 2019; McDonald et al. 2018; Schmidt-Hieber et al. 2018; van de Beek et al. 2016)

#### 36.5.1 Central Line-Related BSI (CRBSI)

CRBSI should be suspected when blood cultures are persistently positive, at the presence of exit site or tunnel infection, and when fever and chills develop during CVC flushing. This can be proved by a differential time to positivity of >120 min in blood cultures simultaneously drawn from the CVC and a vein; or positive semiquantitative or quantitative cultures. Catheter removal, in addition to systemic antimicrobial therapy, is recommended. CVC salvage in stable patients without complications can be attempted by an antimicrobial lock.

#### 36.5.2 Pneumonia

Bacterial pneumonia occurs in 11–24% of patients, its specific etiology frequently unidentified. During neutropenia, it results from GNB (including PA) and GPC typical to this stage. Specific entities include *Stenotrophomonas maltophilia* hemorrhagic pneumonia, VS-associated ARDS, and nosocomial legionellosis. In the late post-engraftment phase, IPD and *Haemophilus influenzae* should be considered. With symptoms and signs often atypical and scarce, it can progress rapidly. Hypoxemia can be the sole finding and should prompt chest CT, diagnostic bronchoscopy if feasible, and immediate empirical antibiotic therapy, reflecting the colonization history and local resistance patterns. Nebulized antibiotic use for the DTR-*P. aeruginosa* pneumonia treatment is not routinely recommended.

#### 36.5.3 Diarrhea

*Clostridioides difficile*-associated infection (CDI) occurs in 5–30% of HCT patients follow-

ing exposure to broad-spectrum antibiotics and chemotherapy. Clinical manifestation may be paradoxically mild, but severe complications, such as toxic megacolon and perforation, can occur. Diagnosis is usually based on a combination of a glutamate dehydrogenase enzyme immunoassay (EIA), a nucleic acid amplification test (NAAT), and a toxin A and B EIA. Treatment choice is determined by the CDI severity, the patient's ability to take oral treatment and whether it is a recurrent infection.

Bacterial diarrhea due to *Shigella*, *Salmonella*, *Yersinia*, *Campylobacter* spp., and enterohemorrhagic *E. coli* is rare, usually occurring in a community-acquired setting. Routine stool culture is thus recommended for patients with diarrhea only within 3 days of admission.

Hygiene measures and contact precautions are important to prevent intestinal pathogen spread.

#### 36.5.4 CNS Infections

Bacteria rarely cause brain abscesses (*Streptococcus viridans*, *Staphylococcus aureus*, *Klebsiella pneumoniae*) or meningoenzephalitis (*Listeria*, IPD). Clinical manifestations include fever, headache, altered mental state, and focal neurological signs and seizures. These can be subtle due to decreased inflammation. MRI is more sensitive than CT in identifying CNS infection. When bacterial meningitis is suspected, empirical therapy should include cefotaxime/ceftriaxone plus amoxicillin or ampicillin or penicillin G. Vancomycin addition can be considered based on local rates of *Streptococcus pneumoniae* ceftriaxone resistance.

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### 36.6 General Principles of Management of Bacterial Infections (Freifeld et al. 2011; Averbuch et al. 2013)

1. Empirical antibiotic therapy should be started immediately when bacterial infection is suspected. It should reflect the patient's clinical condition, prior colonization or infection with resistant bacteria, and local epidemiology.



Monitoring local bacterial resistance patterns and patient colonization status in endemic settings is important.

2. A broad-spectrum antibiotic regimen (e.g., meropenem or  $\beta$ -lactam with aminoglycoside with/without vancomycin) should be used if the patient is unstable or previously colonized/infected with resistant bacteria. This should be followed by de-escalation to narrower spectrum monotherapy in stable patients once a resistant infection is ruled out. Novel laboratory techniques (e.g., matrix-assisted laser desorption/ionization-time of flight, MALDI-TOF) speed pathogen identification and antibiotic susceptibility testing.
3. Targeted therapy should be based on the susceptibility profile using the narrowest spectrum and least toxic active antibiotic. Consultation with a specialist in infectious diseases is recommended, especially when treating infections due to resistant bacteria.
4. *Antimicrobial stewardship* is important in limiting unnecessary antibiotic exposure and in optimizing antimicrobial therapy based on pharmacokinetic/pharmacodynamic principles with TDM, whenever available. A loading dose, followed by prolonged or continuous infusion of time-dependent antibiotics (e.g.,  $\beta$ -lactams) has been associated with lower mortality than short-term infusion, along with lower rates of resistance development on treatment.
5. Source control is important. CVC removal is recommended in:
  - (a) Infections due to *S. aureus*, *P. aeruginosa*, fungi, or mycobacteria
  - (b) Severe sepsis with hemodynamic instability
  - (c) Suppurative thrombophlebitis
  - (d) Endocarditis
  - (e) Persistently positive (>72 h) blood cultures under appropriate antibiotics
  - (f) Tunnel infection or port pocket site infection
6. Antibiotic treatment should be continued for at least 7 days until the infection is microbiologically eradicated and all clinical signs resolved, with the patient afebrile for at least

4 days. Several studies and meta-analyses have failed to demonstrate difference in mortality in patients who received short (7–10 days) vs. long (<10 days) antibiotic treatment for GNB BSI.

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### 36.7 Prevention of Bacterial Infections (Tomblyn et al. 2009; Mikulska et al. 2018; Egan et al. 2019; Buetti et al. 2022)

*General infection prevention measures* include personal patient hygiene, bathing with chlorhexidine-impregnated washcloths, and use of single-patient rooms. Important infection control measures include standard precautions, especially hand hygiene, use of gloves and gowns when soiling is likely, and environmental cleaning. Multifaceted interventions should be practiced preventing MDR bacteria spread, including patient screening for colonization in the epidemic setting, using contact precautions, isolation, and cohorting of colonized and/or infected patients and staff (this last, for CPE-colonized patients). Routine CPE-targeted decolonization with non-absorbable oral antibiotics is not supported.

The main elements of *CRBSI prevention bundles* include sterile insertion by a specialized team, avoidance of femoral sites, chlorhexidine cleaning during use, antiseptic- or antimicrobial-impregnated CVC, and removal of unnecessary catheters.

*FQ prophylaxis (FQP)* is currently recommended by several national and international guidelines in adult patients with expected neutropenia  $\geq 7$  days, aiming to reduce all-cause mortality, febrile episodes, and GNB-BSI rates. An increase in FQ-resistant GNB affects FQP efficacy, however. Meta-analysis of studies published 1980–2018 shows no reduction in mortality on FQP. Febrile neutropenia rates fell by ~15% on FQP, and reduction in BSI rates was observed mainly in acute leukemia and auto-HCT patients, but not in allo-HCT patients. Possible FQP benefits should be weighed against its potential harm, including CDI, microbiome alterations predisposing to increased GVHD risk,



side effects, and association with colonization/infection with FQ-resistant or MDR GNB.

*Late infection prevention* (>100 days post-HCT), targeting mainly encapsulated bacteria, includes:

1. Oral prophylaxis with penicillin (or other agents, according to local antibiotic resistance patterns) in patients with cGVHD or hypogammaglobulinemia.
2. IVIg in patients with severe hypogammaglobulinemia (serum IgG level < 400 mg/dL);
3. Vaccination.

### Key Points

- An increase in infections due to resistant GNB, such as ESBL Enterobacterales, carbapenemase-producing Enterobacterales (CPE), MDR GNB, or DTR *Pseudomonas aeruginosa*, leads to delay in appropriate therapy and increases mortality.
- Main targeted therapy options for severe infections caused by resistant GNB include:
  - ESBL Enterobacterales: carbapenems.
  - KPC-producing Enterobacterales: meropenem-vaborbactam or ceftazidime-avibactam.
  - OXA-48-like-producing Enterobacterales: ceftazidime-avibactam.
  - Metallo- $\beta$ -lactamases-producing Enterobacterales: aztreonam plus ceftazidime-avibactam, or cefiderocol.
  - DTR *Pseudomonas aeruginosa*: ceftolozane-tazobactam.
  - Routine combination therapy of  $\beta$ -lactams with aminoglycosides/FQ/polymyxins for infection due to MDR GNB susceptible to  $\beta$ -lactam is not recommended (with a possible exception of a severe infections due to *Pseudomonas aeruginosa* in neutropenic patients).

- High-dose prolonged  $\beta$ -lactam infusion can maximize efficacy.
- Antimicrobial stewardship aims to individualize an empirical approach to patients with suspected infection (escalation vs. de-escalation), limiting unnecessary antibiotic use, and optimizing treatment based on pharmacokinetic/pharmacodynamic principles.
- Infection control is crucial to limit the spread of MDR pathogens.
- Fluoroquinolone prophylaxis is controversial.
- Encapsulated bacteria (*Streptococcus pneumoniae* and *Haemophilus influenzae*) cause infection during the late post-engraftment period. Preventive measures include oral prophylaxis, IVIg, and vaccinations.

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Johan A. Maertens

## 37.1 Epidemiology

Invasive fungal diseases (IFDs) are frequent infectious complications of HCT and (to a lesser extent) following chimeric antigen receptor-modified T-cell (CAR-T) treatment. The 12 month cumulative incidence approaches 8–10% in unrelated or mismatched allogeneic HCT, 6% in matched related allogeneic HCT, and less than 2% following autologous HCT (Kontoyiannis et al. 2010). However, higher incidences (up to 17%) have been reported in haplo-identical HCT and cord blood transplantation. Classical risk periods for IFD include: (a) the pre-engraftment period when neutropenia and mucosal damage is most profound, (b) the early post-engraftment period (days +40 to +100) when patients are at highest risk for acute GvHD and viral reactivations due to defective T-cell immunity, and (c) the late post-engraftment period (beyond day +100) complicated by chronic GvHD, delayed immune reconstitution, and occasionally secondary neutropenia. The Gruppo Italiano Trapianto Midollo Osseo (GITMO) has identified period-specific risk factors for proven and probable IFD (Girmenia et al. 2014). The presence of a proven or probable IFD is an independent and strong negative predictor

of overall mortality at 1 year after allogeneic HCT. Following CAR-T treatment, mechanical ventilation, high-grade cytokine release syndrome (CRS) and prolonged lymphocyte deficiency within 60 days after CAR-T infusion have been identified as major risk factors. However, invasive fungal infection was not a risk factor for death within 1 year of CAR-T therapy (Yang et al. 2022).

Before the introduction of antifungal prophylaxis, *Candida* infections were prevalent in as many as 18% to 20% of HCT recipients. However, the widespread use of fluconazole prophylaxis since the late 1990s has significantly reduced the incidence of systemic *Candida* infections and has decreased the transplant-related mortality secondary to *Candida* infections and to gut GvHD. However, this successful approach has also resulted in an epidemiological shift from fluconazole-susceptible *Candida albicans* infections to predominantly fluconazole-resistant non-*albicans Candida* infections (including *Nakaseomyces glabrata* [formerly *Candida glabrata*] and *Candida krusei*). Based on a recent EBMT study, the incidence of candidemia by day +100 has now dropped to 1.2% but remains associated with increased NRM and lower short- and long-term OS (with candidemia being an independent risk factor for NRM and OS) (Cesaro et al. 2018). Regional patterns suggest that *N. glabrata* is a major problem in northern Europe, the United States, and Canada, while *C. parapsilosis* is more predominant in southern Europe,

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Asia, and South America. Recently, *Candida auris* has been found to be an emerging fungal pathogen which is related to high mortality rates, persistent candidemia, inconsistencies in susceptibility testing results, and misidentification by available commercial identification systems. Multidrug-resistant, even pandrug-resistant strains are detected. With changes in the epidemiology of invasive *Candida* infections and the emerging resistance to azoles and echinocandins, antifungal resistance testing has become vital.

Over the past two decades, respiratory mould infections caused by *Aspergillus* species (and to a much lesser extent non-*Aspergillus* moulds such as Mucorales, *Fusarium* species, and some rare other pathogens) have become much more prevalent. Unlike yeasts, which are acquired through indwelling lines or via intestinal translocation, mould infections are usually acquired by inhalation of air-borne spores. In HCT recipients, the primary lines of defence, including phagocytosing alveolar macrophages and neutrophils, are often nonfunctional in the presence of immunosuppressive drugs and/or corticosteroids. Hence, *Aspergillus* spores may germinate and produce hyphae, which then invade blood vessels, followed by vascular occlusion and infarction and dissemination to distant organs. The crude mortality rate of invasive mould disease in HCT recipients can be as high as 60%.

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## 37.2 Diagnosis of Fungal Disease

- Mould infections

Despite a high index of clinical suspicion, diagnosing invasive mould disease remains challenging. The clinical presentation in HCT and CAR-T patients is often nonspecific and difficult to distinguish from nonfungal infections and even non-infectious complications. A diagnosis of mould disease is based on histopathological examination of infected tissue, imaging (in particular chest CT-scan) and microbiological tests, both culture-based and non-culture-based.

Although histopathology remains the gold standard for making a definite diagnosis,

many clinicians are reluctant to ask for invasive procedures with biopsy in these vulnerable patients with underlying coagulation problems. As a result, the majority of invasive mould diseases are categorized as probable or even possible.

Culture and direct microscopic examination of sputum, BAL and other body fluids, and skin samples, using staining techniques that allow diagnosis on the same day (e.g. optical brighteners such as Calcofluor White), have been the cornerstones for making a microbiological diagnosis of invasive mould disease. Culture has the additional advantage of allowing fungal species identification and determining antifungal susceptibility. Unfortunately, culture is time-consuming and requires considerable expertise. In addition, blood cultures are notoriously negative for moulds, even in disseminated disease, and culture from any respiratory specimen has only low to moderate sensitivity and predictive value.

The (ongoing) development of serological tests has been a major advance in the field (Maertens et al. 2016a, b, c). Galactomannan (GM), a fungal cell wall molecule that is released during fungal growth, can be detected by a commercial enzyme immunoassay (BioRad Platelia™ *Aspergillus* EIA). Earlier studies used an index of  $\geq 1.5$  to define positivity. The European Conference on Infections in Leukemia (ECIL) guidelines now support the use of a single serum or plasma value of  $\geq 0.7$  or multiple (consecutive) values of  $\geq 0.5$  to define positivity. These lower cut-offs permit detection of fungal infection before the clinico-radiological manifestations appear. However, improved sensitivity with the use of lower cut-offs comes with a loss of specificity. In addition, false positive results as well as false negative results are not uncommon (Table 37.1) and cross-reactivity with non-*Aspergillus* moulds (including but not limited to *Fusarium* spp., *Penicillium* spp., *Acremonium* spp., *Alternaria* spp., and *Histoplasma capsulatum*) may occur, although the assay does not detect Mucorales. GM testing can also be applied to

**Table 37.1** Limitations of antigen assays in the diagnosis of invasive fungal disease

	Galactomannan	B-D-glucan
Reactivity with fungal species	<i>Aspergillus</i> spp., <i>Fusarium</i> spp., <i>Paecilomyces</i> spp., <i>Acremonium</i> spp., <i>Penicillium</i> spp., <i>Alternaria</i> spp., <i>Histoplasma capsulatum</i> , <i>Blastomyces dermatitidis</i> , <i>Cryptococcus neoformans</i> , <i>Emmonsia</i> spp., <i>Wangiella dermatitidis</i> , <i>Prototheca</i> , <i>Myceliophthora</i> , <i>Geotrichum capitatum</i> , <i>Chaetomium globosum</i>	<i>Pneumocystis jirovecii</i> , <i>Aspergillus</i> spp., <i>Fusarium</i> spp., <i>Histoplasma capsulatum</i> , <i>Candida</i> spp., <i>Acremonium</i> spp., <i>Trichosporon</i> sp., <i>Sporothrix schenckii</i> , <i>Saccharomyces cerevisiae</i> , <i>Coccidioides immitis</i> , <i>Prototheca</i>
False-positive test results	Semi-synthetic $\beta$ -lactam antibiotics <sup>a</sup> Multiple myeloma Blood products collected using Fresenius Kabi bags Gluconate-containing plasma expanders Flavoured ice-pops/frozen desserts containing sodium gluconate <i>Bifidobacterium</i> spp. (gut) Severe mucositis or gastrointestinal GvHD Enteral nutritional supplements	Semi-synthetic $\beta$ -lactam antibiotics Human blood products, including IVIg, albumin, plasma, coagulation factor infusions, filtered through cellulose membranes Cellulose haemodialysis/haemofiltration membranes Exposure to (surgical) gauze Bacterial bloodstream infections (e.g. <i>Pseudomonas aeruginosa</i> )
False-negative test results	Concomitant use of mould-active antifungal agents Mucolytic agents	Concomitant use of antifungal agents

<sup>a</sup> Including ampicillin, amoxicillin-clavulanate and piperacillin/tazobactam (although this problem seems largely abated compared with previous experience)

other types of specimens, including BAL fluid. A cut-off value of 1.0 has been recommended, although it is likely that higher thresholds are needed. Recently, an index cut-off of 1.0 has also been suggested for analysing cerebrospinal fluid samples from patients with (suspected) cerebral aspergillosis.

Since a few years, lateral flow assays (LFAs) have been introduced, providing the possibility for rapid single sample (blood and/or BAL fluid) testing. More and more LFAs are entering the market, but, although often being equated, all use their own antibodies, procedures, and interpretation criteria. For some of these assays, very little (if any) validation data is available; hence, each laboratory must check the available performance information of the specific test and should, whenever needed, perform an implementation verification study.

Unlike GM,  $\beta$ -D-glucan (BDG) is a component of the cell wall of many pathogenic fungi including *Candida* spp., *Fusarium* spp.,

and *Pneumocystis* (Maertens et al. 2016a, b, c). The main exceptions are Mucorales and some *Cryptococcus* species. The Fungitell® assay (Associates of Cape Cod) has been approved by the US FDA and carries the European CE label for the presumptive diagnosis of invasive fungal infection. Most studies report good sensitivity, but the specificity and positive predictive values are poor due to a high rate of false positive results (Table 37.1), regardless of the specimen. However, the negative predictive value is around 80–90%. Similar to the GM assay, single sample devices (e.g. WAKO assay) have recently become available for centres with lower volumes of high-risk patients.

Polymerase chain reaction (PCR)-based methods have also been developed. Lack of standardisation has for a long time hampered the acceptance of these diagnostic assays. Fortunately, over the past decade, the European *Aspergillus* PCR Initiative (EAPCRI) has made tremendous progress in standardising



protocols for efficient DNA extraction and amplification (White et al. 2015).

The sensitivity and specificity of conventional radiology are too low to diagnose or to exclude a fungal infection. Thin-section multislice CT-scan nowadays is the preferred imaging technique; more recently, computed tomography pulmonary angiography is rapidly gaining popularity as an alternative diagnostic technique (Stanzani et al. 2015). Nodules, with or without a halo-sign, are suggestive of invasive mould disease; this 'halo-sign' appears early in the course of the infection; thereafter, the lesions become more nonspecific. Following neutrophil recovery, an air crescent sign may develop, usually associated with a good outcome. An inversed halo-sign has been described as more suggestive of invasive mucormycosis. FDG PET/CT has an emerging role in the diagnostic and monitoring pathway for complex infections (including fungal) in these high-risk immunocompromised patients.

- Yeast infections

Cryptococcal Ag assays have become very sensitive and should be used where cryptococcal meningitis is suspected.

Microbiologic cultures, the gold standard diagnostic method for invasive *Candida* infections and candidemia, have low sensitivity (especially for chronic disseminated candidiasis) and take up to 2–5 days to grow (from blood samples). The T2Candida panel is a novel, fully automated qualitative diagnostic platform for the diagnosis of candidemia in whole blood specimens with a mean time to species identification of less than 5 h. The negative predictive value is almost 100% in a population with 5–10% prevalence of candidemia (Mylonakis et al. 2015). Unfortunately, the assay detects only five different *Candida* species.

- *Pneumocystis jirovecii* pneumonia

Immunofluorescence assays remain recommended as the most sensitive microscopic method. Real-time PCR on BAL fluid can be used to rule out the diagnosis of PCP. However, a positive PCR test does not necessarily mean that the patient has PCP, since low fungal

loads will be picked up in colonised patients. BDG positivity in serum can further contribute to the diagnosis, although false-negative results have been seen and a positive test result may also indicate other fungal infections (Alanio et al. 2016).

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### 37.3 Prevention and Prophylaxis

Protective environment measures (such as the use of HEPA-filtered isolation rooms or the use of portable HEPA filters) are useful to prevent in hospital acquisition of air-borne fungal pathogens. However, many patients develop IFD during the out-patient follow-up period, when these isolation measures are not applicable.

Updated ECIL recommendations regarding pharmacological antifungal prophylaxis are phase-specific (10):

- During the (neutropenic) pre-engraftment phase

Fluconazole (400 mg/day) is still recommended for centres with a low incidence of mould infections (i.e. below 5%) but only when combined with a mould-directed diagnostic approach (biomarker and/or CT-scan based) or a mould-directed therapeutic approach (empirical antifungal therapy). Centres with a higher incidence of mould infections are advised to adopt an alternative approach. Voriconazole (400 mg/day following loading) failed to show a difference in fungal-free survival, overall survival, incidence of IFD, invasive aspergillosis, empirical use of antifungals, and toxicity compared with fluconazole. When tested against itraconazole oral solution, voriconazole was superior for the composite end point, but the difference was driven by a lower use of systemic antifungals with voriconazole, which could be given for a longer duration than itraconazole, not by better efficacy. Itraconazole (200 mg IV q24h, followed by oral solution 200 mg q12) provided better protection against invasive mould infections than fluconazole. However, drug toxicities and tolerability limited its usefulness as a prophylactic agent. Therefore, voriconazole

and itraconazole were both given a B-I recommendation. Data for the echinocandins are limited to micafungin (50 mg IV q24h). The study comparing micafungin versus fluconazole had significant shortcomings, including the overrepresentation of a low-risk population and the lack of a pre-defined work-up for diagnosing IFD. Hence, prophylaxis with micafungin received a B-I recommendation for centres with a low incidence of mould infections and C-I for those with a high incidence. The addition of aerosolised liposomal amphotericin B to fluconazole is not recommended for centres with a low incidence of mould infections, although there is some evidence to do so in higher risk centres (B-II). Intravenous liposomal amphotericin B for prophylaxis was given a C-II recommendation. Although there are no specific studies of posaconazole prophylaxis during the pre-engraftment phase, the drug (oral solution 200 mg q8h or gastro-resistant tablet/intravenous formulation 300 mg q24h following a loading dose of 300 mg q12h on the first day) was given a B-II recommendation based on results inferred from data during the neutropenic phase in AML/MDS patients.

- During the (GvHD) post-engraftment phase
 

Given the significantly increased risk of invasive mould infection during GvHD (and its associated high mortality), ECIL strongly recommends against the use of fluconazole for prophylaxis in patients with high-risk GvHD. Based on the results of a large, double-blind study, posaconazole (oral solution or gastro-resistant tablet/intravenous formulation) is the drug of choice for antifungal prophylaxis (A-I), although no difference was observed in patients with chronic GvHD.
- PCP prophylaxis
 

Oral trimethoprim/sulfamethoxazole given two to three times weekly is the drug of choice for the primary prophylaxis of PCP and should be given during the entire period at risk (from engraftment to  $\geq 6$  months and as long as immunosuppression is ongoing). All other drugs, including aerosolised or intravenous pentamidine, atovaquone, and dapsone, are considered second-line alternatives when trimethoprim/sulfamethoxazole is poorly toler-

ated or contra-indicated (Maertens et al. 2016a, b, c).

## 37.4 Treatment of Fungal Disease

Over the last few decades, three basic strategies (apart from prophylaxis) have been developed and investigated in clinical studies to deal with IFD (Mercier and Maertens 2017). For a long time, profound and prolonged neutropenia accompanied by persistent or relapsing fever after 5–7 days of adequate antibacterial coverage has been regarded as a sufficient trigger for starting broad-spectrum antifungals; a strategy referred to as *empirical* antifungal therapy. This practice has never been supported by robust scientific evidence and has important drawbacks, including drug-related toxicity and increased cost due to overtreatment. In spite of this, the empirical use of antifungals became standard of care in many centres. It was also endorsed by consensus guidelines and is relied on by centres that have limited or no access to radiological and mycological diagnostic tools. If relying on this approach, ECIL guidelines recommend the use of caspofungin (50 mg/day following 70 mg on day 1) or liposomal amphotericin B at 3 mg/kg (both have an AI recommendation).

A *diagnostic-driven* approach (also called ‘*pre-emptive*’) has been advocated by some centres and guidelines following recent improvements in diagnostic techniques. The aim is to start antifungal therapy in at-risk patients only when they present with an early marker of fungal infections, such as a positive GM, BDG, or PCR screening assay, or a suggestive lesion on imaging. A recently completed randomized EORTC study comparing the empirical and pre-emptive approach found no difference in day 42 all-cause mortality or rates of fungal diseases but a significant reduction in antifungal usage favouring the pre-emptive approach (Maertens et al. 2023). Unfortunately, such strategy is restricted to centres that perform non-culture-based testing twice weekly and readily have access to imaging modalities.

*Directed* antifungal treatment is used for patients with documented fungal disease, either proven or probable (Table 37.2).

**Table 37.2** ECIL-6 guidelines for the *first-line antifungal treatment* of invasive aspergillosis and mucormycosis in HCT patients

	Grade	Comments
<b>Invasive aspergillosis</b>		
Voriconazole	AI	Daily adult dose 2 × 6 mg/kg on day 1 followed by 2 × 4 mg/kg (initiation oral therapy: CIII) Need for therapeutic drug monitoring Check for drug–drug interactions
Isavuconazole	AI	Adult dose 200 mg t.i.d. for 2 days, thereafter 200 mg daily As effective as voriconazole but better tolerated
Liposomal amphotericin B	BI	Daily adult dose: 3 mg/kg
Amphotericin B lipid complex	BII	Daily adult dose: 5 mg/kg
Amphotericin B colloidal dispersion	CI	Not more effective than amphotericin B deoxycholate but less nephrotoxic
Caspofungin	CII	
Itraconazole	CIII	
Combination anidulafungin + voriconazole	CI	
Other combinations	CIII	
Recommendation against the use of amphotericin B deoxycholate	AI	Less effective and more toxic
<b>Invasive mucormycosis<sup>a</sup></b>		
Amphotericin B deoxycholate	CII	
Liposomal amphotericin B	BII	Daily adult dose: 5 mg/kg. Liposomal amphotericin B should be preferred in CNS infection and/or renal failure
Amphotericin B lipid complex	BII	
Amphotericin B colloidal dispersion	CII	
Posaconazole	CIII	No data to support its use as first-line treatment.
Combination therapy	CIII	

<sup>a</sup> Management of mucormycosis includes antifungal therapy, surgery and control of the underlying condition

- Voriconazole and isavuconazole are recommended as first line treatment for invasive aspergillosis (IA), including cerebral aspergillosis (Tissot et al. 2017). In a randomised clinical trial, voriconazole and isavuconazole had the same efficacy (all-cause mortality at day 42 around 20%), although isavuconazole has a better toxicity profile (including hepatotoxicity) and somewhat fewer drug–drug interactions compared to voriconazole (Maertens et al. 2016a, b, c). Following the 2017 ECIL recommendations, a study comparing voriconazole and posaconazole demonstrated similar efficacy but better safety profile with posaconazole; the latter drug is now also considered a ‘drug of choice’ option for the primary treatment of IA (CNS penetration is limited) (Maertens et al. 2021). The upfront combination of antifungals with different mechanisms of action (e.g. an azole plus an echinocandin) is not recommended because superiority over monotherapy could not be demonstrated (Marr et al. 2015). Liposomal amphotericin B at 3 mg/kg is the recommended alternative for primary therapy if these azoles cannot be used due to intolerance, drug interactions, prior exposure to broad-spectrum azoles (e.g. prophylaxis), or due to documented azole-resistance (Resendiz Sharpe et al. 2018), an emerging problem in some European centres. For salvage therapy, the global response is around 40%, irrespective of the antifungal used. Treatment duration is typically between 6 and 12 weeks, followed by secondary prophylaxis in patients with ongoing immunosuppressive therapy. During the first week of treatment, pulmonary lesions can grow on imaging; this is in line with the normal kinetics of the disease and does not correlate with a poor outcome. When elevated at baseline, reduction in serum GM correlates with treatment response. Expert-panel-based recommendation on when to change therapy in acute invasive aspergillosis is available (Slavin et al. 2021).
- Treatment of mucormycosis includes control of the underlying condition, surgical debridement (often destructive), and antifungal ther-

apy. At present, lipid-based formulations of amphotericin B (at doses of 5–10 mg/kg) are the first line therapy of choice (Tissot et al. 2017; Cornely et al. 2014). Both posaconazole and isavuconazole can be used for oral outpatient therapy following initial stabilisation of the disease (preferably guided by susceptibility testing results).

- Hyalohyphomycosis constitutes a heterogeneous group of fungi, including (but not limited to) *Fusarium*, *Scedosporium*, *Acremonium*, and *Scopulariopsis* species. Clinical manifestations range from colonisation to localised infections to acute invasive and/or disseminated disease. First-line therapy of fusariosis should include voriconazole and surgical debridement where possible; posaconazole can be used as salvage treatment. Voriconazole is also the recommended first-line treatment of scedosporium infections (except for *Lomentospora prolificans*, previously named *S. prolificans*, for which there is currently no standard treatment available). The optimal antifungal treatment has not been established for *Acremonium* spp., *Scopulariopsis* spp, and other hyalohyphomycosis (Tortorano et al. 2014). Olorofim, a first-in-class orotomide antifungal, displays excellent activity against some of these difficult-to-treat fungal pathogens (including *Lomentospora prolificans*) and is currently in late-stage clinical development.
- Echinocandins are the drugs of choice for the first line therapy of invasive candidiasis/candidemia, followed by a step-down approach in clinically stable patients upon receipt of the species identification and antifungal susceptibility testing results (Andes et al. 2012). Catheter removal is strongly recommended in patients with candidemia or with *C. parapsilosis* bloodstream infection. Treatment duration typically is 14 days after the last positive blood culture.
- High-dose trimethoprim/sulfamethoxazole is the treatment of choice for patients with documented PCP; the combination of primaquine plus clindamycin is the preferred alternative. Treatment duration is typically 3 weeks, and

secondary anti-PCP prophylaxis is indicated thereafter. The administration of glucocorticoids must be decided on a case-by-case basis (Maschmeyer et al. 2016).

- Of note, uncertainty about exposure and drug interactions are common when using azole antifungals. Therapeutic drug monitoring for voriconazole (plasma target 1–6 mg/L for prophylaxis and treatment), isavuconazole (target 2–4 mg/L), and posaconazole (plasma target >0.7 mg/L for prophylaxis; >1 mg/L for treatment) is therefore recommended (ECIL-6 guidelines).

### Key Points

- *Aspergillus*, *Candida*, and *Pneumocystis jirovecii* are the cause of almost 90% of the invasive fungal diseases following HCT. Most infections are diagnosed post-engraftment during episodes of acute and/or chronic GvHD.
- Chest and sinus CT scan and noninvasive mycological tools (serology, PCR) are crucial for making an early diagnosis.
- Antifungal prophylaxis, targeting yeast and/or mould infections depending on the post-transplant risk period, is highly recommended. Trimethoprim-sulfamethoxazole remains the drug of choice for preventing PJP.
- Echinocandins are the preferred first line therapy for invasive *Candida* infections and candidemia. Voriconazole, isavuconazole, and posaconazole are the recommended first-line options for invasive aspergillosis, whereas lipid-based formulations of amphotericin B are the recommended first-line option for mucormycosis.
- Novel antifungal agents with extended spectrum of activity and fewer drug-drug interactions are undergoing clinical evaluation in HCT and CAR-T patients, including the long-acting echinocandin rezafungin (in prophylaxis),

olorofim (mould infections with limited or no treatment options, excluding mucormycosis), and opelconazole, an azole developed for inhalation (as an adjunct to salvage therapy).

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## 38.1 Herpes Viruses

### 38.1.1 Cytomegalovirus (CMV)

#### 38.1.1.1 Clinical Symptoms

CMV can cause symptoms from almost any organ as well as nonspecific symptoms such as fever, malaise, and bone marrow suppression in stem cell transplant patients. However, the most important clinical entities in allo-HCT patients are pneumonia and gastroenteritis.

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The likelihood for symptomatic infection is much higher after allo-HCT compared to auto-HCT. Being CMV seropositive (CMV (+)) has traditionally been associated with decreased OS after allo-HCT. In addition, CMV replication is also associated with decreased OS and increased NRM. The situation has changed with the introduction of effective antiviral prophylaxis (see below). In patients undergoing MAC allo-HCT, the use of a CMV (–) donor to a CMV (+) patient has been associated with an increased risk for NRM and decreased OS.

#### 38.1.1.2 Diagnostics

CMV antibody status should be determined pre-transplant in all patients undergoing HCT and in allogeneic stem cell donors.

Allo-HCT patients should be monitored weekly for CMV at least during the first 3 months after HCT. Patients with GVHD and those with documented CMV replication should be monitored longer. There is no need to routinely monitor patients after autologous HCT.

Today, the most commonly used technique is qPCR. Recently tests detecting CMV-specific T-cells have become available, but further evaluation of these tests' usefulness in routine care is necessary.

To diagnose CMV disease, it is important to combine symptoms and signs with documentation of the presence of CMV in affected tissue. An exception is CMV retinitis where ophthalmologic findings are characteristic although to detect CMV

in vitreous fluid is helpful. Established techniques for the detection of CMV in tissue are histopathology, immunohistochemistry, and DNA hybridization. High levels of CMV DNA in BAL are associated with CMV pneumonia, while its absence almost excludes CMV pneumonia. PCR in CSF supports the diagnosis of CMV encephalitis. For other end-organ diseases, qPCR needs additional study.

### 38.1.1.3 Prophylaxis

If possible, a CMV seronegative donor should be chosen for a CMV seronegative patient. CMV safe blood products should be used.

Letermovir given for 3 months after HCT reduces the risk for clinically significant CMV infection (need for preemptive antiviral therapy and/or CMV disease) and also all-cause mortality in CMV (+) patients (Marty et al. 2017). Longer duration of prophylaxis can be given in high-risk patients. It is important to monitor patients after stopping letermovir prophylaxis since reactivations are then common. Ganciclovir can reduce the risk for CMV disease but is associated with significant toxicity. The data regarding prophylactic Ig is conflicting, and its use is not recommended.

### 38.1.1.4 Treatment

Ganciclovir, valganciclovir, and foscarnet have all been shown to be effective to prevent the development of CMV disease in allo-HCT recipients when given pre-emptively after detection of CMV in blood. Their efficacy is similar, so the choice should be based on the risk for side effects and practical aspects.

It is not possible to give a recommendation on what CMV DNA level preemptive therapy should be initiated since this depends on patient factors, the material used for monitoring (plasma/whole blood), and the performance of the assay used (Ljungman et al. 2019).

Therapy is usually given for at least 2 weeks, but longer therapy courses might be needed. Repeated reactivations are not uncommon, and either of the drug mentioned above can be used for retreatment. Maribavir was in a randomized controlled trial shown to be more effective than other alternatives against resistant or refractory CMV infection and associated with less toxicities (Avery et al. 2022).

Ganciclovir (valganciclovir) and foscarnet have been the most used drugs for CMV disease. The addition of high-dose Ig for treatment of CMV pneumonia has been commonly used, but the data supporting this combination is limited. There is no data supporting the addition of Ig to antiviral treatment for other types of CMV disease. Maribavir is an alternative for resistant or refractory disease. Cidofovir has been used when other antiviral therapies failed (Ljungman et al. 2019).

The duration of therapy has to be decided on a case-by-case basis, but normally longer therapy is needed compared to preemptive therapy (6–8 weeks).

### 38.1.1.5 Cellular Immunotherapy

Transfer of CMV-specific T-cells has in several non-controlled studies been used to manage resistant/refractory CMV infection and disease following allo-HCT. The T-cells were initially derived not only from the HSC donor but also either uni- or multispecific T cells from a third-party donors have been used. The efficacy in patients receiving high-dose ( $\geq 2$  mg/kg) corticosteroids is likely to be low.

## 38.1.2 HHV-6 A and B

### 38.1.2.1 Clinical Symptoms

HHV-6B primary infection is the main cause of *exanthema subitum* in young children. It has also been associated with febrile seizures. Almost all children are infected by the age of 2 years. HHV-6A primary infection has so far not been associated with specific symptoms.

HHV-6B is the main cause of viral encephalitis after allo-HCT, but HHV-6A has also been documented. Patients undergoing CBT are at an increased risk. Other symptoms suggested to be associated with HHV-6 are bone marrow suppression, pneumonia, and acute GVHD.

### 38.1.2.2 Diagnostics

Serology is not useful. HHV-6 DNA can be analyzed in blood by qPCR. However, the usefulness of monitoring is not established. HHV-6 can be integrated in germline. These individuals are strongly

positive in qPCR, but this is not a proof of viral replication.

MRI is recommended for diagnosis of HHV-6 encephalitis. The typical finding is of limbic encephalitis, but other patterns are also seen. HHV-6 DNA is usually positive in the CSF in patients with encephalitis.

### 38.1.2.3 Prophylaxis

Foscarnet has been used, but its usefulness is not established.

### 38.1.2.4 Treatment

Either ganciclovir or foscarnet can be used for treatment of HHV-6 encephalitis. There is no established treatment for HHV-6 infection or patients with other suspected HHV-6-associated complications. Cellular immunotherapy was only performed in a few patients.

## 38.1.3 HHV-7

### 38.1.3.1 Clinical Symptoms

HHV-7 primary infection is very common in young children occasionally causing *exanthema subitum* (*roseola*) and rarely status epilepticus with fever. HHV-7 detection after HCT is infrequent, with rare cases in which HHV-7 has been associated with CNS disease (encephalitis, myelitis).

### 38.1.3.2 Diagnostics

HHV-7 DNA by qPCR. HHV-7 might be a cofactor of CMV reactivation.

### 38.1.3.3 Prophylaxis

Not used.

### 38.1.3.4 Treatment

Infection by HHV-7 does not require specific treatment.

## 38.1.4 HHV-8

### 38.1.4.1 Clinical Symptoms

HHV-8 (KSHV, Kaposi's sarcoma-associated herpesvirus) is the cause of Kaposi's sarcoma (KS), primary effusion lymphoma, and multicen-

tric Castleman's disease. KS is very rare after HCT. Fever and marrow aplasia with plasmacytosis can occur. Skin involvement is the dominant clinical presentation in adults, while pediatric cases can have visceral involvement.

### 38.1.4.2 Diagnostics

Detection of HHV-8 DNA by qPCR. KS can be clinically defined on the basis of characteristic skin lesions or histopathologically defined in a malignant tumor.

### 38.1.4.3 Prophylaxis

Not recommended.

### 38.1.4.4 Treatment

In disease limited to the skin only, surgical excision or electrochemotherapy is the most preferable approach. For visceral or disseminated disease, possible options include the use of interferon alpha or chemotherapy. The use of antiviral treatment is considered without benefit. Imatinib showed promising results in HIV-related KS patients.

## 38.1.5 EBV

### 38.1.5.1 Clinical Symptoms

Syndromes caused by primary EBV infection include infectious mononucleosis, chronic active EBV infection, and X-linked lymphoproliferative syndrome.

In HCT patients, EBV can cause life-threatening complication: posttransplant lymphoproliferative disorder (PTLD) or end-organ diseases such as encephalitis/myelitis, pneumonia, or hepatitis. Details on EBV-PTLD are presented in Chap. 45.

Donor EBV seropositivity also contributes to the risk of cGVHD in patients with acute leukemia.

### 38.1.5.2 Diagnostics

All allo-HCT patients and donors should be tested for EBV Ab before HCT.

### 38.1.5.3 Prophylaxis

Since EBV sero-mismatch is a risk factor for PTLD, the selection of an EBV-matched donor,

if possible, might be beneficial. As EBV-PTLD after HCT is usually of donor origin and EBV might be transmitted with the graft, the risk of EBV-PTLD is higher when the donor is seropositive. Anti-CD20 antibodies have been used to prevent EBV reactivations in high-risk patients.

#### 38.1.5.4 Treatment

Most EBV reactivations are subclinical and require no therapy. Antiviral therapy is not effective. Pre-emptive therapy with anti-CD-20 antibodies is one option to decrease the risk for EBV-PTLD. Treatment of EBV-PTLD is discussed in Chap. 45.

### 38.1.6 Herpes Simplex Virus (HSV)

#### 38.1.6.1 Clinical Symptoms

HSV reactivations can be caused by either type 1 or 2 and is usually associated with localized mucocutaneous disease in the orofacial region (85–90%) and less frequently in the esophageal and genital area. Uncommon manifestations are pneumonia, hepatitis, meningitis (HSV-2), and encephalitis (HSV-1).

#### 38.1.6.2 Diagnostics

All patients should be tested for HSV antibodies before HCT. The diagnosis of mucocutaneous HSV disease is suspected on clinical grounds, and the diagnosis is usually verified by PCR. PCR in CSF is the technique of choice for the diagnosis of HSV meningitis and encephalitis.

#### 38.1.6.3 Prophylaxis

Primary HSV infection in HCT patients is unusual, and antiviral drug prophylaxis is thus not recommended in HSV-seronegative patients (but might be needed against VZV; see below). HSV-seropositive patients undergoing allo-HCT should receive antiviral drug prophylaxis. IV acyclovir 250 mg/m<sup>2</sup> or 5 mg/kg q12h, oral acyclovir 3 × 200 to 2 × 800 mg/day, oral valaciclovir 2 × 500 mg/day, or famciclovir 2 × 500 mg/day can be used.

The duration of prophylaxis depends on if prophylaxis against VZV is also indicated (see below) but should be given for at least 4 weeks after HCT in VZV-seronegative patients.

#### 38.1.6.4 Treatment

IV acyclovir 250 mg/m<sup>2</sup> or 5 mg/kg q8h for 7–10 days is the therapy of choice for severe mucocutaneous or visceral HSV disease.

Oral acyclovir, from 5 × 200 to 5 × 400 mg/day, valaciclovir 2 × 500 mg/day, or famciclovir 2 × 500 mg/day for 10 days are considered as alternatives for less serious manifestations of HSV disease.

For HSV pneumonia or HSV meningitis and encephalitis, IV acyclovir 500 mg/m<sup>2</sup> or 10 mg/kg q8h for at least 14–21 days is recommended.

HSV resistance occurs in approximately 5–15% of patients and is mediated through mutation in the HSV thymidine kinase. Foscarnet or cidofovir is second-line therapies.

### 38.1.7 Varicella-Zoster Virus (VZV)

#### 38.1.7.1 Clinical Symptoms

Primary infection (varicella) rarely occurs after HCT, but it might cause severe visceral disease.

Reactivations are common unless long-term antiviral prophylaxis and usually present as herpes zoster (shingles) and can be complicated by prolonged neuralgia. However, severe symptoms including disseminated infection similar to varicella, visceral disease presenting as severe abdominal pain or acute hepatitis, and rarely encephalitis, retinal necrosis, or pneumonitis can occur.

#### 38.1.7.2 Diagnostics

Patients should be tested for VZV antibodies before HCT. The rash in clinical varicella or zoster is usually characteristic. However, in some cases, disseminated HSV can have a similar appearance. PCR on vesicular material for VZV and HSV can differentiate.

Visceral VZV disease can occur without rash and then PCR on blood is diagnostic.

#### 38.1.7.3 Prophylaxis

VZV-seropositive patients should be given antiviral prophylaxis for at least 12 months or up to the end of IS therapy.

Prophylaxis can be given with acyclovir (2 × 800 mg; in children 2 × 20 mg/kg) or valacyclovir (2 × 500 mg).

Vaccination with recombinant VZV vaccine can be given to prevent late reactivations after discontinuation of antiviral prophylaxis but data is still limited in allo-HCT recipients.

In seronegative patients exposed to VZV, post-exposure prophylaxis with acyclovir or valacyclovir is recommended. Prophylaxis should be started as soon as possible and continued until 21 days after exposition.

#### 38.1.7.4 Treatment

First-line therapy for varicella, disseminated zoster, and visceral disease is acyclovir  $3 \times 500$  mg/m<sup>2</sup>/day IV.

For localized or limited infections, oral valacyclovir ( $3 \times 1000$  mg), acyclovir ( $5 \times 800$  mg; in children  $4 \times 20$  mg/kg), or famciclovir ( $3 \times 500$  mg) can be given until the lesions have crusted over (usually 7–10 days).

In case of resistance to acyclovir (rare), second-line therapies are foscarnet (60 mg/kg q12h) or cidofovir (5 mg/kg weekly, together with probenecid and hydration).

VZIg is not recommended. Only case reports exist on cellular therapy for VZV infection.

## 38.2 Community-Acquired Respiratory Viruses (CARVs) (Excluding SARS-CoV-2)

### 38.2.1 Epidemiology

HCT recipients, in particular allo-HCT recipients, are likely to contract CARV infections (by rhinovirus/enterovirus, RSV, seasonal coronavirus, parainfluenza virus, influenza, metapneumovirus, bocavirus, and adenovirus) with similar seasonality as in the community (Fontana and Strasfeld 2019). Respiratory symptoms are mostly mild causing only URI (above the larynx) but LTD (below the larynx) occurs in 10–30% of cases, and some recipients can develop life-threatening symptoms. CARV LTD-related mortality is usually low (<5%), but it could be >30% in recipients with profound immunosuppressed status at the time of infection. Multiple CARVs co-infections are common, likely due to seasonal

overlap of CARV circulation in the community along with the characteristic long viral shedding which occurs in up to 20%, in particular in those under corticosteroids and profound lymphopenia. Bacterial and fungal coinfections are not uncommon and should be ruled out during the diagnostic workup.

### 38.2.2 Diagnostics

A CARV surveillance program in HCT units could be of value in reducing direct and indirect effects by these infections (Piñana et al 2020). Currently, there is no firm evidence of poorer outcome in allo-HCT recipients infected with an specific CARV as compared to others. Thus, it seems reasonable that CARV screening would be based on syndromic multiplex PCR platforms.

### 38.2.3 Risk Factors (RFs)

RFs for progression to LTD and mortality included lymphopenia, neutropenia, corticosteroids use, active GvHD, timing of infection from transplant, older age, and coinfections. These immunosuppression conditions should be assessed at the time of CARV infections for treatment decision-making and/or close clinical monitoring.

### 38.2.4 Management and Prevention

#### 38.2.4.1 Pretransplant

Routine pretransplant radiology is advisable before transplant in symptomatic transplant candidates with CARV to rule out the presence of LTD. Transplant should be delayed until resolution in symptomatic candidates with LTD.

If antiviral therapy is available, recipients with UTD should be treated to shorten clinical symptoms before transplant (i.e., influenza virus, RSV, and SARS-CoV-2).

For CARVs UTD, with the exception of rhinovirus and common coronavirus, it is reasonable to delay transplant until symptoms resolution.

However, for rhinovirus and common coronavirus UTD, a risk/benefit should be considered before delaying HCT. There is no clear clinical benefit in vaccinating HCT recipients before transplant.

#### **38.2.4.2 Posttransplant**

For most of the CARVs, there are no effective antiviral drugs and/or vaccines and management and prevention should be focused in supportive care, bacterial or fungal LTD coinfection therapy, and preventive transmission measures to limit outbreak situations.

#### **38.2.4.3 Influenza**

##### **Prophylaxis**

The most important prophylactic measure is yearly vaccination with inactivated influenza vaccine preferably given at 6 months after HCT, although it can be considered earlier in outbreak situations. A second dose of vaccine can be considered.

##### **Treatment**

Standard therapy is with neuraminidase inhibitors, mainly oseltamivir or zanamivir, as soon as possible during the course of the disease. It should be recognized that the normally recommended duration of 5 days often is too short since viral excretion might continue for a long time. Resistance to oseltamivir is not rare although variable with the strain circulating in that particular season.

#### **38.2.4.4 RSV**

##### **Prophylaxis**

Recently, positive results have come out from trials with RSV vaccines against RSV in adults. None is currently available. There is no indication for prophylaxis with anti-RSV monoclonal antibodies.

##### **Treatment**

Ribavirin either given as inhalation or systemically +/- iv Ig has been suggested to reduce the risk for progression of RSV UTI to LTD and possibly to reduce mortality in RSV pneumonia.

#### **38.2.4.5 Human Parainfluenzavirus (hPIV) and Metapneumovirus (HMPV)**

hPIV exists in four different types of which especially type 3 can produce higher LRTD and mortality. There is no effective prophylaxis. Ribavirin could be considered in high-risk patients although data regarding efficacy is weak.

Similarly, HMPV has comparable LRTD and mortality rates to influenza and RSV. There is no effective prophylaxis available. Ribavirin's effectiveness as therapy is still uncertain.

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### **38.3 Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Infection and Coronavirus 2019 Disease (COVID-19)**

#### **38.3.1 Clinical Symptoms**

COVID-19 is caused by SARS-CoV-2, which was responsible for the recent pandemic causing millions of deaths worldwide. It is spread not only by droplets but also by contact with infected fluids. The usual clinical presentation is a flu-like syndrome with fever, cough, and fatigue. Atypical symptoms are diarrhea, vomiting, confusion, loss of appetite, taste, and/or smell. Asymptomatic infections are common.

COVID-19 may progress to a severe or critical course with breathlessness, interstitial pneumonia with or without consolidations, need for oxygen support, and intensive care. Death due to respiratory insufficiency or multiorgan failure has been reported in 12–25% of HCT patients, although it became substantially lower after introduction of vaccination and the emergence of the Omicron variants (Ljungman 2023). In patients who recovered from COVID-19 from more than 4 weeks, the persistence of fatigue, dyspnea, cough, chest pain, sleep disturbance, and declined quality of life after more is defined as long-COVID (or post-acute COVID-19 syndrome).



### 38.3.2 Diagnostics

The reference diagnostic test is the search of viral RNA on nasopharyngeal specimen by RT-PCR. The use of rapid antigen test is an alternative to RT-PCR test although less sensitive. Testing of lower respiratory tract fluids is recommended only for differential diagnosis for other clinical etiologies or in case of suspicion of coinfections. Assessment of SARS-CoV-2 serum antibody titers is not useful for diagnostic purposes, while it can be used for epidemiological investigations of seroprevalence or to assess the duration of immunity induced by wild infection or vaccine. Despite both natural infection and vaccination elicit a T-cell response, the role T-cell assays in the routine uses is not defined yet.

### 38.3.3 General Management

Preventing infection is based on the control measures recommended for aerosol-droplet-contact transmission: hand hygiene, social and physical distancing, face masks, and ventilation of rooms. Patients hospitalized for the treatment of COVID-19 should be cared for out of HCT ward and to prevent hospital outbreaks, possibly in single rooms with negative or neutral positive air pressure; moreover health care workers must wear protective equipment such as gloves, gowns, face shield, FFP2 mask, and practice careful disinfection of hands (Cesaro et al 2022).

In the SARS-CoV-2 positive patient, the deferral of cellular therapy (HCT, CAR-T) until the achievement of clinical and virological negativity is recommended to reduce the risk for severe-critical COVID-19. The resumption of cellular therapy program in the asymptomatic patient with persistent shedding of the virus requires a case-by-case risk/benefit assessment (Cesaro et al 2022).

### 38.3.4 Prophylaxis

Vaccination with mRNA vaccines represents the main measure to reduce the risk of SARS-COV-2

infection and to prevent severe and critical form of COVID-19. The primary immunization schedule is of 3 vaccine doses, starting from 3 to 6 months after transplant, followed by a booster dose after 3–4 months from the primary vaccine schedule. Additional booster doses may be scheduled according to country recommendations and epidemiological data.

In patients where the vaccination is contraindicated or considered ineffective and are at high risk of progression to severe critical COVID-19, monoclonal antibodies against anti-Spike protein are recommended as pre-exposure or post-exposure prophylaxis, provided that they are active against the circulating variant (Table 38.1).

### 38.3.5 Therapy

The therapy varies according to the risk profile of the patient and the phase of infection: directed to prevent or contain the viral replication in the early phase; directed to reduce the inflammatory response in the advanced phase. Moreover, supportive intensive cares are fundamental in the cases of severe-critical COVID-19. Table 38.1 shows the specific interventions recommended by ECIL 9 conference (Cesaro et al 2022).

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## 38.4 Adenovirus (ADV)

Human ADV is double-stranded DNA viruses with worldwide distribution. They are divided into seven species (A-G), comprising more than 100 different virus types. Infections occur throughout the year. Nearly all people have evidence of prior infection by 10 years of age. Transmission can occur through fomites, aerosolized droplets, fecal-oral spread, infected tissue, or blood.

Following primary infection, ADVs can persist in different tissues, particularly tonsillar and adenoidal T-lymphocyte, from where active infection may recur in the presence of immunosuppression. In contrast to the rest of community-acquired respiratory viruses, adenoviral

**Table 38.1** Management of patients with COVID-19

Pre-exposure prophylaxis (patient at high risk of severe-critical COVID-19)	<ul style="list-style-type: none"> <li>• Monoclonal antibodies against anti-Spike protein, as long as they are active against the circulating variant(s)</li> </ul>
Post-exposure prophylaxis (patient at high risk of severe-critical COVID-19)	<ul style="list-style-type: none"> <li>• Monoclonal antibodies against anti-spike protein, as long as they are active against the circulating variant(s)</li> </ul>
Mild/moderate COVID-19, no O <sub>2</sub> requirement	Early treatment with: <ul style="list-style-type: none"> <li>• Antivirals (nirmatrelvir/ritonavir or remdesivir or molnupiravir</li> </ul> or <ul style="list-style-type: none"> <li>• Monoclonal antibodies against anti-spike protein, as long as they are active against the circulating variant(s)</li> </ul>
Moderate COVID with O <sub>2</sub> required/severe COVID-19	<ul style="list-style-type: none"> <li>• Dexamethasone (in case of worsening add a second immunosuppressant: anti-IL-6 (tocilizumab, sarilumab), or JAK—inhibitor JAK—inhibitor (baricitinib, tofacitinib), or anti-IL1 (anakinra)</li> <li>• Remdesivir</li> <li>• Monoclonal antibodies against anti-Spike protein, as long as they are active against the circulating variant(s) or high titer of convalescent plasma if monoclonals not available</li> </ul>
Critical COVID-19	<ul style="list-style-type: none"> <li>• Dexamethasone (in case of worsening add a second immunosuppressant: anti-IL-6 (tocilizumab, sarilumab)</li> <li>• Remdesivir</li> <li>• Monoclonal antibodies against anti-pike protein, as long as they are active against the circulating variant(s)</li> </ul>

infections can occur by exogenous acquisition or by reappearance of persistent endogenous virus.

### 38.4.1 Clinical Symptoms

Invasive ADV infections are more common in pediatric HCT patients (6%–42%) than in adults (3%–15%), but the clinical manifestations can be equally severe. The true incidence may be under-

estimated, particularly in adults, due to lack of routine monitoring.

The spectrum of ADV disease in HCT patients ranges from mild gastroenteric or respiratory symptoms to severe hemorrhagic enteritis, hemorrhagic cystitis, nephritis, hepatitis, pneumonia, encephalitis, myocarditis, and multiple organ involvement. ADV disease is most commonly diagnosed within 100 days of HCT.

Risk factors for ADV infection/disease include haploidentical or URD graft, CBT, TCD, GVHD III–IV, severe lymphopenia, and treatment with alemtuzumab or anti-thymocyte globulin. Studies in children showed that the onset of invasive ADV infection is almost invariably preceded by the appearance and expansion of the virus in the gastrointestinal tract. But in adults, the role of the gastrointestinal tract as an important site of ADV reactivation and expansion remains unclear (Hiwarkar et al 2018).

### 38.4.2 Diagnostics

ADV-DNA by qPCR. Monitoring with qPCR of ADV viremia in PB is recommended on at least a weekly basis for patients with at least one risk factor, starting immediately posttransplant until immune reconstitution (CD3 > 300/mm<sup>3</sup>). qPCR is also recommended in case of clinical suspicion of ADV infection/disease. The great majority of pediatric centers performs routine screening and preemptive approach, but in adult centers, the screening is normally done when risk factors are present (Hiwarkar et al 2018).

### 38.4.3 Prophylaxis

Nonpharmacological prophylaxis is mandatory: strict isolation and hygienic measures in patients shedding the virus are absolutely necessary to prevent horizontal transmission and nosocomial outbreaks. Prophylactic antiviral therapy is not recommended.

### 38.4.4 Treatment

There is no approved therapy for ADV. Tapering of immunosuppression should be considered, whenever possible.

Patients, especially children, with increasing viral load and at least one risk factor, should receive preemptive antiviral treatment with cidofovir 3–5 mg/kg/week for 2–3 weeks and, thereafter, every other week. Patients with probable or proven ADV disease should be treated with IV cidofovir (5 mg/kg weekly for at least three doses; thereafter, every other week), together with hyperhydration and oral probenecid. Ribavirin is not recommended for ADV, but if ADV species C is detected, the addition of ribavirin to cidofovir may be beneficial.

Donor-derived ADV-specific CTLs are an option for clinically non-responding patients. Intravenous brincidofovir is under development for therapy of adenovirus infection.

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## 38.5 Polyomaviruses

The human polyomaviruses are an increasing family of virus now including 14 members, with a high seroprevalence in the general population for most of them (60–100%). In immunocompromised individuals, several polyomaviruses can cause severe disease.

The two classic human polyomaviruses BK (BKV) and JC (JCV) are the polyomaviruses with recognized clinical implications in HCT. WU and KI polyomaviruses, although frequently detected in the respiratory tract, have shown no association with respiratory disease. Merkel cell polyomavirus (MCPyV), discovered in 2008, is the only human oncovirus in the Polyomaviridae family (associated with the Merkel cell carcinoma, a rare aggressive cutaneous neuroendocrine carcinoma). Thereafter, nine new human polyomaviruses were discovered with three of them related to skin diseases (Bartley et al 2023): trichodysplasia spinulosa virus (TSPyV (the etiologic agent of Trichodysplasia spinulosa, also known as cyclosporine-induced folliculodystrophy, a rare, disfiguring skin disorder); human polyomavirus 6 and 7).

### 38.5.1 Polyoma JCV

#### 38.5.1.1 Clinical Symptoms

Reactivation of the ubiquitous, neurotropic John Cunningham polyomavirus (JCV), under conditions of impaired cellular immunity may cause progressive multifocal leukoencephalopathy (PML); a rare, opportunistic, and severe disease of the CNS. Profound suppression in cellular immunity may constitute a primary PML risk factor. In HCT, the estimated prevalence is 35 per 100,000 person-years. In allogeneic-HCT patients, the time to the manifestation of symptoms ranged from one to 60 months (median: 8 months).

PML awareness increased following the introduction of several new immunomodulatory treatments including natalizumab, rituximab, efalizumab, infliximab, brentuximab, tacrolimus, and MMF. More recently, it has been associated with CAR T therapy with a deduced incidence of 0.9 cases per 1000 CAR T patients, similar to the incidence of PML after natalizumab.

#### 38.5.1.2 Diagnostics

JCV-DNA by PCR in CSF. Brain biopsy with demonstration of JCV DNA or positive histological evidence of JCV antigens in the CNS is required for a definitive diagnosis of PML.

A definite PML diagnosis requires all the following three criteria: compatible clinical features, compatible neuroimaging findings, and a positive PCR result for JCV in cerebrospinal fluid (CSF). For probable PML diagnosis, clinical or imaging criteria are allowed to be omitted requiring a positive PCR result for JCV CSF. Possible PML diagnosis consists either of CSF PCR positive result alone or if negative, compatible clinical features with compatible neuroimaging findings.

#### 38.5.1.3 Prophylaxis

Not used.

#### 38.5.1.4 Treatment

No specific treatment is available. Infection control relies on restoration of the host's immune competence, which cannot be attained in the majority of cases. The application of G-CSF may

facilitate immune reconstitution and JCV clearance in the CSF. JCV-specific CTLs have been used with promising results in a few patients. There is also positive preliminary experience with immune checkpoint-blocking antibodies (nivolumab and pembrolizumab) in PML.

### 38.5.2 BKV

*BKV* (See Chap. 51: Hemorrhagic Cystitis and Renal Dysfunction).

## 38.6 Norovirus

### 38.6.1 Clinical Symptoms

Noroviruses are the most common cause of food-borne disease and acute nonbacterial gastroenteritis worldwide.

Its prevalence was 2% in adults and up to 22% among pediatric transplant recipients with diarrhea, requiring hospitalization in 55% and ICU admission in 27%. Recurrence rate was 29%.

Risk factors: second HCT, intestinal GVHD, children.

Norovirus can cause severe, prolonged disease complicated by enteritis, fever, recurrent hospitalizations for dehydration, chronic diarrhea, acute renal failure, weight loss, malnutrition, pneumatosis intestinalis, peritonitis, secondary bacteremia, and death.

### 38.6.2 Diagnostics

Viral RNA by RT-PCT in the stool.

The major clinical concern is that misdiagnosis of norovirus gastroenteritis as GVHD would lead to an inappropriate increase in immunosuppression. Even with a biopsy, the differentiation could be difficult as the characteristic histologic feature of GVHD, namely, crypt apoptosis, is also sometimes seen in norovirus infection, although it should be limited to the small intestine as opposed to gut GVHD, which usually affects both the small and large bowel.

### 38.6.3 Prophylaxis

Nonpharmacological prophylaxis is mandatory. Strict isolation and hygiene measures in patients shedding the virus are necessary to prevent horizontal transmission and nosocomial outbreaks.

### 38.6.4 Treatment

Symptomatic. Some reports indicate oral human immunoglobulin therapy. Specific therapies are not available. When feasible, immunosuppression should be decreased.

## 38.7 Flaviviruses

Flaviviruses are RNA-positive single-stranded viruses. These include dengue viruses, Tick Borne Encephalitis virus (TBE), Zika virus, and yellow fever. The diagnosis and management of these viruses are not well documented in the HCT (Muhsen et al 2023). These viruses are mainly transmitted by mosquitoes or ticks. Therefore, HCT patients and potential donors should avoid travelling to endemic areas when possible. Vaccines are available against TBE or yellow fever.

### 38.7.1 Zika Virus (ZIKV)

#### 38.7.1.1 Clinical Symptoms

It is transmitted mainly by *Aedes aegypti* mosquitoes but also by sexual contact, maternal-fetal, or blood transfusion and possibly by other tissues or organs donated by infectious donors. Infection in the healthy population typically results in a mild, asymptomatic, or a self-limiting febrile illness lasting 4–7 days. Infection can be followed by neurological consequences including Guillain-Barre syndromes. The main problem worldwide is the development of microcephaly or other congenital neurological syndromes in children whose mothers have been infected during pregnancy.

### 38.7.1.2 Diagnostics

Direct detection of ZIKV-RNA in the initial period (first 7 days of symptoms) or serological testing.

### 38.7.1.3 Prevention

Blood, tissues, and cells should not be imported from areas of ZIKV transmission or should be tested negative for the presence of ZIKV. A donor diagnosed with ZIKV infection or who has just returned from an affected area should be deferred for at least 28 days after cessation of symptoms following the WHO recommendations. The deferral should be at least 3 months after sexual contact with person at risk.

### 38.7.1.4 Treatment

No specific prophylaxis or therapy is available.

## 38.7.2 Tick-Borne Encephalitis Virus (TBEV)

TBEV is a zoonotic disease transmitted by the bite of infected ticks (*Ixodes Ricinus*, *I. persulcatus*) found in forests and in rare instances may be acquired by consumption of infected unpasteurized dairy products. Vertical transmission from an infected mother to the fetus is possible. Transmission by organ transplantation has been described (3 cases). It is endemic in many parts of Europe (with the highest incidence and increasing in the Nordic, Baltic, and Central European countries) and Asia. In Europe, most cases occur from May to November, with a peak in July.

### 38.7.2.1 Clinical Symptoms

TBEV causes Tickborne encephalitis (TBE). In healthy people, two-thirds of TBEV infections are asymptomatic. Symptomatic cases have two phases. The first viremic phase has non-specific symptoms (fever, fatigue, headache, myalgia, and nausea) and lasts approximately 5 days (2–10). It is followed by an asymptomatic period (7 days, range 1–33) that precedes the second “encephalitis phase,” when the symptoms and signs of central nervous system involvement

appear (meningitis, meningoencephalitis, myelitis, paralysis, and radiculitis). In Europe, 20–30% of patients experience the second phase with a mortality of 0.5–2%, but severe neurological sequelae are frequent and occur in up to 10% of patients. Risk factors for severe disease are age (>40 years) immunocompromised state. The course of the disease in HCT is unknown.

### 38.7.2.2 Diagnostics

The diagnosis of TBE is based on the detection of specific IgM antibodies in cerebrospinal fluid and/or serum or detection by PCR. Specific IgM antibodies can persist for up to 10 months in vaccinees or naturally infected individuals.

### 38.7.2.3 Prevention

TBEV can be prevented by avoiding tick bites (wearing protective clothing, using tick repellents) and avoiding consumption of unpasteurized dairy products in risk areas.

The most effective means of preventing TBE in endemic countries is by vaccination against TBEV (inactivated vaccine) particularly indicated in patients engaged in outdoor activities. There has been a clinical trial with the vaccine in HCT recipients starting at 9 months after transplantation, showing that it is safe although the response is lower compared to healthy individuals.

### 38.7.2.4 Treatment

Supportive care. No specific therapy is available.

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## 38.8 West Nile Virus (WNV)

WNV infection is a mosquito-borne zoonosis, transmitted among birds via the bite of infected culex-species mosquitoes and incidentally to humans. The virus can also be transmitted by blood transfusion and organ donation, and even hematopoietic progenitors for HCT. Europe is endemo-epidemic and affects countries in southern, eastern, and western Europe. The incidence of WNV waxes and wanes. About 80% of WNV infections in humans are asymptomatic. West Nile fever (20%) is the most common clinical

presentation. The elderly and immunocompromised persons are at higher risk of developing West Nile neuroinvasive disease. Mortality in patients with neuroinvasive disease may reach up to 10%. The incidence of neuroinvasive disease in HCT is unknown. WNV should be included in the differential diagnosis of meningoencephalitis or lower extremity paralysis in HCT patients.

According to an EU-Directive, prospective blood donors should be deferred for 28 days after leaving a risk area for locally acquired WNV infection, unless the result of an individual nucleic acid test is negative. The same should be applied for a donor for HCT.

The diagnosis of WNV depends on a high index of suspicion and laboratory testing (serum and CSF WNV IgM and IgG antibodies and viral nucleic acid testing). For the diagnosis of WNV neuroinvasive disease, CSF should be studied.

No specific prophylaxis or treatment exists against the disease. The primary treatment of WNV is supportive care, although IVIG with high titres of WNV antibodies can be considered. Temporary reduction in immunosuppression should be considered.

### 38.9 Human T-Cell Lymphotropic Virus (HTLV)

HTLV-1 (human T-cell leukemia/lymphoma virus type 1) was the first oncogenic human discovered. It is present worldwide, with an estimated 5–10 million people infected. It has a high endemicity in Japan, the Caribbean region, areas of South America and tropical Africa and foci in the Middle East, Australia, and Melanesia. In Europe, the only country with an endemic HTLV-1 region is Romania. HTLV-1 is transmitted by mother-to-child, mainly linked to prolonged breast-feeding, sexual, and via transplantation of organs, tissues, and leucocyte-rich blood components.

HTLV-1 is the etiological agent of two diseases: adult T-cell leukemia/lymphoma (ATLL) and Tropical Spastic Paraparesis/HTLV-1-associated myelopathy.

The relevance to HCT comes from 2 sides. First, this virus can be transmitted by blood and hematopoietic progenitors. That affects the screening of donors. And second, for ATLL only, allogeneic HCT appears to be curative. Persons with HTLV-1 infection should be permanently deferred from donation of blood and blood components, although routine screening of blood donation is not recommended. Anti-HTLV-1 screening should be attempted in donors from geographical regions with a high prevalence of HTLV-1 infection or with sexual partners originating from those areas or where the donor's parents originate from those areas.

#### Key Points

- Epidemiology: Latent (especially CMV) and endemic (especially CARV including SARS-CoV-2 and ADV) viruses are important pathogens after HCT
- Diagnosis: Viral diagnostics after HCT require qPCR or multiplex PCR
- Prophylaxis and treatment: Prophylaxis (pharmacological or environmental) or preemptive treatment (if available) is necessary. All patients after HCT should undergo vaccinations according to current recommendations
- Outcome: Viral infections contribute to non-relapse mortality after HCT
- Age: new innovative drugs (letermovir) not yet approved for children



## 38.10 Viruses Covered in Other Chapters

**BKV** (See Chap. 51: Hemorrhagic Cystitis and Renal Dysfunction).

**HIV** (see Chap. 88: *Other T- and B-Aggressive Lymphomas and Lymphomas Associated with HIV*).

**Hepatotropic Viruses** (See Chap. 49: Hepatic Complications).

**EBV** (See Chap. 45: Posttransplant Lymphoproliferative Syndromes).

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Rodrigo Martino

### 39.1 Toxoplasmosis

#### 39.1.1 General Concepts

*Toxoplasma gondii* is a protozoan that commonly infects animals and birds. Primary *T. gondii* infection in humans and other mammals is usually asymptomatic but leads to lifelong latent infection. Transmission to humans occurs by ingesting tissue cysts from undercooked meat or oocysts (released in the feces of cats). Latent cysts can give rise during immunosuppression to a severe localized reactivation producing, for example, toxoplasma encephalitis or chorioretinitis, with dissemination being common. (Martino et al. 2000, 2005; Tomblyn et al. 2009; Martino 2016).

Although toxoplasmosis is the most common systemic parasitic infection in EBMT centers, it is a relatively rare opportunistic infection following HCT. Currently, we are aware that the patients' seroprevalence explains the wide range of incidences published. Table 39.1 summarizes selected case series of toxoplasmosis in HCT published to date.

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#### 39.1.2 Risk Factors and Incidence in HCT

The seroprevalence for *T. gondii* varies greatly between and even within countries, ranging from <15% in Japan and in pediatric wards, 30% in urban adults in North America and the UK, and to 40–80% of adult HCT recipients in countries with high endemicity such as France or Turkey. This varying seroprevalence is the main reason for the great variability in the incidence of toxoplasmosis after HCT, which has been estimated to average 0.8%, with <0.4% in areas of low endemicity to 2–3% in those with high-antibody prevalence.

Toxoplasmosis occurs mainly in allo-HCT recipients, although cases after auto-HCT have been published. Reactivation of latent tissue cysts in previously infected individuals is the usual mechanism implicated. Thus, it is important to determine the patients' serostatus prior to transplant. However, the disease may also develop if primary (or re-)infection after the transplant may occur.

Ninety-five percent of the cases occur within the first 6 months after the procedure, and acute GVHD and its treatment are the main risk factors. Late cases may occur, again usually in patients with chronic GVHD requiring IST. In addition, seropositive patients without GVHD but with severe cellular IS due to in vivo or ex vivo TCD are also at risk.

**Table 39.1** Selected case series of toxoplasmosis after HCT up to 2021 (see updated detailed information in Aerts et al. 2023)

Author (year) <sup>a</sup>	Cases	Number of HCT (% frequency)	% of sero (+) pre-HCT	Median (range) day onset
Derouin et al. (1992)	7	296 allo (2.4)	65	74 (55–180)
Slavin et al. (1994)	12	3.803 allo (0.31) 509 auto (0)	15	59 (35–97)
Bretagne et al. (1995)	2	550 allo (0.3)	70	NS
Maschke et al. (1999)	20 <sup>b</sup>	655 (3.1)	NS	73 (14–689)
Martino et al. (2003)	41	4.391 allo (0.93) 7.097 auto (0)	Variable (multinational study)	64 (4–516)
Small et al. (2000)	10	463 allo (2.2)	23	78 (36–155)
Aoun et al. (2006)	7	121 allo (5) 204 auto (0.4)	69	45 (13–140)
de Medeiros et al. (2001)	9	789 allo-HCT (1.14)	NS	69 (13–265)
Mulanovich et al. (2011)	9	3.626 Allo (0.25) – US pt 0.15% – Non-US pt 1.6%	18% US pt >50% non-US pt	56 (12–122)
Bautista et al. (2012) and Martino et al. (2015)	9	148 adult CBT (4%)	45	39 (7–98)
Sumi et al. (2013)	6	279 allo (1.8%) 87 auto (1.1%)	10	NS
Hakko et al. (2017)	5	170 allo (2.9%)	70	42 (26–119)

<sup>a</sup> Not all in references

<sup>b</sup> Four definite and 16 possible cases of toxoplasmosis

### 39.1.3 Most Common Clinical Presentations

The CNS is the main site of disease, but pneumonitis and myocarditis are also frequent findings.

Toxoplasma encephalitis typically presents with focal neurologic abnormalities of subacute onset, frequently accompanied by nonfocal signs and symptoms such as headache, altered mental status, and fever. Meningeal signs are very rare. CT brain scans often show multiple bilateral cerebral lesions, although MRI is more sensitive than CT in the early diagnosis of this infection. Toxoplasma pneumonitis may develop in the absence of extrapulmonary disease. Toxoplasma chorioretinitis is rare compared to AIDS patients.

### 39.1.4 Diagnosis

In HCT recipients, the utility of serology is mainly to identify those at risk for developing toxoplasmosis posttransplant.

PCR techniques are currently the standard method for its diagnosis. These techniques are

applicable in blood, CSF, and BAL, the usual samples that are available in HCT recipients with this infection. Most centers use qPCR with a level of detection as low as 20 parasites/mL, with parasite loads of >600/mL reported in most patients with toxoplasmosis.

Since histologically proven toxoplasmosis is a very difficult-to-obtain diagnosis, various levels of diagnostic certainty have been proposed. Histologically defined cases are considered as definite cases of toxoplasma disease, PCR-defined cases as probable, and CNS imaging-defined cases as possible ones.

### 39.1.5 Treatment and Prognosis

Table 39.2 details the recommended treatment and prophylaxis of toxoplasmosis in HCT recipients. Most patients respond to one or another of these regimens, and neurologic improvement of toxoplasma brain involvement usually occurs within 7 days. If appropriately treated, up to 60% of patients may show clinical–radiologic improvement or even a complete response to

**Table 39.2** Suggested treatment and prophylaxis for toxoplasmosis in HCT recipients

Treatment	Dose
Pyrimethamine (plus folinic acid)	Oral, 200 mg loading dose, then 50–75 mg q.d. (folinic acid, oral or IV, 10–15 mg q.d.) + <i>one of the following</i>
Sulfadiazine	Oral, 1–1.5 g q6–8h, <i>OR</i>
Clindamycin	Oral or IV, 600 mg q6h
Prophylaxis	Dose
TMP/SMX <sup>a,b</sup>	1 double-strength tablet (160/800 mg)/day, 4 day × week, <i>OR</i>
	2 double-strength tablets (160/800 mg)/day, 3 day × week, <i>OR</i>
	1 standard-dose tablet (80/400 mg) daily, <i>OR</i>
Pyrimethamine and sulfadoxine (fansidar) <sup>a</sup>	2–3 tables per week
Dapsone <sup>b</sup>	100 mg daily
Atovaquone <sup>b</sup>	1500 mg daily

<sup>a</sup>Also effective for PJP prophylaxis, and possibly listeriosis, nocardiosis, and, in some geographic areas, partly effective in preventing gram-positive cocci and gram-negative bacillary (enterobacterial and nonglucose fermenting) infections

<sup>b</sup>The dose can be reduced in patients with mild renal insufficiency

therapy. This highlights the importance of a high index of suspicion for toxoplasmosis in immunocompromised patients.

### 39.1.6 Specific Screening and/or Prophylactic Strategies Available

Current data suggest that infection may precede disease in most cases of toxoplasmosis. Thus, monitoring sero(+) patients with weekly qPCR of blood samples has been advocated, especially when prophylaxis is not being used, in an effort of using a preemptive-type therapeutic approach, as used for CMV infection. Although an optimal qPCR technique has not been standardized, several studies support the usefulness of this approach. Patients on TMP/SMX prophylaxis should not be monitored.

TMP/SMX is useful in minimizing the risk of reactivation of toxoplasmosis, although there are well-reported cases of toxoplasmosis breaking through this prophylaxis in HCT recipients. Suboptimal dosing may have contributed to some of these “breakthrough” infections, since these cases occur when TMP/SMX is taken less than 3 days per week. Thus, using either one standard-dose tablet (80/400 mg) daily or a double-strength tablet (160/800 mg) 4 days per week is the recommended dosing, as shown in Table 39.2.

Avoiding primary or reinfection after HCT is always important, avoiding the most common sources of infection: uncooked meats of any type and drinking contaminated water.

## 39.2 Tuberculosis (TBC)

de la Cámara et al. (2000), Cordonnier et al. (2004), Yao-Chung et al. (2016), Young and Weisdorf (2016), Beswick et al. (2018), and Bergeron et al. (2022).

### 39.2.1 General Concepts

TBC, and especially, multidrug-resistant (MDR) TBC, continues to be a worldwide major health problem. This may surprise many EBMT HCT physicians, who may have never seen a case of TBC.

#### 39.2.1.1 Mycobacterium Tuberculosis

*Mycobacterium tuberculosis* causes nearly all cases of TBC, and these acid-fast bacilli differ from other bacteria in that they can live only in an infected human. Outside of the human body, they have a very short survival, and infection is transmitted by the inhalation of aerosolized particles from a patient. In addition, its isolation from clinical samples should never be considered as a colonization or sample contamination. TBC is not an opportunistic infection, and thus its detailed description is outside the scope of this manual.

### 39.2.2 Risk Factors and Incidence in HCT

The risk of developing TBC is directly proportional to the TBC present in the geographic area of the HCT center and the patients' residence (Fig. 39.1). A few studies have analyzed its incidence with respect to the general population, and most have found that allo-HCT recipients have 2–10 times higher risk than the general population, while auto-HCT recipients do not have a significantly higher risk (De la Cámara et al. 2000) (Table 39.3).

### 39.2.3 Most Common Clinical Presentations

The clinical presentation of TBC in HCT recipients is the same as in the general population, although it may have a more rapid progression, and the ratio of pulmonary to extrapulmonary disease has been reported 34/5 to 11/10, which surely represents a publication bias, with a median of 75%/25%. The most common extrapulmonary disease is meningitis.

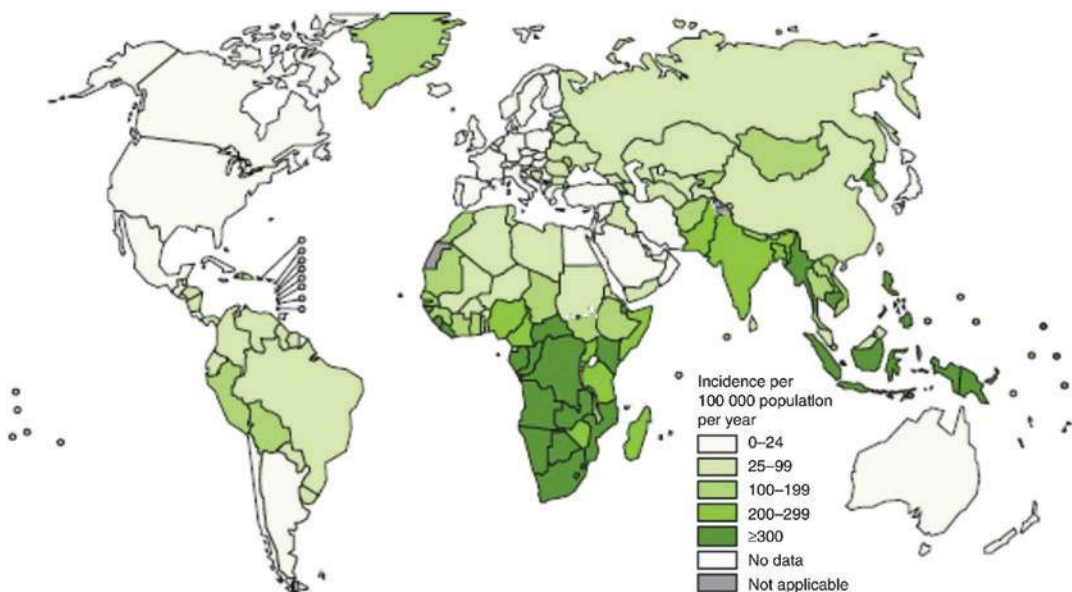
### 39.2.4 Diagnosis

The culture of even a single colony from an affected organ is diagnostic for TBC. Direct microbiologic examination for acid-fast bacilli is of course mandatory, but its sensitivity is probably low. In addition, the results of positive cultures take many days to weeks, and the use of highly sensitive and specific PCR methods is now the usual method for the initial diagnosis.

The quantiFERON-TB Gold test (and other gamma-interferon release assays) is not reliable in the diagnosis of TBC in HCT recipients due to their T-cell immunodeficiency.

### 39.2.5 Treatment and Prognosis

With appropriate treatment, TBC in HCT recipients has a low attributable mortality (<30%). The author suggests that HCT physicians contact ID physicians immediately when the diagnosis of TBC is made. Empirical treatment should be started if this consultation will not be replied immediately, but herein we cannot recommend a "one-fits-all" drug combination, since this varies



**Fig. 39.1** TBC estimate incidence rate 2016 (WHO webpage)

**Table 39.3** Selected case series of mycobacterial infections after HCT before 2018

Author (year) <sup>a</sup>	Country	TBC/HCT × risk with GP <sup>b</sup>	NTM/HCT × risk with GP	Outcome of infection
Lee et al. (2017)	Korea	21/824 (Allo) × 9.1 GP	NA	1 died
Liu et al. (2016) <sup>c</sup>	Taiwan	5/422 (allo)	21/422 (allo)	11 died
Beswick et al. (2018)	Canada	NA	30/1097 (allo) × 35 GP	NA
Fan et al. (2015)	Taiwan	32/1368 (allo) × 7 GP 7/672 (auto) × 2.5 GP	0	20 died
Garces-Ambrossi et al. (2005)	USA	4/577 (allo) × 10 GP	0	NA
Cordonnier et al. (2004)	Multiple (EBMT)	23/1513 (allo) 8/3012 (auto)	8	5 died
Ku et al. (2001)	Taiwan	8/255 (allo) × 13.1 GP 0/95 (auto)	0	ND
de la Cámara et al. (2000)	Spain	12/2866 (allo) × 2.2 GP 8/5147 (auto) = to GP	NA	3 died
Budak-Alpdogan et al. (2000)	Turkey	5/351 (allo) × 3.9 GP	0	No deaths
Gaviria et al. (2000)	USA	3/6529 (3allo)	0	No deaths
Aljurf et al. (1999)	Saudi Arabia	4/641 (allo)	0	2 died
Roy et al. (1997)	USA	2/1486 (allo) 0/755 (auto)	7/1486 (allo) 2/755 (auto)	No deaths
Martino et al. (1996, 2011)	Spain	2/698 (allo) 0/637(auto)	0	No deaths

NA Details not available in the study

<sup>a</sup>Not all in references

<sup>b</sup>x risk with GP, studies in which the relative risk of suffering TBC was compared to that in age-/sex-matched normal individuals from the general population

<sup>c</sup>Abstract

greatly according to the level of drug resistance in each geographical area. Specific recommendations can be found in the recent ECIL-EBMT guidelines (Bergeron et al. 2022).

### 39.2.6 Specific Screening and/or Prophylactic Strategies (Bergeron et al. 2022)

Even in areas where TBC is endemic, pre-HCT screening with the tuberculin skin test or the gamma-interferon quantiFERON-TB Gold test is not done in most HCT centers, and in a recent ECIL-EBMT questionnaire, only 32/88 (36%) had a pre-HCT latent TBC screening routine protocol (Bergeron et al. 2022). In addition, specific antibiotic prophylaxis in patients with past and cured TBC is not warranted.

Recent ECIL-EBMT recommendations (Bergeron et al. 2022) basically emphasize several special scenarios that do, however, require contacting an ID specialist pre- or post-HCT in

order to analyze whether screening or “prophylaxis” may be indicated, the drugs to use, and their duration:

1. Highly IS HCT recipients or candidates who have been substantially exposed to someone with active pulmonary or laryngeal TBC, either before or after the transplant procedure, irrespective of the results of the tuberculin skin test or the gamma-interferon quantiFERON-TB Gold test.
2. HCT recipients or candidates with a positive tuberculin skin test or the gamma-interferon quantiFERON-TB Gold test who were not previously treated and have radiological evidence of TBC lung disease (pleuroparenchymal abnormalities, especially of the upper lobes).
3. Patients with prior TBC which was appropriately treated do not need screening nor prophylaxis, but if there is any doubt as to whether appropriate treatment was indeed given, please consult with an ID specialist.



### 39.3 Nontuberculous (or Atypical) Mycobacterial (NTM) Infections

(Cordonnier et al. 2004; Young and Weisdorf 2016; Beswick et al. 2018; Bergeron et al. 2022).

#### 39.3.1 General Concepts

Atypical mycobacteria are fastidious microorganisms that are ubiquitous in nature and can simply colonize any body surface and secretions and often contaminate clinical samples from the environment. There are a very large number of NTM species with varying geographical distributions. However, with respect to infections in HCT recipients, NTM can be divided into two different categories:

1. *Mycobacterium avium-intracellulare* complex, which are slow-growing mycobacteria.
2. Anonymous or atypical NTM, subdivided into the rapidly growing NTM and the slow-growing NTM: the most commonly reported species from EBMT centers are *M. fortuitum*, *M. abscessus-chelonae* complex, *M. haemophilum*, *M. xenopi*, and *M. kansasii*.

#### 39.3.2 Most Common Clinical Presentations and Risk Factor

A large number of atypical NTM infections are CVC infections (40%), followed by skin infections (30%) and pleuropulmonary infections (20%). However, in patients with severe cGVHD, severe infections of any organ can occur, as well as disseminated cases. *M. avium-intracellulare* complex, on the other hand, usually causes pulmonary disease or disseminated infections, with blood cultures being positive in >50% of cases. Such infections almost always occur in severely immunocompromised allo-HCT recipients, such as those with severe steroid-dependent cGVHD.

#### 39.3.3 Diagnosis

Diagnosis requires the isolation of a NTM from the affected organ(s). In cases of pleuropulmonary infection, differentiating colonization from true active infection and disease can be difficult with NTM. Depending on the species, cultures can be positive in very few days or take up to 9 weeks in slow-growing mycobacteria, as with TBC. Thus, the use of specific PCR methods and/or special biochemical methods is now the usual method for the diagnosis of NTM infections.

#### 39.3.4 Treatment and Prognosis

With appropriate treatment, most NTM infections have a good outcome and a low attributable mortality, although the data are very scarce (Table 39.3). When prolonged antimicrobial treatment is required (which is not always the case, depending on the site of infection and species involved), the drugs most commonly used are macrolides, quinolones, aminoglycosides, and rifamycins (the latter rarely in HCT recipients due to their drug interactions).

As in the case of TBC, the author suggests that HCT physicians contact ID physicians immediately when the diagnosis of NTM infection is made. In CVC infections, the catheter should probably always be removed. While awaiting for the ID specialists, empirical therapy with a macrolide (clarithromycin or azithromycin) plus moxifloxacin or levofloxacin can be started.

#### 39.3.5 Specific Screening and/or Prophylactic Strategies Available

Screening and prophylaxis have no role in NTM infections.

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### 39.4 Listeriosis

Safdar et al. (2002), Boyle (2014), Martino et al. (1996), and Averbuch et al. (2022a, b).

### 39.4.1 General Concepts

Only one species, *Listeria monocytogenes*, produces all cases of this mostly “bacterial food-borne” infection. *L. monocytogenes* is a pseudo-“diphtheroid” gram-positive bacillus. This organism is widespread in nature and in tap water, sewage, the microbiota of pets and farm animals, and nearly all types of fresh foods. The fact that it grows well in refrigerator temperatures adds yet another variable that favors ingestion by humans, which appears to be universal worldwide. At any specific moment, 5% of healthy humans have *L. monocytogenes* in feces. With these premises, it is surprising that listeriosis is an uncommon infection in HCT recipients.

### 39.4.2 Risk Factors and Incidence in HCT

The only risk factor is the combination of ingesting colonized food or water and having a severe cellular IS.

Its incidence is unknown, and only two studies are available. At the MSKCC in New York, six cases occurred in 1315 allo-HCT recipients from 1985 to 1997, with an incidence of 0.47% (Safdar et al. 2002). At the FHCRC in Seattle, three cases occurred among 4069 HCT recipients (<0.1%) during the first 100 days posttransplant (Boyle 2014). Finally, in our center, we have had three cases of listeriosis among 2360 adult HCT recipients (0.1%) (Martino et al. 1996). All other information has been reported as isolated case reports.

### 39.4.3 Most Common Clinical Presentations

Listeriosis in HCT recipients is almost always a sepsis syndrome with bloodstream infection, with CNS involvement in 40–60% of cases, which can present as meningitis, encephalitis, or brain abscess, and with several cases of rhombencephalitis reported (Chang et al. 1995).

### 39.4.4 Diagnosis

The diagnosis is made after the bacterial microbiology laboratory informs the clinicians that the patient has positive blood and/or CSF cultures for this organism. The putative source of the infection cannot be identified in outpatients.

### 39.4.5 Specific Screening and/or Prophylactic Strategies Available

Screening has no role in preventing listeriosis. Standard approaches to food safety handling and preparation are, of course, the main preventive measures.

The routine use of TMP/SMX prophylaxis after HCT surely has a role in preventing listeriosis, but its low incidence makes this impossible to prove.

Cases of listeriosis in long-term inpatients should, of course, activate the rapid intervention of the hospital infection control/prevention unit in the HCT ward.

### 39.4.6 Treatment and Prognosis

The treatment of choice is high-dose ampicillin (or high-dose TMP/SMX in those allergic to penicillin) combined with an aminoglycoside for 3 weeks or 6 weeks in case of CNS infection. We also recommend consultation with ID specialists.

The prognosis of listeriosis in HCT recipients is unknown, although 20% of the reported cases died, while 10% had a CNS recurrence. In an ongoing EBMT study, survival in patients with listeriosis ( $n = 42$ ) and controls ( $n = 62$ ) was 48% and 72%, respectively (Averbuch et al. 2022a, b).

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## 39.5 Nocardiosis

Averbuch et al. (2022a, b), Coussement et al. (2017), Shannon et al. (2016), and Bambace et al. (2013).

### 39.5.1 General Concepts

*Nocardia* spp. (any of the dozens of currently accepted species may be involved, but most cases in Europe appear to be due to *N. asteroides*, *N. brasiliensis*, and *N. nova*) are aerobic gram-positive rods that grow in characteristic filamentous, branching chains and being acid fast, and their appearance makes them easily identifiable by microbiologists, with their acid-fast staining properties differentiating them from *Actinomyces* spp. *Nocardia* spp. grow in soil and decaying matter, and human infection usually occurs from inhalation of airborne bacilli.

### 39.5.2 Risk Factors and Incidence in HCT

Nocardiosis is a late post-HCT infection, occurring months to years after HCT, mostly allo-HCT. Patients usually have steroid-dependent chronic GVHD, secondary diabetes mellitus, and/or bronchiolitis obliterans or bronchiectasis from the numerous post-HCT infections suffered. There are no specific risk factors in HCT, although being at the right time in a place where soil-living bacilli are made massively airborne is a common-sense mechanism of infection. Similar to *M. tuberculosis*, *Nocardia* spp. do not colonize the airways.

The incidence of nocardiosis has been reported to range from 0.3 to 1.7% in allo-HCT, although many large centers have not had a single case. In auto-HCT the median incidence is 0%, although occasional cases have been reported and surely occur in many centers.

### 39.5.3 Most Common Clinical Presentations

Pulmonary infection, with its accompanying signs and symptoms, and radiologically one or more nodular lesions with a tendency to cavitate occur in 90% of patients with nocardiosis. At pre-

sentation, however, around half of the patients have disseminated disease, usually to the skin and osteoskeletal organs, but around 1/3 will have CNS involvement up front. Since CNS involvement is so common and can initially be asymptomatic, a CNS CT or MRI scan is mandatory in all HCT recipients with pulmonary nocardiosis (in any IS host, in fact). Brain abscesses are the usual presentation, although severe hyponatremia due to SIADH is also common due to basal meningitis.

### 39.5.4 Diagnosis

Diagnosis, of course, requires the culture of an affected organ, usually the lungs. Often, the characteristic ramified bacilli can be directly observed from sputum or a directed BAL, but culture-based diagnosis is made in at least 1/3 of the cases. This is of utmost importance, since cultures become positive at a median of 9 days after sampling but can take up to 2–4 weeks. Molecular-based methods are useful only to identify uncommon species of *Nocardia* with known multidrug resistance, but this is rarely required in clinical practice. The most common differential diagnosis is with invasive pulmonary mold infections.

### 39.5.5 Specific Screening and/or Prophylactic Strategies Available

Screening has no role in preventing nocardiosis, but its rapid diagnosis does have an impact on patient outcome.

The routine use of TMP/SMX prophylaxis after HCT may prevent more cases of nocardiosis, but the 2–3-day per week schedules are not effective in preventing it. Of note, *Nocardia* spp. isolated in patients taking single-strength TMP/SMX prophylaxis 5–7 days per week have had a good in vitro susceptibility to TMP/SMX and have responded well to high doses of the drug.

### 39.5.6 Treatment and Prognosis

High-dose TMP/SMX is still the treatment of choice, although there have been good results with carbapenems, amikacin, second-generation cephalosporins, and/or linezolid.

When treated promptly, nocardiosis usually resolves with prolonged antibiotic therapy, but directly attributable mortality has been reported in up to 40% of cases; these are, of course, those cases that affect extremely debilitated allo-HCT recipients due to prolonged severe GVHD and its numerous complications, as well as those with disseminated infection and extensive CNS involvement, including the brain stem. Overall mortality, however, is high, since around 40% of patients have severe coinfections when nocardiosis joins the club.

Treatment of nocardiosis usually requires at least 6 months of specific antibiotic therapy, and it is of course recommended that ID specialists are actively involved in the treatment and follow-up. Of note, most *Nocardia* isolates are susceptible to most of the too-often empirically/prophylactically used antibiotics in HCT recipients (levofloxacin, moxifloxacin, and amoxicillin-clavulanate), as well as tetracyclines and tigecycline.

#### Key Points

- The intense IS associated with allo-HCT, especially when there is a chronic GVHD that requires a prolonged IST, favors the development of infections by very unusual pathogens.
- Despite its low incidence, it is necessary to know these pathologies in order to make an early diagnosis and to adapt the therapy to the causal pathogen.

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# Bleeding and Thrombotic Complications

# 40

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## 40.1 Introduction

Bleeding and thrombotic complications are an important cause of morbidity and mortality in patients undergoing HCT. The major thrombotic complications include venous thromboembolism (VTE) including catheter-related thrombosis (CRT), sinusoidal obstruction syndrome (SOS), and transplant-associated thrombotic microangiopathy (TA-TMA), while bleeding commonly involves the gastrointestinal or respiratory tracts and is most common in thrombocytopenic patients or those with GVHD. HCT is associated with multiple risk factors for both thrombosis and bleeding including the underlying malignancy, thrombocytopenia, high-dose myeloablative chemotherapy (MAC) and immune-modulatory drugs, GVHD, infections, indwelling vascular catheters, and prolonged immobilization (Chiu

and Lazo-Langner 2023; Gerber et al. 2008; Chaturvedi et al. 2016; Nadir and Brenner 2007). In addition, HCT is also associated with alterations in the coagulation system with activation of endothelium-dependent coagulation factors, increase in von Willebrand factor (vWF) and platelet adhesion, increased thrombin generation, decreased antithrombin levels, and decreased levels of anticoagulant proteins such as protein C (Vannucchi et al. 1994). Collectively, major patient-, disease-, and therapy-related factors contribute to hemostatic complications in HCT patients. Thrombotic and bleeding complications in HCT are discussed separately in the following section.

Over the past few years, chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized the field of malignant hematology, particularly for relapsed and refractory B-cell lymphomas, acute lymphoblastic leukemia, and multiple myeloma (MM). Along with these advances, bleeding and thrombosis have emerged as important toxicities associated with CAR-T therapy and are attributed to slow hematopoietic reconstitution, hemostatic defects, and a tendency to thrombosis due to thromboinflammatory influences that are potentiated in the setting of cytokine release syndrome or disseminated intravascular coagulation. We also review the emerging evidence regarding recognition and management of CAR-T-associated bleeding and thrombotic complications in the following section.

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## 40.2 Thrombotic Complications in HCT

### 40.2.1 Epidemiology and Risk Factors

Thromboembolic complications in HCT recipients include venous thromboembolism (VTE), catheter-related thrombosis (CRT), sinusoidal obstruction syndrome, and TA-TMA. VTE is the most common of these complications, and retrospective studies have reported VTE incidence ranging from 4.6% over 180 days in patients undergoing HCT (Gerber et al. 2008). The rate of VTE is higher with allo-HCT than auto-HCT and in the presence of GVHD with 1-year VTE rates of 4.8%, 6.8%, and 8.1% reported with auto-HCT, allo-HCT without GVHD, and allo-HCT with GVHD, respectively (Pihusch et al. 2002). A retrospective series of 447 patients undergoing bone marrow transplantation reported a 5.7% incidence of VTE in the first 100 days following transplant despite being on heparin prophylaxis (100 U/kg iv daily) for hepatic SOS (Pihusch et al. 2002). Finally, Gonsalves et al. reported a 1-year symptomatic VTE incidence of 3.7% in patients undergoing HCT in an *ambulatory* care setting (Gonsalves et al. 2008).

VTE occurs most frequently following engraftment in patients undergoing allogeneic HCT and those with a history of previous VTE or GVHD (Labrador et al. 2013; Gerber et al. 2008). An increased risk of VTE can be observed in both acute and chronic GVHD and is the result of systemic inflammation and endothelial injury (Chiu and Lazo-Langner 2023). The majority of VTE episodes in these studies were catheter-related thromboses. Cortelezzi et al. have previously reported a 12% incidence of catheter-related thromboembolic complications in a cohort of 416 patients with hematologic malignancies (Cortelezzi et al. 2005). Twenty-one percent of these patients were HCT recipients, and 81.2% had platelet counts less than  $50 \times 10^9/L$ . There was a nonstatistically significant trend toward lower rates of thrombotic complications with thrombocytopenia. In a report that included 1514 patients undergoing inpatient HCT, 70 patients

(4.6%) had 75 symptomatic VTE events, of which 55 (73.3%) were catheter-associated. In the same cohort, clinically significant bleeding occurred in 230 patients (15.2%; 95% CI, 13.4–17.1%); 55 patients (3.6%; 95% CI, 2.7–4.7%) had fatal bleeding (Gerber et al. 2008). Overall, in individuals undergoing HCT, the risk of significant bleeding appears to be greater than the risk of serious complications from thrombosis, which greatly influences the weighing of risk versus benefits of thromboprophylaxis, particularly in the setting of thrombocytopenia.

### 40.2.2 VTE Prophylaxis

#### 40.2.2.1 Randomized Studies

Randomized studies have not evaluated empiric prophylactic anticoagulation in HCT recipients; however, studies in patients with cancer provide the next best evidence that can be extrapolated. The PROTECHT (nadroparin versus placebo) and SAVE-ONCO (semuloparin versus placebo) trials showed a significant reduction in the relative risk of venous thromboembolism with prophylactic anticoagulation in patients with cancer; however, the absolute risk reduction is small, and no survival benefit has been demonstrated. The American Society of Clinical Oncology (ASCO) guidelines advise against the use of routine prophylactic anticoagulation in ambulatory patients with cancer (Key et al. 2020). We do not generally recommend prophylactic anticoagulation in thrombocytopenic HCT recipients with the exception of those with multiple myeloma (MM) receiving thalidomide or lenalidomide or hospitalized patients at higher risk of thrombosis (Table 40.1).

#### 40.2.2.2 Multiple Myeloma

Patients with multiple myeloma have a high baseline risk of thrombosis of 5–10% which increases several-fold in patients being treated with the immunomodulators (IMiDs) thalidomide and lenalidomide with dexamethasone or chemotherapy. Consolidation therapy with the thalidomide or lenalidomide after HCT has been shown to improve complete remission rates and

**Table 40.1** Recommendations for prophylaxis and treatment of VTE in HCT recipients<sup>a</sup>

VTE prophylaxis	VTE treatment
<b>Indications for prophylaxis</b> <ul style="list-style-type: none"> <li>– Patients with MM receiving IMiDs</li> <li>– During hospitalization or postoperatively, as long as platelet count is <math>&gt;50 \times 10^9/L</math>.</li> <li>– Prophylaxis is not recommended in outpatients with indwelling vascular catheters</li> </ul>	<b>General principles</b> <ul style="list-style-type: none"> <li>– Start therapeutic doses of LMWH or IV UFH in patients who have platelet count <math>&gt;50 \times 10^9/L</math> and no active bleeding. UFH is preferred in case of renal impairment (GFR <math>&lt;30</math> mL/min) or high bleeding risk</li> <li>– Continue LMWH or transition to warfarin (if LMWH is contraindicated) for maintenance therapy</li> <li>– DOACs are not currently recommended in patients undergoing HCT</li> </ul>
<b>Prophylaxis strategy</b> <ul style="list-style-type: none"> <li>– Aspirin in low-risk patients with MM receiving IMiDs</li> <li>– LMWH (prophylactic dose of 40 mg SC daily) for patients with MM on IMiDs and <math>&gt;1</math> risk factor for VTE</li> <li>– Prophylactic doses of UFH or LMWH in hospitalized patients</li> </ul>	<b>Duration of anticoagulation</b> <ul style="list-style-type: none"> <li>– <i>General</i>: 3–6 months or as long as malignancy or use of IMiDs persists, whichever is longer</li> <li>– <i>Catheter-related thrombosis</i>: 3 months or as long as catheter is in place</li> </ul>
	<b>Inferior vena cava filter</b> Only use to patients in whom anticoagulation is contraindicated, or those who develop pulmonary embolism on anticoagulation. Remove as soon as anticoagulation can be started

DOACs, direct oral anticoagulants; *IMiDs*, immunomodulatory drugs; *LMWH*, low-molecular-weight heparin; *MM*, multiple myeloma; *UFH*, unfractionated heparin

<sup>a</sup>Adapted from Chaturvedi et al. (2016)

prolong event-free survival and is thus rapidly becoming the standard of care (McCarthy et al. 2012; Barlogie et al. 2006). In patients receiving thalidomide consolidation after auto-HCT for MM, the rate of VTE was 24% and 6% in the induction and consolidation periods, respectively, despite thromboprophylaxis with low-molecular-weight heparin (LMWH) (Barlogie et al. 2006). McCarthy et al. reported no episodes of VTE in

patients receiving consolidation therapy with lenalidomide; however, these patients also received prophylactic anticoagulation (McCarthy et al. 2012). Based on studies showing the benefit of thromboprophylaxis in patients with newly diagnosed multiple myeloma receiving lenalidomide- or thalidomide-based treatment (Palumbo et al. 2011) and the ASCO recommendation for thromboprophylaxis in this population (Lyman et al. 2015; Key et al. 2020), we recommend either aspirin or LMWH for lower risk patients and LMWH for higher-risk patients receiving thalidomide or lenalidomide.

#### 40.2.2.3 Hospitalized Patients

Though there is a clear benefit of pharmacologic thromboprophylaxis in medically ill hospitalized patients (Samama et al. 1999), randomized trials have not evaluated thromboprophylaxis in HCT patients. The potential benefit from VTE prophylaxis is proportional to VTE risk, and therefore this is particularly important in patients with reduced mobility and with a history of VTE (if not on long-term anticoagulation) due to an even higher risk of thrombosis. Our practice is to start prophylactic anticoagulation for hospitalized patients in the posttransplant period once the platelet count is  $>50 \times 10^9/L$ , and there is no active bleeding. For very high-risk patients, anticoagulation can be considered if the platelet count is  $>30 \times 10^9/L$ ; however, this must be balanced with the risk of bleeding.

Risk models developed recently have sought to predict VTE risk after allogeneic transplantation to inform which patients may benefit from VTE prophylaxis after platelet engraftment. Martens et al. developed the HIGH-2-LOW score which is calculated from seven predictors (history of CRT, inpatient at day 30, GVHD grade 3 or 4, history of PE or lower extremity DVT, lymphoma diagnosis, BMI  $\geq 35$ , and WBC count  $\geq 11 \times 10^9/L$ ) evaluated at day 30 after allogeneic transplant that each contributes one point to stratify patients into low (0 points), intermediate (1 point), or high risk ( $\geq 2$  points) for VTE between day 30 and 100 after allogeneic transplant (Martens et al. 2021). Patients with a high, intermediate, and low risk had a 10.3%, 3.6%, and 1.5% risk of

incident VTE, respectively, between day 30 and day 100.

The HiGHS2 score is another risk model that evaluates VTE risk at 2 years after transplantation and includes the history of stroke (3 points), chronic GVHD (1 point), hypertension (2 points), male sex (2 points), peripheral blood stem cell source (3 points) as predictors that classify patients into high ( $\geq 5$  points) and low VTE ( $< 5$  points) risk, with a 9.3% and 2.4% incident risk of 10-year VTE, respectively (Gangaraju et al. 2021).

#### 40.2.2.4 Prophylaxis of Catheter-Related Thrombosis

HCT patients, especially those undergoing ambulatory HCT, frequently have indwelling vascular catheters with the potential of catheter-related thrombosis (CRT). Despite multiple randomized and observational studies, thromboprophylaxis for the prevention of catheter-related thrombosis in patients with cancer remains controversial. The largest study of thromboprophylaxis in central venous catheters randomized 1590 cancer patients undergoing chemotherapy to adjusted-dose warfarin (international normalized ratio, 1.5–2.0), fixed-dose warfarin (1 mg/day), and no prophylaxis (Young et al. 2009). Symptomatic CRT was less frequent in the patients given adjusted-dose warfarin than in those who received no prophylaxis (2.7% versus 5.9%,  $P = 0.019$ ); however, both adjusted-dose and fixed-dose warfarin were significantly associated with increased risk of major bleeding (Young et al. 2009). Recent meta-analyses of randomized trials concluded that prophylactic warfarin and LMWH do not significantly reduce symptomatic CRT in patients with cancer (Akl et al. 2007). Based on the available evidence, we do not routinely recommend prophylactic anticoagulation to prevent catheter-related thrombosis.

#### 40.2.3 VTE Diagnosis and Treatment

Venous duplex ultrasonography should be performed in patients presenting with extremity swelling, redness or tenderness, or pulmonary

angiography in patients with chest pain, dyspnea, or unexplained tachycardia. A clinical assessment of bleeding risk is necessary in patients who are diagnosed with VTE. Patients with no increased risk based on bleeding history and platelet count  $> 50 \times 10^9/L$  should be started on therapeutic anticoagulation with either LMWH or unfractionated heparin (UFH). The use of LMWH is restricted to patients with glomerular filtration rate  $> 30$  mL/minute, while UFH is used in patients with impaired renal function (glomerular filtration rate  $< 30$  mL/min) or those with high bleeding risk. Following initiation of anticoagulation with LMWH or UFH, patients may be continued on LMWH or transitioned to an oral anticoagulant such as warfarin or possibly a direct oral anticoagulant. The direct oral anticoagulants (DOACs) have not been evaluated specifically in HCT recipients; however, given their widespread use in clinical practice, and data supporting their use in patients with malignancy, they may be considered on a case-by-case basis in patients who have platelet counts stably over  $> 50 \times 10^9/L$ , no recent bleeding, and adequate renal function. The optimal duration of anticoagulation for VTE in HCT patients has not been evaluated in prospective studies. The recommendation for patients with cancer-related VTE is anticoagulation for 3–6 months, with ongoing therapy if the malignancy persists (Key et al. 2020; Kearon et al. 2012). We follow an analogous strategy in HCT patients with the caveat that extended anticoagulation is often not feasible in patients with relapsed disease and a high likelihood of disease-related or treatment-related thrombocytopenia (Table 40.1).

The use of inferior vena cava (IVC) filters should be restricted to patients with acute deep vein thrombosis and a contraindication to anticoagulation and possibly patients who develop pulmonary embolism while on therapeutic anticoagulation (Kearon et al. 2012). IVC filters should not be used for primary prophylaxis of pulmonary embolism. In patients with large, symptomatic thrombosis and severe thrombocytopenia, we sometimes follow a strategy of platelet transfusions to reach a threshold of  $50 \times 10^9/L$  to allow safer anticoagulation with heparin. In the

setting of thrombocytopenia with platelets  $25\text{--}50 \times 10^9/\text{L}$ , some centers have used reduced-dose LMWH for VTE treatment and may be a viable strategy after weighing the patient-specific benefits of anticoagulation with the risks of bleeding (Ibrahim et al. 2005; Lam et al. 2021; Mantha et al. 2017).

#### 40.2.4 Treatment of Catheter-Related Thrombosis

The rate of PE and mortality from CRT is low, and the objectives of CRT treatment are to reduce symptoms, prevent extension into more central veins, preserve access, and prevent chronic venous stenosis. There is no evidence that removal of the catheter improves outcomes. Therefore, it is reasonable not to remove the catheter unless it is nonfunctional, is no longer needed, or may be infected. Thrombus reduction by catheter-directed thrombolysis is relatively safe and effective and may be tried in an attempt to preserve the catheter. Anticoagulation is required in patients with acute CRT regardless of whether the catheter is removed (Kearon et al. 2012; Lyman et al. 2015). We prefer LMWH, though vitamin K antagonists (VKA) may be used if LMWH is contraindicated. In a prospective study of 78 patients with CRT treated with full-dose dalteparin bridged to warfarin, there were no new thrombotic events at 3 months and 57% of catheters were still functional (Kovacs et al. 2007). The optimum duration of anticoagulation has not been evaluated in prospective studies. Current ACCP guidelines recommend anticoagulation for 3 months or until the catheter is removed, whichever is longer (Kearon et al. 2012). Several clinicians prefer to continue anticoagulation for 1–2 weeks after the catheter is removed.

#### 40.2.5 Sinusoidal Obstruction Syndrome (SOS)

SOS is a life-threatening complication that presents usually within the first 45 days after HCT with elevated serum bilirubin levels, painful hep-

atomegaly, and fluid retention (Carreras 2015). Endothelial injury of the hepatic sinusoids in SOS initiates hepatocyte injury and liver failure. SOS can occur in as high as 8–13% of HCT recipients, and mortality is in excess of 80% (Carreras 2015). Myeloablative conditioning, preexisting liver disease, younger age, and poor performance status are associated with an increased risk of SOS (McDonald et al. 1993). Ursodeoxycholic acid is recommended as prophylaxis for SOS in patients undergoing allo-HCT. Anticoagulation with low-dose heparin has also been studied and is sometimes prescribed to patients undergoing auto-HCT. Defibrotide, a pro-fibrinolytic agent, is a new agent approved for the treatment of severe SOS in both children and adults and is associated with higher rates of survival than historical controls (20–30% at day 100) (Richardson et al. 2016). Defibrotide prophylaxis has been shown to have some efficacy in preventing SOS in high-risk children, but whether this benefit translates for adults is not known.

#### 40.2.6 Transplant-Associated TMA

TA-TMA is a heterogenous, frequently fatal disorder that occurs within 100 days after HCT and is caused by treatment- and disease-related endothelial damage, coagulation activation, and microvascular thrombosis (Nadir and Brenner 2007). It is characterized by thrombocytopenia, microangiopathic anemia with schistocytes on the blood smear, and varying organ impairment such as renal failure and neurological symptoms. The diagnosis can be challenging, since the clinical symptoms overlap with other common complications including GVHD and infections (Rosenthal 2016). Risk factors for developing TA-TMA include exposure to calcineurin inhibitors, high-dose chemotherapy, GVHD, infections, advanced age, female sex, and non-MAC (Elsallabi et al. 2016). Elevated levels of vWF and inflammatory mediators, such as IL-1, TNF- $\alpha$ , and thrombomodulin, and neutrophil extracellular traps have been implicated as causing the endothelial damage in TA-TMA. Treatment of TA-TMA is mostly supportive; however,

recent data show that some patients with severe TA-TMA harbor complement gene mutations, and uncontrolled complement activation has been demonstrated in TA-TMA, which is a potential therapeutic target. The complement inhibitor eculizumab has been successfully used in some cases of TA-TMA (Rosenthal 2016).

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### 40.3 Bleeding Complications

Bleeding in HCT recipients is closely associated with prolonged and severe thrombocytopenia. In retrospective studies, the rate of bleeding in HCT recipients ranges from 15.2% to 27.1%, and life-threatening or fatal bleeding occurred in 1.1% to 3.6% of patients (Gerber et al. 2008; Pihusch et al. 2002; Labrador et al. 2013). Gerber et al. reported that the initiation of therapeutic anticoagulation during days 1–180 after HCT was the strongest predictor of bleeding [OR 3.1 (95% CI 1.8–5.5)] (Gerber et al. 2008). Furthermore, GVHD [OR 2.4 (95% CI 1.1–3.3)] increased the risk of bleeding, while auto-HCT (versus allo-HCT) was protective [OR 0.46 (95% CI 0.33–0.64)]. Bleeding can take any form, including gastrointestinal hemorrhage in patients with GVHD of the gut, hemorrhagic cystitis in patients with genitourinary involvement by GVHD, viral reactivation, and alkylating agent therapy, or spontaneously. Diffuse alveolar hemorrhage (DAH) is a devastating bleeding complication that occurs in 2%–14% of HCT recipients and presents with progressive hypoxia, pulmonary infiltrates, and bloody alveolar lavage (Nadir and Brenner 2007). DAH is more common in thrombocytopenic patients and those with acute GVHD, and the effects of inflammatory cytokines on the alveolar lining have been implicated. There are no evidence-based prophylactic and therapeutic strategies, and reported mortality is around 80% (range 64 to 100%) (Afessa et al. 2002). Platelet transfusions, systemic corticosteroids, antifibrinolytics, and recombinant factor VIIa have all been used with inconsistent results. It is general practice to administer prophylactic platelet transfusions for platelet counts less than  $10 \times 10^9/L$  in patients undergoing myeloablative

chemotherapy or HCT, though the superiority of prophylactic over therapeutic platelet transfusions is supported by low- to moderate-grade evidence. Given the competing risks of bleeding and thrombosis, identifying patients at high risk for these outcomes can optimize strategies for prophylaxis. The timing of hemostatic complications is an important consideration, since bleeding events are more likely to occur early in the post-transplant course when patients are profoundly thrombocytopenic, while thrombotic events occur more frequently after hematopoietic recovery (Gerber et al. 2008; Labrador et al. 2013).

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### 40.4 Thrombosis and Bleeding in CAR-T

Thrombotic and bleeding events are also common complications seen in patients after chimeric antigen receptor T-cell (CAR-T) therapy. In a retrospective report of 148 patients receiving CD19 CAR T-cell therapy for large B-cell lymphoma, the incidence of VTE was 11% between day 0 and day 100 of CAR T-cell infusion (Hashmi et al. 2020). Half of all new thrombotic events were deep vein thromboses and half of which were catheter-related. Twenty-five percent were pulmonary emboli and the remaining quarter were categorized as other (mesenteric, cerebral, and renal). Risk factors associated with VTE were bulky disease, use of bridging therapy, worse performance status, severe cytokine release syndrome, and immune effector cell-associated neurotoxicity syndrome (ICANS). Most of these patients with VTE were treated with anticoagulation, either heparin products or direct oral anticoagulants, which was later held if thrombocytopenia developed. There were no major bleeding events or deaths from VTE or bleeding.

Another retrospective study by Parks et al. reported a 9% incidence of VTE within 60 days of CAR-T cell infusion in 91 patients with relapsed/refractory non-Hodgkin lymphoma or multiple myeloma (Parks et al. 2021). Of these patients, a majority were started on anticoagulation with direct oral anticoagulants or LMWH, one of whom had to stop therapy due to thrombo-



cytopenia. None experienced bleeding events or recurrent thromboses.

In a cohort of 127 patients, Johnsrud et al. found that 6.3% of patients developed VTE and 9.4% developed bleeding complications within the first 3 months after CAR T-cell infusion (Johnsrud et al. 2021). Bleeding events included gross hematuria, soft tissue bleeding, gastrointestinal hemorrhage, hemoptysis, and subdural hematoma. Older age, elevated pre-lymphodepleting chemotherapy lactate dehydrogenase, and lower baseline platelet count were associated with bleeding. Patients with bleeding also had lower platelet and fibrinogen nadirs compared to patients without bleeding events. High-grade ( $\geq 3$ ) ICANS was associated with both bleeding and thrombosis, and CRS was not associated with either.

There are no large, prospective studies that evaluate prophylaxis and management of hemostatic complications in patients undergoing CAR T-cell therapy. Preliminary reports indicate that thrombotic and bleeding events are common, that anticoagulation for the treatment of VTE may be safe when platelets are above  $50 \times 10^9/L$ , and that hemostatic complications may be correlated with other CAR-T adverse events such as cytokine release syndrome, neurotoxicity, thrombocytopenia, and hypofibrinogenemia.

### Key Points

- Hemostatic complications, including both thrombosis and bleeding, are common in HCT recipients and contribute to morbidity and mortality.
- Indwelling vascular catheters, GVHD-associated inflammation, and certain medications are important risk factors for VTE, while prolonged severe thrombocytopenia and GVHD predispose to bleeding.
- Pharmacologic thromboprophylaxis is recommended for patients with MM receiving IMiDs and hospitalized patients with platelet count  $>50 \times 10^9/L$ , but not for routine prophylaxis of CRT.

- LMWH (or UFH) is the treatment of choice for VTE in HCT recipients.
- Ursodiol and defibrotide are recommended for the prevention and treatment of SOS, respectively. Defibrotide may also have a role in the prophylaxis of high-risk patients.

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## 41.1 Introduction

The current definition for hematological recovery includes neutrophil recovery, defined as the first of three consecutive days with an absolute neutrophil count  $\geq 0.5 \times 10^9/L$  and platelet recovery which is defined as a platelet count of  $\geq 20 \times 10^9/L$  in the absence of platelet transfusion for 7 consecutive days.

In allogeneic transplantation, chimerism evaluation is essential to confirm that cells are donor-derived, particularly in reduced-intensity and non-myeloablative conditioning regimens. A common cutoff for complete donor chimerism is  $\geq 95\%$  of donor-derived cells (typically ranging from 90% to 97.5% in most centers). It is advisable to assess multiple lineages, including T-cells and granulocytes, if feasible.

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The incidence of graft failure (GF) is  $<3\text{--}5\%$  in the auto- and matched allo-HCT setting, but it increases up to 10% in the cases of haploidentical or CBT. The prognosis of GF is poor, and most patients die due to infections or bleeding, with an OS at 3–5 years after the diagnosis of GF in the range of 20–30%.

## 41.2 Definitions (Kharfan-Dabaja et al.2021)

Primary graft failure (GF)	ANC $<0.5 \times 10^9/L$ by day +30 with associated pancytopenia. Donor chimerism testing is also done to confirm the suspicion of graft failure. CBT: Up to day +42, with associated pancytopenia
Secondary GF	A decline in hematopoietic function (may involve hemoglobin and/or platelets and/or neutrophils) necessitating blood products or growth factor support, after having met the standard definition of hematopoietic (neutrophils and platelets) recovery. This assumes donor chimerism testing is also done to confirm the suspicion of graft failure.
Poor graft function	Frequent dependence on blood and/or platelet transfusions and/or growth factor support in the absence of other explanations, such as disease relapse, drugs, or infections. This assumes that donor myeloid and lymphoid chimerism are within a desirable target level.
Graft rejection	GF caused by the <i>immune rejection</i> of donor cells mediated by host cells.

### 41.3 Causes and Risk Factors

The etiology of GF is multifactorial in most of the cases (Fig. 41.1, Table 41.1).

#### 41.3.1 Donor Type, HLA Matching, and Graft Source

Classical studies showed a close relationship between the degree of HLA mismatch and the incidence of GF, but it is difficult to draw conclusions because most of them used a limited HLA matching, including only low-resolution A, B, and DR locus (Anasetti et al. 1989; Petersdorf et al. 2001). More recent studies, using high-resolution techniques for HLA typing and including 10–12 loci (A, B, C, DR, DQ, and DP), did not find differences in GF rates between no HLA antigen mismatch and a single HLA mismatch in

both conventional MAC (Lee et al. 2007) and RIC (Passweg et al. 2011).

URD transplant was associated with a higher risk of GF (HR 1.38,  $p < 0.001$  compared to HLA identical sibling) that was even higher when there were two or more mismatches (HR 1.79,  $p < 0.001$ ) (Olsson et al. 2015).

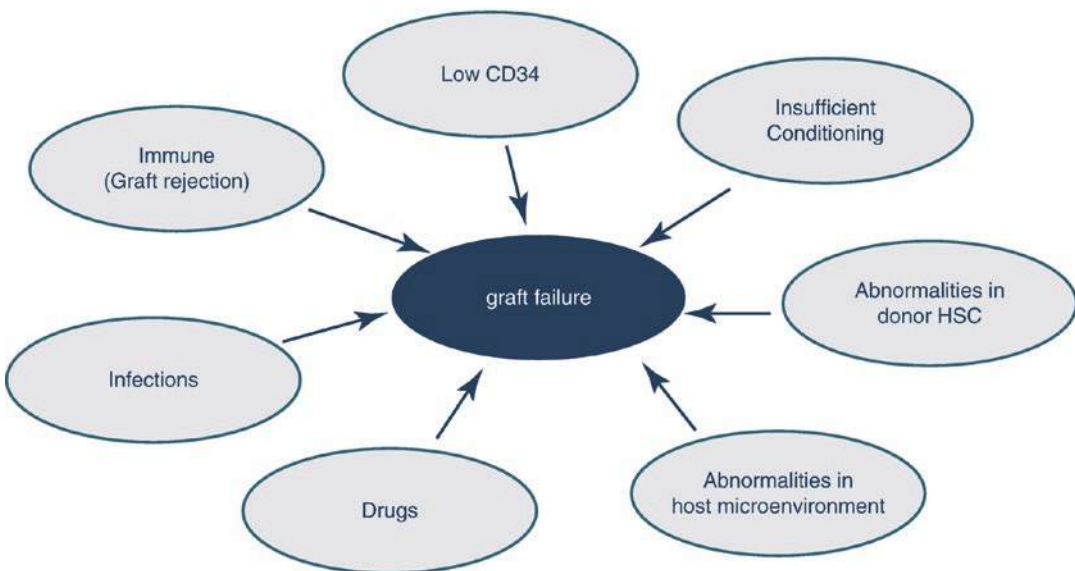
In the haploidentical setting, the incidence of GF is around 10%, which seems higher than the 3–5% currently reported for MSD or URD HCT, although there are not well-designed comparative studies.

#### 41.3.2 Graft Source and Cellular Content

BM is consistently associated with delayed neutrophil and platelet engraftment across all types of transplants; the impact on GF depends on the

**Table 41.1** Risk factors for GF

Pretransplant (difficult to modify)	Pretransplant (easy to modify)	Peri- and post-transplant
HLA mismatches	Graft source	CD34+ cell count
Non-malignant disease	Conditioning	Viral infections
Advanced disease	T-cell depletion	GVHD
Extensive marrow fibrosis		Drug toxicity
Donor age		
Splenomegaly		
Iron overload		
HLA antibodies		
Transfusion history		



**Fig. 41.1** Causes associated with the development of GF

**Table 41.2** Minimum cell content recommended

Progenitors	Type of transplant	Amount of cells
BM progenitors	Autologous	TNC: $2 \times 10^8/\text{kg}$
	Allogeneic	TNC: $3 \times 10^8/\text{kg}$
PB progenitors	Autologous	Minimum: CD34 $> 1 \times 10^6/\text{kg}$ Optimum: CD34 $> 2 \times 10^6/\text{kg}$
		Allogeneic MAC
	Allogeneic RIC	Minimum: CD34 $> 2 \times 10^6/\text{kg}$ Optimum: CD34 $4\text{--}8 \times 10^6/\text{kg}$
Cord blood	HLA 4–6/6	TNC $> 2.5\text{--}3 \times 10^7/\text{kg}$ CD 34 $> 1 \times 10^5/\text{kg}$

TNC total nucleated cells; MAC myeloablative conditioning; RIC reduced-intensity conditioning regimen

donor type. GF incidence is not different for HLA MRD (Bensinger, 2012), but it is higher in the setting of URD (9% vs 3%, for BM and PB, respectively,  $p < 0.001$ ) (Anasetti et al. 2012). There are no prospective randomized data either looking at MAC or RIC, but retrospective results from EBMT and CIBMTR suggested there were no differences in GF between BM and PB (less than 5% in all cases). In contrast, in a study evaluating donor characteristics, the use of BM was the only factor associated with GF after RIC (HR 2.3;  $p = 0.02$ ) (Passweg et al. 2011).

The minimum cellular content required is still a matter of debate. Table 41.2 depicts a conservative proposal based on the literature review.

### 41.3.3 Anti-HLA Antibodies

The presence of donor-specific anti-HLA antibodies (DSAs) is associated with a higher risk of GF in the context of haploidentical CBT and URD transplants, and it may in fact translate into a reduced OS (Spellman et al. 2010; Ciurea et al. 2009; Ciurea et al. 2015). The high prevalence of anti-HLA antibodies (10–40%) (Morin-Zorman et al. 2016) and the increasing use of mismatched donors prompted the EBMT to write a set of advice and recommendations on this issue (Table 41.3) (Ciurea et al. 2018).

**Table 41.3** Considerations regarding the presence of anti-HLA antibodies

Anti-HLA and DSA prevalence	Anti-HLA: 10–40% DSA: 10–20%. Higher in females (increase with each pregnancy)
Detection methods	<ul style="list-style-type: none"> <li>Cell-based (direct test): donor viable lymphocytes and patient serum are needed. Complex and time-consuming technique. Low specificity and variable sensitivity (higher with flow cytometry assays than complement-based assays)</li> <li>Solid-phase immunoassays (virtual test): only require patient serum, and the technique is easy and fast. Sensitivity and specificity are intermediate/high depending on the type of assay. Modified techniques such as C4d or Cq1 assays allow to detect complement-fixing antibodies, which are at higher risk of inducing GF. These are the tests most commonly used nowadays; initial DSA testing and complement assay in case of positivity are recommended</li> <li>Although not well validated, the threshold of positivity for DSA can be considered <math>&gt;1000</math> and especially <math>&gt;5000</math> MFI, which is probably associated with the presence of complement-binding antibodies</li> <li>DSA study should be done during donor identification to select a donor and also within the month prior to transplant</li> </ul>
Management and desensitization treatment	<ul style="list-style-type: none"> <li>No standard scheme is widely accepted; different combinations have proven to be efficacious                             <ul style="list-style-type: none"> <li>Ab removal: plasmapheresis 1–4 procedures days –10 to –17 and even after transplant</li> <li>Inhibition of ab production: rituximab 375 mg/m<sup>2</sup> IV</li> <li>Ab neutralization: infusion of 20–40 platelet units selected to share donor antigens or buffy coat from 1 unit of blood, on day 1. IVIg can also be used</li> </ul> </li> </ul> <p><i>Avoid complement activation: IVIg, eculizumab</i></p>

DSA donor-specific antibodies; MFI mean fluorescence intensity; Ab antibodies; IVIg intravenous immunoglobulins

### 41.3.4 Conditioning Regimen

Increasing the intensity of MAC conditioning protocols does not reduce the incidence of GF. In contrast, RIC may be associated with a higher risk of GF.

Although it is well accepted that TBI reduces the risk of GF, there are no comparative studies that confirm this latter point. In combination with CY, the use of full-dose TBI does not seem to reduce GF in comparison with BU. The use of ATG in the preparative regimen in combination with CY seems to reduce the incidence of GF in patients with aplastic anemia. Also, in aplastic anemia patients, the addition of 2 Gy TBI to FLU/CY did not reduce the incidence of this complication.

### 41.3.5 Other Factors Associated with the Development of GF

**ABO mismatch:** Major incompatibility was associated with primary GF (HR 1.24;  $p = 0.012$ ).

**Cryopreservation:** Associated with primary GF (HR 1.43;  $p = 0.013$ ).

**Female donor to male recipient:** Associated with primary GF (HR 1.28;  $p = 0.001$ ).

**Splenomegaly:** Associated with primary GF in MPN (HR 3.92;  $p = 0.001$ ) and MDS (HR 2.24;  $p = 0.002$ ).

**Use of G-CSF:** Associated with reduced risk of primary GF (HR 0.36;  $p < 0.001$ ) vs no growth factors.

**Underlying disease:** Non-malignant diseases are associated with a higher incidence.

**Previous treatments:** On the one hand, the use of numerous lines of treatments prior to the transplant may impair engraftment through the damage of the marrow microenvironment, while on the other hand, the absence of treatments may facilitate graft rejection.

**Graft manipulation:** Ex vivo TCD is associated with a higher risk of primary GF in most studies.

## 41.4 Management of GF

OS after GF remains consistently low, even in patients who undergo salvage transplant. Therefore, the focus should be on preventing GF and prompting its identification to adopt the measures to revert it.

### 41.4.1 Prevention and Early Diagnosis of GF

The identification of DSA is of utmost importance in the mismatch setting. Desensitization treatment for patients at higher risk seems reasonable. Although barely supported by well-designed studies, we recommend the following measures to be adopted in patients at high risk of GF: use PB as a stem cell source, include low-dose TBI and/or ATG in the conditioning regimen, consider the use of G-CSF posttransplant, and closely evaluate the engraftment including marrow chimerism studies shortly after transplant (day +14). In a single-CBT study, a level of donor chimerism in BM lower than 65% was associated with a higher risk of GF (Moscardó et al. 2009); these results cannot be directly extrapolated to other types of transplants.

Olson and colleagues developed a score to predict GF in patients at risk at day +21 post-HCT (Olsson et al. 2015): age (<30, 1 point), Karnofsky status (<90%, 1 point), disease (MDS, 1; CLL or CML, 2; and MPN, 3 points), status (advanced, 1 point), HLA matching (mismatched, 2 points), graft (BM  $< 2.4 \times 10^8/\text{kg}$ , 1 point; PB, 2 points), conditioning (no TBI, 2 points), and GVHD prophylaxis (no CNI + MTX, 2 points; TCD, 3 points). A score  $> 6$  at day +21 had a positive predictive value of 28–36%, while the negative predictive value of a score  $< 7$  was 81% for GF.

### 41.4.2 Initial Measures

It is important to apply them as soon as GF is suspected.



- Stop as many toxic drugs as possible; treat infections; although of limited utility, it would be reasonable to trial use of G-CSF.
- Adjust posttransplant IS. Maintain correct IS levels in the early posttransplant period. Later on, after the third/sixth month and if mixed chimerism is present, especially after a RIC transplant, a faster tapering of IST could overcome mixed chimera (in patients with SAA, it is commonly recommended to increase IST). As the best approach is influenced by multiple variables (chimerism dynamics, cell lineage affected by the mixed chimerism, disease type and status, presence of GVHD, etc) it is important to underline the absence of consensus regarding the best management in this situation, as it is highlighted in a recent report on behalf of the ASTCT (Kharfan-Dabaja et al. 2021).
- Data regarding the use of TPO analogs after transplant are scarce, but the results of eltrombopag in aplastic anemia and its favorable toxicity profile would support, in our view, a trial with this drug before considering more complex and risky options such as DLI or a second transplant. Recent studies in the transplant setting suggest that TPO receptor agonists are safe and may be useful to revert cytopenias, especially thrombocytopenia (Bento et al. 2019).

#### 41.4.3 DLI, CD34 Boost, and Mesenchymal Stem Cells (MSCs)

DLI could be recommended if decreasing levels of donor chimerism are observed. A careful risk/benefit evaluation is warranted, as this is not a risk-free approach and a high risk of development of GVHD is anticipated.

In patients with poor graft function, the use of CD34 boost can be offered, with a recent systematic review and meta-analysis supporting it is relatively safe and suggesting a possible benefit in survival (Shahzad et al. 2021). Unfortunately, it is not clear when to perform it, but probably 2–3 months without improvement after the initial measures would be a reasonable cutoff.

Some recent experiences have also shown the safety and potential utility of the infusion of MSC in the context of PGF or GF (Servais et al. 2023).

#### 41.4.4 Second Transplant

The limited utility and low success of cryopreserved autologous stem cells do not allow to formally recommend to perform auto-HSC harvest in any type of transplant procedure.

Results and recommendations for second allogeneic transplantation are detailed in Tables 41.4 and 41.5.

**Table 41.4** Second allogeneic hematopoietic cell transplant in patients with GF

Author (year)	n patients diagnosis	Donor (same/different) source	Engraftment (median d)	OS
Gaziev (1999)	32 (1°, 4; 2°, 28) Thalassemia	28/4 All BM	67.7% (+19)	3 years: 60%
Guardiola (2000)	82 (1°, 7; 2°, 54) Hem Neo, AA	56/26 72 BM; 10 PB	62% (+17)	3 years: 33%
Min (2000)	20 (1°, 7; 2°, 10) Hem Neo, AA	20/0 6 BM, 14 PB	75% (NR)	3 years: 70%
Chewning (2007)	16 (1°, 11; 2°, 5) Hem Neo, FA	6/16 13 PB (8 TCD), 2 BM, 1 CB	100% (+12)	3 years: 35%
Gyurkocza et al. (2009)	38 (1°, 18; 2°, 20) Hem Neo, AA	14/24 36 PB, 1 BM, 1 CB	87% (+15)	4 years: 42%
Schreiber (2010)	122 (1°, 122) Hem Neo, AA	98/24 60 PB, 62 CB	66% (NR)	1 year: 11%
Remberger (2010)	20 (1°, 6; 2°, 14) Hem Neo, Non-Mal	11/9 7 PB, 11 BM, 2 CB	90% (+20)	3 years: 60%
Fuji (2012)	220 (1°, 200; 2°, 19) Hem Neo, Non-Mal	0/220 24 PB, 16 BM, 180 CB	CB 30% (21) PB-BM 70–75% (18–14)	1 year PB:58% CB: 28%
Ferrá (2014)	89 (1°, 49; 2°, 40) Hem Neo, Non-Mal	38/37 61 PB, 6 BM, 8 CB	85% (+15)	5 years: 31%
Nagler (2023)	243 (all 1° GF and AL)		77.3%	5 years: 30.7%

*Hem Neo* hematological neoplasias; *AA* aplastic anemia; *AL* Acute leukemia; *FA* Fanconi anemia; *Non-Mal* nonmalignant disorders; *PB* peripheral blood; *BM* bone marrow; *CB* cord blood; *TCD* T-cell depletion

**Table 41.5** Recommendations to perform a second allogeneic HCT as treatment for GF

Type of donor	Similar results using the same/different donor. Consider a different donor if it is not associated with significant delays. Consider haploidentical donors. Always avoid donors if positive DSA
Conditioning regimen	It is always required. Better RIC
Posttransplant IS	It is required; CNI-based schemes are the most commonly used
Stem cell source	PB or BM show similar results and should be preferred to CB
T-cell depletion	– avoid ex vivo T-cell depletion, especially if with immune graft rejection – in cases of poor graft function, it can be a good option as it reduces the potential risk of GVHD – ATG or alemtuzumab have been used to foster IS and also to reduce the GVHD risk

*DSA* donor-specific antigens; *BM* bone marrow; *PB* peripheral blood

**Key Points**

- Graft failure is an infrequent but often fatal complication of HCT.
- Etiology is complex and usually multifactorial.
- Preventive measures and early identification of potential causes in order to try to revert them are the key aspects to treat it.

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# Early Complications of Endothelial Origin

# 42

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## 42.1 Early Complications of Endothelial Origin

### 42.1.1 Introduction

Endothelial dysfunction is a common pathophysiology during inflammation and allo-immunity. The endothelium is the first contact for immunological effector cells in the blood and key to the regulation of various inflammatory processes. It is well known that during inflammatory diseases and allo-immune responses, activation of endothelial cells (ECs) occurs, leading to increased expression of adhesion molecules, release of chemokines, production of growth factors, and activation of coagulation factors. Published data indicate that endothelial

dysfunction may be related to donor T-cell recognition of host HLA molecules on endothelial cells (ECs). In murine models, it was shown that the transfer of allogeneic lymphocytes leads to EC activation and damage (Deschaumes et al. 2007).

Additionally, the animal models of the most well-known endothelial syndrome, SOS/VOD (see Chap. 49), evidence that the first morphological alterations occur in endothelial cells (ECs) of the hepatic sinusoids (DeLeve et al. 1996). Similarly, multiple ex vivo and in vitro studies have shown that, in auto- and allo-HCT, there is a pro-inflammatory, prothrombotic, and pro-apoptotic state secondary to endothelial damage (Palomo et al. 2009, 2010; Carreras and Diaz-Ricart 2011).

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### 42.1.2 Main Characteristics of These Early Complications

1. They appear early after HCT (between days 0 and +100).
2. Their diagnosis is based on the presence of overlapping medical signs and symptoms, and consequently, they are classified as syndromes.
3. They seem to begin at the capillary level, in a systemic way or in one or more affected organs.
4. If not properly treated, they can evolve into an irreversible MODS/MOF.

### 42.1.3 Classification of Vascular Endothelial Syndromes

Nowadays, the following entities are considered secondaries to endothelial damage during HCT (Carreras 2020):

- Fluid overload syndrome (FOS)
- Capillary leak syndrome (CLS)
- Sinusoidal obstruction syndrome (SOS/VOD) (see Chap. 49)
- Pre-engraftment syndrome (pES) and engraftment syndrome (ES)
- Thrombotic microangiopathy associated with HCT (TMA)
- Vascular idiopathic pneumonia syndrome (vIPS) (see Chap. 52) including diffuse alveolar hemorrhage (DAH), pulmonary capillary hyperpermeability syndrome, and; peri-engraftment respiratory distress syndrome (PREDS).
- Posterior reversible encephalopathy syndrome (PRES)
- Acute graft-versus-recipient disease (aGVHD) (see Chap. 43)

### 42.1.4 Biomarkers

Given the difficulty in establishing a correct differential diagnosis of these syndromes, attempts have been made for years to find panels of biomarkers that facilitate their diagnosis, but for the moment, except in aGVHD, little progress has been made since many of these syndromes share biomarkers that only reflect endothelial damage. For example, we could mention:

<b>SOS</b>	vWF; sTM; P-selectin; PAI-1; sICAM-1; L-Ficolin; Ang-2; hyaluronan; IGF-1
<b>TMA</b>	vWF; t-PA; sTM; Ab. anti-CFH; sC5b-9; ds-DNA; sST2
<b>CLS</b>	VEGF; Ang-2
<b>ES</b>	CRP; procalcitonin; elafine; sST2; IL2R- $\alpha$ ; TNFR1; IL-6
<b>GVHD</b>	vWF; sICAM-1; sVCAM-1; sTM, TNFR1, E-selectin; Ang-2; EMP; sST2; REG3- $\alpha$ ; CEC; Follistatin; PIGF; (panel Ang-2, sTM, dimer D, CRP)

Adapted from Luft et al. (2020), Hildebrandt and Chao (2020), and Putta et al. (2023). *vWF* von Willebrand factor; *sTM* soluble thrombomodulin; *PAI-1* plasminogen activator inhibitor-1; *sICAM-1* soluble intercellular adhesion molecule-1; *IGH-1* insulin-like growth factor; *t-PA* tissue plasminogen activator; *CHF* complement factor H; *sC5b-9* soluble complement C5b-9 fraction; *ds-DNA* double-stranded DNA; *sST2* soluble tumorigenicity suppressor-2; *VEGF* vascular endothelial growth factor; *Ang-2* angiopoietin-2; *CRP* C-reactive protein; *IL2R* interleukin-2 receptor; *TNFR1* tumor necrosis factor receptor 1; *IL* interleukin; *sVCAM-1* soluble vascular adhesion molecule-1; *EMP* endothelium-derived microparticles; *REG3* pancreatic islet-derived regenerating protein-3-alpha; *CEC* circulating endothelial cells; *PIGF* placental growth factor

### 42.1.5 EASIX

Despite all these efforts to have specific biomarkers available, today the “biomarker panel” that is proving most successful for the suspicion, diagnosis, and prognosis of endothelial dysfunction syndromes is the so-called EASIX (endothelial activation and stress index), which is obtained by applying the formula:

$$\text{LDH (U/L)} \times \text{Creatinine (mg/mL)} / \text{Platelets} (\times 10^9/\text{L})$$

EASIX has been shown to be an excellent surrogate marker to quantify endothelial damage. Measured before HCT, it predicts TMA and TRM. Measured at day 0, it predicts SOS. Measured at the onset of aGVHD, it predicts TRM. In addition, before HCT and on day 0, it predicts early water retention and early hyperbilirubinemia (without the need for SOS). Its value in other clinical situations such as MDS, MM, CAR-T cell therapy, and COVID-19 is being analyzed (Luft et al. 2020; Korell et al. 2022).



## 42.2 Fluid Overload Syndrome (FOS)

FOS is believed to be a consequence of increased vascular permeability caused by endothelial activation, as well as the usual hyperhydration used in conditioning. Despite its high frequency (up to 60% around day 0 of HCT), few publications recognize this entity (Rondón et al. 2017).

This diagnosis should be suspected when very early (even before the administration of stem cells), the patient presents weight gain, moderate dyspnea, nonproductive cough, and moderate hypoxemia in the absence of renal or cardiac insufficiency. Pulmonary auscultation shows bibasal moist rales, and radiology shows increased cardiothoracic index and diffuse alveolar/interstitial infiltrates. Central venous pressure is elevated. An elevated EASIX index on day 0 predicts this complication. If recognized and correctly managed with diuretics, it has no impact on TRM.

## 42.3 Capillary Leak Syndrome (CLS)

Idiopathic systemic capillary syndrome was described in healthy patients who presented episodic crisis of hypotension/hypoperfusion, hypoalbuminemia, and severe generalized edema (Clarkson disease). Usually, these manifestations could be reversed with steroids, vasopressors, fluid, and colloids, but some patients could die during the recovery phase due to cardiopulmonary failure. Very similar episodes have also been described after the administration of IL-2, IL-4, TNF- $\alpha$ , GM-CSF, and G-CSF and in the context of HCT (Nürnberg et al. 1997; Lucchini et al. 2016).

**Pathogenesis:** Many mechanisms have been suspected but, nowadays, due to the duration of the capillary leak and its reversibility, the endothelial injury seems to be the main cause of the capillary damage. The high levels of VEGF and angiopoietin-2 (potent inducers of vascular permeability) observed in these patients could play a role (Xie et al. 2012).

**Diagnosis** of CLS post-HCT is accepted when there is (Lucchini et al. 2016):

- Weight gain >3% in 24 h, not justifiable by hydric overload.
- Generalized edema (ascites, pericardial, or pleural effusion).
- Absence of response to 24 h of furosemide treatment (at least 1 mg/kg).

it is not uncommon to observe tachycardia, hypotension, prerenal renal failure, and hypoalbuminemia.

No clear **risk factors** have been evident, although G-CSF has always been suspected. What is clear is that patients with CLS have a higher TRM and a higher incidence of aGVHD.

Its true **incidence** is unknown (due to the variable diagnostic criteria used) but mainly observed in children with 5.4% in the largest series (similar incidence between MAC and RIC).

**Treatment:** Only supportive measures are available, such as immediate withdrawal of growth factors, steroid treatment, hemodynamic support (catecholamines, colloids, and plasma), and even mechanical ventilation. Administration of C1-starchase inhibitor concentrates has been shown to be ineffective. In the only case described, the administration of Bevacizumab (MoAb against VEGF) was effective (Yabe et al. 2010), as was colchicine in the only two published cases (Cocchi et al. 2019).

## 42.4 Engraftment Syndrome (ES)

This syndrome has also been referred to as: implant CLS; auto-aggression syndrome; engraftment respiratory distress; aseptic septic shock, and autologous GVHD. It appears to result from endothelial dysfunction produced by the massive release of activated leukocytes and pro-inflammatory cytokines during the complex process of engraftment, plus all the previously mentioned factors that damage the endothelium during conditioning.

**Risk factors:** it has always been thought that ES may be related to infused cellularity (higher incidence with PB), concomitant administration of

G-CSF/GM-CSF (potent endothelial toxicants), or the use of DMSO (in auto-HCT or CBT), without clear evidence of this. There does appear to be a correlation with the intensity of previous treatments received (Carreras et al. 2010) (see below).

**Diagnostic.** The existence of three very different diagnostic criteria (Spitzer 2001; Maiolino et al. 2003; Grant et al. 2020) has made it difficult to know the true incidence (7% to 59% in auto- and 10% to 25% in allo-HCT).

Author	Spitzer (2001)	Maiolino et al. (2003)	Grant et al. (2020)
<b>Clinical criteria observed</b>	3 M or 2 M + ≥1 m	1 M + 1 m	2 M or 1 M + ≥2 m
Hours before/after engraftment (E)	96 < E < 96	24 < E < any after	96 < E < 24
Noninfectious fever	M	M	M
Skin rash >25% body surface	M [2]	m	M
Pulmonary edema/hypoxia [1]	M	m	m
Weight gain >2.5% of basal	m	m (>3% of basal)	m
Hepatic/renal dysfunction	m	–	–
Encephalopathy	m	–	–
Diarrhea (≥2 episodes)	–	m	m

M MAJOR criteria; m MINOR criteria. A CRP >6 mg/dL (usually up to 10–15 mg/dL) at fever onset has an excellent positive and negative prognostic value (90% and 90%, respectively) and serves to monitor the response to treatment (Carreras et al. 2010). [1] Without cardiac failure, thromboembolism, or infection. [2] Without histological data of acute GVHD (Spitzer 2015)

From a descriptive point of view, it is advisable to separate the ES observed after auto-HCT (or syngeneic) and the ES observed after allo-HCT (excluding CBT → see preES).

- **ES in auto-HCT.** In these cases, the diagnosis is very simple allowing rapid treatment and resolution. For this reason, it is advisable to apply Maiolino’s diagnostic criteria. If Spitzer’s criteria are applied, up to 50% will not be diagnosed with ES.
- **ES in allo-HCT.** The diagnosis of ES is complex to establish in this context. In allo-HCT, the phenomena of alloreactivity, the use of CNI and the increased risk of infections require a broad differential diagnosis. Therefore, if suspected, it is advisable to use the Spitzer’s diagnostic criteria, which are much stricter and require that there be no histological alterations of GVHD to avoid possible confusion with an early aGVHD.

**Treatment:** (1) Stop G-CSF immediately, obtain cultures, and start broad-spectrum ATB. (2) After, 48 h to see the effect of ATBs and to know the result of cultures, and without with-

drawing ATBs, start methyl-PDN 1 mg/kg q12h IV (×3 days) and taper out over a week. Remember that delay in starting treatment can lead to MOF (Dispenzieri et al. 2008). If early steroid treatment, rapid resolution of the picture in >90% of cases (CRP levels are good indicators of response). If no rapid improvement, consider other diagnoses. Occasional relapses when steroids are stopped.

## 42.5 Pre-engraftment Syndrome (pES)

Initially called early inflammatory syndrome with a similar pathogenesis to ES + an alloreactivity component. pES is clinically similar to ES, but with three relevant differences (Lee and Rah 2016):

- It is characteristic of CBT, especially if they receive MAC.
- It occurs well before engraftment (approx. Day +7; 10–11 days before).
- Water retention is more frequent than in ES (30%).

Other aspects of interest are:

- It is more frequent than ES, with an incidence between 20% and 70% in CBT.
- The use of G-CSF, DMSO, and the higher percentage of NK cells in the inoculum are considered risk factors.
- It is associated with a lower incidence of engraftment failure, a higher incidence of GVHD and early bacterial infections, and an equal incidence of TRM, REL, or SRV (Park et al. 2013).
- Its treatment is the same as ES.

### 42.6.2 Pathogenesis

Like in the other vascular–endothelial syndromes after HCT, the endothelial injury due to the action of different factors (conditioning, lipopolysaccharides, CNI, alloreactivity, and GVHD) plays a crucial role in its development. Endothelial injury generates a prothrombotic and pro-inflammatory status that favors capillary occlusion.

However, unlike in other endothelial syndromes, the dysregulation of the complement system and the possible presence of specific antibodies (donor- or recipient-specific Ab, as anti-factor H Ab) could play a relevant role in some TA-TMA. The activation of the classical pathway of the complement system (by chemotherapy, infections, and GVHD) and the activation of the alternative pathway (favored by a genetically determined mutation of several genes (CFH, CFI, CFB, and CFHR1,3,5) produce deposits of C4d or C5b-9 (membrane attack complex) fractions, respectively (Jodele et al. 2016).

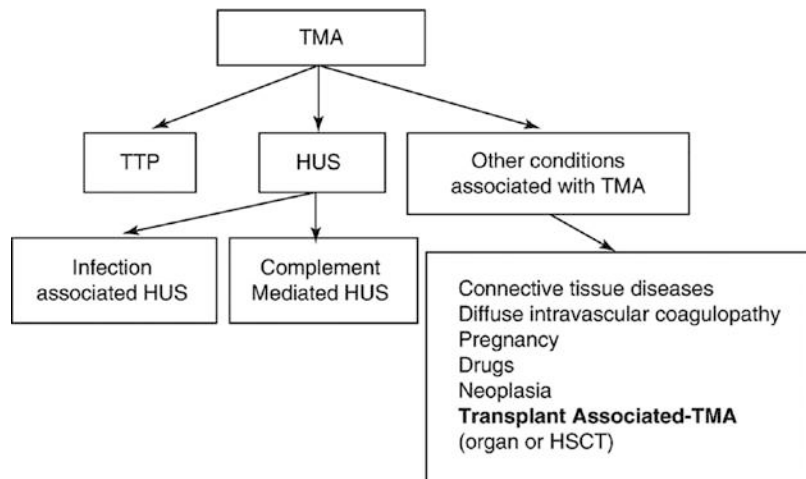
Recently, a “three-hit hypothesis” was proposed in which patients with either an underlying predisposition to complement activation or pre-existing endothelial injury (Hit 1) undergo a toxic conditioning regimen causing endothelial injury (Hit 2), and then additional insults are triggered by medications, alloreactivity, infections, and/or antibodies (Hit 3) (Dvorak et al. 2019).

## 42.6 Thrombotic Microangiopathy Associated with HCT (TA-TMA)

### 42.6.1 Definition and Classification

TMA is a heterogeneous group of diseases characterized by microangiopathic hemolytic anemia and thrombocytopenia due to platelet clumping in the microcirculation leading to ischemic organ dysfunction. As this phenomenon could be observed in different clinical situations, a consensus on the standardization of terminology has been recently proposed by an International Working Group (Scully et al. 2017) (Fig. 42.1).

**Fig. 42.1** Terminology of TMA



TTP=Thrombotic Thrombocytopenic Purpura; HUS=Hemolytic Uremic Syndrome

Understanding this cycle of injury permits the development of a specific TA-TMA treatment algorithm designed to treat both the triggers and the drivers of the endothelial injury.

Blood and Marrow Transplantation, and European Society for Blood and Marrow Transplantation experts proposed consensus criteria for TA-TMA diagnosis and prognosis (Schoettler et al. 2023).

### 42.6.3 Clinical Manifestations

Manifestations of microangiopathic hemolytic anemia	
De novo anemia	
De novo thrombocytopenia	
Increased transfusion requirements	
Elevated LDH	
Schistocytes in the blood	
Decreased haptoglobin (may be increased early in the disease process)	
Increased free plasma hemoglobin	
Manifestations of organ damage	
Kidney	Decreased glomerular filtration rate (by nuclear GFR or cystatin C GFR) Proteinuria as measured by random urine protein/creatinine ratio (rUPCR) Microhematuria Hypertension; ≥2 medications
Lungs	Hypoxemia, respiratory distress
Cardiovascular	Pulmonary hypertension, right-sided heart failure
GI tract	Abdominal pain/GI bleeding/ileus
CNS	Headaches/confusion Hallucinations/seizures Posterior reversible encephalopathy syndrome (PRES)
Skin	Purpura, ecchymoses
Testes	Painless vasculopathy
Polyserositis	Refractory pericardial/pleural effusion, and/or ascites, without generalized edema

Schoettler et al. (2023)

### 42.6.4 Diagnostic Criteria

The gold standard for diagnosis is a biopsy of the damaged organ. However, obtaining these samples is almost impossible in these patients, so TA-TMA remains a clinical diagnosis. Multiple diagnostic criteria have been proposed without universal application. To address this urgent need, the American Society for Transplantation and Cellular Therapy, Center for International Bone Marrow Transplant Research, Asia-Pacific

Harmonization panel consensus TA-TMA diagnostic criteria	
1. Biopsy-proven disease of ANY organ <b>OR</b>	
2. Clinical diagnosis. <b>Diagnostic criteria</b> must meet <b>≥4/7 of the following within 14 days at two consecutive time points</b>	
Anemia <sup>a</sup>	Defined as one of the following: 1. Failure to achieve transfusion independence for pRBCS despite evidence of neutrophil engraftment 2. Hemoglobin decline from the patient’s baseline by 1 g/dL 3. New onset of transfusion dependence. <i>Rule out other causes of anemia such as AIHA and PRCA</i>
Thrombocytopenia <sup>a</sup>	Defined as one of the following: 1. Failure to achieve platelet engraftment 2. Higher than expected platelet transfusions needs 3. Refractoriness to platelet transfusions 4. 50% reduction in baseline platelet count after full platelet engraftment
Elevated LDH	Above the upper limit of normal for age
Schistocytes	Present
Hypertension	>99th percentile for age (<18 years old), or systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg (≥18 years old)
Elevated sC5b-9	Greater than the upper limit of normal
Proteinuria	≥1 mg/mg random urine protein to creatinine ratio (rUPCR)

<sup>a</sup> Clarification from published Jodele et al., 2015

### 42.6.5 Clinical Forms, Incidence, Risk Factors, and Prognosis

- Clinical manifestations: onset day, median time day +32 to +40 (>92% before day +100).
- Incidence: Prospective multi-institutional study in 13 pediatric centers showed TA-TMA incidence of 19% in allo-HCT recipients. In auto-HCT recipients, TA-TMA was observed exclusively in children with neuroblastoma after the second tandem transplant (25%). TA-TMA incidence in adult HCT recipients is not well defined, but large centers screening for TA-TMA report ~12–15% incidence (Dandoy et al. 2021).
- Risk factors: Use of CNI, the combination of sirolimus with CNIs (Chen et al. 2021), viral (CMV, ADV, BK virus, etc.) or fungal infection, active GVHD, URD/mismatch HCT (probably due to more infections and GVHD), and several gene polymorphisms (predominant in non-Caucasian).
- Risk stratification: TA-TMA is currently stratified into standard risk and high risk. Patients with TA-TMA presenting at least with one high-risk feature are assigned to high-risk group and should be considered for TA-TMA-targeted therapy.
- Prognosis: Despite the resolution of TA-TMA, these patients have an increased relative risk (RR) of chronic kidney disease (4.3); arterial hypertension (9); and TRM (5).

International Consensus Risk stratification is a modification of Jodele et al., 2015 with the additional risk factors indicated by an asterisk (\*) (Schoettler et al. 2023). Kidney disease improving global outcomes (KDIGO) stage 1 kidney injury is defined as serum creatinine of 1.5–1.9-time baseline. Lactate dehydrogenase (LDH), random urine protein to creatinine ratio (rUPCR)

### 42.6.6 Recommended Screening and Diagnostic Work-Up for TA-TMA (Fig. 42.2)

There is sufficient evidence supporting routine screening of all allo- and pediatric auto-HCT recipients with an underlying diagnosis of neuroblastoma through day 100 post-HCT. Screening also should be considered after day 100 in patients who develop a known risk factor for TA-TMA, including acute GVHD, chronic GVHD, or infection. In patients with >3 abnormal screening laboratory test results or clinical manifestations or organ dysfunction concerning TA-TMA, additional testing should be done. In patients who meet the criteria for high-risk TA-TMA, treatment with TA-TMA-directed therapy should be considered. Patients who do not meet high-risk criteria should be monitored closely, and treatment may be initiated at the discretion of the clinician if cytopenia or other manifestations persist. Triggers of TA-TMA (e.g., infection, GVHD) should be aggressively managed. If sC5b-9 testing is not available, screening of urine and other organ function should continue, and treatment offered to patients meeting high-risk TA-TMA criteria.

### 42.6.7 Treatment

Supportive therapy
Red cell and platelet transfusion support
Discontinue causative agents, if feasible
Modify immunosuppression, if risk/benefit assessment is in favor (be cautious not to provoke acute GVHD)
Treat GVHD, infections
Aggressively treat hypertension
Monitor for organ injury
Nutrition, vitamin support (Vit D, C)

Risk stratification of TA-TMA by harmonization of definitions subcommittee	
Standard-risk TA-TMA	High-risk TA-TMA
- Peak LDH <2x ULN	- Peak LDH ≥ 2x ULN*
- rUPCR <1 mg/mg	- rUPCR ≥1 mg/mg
- KDIGO stage I acute kidney injury	- any organ dysfunction that develops in the setting of TMA except KDIGO stage I acute kidney injury
- Normal C5b-9	- Elevated soluble C5b-9 (> ULN)
	- Concurrent acute GVHD grade II-IV *
	- Concurrent systemic infection (bacterial or viral)*



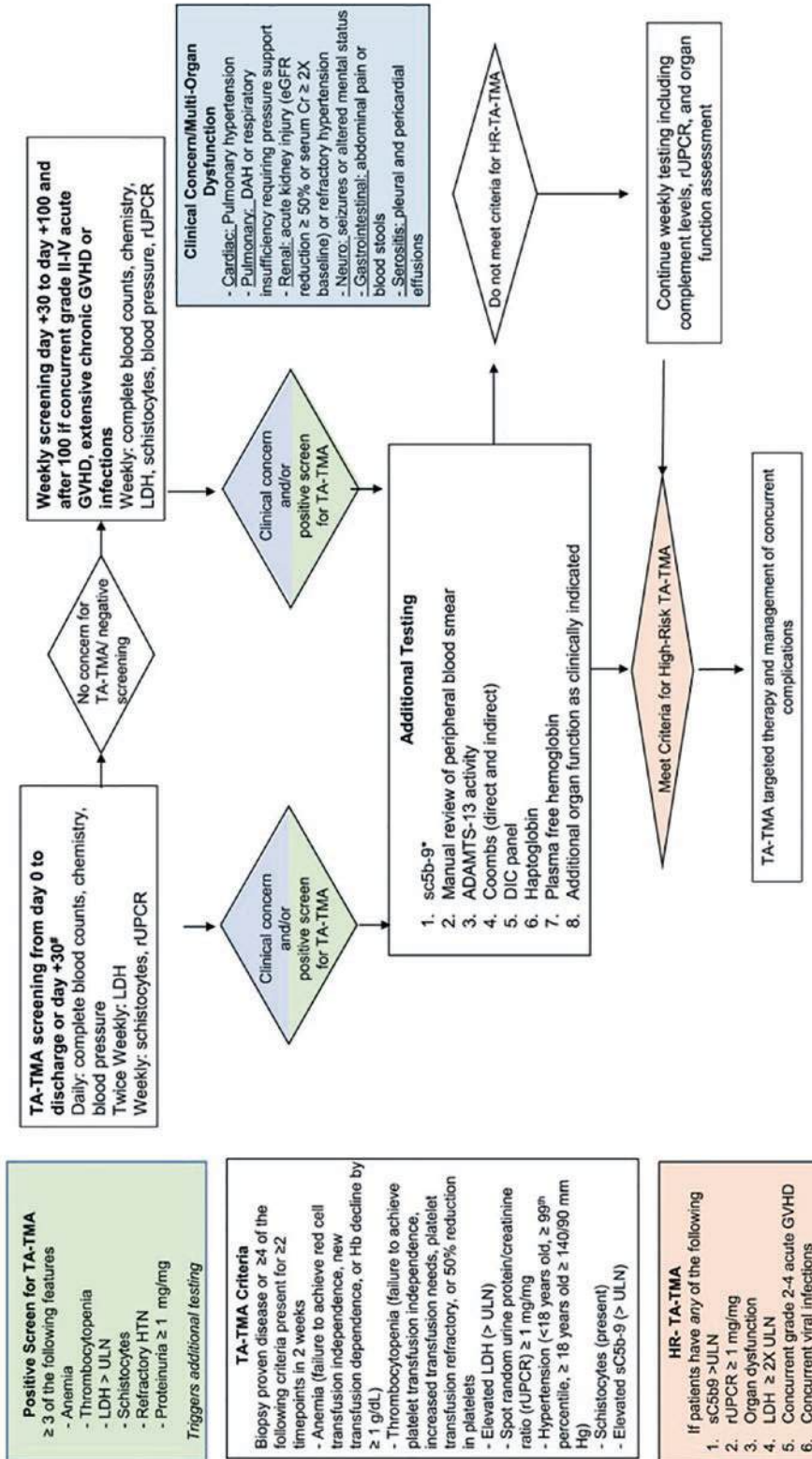


Fig. 42.2 Recommended screening and diagnostic work-up



## Supportive therapy

## TA-TMA-targeted therapy

- Complement blocking agents with reported clinical benefit:
  - eculizumab (C5 blocker): most promising targeted agent for high-risk complement-mediated TA-TMA in children and adults, demonstrating increased survival to 70% in high-risk TA-TMA and recovery of organ function in survivors (Jodele et al. 2020).
  - narsoplimab (MASP2 inhibitor): most experience in adults demonstrating 68% survival at 100 days (Khaled et al. 2022).
- Complement-blocking agents currently being evaluated in clinical studies for TA-TMA:
  - Ravulizumab (C5 blocker)
  - Nomacopan (C5 blocker)
  - Pegcetacoplan (C3 blocker)
- Therapeutic plasma exchange (TPE): TA-TMA responses had been reported with early initiation of TPE. It may serve as an alternative option for selected patients when complement-blocking agents are not available. In patients with ab anti-factor H, responses had been reported in combination with rituximab.
- Rituximab: Reported 12/15 responses to Rituximab + TPE. (Uderzo et al. 2014)
- Defibrotide: responses documented in children and adults after HCT in retrospective multi-institutional review (Yeates et al. 2017)

## TA-TMA prophylaxis

- Defibrotide: has been studied as prophylactic agent in pediatric patients with high-risk TA-TMA (Higham et al. 2022; Richardson et al. 2021)
- N-acetylcysteine: potential benefit was demonstrated in the prospective randomized study (Pan et al. 2022)
- Statins: Statin-based endothelial prophylaxis (SEP) has been shown beneficial in adult HCT recipients with the reduction of GVDH and TA-TMA (Pabst et al. 2023).

## TA-TMA key points

- TA-TMA is now a well-recognized endothelial injury syndrome after HCT, resulting in high mortality and morbidity.
- Prospective screening for TA-TMA allows timely disease diagnosis and risk stratification.
- Early diagnosis is essential to prevent TA-TMA-associated organ injury and improve outcome.
- Complement-blocking therapies are the most promising targeted interventions for high-risk TA-TMA and have been shown to be safe in HCT recipients.
- Given the limited availability of complement-blocking agents, all modifiable risk factors that may favor the development of TA-TMA should be avoided.
- Prophylactic and preventative strategies to reduce or severity of TA-TMA are needed

## 42.7 Posterior Reversible Encephalopathy Syndrome (PRES)

**Pathogenesis:** There are two theories (Fischer and Schmutzhard 2017): 1) Rapid rise in blood pressure (BP) as a trigger. However, about 30% of patients with PRES show normal or minimally elevated BP values. 2) Triggered by endothelial dysfunction leakage caused by endogenous circulating toxins. In favor of this hypothesis, PRES is frequently observed in patients with (pre) eclampsia, sepsis, or during HCT or cytotoxic treatments (Geocadin 2023).

**Risk factors** for this complication are not clearly established but have been described: hypertension (sometimes just an elevated mean blood pressure), fludarabine, HCT for Hbpathies, CNI, hypoMg, and presence of aGVHD II-IV. The **incidence** is variable among the published series and appears to be around 8% in allo-HCT with a wide range from 1% following haplo-HCT (Chen et al. 2021) to 25% in HCT due to sickle cell disease (Shenoy et al. 2017).

The most frequent PRES **clinical manifestations** are (Fischer and Schmutzhard 2017) encephalopathy, disturbances in consciousness, hypertension (sometimes simply elevated mean arterial blood pressure), seizures, visual disturbances, headache, and occasionally neurological focality.

CSF is essential for differential diagnosis but normal in PRES. EEG: it is useful for the detection of epileptic seizures (nonconvulsive) and status epilepticus and for evaluating encephalopathy.

**MRI of the brain:** evidence of bilateral parieto-occipital vasogenic edema (more evident in T2 or FLAIR sequences) although they may be distributed asymmetrically. Due to the lower density of the white matter, the subcortical areas are the most affected but cortical involvement has also been described. Lesions in other areas are infrequent.

**PRES treatment** is symptomatic as there are no specific therapies. Treat hypertension (if present), anticonvulsant, or hypomagnesemia treatment if necessary. Act on possible triggers: it has always been said that stopping CNIs was the best measure and several isolated cases reported in the

literature seem to indicate this. But in the few studies in which stopping (or changing) the CNI while maintaining it (Hammerstrom et al. 2013; Singer et al. 2015), no different evolution of PRES has been observed.

In more than 80% of cases, all manifestations of PRES disappear rapidly. Neurological sequelae may be present in a few cases, but the majority of patients who die do so from intercurrent causes. PRES does not seem to affect TRM or SRV.

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# Acute Graft-Versus-Host Disease

# 43

Ernst Holler, Hildegard Greinix, and Robert Zeiser

## 43.1 Introduction

Graft-versus-host disease (GvHD) was first recognized in murine models of HCT, and in the absence of knowledge of the HLA system, it was termed “secondary” (secondary to recovery from irradiation damage) or “runt” disease. Billingham established the criteria for the occurrence of secondary disease in the 1960s, i.e.:

- The administration of a graft containing immunocompetent cells
- Immunological disparity between host and donor
- The administration of the graft to an immunosuppressed host unable to reject the graft cells

In the human setting, we traditionally recognize two forms of GvHD, acute (aGvHD) and chronic (cGvHD). The original distinction of acute from chronic GvHD, namely, the occurrence before or after day 100 post stem cell infusion, has become blurred due to occurrence of aGvHD symptoms beyond day 100 after RIC regimens and/or after DLI (usually given after day 100). Nevertheless, the underlying combination of symptoms and signs affecting the skin, liver, and gastrointestinal tract forms a classical clinical syndrome enabling the diagnosis, and a helpful guide to the appropriate terminology is provided in Table 43.1 (Filipovich et al. 2005).

**Table 43.1** Current classification of acute and chronic GvHD

Classification	Day after HCT	Features of acute GvHD	Features of chronic GvHD
<i>Acute GvHD</i>			
– Classic acute	<100 days	Yes	No
– Persistent, recurrent, or late onset	>100 days	Yes	No
<i>Chronic GvHD</i>			
– Classic chronic	No time limit	No	Yes
– Overlap syndrome	No time limit	Yes	Yes

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## 43.2 Definition

aGvHD remains, directly or indirectly, the major cause of short-term (day 100 and 1 year) mortality after allo-HCT. The pathophysiology of aGvHD has been attributed to a three-phase process comprising initial tissue damage from the conditioning regimen which in turn leads to activation of host antigen-presenting cells by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and activation and proliferation of donor T cells (afferent phase) and finally to the effector phase characterized by cytotoxic cell damage and release of inflammatory cytokines such as interleukin-1 (IL-1) and tissue necrosis factor-alpha (TNF $\alpha$ ) that eventually produce tissue necrosis (efferent phase). The action of this pathogenetic process in the induction of aGvHD is modulated in part by the presence of cells capable of inhibiting immune response, such as T-regulatory cells (Tregs), type 1 regulatory T cells (Tr1 cells), invariant NKT cells, and myeloid-derived suppressor cells (MDSCs) (Ferrara et al. 2009; Teshima et al. 2016).

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## 43.3 Risk Factors

As aGvHD is a result of an alloimmune effect, the major risk for occurrence is the presence of HLA disparity and increasing degrees of HLA mismatching increase the probability of more severe disease. Other important and consistent risk factors include older patient age, the use of female donors for male recipients, prior alloimmunization of the donor, and the nature of GvHD prophylaxis. A number of publications have variously reported risk factors such as increasing donor age, increasing intensity of the preparative regimen, the use of PBSC as opposed to BM, and recipient seropositivity for CMV.

A recent study of 2941 recipients of allo-HCT in Seattle confirmed the importance of the degree of HLA mismatching, the use of URD, and the administration of high-dose TBI in predicting the occurrence of moderate to severe aGvHD. In contrast they found that increasing donor age,

cytokine-mobilized stem cells, and the use of female donors for male recipients did not impact on the likelihood of aGvHD but were associated with the occurrence of cGvHD (Flowers et al. 2011).

More recently, we have begun to appreciate the importance of non-HLA genetic factors in the development of GvHD. Examples include polymorphisms in the genes encoding cytokines such as the tumor necrosis factors, the interleukins (IL-1, IL-6, and IL-10), interferon gamma (IFN- $\gamma$ ), and transforming growth factor- $\beta$ 3 (TGF- $\beta$ 3) and the expression of the killer cell immunoglobulin-like receptors (KIR). Interestingly, one of the common features of the organs involved in aGvHD is that they are all exposed to microbial pathogens through the intestinal mucosa, epidermis, and portal circulation, and early murine studies confirmed a reduction in the severity and incidence of GvHD in animals that received antibiotic prophylaxis to “decontaminate” the GI tract or those kept in germ-free environments. This has led to the speculation that potential differences within individuals in the interactions of antigens derived from infective organisms and pathogen recognition receptors (PRR) might protect or predispose to the occurrence of GvHD. This concept was further supported by studies on SNPs within NOD2/CARD15, a pathogen receptor detecting bacterial muramyl peptide, as a risk factor of GvHD; however, extensive studies were conflicting (reviewed in Penack et al. 2010).

More recently, the availability of noncultural methods to analyze the whole set of bacteria (called microbiota) has broadened our view as the presence of commensal microbiota and a high diversity of the patients’ microbiota associated with substantial protection not only from GvHD but also from systemic and pulmonary infectious complications (Peled J et al, 2016). The exact mechanisms of this protection need still to be defined before translation into new preventive approaches, but beneficial effects of microbial metabolites (such as short-chain fatty acids and indoles) both on epithelial integrity and on immunoregulation have been shown (Swimm et al. 2018; Shono and van den Brink 2018).



### 43.4 Diagnosis and Scoring

aGvHD is manifested by one or more of the following features: an erythematous skin reaction, cholestatic liver disease, and gastrointestinal dysfunction. The variety of presentations in each organ is provided in more detail in Table 43.2; the syndrome ranges from a mild self-limiting condition to a serious and potentially fatal disorder. Because of the complexity of care of an allo-HCT recipient, it is often very difficult to distinguish the characteristic features of aGvHD from those of other complications such as VOD/SOS, conditioning, and general drug toxicity and infection and consequently to determine the appropriate choice of treatment.

For this reason, it is essential to establish the diagnosis by biopsy of one or more affected organs and confirmation of the characteristic histopathological features (Table 43.3). The targets of the immune response in aGvHD are the epithelial cells including basal and suprabasal cells of the epidermis, the intestinal epithelium, and the biliary duct epithelium, and the characteristic feature is identical, i.e., the presence of infiltrating immune cells close to apoptotic cells known as “satellite cell necrosis.”

**Table 43.2** Clinical manifestations of acute GvHD

Organ	Clinical manifestation
Skin	Erythematous maculopapular rash, often initially involving the palms and soles May progress to involve the entire body surface and may be pruritic and/or painful In severe cases, bullae may form leading to desquamation
Liver	Cholestasis with or without frank jaundice Cholestatic enzymes comparatively more deranged than transaminases
Gastrointestinal (GI) tract	<i>Upper:</i> anorexia, nausea, and vomiting <i>Lower:</i> diarrhea, typically green and watery; in severe case diarrhea contains fresh blood and mucosa and is accompanied by abdominal cramps and, on occasion, paralytic ileus

The first classification of aGvHD was developed by Glucksberg et al. (1974). Each organ was staged from 0 to 4 (Table 43.4), and the resultant stages were combined to provide an overall grade (Glucksberg et al. 1974). In 1994 Przepiorka et al. described the outcome of a consensus workshop to develop an improved scoring system that retained most of the characteristics of Glucksberg but dropped the use of the clinical performance score and included upper intestinal symptoms within the definition of aGvHD (Przepiorka et al. 1995). Subsequently, the IBMTR prospectively evaluated a “severity index” against the

**Table 43.3** Histopathological findings in acute GvHD

Organ	Histopathological features
Skin	The diagnostic feature is a lichenoid infiltration of the upper dermis and lower epidermis with vacuolation, degeneration, and individual cell necrosis of the cells of the basal layer of the epidermis Grade I: vacuolation of epidermal basal cells Grade II: presence of individually necrotic keratinocytes Grade III: confluent areas of keratinocyte necrosis forming bullae Grade IV: sloughing of the epidermis
Liver	The most consistent histological feature is small bile duct damage, which is usually seen in association with cholestasis and is rare in other complications of HCT The biliary epithelial cells have enlarged hyperchromatic nuclei or small pyknotic nuclei and vacuolated cytoplasm Periportal and midzone hepatocellular necrosis and minimal lymphocytic infiltrates in the portal tract Although there is a histological grading for liver histology, it has no proven prognostic value
Gastrointestinal	“Exploding crypts” within which are necrosis of individual epithelial cells at the periphery of the crypts leaving fragments of nuclear and cytoplasmic debris Grade I: individual cell necrosis Grade II: loss of individual crypts Grade III: loss of two or more adjacent crypts with ulceration Grade IV: denudation of epithelium



**Table 43.4** Staging of acute graft-versus-host disease (according to Harris et al. 2016)

GVHD target organ staging				
Stage	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: <500 mL/day or <3 episodes/day Child: <10 mL/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2–3 mg/dL	Persistent nausea, vomiting, or anorexia	Adult: 500–999 mL/day or 3–4 episodes/day Child: 10–19.9 mL/kg/day or 4–6 episodes/day
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dL	–	Adult: 1000–1500 mL/day or 5–7 episodes/day Child: 20–30 mL/kg/day or 7–10 episodes/day
3	Maculopapular rash >50% BSA	6.1–15 mg/dL	–	Adult: >1500 mL/day or >7 episodes/day Child: >30 mL/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation >5% BSA	>15 mg/dL	–	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume)

Glucksberg criteria but were unable to identify any particular advantage for the new system (Rowlings et al. 1997). In fact, the Glucksberg score was a better predictor of survival and remains in regular use (Cahn et al. 2005). Currently, electronic applications are developed supporting the accuracy of staging and grading of acute GvHD (Schoemans et al. 2018).

Overall clinical grade (based upon most severe target organ involvement):

- Grade 0: No stage 1–4 of any organ
- Grade I: Stage 1–2 skin without liver, upper GI or lower GI involvement
- Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI
- Grade III: Stage 2–3 liver and/or stage 2–3 lower GI, with stage 0–3 skin and/or stage 0–1 upper GI
- Grade IV: Stage 4 skin, liver or lower GI involvement, with stage 0–1 upper GI

### 43.5 Epidemiology

Moderate to severe aGvHD occurs in approximately 40% of all recipients of allo-HCT, but the precise incidence varies considerably depending

predominantly on the nature of the donor and the method of GvHD prophylaxis. Without effective prophylaxis, it is an almost inevitable and frequently deleterious complication at least in unrelated matched donor and mismatched family grafts.

### 43.6 Prevention (also See Chap. 26)

Grade III–IV aGvHD, especially if it turns out to be resistant to first-line treatment, has an extremely poor prognosis despite therapeutic intervention, and consequently, considerable efforts are made to try and prevent its occurrence. The rationale of prophylaxis was originally directed toward prolonged IS of donor T-cell function through the peri- and posttransplant administration of IS agents. Early studies identified the superiority of a combination of the CNI and CSA, with MTX over MTX alone. In practice this combination remains the most frequently used method of prophylaxis although some investigators have replaced CSA with tacrolimus (TAC) since large two phase III randomized studies reported a reduction in the incidence of grade II–IV aGvHD at 32% in recipients of sibling

transplants and 56% in those who received unrelated donor grafts in patients who received TAC plus MTX compared to 44% (sibling) and 74% (unrelated) in those who were randomized to CSA and MTX. However, there was no difference in survival that could be attributed to the nature of the GvHD prevention (Ratanatharathorn et al. 1998; Nash et al. 2000). Recently, investigators have also reported the efficacy of newer agents such as mycophenolate mofetil (MMF) and sirolimus (SIR). Whereas MMF has not been tested in large randomized trials, the combination of TAC and SIR was compared with that of TAC and MTX in a phase III randomized study showing equivalent efficacy but differences in toxicity (Törlén et al. 2016).

An alternative approach to GvHD prophylaxis is to consider removal of donor T cells either *ex vivo* prior to infusion or *in vivo* before and/or after infusion using polyclonal (anti-thymocyte globulin [ATG]) or MoAb. A similar effect can also be achieved by positive selection of CD34+ stem cells. These techniques, collectively known as TCD, are extremely efficient in preventing acute and chronic GvHD and were in widespread use in the 1980s and 1990s. Unfortunately, they were rapidly identified as contributing to an increased risk of infection and disease relapse and subsequently became confined to situations in which the risk of GvHD is increased, e.g., recipients of mismatched and haploidentical transplants where the risk of death from GvHD outweighs the risk of later disease recurrence. In unrelated donor HCT, polyclonal ATG has become a major player as two randomized trials showed positive effects mainly on chronic GvHD (Finke et al. 2009; Walker et al. 2016).

Recently, a prospective, double-blind phase III trial to investigate the effect of ATG in the setting of MUD HCT showed that grade II–IV acute GvHD and moderate–severe cGvHD was lower in ATG recipients but the overall survival was lower in ATG recipients (Soiffer et al. 2017). This could be related to higher ATG levels in patients with low lymphocyte counts following TBI which might translate in subsequent infectious complications and EBV-related posttransplant lymphoproliferative disease. Thus, balancing

suppression of long-term GvHD versus suppressing anti-infectious defense is an ongoing challenge in GvHD (Gagelmann et al. 2017).

Other studies have explored alternative methods of aGvHD prophylaxis including the infusion of an expanded population of T-regulatory cells at the time of stem cell infusion and partial TCD such as depletion of  $\alpha/\beta$  T cells or elimination of alloreactive T cells after *in vitro* or *in vivo* activation. In this context, the administration of PT-CY in order to eliminate early activated donor T cells has gained substantial interest particularly in the context of haploidentical transplantation, and further studies comparing the more complex T-cell depletion approach with the simple approach of PT-CY are currently performed (Kanakry et al. 2016). In a prospective, randomized study on 431 adults given reduced-intensity conditioning and 6/6 related or 7–8/8 unrelated donor grafts, GvHD prophylaxis consisted of either PT-CY, tacrolimus and MMF, or tacrolimus and MTX (Holtan SG Blood 2022; LBA). One-year GvHD relapse-free survival (GRFS) was significantly better in the PT-CY study arm (52.7% vs. 34.9%,  $p < 0.001$ ) due to significantly lower incidences of acute and chronic GvHD.

Recent trials showed the activity of abatacept for aGvHD prevention (Watkins et al. 2021) leading to the FDA approval of abatacept in combination with a CNI and MTX for allo-HCT with a matched or 1-allele mismatched unrelated donor.

Also vedolizumab, an antibody directed against  $\alpha4/\beta7$  integrin which is selectively expressed in the GI tract, was tested in a prospective phase III trial and showed a significant reduction of GI aGvHD (Chen YB et al. 2019, Floisand Y et al. 2021).

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## 43.7 Treatment

Grade I aGvHD, by definition affecting only the skin, can often be effectively treated with topical steroids alone. Early systemic treatment of grade I GvHD has been tested but showed no long-term advantage. More advanced grades require systemic therapy, and the mainstay of treatment

remains high-dose methylprednisolone (or equivalent), usually at a dose of 2 mg/kg/day, continued for 7–14 days and followed by a gradual reduction in dose (Penack et al. 2020). Patients with mild upper GI GvHD may start on lower doses with concomitant topical treatment; higher doses of steroids resulted in more infectious complications without superior long-term response. The chance of response decreases with increasing grade of GvHD, but in general approximately 40–50% of patients will demonstrate a response. Reductions in steroid doses may be followed by an exacerbation of symptoms that can sometimes be settled by simply increasing the dose and reducing more slowly on the second occasion. Achieving a balance between the levels of IS required to control aGvHD and retaining a degree of immunocompetence against microbial infection is challenging, and viral and fungal infections are frequent complications of prolonged steroid therapy. Anti-infective prophylaxis should be considered for all such patients. Among several candidates for first-line combination treatments, the most promising combination of steroids and MMF has been taken forward to a phase III study against steroids alone but failed to show superiority for the combination again due to an increased rate of infectious complications. Thus, so far no single agent has shown superiority of results when combined with corticosteroids for first-line treatment (Martin et al. 2012; Rashidi et al. 2016).

Failure to respond to standard steroid doses (defined as progression within 3–5 days of starting treatment or an incomplete response by 7–14 days) or recurrence after initial dose reduction (steroid dependence) will necessitate second-line treatment. In this context many agents have been tried alone or in combination with corticosteroid.

A multicenter phase 3 trial reported that ruxolitinib was superior to standard care for grade 2–4 SR-aGvHD (Zeiser et al. 2020). In this trial, 309 patients  $\geq 12$  years old were randomly assigned (1:1) to ruxolitinib (10 mg by mouth, twice daily) versus the investigator's choice of therapy; control therapy was chosen by the investigator at the time of randomization from the following: anti-thymocyte globulin, extracorporeal photopheresis, mesenchymal stromal cells, low-

dose methotrexate, mycophenolate mofetil, everolimus, sirolimus, etanercept, or infliximab. At day 28, compared with the control group, ruxolitinib achieved superior overall response rate (ORR; 62 versus 39%; odds ratio 2.64 [95% CI 1.65–4.22]) and complete response (CR; 34 versus 19%) (Zeiser et al. 2020). Superiority of ruxolitinib was maintained at day 56 (40 versus 22%) with all grades of disease and affected organs. There is no consensus treatment for SR-aGvHD beyond ruxolitinib. The preferred treatment varies among institutions and is influenced by availability, cost, and patient preference.

Most agents often result in short-term control, but durable effects are relatively infrequent, and the outcome of refractory aGvHD is dismal with approximately 80% mortality, especially if the lower GI tract is involved.

Responses have been reported with extracorporeal photopheresis administered at least twice a week on a weekly basis, and outcome seems to be superior with less toxicities occurring (Jagasia et al. 2013) (see Chap. 66).

In 2006, Ringden et al. reported the successful use of mesenchymal stromal cells (MSC) in a small group of patients with refractory severe aGvHD, and later this group described a response rate of >50% in a larger group of patients (Munneke et al. 2016). MSC exert immunosuppressive effects in a non-HLA-restricted manner and like Tregs offer interesting and novel strategies for the management of this potentially fatal complication although long-term results need to be established in future trials (Le Blanc et al. 2008). Currently, a prospective phase III trial evaluates the efficacy of MSC versus ruxolitinib in patients with SR-aGvHD (IDUNN trial).

The administration of pooled fecal allogeneic microbiotatherapy (FMT) in 81 patients with SR-aGvHD of the GI tract reportedly resulted in an ORR of 56% including 37% CR after a median of three doses and translated to increased OS in responding patients (Malard F et al, 2023). Currently, a phase III prospective study in SR-aGvHD patients is ongoing to evaluate the efficacy of FMT.

While classical IS regimens inhibit a signal pathway or cytokine receptor, novel strategies target CD28 (e.g., Aurora kinase), cell migration (ROCK), or growth factor signaling (e.g., MEK)

(Zeiser et al. 2017). These inhibitors were tested in preclinical studies and showed promising activity (Zeiser R, Blazar B 2017, Hill et al. 2018).

Another novel approach is infusion of alpha-1 anti-trypsin which exerts anti-inflammatory effects and stimulates regulatory T cells. Two recent phase II trials showed CR rates of 35% and OR rates of 60% of the patients on day 28 after treatment starts (Marcondes et al. 2016; Magenau et al. 2018).

### 43.8 Future Perspectives: Biomarkers, Risk-Adapted Treatment, and Tissue Regeneration

The difficulties to improve results in SR-aGvHD underline that steroid resistance might not just represent resistance of alloreactive T cells but loss of immunoregulation and tissue tolerance (Wu and Reddy 2017) which is difficult to overcome by classical immunosuppressants. Besides new approaches of modulation, potential solutions might be earlier risk adapted or even preemptive treatment strategies which require, however, reliable and reproducible identification of these patients. Recently, clinical risk scores (MacMillan et al. 2015) and novel biomarkers have been reported. The strength of these biomarkers for early identification of high-risk patients at day 7 after HCT or at onset of GvHD has been proven in large multicenter consortia and needs now confirmation by trials on biomarker-guided treatment strategies (Vander Lugt et al. 2013; Hartwell et al. 2017; Levine et al. 2015). The strength of the current biomarkers and scores are partially explained by the fact that they identify GI GvHD as the most severe and deleterious manifestation in an early phase of the disease. Besides the immunosuppressive approaches for aGVHD novel treatment that aim at regenerating the intestinal tract including IL-22 and glucagon-like peptide 2 (GLP-2) have been tested in mouse models of GVHD (Norona et al. 2020; Hanash et al. 2012) and in early clinical trials (Ponce et al. 2023). The data show acceptable toxicity and signs of efficacy that need to be validated in prospective phase III trials.

#### Key Points

- Acute GvHD occurs until day 100 as classical acute GvHD and beyond day 100 as delayed acute GvHD.
- As treatment options are limited beyond the use of corticosteroids, careful selection of GvHD prevention is essential.
- In the USA abatacept has been approved for GvHD prevention in allo-HCT with unrelated donors.
- Besides classical IS agents like CNI, MTX or MMF, and m-ToR inhibitors, partial TCD (IV serotherapy, depletion of T-cell subpopulations, elimination of alloreactive T cells, or PT-CY) are possible options for prophylaxis.
- Ruxolitinib has received EMA/FDA approval for treatment of SR-aGvHD.
- Institutional standards of treatment and supportive care are essential.

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Daniel Wolff, Zinaida Peric, and Anita Lawitschka

## 44.1 Introduction

Chronic GVHD (cGVHD) is the most relevant cause of late non-relapse morbidity and subsequent mortality (approximately 25%) following allo-HCT (Grube et al. 2016). Its incidence is approximately 50% among all patients following allo-HCT and has increased during the last two decades due to increasing patient age and increasing use of unrelated and/or mismatched donors, RIC regimens, PBSC with application of standard GVHD prophylaxis (calcineurin inhibitor [CNI] + MMF or MTX) only (Arai et al. 2015). While the incidence of cGVHD is lower (6–40%) in children, its incidence rises to 60% as age increases (Baird et al. 2010; Sobkowiak-Sobierajska et al. 2022).

The *pathophysiology* of cGVHD is different from aGVHD and mainly characterized by impaired immune tolerance mechanisms affecting innate and adaptive immunity. Both autoreactive and alloreactive donor-derived T and B cells play a role (Cooke et al. 2017). Other pathophysiological factors are indirect presentations of allo-antigens through antigen-presenting donor cells and mechanisms of chronic inflammation with subsequent scar formation and fibrosis. One important aspect of GVHD pathophysiology is the variability of immune reconstitution, which is age-related and dependent on thymic function and hormones (Sobkowiak-Sobierajska et al. 2022). This adds to the unpredictability of the effects of transplant procedures and complications in a very heterogenous cohort of children and adolescents with malignant and nonmalignant diseases.

Main *risk factors* for adult and pediatric cGVHD are unrelated and/or mismatched donor, PBSCs as donor source, older donor age, older patient age (>12 years), and female donor into male recipient combinations (Baird et al. 2010). By far the strongest predictor is the history and severity of acute GVHD and children below the age of 12 years rarely develop de novo cGVHD (Cuvelier et al. 2019).

In addition to the harm it causes, cGVHD also has a *protective effect*, as patients with cGVHD have lower rates of recurrence of their underlying malignant disease (Grube et al. 2016). Overall survival of patients transplanted for malignant

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diseases developing mild cGVHD is therefore better compared to patients without cGVHD. Even OS of patients with moderate cGVHD is not different from patients without cGVHD, as the slightly increased mortality associated with cGVHD is counterbalanced by lower disease-associated mortality (Kuzmina et al. 2012).

In contrast, the *long-term mortality* rate of patients with severe cGVHD is as high as 50% taking into account that the severity is less relevant compared to certain risk factors for mortality consisting of low platelets at diagnosis of cGVHD, the direct progression of acute GVHD into cGVHD (progressive onset), and certain organ manifestations (lung, gastrointestinal, and cholestatic liver involvement) (Grube et al. 2016). One important pediatric aspect involves the high proportion (up to 50%) of nonmalignant underlying diseases as HCT indication. While malignant diseases benefit from the graft-versus-malignancy effect induced by GVHD, it only offers harm for the nonmalignant diseases. In daily clinical routine, this fact influences GVHD prophylaxis and treatment both in regard to intensity and duration of immunosuppressants (Lawitschka et al. 2020).

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## 44.2 Clinical Manifestations

cGVHD usually begins between 3 months and 2 years after HCT, but earlier onset (at least 1 month after transplantation) is possible (Jagasia et al. 2015).

While involvement of the organ systems mentioned below is regarded as NIH-defined classic cGVHD, almost every other organ can potentially be impaired by atypical forms of cGVHD or atypical manifestations can involve organs otherwise regarded as classic manifestation of cGVHD (restrictive forms of pulmonary cGVHD). The most frequent manifestations are immune-mediated cytopenias, polyserositis, renal manifestations, involvement of the central and peripheral nervous system, rheumatological including myositis, and autoimmune thyroiditis (Cuvelier et al. 2022). As cGVHD can affect a

number of organs, and patients often do not report changes until functional impairment is recognized, regular examination of all organs potentially affected is essential. The following section describes the most common clinical organ manifestations of cGVHD. In general, pediatric manifestations are similar to adult cGVHD; when indicated, specific aspects are shortly described.

### 44.2.1 Skin

The skin is the most frequently involved organ with different morphology, depending on the different skin layers (epidermis, cutis, subcutis, and fasciae) involved. Some manifestations may overlap with acute GVHD like erythema, maculopapular rash, and pruritus. Cutaneous cGVHD may show many different non-sclerotic and sclerotic phenotypes often simulating well-known chronic inflammatory and autoimmune diseases (Strong Rodrigues et al. 2018).

Diagnostic features of NIH-defined cGVHD include poikiloderma, lichen planus-like, lichen sclerosus-like, morphea-like, and deep sclerotic eruptions, and no biopsy is needed to confirm the diagnosis. Distinctive for cGVHD, other or common skin manifestations like depigmentation and papulosquamous lesions or ichthyosis, keratosis pilaris, pigmental changes, loss of skin appendages, and sweat impairment are not sufficient for diagnosis and require histopathological confirmation if no diagnostic signs in the skin or other organs are present (Jagasia et al. 2015).

In pediatric patients, the incidence of viral reactivation and infection seems higher (although only proven for some viruses), and therefore, infection has to be ruled out. Viral skin infections can worsen or activate cGVHD (Jacobsohn 2010). Premature graying of the hair is even in small children common, possibly together with seborrheic scalp changes. Of note, if sweat glands are destroyed, this may be of importance for phototherapy because of the inability to sweat with consequent hyperthermia. Of note, long-lasting cutaneous cGVHD is a risk factor for secondary cutaneous malignancies.

### 44.2.2 Eyes

cGVHD of the eyes usually manifests as keratitis sicca. In addition to atrophy of the lacrimal gland with subsequent tear deficiency (sicca syndrome), the meibomian glands and eyelids are often affected by severe blepharitis which may initially present with tearing. Around the conjunctiva there are often not only fibrotic alterations but also chronic persistent inflammation with visible erythema of the conjunctiva. As dry eye symptoms are rarely communicated by children, light sensitivity is the predominant symptom, sometimes with excessive eye rubbing. Infections have to be ruled out. Referral to a pediatric experienced ophthalmologist is recommended.

### 44.2.3 Oral Mucosa

Oral manifestations may appear as erythema or lichenoid changes (the latter are regarded as diagnostic) of the oral mucosa as well as ulcers and mucocoeles. Sicca symptoms may result from destruction of the salivary glands. Long-term cGVHD may lead to gingivitis, periodontitis, increased tooth decay, tooth loss, and secondary malignancies of the oral mucosa. In children excessive drinking during eating may be the first symptom of oral involvement. Not only mucosal problems but abnormal teeth development (e.g., hypodontia, root malformation, enamel hypoplasia) and caries are often seen as secondary symptoms in infants.

### 44.2.4 Liver

Liver involvement manifests as cholestasis and may resemble primary biliary cirrhosis, but hepatic forms with high transaminases are also possible. Other factors, such as viral infections (hepatitis A, B, C, and E, CMV, EBV, ADV, and HHV6/7), drug toxicity, or total-parenteral nutrition-related cholestasis, should be excluded, but liver biopsy may be required to confirm the diagnosis, particularly in patients with no other symptoms of cGVHD and failure to respond to initial treatment of suspected GVHD (Stift et al. 2014).

### 44.2.5 Gastrointestinal Tract

GI manifestations can lead to dysphagia (esophagus), nausea and vomiting (stomach), or chronic diarrhea and malabsorption syndrome (intestines, pancreas). Occasionally, cGVHD may also manifest as immune-mediated pancreatitis. Of note, except esophageal involvement, intestinal involvement is regarded as manifestation of acute GVHD, and patients are therefore classified as suffering from overlap syndrome in which concomitant symptoms of chronic and acute GVHD occur.

Infections like ADV or CMV gastroenteritis, secondary gluten or lactose intolerance, pancreatic insufficiency, and drug-related side effects (e.g., mycophenolate mofetil) have to be ruled out.

Malnutrition and enteral fluid and protein loss in small children require regular laboratory monitoring.

### 44.2.6 Genitals

The symptoms of cGVHD are similar to those of genital lichen planus which may occur in males and females. Vaginal synechia, ulceration, and fissures can subsequently occur. Genital manifestations are often associated with oral manifestations of cGVHD. As symptoms may not be reported spontaneously, females suffering from cGVHD require regular gynecological follow-up including screening for secondary malignancies. In girls cGVHD may manifest with vulvovaginitis, in boys with balanitis or balanoposthitis. Of note, healing may occur with fibrosis possibly leading to synechia with the risk of hematocolpos during puberty in females and of phimosis in males.

### 44.2.7 Lung

Pulmonary manifestations occur as progressive, irreversible obstruction (bronchiolitis obliterans syndrome) and less frequently as restrictive manifestation with bronchiolitis obliterans organizing pneumonia (BOOP) which may progress towards

fibrosis with nonspecific interstitial pneumonia pattern (see Chap. 52).

Since the onset of pulmonary symptoms may not be symptomatic and obstruction may be irreversible, regular evaluations of a serial pulmonary function test (PFT) with body plethysmography (from the age of 4–6 years on) and diffusion capacity (usually from 8 to 10 years of age on) are required in asymptomatic patients.

Patients require follow-up by a pediatric experienced pulmonologist. Of note, the possible overlap of (1) myopathy/hypotrophy of the respiratory muscles (glucocorticoid induced,  $\pm$ central obesity, and/or physical inactivity), (2) restriction of the chest wall in the context of dermal sclerosis, and (3) unproportional chest growth after TBI and/or local irradiation may contribute to a restrictive ventilator dysfunction leading to a mixed picture.

Finally, a thorough diagnostic evaluation includes a lung CT scan and a BAL to rule out viral, bacterial, fungal, and mycobacterial infections.

Coexisting IgA deficiency and chronic sinusitis or sinubronchial syndrome should be considered in the diagnostic workup (Hildebrandt et al. 2011).

#### 44.2.8 Joints and Fasciae

cGVHD-associated fasciitis (diagnostic for cGVHD) can result in restricted mobility of joints. This can also be caused by deep cutaneous sclerosis. Moreover, rheumatoid complaints may

be associated with cGVHD. In children, myositis, muscle weakness, cramping, edema, and pain are quite common. However, iatrogenic glucocorticoid-induced myopathy may overlap with fasciitis. Photographic range-of-motion (P-ROM) examinations are recommended at baseline and at serial intervals with the P-ROM scale providing an easy-to-apply tool. (There is a pediatric adaptation, ped P-ROM; see Addendum.)

### 44.3 Diagnosis

cGVHD is diagnosed on the basis of cGVHD symptoms of eight organs, laboratory values (for hepatic manifestations), and PFTs. Each organ is graded between 0 and 3. The overall severity of cGVHD is classified as mild, moderate, or severe based on this organ-specific grading (number of organs and severity). Overall severity is calculated on the basis of the number of organs affected and the severity of their involvement. Only in case that functional involvement is solely due to none GVHD causes, the impairment is not scored (Jagasia et al. 2015). Biomarkers of cGVHD are currently explored but require validation before clinical use.

#### 44.3.1 Organ Grading of cGVHD for Adults and Children (See Annex 1 and Addendum)

##### 44.3.1.1 Grading of Overall Severity of cGVHD (Jagasia et al. 2015)

Overall severity	Mild	Moderate	Severe
Number of involved organs	1–2	>3	$\geq 3$
Severity of involved organs	Mild (excluding lung)	Mild–moderate (lung only mild)	Severe (lung moderate or severe)

If diagnostic symptoms of cGVHD are absent, histological confirmation of diagnosis may be required. This may be particularly the case in gastrointestinal, nonspecific cutaneous, hepatic, and pulmonary manifestations to rule out toxic or infectious causes or comorbidity. Clinicopathologic series indicate a significant risk for inappropriate diagnosis and subsequent treatment if diagnosis has been made solely by clinical manifestations (and lacking diagnostic symptoms) without histological confirmation.

## 44.4 Treatment

### 44.4.1 First-Line Therapy

First-line treatment (see Table 44.1) consists of steroids given alone or in combination with CNI and is based on randomized trials.

As *mild cGVHD* does not impair organ function, the use of topical IS (topical steroids, topical

CNI, or phototherapy) should be considered. If this is impossible, PRD treatment at an initial dose of 0.5–1 mg/kg body weight/day is recommended. Topical IS can be used in addition to systemic IS, to improve efficacy, or to reduce systemic IS, but lack systemic efficacy.

For *moderate or severe cGVHD*, systemic treatment with PRD or methylPRD at an initial dose of 1 mg/kg body weight/day should be used. In individual cases lower doses of 0.5–1 mg/kg may be used (Jacobsohn 2010). The combination of steroids with a CNI (CSA or TAC) is particularly worth considering for severe cGVHD. Rituximab has been explored in the first-line treatment of cGVHD in combination with steroids and CNI demonstrating an increased response rate on the expense of an increased risk for late infectious complications and delayed B-cell recovery. In addition, sirolimus may also be combined with prednisone showing identical response rates compared to CNI (Carpenter et al. 2018). Other combination partners like ECP, itaci-

**Table 44.1** First-line treatment of cGVHD

Drug	Recommendation		Side effects in >25% patients	Response rate	Comment
	Grade	Evidence			
Steroids	A	I	Osteoporosis, osteonecrosis, diabetes mellitus	~30 to 50% CR	Main drug; strategies to reduce use due to SEs very important
CNI + steroids	C-1	II	Renal toxicity, hypertension	~30 to 50% RC	Reduces steroid use, reduced incidence of osteonecrosis
Rituximab + steroids/ CNI	C-1	III-1	Increased risk for late infectious complications	~75%	Randomized data are lacking
Sirolimus + steroids	C-1	II	Hypercholesterinemia (Carpenter et al. 2018), cytopenia	~50%	Equal efficacy compared to combination with CNI within a randomized trial
MMF + CNI/steroids	D	II	GI complaints, infections		No increased efficacy compared to CNI and steroids, increased risk of relapse of malignancy
Azathioprine	D	II	Cytopenia, risk of infection		Increased mortality
Thalidomide	D	II	Neurotoxicity, drowsiness, constipation		Very little effect in first-line therapy

A, should always be used; C-1, use in first-line therapy justified; D, moderate evidence of lack of efficacy or unacceptably high risks, should generally not be offered; I, evidence from ≥1 properly randomized, controlled trials; II, evidence from more than one well-planned nonrandomized clinical trial, from cohort or case-controlled, analytic studies (preferably at several sites); III-1, only one noncontrolled study; III-2, only one retrospective, noncontrolled study or retrospective evaluation. (Evidence and recommendations graded according to the 2005 NIH Consensus)

SE side effect, NIH US National Institutes of Health, MMF mycophenolate mofetil

Adapted from Wolff et al. (2011)

tinib, and ibrutinib have been evaluated in the first-line treatment of cGVHD within randomized clinical trials but did not result in significantly higher response rates compared to steroids alone.

As cGVHD often takes time to respond to IS treatment, response should not be assessed until at least 8 weeks have elapsed or until 3–6 months have elapsed in the presence of deep cutaneous sclerosis. Long-term IS treatment lasting at least 3–6 months is often required. Dose reduction of IS agents should be performed stepwise. Depending on the patient population, first-line therapy achieves complete remission of cGVHD in approximately 20% (adults) to 50% (children) of cases. If symptoms progress during the first 4 weeks of first-line therapy or there is no improvement in symptoms within 8–12 weeks, second-line therapy should be initiated.

#### 44.4.2 Topical Therapy and Supportive Care

In principle, there is no difference between cGVHD treatment for children and adults. However, long-term steroid therapy in children causes major side effects in terms of growth, bone density, osteonecrosis, and organ development, making agents that reduce steroid use, entailing the use of topical drugs, particularly important. Age-based ancillary supportive care is essential in the management of pediatric cGVHD with the chance of sparing systemic therapy, often supported by highly compliant parents and/or family members as caregivers (Carpenter et al. 2015; Sobkowiak-Sobierajska et al. 2022). In small children, the risk of systemic effects of topical steroid and CNI treatment must be considered. cGVHD is by itself remarkably immunosuppressive intensified by its treatment (especially high-dose corticosteroids) leading to a high risk for infections: (a) for viral reactivation like CMV, ADV, and EBV and (b) for fungal infection like candida and aspergillosis. Functional asplenia with occurrence of Howell-Jolly bodies and a higher incidence of pneumococcal sepsis has to be considered also. Breakdown of skin and mucosal barriers adds to this risk.

Revaccinations (see Chap. 29) with inactivated vaccines are strongly recommended after

consolidation of cGVHD (Hilgendorf et al. 2011). Live vaccines should be avoided in this patient population. Ursodeoxycholic acid reduced liver GVHD and improved survival (Ruutu et al. 2014). Supplemental IG replacement is recommended in cGVHD patients with IgG <400 mg/dL or recurrent infections which is of special importance in children but does also apply to adults.

#### 44.4.3 Second-Line Therapy

While first-line therapy is based on randomized trials, second-line treatment is mostly based on phase II trials and retrospective analyses taking into account that ruxolitinib has been recently evaluated within a phase III trial and additional randomized trials evaluated ECP, rituximab, and imatinib (see Table 44.2). In addition, because the data on disease severity and patient populations are very heterogeneous (in terms of age, treatment line, conditioning, and stem cell source), the published response rates cannot be fully extrapolated to the majority of patients currently treated for cGVHD. Moreover, many substances have been used almost exclusively in combination with steroids.

In general, no more than three IS agents should be combined, as combinations of more drugs often does not lead to improved efficacy but results in a significantly increased risk of side effects and infections. Because of the substantial toxicity of long-term steroid treatment, strategies for dose reduction are very important. Since no predictors of response for a single agent in individual patients are yet available, the choice of agent depends mainly on side effect profiles, availability including approval status, evidence, biology of the disease (overlap vs. classic), and patients' medical history. The response rates for specific agents range between 20 and 70%.

Response is assessed as for first-line therapy. Administration of drugs that have been shown to be ineffective should be stopped. As a rule, drugs shown to be ineffective should be tapered off stepwise with no more than one drug to be changed at a time in order to be able to evaluate their efficacy.



**Table 44.2** Second-line treatment of cGVHD (Wolff et al. 2021)

Drug	Recommendation		Response rate	Side effects in >25% of patients	Comments
	Grade	Evidence			
Steroids	B	III-1	n.a.	Osteoporosis, osteonecrosis, diabetes mellitus	Main drug, strategies to reduce use due to SEs very important
Ruxolitinib	C-1	II	~50%	Infections, cytopenia	FDA and EMA approved in second-line treatment
Ibrutinib	C-1	III-1	~50 to 75% ~16 to 25% CR	Bruising, diarrhea, infections	FDA approved in second-line treatment of cGVHD
Photopheresis	C-1	II	~60 to 70% ~30% CR	Infections of the CVC (if applicable)	Venous access required, steroid-saving effect, good tolerability
mTOR-inh (sirolimus, everolimus)	C-1	III-1	~60% ~20% CR	TMA, hyperlipidemia, cytopenia	Increased risk of TMA when combined with CNI, regular blood levels required
MMF	C-1	III-1	~50% ~10% CR	GI SEs, risk of infection (viral) and increased risk of relapse	Steroid-sparing activity
CNI	C-1	III-1	n.a.	Renal toxicity, hypertension	Reduces steroid use, regular blood levels required
Belumosudil	C-2	III-1	~75%	Liver toxicity	FDA approved in third-line treatment
MTX	C-2	III-1	~50% ~10 to 20% CR	Cytopenia, mucositis	Best results in mucocutaneous cGVHD, reduces steroid use, contraindicated in the presence of pleural effusions or ascites
IL-2	C-2	III-1	~65% (only PR)	Fever, malaise, and fatigue	Applied in sclerodermitoid skin disease
Ixazomib	C-2	III-2	~40%	Nausea, infection, cytopenia	Evaluated in advanced cGVHD
Bortezomib	C-2	III-1	n.a. for second-line Tx	Cytopenia, neuropathy	Trial was performed in first-line treatment
High-dose steroids	C-2	III-2	50–75% (only PR)	Infections	Rapid control of cGVHD
Total nodal irradiation	C-2	III-2	~50% ~25% CR	Cytopenia	Best results for fasciitis and mucocutaneous cGVHD
Abatacept	C-2	III-1	~45%	Airway infection	Best results in lung and mucocutaneous cGVHD
Hydroxychloroquine	C-2	III-2	~25% ~10% CR	GI side effects	Best results for mucocutaneous and hepatic cGVHD
Pentostatin	C-2	II	~50% ~10% CR	Cytopenia, risk of infection	Best results in children
Rituximab	C-2	II	~50% ~10% CR	Risk of infection	Effective in manifestations associated with autoAb and sclerodermitoid cutaneous involvement
Imatinib	C-2	III-1	~50% ~20% CR	Fluid retention	Efficacy demonstrated mainly in sclerodermitoid cGVHD and bronchiolitis obliterans
Pomalidomid	C-2	III-1	~60%	Cytopenia	Evaluated in late cGVHD only

(continued)

**Table 44.2** (continued)

Drug	Recommendation		Response rate	Side effects in >25% of patients	Comments
	Grade	Evidence			
Axatilimab	C-3	III-1	~50%	Elevated liver enzymes, periorbital edema	Available in clinical trials only
Fostamatinib	C-3	III-2	~70%	Risk of infection	Phase I trial only
Thalidomide	C-3	II	~20 to 30% (only PR)	Neurotoxicity, drowsiness, constipation	Treatment for simultaneous cGVHD and recurrent multiple myeloma
Azathioprine	C-3	III-1	n.a.	Cytopenia, risk of infection, secondary malignancies	Increased risk of malignant disease of the oral mucosa
Retinoids	C-3	III-2	~60% (only PR)	Skin toxicity, hyperlipidemia	Effective in sclerodermoid cutaneous involvement
Tocilizumab	C-3	III-2	n.a.	Cytopenia, lack of acute phase reaction	Effective in sclerosing skin involvement
Regulatory T cells	C-4			None	Currently explored in several clinical trials
Mesenchymal stem cells	C-4	III-2	~50%	None	Repetitive application required
Alemtuzumab	C-4	III-3	n.a.	Infectious risks	Last resort for refractory cGVHD
Etanercept	C-4	III-3	n.a.	Infectious risks	May be used to treat mixed acute and chronic GVHD or pulmonary or GI manifestations of cGVHD

B, should generally be used; C-1, use in second-line therapy justified; C-2, use after failure of second-line therapy justified; C-3, should only be used in specific circumstances, due to unfavorable risk profile; C-4, experimental, should only be used in clinical trials and individual cases; II, evidence from >1 well-designed clinical trial without randomization, from cohort or case-controlled analytic studies (preferable from >1 center) or from multiple time series; III-1, several reports from retrospective evaluations or small uncontrolled clinical trials; III-2, only one report from small uncontrolled clinical trial or retrospective evaluations; III-3, only case reports available  
*SE* side effect, *n.a.* not available  
 Adapted from Wolff et al. (2011)

## Appendix A

Annex 1 - Organ Scoring of Chronic GVHD

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
<b>PERFORMANCE SCORE:</b> <b>KPS ECOG LPS</b>	<input type="checkbox"/> Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	<input type="checkbox"/> Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	<input type="checkbox"/> Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	<input type="checkbox"/> Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
<b>SKIN†</b>  <b>SCORE %BSA</b>				
<u>GVHD features to be scored by BSA:</u> <b>Check all that applies:</b> <ul style="list-style-type: none"> <li><input type="checkbox"/> Maculopapular rash/erythema</li> <li><input type="checkbox"/> Lichen planus-like features</li> <li><input type="checkbox"/> Sclerotic features</li> <li><input type="checkbox"/> Papulosquamous lesions or ichthyosis</li> <li><input type="checkbox"/> Keratosis pilaris-like GVHD</li> </ul>	<input type="checkbox"/> No BSA involved	<input type="checkbox"/> 1-18% BSA	<input type="checkbox"/> 19-50% BSA	<input type="checkbox"/> >50% BSA
<b>SKIN FEATURES SCORE:</b>	<input type="checkbox"/> No sclerotic features		<input type="checkbox"/> Superficial sclerotic features "not hidebound" (able to pinch)	<b>Check all that applies:</b> <ul style="list-style-type: none"> <li><input type="checkbox"/> Deep sclerotic features</li> <li><input type="checkbox"/> "Hidebound" (unable to pinch)</li> <li><input type="checkbox"/> Impaired mobility</li> <li><input type="checkbox"/> Ulceration</li> </ul>
<u>Other skin GVHD features (NOT scored by BSA)</u> <b>Check all that applies:</b> <ul style="list-style-type: none"> <li><input type="checkbox"/> Hyperpigmentation</li> <li><input type="checkbox"/> Hypopigmentation</li> <li><input type="checkbox"/> Poikiloderma</li> <li><input type="checkbox"/> Severe or generalized pruritus</li> <li><input type="checkbox"/> Hair involvement</li> <li><input type="checkbox"/> Nail involvement</li> </ul>				
<input type="checkbox"/> Abnormality present but explained entirely by non-GVHD documented cause (specify): _____				
<b>MOUTH</b> Lichen planus-like features present: <ul style="list-style-type: none"> <li><input type="checkbox"/> Yes</li> <li><input type="checkbox"/> No</li> </ul>	<input type="checkbox"/> No symptoms	<input type="checkbox"/> Mild symptoms with disease signs but not limiting oral intake significantly	<input type="checkbox"/> Moderate symptoms with disease signs with partial limitation of oral intake	<input type="checkbox"/> Severe symptoms with disease signs on examination with major limitation of oral intake
<input type="checkbox"/> Abnormality present but explained entirely by non-GVHD documented cause (specify): _____				

**Annex 1 - Organ Scoring of Chronic GVHD (continued)**

	<b>SCORE 0</b>	<b>SCORE 1</b>	<b>SCORE 2</b>	<b>SCORE 3</b>
<b>EYES</b>  <i>Keratoconjunctivitis sicca (KCS) confirmed by Ophthalmologist:</i> <input type="checkbox"/> <b>Yes</b> <input type="checkbox"/> <b>No</b> <input type="checkbox"/> <b>Not examined</b>	<input type="checkbox"/> No symptoms	<input type="checkbox"/> Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤3 x per day)	<input type="checkbox"/> Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), <b>WITHOUT</b> new vision impairment due to KCS	<input type="checkbox"/> Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) <b>OR</b> unable to work because of ocular symptoms <b>OR</b> loss of vision due to KCS
<input type="checkbox"/> <i>Abnormality present but explained entirely by non-GVHD documented cause (specify):</i> _____				
<b>GI TRACT</b> <b>Check all that applies:</b> <input type="checkbox"/> Esophageal web/proximal stricture or ring <input type="checkbox"/> Dysphagia <input type="checkbox"/> Anorexia <input type="checkbox"/> Nausea <input type="checkbox"/> Vomiting <input type="checkbox"/> Diarrhea <input type="checkbox"/> Weight loss* <input type="checkbox"/> Failure to thrive	<input type="checkbox"/> No symptoms	<input type="checkbox"/> Symptoms without significant weight loss* (<5%)	<input type="checkbox"/> Symptoms associated with mild to moderate weight loss* (5-15%) <b>OR</b> moderate diarrhea without significant interference of daily living	<input type="checkbox"/> Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs <b>OR</b> esophageal dilation <b>OR</b> severe diarrhea with significant interference of daily living
<input type="checkbox"/> <i>Abnormality present but explained entirely by non-GVHD documented cause (specify):</i> _____				
<b>LIVER</b>	<input type="checkbox"/> Normal total bilirubin and ALT or AP <3 x ULN	<input type="checkbox"/> Normal total bilirubin with ALT ≥3 to 5 x ULN or AP > 3 x ULN	<input type="checkbox"/> Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	<input type="checkbox"/> Elevated total bilirubin > 3 mg/dL
<input type="checkbox"/> <i>Abnormality present but explained entirely by non-GVHD documented cause (specify):</i> _____				
<b>LUNGS**</b> <b>Symptoms score:</b>	<input type="checkbox"/> No symptoms	<input type="checkbox"/> Mild symptoms (shortness of breath after climbing one flight of steps)	<input type="checkbox"/> Moderate symptoms (shortness of breath after walking on flat ground)	<input type="checkbox"/> Severe symptoms (shortness of breath at rest; requiring O <sub>2</sub> )
<b>Lung score:</b> FEV1 <input style="width: 40px; height: 20px;" type="text"/>	<input type="checkbox"/> FEV1 ≥80%	<input type="checkbox"/> FEV1 60-79	<input type="checkbox"/> FEV1 40-59%	<input type="checkbox"/> FEV1 ≤39%
<i>Pulmonary function tests</i> <input type="checkbox"/> Not performed				
<input type="checkbox"/> <i>Abnormality present but explained entirely by non-GVHD documented cause (specify):</i> _____				

**Annex 1. Organ scoring of chronic GVHD (continued)**

	<b>SCORE 0</b>	<b>SCORE 1</b>	<b>SCORE 2</b>	<b>SCORE 3</b>
<b>JOINTS AND FASCIA</b>  <i>P-ROM score (see below)</i> Shoulder (1-7): ____ Elbow (1-7): ____ Wrist/finger (1-7): ____ Ankle (1-4): ____	<input type="checkbox"/> No symptoms	<input type="checkbox"/> Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) <b>AND</b> not affecting ADL	<input type="checkbox"/> Tightness of arms or legs <b>OR</b> joint contractures, erythema thought due to fasciitis, moderate decrease ROM <b>AND</b> mild to moderate limitation of ADL	<input type="checkbox"/> Contractures <b>WITH</b> significant decrease of ROM <b>AND</b> significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
<input type="checkbox"/> Abnormality present but explained entirely by non-GVHD documented cause (specify): _____				
<b>GENITAL TRACT</b> <i>(See Supplemental figure†)</i> <b>Check all that applies</b> <input type="checkbox"/> Not examined Currently sexually active <input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> No signs	<input type="checkbox"/> Mild signs‡ and females with or without discomfort on exam	<input type="checkbox"/> Moderate signs‡ and may have symptoms* with discomfort on exam	<input type="checkbox"/> Severe signs‡ with or without symptoms
<input type="checkbox"/> Abnormality present but explained entirely by non-GVHD documented cause (specify): _____				
<b>Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable none -0, mild -1, moderate -2, severe -3)</b>				
<input type="checkbox"/> Ascites (serositis) ____		<input type="checkbox"/> Myasthenia Gravis ____		
<input type="checkbox"/> Pericardial Effusion ____		<input type="checkbox"/> Peripheral Neuropathy ____		<input type="checkbox"/> Eosinophilia > 500µl ____
<input type="checkbox"/> Pleural Effusion(s) ____		<input type="checkbox"/> Polymyositis ____		<input type="checkbox"/> Platelets <100,000/µl ____
<input type="checkbox"/> Nephrotic syndrome ____		<input type="checkbox"/> Weight loss* without GI symptoms ____		<input type="checkbox"/> Others (specify): _____
<b>Overall GVHD Severity</b> <i>(Opinion of the evaluator)</i>	<input type="checkbox"/> No GVHD	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
<b>Photographic Range of Motion (P-ROM)</b>				

Adapted from Jagasia, 2015.

† Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, **OR** if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring.

\* Weight loss within 3 months.

\*\* Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.  
**Abbreviations:** ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); NUL (normal upper limit).

‡ To be completed by specialist or trained medical providers (see Supplemental Figure).

## Appendix B

### Diagnosis and staging cGVHD in children

Jagasia et al BBMT 2015  
 pediatric adaptation A. Lawitschka 11/2015

patient name

date:

patient name

- ▶ please score/check the worst manifestation
- ▶ diagnostic features are marked **bold**

- classification:actual**
- feat. of acute GVHD
  - feat.of classic cGVHD
  - both

- onset type ONLY at diagn.:**
- de novo
  - quiescent
  - progressive

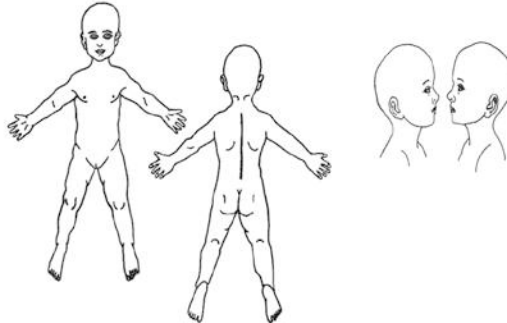
symptoms/features	Score 0	Score1	Score 2	Score 3
<b>KPS/LPS:</b> %	<input type="checkbox"/> asymptomatic and fully active (KPS/LPS 100%)	<input type="checkbox"/> sympt., fully amb., restricted only in physically strenous activity (KPS/LPS 80-90%)	<input type="checkbox"/> sympt., amb., capable of self-care, >50% of waking hours out of bed (KPS/LPS 60-70%)	<input type="checkbox"/> sympt., limited self-care >50% of waking hours in bed (KPS/LPS < 60%)

**SKIN**

Feat. scored by BSA:	no BSA involved	1-18% BSA	19-50% BSA	> 50% BSA
<input type="checkbox"/> maculopapular rash/erythema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> <b>lichen planus-like features</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> <b>sclerotic features:</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> lichen sclerosus-like	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> morphea-like	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> papulosquamous lesions	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> ichthyosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> keratosis pilaris-like GVHD	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Feat. not scored by BSA:

- hyperpigmentation
- hypopigmentation/ depigmentation
- poikiloderma**
- severe pruritus
- hair involvement
- nail involvement
- sweat impairment
- abnormality present but explained entirely by non-GVHD cause (specify):



%BSA:  
 child: head front/back 9 / 9  
 back 18, chest 18,  
 arm left 9, arm right 9  
 leg left 13,5, leg right 13,5  
 adult: head front/back 4,5 / 4,5  
 back 18, chest 18  
 arm left 9, arm right 9  
 leg left 18, leg right 18  
 palm: 1,5

- ▶ feature decisive for diagnosis /scoring:

<b>sclerotic features:</b>	<input type="checkbox"/> no sclerotic features	<input type="checkbox"/> superficial sclerotic features "not hidebound" (able to pinch)	<input type="checkbox"/> deep sclerotic features "hidebound" (unable to pinch) impaired mobility ulceration
----------------------------	--	---	---

**MOUTH**

	no symptoms	mild sympt with disease signs but not limiting oral intake significantly	moderate sympt. with disease signs with partial limitation of oral intake	severe sympt. with disease signs on examination with major limitation of oral intake
<input type="checkbox"/> erythema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> <b>lichen planus-like features</b>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> hyperkeratot. plaques	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> mucocelles <input type="checkbox"/> pseudomembranes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> ulcers <input type="checkbox"/> mucosal atrophy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> dryness <input type="checkbox"/> pain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

- ▶ feature decisive for diagnosis /scoring:



**Appendix 2 - Diagnosis and staging cGVHD in children (continued)**

symptoms/features	Score 0	Score 1	Score 2	Score 3
<b>EYES</b>				
<input type="checkbox"/> keratokonjunktivitis sicca (KCS)	<input type="checkbox"/> no symptoms	<input type="checkbox"/> mild dry eye sympt.	<input type="checkbox"/> moderate dry eye sympt.	<input type="checkbox"/> severe dry eye sympt.
<input type="checkbox"/> confirmed by ophthalmologist		not affecting ADL	partially affecting ADL	significantly affecting ADL
<input type="checkbox"/> dryness <input type="checkbox"/> pain		(requirement of	(lubricant eye drops	(special eyewear to relieve pain) or
<input type="checkbox"/> photophobia <input type="checkbox"/> blepharitis		lubricant eye drops	>3 x/d or punctual plugs)	unable to work because of ocular
<input type="checkbox"/> pseudomembranes <input type="checkbox"/> ulcers		≤ 3 x per day)	without new vision	sympt or loss of vision due to KCS
			impairment due to KCS	
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):				
▶ feature decisive for diagnosis /scoring:				
<b>GI TRACT</b>				
<input type="checkbox"/> esophageal web/ prox stricture or ring	<input type="checkbox"/> no symptoms	<input type="checkbox"/> symptoms without significant weight loss (5%)	<input type="checkbox"/> sympt. associated with mild to moderate weight loss (5-15%) or moderate diarrhea without significant interference with daily living	<input type="checkbox"/> symptoms associated with significant weight loss (> 15%) requires nutritional supplement for most calorie needs or esophageal dilatation or severe diarrhea with signif. Interference with daily living
<input type="checkbox"/> dysphagia <input type="checkbox"/> abdominal pain				
<input type="checkbox"/> anorexia <input type="checkbox"/> failure to thrive				
<input type="checkbox"/> nausea <input type="checkbox"/> vomiting				
<input type="checkbox"/> diarrhea <input type="checkbox"/> weight loss ≥ 5%				
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):				height:
▶ feature decisive for diagnosis /scoring:				weight:
<b>LIVER</b>				
<input type="checkbox"/> hepatic pattern	<input type="checkbox"/> normal total bili	<input type="checkbox"/> normal total bili	<input type="checkbox"/> elevated total bili	<input type="checkbox"/> elevated total bili > 3 mg/dl
Bili: _____ AST: _____ ALT: _____	and ALT or AP	with ALT ≥ 3-5x ULN	but ≤ 3 mg/dl or	
GGT: _____ AP: _____	< 3 ULN	or AP ≥ 3 x ULN	ALT > 5 ULN	
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):				
▶ feature decisive for diagnosis /scoring:				
<b>LUNGS</b>				
FEV1: _____ %      MEF25: _____ %	<input type="checkbox"/> no symptoms	<input type="checkbox"/> mild symptoms	<input type="checkbox"/> moderate symptoms	<input type="checkbox"/> severe symptoms
FVC: _____ %      MEF50: _____ %	FEV1 ≥ 80%	(shortness of breath	(shortness of breath	(shortness of breath at rest;
DLCO: _____ %      MEF75: _____ %		after climbing one	after walking on	requiring O2)
RV: _____ <input type="checkbox"/> RV/TLC > 120%		flight of steps)	flat ground)	FEV1 ≤ 39%
CT: _____		FEV1 60-79%	FEV1 40-59%	
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):				
▶ feature decisive for diagnosis /scoring:				
<b>JOINTS AND FASCIA</b>				
ped P-ROM score (see below)	<input type="checkbox"/> no symptoms	<input type="checkbox"/> mild tightness,	<input type="checkbox"/> tightness or joint	<input type="checkbox"/> contractures, fasciitis
<input type="checkbox"/> edema <input type="checkbox"/> fasciitis		normal or mild ↓ of	contractures, fasciitis,	significant ↓ of ROM,
<input type="checkbox"/> muscle cramps <input type="checkbox"/> athermalgia		range of motion (ROM)	moderate ↓ of ROM,	significant ↓ of ADL
		not affecting ADL	mild - moderate ↓ of ADL	
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):				
▶ feature decisive for diagnosis /scoring:				
<b>GENITAL TRACT</b>				
<input type="checkbox"/> erosions, fissures	<input type="checkbox"/> no signs	<input type="checkbox"/> mild signs	<input type="checkbox"/> moderate signs	<input type="checkbox"/> severe signs with or without symptoms
<input type="checkbox"/> lichen planus-like features				
<input type="checkbox"/> lichen sclerosus-like features				
<input type="checkbox"/> labial/ vaginal scarring <input type="checkbox"/> phimosis				
<input type="checkbox"/> abnormality present but explained entirely by non-GVHD cause (specify):				
▶ feature decisive for diagnosis /scoring:				

**Overall GVHD severity**

- no cGVHD
- mild: max. score of 1 in any affected organ, max. 2 organs affected, no lung involvement
- moderate: ≥3 organ with max score 1 or max. score of 2 in any affected organ, lung score max 1
- severe: score 3 in any affected organ, lung score 2-3

**Appendix 2 - Diagnosis and staging cGVHD in children (continued)**

**Other indicators, clinical features or complications related to cGVHD**

check all that apply and assign a severity score (0-3) based on functional impact

- |   |   |   |
|---|---|---|
| <input type="checkbox"/> ascites (serositis)  | <input type="checkbox"/> myasthenia gravis                | <input type="checkbox"/> eosinophilia >500 /ul  |
| <input type="checkbox"/> pericardial effusion | <input type="checkbox"/> peripheral neuropathy            | <input type="checkbox"/> platelets <100 000/ul  |
| <input type="checkbox"/> pleural effusion     | <input type="checkbox"/> polymyositis                     | <input type="checkbox"/> hypo/hyperglobulinemia |
| <input type="checkbox"/> nephrotic syndrome   | <input type="checkbox"/> weight loss >5% without GI sympt | <input type="checkbox"/> auto-antibodies        |
| <input type="checkbox"/> others (specify)     | <input type="checkbox"/> diabetes                         |   |

**biopsy:**

organ:  
GVHD confirmed?

**pediatric photographic range of motion (adapted ped P-ROM):**

please mark appropriate number

shoulder:	1 (worst)	2	3	4	5 (normal)
elbow:	1 (worst)	2	3	4 (normal)	
wrist / finger:	1 (worst)	2	3	4 (normal)	
global flexion:	1 (worst)	2	3	4 (normal)	
ankle:	1 (worst)	2	3 (normal)		

>

## Appendix C

### Genital Tract GVHD Assessment and Scoring Form

Name: \_\_\_\_\_ Date of birth: \_\_\_\_\_

Assessment date: \_\_\_\_\_

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
<b>GENITAL TRACT (male or female)</b>	<input type="checkbox"/> No signs	<input type="checkbox"/> Mild signs and females may have symptoms* WITH discomfort on exam	<input type="checkbox"/> Moderate signs and may have symptoms* with discomfort on exam	<input type="checkbox"/> Severe signs with or without symptoms*
Currently sexually active: <input type="checkbox"/> Yes <input type="checkbox"/> No <b>Check all signs that applies:</b> <input type="checkbox"/> Lichen planus -like features <input type="checkbox"/> Lichen sclerosis-like features <input type="checkbox"/> Vaginal scarring (female) <input type="checkbox"/> Clitoral/labial agglutination (female) <input type="checkbox"/> Labial resorption (female) <input type="checkbox"/> Erosions <input type="checkbox"/> Fissures <input type="checkbox"/> Ulcers <input type="checkbox"/> Phimosis (male) <input type="checkbox"/> Urethral meatusscarring/ stenosis (male)				
<input type="checkbox"/> Abnormality present but <u>NOT</u> thought to represent GVHD (specify cause): _____				
<input type="checkbox"/> Abnormality thought to represent GVHD <u>PLUS</u> other causes (specify cause): _____				

\* Genital symptoms are not specific to cGVHD and can represent premature gonadal failure or genital tract infection.

If a gynecologist is unavailable, external examination may be performed to determine "discomfort on exam" as follows:

- Spread the labia majora to inspect the vulva for the above signs. Touch the vestibular gland openings (Skene's and Bartholin's), labia minora and majora gently with a qtip. Vulvar pain elicited by the gentle touch of a qtip is classified as discomfort on examination. Palpate the vaginal walls with a single digit to detect bands, shortening, narrowing or other signs of vaginal scarring.
- If the woman is sexually active, determine whether qtip palpation or gentle palpation of scarred ridges elicits pain similar to that which the woman experiences during intercourse.

**Female genitalia:** Severity of signs:

- Mild (any of the following); erythema on vulvar mucosal surfaces, vulvar lichen-planus or vulvar lichen-sclerosi.
- Moderate (any of the following); erosive inflammatory changes of the vulvar mucosa, fissures in vulvar folds.
- Severe (any of the following); labial fusion, clitoral hood agglutination, fibrinous vaginal adhesions, circumferential fibrous vaginal banding, vaginal shortening, synechia, dense sclerotic changes, and complete vaginal stenosis.

**Male genitalia:** Diagnostic features include lichen planus-like or lichen sclerosis-like features and phimosis or urethral scarring or stenosis. Severity of signs:

- Mild: lichen planus-like feature;
- Moderate: lichen sclerosis-like feature or moderate erythema;
- Severe: phimosis or urethral/meatal scarring.

Biopsy obtained:	<input type="checkbox"/> Yes <input type="checkbox"/> No	Site biopsied: _____	GVHD confirmed by histology:	<input type="checkbox"/> Yes <input type="checkbox"/> No
Change from previous evaluation:	<input type="checkbox"/> No prior or current GVHD	<input type="checkbox"/> Improved	<input type="checkbox"/> Stable	<input type="checkbox"/> Worse <input type="checkbox"/> N/A (baseline)

Completed by (spell out name): \_\_\_\_\_

Date form completed: \_\_\_\_\_

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# Posttransplant Lymphoproliferative Syndromes

# 45

Jan Styczynski and Sebastian Giebel

## 45.1 Definitions

Posttransplant lymphoproliferative disorders (PTLD) constitute a heterogeneous group of lymphoproliferative diseases that occur in the setting of transplantation and result from the uncontrolled neoplastic proliferation of lymphoid or plasmacytic cells in the context of extrinsic immunosuppression after transplantation.

PTLD in the HCT setting are largely caused by latent Epstein-Barr virus (EBV, HHV-4), belonging to the herpesviruses family. It is one of the most common viruses in humans, with prevalence of 82–84% in the overall population (Styczynski et al. 2016a). EBV is associated with the development of various diseases, which can be categorized as primary syndromes, EBV-associated tumors, and EBV-associated posttransplant diseases: PTLD and other end-organ diseases (encephalitis/myelitis, pneumonitis, hepatitis, or hemophagocytic lymphohistiocytosis).

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Comparably to other herpesviruses, there are two types of EBV infection: primary and recurrent. Primary EBV infection is diagnosed when EBV is detected (nucleic acid or serologically) in an EBV-naïve individual. Recurrent EBV-DNAemia (previously: latent infection) is diagnosed by detection of EBV-DNA in the blood, in a previously infected individual (Dharnidharka et al. 2016).

## 45.2 Types of PTLD

PTLD or end-organ EBV-associated posttransplant disease can be diagnosed at the probable or proven level. Probable EBV disease is diagnosed in case of significant lymphadenopathy, hepatosplenomegaly, or other end-organ manifestations (without tissue biopsy, but in the absence of other documented cause) together with high EBV-DNAemia. Proven EBV disease (PTLD or other end-organ disease) is diagnosed in case of symptoms and/or signs from the affected organ together with the detection of EBV-encoded RNA by in situ hybridization (EBER-ISH) in a tissue specimen (immunohistochemistry for EBV proteins have good specificity but lower sensitivity; these proteins are variably expressed in PTLD biopsies). Histological WHO 2016 classification included six types of morphological PTLD: plasmacytic hyperplasia, infectious mononucleosis-like, florid follicular hyperplasia, polymorphic, monomorphic (B-cell or T-/NK-cell types), and



classical Hodgkin lymphoma PTLD. In the 2022 WHO classification of lymphoid malignancies, PTLD are not any longer listed and are defined as immunodeficiency-associated lymphoproliferative disorders (Marjanska et al. 2023).

### Atypical PTLD

- **EBV-negative PTLD:** A growing number of cases of EBV-negative PTLD have been reported, mainly in SOT recipients. These cases tend to present later (>5 years) after transplant, and an increased risk is observed as long as 10 years after transplantation. These cases should be regarded as malignant lymphoma rather than as PTLD.
- **T-lineage PTLD:** T-PTLD is usually EBV-negative and the relatively long latency between transplantation and T-PTLD onset may be explained by molecular events. The frequency of T-PTLDs ranges 4–15% of all PTLD cases. EBV is present in approximately one-third of T-PTLDs.
- **Composite B-cell and T-cell lineage PTLD:** harboring both B- and T-cell clones either concurrently or successively in the same patient is extremely rare and only a few cases have been reported in the literature, exclusively after SOT, with poor outcome.

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## 45.3 Pathogenesis

The pathogenesis of PTLDs is a result of EBV-induced transformation of B-cells in the setting of impaired anti-EBV cellular immunity due to iatrogenic immunosuppression and resulting in an outgrowth of EBV-infected B-cells. GVHD prevention strategies that indiscriminately remove T-cells from the graft increase the risk of PTLD.

Recurrent EBV infection preceding clinically overt PTLD is the consequence of viral latency, which is the stage in the viral life cycle in which no virions are produced, as opposed to the lytic stage. During viral latency of EBV, three associ-

ated patterns of viral protein expression, so-called latency programs, may be expressed. During infection of the B-cell, these latency programs guide the B-cell through the germinal center reaction pushing it towards the resting memory cell stage. Different latency proteins are implicated in EBV-driven lymphomagenesis demonstrated by the expression of a particular latency program in different lymphoma subtypes.

B-cells in PTLD express a number of latency proteins which are highly immunogenic and are vigorously targeted by T lymphocytes in immunocompetent hosts. This viral gene program in EBV-PTLD is called type III (infected cells express EBNA1, 2, 3A, 3B, and 3C, EBNA-LP, LMP 1 and 2, EBER 1 and 2, and the microRNAs [miRNA] miR-BHRF1 and miR-BART3) of latency and is different and less immunogenic than in other EBV-related diseases with type I (EBNA1, EBV-encoded small RNA [EBER], and BamHI fragment A rightward transcripts [BART] transcripts) or II (latent membrane proteins, LMP1/2EBNA2, EBNA3, and EBNA-leader protein LP) of latency.

EBV plays also an important role in the pathogenesis and epidemiology of acute and chronic GVHD. As B-cells also play a role in the pathophysiology of chronic GVHD, and B-cells are stimulated into activity by EBV infection and B-cell recovery occurs usually after day +100, the impact of EBV-infected B-cells is stronger for the development of chronic and, to a lesser extent, acute GVHD.

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## 45.4 Clinical Manifestations

Lymphadenopathy and fever are the most common symptoms of EBV-PTLD. Rare EBV-associated PTLD manifestations, also referred to as EBV end-organ disease, include encephalitis/myelitis, pneumonitis, hepatitis, and hemophagocytic lymphohistiocytosis (Table 45.1).



**Table 45.1** Clinical manifestations of PTLD

Time to PTLD	<ul style="list-style-type: none"> <li>• Median time of PTLD development: 2–4 months</li> <li>• 6% PTLD cases are diagnosed within the first month</li> <li>• 90% diagnosed within the first 6 months after HCT</li> <li>• Rarely: &gt;5 years post-HCT (more likely representing lymphoma)</li> </ul>
Incidence of EBV-DNA-emia <sup>a</sup>	<ul style="list-style-type: none"> <li>• Median: 29.4% (range: 0.1–63%)</li> <li>• Highest in MUD/MMUD-HCT, haplo-HCT without PTCy</li> <li>• Lowest in haplo-HCT with PTCy (post-HCT CY)</li> </ul>
Incidence of PTLD <sup>a</sup>	<ul style="list-style-type: none"> <li>• All allo-HCT: 3.22%</li> <li>• MFD-HCT: 1.16%</li> <li>• MMFD-HCT: 2.86%</li> <li>• MUD-HCT<sup>b</sup>: 3.97%</li> <li>• MMUD-HCT<sup>b</sup>: 11.24%</li> <li>• CBT: 4.06%</li> <li>• Auto-HCT without TCD: casuistic</li> </ul>
Target organs	<ul style="list-style-type: none"> <li>• Frequently: lymph nodes</li> <li>• Rarely: CNS, GI tract, lungs, liver</li> </ul>

<sup>a</sup> Recent data (during the COVID-19 pandemic) suggest lower incidence of EBV-DNA-emia and EBV-PTLD

<sup>b</sup> Level of donor match determined locally as 8/8 or 10/10

## 45.5 Diagnosis

The diagnosis of EBV-PTLD must be based on symptoms and/or signs consistent with PTLD together with detection of EBV by an appropriate method applied to a specimen from the involved tissue. Definitive diagnosis of EBV-PTLD requires noninvasive and invasive techniques (biopsy and histological examination) (Table 45.2).

**PET imaging:** By definition, PTLD is a neoplastic lymphoproliferation. Malignant lymphomas have the ability to metabolize 18F-fluorodeoxyglucose (FDG), which is used by PET imaging. In most cases, PTLD has FDG-avid histology; thus, FDG-PET is an important diagnostic tool for this disease (Dierickx et al. 2013; Dierickx et al. 2018).

**Monitoring EBV-DNA-emia:** Serial quantitative measurement of EBV viral load posttransplant is currently the method of choice for early detection and monitoring progression and response to treatment of EBV-PTLD. Although the value of the viral load in PTLD risk assessment is uncertain, it is recommended to begin the screening in patients with risk factors after hematological

**Table 45.2** Diagnostic methods for PTLD

<b>Noninvasive diagnostic methods</b>
<ul style="list-style-type: none"> <li>• Quantitative determination of EBV-DNA-emia</li> <li>• Imaging: CT or PET-CT (for avid structures: localized in lymph nodes, spleen, liver, GI tract, skin, lungs, bone, BM) or MRI (in CNS disease; and non-avid histologies)</li> </ul>
<b>Invasive diagnostic methods</b>
<ul style="list-style-type: none"> <li>• Biopsy of the lymph node and/or other suspected sites</li> <li>• Endoscopy: when with GI symptoms</li> <li>• Histological examination:               <ol style="list-style-type: none"> <li>(a) Detection of viral antigens or in situ hybridization for EBV (EBV-encoded-RNA) transcripts</li> <li>(b) Immunohistochemistry</li> <li>(c) Flow cytometry for B-cell, T-cell, and plasma cell lineage-specific antigens</li> </ol> </li> </ul>

recovery and no later than 4 weeks after the day of HCT. In EBV-DNA-negative patients, frequency of screening should be once a week, while in patients with rising EBV-DNA-emia, more frequent sampling might be considered, as the calculated doubling time for EBV might be as short as 56 h. The screening should be continued at least 4 months in high-risk patients. Longer monitoring is recommended in patients considered to have poor T-cell reconstitution, with severe GVHD, after haplo-HCT, with the use of TCD, after conditioning with ATG/alemtuzumab or in those having experienced an early EBV reactivation.

## 45.6 Risk Factors

Risk of development of PTLD is essentially proportional to the degree of T-cell depletion/impairment and this should be regarded as the principal risk factor. Thus, the type of donor and type of conditioning have secondary value as risk factors. Since in the HCT setting PTLD usually originates from the donor, and the risk of PTLD is obviously higher when the donor is seropositive. Risk factors for PTLD in match family donor (MFD) transplants include TCD ex vivo or in vivo, EBV serology mismatch between donor and recipient, and splenectomy (Uhlin et al. 2014). ECIL-6 classified HCT patients into three groups of the risk for EBV-PTLD: low, standard, and high risk (Table 45.3) (Styczynski et al. 2016b).

**Table 45.3** Risk factors for PTLD

Risk group	Patients
High	<ul style="list-style-type: none"> <li>• MUD/MMUD</li> <li>• Alternative donors including CBT</li> <li>• MFD-HCT with at least one risk factor</li> </ul>
Standard	<ul style="list-style-type: none"> <li>• MFD-HCT without risk factors</li> <li>• Haplo-PTCy-HCT</li> </ul>
Low	<ul style="list-style-type: none"> <li>• Auto-HCT</li> </ul>

## 45.7 Grading

No grading system currently exists for PTLD. It seems that apart from the findings from biopsy material with a histological examination, the diagnostic criteria of tissue involvement in PTLD should be consistent with those for lymphoma (the Ann Arbor and the Lugano classifications). Nowadays, the use of FDG-PET-CT has emerged as an important imaging tool for PTLD diagnosis and staging.

Possible staging of PTLD includes the following:

- Clinical end-organ staging: nodal vs extranodal disease.
- Clinical severity staging: limited (unifocal) vs advanced (multifocal) disease.
- ECIL-6 staging (based on: the Lugano lymphoma classification by PET-CT imaging): limited (stages I-II); advanced forms (stages III-IV).

## 45.8 Treatment

### 45.8.1 Prevention: Donor and Recipient Issues

As EBV might be transmitted with the graft, selection of EBV-seronegative donor might be beneficial for EBV-seronegative recipient, if possible. For EBV-seropositive patients, selection of an EBV-seropositive donor might be justified, as transmission of EBV-specific CTLs outweighs the risk of transmission of EBV-positive B-cells from the donor. However, recent data suggest lack of transfer of EBV-CTL from EBV-seropositive donor to recipient.

### 45.8.2 Treatment Strategies

PTLD has to be regarded as disseminated disease at diagnosis. This is because of the involvement of lymphoid tissue, which localized throughout the whole body. Therapeutic approaches applied in the prevention and treatment of EBV-PTLD include administration of rituximab, reduction of immunosuppression (RIS), use of EBV-CTL, DLI, and chemotherapy, while other methods have only historical value. No antiviral drug is currently effective against EBV. There are three major approaches to EBV infection after HCT: prophylaxis, preemptive therapy (also known as preemptive prophylaxis), and treatment of established EBV-PTLD.

#### 45.8.2.1 Prophylaxis

- Prophylaxis of EBV disease is defined as drug or cellular therapy given to an asymptomatic EBV-seropositive patient to prevent EBV-DNA-emia. This strategy is administered rarely, with the use of rituximab or EBV-CTL. Antiviral drugs are not justified as anti-EBV prophylaxis.
- The rationale for prophylactic use of rituximab before or early after allo-HCT is B-cell depletion. Prophylactic use of posttransplant rituximab reduced the risk of EBV-DNA-emia, with no impact on PTLD incidence, TRM, or OS in comparison to preemptive therapy.
- The prophylactic use of EBV-CTLs resulted in excellent efficacy in patients at a high-risk group for EBV-PTLD. The obstacle for the use of this approach is the limited availability of CTLs in most transplant centers.
- Low risk of EBV-DNA-emia and EBV-PTLD was observed after the use of PT-Cy and sirolimus for GVHD prophylaxis.

#### 45.8.2.2 Preemptive Therapy

- Preemptive therapy denotes drug or cellular therapy given to a patient with EBV-DNA-emia in order to prevent EBV disease.
- Monitoring for EBV-DNA-emia is essential in all patients with risk factors for EBV-PTLD. Significant EBV-DNA-emia without clinical symptoms of disease in high-risk

patients for EBV-PTLD is usually an indication for preemptive therapy.

- The goal of preemptive therapy is to obtain a negative EBV PCR or EBV-DNA-emia below the initial threshold without relapse.
- Usually, EBV-DNA-emia occurs prior to the onset of clinical symptoms. There is a correlation between rising or high EBV-DNA-emia in PB and the development of EBV-PTLD but this not the rule.
- Currently available data does not allow for the determination of an unambiguous EBV-DNA threshold value for the diagnosis of EBV-PTLD or other end-organ EBV disease in HCT patients.
- Apart from the EBV-DNA value, also the kinetics of a rising EBV-DNA-emia, together with an assessment of an individual patient's immune function, are very important when appraising the need for preemptive therapy. Local experience based on the correlation of clinical and laboratory data might be a rationale for center-specific cutoff value.
- The primary method for preemptive therapy includes rituximab, once weekly until EBV-DNA-emia negativity. Usually, 1–2 doses of rituximab are sufficient. Rituximab should be combined with reduction of immunosuppression (RIS), if possible. A contraindication for RIS is severe, uncontrolled acute or chronic GVHD. This approach might have additional benefit of rituximab administration, as rituximab possibly reduces the risk of acute/chronic GVHD. Donor or third-party EBV-CTL is another option, although it is not widely available.

#### 45.8.2.3 Treatment of Established EBV-PTLD

- Treatment of established EBV-PTLD means therapeutic interventions for patients with probable or proven EBV disease. Due to the consequential risk of a rapidly growing high-grade lymphoid tumor, together with the potential for EBV to cause rapid MOF, therapy should be implemented as soon as possible (Styczynski et al. 2013).
- For first-line therapy three options are recommended: (a) rituximab, 375 mg/m<sup>2</sup>, once

weekly; (b) RIS, if possible, usually together with administration of rituximab; (c) adoptive immunotherapy with cellular therapy with in vitro-generated donor or third-party EBV-CTL, if available.

- For the second-line therapy, in case of rituximab failure the following are recommended: (a) cellular therapy (nonspecific DLI or specific EBV-CTLs, if available) and (b) chemotherapy ± rituximab. Unselected DLI from EBV-positive donor is used in order to restore broad T-cell reactivity, including EBV-specific responses. In 2022, EMA approved tabellecleucel (“off-the-shelf” third-party donor EBV-specific VST, viral-specific T-lymphocytes).
- Not recommended: IVIG, interferon, and antiviral agents should not be used for any anti-EBV therapy of PTLD (Table 45.4).

#### 45.8.2.4 Treatment in CNS Disease

- CNS localization of PTLD is a special form of the disease, due to the risk of neurological consequences even in case of successful eradication of EBV from CNS. No standard therapy has been accepted up to date.
- Possible therapeutic options include the following: (a) rituximab, either systemic or intrathecal (in the latter case, dose of rituximab was 10–30 mg in 3–10 mL saline administered weekly), (b) T-cell therapy with EBV-CTLs, (c) radiotherapy, and (d) chemotherapy ± rituximab according to primary CNS lymphoma protocols based on a high dose of methotrexate ± cytarabine (Czyzewski et al. 2013).

**Table 45.4** Response rates to anti-EBV-PTLD therapy

Treatment strategy	Preemptive therapy	Therapy of PTLD
Rituximab	90%	65%
Rituximab + RIS		78%
EBV-CTL	94–100%	71–75%
RIS	68%	61%
DLI		58% <sup>a</sup>
Chemotherapy		26% <sup>a</sup>
Antivirals (cidofovir)		34% <sup>a</sup>

RIS reduction of immunosuppression

<sup>a</sup> With other therapies

#### 45.8.2.5 Criteria of Response to Therapy in EBV-PTLD

- The treatment goal in the EBV-PTLD setting is resolution of all signs and symptoms of PTLD together with negative EBV-DNA-emia.
- The response to rituximab therapy can be identified by a decrease in EBV-DNA-emia of at least 1 log of magnitude in the first week of treatment.
- Positive prognostic factors for outcome to rituximab therapy include age below 30 years, underlying nonmalignant disease, no acute GVHD  $\geq$ II, RIS at time of PTLD diagnosis, and decrease of viral load after 1 or 2 weeks of therapy.
- Complete remission of PTLD can be defined as resolution of all symptoms of PTLD, including clearance of EBV-DNA-emia. Partial response of PTLD can be stated with the decrease of at least 50% of initial changes, including decrease of EBV-DNA-emia.
- The response to therapy can be confirmed by achievement of a PET-negative complete remission for avid lymphomas and CT/MRI for non-avid histologies or CNS localization.

- Management strategies: prophylaxis, preemptive treatment, and therapy of established EBV-PTLD. Therapeutic approaches include administration of rituximab, reduction of immunosuppression (RIS), use of EBV-CTL (including third-party donor), or DLI and chemotherapy, while other methods have only a historical value.
- EBV-DNA-emia threshold value: No value determines diagnosis of EBV-PTLD or other end-organ EBV disease in HCT patients. In order to initiate preemptive therapy, transplant centers should use own threshold values of EBV-DNA-emia.
- Outcome: Administration of rituximab results in a positive outcome for over 90% of patients treated preemptively and over 65% when it is used as targeted therapy for EBV-PTLD. RIS when applied in combination with rituximab: over 80%. The use of EBV-CTLs: >90% of patients treated preemptively and approximately 75% in therapy of EBV-PTLD.

#### Key Points

- Definition: PTLD results from an uncontrolled neoplastic proliferation of lymphoid or plasmacytic cells in the context of extrinsic immunosuppression after transplantation. PTLD in the HCT setting are largely caused by latent EBV. Risk factors for EBV-PTLD are proportional to the degree of T-cell impairment.
- Diagnosis: should be based on invasive techniques including biopsy of the lymph node and/or other sites suspected for EBV disease. Noninvasive diagnostic methods have an accessory value and include the quantitative determination of EBV-DNA-emia in blood, plasma, or serum and PET-CT/CT/MRI.

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## 46.1 Introduction

Iron overload/toxicity is an unavoidable consequence in several diseases characterized by anemia and red blood cell transfusion requirement.

It is now clear that iron-related damage is due not only to iron level “per se” but to the presence in the serum of non-transferrin forms of iron (non-transferrin-bound iron [NTBI]). A component of NTBI, called labile plasma iron (LPI), is a potent redox-active agent capable of permeating into cells in an uncontrolled way, thus inducing cellular iron overload and impacts the delicate equilibrium of labile cellular iron (LCI). The breakage of LCI balance catalyzes the formation of reactive oxygen species (ROS), which leads to cytotoxic cell injury (DNA damage, lipid peroxidation, protein modification, and mitochondrial damage). Of course iron overload is a source of NTBI/LPI production.

Other factors significantly impact on iron toxicity: the quantity of the abovementioned toxic iron-related species, individual’s antioxidant genetics, environmental factors, and, most importantly, duration of exposure (Coates 2014).

Several cellular pathways are sensitive to the detrimental action of ROS in a non-dose-dependent manner. Different human tissues have a different capacity to respond to iron-mediated toxicity, indicating that the toxicity thresholds are disease-specific and patient-dependent (Pilo et al. 2022).

Notably, NTBI and LPI appear in the serum only when transferrin saturation exceeds 70% (de Swart et al. 2016) and are cheatable forms of iron.

NTBI and LPI measurement is today available in selected laboratories for research purposes only. Transferrin saturation is at the moment a valid surrogate indicating, when exceeding 70%, the presence of NTBI /LPI in patient serum. Table 46.1 reports today available methods to evaluate iron toxicity and iron load.

The mechanisms that impact on the outcome of the transplant, and clinical implications, are different in the different temporal phases of HCT—before, during, and after (Angelucci and Pilo 2016)—and will be here discussed separately:

- *Before transplant*: any time before the starting of the conditioning regimen.
- *During transplant*: from the start of conditioning regimen up to a sustained engraftment is achieved.
- *After transplant*: after sustained engraftment has been achieved.

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**Table 46.1** Currently available methods to evaluate risk of iron toxicity and quantify iron overload

Method	Advantages	Limitations
Transferrin saturation	Only available test that indirectly reflects toxic NTBI/LPI pool >70% definitely indicate NTBI/LPI presence in the serum	No quantitative correlation to iron pool
	Inexpensive and easy to use for repeated assessment	Limited reliability for poly-transfused patients
Serum ferritin	Inexpensive and easy to use for repeated assessment	Indirect estimate of iron burden
	Possible to identify trends	Nonlinear response to iron load at high levels
	Correlates with total body iron stores and clinical outcome	No decrease does not exclude chelation response
	Only method available for several centers	Strongly influenced by not iron condition (inflammation, infections liver disease)
Non-transferrin-bound iron (NTBI)/labile plasma iron (LPI)	Correspond to potentially toxic form of circulating iron	Not a routine test. Unvalidated. Possible only in specialized research laboratory
	Normalizing NTBI/LPI is a really important goal to prevent iron toxicity	Highly labile. Highly rebound after a chelator is cleared
		Complexity of interpreting in several circumstances like ineffective erythropoiesis, phase of transfusion cycle, and different rates of transfusions
Liver biopsy	Direct quantification of iron overload. Still gold standard for liver iron concentration	Invasive, risk of complications (very low in expert centers)
	Evaluation of liver histology	Poorly accepted by patients
	Still the only method in the absence of 1.5 Tesla MRI scanner	Risk of inadequate sample size (adequate $\geq 1$ mg dry weight)
	Inexpensive	Not useable for repeated follow-up
Magnetic resonance imaging—MRI (current standard of care)	Noninvasive and safe	Indirect liver iron concentration measurement
	Reciprocal of T2/T2* linearly related to liver iron concentration	Require calibration to convert measured T2/T2* into iron concentration
	Validated and standardized. Readable precision	Calibration is organ specific
	Widely used worldwide	Patients with ferromagnetic insert cannot undergo test
	Inter- and intra-scanner reproducibility	Trained expert personnel required for data acquisition and interpretation
	Possible multiorgan evaluation	Expensive and not always available

## 46.2 Iron Overload Before HCT (Before the Start of Conditioning)

In thalassemia it has been very well demonstrated that HCT outcome is significantly impacted by a story of irregular chelation, presence of liver fibrosis, and hepatomegaly (Angelucci 2010a). Now we can recognize that all the three risk factors are related not only to the accumulated iron “per se” but to the intensity and duration of tissue exposition to the abovementioned iron toxic-related species (Angelucci et al. 2017).

Iron-mediated toxicity causes tissue damage that accumulates over the years and adds to the transplant-related toxicity, thus resulting in a cumulative additive effect. In this situation organs and tissues are less resistant to transplant-related morbidity. For example, in thalassemia there is no difference in the incidence of graft versus host (GvHD) in the different risk classes, but there is a dramatic difference in survival of grade III–IV GvHD in higher risk patients (Gaziev et al. 1997). In a GITMO study (MDS and acute leukemias) transfusional burden impacted in patients who had received a myeloablative conditioning and

**Table 46.2** Available iron chelators. Indications and dosages depend on the registration and formulation which are different in different countries

Chelators	Pharmacologic characteristics	Administration route	Plasma half-life and daily schedule	Main adverse event
Deferoxamine	Hexadentate iron chelator. Molecular weight 560	Parenteral	30 min. Require continued intravenous or subcutaneous infusion	Infusion site reaction, allergic reaction, bone abnormalities, growth failure, auditory, ophthalmologic, Yersinia infection
Deferasirox	Tridentate iron chelator molecular weight 373	Oral	8–16 h. Once/day, twice/day schedule under investigation	Renal toxicity, increase liver enzymes, skin rash, gastrointestinal disturbances
Deferiprone	Bidentate iron chelator molecular weight 139	Oral	3 h. Three times/day	Gastrointestinal disturbances, increased liver enzymes, neutropenia, agranulocytosis

not in those who had received a reduced intensity conditioning (Alessandrino et al. 2010).

Therefore, any effort should be made to prevent tissue/organ damage by regularly suppressing NTBI/LPI in the years before transplant. This target can be achieved with early, regular, and consistent iron chelation. Thus, in any patient receiving transfusion therapy who may have an HCT in the future, the decision of starting chelation is critical and should be undertaken as soon as possible. Moreover, iron chelation must be taken regularly in the long term. Table 46.2 shows the iron chelators available on the market today. The indications depend on the registration which is different in different countries. Because of reported cases of agranulocytosis, deferiprone is usually not used in hemopoietic stem cell disorders.

Limited data are available on the rationale for intensive pre-HCT chelation therapy unless sufficient time is available to correct iron overload and warrant tissue lesion repair likely only in young patients.

### 46.3 Iron Overload During HCT (from the Start of Conditioning up to Sustained Engraftment)

During conditioning regimen, a huge amount of NTBI and LPI enter the circulation due to massive erythroid marrow lysis (Dürken et al. 1997). Moreover, until the erythroid recovery begins, no iron can be released by serum transferrin to the

erythroid system. Once erythroid recovery initiates, transferrin iron is greedily captured by the recovering erythroid system and unbound transferrin—a natural iron chelator—able to receive iron from the reticular endothelial system appears in the serum. NTBI and LPI disappear from the circulation by this natural mechanism in 3–4 weeks unless iron overload is present (Duca et al. 2018).

Transplant animal studies demonstrated that iron toxicity could impair the hematopoietic niche by damaging hematopoietic stem cells' self-renewal potential, proliferation, and differentiation and the marrow microenvironment (Pilo and Angelucci 2018). These data suggest that iron can impact the HSC engraftment, the hemopoietic recovery, and possibly transplant outcome.

Inclusion of chelation therapy during the transplant phase to suppress NTBI/LPI should be considered an experimental treatment; however, in case of slow, delayed, or incomplete marrow recovery and high transferrin saturation, iron chelation can be considered.

### 46.4 Iron Overload After HCT (After Sustained Engraftment Has Been Achieved)

After successful transplantation, patients are usually free from transfusion support but affected by the already acquired iron overload that cannot be eliminated without active intervention. In this condition the already acquired iron overload con-

tinues to disrupt the delicate LCI equilibrium and promotes ROS generation. It has been prospectively demonstrated in transplanted thalassemia patients that elevated transferrin saturation persists indefinitely without treatment (Angelucci et al. 1998) and liver disease progresses even in the absence of other comorbidities (Angelucci et al. 2002). Of course, the deleterious effect can be worsened by the presence of comorbidities even with a low level of iron accumulation (Angelucci et al. 2002).

Therefore, even because of the results of epidemiologic studies in thalassemia (Coates et al. 2016), in rare transfusion-dependent anemias (Puliyel et al. 2015), and in the normal population (Ellervik et al. 2011) in the posttransplant setting, the target iron level should be a normal iron level. Normal transferrin saturation excluding the presence of toxic iron-reactive species should be the target level of posttransplant iron removal (Table 46.1).

Because of the acquired effective erythropoiesis, phlebotomy (Angelucci et al. 1997a; Inati et al. 2017) can be an alternative to chelation. The standard chelation program consists of blood sampling of 6 mL/kg every 14 days (Angelucci et al. 1997a). Table 46.3 reports the pros and cons for selecting phlebotomy or iron chelation for post-HCT iron removal.

**Table 46.3** Factors to be considered in selecting the appropriate post-HCT iron removal strategy

	Phlebotomy	Chelation
Pros	<ul style="list-style-type: none"> <li>– Efficient</li> <li>– Safe</li> <li>– Inexpensive</li> <li>– Permits complete iron removal and normalizes iron body content</li> </ul>	<ul style="list-style-type: none"> <li>– Efficient</li> <li>– Safe</li> <li>– Immediate effect on NTBI/LPI</li> <li>– Hospital access not required</li> <li>– Cheap</li> </ul>
Cons	<ul style="list-style-type: none"> <li>– Requires sustained engraftment (not usable in the early post-HCT period)</li> <li>– Immediate effect on NTBI/LPI still remains to be verified</li> <li>– Hospital access required</li> </ul>	<ul style="list-style-type: none"> <li>– Warning of renal toxicity in the case of concomitant use of CSA</li> <li>– Possible increase in toxicity for low level of iron burden</li> </ul>

**Key Points**

- Iron toxicity depends on several factors in addition to iron overload. The most important is the duration of exposition to free iron species: NTBI and LPI inducing oxidative stress and tissue damage.
- Prevention of tissue damage by regularly and consistently suppressing tissue reactive iron species in the years before HCT is the key factor to improve transplant outcome.
- Iron toxicity can impair the bone marrow microenvironment, the quantity and quality of bone marrow mesenchymal stem cells, the ratio of immature HSC, and the clonogenic capacity of hemopoietic stem and progenitor cells, thus likely impacting hemopoietic recovery and possibly transplant outcome.
- After successful HCT, one should aim to achieve normal iron levels (i.e., normal transferrin saturation).

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# Secondary Neoplasia (Other Than PTLPS)

# 47

André Tichelli and Alicia Rovó

## 47.1 Definitions

Secondary neoplasia (SN) after HCT includes any malignant disorder occurring after HCT, irrespectively, if related or not to transplantation. For an individual patient, a clear relationship between HCT and SN often cannot be

demonstrated. Posttransplant lymphoproliferative disorders are discussed elsewhere (see Chap. 45).

## 47.2 Types of Secondary Neoplasia After HCT

	Therapy-related myeloid neoplasms (t-MN) (Pedersen-Bjergaard et al. 2000)	Donor-derived malignancy (DDM) (Sala-Torra et al. 2006; Wiseman 2011; Engel et al. 2019)	Second solid neoplasms (SSN) (Kolb et al. 1999; Rizzo et al. 2009)
Definition	t-MDS or t-AML after exposition chemo- or radiation therapy	Hematologic neoplasms occurring in grafted donor cells	Solid cancers of any site and histology occurring after HCT
Occurrence	Mainly after auto-HCT Not excluded after allo-HCT (Yamasaki et al. 2017)	After allo-HCT only	After allo-HCT and auto-HCT
Appearance	Within the first 10 years mainly	Variable	Increasing incidental rate with longer follow-up
Prognosis	Poor	Poor	Depends mainly on the cancer type

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## 47.3 Pathophysiology

### 47.3.1 Therapy-Related Myeloid Neoplasms

t-MN are mainly associated with cytotoxic chemotherapy and radiation therapy that the patient has received either before HCT or as conditioning. The causal role of ionizing radiation in the development of myeloid neoplasms has been demonstrated in atomic bomb survivors of Hiroshima/Nagasaki and in medical radiation workers employed before 1950.

Responsible cytotoxic drugs:

- Alkylating agents, anthracyclines, and topoisomerase II inhibitors.
- To a lesser extent antimetabolites and purine analogs.
- Controversy exists on the role of azathioprine, methotrexate, hydroxyurea, and 6-mercaptopurines used for the treatment of malignant and nonmalignant diseases.

t-MN occur mainly after auto-HCT, where the healthy HCT has been exposed to cytotoxic effect (Diamond et al. 2023). Detecting clonal hematopoiesis mutations in cryopreserved cells before auto-HCT has been associated with a higher risk of t-MN (Gramegna et al. 2022). Rarely t-MN can be observed after allo-HCT. Persistent microchimerism with exposed residual recipient cells or autologous recovery may explain the development of t-MN after allo-HCT (Hoshino et al. 2022). After HCT for sickle cell disease, the 10-year incidence of leukemia and MDS is increased, mainly when conditioned with reduce-intensity and TBI-containing regimen. The onset of t-MN and graft failure after HCT are closely related (Eapen et al. 2023).

Today, increasingly cytotoxic drugs are applied after the allo-HCT, either as GVHD prophylaxis (posttransplant CY) or to prevent disease recurrence (posttransplant maintenance). Posttransplant CY does not seem to increase the risk of t-MN (Majzner et al. 2017; TCT). We do not yet know whether posttransplant maintenance therapy could increase the risk of t-MN after allo-HCT.

A germline cancer predisposition has been demonstrated in 15–20% of t-MNs, and acquired mutagenic effect of cytotoxic therapy with clonal hematopoiesis of indetermined potential (CHIP) is frequently the first step in the multihit development of t-MNs (Voso et al. 2021).

### 47.3.2 Donor-Derived Malignancy (DDM)

The pathogenesis of donor-derived hematological malignancies is not fully understood but is likely multifactorial (Sala-Torra et al. 2006; Wiseman 2011; Williams et al. 2021; Gibson et al. 2022):

- Treatment damage to bone marrow microenvironment from previous chemotherapy, radiation, and treatments to prevent GVHD
- Transplantation of a malignant clone, or germline or somatic mutations from the donor
- Stress of rapid clonal expansion after transplant

Reported donor cell leukemia are AML, MDS, ALL, CML, and lymphoid neoplasms including CLL (Engel et al. 2019). The risk of DDM in allogeneic hematopoietic cell transplantation is driven by somatic myelodysplastic syndrome-associated mutations or germline predisposition in donors (Gibson et al. 2022).

Clonal hematopoiesis can be transmitted from a donor to a recipient during allo-HCT. Exclusion of candidate donors with clonal hematopoiesis is controversial since its impact on recipient outcomes and graft alloimmune function is uncertain.

Over 20% of donor-derived myeloid neoplasms carry chromosome 7 abnormalities. Gene sequencing of the donor cells allowed to detect a number of candidate genes that could contribute to the development of DDM (Williams et al. 2021). Malignant clones transferred to the recipient can be of lymphoid origin, observed in older donors, and may evolve into a lymphoid neoplasm in the immunosuppressed host.



### 47.3.3 Second Solid Neoplasms (SSN)

Little is known about pathogenesis of SSN after HCT. An interaction between cytotoxic treatment, genetic predisposition, environmental factors, viral infections, GVHD, and its immunosuppression may play a role.

Three main types of SSN (Rizzo et al. 2009):

- Radiation-related SSN
  - Proven for thyroid, breast, and brain cancers
  - Occur after a long latency ( $\geq 10$  years after radiation)
  - Is dose related
- GVHD/immunosuppression-related SSN
  - Squamous cell carcinoma of the skin and oropharyngeal area
  - Short latency
  - Can occur at different localizations
- Association with viral infection

- HCV infection associated with hepatocellular cancer
- HPV-related precancer or second cancer, with increased cumulative incidence for cervical, head and neck, vulvar, vaginal, anal, and penile second cancer (Zhao et al. 2021)

## 47.4 Frequency and Risk Factors (See Table 47.1)

### 47.4.1 Remarks on SSN

The CI of second solid cancer is 2.2% at 10 years and 6.7% at 15 years (Rizzo et al. 2009).

Increased risk for SSN after HCT has been demonstrated from breast, thyroid, skin, liver, lung, oral cavity and pharynx, brain and CNS, bone and connective tissue cancers and malignant melanoma.

**Table 47.1** Frequency and risk factors

Type of SN	Frequency	Risk factors
t-MN	Great variability on the CI of t-MN after auto-HCT <ul style="list-style-type: none"> <li>• In lymphoma patients between 1% at 2 years up to 24% at 43 months</li> <li>• Lower CI for patients treated for breast cancer, germ cell tumor, and multiple myeloma</li> <li>• Rare n-MN after HCT for AID</li> </ul> CI depends mainly on pretransplant cytotoxic and radiation therapy and the use of TBI CI of t-MN after allo-HCT: 0.06–0.67% at 3 years (Yamasaki et al. 2017)	<ul style="list-style-type: none"> <li>• Quantity of pretransplant chemotherapy (see pathogenesis) and local radiotherapy</li> <li>• Conditioning with TBI</li> <li>• Older age at HCT</li> </ul> t-MN are mainly observed after HCT for lymphoma (NHL, HL), probably because lymphoma patients often receive more cytotoxic therapy before HCT The use of lenalidomide (also post-transplant) for multiple myeloma is associated with an increased risk of t-MN among auto-HCT recipients (Rosenberg et al. 2021; Inamoto et al. 2015)
DDM	Rare complication, with a CI <1% at 15 years Possibly underestimated (difficulty to prove donor type of malignant cells) Could represent up to 5% of posttransplant leukemia “relapses”	No clear risk factor defined (too few, heterogeneous DCL) Possible risk factors (Engel et al. 2019) <ul style="list-style-type: none"> <li>• Malignant donor clone in the transplant</li> <li>• G-CSF therapy</li> <li>• In vivo T cell depletion</li> <li>• Multiple transplantations</li> </ul>
SSN		
Breast, thyroid, bone, melanoma, connective tissue, brain, BCC	Breast cancer: 11% at 25 years (Friedman et al. 2008) Thyroid cancer: SIR 3.2 compared to general population (Cohen et al. 2007) BCC: 6.5% at 20 years (Leisenring et al. 2006)	Radiation before HCT or TBI Younger age at radiation Longer follow-up Light-skinned patients (BCC) T-cell depletion (melanoma)

(continued)

**Table 47.1** (continued)

Type of SN	Frequency	Risk factors
SSC of skin, oral cavity, and esophagus	SCC of the skin: 3.4 at 20 years (Curtis et al. 2005) Increased risk of atypical melanocytic proliferations and nonmelanoma skin cancer in pediatric HCT recipients (Song et al. 2017)	Chronic GVHD Prolonged GvHD therapy IS including azathioprine Male sex Unrelated with radiation At any time after HCT
Hepatocellular carcinoma	Patients with HCV infection: CI 16% at 20 years (de Latour et al. 2004)	Chronic HCV infection Cirrhosis
Lung cancer	SIR 2.59 after BuCy (Majhail et al. 2011)	Conditioning with Bu-Cy Smoking prior to HCT
Genital and anal cancer	Cervical SIR 2.13; anal SIR 4.85; vulvar and vaginal SIR 22.76; penile SIR 22.76 compared to general population (Zhao et al. 2021)	HPV reactivation (in female and male)

*BCC* basal cell carcinoma of the skin, *SSC* squamous cell carcinoma, *CI* cumulative incidence, *AID* autoimmune disorders, *SIR* standardized incidence ratio

An individual patient can present several subsequent different SSN after HCT. Up to five different solid cancers have been observed in a patient treated with allo-HCT.

Digestive system and particularly colorectal cancers have not been proven to be increased after HCT (Heydari et al. 2020). In non-transplanted cancer patients, second colorectal cancers are increased when treated with abdominal radiation (Henderson et al. 2012; Rapiti et al. 2008; van Eggermond et al. 2017).

So far there are few long-term data on SSN after RIC. A single-center study shows an increased rate of SSC compared to MAC during the first 10 years post-HCT (Shimoni et al. 2013). The 10-year cumulative incidence of SSC following RIC reached 12.9%, with an overall SIR of 1.03–1.9, mainly significant for head and neck and bladder cancers. Second malignancy seems to be higher than expected in treated allo-HCT recipients conditioned with RIC but still longer follow-up time with larger cohorts is needed for definitive assessment (Del Galy et al. 2022).

The outcome of SSC is mainly dependent on the type of second cancer. Standardized mortality ratio was higher, compared with de novo solid cancers, for melanoma, prostate, breast, kidney, bladder, colorectal, and endometrial cancers but not for the other cancers (Tichelli et al. 2019).

Most subsequent solid cancers occurred at younger ages than primary cancers, emphasizing the need for cancer screening at younger ages (Inamoto et al. 2018).

## 47.5 Screening (Majhail et al. 2012; Inamoto et al. 2015) (See Also Chap. 21)

### 47.5.1 Therapy-Related Myeloid Neoplasms

Annual monitoring of full peripheral blood counts during the first 10 years after auto-HCT (most t-MN occur within 10 years after HCT).

If unexpected abnormalities in the blood count are observed (increased MCV, cytopenia, dysplasia in peripheral blood, thrombocytosis, leukocytosis, monocytosis), extended analysis of blood and bone marrow is warranted, including cytogenetics and NGS (Bachiashvili et al. 2022).

### 47.5.2 Donor-Derived Malignancy

Chimerism monitoring of the malignant cells is needed in case of “relapse” or new hematological malignancy after allo-HCT.

Whether to search for clonal hematopoiesis, lymphoid malignant clone, and germline muta-

tions in the donor in case of DDM remains controversial.

- During annual control, clinical screening and reviewing for possible symptoms of SSN.
- Follow country-specific or international guidelines for cancer screening recommendations for the general population.
- Be informed and counselled about the risk of SSN.

**47.5.3 Second Solid Cancer (Socie and Rizzo 2012; Inamoto et al. 2015)**

Lifelong screening for SSN is recommended after auto-HCT and allo-HCT.

General recommendations are as follows:

Specific recommendations are included in Table 47.2.

**Table 47.2** Screening for second solid cancer after HCT

Skin	<p><i>All patients</i> Encouraged to:</p> <ul style="list-style-type: none"> <li>• Perform regularly genital/testicular and skin self-examination</li> <li>• Avoid unprotected UV skin exposure</li> </ul> <p>Skin examination by dermatologist every 1–2 years <i>Patients at risk</i> More frequent examination by dermatologist</p> <ul style="list-style-type: none"> <li>• After first skin cancer</li> <li>• Patients with chronic skin GvHD</li> </ul>
Oral cavity and pharynx	<p><i>All patients</i> Examination during annual control <i>Patients at risk</i> Annual control by specialist if severe oral and pharynx GvHD Histology in case of suspicious lesion</p>
Thyroid	<p><i>All patients</i> Annual thyroid palpation to identify suspicious thyroid nodules <i>Patients at risk</i> (after TBI or local radiation) Regular thyroid ultrasound Fine-needle aspiration in case of a suspicious nodule</p>
Breast	<p><i>All patients</i> Discuss breast self-examination with their physician <i>Patients at risk</i> (after TBI or local radiation) Screening mammography every 1–2 years starts at the age of 25 years or 8 years after radiation, whichever occurs later, but not later than age of 40 years</p>
Vagina, Cervix	<p><i>All patients</i> Screening with pap smears every 1–3 years in women older than 21 years old or within 3 years of initial sexual activity, whichever occurs earlier <i>Patients at risk (HPV positive)</i> Regular control by specialist of genital organ and anal region</p>
Lung	<p><i>All patients</i> Encouraged to avoid smoking and passive tobacco exposure <i>Patients at risk</i> Patients at risk (high-dose busulfan conditioning and smoking), chest CT</p>
Liver	<p><i>Patients at risk</i> Patients with known HCV infection should be assessed for fibrosis/cirrhosis of the liver 8–10 years after HCT (biopsy; fibroscan)</p>
Colorectal	<p><i>All patients</i> Screening should start at age 50 years in the absence of a family history (first-degree relative diagnosed with colorectal cancer before age 60 years): annual fecal occult blood testing, sigmoidoscopy every 5 years, with fecal occult testing every 3 years, or colonoscopy every 10 years</p>
Prostate	<p><i>All patients</i> No specific recommendations</p>

## 47.6 Treatment

Neoplasm	Treatment
t-MN	Same treatment than de novo myeloid neoplasms Early donor search and rapid allo-HCT (Finke et al. 2016; Kroger et al. 2011; Metafuni et al. 2018) Decision-making including consideration of cumulative toxicity due to previous HCT, age, and comorbidity
DDM	No standard treatment Treatment depends on the nature of disease Reported treatments (Engel et al. 2019) <ul style="list-style-type: none"> <li>• Retransplantation</li> <li>• Conventional chemotherapy</li> <li>• DLI</li> <li>• Palliation</li> </ul>
SSN	Should be treated as de novo cancers of the same type

## 47.7 Outcome

Neoplasm	Outcome
t-MN	Generally very poor Median survival of 6 m Identical outcome than t-MN in general
DDM	Few data available In most cases, mortality high and OS poor In a small series of 47 DDM, median survival 32.8% months Death mainly due to progression or relapse of DDM
SSN	Mainly dependent on the type of SSN (Ehrhardt et al. 2016; Tichelli et al. 2019; Inamoto et al. 2018) Favorable outcome <ul style="list-style-type: none"> <li>• Thyroid, breast, prostate, melanoma, cervix</li> </ul> Intermediate outcome <ul style="list-style-type: none"> <li>• Oropharyngeal, colorectal, bladder, renal, ovarian, endometrial</li> </ul> Poor outcome <ul style="list-style-type: none"> <li>• Pancreas, lung, brain, hepatobiliary, esophageal</li> </ul>

### Key Points

- Three types of secondary neoplasia may occur after HCT: therapy-related myeloid neoplasms (t-MN), mainly after auto-HCT; donor-derived malignancy (DDM) after allo-HCT; and second solid neoplasia (SSN) after auto-HCT and allo-HCT.
- Pretreatment or conditioning with radiation and/or chemotherapy including alkylating agents, anthracyclines, and topoisomerase II inhibitors is mainly responsible for t-MN.
- DDM are extremely rare and are either transmitted from the donor or newly transformed in the host.
- Non-squamous second solid cancers (breast, thyroid, brain, etc.) are strongly related to local radiation or TBI and occur with long delay after HCT. Squamous cell carcinoma of the skin, the oral cavity, and the pharynx is related with chronic GVHD and can occur early after HCT. Conditioning with RIC does not seem to reduce the risk of second cancer.
- Outcome of t-MN is poor, and allogeneic HCT represents the only curative treatment.
- Outcome of SSN depends mainly on the type of second cancer; second solid cancer should be treated as a de novo cancer of the same type.

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## **Part VI**

### **Specific Organ Complications**

**Topic Leaders: Raffaella Greco  
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## 48.1 Ocular Complications

### 48.1.1 Introduction

Ocular complications in HCT patients include both ocular GVHD- and non-GVHD-related late effects which, if left untreated, can lead to ocular discomfort and blindness. Non-GVHD-associated complications include cataracts, glaucoma, infections particularly (viral retinitis, fungal endophthalmitis), and posterior segment abnormalities. Ocular GVHD (oGVHD) is a rapidly progressing highly inflammatory condition that mainly affects the ocular surface and adnexae, but also the posterior segment in rare cases.

The World Health Organization considers blindness as one of the top causes of disability-adjusted life years, and it significantly affects the QoL. Thus, prevention and prompt management

of ocular complications should be a priority of a transplant program given the morbidity associated with it.

### 48.1.2 Ocular GVHD

Ocular GVHD occurs as acute, chronic, and overlapping forms with chronic oGVHD representing the most common form with an onset between 5 and 24 months after allo-HCT (Kitko et al. 2021; Wolff et al. 2021).

The occurrence of oGVHD is variable in different series but is approximately 40–60% of patients receiving HCT (Nassar et al. 2013). The risk factors for oGVHD include donor-recipient HLA and gender disparity (female donor to male recipient), an older donor age, preexisting diabetes, and environmental stress (Gehlsen et al. 2022a).

The 2020 NIH consensus development project on cGVHD describes distinct differences of oGVHD from the dry-eye disease such as rapid manifestation, activation of donor hematopoietic/mesenchymal stem cells, early fibrosis, and potential intraocular involvement, although clinical phenotypes may be initially similar. The NIH cGVHD consortium published diagnostic best practice components that included eye care exams within a month prior to hematopoietic cell transplantation or within 3 months afterward and at regular intervals (e.g., every 3 months) through an ophthalmologist. These exams should include

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surveillance for infection, cataract formation, and increased intraocular pressure for prevention of ocular complications in GVHD.

The principles of oGVHD *management* include lubrication, drainage control, evaporation control, minimization of ocular surface inflammation, and improving epithelial wound healing. Based on the 2014 NIH chronic GVHD consensus panel, current recommendations for stepwise treatment of oGVHD include preservative-free artificial tears or ointments as a basic therapy, together with early application of topical cyclosporine and/or steroid eye drops for mild/moderate oGVHD. For severe forms, dosages are increased and serum eye drops and contact lenses are added (Carpenter et al. 2015). Among surgical procedures for moderate/severe oGVHD, amniotic membrane transplantation, punctal occlusion, and partial tarsorrhaphy have been recommended. There is also some evidence on other modalities of treatment which include occlusive eye wear, lid care/warm compresses, and humidified environment. Corneal melting, superinfection, and perforation should be avoided at all costs as corneal transplantations frequently fail due to graft rejection and disturbed wound healing.

In addition, special attention should be given to other risk factors for ocular complications.

### 48.1.3 Posterior Segment Complications

Retinal and vitreous hemorrhages are not uncommon in HCT patients and may happen with or without the presence of oGVHD (Yoo et al. 2017). This is complicated by the presence of thrombocytopenia early in transplant but also later in the course since both drugs and chronic GVHD can be associated with thrombocytopenia. Prompt referral to an ophthalmologist is the key to preventing blindness; therefore, the practicing transplant clinician should have a high sus-

picion of retinopathy, retinal tears, or vitreous hemorrhages when a patient complains of “floaters” or just “decreased vision,” which happens suddenly.

### 48.1.4 Ocular Infections

CMV infection is one the most widely studied ocular infections and can rapidly lead to retinitis, and since quite often IV or intraocular drug applications are required, prompt referral to an ophthalmologist is mandatory. Apart from CMV, adenovirus is also a common virus and can lead to viremia if untreated. Moreover, unlike immunocompetent individuals, varicella zoster infection within hours or a couple of days can lead to dissemination as well as postherpetic neuralgia, cranial nerve palsies, zoster paresis, meningoencephalitis, cerebellitis, myelopathy, and irreversible blindness.

Fungal infections in severely IS HCT patients (particularly those on multiple IS for GVHD) can quickly lead to mortality; thus, prompt referral for IV antifungals is indicated. Aspergillosis, mucormycosis, and candida have been reported in GVHD patients affecting the ocular tissues.

### 48.1.5 Glaucoma

Since the most common subtype of glaucoma (primary open-angle glaucoma) presents with gradual symptoms, its diagnosis is frequently missed in early phases. However, many risk factors in HCT can predispose to glaucoma and can lead to blindness which includes diabetes (allo-HCT patients have a four times higher risk of diabetes), retinopathy, and steroid use (for GVHD). Since the diagnosis of glaucoma is based on tonometry, gonioscopy, perimetry, and ophthalmoscopy, regular screening by the ophthalmologist is indicated.

### 48.1.6 Cataract

Cataract is the most common cause of blindness in the developed world. Risk factors in the HCT patients include steroid use, total body irradiation, and diabetes. Since intraocular lens implantation (particularly via phacoemulsification) has become a widely performed procedure worldwide for the treatment of cataracts, early recognition and prompt treatment can help in the preservation of vision. Although generally associated with a low rate of complications in oGVHD, complication rate can be elevated (Gehlsen et al. 2022b). Therefore, special attention should be applied perioperatively and prevention of cataracts by controlling risk factors should be a management strategy in HCT survivors if possible (Tables 48.1 and 48.2).

#### Key Points

- Ocular complications of HCT are not restricted to GVHD, since both non-GVHD allo-HCT and auto-HCT recipients can suffer from cataracts, viral/fungal infections, glaucoma, and retinopathies.
- Early detection and aggressive therapy by ophthalmologists familiar with ocular GVHD are paramount due to ensuing blindness.
- Transplant centers should consider a close collaboration with ophthalmology teams both for treatment and preventative strategies. This could ideally be achieved in a multidisciplinary team in a long-term follow-up or a survivorship clinic.

**Table 48.1** Ocular complications of HCT

	Risk factors/manifestations	Management	Survivorship issues
GVHD-associated	<ul style="list-style-type: none"> <li>– Acute and chronic ocular GVHD with dry-eye phenotype</li> <li>– Retinopathies</li> <li>– Cataracts</li> <li>– Infections</li> </ul>	<ul style="list-style-type: none"> <li>– Topical: artificial tears, steroids, CSA, steroids, scleral lenses, serum eye drops, surgical procedures</li> <li>– Systemic: ECP, rituximab, ibrutinib</li> </ul>	Presence of ophthalmologist preferentially pre-allo-HCT and in the long-term follow-up or survivorship clinic
Non-GVHD-associated	<ul style="list-style-type: none"> <li>– Glaucoma</li> <li>– Cataract</li> <li>– Infection</li> <li>– Retinal/vitreous hemorrhage</li> </ul>	<ul style="list-style-type: none"> <li>– Removal of risk factors</li> <li>– Surgery</li> <li>– Antimicrobials</li> </ul>	

**Table 48.2** Oral complications of HCT

	Risk factors/manifestations	Management	Survivorship issues
GVHD-associated	Risk factors; poor oral hygiene Manifestations: <ul style="list-style-type: none"> <li>– Lichen planus-like patterning</li> <li>– Hyperkeratosis</li> <li>– Dry mouth</li> <li>– Oral ulcers</li> <li>– Oral sensitivity</li> <li>– Mucocele</li> <li>– Mucosal erythema or atrophy</li> </ul> Long-term risks: oral squamous cell carcinoma, salivary gland dysfunction	<ul style="list-style-type: none"> <li>– Topical: steroids of progressive potency, tacrolimus for lip lesions</li> <li>– Systemic: steroids, ruxolitinib, belumosidil, extracorporeal photopheresis,</li> <li>– Physical therapy for reduced oral opening</li> <li>– Dry mouth: palliative aids, topical fluoride</li> <li>– Regular dental care</li> </ul>	<ul style="list-style-type: none"> <li>– Regular dental care</li> <li>– Regular oral cancer screening</li> <li>– Participation of a dentist with HCT expertise in the long-term follow-up or survivorship clinic</li> </ul>
Non-GVHD-associated	<ul style="list-style-type: none"> <li>– Dysgeusia, temporomandibular joint disorders, mucositis, dental caries</li> </ul>		

## 48.2 Oral Complications

### 48.2.1 Introduction

Oral complications in HCT patients include both oral GVHD and non-GVHD-related late effects which can adversely affect patient nutrition, quality of life (QoL), and health.

### 48.2.2 Oral GVHD

Three domains of the craniofacial complex are impacted by oral cGVHD: oral mucosa, salivary glands, and oral opening. Symptoms often begin with new-onset dry mouth and sensitivity to spice and mint flavors. At the onset, oral cGVHD may appear mucositis-like, but mature lesions can progress to tissue fibrosis and restrict oral opening and oral function and lead to permanent salivary gland dysfunction.

A diagnosis of oral cGVHD can be made clinically or with the aid of an oral mucosal biopsy. Diagnostic clinical features of oral cGVHD include the presence of lichen planus-like features which resemble white lacelike hyperkeratosis and may occur on any oral mucosal surface (Jagasia et al. 2015). Distinctive features include erythema, ulceration, oral dryness, mucocelles, mucosal atrophy, and pseudomembranes. In the case of distinctive features only, other potential causes of these lesions should be diagnostically excluded per the 2014 National Institutes of Health chronic GVHD consensus criteria (Jagasia et al. 2015). Other manifestations of oral cGVHD include low saliva production, gingivitis, oral sensitivity, oral pain, and mucosal fragility. These can lead to dental caries and chronic ulcers. Sclerosis of the perioral skin, fibrosis within the cheek tissues, and GVHD manifestations in the temporomandibular joints can reduce oral opening which impairs oral hygiene and nutrition and may impede oral access for necessary medical and dental procedures. Finally, avascular necrosis (AVN) of the temporomandibular joint can occur as a complication of steroid therapy for GVHD (Treister et al. 2012).

The 2020 National Institutes of Health chronic GVHD consensus criteria recommend oral exam

prior to transplant and at day +100 that includes assessment of linea alba, lichen planus-like changes, and mucosa abnormalities (Kitko et al. 2021). Regular oral exams starting at day +100 posttransplant should include evaluation for any lichen-planus like changes, ulcers, erythema, and restriction of mouth opening. This exam should include an inquiry about patient-reported pain, difficulty swallowing, or oral dryness. Identification of new lesions suggestive of oral GVHD as detailed above should be followed with a referral to a dentist with experience in dealing with long-term HCT complications, often an oral medicine specialist.

The *management of oral GVHD* may require both topical treatments and systemic therapy. Topical treatments may include topical steroids of increasing potency as needed and calcineurin inhibitors on the vermilion lip where steroids are contraindicated. Emerging topical therapies include photobiomodulation and autologous blood products (Baumerin et al. 2022). Adjunctive medications for pain and symptom management are recommended to improve oral intake and QoL. Physical therapy may help to restore and maintain oral function. Among nonsteroidal systemic treatments for oral GVHD, good oral GVHD response rates have been reported for ruxolitinib, belumosudil, and extracorporeal photopheresis (Kaurinovic et al. 2022; Jagasia et al. 2021; Malik et al. 2014).

### 48.2.3 Subsequent Oral Cancers

Long-term survivors of HCT are at risk of subsequent cancer development. Oral squamous cell carcinoma is the most frequent subsequent cancer of the oral cavity (Inamoto et al. 2015). Risk factors include transplant for nonmalignant disease and GVHD with the highest increased risk in patients with a history moderate–severe oral GVHD and in those with >15-month duration of oral GVHD (Santarone et al. 2021; Schaar et al. 2021). Accordingly, aggressive and early treatment of oral GVHD is essential to reduce risk. Oral exam including an oral cancer screening is recommended at least every 6 months while HCT patients remain on immunosuppression

(Carpenter et al. 2015). Treatment of these lesions often includes surgical resection and adjuvant therapy in cases with high-risk pathologic features (Hanna et al. 2018). Lifestyle factors that contribute to oral cancer risk include radiation exposure, tobacco use, alcohol intake, and betel nut chewing and should be avoided in HCT survivors.

#### 48.2.4 Non-GVHD-Associated Oral Complications

HCT patients with reduced immunity have increased susceptibility to oral infections. New lesions in the oral cavity should be screened for viruses including herpes simplex virus, fungal involvement including *Candida albicans* overgrowth, and drug reactions. Patients on systemic mTOR inhibitors may develop mTOR inhibitor stomatitis when blood levels of drug are elevated.

Salivary gland dysfunction may occur independent of GVHD following HCT and contributes to the rapid progression of dental decay and reduced QoL. Patients with myeloablative conditioning regimens frequently present with salivary gland dysfunction in the posttransplant period. Loss of saliva production may result from a history of total body irradiation or targeted irradiation due to progressive irreversible fibrosis of the acinar units and ducts within the salivary glands. Similarly, temporary reduction in saliva production may result from the use of specific medication classes including antidepressants, immune checkpoint inhibitors, antihistamines, opioids, and others. Dry mouth, or xerostomia, may be reported by patients if the quality or mucous content of saliva decreases even if the rate of its production remains steady.

Dysguesia following HCT is not well understood. Taste disturbances in both the flavor and texture experience are associated with specific chemotherapy regimens, mucosal damage, and alteration in the oral microbiome as well as with GVHD onset and generally result in lower caloric intake and reduced QoL in patients (Scordo et al. 2022). Dysguesia in most patients resolves with time.

#### 48.2.5 Dental Requirements of the HCT Patient

Dentists with expertise in oral medicine should be part of the multidisciplinary team managing late effects in both autologous and allogeneic HCT survivors as endorsed by the National Institutes of Health's Late Effects Initiative (Hashmi et al. 2017). Once the primary indication for HCT is cured, it is essential that surveillance and preventative strategies be undertaken to alleviate the burden of comorbidities in these survivors. Oral hygiene and health are important for these patients as mucosal inflammation from gingivitis and periodontitis increases susceptibility to infection, with a less clear relationship with GVHD risk (Williams et al. 2021). Regular oral cancer screening is required, and intensive management may be needed for GVHD and non-GVHD oral complications after HCT. Thus, a long-term follow-up clinic should optimally have dentistry services available ad hoc if not on a routine surveillance basis.

##### Key Points

- Oral cGVHD typically presents first as new-onset dry mouth and oral sensitivity and may resemble mucositis. Mature lesions can develop tissue and salivary gland fibrosis.
- Patients with a history of oral cGVHD have an increased risk of subsequent oral cancer. Thus, early and aggressive treatment is recommended along with regular screening.
- Non-GVHD complications should be vigilantly managed including dental caries, xerostomia, gingivitis, dysgeusia, drug reactions, and oral infections.
- Dentists with expertise in oral medicine should be part of multidisciplinary teams of a long-term HCT follow-up/survivorship clinic.



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# Hepatic Complications

# 49

Enric Carreras, Tapani Ruutu, Mohamad Mohty, Selim Corbacioglu, and Rafael de la Cámara

## 49.1 Introduction

The frequency and severity of hepatic complications during hematopoietic cell transplantation (HCT) markedly decreased in the last decade, thanks to improvements in preventive and therapeutic measures for most frequent complications, as fungal infections, hepatotropic viruses, sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD), hepatic graft-versus-host disease (GVHD), and improved management of hepatotoxic drugs and iron overload (Hockenbery et al. 2016).

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## 49.1.1 Classification

The origin of hepatic HCT complications can be:

Multifactorial	<b>Sinusoidal obstruction syndrome (SOS/VOD)</b>
Infectious	Bacteria (see Chap. 36), fungi (see Chap. 37), or viruses (CMV, VVZ, and ADV) (see Chap. 38)
Infectious/immune	<b>Due to hepatotropic viruses (HBV, HCV, and HEV)</b>
Immune	Acute or chronic GVHD (see Chaps. 43 and 44) or <b>autoimmune hepatitis</b>
Toxic	Due to <b>hepatotoxic agents</b> used in HCT
Degenerative	<b>Cirrhosis and hepatocellular carcinoma</b>
Iron overload	Hemosiderosis (see Chap. 46)
Ischemic	<b>Ischemic hepatitis</b>
Not well established	<b>Cholangitis lenta, nodular regenerative hyperplasia, focal nodular hyperplasia, and idiopathic hyperammonemia</b>

In **bold** pathologies analyzed in this chapter

## 49.2 Veno-Occlusive Disease in Adults

Mohamad Mohty

### 49.2.1 Introduction

Sinusoidal obstruction syndrome (SOS), also known as veno-occlusive disease (VOD, referred

to as SOS/VOD hereafter) is a life-threatening complication occurring after HCT (Mohty et al. 2015). Clinical manifestation includes hepatomegaly, hepatalgia, fluid retention with ascites, weight gain, transfusion refractory thrombocytopenia (RT), and jaundice. SOS/VOD usually resolves progressively within a few weeks; nevertheless, in patients with a severe form, the mortality rate is very high (>80%) (Coppell et al. 2010; Richardson et al. 2016). The overall incidence of SOS/VOD in adults can be estimated at around 5–15%, but it varies considerably depending on the presence of risk factors and the conditioning regimen intensity (Mohty et al. 2015; Coppell et al. 2010; Carreras et al. 1998, 2011).

### 49.2.2 Pathophysiology

Pathophysiology of SOS/VOD is not well known. Conditioning regimens generate toxic metabolites that damage the hepatocytes and activate sinusoidal endothelial cells mainly in zone 3 of the hepatic acinus (Carreras and Diaz-Ricart 2011; Mohty et al. 2016). Activated sinusoidal endothelial cells swell up, leading to the formation of gaps in the sinusoidal barrier. Formed elements of the blood (red blood cells and leukocytes) as well as cellular debris can then pass through these gaps between endothelial cells into the space of Disse and dissect the endothelial lining. This results in a progressive narrowing of the venous lumen, a reduced sinusoidal venous outflow, and ultimately postsinusoidal portal hypertension (Mohty et al. 2015).

Additional information on biomarkers and EASIX index in SOS/VOD could be seen in Sect. 42.1 of the Handbook. EASIX on day 0 seems to be a promising biomarker to identify populations at high risk of SOS/VOD, and studies analyzing correlation with established SOS/VOD risk factors and severity would be important in order to establish how EASIX-d0 can be implemented in routine practice for SOS/VOD diagnosis, severity grading, and treatment initiation (Jiang et al. 2021).

### 49.2.3 Risk Factors

The accurate definition of SOS/VOD risk factors is indispensable, particularly since they are taken into account in the severity grading. Thus, it is important to classify risk factors as modifiable or unmodifiable to provide some guidance on reducing risk factors and improving patients' management (Table 49.1).

**Table 49.1** Unmodifiable and modifiable SOS/VOD risk factors (adults)

<b>Unmodifiable risk factors (in bold the factors with the highest relative risk)</b>
Second HCT
Advanced disease (beyond second CR or relapse)
Primary immunodeficiency diagnosis
Genetic factors (GSTM1 polymorphism, C282Y allele, and <i>MTHFR</i> 677CC/1298CC haplotype)
<b>Older patient age</b>
Increased serum transaminase
Karnofsky score below 90%
Metabolic syndrome
<b>Female receiving norethisterone</b>
<b>Previous use of gemtuzumab ozogamicin or inotuzumab ozogamicin</b>
Hepatotoxic drugs
Iron overload (>1.000 ng/mL)
<b>Serum bilirubin &gt; 1.5 mg/L (&gt;26 μmol/L),</b>
Transaminase >2.5 ULN
<b>Preexisting liver disease:</b> hepatic fibrosis, cirrhosis, and active viral hepatitis
Abdominal or hepatic irradiation
<b>Modifiable risk factors and recommendable preventive measures</b>
Conditioning:
High-dose (myeloablative) regimens
Oral or high-dose busulfan
High-dose treosulfan
High-dose TBI-based regimen
Donor:
Unrelated donor
HLA-mismatched donor
GVHD prophylaxis:
Sirolimus + methotrexate + tacrolimus
Methotrexate + cyclosporin or tacrolimus
Non T-cell-depleted transplant
Nutrition:
Use of parenteral nutrition

*HCT* hematopoietic cell transplantation, *CR* complete remission, *HLA* human leukocyte antigen, *TBI* total body irradiation, *ULN* upper limit of normal, *GVHD* graft-versus-host disease

### 49.2.4 Diagnosis Criteria

For a very long time, two definitions of SOS/VOD have coexisted, based on the Seattle criteria, reported by McDonald et al. in 1984, and the Baltimore criteria, reported by Jones et al. in 1987. While these definitions were used in clinical practice and research studies, they were not suitable criteria for early diagnosis, and they missed late-onset SOS/VOD. Therefore, in 2016, the European Group for Blood and Marrow Transplantation (EBMT) revised criteria for SOS/VOD were published. Since hyperbilirubinemia and jaundice are almost invariably present in classic SOS/VOD in adult patients, more recently, it was decided to keep the classical original Baltimore criteria for diagnosis of classical SOS/VOD (within 21 days after HCT) in the revised EBMT criteria (Mohty et al. 2016). Indeed, contrary to the Seattle criteria, bilirubin  $\geq 2$  mg/dL is mandatory in the Baltimore criteria. In addition, we need to distinguish late-onset SOS/VOD (beyond day 21), where hyperbilirubinemia is less consistent and therefore not mandatory for diagnosis, provided patients present with at least two clinical manifestations (hyperbilirubinemia, painful hepatomegaly, weight gain  $>5\%$ , and/or ascites) as well as hemodynamic and/or ultrasound evidence of SOS/VOD.

While those criteria have been recently established and no data suggest they should be challenged, we would like to acknowledge that early diagnosis of SOS/VOD can remain difficult in some patients who do not fulfill all SOS/VOD

criteria, despite having severe disease. This situation can lead to a delayed initiation of treatment that may have life-threatening consequences. Therefore, the EBMT has updated the previously published criteria, with the addition of a new category of probable SOS/VOD diagnosis (Mohty et al. 2023). Probable SOS/VOD would be defined by two or more of the following five criteria: hyperbilirubinemia, painful hepatomegaly, weight gain  $>5\%$ , ascites, and/or ultrasound and/or elastography suggestive of SOS/VOD (Table 49.2). SOS/VOD diagnoses based on the previously published EBMT SOS/VOD criteria: association of hyperbilirubinemia with 2 of the following criteria (painful hepatomegaly, weight gain  $>5\%$ , and/or ascites) will be considered as clinical SOS/VOD, and histologically or hemodynamically proven SOS/VOD will be considered proven SOS/VOD.

Importantly, these criteria overlap with the revised EBMT criteria for late-onset SOS/VOD; therefore, the distinction probable/clinical/proven will also be applied and the only difference for diagnosis between classical and late-onset SOS/VOD will be time of onset (up to day 21 or after day 21).

Diagnostic imaging techniques include hemodynamic, ultrasound, and elastography. Measurement of the hepatic venous pressure gradient (HVPG) through the jugular vein is the most accurate method to confirm the diagnosis of SOS/VOD, since an HVGP  $>10$  mmHg has an extremely high specificity and sensitivity for SOS/VOD diagnosis in patients without previous liver disease

**Table 49.2** SOS/VOD criteria for diagnosis (adults)

Probable	Clinical	Proven
Two of the following criteria must be present: <ul style="list-style-type: none"> <li>– Bilirubin <math>\geq 2</math> mg/dL</li> <li>– Painful hepatomegaly</li> <li>– Weight gain <math>&gt;5\%</math></li> <li>– Ascites</li> <li>– Ultrasound and/or elastography suggestive of SOS/VOD</li> </ul>	Bilirubin $\geq 2$ mg/dL and two of the following criteria must be present: <ul style="list-style-type: none"> <li>– Painful hepatomegaly</li> <li>– Weight gain <math>&gt;5\%</math></li> <li>– Ascites</li> </ul>	Histologically proven SOS/VOD or hemodynamically proven (HVPG $\geq 10$ mmHg)
	Onset	
In the first 21 days after HCT: classical SOS/VOD	$>21$ days after HCT: late-onset SOS/VOD	

For any patient, these symptoms/signs should not be attributable to others causes

(Carreras et al. 1993; Carreras 2015; Shulman et al. 1995; Gressens et al. 2022). However, this technique is invasive, requires experienced staff, and is not routinely available in most centers. Therefore, noninvasive techniques have been developed including ultrasound and elastography. Ultrasound can detect nonspecific abnormalities in SOS/VOD, including hepatomegaly, splenomegaly, gallbladder wall thickening, ascites, and portal venous flow abnormalities (Mahgerefteh et al. 2011; Lassau et al. 1997). A decrease in velocity or reversal of the portal venous flow is considered more specific for SOS/VOD but is inconsistent and usually occurs late in the disease (Mahgerefteh et al. 2011; Lassau et al. 1997; Brown et al. 1990). Importantly, in a study among 106 patients post-allo-HCT, including 10 (9.4%) diagnosed with SOS/VOD, a novel ultrasound scoring, HokUS-10, was established that consisted of 10 parameters (Nishida et al. 2018). The sensitivity and specificity were 100% and 95.8%, respectively. While this score remains to be validated in a larger cohort, it can be useful for ultrasound assessment of SOS/VOD. Of note, there is a direct correlation between the hepatic arterial early acceleration index and HVPG (Tasu et al. 2002), which could be helpful for SOS/VOD diagnosis. Nevertheless, this noninvasive technique requires expertise and is not available routinely.

Liver stiffness measurement (LSM) has been reported as a possible surrogate for portal hypertension and its complications and prompted the

evaluation of this technique for the diagnosis of SOS/VOD. Two recent studies have evaluated the impact of LSM in HCT showing excellent specificity and sensitivity to early detect SOS/VOD (Colecchia et al. 2019; Debureaux et al. 2021). Additionally, LSM gradually decreased following successful specific SOS/VOD treatment. LSM can also be evaluated through magnetic resonance imaging (MRI), and increased LSM using magnetic resonance elastography was also reported in patients who developed SOS/VOD after chemotherapy treatment with oxaliplatin, further confirming the role of LSM for SOS/VOD diagnosis (Poker et al. 2022).

Overall, elastography for LSM is sensitive and specific for SOS/VOD diagnosis and is relevant for inclusion in the SOS/VOD diagnostic criteria in addition to hemodynamic and/or ultrasound techniques.

The use of other imaging techniques, including computed tomography (CT) scans and MRI scans, has been investigated in SOS/VOD with no specific findings (Dignan et al. 2013).

#### 49.2.4.1 Severity Grading

According to the EBMT, SOS/VOD is graded in four stages of severity: mild, moderate, severe, and very severe, based on the following parameters: time since the first clinical manifestation of SOS/VOD, bilirubin level and kinetics, transaminase level, weight gain, and renal function (Table 49.3). In the presence of 2 or more

**Table 49.3** Severity grading of SOS/VOD in adults

	Mild <sup>a</sup>	Moderate <sup>a</sup>	Severe	Very severe—MOD <sup>b</sup>
Time since clinical symptoms of SOS/VOD	>7 days	5–7 days	≤4 days	Any time
Bilirubin (mg/dL)	≥2 and <3	≥3 and <5	≥5 and <8	≥8
Bilirubin kinetic			Doubling within 48 h	
Transaminases	≤2 × normal	>2 and ≤5 × normal	>5 and ≤8 × normal	>8 × normal
Weight increase			≥5%	≥10%
Renal function (creatininemia)	Baseline at transplant	<1.5 × baseline at transplant	≥1.5 and <2 × baseline at transplant	≥2 × baseline at transplant or diagnosis of MOD <sup>b</sup>

Patients belong to the category that fulfilled 2 or more criteria. If patients fulfilled 2 or more criteria in 2 different categories, they must be classified in the most severe category between both

<sup>a</sup> In case of presence of 2 or more risk factors for SOS/VOD, patients should be in the upper grade

<sup>b</sup> Patients with multiple organ dysfunction (MOD) must be classified as very severe, and MOD is defined as ≥2 organs from the SOFA score with a score ≥2 or an increase ≥2 or organ dysfunction for patients with underlying organ involvement

risk factors, patients are classified in the upper grade. These criteria were validated by Yoon et al. 2019, in a group of 203 patients with SOS/VOD. In these patients, very severe SOS/VOD was associated with a significantly lower OS than the others (58.6% versus 89.3%,  $p < 0.0001$ ) and a higher day +100 transplant-related mortality, being 36.7%, versus 8.3% in mild, 8.0% in moderate, and 2.7% in severe ( $p < 0.0001$ ).

These criteria must be applied once the diagnosis of SOS/VOD is performed according to the revised EBMT diagnosis criteria and can be applied for probable, clinical, or proven SOS/VOD. It is important to evaluate SOS/VOD severity at diagnosis; nevertheless, in some patients SOS/VOD worsens, and we must clearly indicate when we assign SOS/VOD severity grading whether we consider severity at diagnosis or the overall highest severity grade, irrespective of the timing of the grading.

Also, the EBMT clarified the definition of multiple organ dysfunction/multiple organ failure (MOD/MOF). This is particularly important since patients with SOS/VOD who develop MOD/MOF will be classified as very severe (Mohty et al. 2023).

### 49.2.5 Prophylaxis and Treatment

Regarding SOS/VOD prophylaxis and treatment, we issued recommendations in 2020 (Mohty et al. 2020) that are still accurate today. Defibrotide remains the only agent for the treatment of severe SOS/VOD and should be initiated as soon as possible in those patients. Furthermore, given that early treatment initiation is associated with a higher day +100 OS, and that moderate SOS/VOD is associated with significant mortality (Kernan et al. 2018), we also recommend early initiation of defibrotide in patients with moderate SOS/VOD. For patients with mild SOS/VOD, supportive care must be pursued with close monitoring of severity criteria to allow early initiation of defibrotide in case of worsening. Importantly, defibrotide must be initiated promptly, based on severity criteria as soon as the diagnosis of SOS/

VOD is confirmed, irrespective of the diagnostic status (probable, clinical, or proven). Defibrotide is administered at a dose of 25 mg/kg/day for at least 14–21 days and until the resolution of all SOS/VOD symptoms.

Regarding prophylaxis, nonpharmacologic measures to reduce SOS/VOD modifiable risk factors are crucial. For the pharmacologic measures, ursodeoxycholic acid administered from initiation of conditioning until day +90 after transplantation is recommended in adults (Ruutu et al. 2014). Regarding the use of prophylactic defibrotide, a prospective randomized phase III clinical trial compared defibrotide versus best supportive care for prevention of SOS/VOD in 372 pediatric and adult patients at high risk of SOS/VOD after transplantation (NCT02851407) (Grupp et al. 2021). No significant difference was observed between defibrotide and best supportive care groups in the primary end point: SOS/VOD-free survival at day +30 (67% versus 73% respectively,  $p = 0.85$ ). Importantly, there were no differences in adverse events between groups.

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## 49.3 SOS/VOD in Children

Selim Corbacioglu

### 49.3.1 Introduction

SOS/VOD in children differs in many aspects substantially from adult patients despite a similarity in the underlying pathophysiology. The primary difference is the hepatic immaturity of infants and toddlers. In part, this affects the incidence and the risk factors. There are also predisposing diseases, the clinical presentation, the diagnostic criteria, and finally the indication for prophylaxis that represent major differences in pediatric transplant physicians must be aware of optimizing their approach to one of the most prevalent and deleterious early posttransplant complications in childhood. (Table 49.4 summarizes the major differences between adults and children).



**Table 49.4**<sup>a</sup>

Criteria	Children	Adults
Incidence	<ul style="list-style-type: none"> <li>• Approximately 15–20%</li> <li>• Up to 60% in high-risk patients</li> </ul>	<ul style="list-style-type: none"> <li>• Approximately 5–10%</li> </ul>
Risk factors	<ul style="list-style-type: none"> <li>• Additional pediatric risk factors:               <ul style="list-style-type: none"> <li>– Infants</li> <li>– Pediatric/genetic diseases with incidences above average</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Established risk factors</li> </ul>
Clinical presentation	<ul style="list-style-type: none"> <li>• Late-onset hepatic VOD in 20%</li> <li>• Anicteric hepatic VOD in 30%</li> <li>• Hyperbilirubinemia, if present:               <ul style="list-style-type: none"> <li>– Is frequently preexistent</li> <li>– Occurs late during hepatic SOS/VOD</li> <li>– Hyperbilirubinemia of <math>\geq 2</math> mg/dL is associated with severe hepatic SOS/VOD</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Late-onset hepatic SOS/VOD is rare</li> <li>• Anicteric hepatic SOS/VOD is approximately 15%</li> </ul>
Need for proper assessment of ascites and hepatomegaly	<ul style="list-style-type: none"> <li>• High incidence of disease-related hepatomegaly and ascites pre-HCT</li> </ul>	
Diagnostic criteria	<ul style="list-style-type: none"> <li>• Pediatric EBMT criteria</li> <li>• Inclusion of refractory thrombocytopenia as sensitive marker</li> </ul>	<ul style="list-style-type: none"> <li>• Adult EBMT criteria (obligatory hyperbilirubinemia <math>\geq 2</math> mg/dL)</li> <li>• Baltimore criteria (obligatory hyperbilirubinemia <math>\geq 2</math> mg/dL)</li> <li>• Seattle criteria</li> </ul>

<sup>a</sup> Adapted from Corbacioglu S, et al. *Bone Marrow Transplant* 2018; 53:138–45

### 49.3.2 Incidence

SOS/VOD remains primarily a pediatric disease. The proper incidence is in fact difficult to capture since the diagnostic criteria used impact significantly on this parameter. According to the current literature, a 15% posttransplant incidence (using Seattle/pediatric EBMT criteria (see below) can be assumed (Coppell et al. 2010; Corbacioglu et al. 2012a, b; Xia et al. 2021; Yoon et al. 2021), but depending on several influencing factors such as the conditioning regimen and the focus of the respective centers, the incidence can reach 30% and more (Felber et al. 2020). In summary, the overall incidence in children can be considered twice as high as in adults.

### 49.3.3 Risk Factors

Knowing the conditions that are associated with a high risk for SOS/VOD helps to “earmark” patients for a closer observation during the posttransplant period. The highest risk population are infants and toddlers (<2 years) (Strouse et al. 2018) where SOS/VOD can occur also during conventional che-

motherapy, for example, with intensified regimens for infant leukemia. Since risk factors have additive effects (Dalle and Giralt 2016), certain diseases such as malignant infantile osteopetrosis (MIO), congenital macrophage activation syndromes (such as hemophagocytic lymphohistiocytosis - HLH), juvenile myelomonocytic leukemia (JMML), and neuroblastoma, all are highly prevalent in infancy, augment the individual risk significantly (Corbacioglu et al. 2012a, b). Severe iron overload of the liver raises the SOS/VOD risk and is most prevalent in patients in chronic transfusion programs, such as transfusion-dependent thalassemia (TDT), certain patients suffering from sickle cell disease, and aplastic anemia. This risk is more prominent when iron overload leads to an inflammatory response in the liver. In cases where a stagnation of the iron depletion despite an aggressive chelation prior to HCT (“downstaging”) is observed, a liver biopsy is highly recommended to assess the underlying inflammatory process. If there is only mild inflammation, the risk for SOS/VOD can be limited despite a high liver iron content. Similarly, patients with an active inflammatory liver disease of an unspecified origin prior to conditioning are at risk. The use of busulfan (inde-

pendent of serum-level measurements) as part of a myeloablative conditioning (MAC) regimen needs to be considered a risk factor, in particular in combination with high-risk diseases such as MIO and HLH (Felber et al. 2020; Strouse et al. 2018). Whereas treosulfan, albeit another alkylator, seems to have a lower detrimental impact on the sinusoidal endothelium with significantly lower incidences of SOS/VOD in high-risk diseases (Shadur et al. 2018; Wustrau et al. 2020).

A prior SOS/VOD and a second MAC-based HCT are additional risk factors. Probably, the group with the highest risk beyond infancy is patients treated with ozogamicin-conjugated monoclonal antibodies preceding a MAC HCT. It is important to mention that even severe SOS/VOD is not limited to patients at risk. In general, awareness of the clinical presentation with or without associated risk factors is pivotal to respond appropriately.

### 49.3.4 Clinical Presentation

The clinical presentation of SOS/VOD in children also differs in several aspects from adults. The incidence of SOS/VOD peaks around day 12 posttransplant, and 80% of the children are present before day 21. Different from adults, the incidence of anicteric SOS/VOD is approximately 30% (Corbacioglu et al. 2012a, b; Naples et al. 2016) twice as high as in adults, occurring in 80% within the first 21 days post-HCT compared to 50% in adults. Furthermore, the incidence of severe anicteric SOS/VOD with multi-organ dysfunction is higher in children compared to adults (74% versus 59%) (Corbacioglu et al. 2020).

It must be considered that many children present with preexistent hepatomegaly and ascites prior to transplant, prevalent in HLH, OP, TDT, and other diseases frequently transplanted in childhood. Therefore, a pretransplant ultrasound to define the baseline for liver size and free fluid, as required by the pediatric EBMT criteria (pEBMT), is recommended. Right upper quadrant pain, part of the Seattle criteria, is imaginably difficult to assess in infants and was therefore not considered part of the pEBMT criteria.

### 49.3.5 Diagnostic Criteria

The high incidence of anicteric SOS/VOD discourages the use of criteria that require an obligatory hyperbilirubinemia (>2 mg/dL) as in the Baltimore and adult EBMT criteria (Mohty et al. 2016). In addition, hyperbilirubinemia is a late finding, where even the 2 mg/dL threshold significantly reduces survival and early intervention with Defibrotide has been demonstrated to have a critical impact on morbidity and mortality (Corbacioglu et al. 2020; Kernan et al. 2018). The Seattle criteria, which were used in two large prospective trials (Corbacioglu et al. 2012a, b; Grupp et al. 2023) involving children and adults, omitted the obligatory 2 mg/dL and were until recently more suitable for children.

The pEBMT criteria introduced in 2018 (Table 49.5) intend to cover the pediatric particularities and trigger early therapeutic intervention. With that regard the obligatory bilirubin threshold was omitted reflecting the high incidence of anicteric SOS/VOD. It was replaced by a dynamic approach of rising bilirubin levels on three con-

**Table 49.5** <sup>a</sup>

Pediatric EBMT criteria
• No limitation for time of onset of SOS/VOD
• The presence of two or more of the following <sup>b</sup> :
– Otherwise unexplained weight gain on three consecutive days despite the use of diuretics or a weight gain >5% above baseline value within 72 h
– Rising bilirubin from a baseline value on three consecutive days or bilirubin $\geq 2$ mg/dL within 72 h
– Hepatomegaly <sup>c</sup> (best if confirmed by imaging) above the baseline value
– Ascites <sup>c</sup> (best if confirmed by imaging) above the baseline value
– Unexplained consumptive and transfusion refractory thrombocytopenia <sup>d</sup>

CT computed tomography, MRI magnetic resonance imaging, US ultrasound

<sup>a</sup> Adapted from Corbacioglu S, et al. *Bone Marrow Transplant* 2018; 53: 138–45

<sup>b</sup> With the exclusion of other potential differential diagnoses

<sup>c</sup> Recommended: imaging (US, CT, or MRI) immediately before HCT to determine baseline value for both hepatomegaly and ascites

<sup>d</sup>  $\geq 1$  Weight-adjusted platelet transfusion/day to maintain institutional guidelines

secutive days. A similar approach was chosen for weight gain omitting the arbitrary 3% or 5% weight gain of the Seattle and Baltimore criteria, respectively. A baseline abdominal imaging, preferably via ultrasound, is required to verify hepatomegaly and ascites as an individual baseline, particularly important in patients with pre-existing conditions.

The most sensitive criterion added was refractory thrombocytopenia. SOS/VOD is a sinusoidal disease, with a consumptive and transfusion refractory thrombocytopenia (RT) being the earliest symptom. Therefore, RT is the most sensitive trigger for intervention. RT has been described by several authors as an early sign and, if prolonged as a predictor of poor outcome, including McDonalds, was never introduced as a criterion (McDonald et al. 1993; Embaby et al. 2020; Roeker et al. 2019). Several publications confirmed the validity of RT as an early trigger by comparing pEBMT with the established criteria. Szmit et al. (2020) showed in a single center that using modified Seattle criteria delayed diagnosis by up to 11 days (median 3 days) compared to pEBMT criteria. Another single-center analysis in 226 pediatric patients found a shortened time-to-diagnosis also by 3 days, with a 75% incidence of RT as the first symptom (Ragoonanan et al. 2021). With regards to scientific objectives, it is pivotal to be aware of the impact of the different diagnostic criteria on the incidence of SOS/VOD, with a doubling or even quadrupling the incidence by using Baltimore versus Seattle criteria (Coppell et al. 2010; Yakushijin et al. 2005) and another rise of approximately 5% from to Seattle to pEBMT criteria.

### 49.3.6 Therapeutic Intervention

The therapeutic interventions in children differ only in parts from adults, with defibrotide being the only licensed drug to specifically treat SOS/VOD. Early intervention demonstrated to have a pivotal impact on morbidity and mortality. A bilirubin level above 2 mg/dL in all ages is a predictor of a poor day 100 survival (Corbacioglu et al. 2020). Earlier initiation with defibrotide was

associated with higher day 100 survival in the subgroup of patients with multi-organ dysfunction (Kernan et al. 2018).

When comparing Seattle criteria-triggered intervention with defibrotide with pEBMT criteria a significantly better overall survival, a five times lower transplant-related mortality and a shortened length of hospitalization in the median of 12 days was observed (Szmit et al. 2020). Next to the established supportive measures of balanced fluid management to avoid prerenal failure, avoidance of hepatotoxic drugs, and careful management of hemostasis, a particular recommendation for toddlers is to consider early paracentesis for ascitic drainage to avoid pulmonary impairment/insufficiency due to infra-diaphragmatic expansion into the thoracic cavity.

### 49.3.7 Prophylaxis

Regarding the efficacy of defibrotide for prophylaxis, the body of evidence seems to be contradictory. Next to several retrospective analyses and case series, the pediatric prevention trial (NCT00272948) was a prospective randomized trial conducted in 360 high-risk pediatric patients that demonstrated efficacy in the prevention of SOS/VOD by reducing the incidence from 20 to 12% ( $p = 0.049$ ; per protocol population:  $p = 0.022$ ) (Corbacioglu et al. 2012a). On the other hand, the Harmony Trial (NCT02851407), another prospective randomized trial with a similar sample size, that also included 50% adults, reported that defibrotide was not effective for the prevention of SOS/VOD. This discrepancy can be solved when the latter trial is scrutinized regarding sample size calculation, end points, and general design (Corbacioglu et al. 2023). Therefore, several aspects encourage a critical discussion on the need for prophylactic intervention with defibrotide in children. The disease is very prevalent, and a delayed intervention can affect morbidity and mortality significantly. The diagnostic criteria remain based on clinical observation only and might under certain circumstances lead to a substantial therapeutic delay. On

the other hand, sensitized diagnostic criteria such as the pEBMT criteria can trigger early/preemptive therapeutic intervention with defibrotide by several days. In conclusion, the question remains if the indication for prophylactic intervention with defibrotide is obsolete or remains an unmet need in children to cover this gap in a high-risk pediatric population.

## 49.4 Hepatotropic Viruses

Rafael de la Cámara

### 49.4.1 Hepatitis A Virus (HAV)

Hepatitis A virus (HAV) is a non-enveloped hepatotropic virus, now named *Hepatovirus A*, classified in the genus *Hepatovirus* within the family *Picornaviridae*.

Most countries of the European Economic Area (EEA) currently experience very low or low HAV endemicity.

HAV is primarily transmitted via the fecal-oral route and is acquired through ingestion of contaminated food or water, sexual, or another direct contact with an infected individual. On rare occasions, HAV has been transmitted by transfusion of blood or blood products collected from donors during the viremic phase of the infection. Blood products and HCT donors are not routinely tested for HAV.

#### 49.4.1.1 Clinical Symptoms

Hepatitis due to HAV is usually mild and self-limited when healthy persons are infected. Disease severity increases in older or immunocompromised, have chronic liver disease, or have other underlying health conditions. No chronic infection is known to occur, although prolonged, relapsing hepatitis occurs in 15% of cases. Very severe disease is unusual. There is no specific series of HAV hepatitis in HCT patients.

#### 49.4.1.2 Diagnostics

Anti-HAV IgM establishes the diagnosis of acute hepatitis A and can remain elevated for

3–12 months following infection. Polymerase chain reaction (PCR) is the preferred method in the HCT setting. Anti-HAV IgG generally persists for the duration of a patient's life following infection or vaccination.

Liver function tests (LFTs) should be performed in donors before HCT harvesting. Donors with abnormal LFT should be tested for anti-HAV Immunoglobulin (Ig) M. If HAV is detected, the donation should be delayed until HAV-RNA is no longer detectable in the donor.

#### 49.4.1.3 Prevention

HCT is not recommended if the donor or the recipient is viremic for HAV because of an increased risk of SOS/VOD (Nelson et al. 2020).

Vaccination should be considered in HAV-IgG-negative patients at risk. There is limited experience of HAV vaccination in HCT. Limited data suggest that doubling of the standard antigen might increase response rates.

In some countries, there are specific HAV Ig products that can be used for both preexposure for travelers to intermediate or high-endemicity areas prior to travel and postexposure prophylaxis of HAV (within 2 weeks of the exposure).

Persons with chronic liver disease have a higher likelihood of fulminant hepatitis if not previously vaccinated or immune against HAV. HCT with chronic liver disease should be tested for antibodies against HAV and, if negative, should be advised to be vaccinated against HAV.

#### 49.4.1.4 Treatment

Symptomatic (Deasy and Kim 2020).

### 49.4.2 Hepatitis B Virus (HBV)

HBV is a double-stranded DNA virus, a species of the genus *Orthohepadnavirus*, within the family *Hepadnaviridae*.

HBV is a frequent infection worldwide, and consequently, HBV infection in HCT candidates is a frequent situation. An infection due to HBV does not preclude an HCT, but the liver disease caused by this virus can.

#### 49.4.2.1 Clinical Symptoms

After primary infection, even in the case of HBsAg seroconversion (became negative with positive anti-HBs), HBV probably persists life-long in the nucleus of hepatocytes and, therefore, can reactivate after treatment-induced loss of immune control as is the case of HCT. Hepatitis, including cases of fulminant hepatic failure, typically occurs after immune system reconstitution, de novo recognition, and destruction of HBV-infected hepatocytes. Reactivation of resolved HBV infection, known as reverse seroconversion (RS), is an important and late complication of HCT, with a median of 18–20 months after transplant. The risk of reactivation is higher among those with HBsAg (+) than in those with HBsAg (–) and anti-HBc (+), in those with low anti-HBs (<10 IU/L), extensive chronic GVHD, and higher in allogeneic compared to auto-HCT. Fibrosing cholestatic hepatitis can be a consequence of HBV reactivation. Nonetheless, HBV infection does not affect 10-year survival.

#### 49.4.2.2 Diagnostics

All donors and recipients must be screened for HBsAg, anti-HBc, anti-HBs, and HBV-DNA (or at least if anti-HBc or HBsAg are detected). The combination of serology, DNA-HBV, and level of transaminases classified the different types of HBV infections (Table 49.6).

#### 49.4.2.3 Prevention

The most important practical issue with HBV in HCT is the prevention of severe or fatal hepatitis due to HBV (Siyahian et al. 2018).

##### (a) DNA-HBV or HBsAg-positive patients

Antiviral prophylaxis should be given to all DNA-HBV or HBsAg-positive patients. Tenofovir (limited experience in HCT patients) or entecavir are the drugs of choice, starting before the conditioning regimen and maintained at least for 1 year after HCT, and longer if GVHD is present or immunosuppression is given. Lamivudine is not recommended. After stopping antiviral prophylaxis, HBV monitoring every 3 months is recommended as late reactivation with fulminant hepatitis has been described.

**Table 49.6** Different types of HBV infection

Not infected, not vaccinated	Immunized by vaccination
HBsAg (–)	HBsAg (–)
Anti-HBs (–)	Anti-HBs (+)
Anti-HBc (–)	Anti-HBc (–)
DNA-HBV (–)	DNA-HBV (–)
Acute infection, window period	Acute infection
HBsAg (–)	HBsAg (+)
Anti-HBs (–)	Anti-HBs (–)
IgM anti-HBc (+)	IgM anti-HBc (+)
DNA-HBV (±)	DNA-HBV (+)
Acute hepatitis in resolution	Immunized after infection (occult HBV infection)
HBsAg (±)	HBsAg (–)
Anti-HBs (±)	Anti-HBs (+)
IgM anti-HBc (–)	IgM anti-HBc (–)
IgG anti-HBc (+)	IgG anti-HBc (+)
DNA-HBV (±)	DNA-HBV (–)
Chronic active hepatitis <sup>a</sup>	Chronic carrier
HBsAg (high +)	HBsAg (low +)
Anti-HBs (–)	Anti-HBs (–)
IgM anti-HBc (–)	IgM anti-HBc (–)
IgG anti-HBc (+)	IgG anti-HBc (+)
HBeAg (+), anti-HBe (–)	HBeAg (–), anti-HBe (+)
DNA-HBV (+) (>2000 UI/mL)	DNA-HBV (+) (<2000 UI/mL)
Elevated transaminases	normal transaminases

Adapted from Carreras E, Rovira M, Valcarcel D (eds) Manual de Trasplante Hematopoyético, 2022. Chap. 4.18  
<sup>a</sup> Chronic hepatitis B can be HBeAg-negative, usually with detectable anti-HBe, and persistent or fluctuating moderate to high levels of serum HBV DNA (often lower than in HBeAg-positive patients). Most of these subjects harbor HBV variants in the precore and/or the basal core promoter regions that impair or abolish HBeAg expression

##### (b) Anti-HBc-positive but HBsAg/DNA-HBV-negative patients

Two strategies can be used for the prevention of HBV hepatitis in these patients: prophylaxis with antivirals or a preemptive therapy with DNA-HBV monitoring.

The ECIL recommends antiviral prophylaxis (with tenofovir or entecavir) to all anti-HBc-positive patients who undergo a HCT (Mallet et al. 2016).

The European Association for the Study of the Liver recommends antiviral prophylaxis when the risk of HBV reactivation is



high (>10%) (EASL 2017). In these patients, lamivudine is also an option. For moderate- (<10%) or low-risk (1%) reactivation patients, preemptive therapy, not prophylaxis, is generally recommended. Preemptive therapy is based on monitoring HBsAg and HBV DNA every 1–3 months and starting antiviral treatment as early as HBV DNA is detectable or HBsAg seroconversion, independently of alanine transaminase (ALT) levels.

(c) HBsAg (or DNA-HBV)-positive donors

An HBsAg (+) donor transmits HBV to the recipient in 22–44% of cases. Antiviral therapy of the donor and, if possible, antiviral prophylaxis of the recipient are recommended. The addition of hepatitis B immune globulin can be considered. Pre-HCT vaccination of the recipient, if possible, could decrease the transmission of HBV from the donor.

#### 49.4.2.4 Vaccination

Vaccination of anti-HBc-negative and anti-HBs-negative patients after HCT is recommended. Double vaccine doses may be required to achieve an anti-HBs response in immunocompromised patients (0–1–2–6 months). Vaccination can reduce the risk of reverse seroconversion in anti-HBc-positive patients (Hammond et al. 2022).

The transfer of HBV immunity from donor to recipient has been described. Vaccination of the donor should be done only in the interest of the donor.

Patients with HBV infection should be tested for antibodies against HAV and, if negative for anti-HAV, should be advised to be vaccinated against HAV.

#### 49.4.2.5 Treatment

In HCT candidates with chronic hepatitis based on biopsy or positive HBsAg or high levels of HBV DNA, transplant procedure should be delayed when possible, and antiviral therapy should be given for 3–6 months before conditioning.

Treatment of patients with acute or chronic HBV hepatitis should be done in collaboration with hepatology. Acute hepatitis after HCT should also be treated to prevent a bad or fatal course.

### 49.4.3 Hepatitis C Virus (HCV)

The hepatitis C virus (HCV), now named *Hepacivirus C*, is a small, enveloped, single-stranded, RNA virus, the member of the genus *Hepacivirus* in the family *Flaviviridae*. HCV is a frequent infection worldwide, and consequently, HCV infection in HCT candidates is a frequent situation. The hepatitis C virus is a bloodborne virus. Transmission of HCV by blood transfusion is an exceptional event in developed countries. In HCT, the most important aspect is the detection of HCV infection in order to implement treatment to avoid liver damage.

#### 49.4.3.1 Clinical Symptoms

HCV can cause both acute and chronic hepatitis infection. The majority of HCV infections are asymptomatic.

An infection due to HCV does not preclude an HCT, but the possible existence of hepatic fibrosis or the presence of cirrhosis and hypertension in HCV-RNA (+) patients should be evaluated prior to the transplant. Liver fibrosis is a risk factor for SOS/VOD and drug toxicity. Cirrhosis and a worse outcome have been documented after HCT. Nonetheless, HCV infection does not affect the 10-year survival.

#### 49.4.3.2 Diagnostics

All donors and recipients must be screened for HCV before transplant by serology and HCV-RNA PCR. In cases of spontaneous or treatment-induced viral elimination, anti-HCV antibodies persist for life in the absence of HCV RNA. Patients who have successfully eliminated HCV in the past are not at risk of reactivation under immunosuppressive therapy, but reinfection with HCV is possible.

Close monitoring of LFT and HCV-RNA is recommended in infected patients.



### 49.4.3.3 Prevention

There is no vaccine for HCV, so prevention of infection depends on reducing the risk of exposure to the virus in healthcare settings and avoiding personal practices that are associated with the risk of transmission (unsafe sex, injecting drugs, tattooing, acupuncture, and body piercing).

SOS-sparing regimens should be considered in HCV RNA-positive patients with significant liver fibrosis.

The presence of HCV-RNA positive in the recipient does not constitute a contraindication for HCT, but antiviral therapy should be considered, if possible, to postpone the HCT to allow completion of a treatment course.

If the donor is HCV-RNA-positive, the infection will be transmitted to the recipient in all cases. An HCV-RNA-positive donor could be considered if other donor options are considered inferior. In this case, the donor should be rapidly evaluated by a hepatologist, and treatment with direct-acting antivirals (DAAs) should be considered.

### 49.4.3.4 Treatment

The treatment of HCV has entirely changed with the incorporation of direct-acting antivirals (DAAs), which obtain cure in over 90% of patients. Antiviral treatment should be considered for all HCV-RNA-positive hematological patients. HCV can be treated concomitantly with chemotherapy if treatment of hematological malignancy is urgent. This should be done in consultation with an expert hepatologist. If possible, all HCV-RNA-positive patients should be treated before the transplant.

## 49.4.4 Hepatitis E Virus (HEV)

Hepatitis E is caused by the HEV, a positive-stranded RNA virus of the *Hepeviridae* family, genus *Orthohepevirus A*.

HEV is divided into 4 genotypes. Genotypes 1 and 2 are more virulent and infect humans only, while genotypes 3 and 4 are zoonotic. In Europe, autochthonous infections are mostly related to HEV-3. The seroprevalence of HEV in Europe varies between and within countries (1 to >50%).

The main source of spread varies between different parts of the world. Hepatitis E is a waterborne infection caused by HEV genotype 1 or 2 in developing countries, while in developed countries, autochthonous hepatitis E is a zoonotic infection (reservoir in pigs or wild boar), caused by HEV genotypes 3 and 4. There is no evidence of sexual transmission of HEV. Transfusion (plasma, platelet concentrates, and red blood cell concentrates) or transplantation-transmitted HEV infections have been observed sporadically. In Europe, where HEV-3 is endemic, the infection is not associated with severe disease in pregnant women, and thus, they are not considered as a risk group (Mikulska et al. 2022).

Patients undergoing HCT might be at risk of acquiring HEV through blood transfusions. There is a possibility for HEV transfer from stem cell donors. Currently, there is no possibility of calculating the risk–benefit ratio of systematically testing donors for HEV RNA.

### 49.4.4.1 Clinical Symptoms

HEV can cause acute or chronic hepatitis. Acute infections cause self-limiting hepatitis but can become chronic in immunocompromised patients, like HCT patients, with the risk of the development of severe liver cirrhosis. In a few cases, the acute infection can result in fulminant hepatitis with acute liver failure. No case of fulminant HEV in HCT recipients has been described so far. Patients with preexisting chronic liver disease are at risk of severe disease progression with liver failure. Several extrahepatic manifestations have been described associated with HEV. The probably more important clinical picture in HCT recipients is chronic hepatitis, usually showing limited symptoms of hepatitis or nonspecific clinical symptoms, since rapid progression to cirrhosis has been reported in IS patients.

### 49.4.4.2 Diagnostics

Nucleic Acid Amplification Testing (NAT) testing should be preferred over serology to diagnose HEV infection, as serological tests vary in sensitivity and specificity. In HCT, no routine screening for antibodies against HEV or RNA-HEV is done either in the donor or in the recipient.

**49.4.4.3 Prevention**

So far, the detection of HEV-RNA neither in the donor nor in the recipient can be considered as an absolute contraindication for HCT. HCT with an HEV-RNA-positive donor could be considered if other donor options are considered inferior. In this case, treatment with Ribavirin (RBV) of the recipient could be considered.

HCT recipients should be informed about the risks of foodborne HEV transmission by avoiding the consumption of undercooked meat. When traveling to countries with poor sanitation, it is advisable to boil water for drinking and for brushing teeth.

A vaccine has been developed and licensed in China (2011) but is not licensed in Europe and the USA or recommended for use by WHO.

**49.4.4.4 Treatment**

For patients with HEV infection, a decrease in the dose of immunosuppressive drugs could be considered. Ribavirin has been suggested as a treatment for chronic infection based on case reports and small case series. However, no controlled data exist.

**49.5 Other Hepatic Complications**

Enric Carreras and Tapani Ruutu

**49.5.1 Autoimmune Hepatitis**

The main problem with these hepatitis is how to differentiate them from hepatic GVHD, as the pathogenesis, clinical manifestations, and biological changes are virtually identical (Dalekos et al. 2002; Ruutu and Carreras 2019) as shown in the table:

Variables	Autoimmune hepatitis	Hepatic GVHD
Jaundice	Generally mild	Various degrees
Other symptoms	Fatigue and malaise, but often asymptomatic	Hepatic tenderness, choloria, acholia, and anorexia, <b>almost always GVHD in other organs</b>

Increased AST	Moderate to severe	Minimal
Increased GGT	Marked	Normal or decreased
Type of auto-antibodies	Characteristic of AIH, Ab type 2 ( <b>ALKM and ALC-1</b> )	Similar Ab but usually of AIH type 1 (ANA and ANCA)
Pathology	Inflammatory infiltrate in the portal area, often penetrating into lobules	Inflammatory infiltrate, <b>bile duct leakage, degeneration of the ductal epithelium</b> , and cholestasis
Cirrhosis	May be present	Exceptional
Steroid resp.	Excellent	Variable according to severity

In **bold** main differential data

*Ab* autoantibodies, *AIH* autoimmune hepatitis, *ANA* antinuclear antibodies, *ANCA* anti-cytoplasm antibodies, *ALKM* microsomal Ab. against liver and kidney, *AC1* anti-cytosol type 1

**49.5.2 Drug-Induced Hepatitis**

Most relevant agents <sup>a</sup>	Presentation
Triazole antifungals <sup>b</sup>	Cholestatic <sup>c</sup> or hepatocellular <sup>d</sup> hepatitis, hepatic insuf.
Echinocandins	Mild-moderate or hepatocellular hepatitis
Fluoroquinolones	Hepatocellular hepatitis
TMP-SMX	Hepatocellular hepatitis
Rapamycin	Hepatocellular damage and increased risk of SOS
Anticonvulsants	Hepatocellular hepatitis or cholestatic hepatitis
NSAIDs	Hepatocellular or cholestatic hepatitis
Paracetamol	Hepatocellular or cholestatic hepatitis
Ranitidine	Cholestatic hepatitis and eosinophilic infiltration
Amoxicillin-clavulanic acid	Cholestatic and/or hepatocellular hepatitis

<sup>a</sup> There are online resources for toxicity and interaction queries such as LiverTox: [www.ncbi.nlm.nih.gov/books/](http://www.ncbi.nlm.nih.gov/books/)

<sup>b</sup> Voriconazole and posaconazole

<sup>c</sup> Liver damage with predominant elevation of transaminases

<sup>d</sup> Liver damage with predominant elevation of cholestasis and bilirubin enzymes

### 49.5.3 Cirrhosis

Based on historical data, it can be said that evolution to cirrhosis has been described in the following cases:

- **HBV:** progression to cirrhosis after HCT is exceptional.
- **HCV:** a case–control study evaluated the prevalence of cirrhosis in 3721 long-term survivors of HCT. In patients who survived  $\geq 1$  year after HCT, cirrhosis (clinical or histological) was detected in 31 of the 3721 patients (1%). The median time from HCT to cirrhosis was 10 years. HCV was the etiology of cirrhosis in 25 of the 31 patients, compared to 14 of the 31 control patients ( $p = 0.01$ ).

In a single-center retrospective study, 96 HCV-infected patients during the HCT period were compared with a control group of 158 HCV patients without HCT ( $p = 0.01$ ). The cumulative incidence of cirrhosis at 20 years of HCT was 24%, and the median time to cirrhosis was 18 years, compared to 40 years in the control group (Peffault De Latour et al. 2004).

In another study, the cumulative incidence of severe liver complications in HCV-infected patients was 11.7% at 20 years in a multicenter cohort (Ljungman et al. 2012).

- **HEV:** the incidence of progression to cirrhosis of HCTs with HEV infection is unknown, but isolated rapidly progressive cases have been reported (Swartling et al. 2020).

Poorly compensated cirrhosis is a formal contraindication for HCT due to the very high risk of developing SOS after MAC (Swartling et al. 2020). Even compensated cirrhosis has a high likelihood of hepatic decompensation even on reduced-intensity conditioning (RIC) or non-myeloablative conditioning (NMC) (Hogan et al. 2004). It is not known whether these historical data remain true after the availability of the new antiviral agents.

### 49.5.4 Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the most common liver cancer and is the fourth leading cause of cancer death. The most important risk factor for developing HCC is cirrhosis. Therefore, chronic liver disease due to HBV or HCV and hepatic steatosis play a key role in the pathogenesis of HCC (reviewed in Gilman et al. 2021).

The cumulative incidence of HCC after HCT is 5%, 20 years after the procedure (Peffault De Latour et al. 2004). Therefore, at-risk patients should undergo surveillance with liver ultrasound scans every 6–12 m, according to international guidelines.

Patients may be asymptomatic or have clinical manifestations of cirrhosis or decompensated liver disease.

Early diagnosis can be made by periodic abdominal ultrasound, with or without levels of alpha-fetoprotein (AFP) levels. If a lesion is detected on ultrasound, imaging should be completed with CT or MRI. Definitive diagnosis should be established by liver biopsy, despite an elevated AFP is practically confirmatory of the diagnosis.

Therapeutic options may be curative or non-curative. Curative options include surgical resection, ablation, and liver transplantation. Noncurative options include embolization, radiation, and systemic chemotherapy.

### 49.5.5 Nodular Regenerative Hyperplasia (NRH)

Nodular regenerative hyperplasia (NRH) is a form of noncirrhotic portal hypertension with small regenerative nodules in the liver (reviewed in Gilman et al. 2021). After HCT, it is occasionally seen in patients with previous SOS. Its pathogenesis is probably the consequence of changes in hepatic blood flow with atrophy of zone 3 of the acinus and hypertrophy of zone 1 (without fibrosis).

The diagnosis may be suspected in the presence of a silent course (except for thrombocytopenia and increased alkaline phosphatase) toward portal hypertension (variceal bleeding, ascites, and hepato-splenomegaly). MRI shows characteristic nodular images. Liver biopsy allows to establish the diagnosis and rule out carcinoma or cirrhosis. This study should not be performed by transjugular or fine needle biopsy because these procedures do not provide sufficient diagnostic material (McDonald 2010). Management includes treatment of the underlying disorder and management of portal hypertension.

### 49.5.6 Focal Nodular Hyperplasia (FNH)

Focal nodular hyperplasia (FNH) is a benign liver lesion being the second most common hepatic tumor (reviewed in Gilman et al. 2021). Its pathogenesis is unknown. In HCT, it is thought to be a consequence of sinusoidal injury from the conditioning regimen or by agents that subsequently cause endothelial damage. Patients with FNH are usually asymptomatic.

Its incidence was assessed in a prospective study of 138 HCT (70% children and 30% adults) who were being followed up for screening for early detection of secondary hemochromatosis. The diagnosis of FNH was made by MRI in 16 (12%) with a median HCT diagnosis time of 6.4 years.

In a retrospective study of 87 pediatric patients after HCT, the diagnosis of FNH was established in 10 patients (11%) with a median time to diagnosis of 7 years. There was no malignant transformation. In a third study with 324 HCT, 17 patients (5.2%) with FNH were identified after a median time of 5.7 years. There was no malignant transformation.

The diagnosis is usually made incidentally by performing an ultrasound scan that detects an isoechoic lesion. The confirmatory diagnosis includes CT or MRI, which may detect a characteristic central scar. On CT, the lesions appear diffuse, homogeneous and hyperdense, reflecting the central scar. On MRI, the lesions have a hypervascular (arterial) appearance, an isointense (portal venous) or hypointense signal on T1 sequences (without contrast), and an isointense or hyperintense signal on T2 sequences (without contrast). Liver biopsy is not routinely indicated to confirm the diagnosis. Treatment should be conservative, and follow-up imaging tests are not needed.

### 49.5.7 Idiopathic Hyperammonemia

A rare but lethal syndrome with hyperammonemia and coma after conditioning for HCT has been described. Patients present with progressive

lethargy, confusion, weakness, incoordination, vomiting, hyperventilation with respiratory alkalosis, and plasma ammonia  $>200 \mu\text{mol/L}$ . The pathogenesis of idiopathic hyperammonemia likely involves a latent genetic disorder similar to ornithine transcarbamylase deficiency (McDonald 2010).

### 49.5.8 Cholangitis Lenta (CL)

CL is a hyperbilirubinemia frequently observed in neutropenic and febrile patients with intestinal mucosal lesions due to the conditioning regimen. Hepatocyte retention of conjugated bilirubin appears to be mediated by endotoxins, IL6 and TNF $\alpha$ . The exact pathophysiologic mechanisms of sluggish cholangitis remain unclear. It is hypothesized that bacterial endotoxin release and subsequent damage may cause ductal proliferation and impaired bile flow (Torous et al. 2017). Although this disorder is often referred to as “cholestasis of sepsis,” it can be seen in patients with isolated fever without focality, or in patients with a localized infection in the lungs and soft tissues (McDonald 2010).

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Grzegorz W. Basak

## 50.1 Introduction

The gastrointestinal (GI) tract is one of the systems most commonly affected by transplant complications. It is due to the high vulnerability of the gut mucosa composed of dividing cells, which are susceptible to chemotherapy-induced damage, rich vasculature, constant contact with intestinal microflora, and high content of immune-competent cells. Therefore, when evaluating symptoms from the GI system, various possible causes must be taken into account, especially drug toxicity, infections, and graft-versus-host disease. In this chapter selected GI complications most frequent after HCT will be presented. The GI aGVHD was already discussed in Chaps. 43 and 44 and infectious causes in Chaps. 38 and 39.

## 50.2 Nausea/Vomiting

### 50.2.1 Definitions

Nausea: a disorder characterized by a queasy sensation and/or the urge to vomit.

Vomiting: a disorder characterized by the reflexive act of ejecting the contents of the stomach through the mouth.

### 50.2.2 Types

Acute onset: within 24 h of chemotherapy administration (peak at 4–6 h) lasting for 24–48 h.

Delayed onset: occurs more than 24 h after chemotherapy (peak at 2–3 days) lasting for a prolonged period of time.

### 50.2.3 Pathophysiology

1. Direct activation of the vomiting center in the brain stem by chemotherapy, which triggers target organs in the GI tract.
2. Damage to the GI mucosa, causing vagal stimulation and neurotransmitter (serotonin, neurokinin-1, dopamine) release causing reflexive stimulation of the vomiting center.
3. Radiotherapy-induced neurotransmitter release stimulating the vomiting center concomitant with brain edema.

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### 50.2.4 Causes

*Induced directly by conditioning chemoradiotherapy*  
 TBI, TLI, cranio-spinal irradiation  
 Chemotherapy drugs (NCCN 2017):

- High emetic risk (frequency >90%): CY > 1500 mg/m<sup>2</sup>, BCNU >250 mg/m<sup>2</sup>
- Moderate emetic risk (frequency 30–90%): bendamustine, BU, BCNU ≤250 mg/m<sup>2</sup>, CY ≤1500 mg/m<sup>2</sup>, MEL
- Minimal to low emetic risk (frequency <30%): VP, TT, FLU, MTX ≤50 mg/m<sup>2</sup>

*Drugs: opioids, CNI, nystatin, AmB, voriconazole, itraconazole, TMP-SMX, MMF*  
*GVHD*  
*Hepatic disease: GVHD, VOD, viral hepatitis*  
*Infection: CMV, HSV, VZV, fungal, bacterial, norovirus, rotavirus, parasites*  
*Adrenal insufficiency*  
*Pancreatitis*

### 50.2.5 Diagnosis

Based on symptoms.

### 50.2.6 Grading (CTCAE v4.0 [National Cancer Institute 2009])

<i>Nausea</i>	
Grade 1	Loss of appetite without alteration of eating habits
Grade 2	Oral intake decreased without significant weight loss, dehydration, or malnutrition
Grade 3	Inadequate oral caloric or fluid intake, tube feeding, TPN, or hospitalization indicated
<i>Vomiting</i>	
Grade 1	1–2 episodes (separated by 5 min) in 24 h
Grade 2	3–5 episodes (separated by 5 min) in 24 h
Grade 3	≥6 episodes (separated by 5 min) in 24 h, tube feeding, TPN, or hospitalization indicated
Grade 4	Life-threatening consequences, urgent intervention indicated

### 50.2.7 Treatment

Prevention of nausea/vomiting is the mainstay of clinical management since treatment frequently proves ineffective. Delayed nausea should be

treated with scheduled antiemetics for 2–4 days after completion of chemotherapy.

Breakthrough treatment	Addition of a different class of antiemetic drug Prochlorperazine (10 mg IV q6h) Haloperidol (1–2 mg q4h) Metoclopramide (0.5–2 mg/kg IV q6h) Olanzapine Scopolamine transdermal patch Corticosteroids Lorazepam
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### 50.2.8 Prophylaxis

Choice of drugs depends on the use of drug with highest emetogenic potential (NCCN 2017):

High emetic risk	Serotonin (5-HT <sub>3</sub> antagonist) (patients should be monitored for QT corrected prolongation) <ul style="list-style-type: none"> <li>• Short-acting: ondansetron 3 × 8 mg IV on days of chemo +24 to 48 h, granisetron, dolasetron</li> <li>• Long-acting: palonosetron 0.25 mg IV, may be repeated every 3 days</li> </ul> Plus Neurokinin-1 receptor antagonists, e.g., aprepitant Plus/minus Dexamethasone 2–10 mg IV (as required for a short duration)
Moderate emetic risk	Serotonin (5-HT <sub>3</sub> ) antagonists (as above) Plus/minus Dexamethasone 2–10 mg IV
Low emetic risk	Serotonin (5-HT <sub>3</sub> ) antagonists (short acting, as above) Metoclopramide Prochlorperazine
TBI	Serotonin (5-HT <sub>3</sub> ) antagonists (short- or long-acting, as above) Dexamethasone (4 mg/day or 4 mg bid)

### 50.2.9 Other Nausea/Vomiting

Anticipatory nausea/vomiting	Prevention of nausea/vomiting by efficient prophylaxis at every treatment Strong smell avoidance Behavioral therapy Lorazepam, alprazolam
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## 50.3 Diarrhea

### 50.3.1 Definitions

A disorder characterized by frequent and watery bowel movements.

### 50.3.2 Physiopathogeny

Depending on the cause.

### 50.3.3 Causes

The diarrhea in the preengraftment period is most frequently caused by toxicity of conditioning. In the posttransplant period, aGVHD must be taken into consideration. The risk of infectious causes persists for the whole time with bacterial causes predominating relatively earlier than viral infections.

#### *Chemotherapy and radiation therapy-related toxicity*

#### *Acute GVHD*

#### *Intestinal infections:*

- *Clostridium difficile*
- Viral (CMV, VZV, rotavirus, norovirus, astrovirus, adenovirus)
- Parasitic (giardia, strongyloides, cryptosporidium)
- Fungal (candida)

*Medications* (antibiotics, mycophenolate mofetil, oral nutritional supplements)

#### *Transplant-associated microangiopathy*

*Other:* pancreatitis/pancreatic insufficiency, lactose intolerance/disaccharidase deficiency, malabsorption, inflammatory bowel disease, liver and gallbladder disease

### 50.3.4 Diagnosis

The standard workup for diarrhea after HCT includes stool cultures, tests for *Clostridium difficile* toxin A and B, *Clostridium* antigen, stool and/or blood tests for viruses, and, when negative, endoscopy with biopsy for aGVHD and CMV. However, when these tests are proven negative, a broad area of causes must be considered (Robak et al. 2017).

#### *Stool examination and microbiological workup*

- *C. difficile* toxin, antigen, culture
- Parasites (giardia, strongyloides, cryptosporidium)
- Viruses (CMV, VZV, rotavirus, norovirus, astrovirus, adenovirus)
- Fungi (culture)

#### *Sigmoidoscopy/colonoscopy ± gastroscopy*

- Histopathology for GVHD, cryptosporidium, and CMV
- Viral, parasitic/bacterial cultures

#### *Biochemistry* (triglycerides, amylase, lipase)

*GVHD biomarkers* (calprotectin, REG3- $\alpha$ ) (Rodriguez-Otero et al. 2012; Ferrara et al. 2011)

*Ultrasound, CT* (in GVHD distal ileum or proximal colon most likely involved)

#### *Capsule endoscopy*

### 50.3.5 Grading

When the diagnosis of gut aGVHD is established or suspected, aGVHD grading should be used as described in Chap. 43. Otherwise, (CTCAE v4.0) grading should be used (National Cancer Institute 2009).

Grade 1	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline
Grade 2	Increase of 4–6 stools per day over baseline; moderate increase in ostomy output compared to baseline
Grade 3	Increase of $\geq 7$ stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care activities of daily living
Grade 4	Life-threatening consequences; urgent intervention indicated

### 50.3.6 Treatment

*Targeted*, according to the known or suspected cause, consider overlap with another pathology (e.g., aGVHD with gut CMV infection)

#### *Ancillary:* modification of diet

- Lactose- or gluten-free
- Restricted diet (low roughage, low residue, low or no lactose)
- Temporarily nothing per os and TPN

*Avoid* fluid loss and dyselectrolytemia

*Monitor* and replace protein losses (albumin, gamma globulin)

*Loperamide* 2–4 mg p.o. every 6 h if associated with toxicity of conditioning or GVHD

#### *Octreotide*

## 50.4 Esophagitis/Gastritis

### 50.4.1 Definitions/Symptoms

Heartburn and/or epigastric pain observed most frequently during conditioning and period of mucositis.

### 50.4.2 Causes

Mucositis, medications, altered gastric pH, peptic ulcer disease, and fungal esophagitis.

### 50.4.3 Diagnosis

Based on clinical symptoms  $\pm$  endoscopy.

### 50.4.4 Treatment

Depending on the cause, elevation of the head of bed, and consideration of proton pump inhibitors and other symptomatic treatments (e.g., alginate, antacid, and topical local anesthetics, such as oxetacaine for mucositis). May require systemic analgesia if patient unable to swallow.

## 50.5 GI Bleeding

### 50.5.1 Definitions/Symptoms

May appear as melena, hematemesis or bloody stool, or emergence of normocytic anemia.

### 50.5.2 Causes

Thrombocytopenia, esophageal trauma, esophagitis, colitis, anal fissures or varices, viral infections, GVHD, and plasma coagulation impairment.

### 50.5.3 Diagnosis

Esophagogastroduodenoscopy, colonoscopy, and angioCT.

### 50.5.4 Treatment

Treatment of underlying disorder

Symptomatic

- Platelet transfusion to  $>50 \times 10^9/L$
- RBC transfusion
- Fresh frozen plasma, fibrinogen concentrates, vitamin K supplementation
- Octreotide
- Endoscopic cauterization or embolization

When massive blood loss

- Desmopressin
- Tranexamic acid
- Recombinant factor VII

## 50.6 Typhlitis

### 50.6.1 Definitions/Symptoms

Necrosis of usually large intestinal wall associated with chemotherapy toxicity and bacterial overgrowth.

Occurs within 30 days after HCT; patients usually complain of pain in the right lower abdominal quadrant, often associated with fever.

Additionally, nausea, emesis, increased abdominal wall tension, and watery bloody diarrhea may occur (Robak et al. 2017).

### 50.6.2 Causes

Toxicity/infection.

### 50.6.3 Diagnosis

Clinical and abdominal ultrasound or CT: bowel wall thickening usually limited to single region, e.g., ileocecal or ascending colon; may be associated with perforation and air within intestinal wall.

### 50.6.4 Treatment

Antibiotics and bowel rest. Avoid surgical intervention.

## 50.7 Pancreatic Disease

### 50.7.1 Definitions/Symptoms

Pancreatic insufficiency and atrophy or acute pancreatitis.

### 50.7.2 Causes

Medications (prednisone, tacrolimus), stones, and pancreatic GVHD.

### 50.7.3 Diagnosis

Insufficiency and atrophy: low serum trypsinogen, high fecal elastase-1, and possible atrophy in imaging. Acute pancreatitis: elevated lipase and amylase, elevated fecal fat, and edema in ultrasound/CT.

### 50.7.4 Treatment

When insufficiency: enzyme replacement.

## 50.8 Chronic Esophageal GVHD

### 50.8.1 Definitions/Symptoms

Dysphagia to solid food, chest discomfort, and aspiration (Jagasia et al. 2015; Robak et al. 2017).

### 50.8.2 Diagnosis

Barium meal: mid-/upper esophageal strictures, webs, rings, bullae, and desquamation. Endoscopy: as above, erythematous, friable sloughed mucosa.

### 50.8.3 Treatment

When severe and chronic, need serial dilations and enteral tube placement or esophagectomy.

#### Key Points

The workup and management of GI complications after HCT follow a general medical approach; however, the most frequent scenarios remain characteristic for this patient population. The most common causes include toxicity of drugs, especially those used for conditioning, infection, and/or graft-versus-host disease:

- Nausea/vomiting or diarrhea occurring before engraftment is most likely caused by toxicity of conditioning, while after engraftment, GVHD needs to be considered, especially in the allo-HCT setting.
- For the whole posttransplant period, infectious causes should also be considered with bacterial or fungal causes predominating in the neutropenic period and viral reactivations/infections in the later phases.
- Importantly, inflammation caused by infection may become a trigger to GVHD, while GVHD is frequently followed by infection; therefore, overlapping scenarios always need to be taken into account.
- GI GVHD is frequently a diagnosis of exclusion (especially in patients with other overlapping causes which may impact on laboratory investigations). However, it should always be considered when symptoms persist despite extensive workup and/or directed treatment.

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# Haemorrhagic Cystitis and Renal Dysfunction

# 51

Simone Cesaro

## 51.1 Haemorrhagic Cystitis

### 51.1.1 Introduction

Haemorrhagic cystitis (HC) is a frequent complication after haematopoietic cell transplantation (HCT). According to the time of occurrence after HCT, HC is defined as early onset and late onset. Early-onset HC occurs typically during or within 48 h after the end of the conditioning regimen, and it is the result of a direct toxic effect of drug metabolites and radiotherapy on the bladder mucosa. Late-onset HC usually starts around the time of neutrophil engraftment (weeks 2–4) up to the second to third month after HCT (Hirsch and Pergam 2016). Haematuria, with or without symptoms, is classified into four categories: microscopic (grade 1), macroscopic (grade 2), macroscopic with clots (grade 3) and macroscopic with clots and renal failure secondary to urinary tract obstruction (Droller et al. 1982; Bedi et al. 1995). HC is associated with haematuria >grade 2.

### 51.1.2 Risk Factors

The presence of pro-haemorrhagic abnormalities of coagulation, severe thrombocytopenia and

mucosal inflammation are predisposing factors for any type of HC.

The main risk factor for late-onset HC is infection by polyomavirus BK (BKPyV), whereas other viruses such as *adenovirus*, *cytomegalovirus* and *JC polyomavirus* have been rarely implicated. BKPyV is a common cause of asymptomatic or mild flu-like infection during early infancy and childhood, and more than 90% of adults are seropositive for BKPyV. The route of transmission is by droplets or contact with oral saliva and respiratory tract secretions. The virus persists latently in renal tubular epithelial and urothelial cell and can replicate as the host virus-specific T-cell response is lost or severely weakened. Mild to moderate asymptomatic BKPyV viruria is seen in 5–10% of healthy individuals, especially the oldest and the pregnant women, whereas high-load BKPyV viruria is detected in 50–60% of patients who underwent allogeneic HCT due to the severe immunosuppression condition.

BKPyV viruria develops in more than 50% of allogeneic HCT, but overt HC occurs in about 20% of patients because several patient- or transplant-related factors affect its occurrence: the type of graft (CB and PB > BM); the type of donor (Haplo > URD > MRD); the type of conditioning regimen (MAC > RIC); the use in the conditioning regimen of ATG, CY, or BU; the occurrence of acute GVHD grade 2–4; and, among the paediatric patients, a recipient age >7 years.

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### 51.1.3 Pathogenesis

The cause of post-HCT HC is multifactorial and includes the combined effects of the extensive viral cytopathic damage of the bladder mucosa, the chemical or actinic damage induced by the conditioning regimen and the immune donor-derived alloreactivity targeting bladder mucosa (Cesaro et al. 2018). In patients receiving allogeneic HCT, BKPyV viremia  $>10^7$  genomic copies/mL and BKPyV viremia  $>10^3$  genomic copies/mL are predictive factors for BKPyV-HC (Cesaro et al. 2015).

### 51.1.4 Diagnosis

The diagnosis of BKPyV-HC is defined by the presence of macrohaematuria ( $>$ grade 2), of clinical symptoms/signs of cystitis (dysuria, increased urinary frequency, lower abdominal pain) and of high-load BKPyV viremia ( $>10^7$ ) (Cesaro et al. 2018). Two-thirds of patients have also BKPyV viremia ( $>10^3$ ) (Erard et al. 2005; Cesaro et al. 2015). Other infectious and non-infectious causes of HC must be excluded. Instrumental examination such as ultrasound or CT scan shows signs of bladder inflammation (oedema and thickening of bladder wall) and the presence of intra-bladder clots or obstruction of urinary tract.

The reduction of both BKPyV viremia and BKPyV viremia has been correlated with clinical recovery from HC. The monitoring of BKPyV viremia and viremia of HCT patients is not recommended since the type of pre-emptive intervention is not established.

### 51.1.5 Prophylaxis

Effective preventive measures are possible only for early-onset HC in patients who receive high dose of CY as part of the conditioning regimen or GVHD prophylaxis (HCT with PTCy). These are based on hyperhydration and the administration of continuous intravenous infusion of mesna, which reduce the exposure of the bladder mucosa to acrolein and other toxic catabolites.

The bladder irrigation through a two- or three-way urinary catheter is no more effective than hyperhydration, and considering the invasiveness and patient discomfort, its use is not recommended.

In the late-onset HC, BKPyV replication has a key role in exacerbating the damage of bladder mucosa through its cytopathic effect and in inducing the donor immune alloreactivity targeting the bladder mucosa. Although fluoroquinolones inhibit *in vitro* BKPyV, the efficacy *in vivo* is weak and do not affect significantly the incidence of HC. Considering the risk of inducing bacterial resistance and tendon and joint damages in children, fluoroquinolones are not recommended for HC prophylaxis.

### 51.1.6 Treatment

Cidofovir is a nucleotide analogue inhibiting several DNA viruses including BKPyV. Its long half-life (15–65 h) allows the administration every 1 or 2 weeks. Given the significant risk of tubular nephrotoxicity, cidofovir has been used only for therapeutic purposes (Cesaro et al. 2009). The nephrotoxicity can be limited by saline hydration and by the use of probenecid that inhibits the capture and transport of cidofovir into the renal tubular epithelial cells. There is no agreement on the optimal dose, modality of administration and frequency of administration. One scheme is the intravenous administration of cidofovir, 3–5 mg/kg body weight, weekly or fortnightly, together with probenecid to prevent nephrotoxicity. Mild to moderate increase in serum creatinine is observed in 18% of the patients. Another scheme is the administration of low-dose intravenous cidofovir, 0.5–1.5 mg/kg body weight, 1–3 times a week, without probenecid (Ganguly et al. 2010). Mild to moderate renal toxicity is reported in 20% of patients. Alternatively, cidofovir can be administered intravesically to reduce the risk of nephrotoxicity, at the dose of 5 mg/kg/body weight/week and left *in situ* for 1–2 h after clamping the vesical catheter, the response rate being about 50% (Bridges et al. 2006).

Some results have been reported with leflunomide, an antimetabolite drug with immunomodulatory and antiviral activity. Anecdotal use of other agents (vidarabine, oral levofloxacin, FXIII concentrate, intravesical sodium hyaluronate and oestrogens) is reported in the literature (Cesaro et al. 2018).

The recovery from HC, whatever the cause, can benefit from treatment aiming to repair and regenerate the urothelial mucosa (hyperbaric oxygen therapy) and to stop bleeding (topical application of fibrin glue or platelet-rich plasma). The main drawback of hyperbaric oxygen is the limited availability, the requirements for dedicated hyperbaric room facilities, the risk of ear barotrauma or pressure intolerance and claustrophobia episodes during the procedure (Zama et al. 2013; Cesaro et al. 2018). Cystoscopic application of fibrin glue to the damaged bleeding bladder mucosa has been associated with a response rate of 83%, with most of cases resolved with one or two applications (Tirindelli et al. 2014).

The mesenchymal stromal cells (MSC) and the adoptive immunotherapy represent the most recent innovative treatment experimented for HC. MSC have the potential to stimulate the tissue repairing process and exercise an immune modulatory and anti-inflammatory effect. The use of third-party MSC infusion into seven patients with BKPyV-HC obtained the resolution of haematuria in five patients (Ringden et al. 2007). This approach needs to be validated further to assess the feasibility and also the safety of MSC.

Adoptive transfer of donor-derived virus-specific T cells (VSTs) has shown efficacy for the treatment of several viral infections although their use on a larger scale is limited by the costs, the complexity of manufacturing, the time needed to obtain the final cell product that is not suitable for the urgent treatment and the prompt availability of a seropositive donor. The use of banked VSTs obtained by a third-party healthy seropositive donor, cryopreserved and used as the patient develops a viral infection refractory to antiviral treatment represents a promising development (Olson et al. 2021). In phase II trials, the use of

VSTs directed against BKPyV obtained an overall response rate of 81–92%. The infusions of VSTs resulted safe with no cases of moderate–severe GVHD. Importantly, the VSTs expanded *in vivo* and the functionality persisted for up to 12 weeks (Tzannou et al. 2017). These results are encouraging and they need the confirmation by prospective larger studies.

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## 51.2 Renal Dysfunction

Acute kidney injury (AKI) occurs in 27–66% of patients who underwent allo-HCT mainly within the first 100 days. The incidence of AKI is lower in autologous than in allogeneic HCT due to several reasons: rapid engraftment, lower incidence of infectious complications, absence of GVHD and its inflammatory cytokines, absence of CMV infection, lower frequency of severe diarrhoea and dehydration and less drug-induced nephrotoxicity (Lopes et al. 2016; Raina et al. 2017).

The diseases associated with AKI act at different renal levels: prerenal (sepsis, engraftment syndrome, SOS/VOD), renal glomerular (transplant-associated microangiopathy), renal tubular (acute tubular necrosis due to dehydration, sepsis, shock, engraftment syndrome, intratubular obstruction due to drugs, or tumour lysis syndrome), renal interstitial (acute GVHD, viral infection by BKPyV or ADV) and post-renal (obstruction by BKPyV or adenovirus cystitis, retroperitoneal fibrosis, lymphadenopathy).

General risk factors for AKI are pre-HCT diabetes, hypertension and renal impairment; the use of nephrotoxic drugs for the conditioning regimen (ifosfamide, CY, carboplatin, cisplatin), for the treatment of GVHD (MTX, cyclosporine, tacrolimus), for the treatment of infections (liposomal amphotericin B, aminoglycosides, vancomycin) and for the treatment of other severe organ damages that require ICU admission and mechanical ventilation (Hingorani 2016).

Clinically, the severity of AKI is defined by serum creatinine (SCr) and urine output (UO) that permits the identification of three groups: patient at risk of AKI (SCr increase of 1.5–2× and

UO <0.5 mL/kg/h for >6 h); patient with kidney injury (SCr increase of 2–3× and UO <0.5 mL/kg/h for >12 h); and patient with kidney failure (SCr increase of >3× and UO <0.3 mL/kg/h for >24 h or anuria >12 h, or initiation of replacement therapy) (Lopes et al. 2016).

AKI represents a risk factor for the development of chronic kidney disease on the medium–long-term period, especially if the acute damage is not completely resolved and proteinuria and hypertension persist and for the increase of non-relapse and overall mortality (Shingai et al. 2015).

### Key Points

	Early-onset HC	Late-onset HC	Comments
Incidence	<3%	7–25%	Early-onset HC is nowadays rare
Pathogenesis	Chemical (drugs) or actinic damage of bladder mucosa	BK virus infection Adenovirus infection Donor alloreactivity	
Diagnosis	Macrohaematuria with dysuria, increased urinary frequency, low abdominal pain	Macrohaematuria with dysuria, increased urinary frequency, low abdominal pain, high load of BK viruria and/or viremia	Signs of bladder inflammation at ultrasound examination
Prevention	Hyperhydration, mesna (if chemo with Cy), forced diuresis	Hyperhydration, forced diuresis	Fluoroquinolones not recommended
Therapy	Hyperhydration Forced diuresis Hyperbaric O <sub>2</sub> therapy (HOT) Application of fibrin glue by cystoscopy	Hyperhydration, forced diuresis IV (or intravesical) cidofovir HOT Application of fibrin glue or platelet-rich plasma by cystoscopy	No agreement On dose and route of Cidofovir administration Limited evidence/experience for HOT, fibrin glue, platelet-rich plasma and other treatments
Experimental	/	Virus-specific T cells (BK virus, Adenovirus) Mesenchymal cells	

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# Noninfectious Pulmonary Complications

Anne Bergeron and Kenneth R. Cooke

## 52.1 Introduction

Lung injury occurs frequently following HCT and significantly contributes to morbidity and mortality in the immediate posttransplant period and in the months and years that follow. In aggregate, pulmonary dysfunction can be observed in 25–55% of recipients (Cooke and Yanik 2016; Haider et al. 2020). In this context, an NIH workshop was recently convened to specifically identify clinical challenges and scientific knowledge gaps regarding pulmonary dysfunction in pediatric HCT recipient (Tamburro et al. 2021).

Historically, approximately half of all pulmonary complications seen after HCT were secondary to infection, but the judicious use of broad-spectrum antimicrobial agents has tipped the balance toward noninfectious causes.

Noninfectious lung injury following HCT may be mediated by immune or nonimmune mechanisms and accounts for up to 50% of noninfectious mortality after allo-HCT.

These complications have been classified by the American Thoracic Society according to the tissue primarily injured and its etiology (Panoskaltzis-Mortari et al. 2011) (Table 52.1).

**Table 52.1** Noninfectious pulmonary complications after HCT<sup>a</sup>

Localization	Entity
Pulmonary parenchyma	– Acute interstitial pneumonitis <sup>b</sup>
	– Acute respiratory distress syndrome (ARDS) <sup>b</sup>
	– BCNU pneumonitis
	– Radiation pneumonitis
	– Delayed pulmonary toxicity syndrome <sup>b</sup>
	– Post-HCT lymphoproliferative disease (see Chap. 45)
	– Eosinophilic pneumonia
	– Pulmonary alveolar proteinosis
Vascular endothelium	– Peri-engraftment respiratory distress syndrome (PERDS) <sup>b</sup>
	– Capillary leak syndrome (CLS) <sup>b</sup> (see Chap. 42)
	– Diffuse alveolar hemorrhage (DAH) <sup>b</sup>
	– Pulmonary VOD
	– Transfusion-associated acute lung injury
	– Pulmonary cytolytic syndrome
	– Pulmonary arterial hypertension
	– Pulmonary thromboembolism

(continued)

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**Table 52.1** (continued)

Localization	Entity
Airway epithelium	<ul style="list-style-type: none"> <li>– Interstitial lung disease including cryptogenic organizing pneumonia (COP)<sup>b, c</sup></li> <li>– Bronchiolitis obliterans syndrome (BOS)</li> </ul>

<sup>a</sup> Importantly, this classification does not include the most frequent lung complication after HCT, i.e., pulmonary edema secondary to fluid overload

<sup>b</sup> All these complications could be categorized as IPS if occurring early after HCT

<sup>c</sup> Formerly called bronchiolitis obliterans organizing pneumonia (BOOP)

## 52.2 Approach to Diagnostic Workup for Pulmonary Complications After HCT

Ideally, any respiratory/pulmonary complication observed after HCT whether early or late onset should be evaluated following predetermined, institutional, standard of practice guidelines (Lucena et al. 2014). Workup requires attention to pulmonary and non-pulmonary causes. Respiratory distress may progress rapidly once identified; hence, a timely workup including assessment of pulmonary and cardiac function, imaging, and procurement of samples to rule out infection is critical for optimizing outcomes (Tamburro et al. 2021; Cooke and Yanik 2016):

1. *Assessment of oxygen saturation/oxygen requirement*: Pulse oximetry or arterial blood gas analysis if indicated. For late-onset noninfectious pulmonary complications (LONIPC), pulse oximetry following a hall walk is suggested.
2. *Imaging*: Chest radiographs (particularly in children) or CT scanning to assess for the presence of lobar, multi-lobar, or diffuse pulmonary infiltrates. For early-onset lung injury, a low-dose CT can suffice. For LONIPC, a CT with inspiratory cuts is mandatory, and expiratory views to assess for air trapping should be discussed. Echocardiography can be considered to identify left-heart dysfunction or pulmonary hypertension.
3. *Pulmonary function testing (PFTs)*: PFTs are rarely indicated in the context of acute lung complications but can be considered. For LONIPC, PFTs including spirometry (without and with albuterol), lung volumes, and DLCO are recommended to assess for signs for either restrictive or obstructive pulmonary dysfunction and to assess severity (Jagasia et al. 2015; Wolff et al. 2021). Assessing PFTs in pediatric patients remains a challenge particularly in young children (Tamburro et al. 2021).
4. *Infectious disease testing*: Blood samples for bacterial culture and detection of viral replication; sputum culture; nasopharyngeal swabs for rapid viral panels including SARS-CoV-2, respiratory syncytial virus (RSV), parainfluenza virus (PIV), adenovirus (ADV), rhinoviruses, influenza A and B (in season), metapneumovirus, and others; urinary antigen tests; blood samples for galactomannan and beta-D-glucan assays.
5. *Fiber-optic bronchoscopy (FOB) with bronchoalveolar lavage (BAL)* (strongly encouraged): Complete in collaboration with specialists in pulmonary medicine and infectious diseases. PCR for *Pneumocystis jirovecii* (PJ), *Legionella*, and *Mycoplasma*. Cultures and stains for bacteria, fungi, and AFB along with galactomannan assay. If BAL cannot be completed, consider approaches to induce and collect sputum for the assays noted above:
  - If positive, treat accordingly.
  - If negative, consider empiric treatment and consideration for idiopathic pneumonia syndrome (IPS) and other etiologies (see below).
6. *Tissue biopsy*: While not encouraged, in some selected cases, e.g., later-onset cases where the diagnosis is not established by noninvasive tests or those poorly responsive to antimicrobial or anti-inflammatory therapy (see below), a tissue biopsy could be considered using either transbronchial (less favored) or minimally invasive, video-assisted thoracoscopic surgery (VATS) (Dieffenbach et al. 2019).

### 52.2.1 Results Reported Using This Methodology (Seo et al. 2015; Lucena et al. 2014; Shannon et al. 2010; Yanik et al. 2008a, b)

FOB/BAL permits an etiological diagnosis in up to 78% of cases.

In suspected IPS, a BAL study may detect a pathogen in ~50% of cases.

For pathogen detection, early FOB (<5 days) offers better yield than late FOB; hence, completing FOB as soon as possible is preferred.

The risk of complications with FOB is <5%.

Note: Identifying an organism in pulmonary secretions does not always imply causality, and infectious and immune-mediated inflammation can occur concurrently.

Diagnosis	PE should be suspected in the context of weight gain, an increased cardiothoracic index on imaging, and crackles/rales. Though rarely necessary, the diagnosis can be confirmed by pulmonary pressure measurements.
Differential diagnosis	<ul style="list-style-type: none"> <li>– Heart failure (prior anthracycline toxicity or conditioning with CY).</li> <li>– Endothelial syndromes: SOS, CLS, ES (see Chaps. 42 and 49).</li> <li>– Respiratory tract infections:             <ul style="list-style-type: none"> <li>• Idiopathic pneumonia syndrome (IPS)</li> </ul> </li> <li>– Posttransfusion reactions (TRALI).</li> </ul>
Treatment	Goal: Re-establish fluid homeostasis/euvolemia via fluid restriction and diuretics.

## 52.3 Acute Noninfectious Pulmonary Complications After HCT

### 52.3.1 Pulmonary Edema due to Fluid Overload

Despite not being included in most classifications of pulmonary complications after HCT, pulmonary edema (PE) as a consequence of a fluid overload (FO) is extremely frequent (Rondón et al. 2017):

Incidence	FO may be observed in up to 60% of patients early after HCT. The exact incidence of PE is not established although it could be higher than 20%.
Symptoms and signs	<ul style="list-style-type: none"> <li>– Weight gain, shortness of breath, nonproductive cough, moderate hypoxemia.</li> <li>– Crackles and rales in both lung bases.</li> <li>– Chest radiology with diffuse alveolar/interstitial infiltrates.</li> </ul>

### 52.3.2 Idiopathic Pneumonia Syndrome

#### 52.3.2.1 Definition

It is a widespread alveolar injury in the absence of active lower respiratory tract infection, cardiac or renal dysfunction, and iatrogenic fluid overload (Clark et al. 1993; Panoskaltzis-Mortari et al. 2011).

#### 52.3.2.2 Clinical Manifestations

Signs and symptoms of IPS classically occur within 120 days (but as early as 3 weeks and as late as 180 days) after HCT and include fever, nonproductive cough, dyspnea, tachypnea, hypoxemia, rales, and diffuse alveolar or interstitial infiltrates on X-rays or CT scans.

#### 52.3.2.3 Diagnosis

All of the following must be present for the diagnosis of IPS:

1. *Evidence of widespread alveolar injury:*
  - (a) Multi-lobar infiltrates on chest radiographs or CT
  - (b) Symptoms and signs of pneumonia (cough, dyspnea, tachypnea, crackles/rales)
  - (c) Evidence of abnormal pulmonary physiology  
Increased alveolar to arterial oxygen difference;  
need for supplemental O<sub>2</sub> therapy  
New or increased restrictive PFT abnormality
2. *Absence of active lower respiratory tract infection based upon:*
  - (a) BAL negative for significant bacterial pathogens including acid-fast bacilli, *Nocardia*, and *Legionella* species  
Routine culture for bacteria, fungi, and AFB and shell vial culture for CMV  
PCR for respiratory viruses and CMV  
Cytology for CMV inclusions, fungi, and *Pneumocystis jirovecii*
  - (b) BAL negative for pathogenic nonbacterial microorganisms  
Routine culture for bacteria, fungi, and AFB and shell vial culture for CMV  
PCR for respiratory viruses and CMV  
Cytology for CMV inclusions, fungi, and *Pneumocystis jirovecii*
  - (c) Other organisms/tests to also consider:  
PCR for human metapneumovirus, rhinovirus, coronavirus, and HHV6  
PCR for *Chlamydia*, *Mycoplasma*, and *Aspergillus* spp.  
Serum and BAL fluid GM for *Aspergillus* species, serum beta-D-glucan
3. *Absence of cardiac dysfunction, acute renal failure, or iatrogenic fluid overload as etiology for pulmonary dysfunction*

Biomarker incidence	<ul style="list-style-type: none"> <li>– sTNFR1, IL-6, MCP-1, Ang2, sCD14, and IL-8 are elevated in patients with IPS (Yanik et al. 2008a, b, 2015).</li> <li>– sTNRF1, IL-6, and ST-2 were most predictive in diagnosing IPS even before clinical signs and symptoms were present (Seo et al. 2017).</li> <li>– In addition, a biomarker panel including ST2, IL-6, and sTNFR1 could, as early as day 7, predict respiratory failure and associated mortality after HCT (Rowan et al. 2022).</li> <li>– The strict definition required to establish a diagnosis and the increased use of RIC have significantly reduced the incidence of IPS from that observed in the past (~25%).</li> <li>– This reduction runs in parallel of the improvement in the diagnostic methodologies to detect infectious pathogens (Seo et al. 2015; Zinter et al. 2019).</li> <li>– Currently, the incidence of IPS is &lt;10% of allo-HCT (7% after MAC with ≥12 Gy TBI; 2% after RIC in adults) (Wenger et al. 2020; Sano et al. 2014).</li> </ul>
Timing	<ul style="list-style-type: none"> <li>– Generally, within first 120 days after BMT but can be observed between days 21 and 180.</li> <li>– Late IPS (up to 180 days post-HCT) can be observed but is rare (Thompson et al. 2017).</li> </ul>
Risk factors (from Cooke and Yanik 2016)	<ul style="list-style-type: none"> <li>– Older age/Karnofsky index &lt;90/ higher interval diagnosis HCT. MAC or TBI (≥12 Gy)/HLA disparity/ GVHD prophylaxis with MTX. Acute GVHD/previous viral infection/ other malignancies than leukemia.</li> </ul>

**52.3.2.4 Pathogenesis, Incidence, Presentation, and Risk Factors**

Pathogenesis	<ul style="list-style-type: none"> <li>– The pathophysiology of IPS is complex. Data generated using experimental models support IPS as a process in which the lung is susceptible to cellular and soluble inflammatory effectors. These distinct but related pathways of inflammation culminate in the recruitment of immune cells to the lung leading to tissue damage and dysfunction (Cooke and Yanik 2016).</li> <li>– Recent work has also underscored a role for the pulmonary microbiome and metatranscriptome to the development of lung inflammation after HCT (Zinter et al. 2021).</li> </ul>
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**52.3.2.5 Treatment and Prognosis**

Supportive measures	<p>Supplemental O<sub>2</sub> therapy Noninvasive [high-flow nasal O<sub>2</sub>, CPAP] or, when needed, invasive (mechanical) ventilatory support:</p> <ul style="list-style-type: none"> <li>– Empiric broad-spectrum antimicrobials</li> <li>– Strict control of fluid balance/ hemofiltration</li> </ul>
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Specific treatment	<p>Lung injury in IPS can occur through two pathways, the TNF-<math>\alpha</math>/LPS-dependent and IL6/IL17-dependent (Tamburro et al. 2021; Cooke and Yanik 2016; Varelias et al. 2015) informing current treatment options:</p> <ul style="list-style-type: none"> <li>• <i>Methyl-PDN</i> <math>\leq 2</math> mg/kg/day; if not clear response, consider as soon as possible.</li> <li>• Anti-TNF<math>\alpha</math>: <i>etanercept</i> 0.4 mg/kg twice weekly (maximum of 8 doses) + systemic steroids (2 mg/kg/day). In a phase II trial in children, the CR rate was 71%, and 1 year survival was 63% (Yanik et al. 2015). This combination has also been shown to be effective in exceptional cases of late IPS with a 42% of CR and a 2-y survival of 62% among responders (Thompson et al. 2017). The randomized study of <i>etanercept</i> + steroids vs. steroids + placebo was terminated prematurely due to slow accrual. In the limited number of patients examined, there were no differences in response rates (<math>\approx 60\%</math>) at day +28. These results do not necessarily imply that this agent is not effective; lack of evidence in this under-accrued trial does not equate to lack of effectiveness (Yanik et al. 2014).</li> <li>• Other <i>investigational agents</i> such as: <ul style="list-style-type: none"> <li>– MoAb anti-IL6: <i>tocilizumab</i> (experimental IPS; Varelias et al. 2015). Note, IL-6 levels were found to be highest in patients not responsive to TNF blockade (Varelias et al. 2015) suggesting that strategies of combinatorial cytokine blockade may warrant future study (Tamburro et al. 2021).</li> <li>– MoAb anti-IL17: <i>brodalumab</i> (experimental IPS; (Varelias et al. 2015).</li> <li>– Strategies that protect/improve pulmonary vascular endothelial integrity (Klein et al. 2023).</li> </ul> </li> </ul>
Evolution	Despite the diagnosis and therapeutic advances, the mortality from IPS in adults remains high (Wenger et al. 2020).

### 52.3.3 Diffuse Alveolar Hemorrhage (DAH)

Diffuse alveolar hemorrhage (DAH) is a relevant cause of acute respiratory failure that occurs in 2–14% of recipients (Fan et al. 2020; Zhang et al.

2021). DAH is likely a consequence of damage to the alveolar capillary basement membrane (see Chap. 42). DAH can be noninfectious or infectious in etiology (Majhail et al. 2006).

#### 52.3.3.1 Clinical Aspects of DAH

Clinical manifestations	Usually observed within the first month after HCT (a median of 23 days), often during the pre-engraftment phase; however, later onset is encountered in up to 42% of cases The clinical manifestations are those of all IPS. Frank hemoptysis is rare.
Diagnosis	Based on BAL: the progressive bloodier return of BAL fluid aliquots, in at least three segmental bronchi, indicating the presence of blood in the alveoli (or 20% hemosiderin-laden macrophage, although their absence does not exclude the diagnosis as it can take 72 h to appear).
Risk factors	– Higher incidence after TBI and high-dose CY.
Differential diagnosis with	– Classic IPS: very difficult, only by means of BAL. IPS usually appears after engraftment, predominates in allo-HCT, and is less responsive to steroids, <i>Note</i> : Noninfectious DAH falls under the “diagnostic umbrella” of IPS (Panoskaltis-Mortari et al. 2011). – PERDS: almost impossible except for LBA progressively bloodier. – DAH associated with infection: impossible without detection of the pathogen (Majhail et al. 2006).

#### 52.3.3.2 Treatment and Prognosis of DAH

Treatment	<ul style="list-style-type: none"> <li>– Traditionally with high doses of methyl-PDN (250–500 mg q6h <math>\times</math> 5 days followed by taper), but the overall response to this treatment is disappointing even when combined with aminocaproic acid (ACA) (Rathi et al. 2015). Of note, two-thirds of cases were noninfectious in origin and could be classified as IPS, but TNF<math>\alpha</math> inhibition was not used in this study.</li> <li>– When combined with high-dose PDN, inhaled, recombinant, factor VIIa has shown benefit in some (Park and Kim 2015) but not all (Elinoff et al. 2014) studies.</li> </ul>
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Prognosis	<ul style="list-style-type: none"> <li>– Poor: Overall mortality as high as 85% by day 100 (Rathi et al. 2015).</li> <li>– Less than 15% of patients die as a direct consequence of DAH, but the frequent evolution to MOF increases mortality to &gt;60% (30% in auto- and 70% in allo-HCT).</li> <li>– DAH that appears after auto-HCT has a better prognosis (Majhail et al. 2006).</li> </ul>
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(BOS) and diffuse interstitial lung diseases (ILD) being the most frequent (Bergeron et al. 2018). Before making the diagnosis of LONIPC, a respiratory infection must be ruled out. However, a respiratory infection (i.e., particularly viral) is often the trigger for LONIPC. Although BOS is currently recognized as the only manifestation of chronic GVHD based on epidemiological data, the question arises whether ILD may be another lung manifestation of cGVHD (Archer et al. 2023).

## 52.4 Late-Onset Noninfectious Pulmonary Complications (LONIPC)

LONIPCs, when defined as occurring beyond 100 days posttransplant, occur in up to 20% of allo-HCT recipients, including a variety of clinical entities, with bronchiolitis obliterans syndrome

### 52.4.1 Bronchiolitis Obliterans Syndrome (BOS)

Pathogenesis, timing, incidence, clinical manifestations, diagnosis, and radiology of BOS are shown in Table 52.2.

**Table 52.2** Main clinical characteristics of BOS

Pathogenesis	The same as cGVHD but specifically involving the lung (Cooke et al. 2017).
Risk factors	Extrathoracic cGVHD, respiratory viral infections, lung function decline (Bergeron et al. 2018; Sheshadri et al. 2019; Jamani et al. 2020; Abedin et al. 2015).
Timing and incidence	Onset after transplant: 6–24 months. Incidence: 3–11%.
Clinical manifestations	<ul style="list-style-type: none"> <li>– Breathlessness, nonproductive cough, and wheezing.</li> <li>– May be asymptomatic requiring routine PFT follow-up for an early detection (pretransplant baseline, then every 3 months in the first year after HCT and in patients who develop extrathoracic GVHD; close spirometry monitoring after a respiratory viral infection (Kitko et al. 2021)).</li> </ul> <p>In &gt;90% of the BOS, there is more or less severe chronic GVHD in other locations.</p>
Diagnosis	<p>Chronic GVHD in other locations<sup>a</sup> + PFT impairment (NIH consensus) (Jagasia et al. 2015; Wolff et al. 2021).</p> <ul style="list-style-type: none"> <li>– New-onset obstructive ventilatory defect with air trapping (FEV1/VC ratio &lt; 0.7 or the fifth percentile of predicted; FEV1 &lt; 75% of predicted with ≥10% decline from baseline; residual volume &gt; 120% of predicted) with no response to bronchodilators.             <ul style="list-style-type: none"> <li>• <i>Definitive</i> (not required): histologic confirmation by VATS (BO).</li> </ul> </li> </ul>
Lung CT scan prognosis	<p>Required at BOS diagnosis to rule out other diagnoses (lung parenchymal abnormalities should suggest either an alternative or an additional diagnosis). Pattern of constrictive bronchiolitis with attenuation in mosaic, bronchiectasis, and bronchial wall thickening and air trapping by expiratory CT<sup>b</sup> Bronchial abnormalities at 3 months posttransplant were found to predict BOS (Bergeron et al. 2018). Overall survival: 65–79% at 2 years and 74% at 5 years in the most recent studies (Arora et al. 2016; Archer et al. 2023). Worse prognosis if early onset after HCT (Bergeron et al. 2013). Morbidity: respiratory disability, respiratory exacerbations.</p>

DLCO transfer capacity of CO, FEV1 forced expiratory volume in the first second, FVC forced vital capacity, VATS video-assisted thoracoscopic surgery, CT computed tomography

<sup>a</sup> If the lung is the only organ with cGVHD, a biopsy is needed to confirm the diagnosis

<sup>b</sup> More than 30% of patients have air trapping on pretransplant lung CT scan limiting the value of expiratory chest CT in the diagnosis of posttransplant BOS (Bergeron et al. 2018). BOS diagnosis mainly relies on PFTs



The latest NIH consensus does not require lung biopsy; the diagnosis of chronic pulmonary GVHD is made on the basis of lung function data alone. Thus, we speak of BOS rather than BO (i.e., constrictive bronchiolitis), which requires histological analysis (Jagasia et al. 2015). BOS phenotypes not included in the current NIH diagnostic criteria do exist. On the one hand, BOS reflects various pathologies of the small airways (Meignin et al. 2018; Holbro et al. 2013), and on the other, PFTs of BOS may show a preserved FEV1/VC ratio (Bergeron et al. 2013; Wolff et al. 2021; Uhlving et al. 2015).

#### 52.4.1.1 Treatment of BOS

Few treatments have been prospectively and specifically evaluated for BOS, including those for chronic GVHD. Low levels of evidence prevent recommendation of any specific treatment algorithm. It is essential to weigh the benefit-risk balance before introducing any treatment, particularly corticosteroids, which are known to be of low efficacy for BOS.

The natural history of BOS is poorly understood (Cooke et al. 2017). FEV1 most often declines abruptly before stabilizing at a more or less severe value (Cheng et al. 2016; Bergeron et al. 2013, 2018). Secondary decline in lung function over time is often associated with infectious exacerbations. In this context, the endpoint for assessing the efficacy of any treatment on BOS is debated: stabilization or improvement of pulmonary function.

Only inhaled budesonide/formoterol has shown improvement of FEV1 for new-onset moderate to severe BOS in a double-blind placebo-controlled randomized trial (Bergeron et al. 2015). The FAM strategy (fluticasone, azithromycin, montelukast) that is commonly used for the treatment of BOS was evaluated in an open-label single-arm trial. In this study, FAM was associated with a stabilization of FEV1 (Williams et al. 2016). Azithromycin administration in allo-HCT recipients should be cautioned against a potential increased risk of cancer, particularly when given early after transplantation (Bergeron et al. 2017; Cheng et al. 2020; Vallet et al. 2022).

Current diagnostic criteria for BOS probably only allow detection of a late stage of the disease. Numerous studies are looking for early biomarkers of BOS (respiratory function, and/or radiology, and/or biology) in the hope of greater treatment efficacy at an earlier stage.

In patients with uncontrolled end-stage BOS, who have no or non-active extrathoracic GVHD and very low risk of hematological relapse, lung transplantation should be considered (Greer et al. 2018).

Respiratory rehabilitation should be offered to dyspneic patients to improve their quality of life. Other support measures include treatment of gastroesophageal reflux, anti-infectious prophylaxis, vaccination against respiratory viral infections, and IVIg in case of both infections and hypogammaglobulinemia.

#### 52.4.2 Interstitial Lung Diseases (ILD)

Three-year cumulative incidence of ILD was found to be 5% (Bergeron et al. 2018). Unlike BOS, the diagnosis of ILD relies on imaging showing the presence of lung parenchymal opacities in the absence of infection. Based on lung CT scan, ILD could be classified into three patterns: organizing pneumonia (OP, 37%), pleuro-parenchymal fibroelastosis (PPFE, 13%), and undetermined (50%) (Archer et al. 2023; Bondeelle et al. 2020). The “undetermined” CT scan pattern corresponds to different clinico-histological diagnoses, including non-specific interstitial pneumonia (Meignin et al. 2018). The clinical presentation may be acute, subacute, or chronic; in a quarter of cases, the diagnosis is made in intensive care (Archer et al. 2023).

Unlike BOS, which is exceptionally diagnosed more than 2 years after transplantation, ILD can occur anytime. Of note, PPFE occurs several years after transplantation (Bondeelle et al. 2020). Most patients present with dyspnea.

Specific risk factors for ILD are prior thoracic irradiation and the absence of immunosuppressive treatment at the time of ILD occurrence (Archer et al. 2023).

In the context of ILD, complete PFTs (including lung volumes and DLCO) are useful for assessing the severity of lung dysfunction and monitoring treatment efficacy. PFTs also allow to diagnose combined ILD and BOS which occurs in 25% of cases of ILD (Archer et al. 2023).

A distinction should be made between inflammatory ILD (OP and undetermined), which are usually sensitive to corticosteroid therapy, and PPFE, which have low inflammation and do not respond to immunosuppressive treatments, including steroids (Table 52.3).

**Table 52.3** Comparative characteristics between BOS and ILD

Median onset after HCT	BOS: 13 months ILD: 15 months <sup>a</sup>
Extrathoracic cGVHD	BOS: >90% ILD: >75% <sup>b</sup>
Thoracic CT scan <sup>c</sup>	BOS: bronchial abnormalities, air trapping, <i>no</i> alveolar and/or interstitial abnormalities ILD: alveolar and/or interstitial abnormalities
PFT pattern <sup>e</sup>	BOS: obstructive pattern – FEV1/FVC < 0.7 and TLC > 80% ILD: normal/restrictive pattern, TLC < 80% and FEV1/FVC > 0.7; mixed ventilatory defect <sup>d</sup> , TLC < 80% and FEV1/FVC < 0.7; DLCO reduced
Treatment	BOS: limited ILD: usual response to steroids <sup>e</sup> , risk of relapse when steroids are tapered off
Prognosis <sup>f</sup>	BOS: 3-year OS 79%; 5-year OS 74% ILD: 3-year OS 81%; 5-year OS 71%

*PFT* pulmonary function testing, *PPFE* pleuro-parenchymal fibroelastosis, *FEV1* forced expiratory volume in 1 s, *FVC* forced vital capacity, *TLC* total lung volume, *DLCO* diffusion capacity of the lungs for carbon monoxide, *OS* overall survival

<sup>a</sup> PPFE occurs several years after HCT

<sup>b</sup> Less than 50% in PPFE

<sup>c</sup> The diagnosis of BOS is based on PFT, whereas those of ILD relies on chest imaging

<sup>d</sup> When combined with BOS

<sup>e</sup> Except for PPFE where the treatment is limited

<sup>f</sup> Morbidity is significant including respiratory disabling and infections

### Key Points

- Noninfectious lung injury occurs frequently following HCT and significantly contributes to morbidity and mortality in the immediate posttransplant period and in the months and years that follow. It can be observed in 25–55% of recipients.
- Noninfectious lung injury following HCT may be mediated by either immune or nonimmune mechanisms and could represent up to the 50% of noninfectious mortality after allo-HCT.
- Most relevant noninfectious early pulmonary complications are pulmonary edema by fluid overflow, IPS, and DAH, a vascular endothelial syndrome.
- The most relevant LONIPCs are BOS and ILD including OP.
- All of them have specific diagnostic criteria, management, treatment, and prognosis.

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# Neurological Complications

# 53

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## 53.1 Definitions and Epidemiology

Neurological complications of hematopoietic cell transplantation (HCT) are frequent and can be highly challenging to manage. Their reported incidence ranges from 8 to 65%, depending on types of manifestations included, transplant setting, and patient population (Maffini et al. 2017). The severity varies widely, ranging from mild transient disorders to life-threatening illnesses. Main factors and causative agents include neurotoxic drugs, infectious pathogens, cerebrovascular illness, metabolic encephalopathy, and immune-mediated diseases. CNS relapse of the underlying disease, thrombotic microangiopathy (TAM), and post-transplant lymphoproliferative disorder (PTLD) should also be ruled out (Table 53.1).

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Neurological complications can also be classified based on their onset after HCT. Early events

**Table 53.1** Main causes of neurological complications of HCT

Neurological complication	Cause
Drug-related	Calcineurin inhibitors (PRES) Methotrexate/cytotoxic agents (busulfan, fludarabine) Anti-infective agents Opioids, benzodiazepines
Infectious pathogens	Viruses (EBV, HHV-6, CMV, VZV, HSV, JCV, West Nile virus, adenovirus) Fungi and parasites ( <i>Toxoplasma gondii</i> , <i>Aspergillus</i> spp., <i>Candida</i> spp., Mucorales, <i>Cryptococcus neoformans</i> , <i>Histoplasma capsulatum</i> ) Bacteria (Gram-negative rods, Gram-positive cocci, <i>Mycobacterium tuberculosis</i> , <i>Nocardia</i> )
Metabolic	Uremic encephalopathy Hepatic encephalopathy Diabetic ketoacidosis
Cerebrovascular	Hemorrhage Ischemic stroke
Immune-mediated	Demyelinating diseases Myositis Myasthenia gravis CNS chronic GVHD CRS
Thrombotic microangiopathy	Calcineurin inhibitors Infectious pathogens
Malignancies	PTLD Hematological disease relapse



are primarily due to drugs administered in the conditioning regimen and IS therapy, while later complications typically arise from the consequences of immunodeficiency, particularly infections (Schmidt-Hieber et al. 2011, 2020). Because clinical manifestations are often misleading and nonspecific, finding the right etiology may be long and difficult. Yet, early diagnosis and treatment are of paramount importance to reduce the risks of irreversible complications, impairment of quality of life, and transplantation-related death.

## 53.2 Causes and Types of Neurological Complications

### 53.2.1 Neurotoxic Drugs

Calcineurin inhibitors (CNIs), antibiotics, antiviral drugs, and cytotoxic agents used in conditioning regimen are the most frequent causes of drug toxicity (Table 53.2). In addition, drug-drug interactions are a common cause of neurotoxicity and must be carefully checked. For example, combining imipenem/cilastatin and ganciclovir can trigger generalized seizures. Similarly, introducing voriconazole can increase the serum concentration of cyclosporin A (CSA)/tacrolimus (TAC), potentially leading to neurological complications, such as posterior reversible encephalopathy syndrome (PRES).

#### 53.2.1.1 Calcineurin Inhibitors

The use of CSA and TAC is associated with neurological complications in 25–59% of patients treated with HCT (Reece et al. 1991). The clinical picture of CNI-induced neurotoxicity ranges from transient isolated symptoms to severe manifestations such as TAM (see Chap. 42) or PRES (Table 53.2).

PRES refers to a disorder of reversible subcortical vasogenic brain edema and is caused by endothelial injury related to abrupt blood pressure changes or direct effects of cytokines on the endothelium. It may occur in 1.6–7.2% of HCT recipients and, if diagnosed early, is reversible

**Table 53.2** Main neurological side effects of the major drugs used in HCT

Drug	Most common symptoms
Acyclovir	Headache, tremor, dysarthria, hallucinations, encephalopathy
Amphotericin B	Headache, encephalopathy, numbness, vision changes
Busulfan	Seizures, headache, dizziness
Blinatumomab	Encephalopathy, headache, aphasia, ataxia, tremor, seizures
Cefepime	Headache, paresthesia, encephalopathy
CSA/TAC	PRES, confusion, tremor, ataxia, seizures, cortical blindness
Fludarabine	Acute toxic leukoencephalopathy
Foscarnet	Headache, vertigo, paresthesia, seizures, tremor, encephalopathy
Ganciclovir	Headache, numbness, tremor, seizures
Imipenem/cilastatin	Seizures, tremor, vertigo, paresthesia, somnolence, encephalopathy
Isavuconazole	Headache, somnolence, encephalopathy, seizures, paresthesia, peripheral neuropathy
Methotrexate	Headache, lethargy, dysarthria, diffuse necrotizing leukoencephalopathy
Posaconazole	Paresthesia, dizziness, somnolence, headache, seizures, tremor
Rituximab	PML
Sorafenib	PRES, peripheral sensory neuropathy
Thiotepa	Headache, encephalopathy, seizures, paresthesia
Treosulfan	Headache, dizziness, intracranial hemorrhage, peripheral sensory neuropathy
Voriconazole	Headache, seizures, vision changes, hallucinations, numbness, encephalopathy

after CNI withdrawal. Symptoms such as headache, visual disturbance, seizures, encephalopathy, or focal neurologic deficit, when associated with renal failure or blood pressure fluctuations, are highly suggestive of PRES (Schmidt et al. 2016).

Although vasogenic edema can be visualized on computed tomography (CT) in some patients, brain magnetic resonance imaging (MRI) is much more sensitive. MRI shows bilateral multifocal areas of hyperintensity in T2-weighted sequences, especially in the white matter of



parieto-occipital regions. Other variations, such as superior frontal sulcus pattern or holo-hemispheric watershed pattern, can be observed. Persistent neurological sequelae have been reported, especially if PRES is not rapidly diagnosed and treated.

### 53.2.1.2 MTX and Cytotoxic Agents

GVHD prophylaxis with short course of methotrexate (MTX) may cause minor neurological symptoms, such as lethargy, dysarthria, and headache, and, very rarely, diffuse necrotizing leukoencephalopathy (Paudyal et al. 2010).

Busulfan (BU) is associated with seizure and requires preventive prophylaxis with benzodiazepines (Eberly et al. 2008).

For fludarabine (FLU), the main neurological complication is acute toxic leukoencephalopathy. The clinical syndrome is characterized by visual disturbance, sensitive defects, and cognitive impairment. Brain MRI shows bilateral white matter areas of T2-weighted hyperintensity, which differ significantly from the MRI findings seen in PRES. Classical PRES arises from subcortical white matter, whereas acute toxic leukoencephalopathy arises from periventricular white matter. Risk factors include poor renal function, older age, higher FLU dose, previously treated CNS disease, or previous FLU-based conditioning regimen. Outcomes are very poor with irreversible neurological sequelae and median OS of 2 months (Beitinjaneh et al. 2011).

### 53.2.1.3 Immunotherapy and Tyrosine Kinase Inhibitors (TKI)

Rituximab, TKI, and bispecific T-cell-engaging antibodies, such as blinatumomab, are increasingly used after HCT. Their most notable neurological side effects are described in Table 53.2.

### 53.2.1.4 Anti-infective Drugs

Anti-infective drugs are among the major causes of neurological complications. Dose adaptation is warranted in case of drug-drug interaction or impaired renal function. Their main neurological side effects are described in Table 53.2.

## 53.2.2 Infectious Pathogens

A wide array of pathogens can potentially lead to CNS infections after HCT (Schmidt-Hieber et al. 2011, 2020). Among them, fungi (e.g., *Aspergillus* spp.) and viruses (mainly EBV and HHV-6) are the most prevalent agents (Schmidt-Hieber et al. 2020; Liu et al. 2021). To identify a potential agent that may be causing unclear neurological disorders, thorough diagnosis investigation should be conducted. This should include MRI-based neuroimaging and cerebrospinal fluid (CSF) analysis. Considering the clinical symptoms and their timing in relation to HCT may be helpful to decipher the accurate diagnosis (Schmidt-Hieber et al. 2011, 2016, 2020). Early preemptive or target treatment of CNS infection is crucial to improve the prognosis. Notably, while isavuconazole or voriconazole may be used to treat CNS-involving infections caused by *Aspergillus* spp. and zygomycosis (Tissot et al. 2017; Schwartz et al. 2020), HHV-6 encephalitis should be treated by foscarnet or ganciclovir (Schmidt-Hieber et al. 2016; Ward et al. 2019).

## 53.2.3 Metabolic Complications

Metabolic complications may include uremic encephalopathy associated with CNI nephrotoxicity or TAM, hepatic encephalopathy associated with SOS/VOD or severe hepatic GVHD, and diabetic ketoacidosis. Pharmacologic sedation with opioids, CNS infections, cytokine release syndrome (CRS), systemic inflammatory response, and hemophagocytic lymphohistiocytosis should be considered in the differential diagnosis of metabolic causes of neurological dysfunction (Maffini et al. 2017).

## 53.2.4 Cerebrovascular Diseases

Cerebrovascular hemorrhagic or thrombotic CNS events represent potentially lethal complications.

Among these, subdural hematoma is one of the most common, occurring in 2.6% of HCT recipients. Risk factors for CNS hemorrhagic

complications include posttransplant falls, prolonged severe thrombocytopenia or platelet transfusion refractoriness, acute grades III–IV GVHD, and arterial hypertension. CT scans usually confirm the diagnosis but can be negative in 20–25% of the patients with CNS hemorrhagic events. Risk factors for CNS thrombotic complications include active infections, atrial fibrillation, hypercoagulable state, previous venous thromboembolism, high-risk malignant disease, TMA, and GVHD (Zhang et al. 2016; Cai et al. 2020).

### 53.2.5 Immune-Mediated Causes

Immune-mediated neurological diseases are rare but potentially severe complications of HCT, which encompasses demyelinating polyneuropathy, myositis, myasthenia gravis, CNS manifestations of chronic GVHD, and CRS.

#### 53.2.5.1 Demyelinating Polyneuropathies

Immune-mediated demyelinating polyneuropathies, which includes Guillain-Barré syndrome, may occur in 1–4% of the patients, especially within the first 3 months after HCT (Yoshida et al. 2016). Progressive symmetrical ascending motor deficiency, numbness, hyporeflexia, and respiratory insufficiency are the most common symptoms. MRI, lumbar puncture, and nerve conduction studies should be performed promptly. Symptoms may resolve with the use of intravenous immunoglobulins. Plasma exchange or rituximab may be used in unresponsive patients.

#### 53.2.5.2 Myositis

Myositis is characterized by proximal muscle weakness and is often associated with chronic GVHD. It can occur in 2–3% of HCT recipients (Limaye and Limaye 2021). Previous exposure to immune checkpoint inhibitor may increase the risk of developing myositis. Levels of creatine phosphokinase are elevated, electromyography shows a myopathic pattern, and MRI is valuable in diagnosing and monitoring the treatment

response. Diagnosis can be confirmed by muscle biopsy. Patients often respond to corticosteroid therapy within 1–6 weeks.

#### 53.2.5.3 Myasthenia Gravis

Myasthenia gravis usually occurs after the onset of GVHD in less than 1% of HCT recipients (Ahmed et al. 2018). The main symptoms include ptosis, facial weakness, diplopia, dysarthria, and dysphagia. The diagnosis is confirmed with electromyography showing a progressive decrease in the muscle action potential or increased jitter. Cholinesterase inhibitors and corticosteroid therapy are the treatments of choice.

#### 53.2.5.4 Central Nervous System GVHD

CNS manifestations of GVHD are considered rare, although their true incidence may be underestimated. Notably, three types of chronic CNS GVHD are recognized by the NIH Consensus Conference on criteria for clinical trials in chronic GVHD: demyelinating diseases, cerebrovascular disease, and immune-mediated encephalitis (Vinnakota and Zeiser 2021).

Demyelinating diseases have been reported in the cerebral white matter, optic nerve, and spinal cord. Symptoms typically follow a relapsing-remitting course, as observed in multiple sclerosis. The treatment consists in corticosteroid pulses. Refractory/relapsing patients may benefit from sphingosine-1-phosphate receptor agonists, such as fingolimod (Gauthier et al. 2018).

Vasculitis, the most common cerebrovascular manifestation of GVHD, affects small- to large-sized arterial vessels of cerebral parenchyma and meninges. Ischemic lesions, microhemorrhages, and multifocal signal changes in the white matter can be observed on MRI. Diagnosis can be confirmed by brain biopsy, and treatment involves corticosteroids in combination with cyclophosphamide.

Finally, cases of immune-mediated encephalitis have been reported, requiring repeated CSF examination to confirm the diagnosis and rule out infectious encephalitis.

### 53.2.5.5 Cytokine Release Syndrome

CRS is a frequent complication of haploidentical HCT, particularly when PBSC are used, and it may occur in up to 90% of patients within the first days following graft infusion. The clinical manifestations of CRS are similar to those observed in patients after chimeric antigen receptor T-cell or bispecific antibody therapies, ranging from isolated fever to potentially life-threatening complications. Headache associated with fever is common in mild forms of CRS. However, severe CRS-related neurological symptoms, such as encephalopathy, occur in less than 10% of patients (Abboud et al. 2021). Patients can be effectively treated with cytokine blockade using monoclonal antibodies targeting the IL-6 receptor, such as tocilizumab, or interleukin-1 receptor antagonist, such as anakinra (Hayden et al. 2022; Gazeau et al. 2023).

## 53.3 Diagnostic Algorithm

When faced with neurological complications of HCT, the following ten steps can be helpful to research the correct diagnosis and start the right treatment promptly:

1. Carefully review the medication history and search for potential metabolic disorders.
2. Determine whether the clinical signs and symptoms are generalized (e.g., altered consciousness, seizures) or focal (e.g., stroke, mass lesion).
3. Assess the timing of neurological signs and symptoms in relation to HCT.
4. Perform CT scan or MRI to rule out PRES, encephalitis (infectious or immune-mediated), parenchymal infiltrates, cerebrovascular events, and hematological disease relapse.
5. Analyze CSF to diagnose infectious complications, demyelinating neuropathy, and underlying disease relapse.
6. Perform electroencephalography in patients exhibiting altered consciousness, hallucinations, or seizures.
7. Perform electromyography in patients exhibiting neuropathy, myopathy, or neuromuscular pattern of weakness.
8. Repeat each of the previous steps as investigations may be negative if performed early and symptoms may evolve or fluctuate after the disease onset.
9. Consider brain or neuromuscular biopsy to confirm or rule out opportunistic infections, PML, vasculitis, PTLD, or other malignancies.
10. A neurology review is crucial at every step and highly recommended, particularly in complex clinical cases.

## 53.4 Conclusions

Neurological complications of HCT, particularly allo-HCT, are frequent and can be fatal. The main causes of these complications include drug-related toxicities, infections, metabolic disorders, cerebrovascular events, immune-mediated disorders, and disease recurrence. Although their management can be highly challenging, early diagnosis and treatment, guided by the expertise of a neurologist, are extremely important to reduce mortality and improve quality of life.

### Key Points

- Neurological complications of HCT require prompt diagnosis and timely treatment to reduce posttransplant mortality and enhance quality of life.
- Their etiology is often multifactorial, involving neurotoxic drugs, infectious pathogens, metabolic encephalopathy, cerebrovascular disorders, and immune-mediated diseases.
- TAM, PTLD with CNS involvement, CRS, and CNS relapse of the underlying hematological disease should be included in the differential diagnosis.
- CNS manifestations of GVHD are considered rare and often pose considerable challenges to manage.
- Consulting with a neurologist is recommended, especially in complex clinical cases.

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# Skin, Hair, and Musculoskeletal Complications

# 54

Francis Ayuk and Bipin N. Savani

## 54.1 Complications Involving the Skin and Hair

### 54.1.1 Introduction

Nearly every recipient of an allo-HCT will at some stage develop complications involving the skin and hair. These complications can be grouped into drug-related toxicities and allergies, GVHD, infections, and malignant conditions.

### 54.1.2 Allergies, Drug-Related, and Other Toxicities

Drug-related toxicities are most often due to the conditioning regimen, antibiotics, or immunosuppressive (IS) agents. Presentation can vary broadly from localized erythema to epidermal necrolysis and Stevens-Johnson syndrome.

Diagnosis may be difficult because the morphological and chronological presentations of the lesions are generally nonspecific. Skin biopsies

and histological examination can help improve diagnostic certainty (Paun et al. 2013).

Management requires discontinuation of suspected causative agents, topical treatment with healing ointments, prevention of secondary infections, and in severe cases (or when other organs are involved) systemic therapy with corticosteroids and antihistamines.

Extensive sun exposure including skin burns may increase the risk of skin GVHD, dryness, and subsequent infections. Regular examination of skin and skin-derived tissue and specialist consultation may enable early detection and better management (Majhail et al. 2012).

### 54.1.3 Graft-Versus-Host Disease

The skin is one of the most frequently affected organs in acute and chronic GVHD. Acute GVHD of the skin mainly affects the epidermis of the skin and adjacent oral, anal, and genital mucosa. Chronic GVHD may affect all layers of the skin including the epidermis, dermis, and subcutaneous tissue and may also manifest as skin dyspigmentation, sweat impairment, alopecia and thinning of scalp hair, hair loss in other areas (e.g., eye brows), and nail dystrophy. Chronic GVHD with sclerosis of the subcutaneous tissue including fasciae, joints, and the musculoskeletal system can severely impact patients' quality of life. Scleroderma lesions of the thorax or abdomen may impair breathing, lesions

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adjacent to joints may impair movement, and genital lesions may cause phimosis, vaginal scarring, and narrowing of the introitus that may cause dyspareunia and even complete obliteration of the vaginal tract.

In the early “inflammatory” phase of GVHD, patients often present with edema and discomfort which later progress to fibrosis and joint contractures. Regular survey of range of motion by patients and physicians may enable early detection of reversible lesions. Regular assessment (e.g., via questionnaire or oral interview) during routine clinical visits may encourage physicians and patients to address issues involving the genital tract, thereby enabling early detection and treatment.

General treatment and management of GVHD are discussed in Chaps. 43 and 44. In patients with chronic GVHD of the skin and subcutaneous tissues, treatment is best initiated in the early phase prior to development of most often irreversible fibrosis and contractures, which otherwise may require protracted immunosuppression and other measures, sometimes over several years. Physiotherapy including deep myofascial massage and stretching exercises is essential to restore or maintain range of motion. In patients with genital involvement, topical treatment with immunosuppressive agents and hormones and use of vaginal dilators should be initiated early to prevent or reduce the degree of irreversible fibrosis and avoid the need for surgical intervention.

#### 54.1.4 Infectious Complications Involving the Skin

Infection-associated skin lesions are often due to viruses. Unexplained fever and rash are more frequent in patients with HHV6 viremia compared to controls (Betts et al. 2011). Due to the lack of effective prophylaxis, HHV6-related complications occur during early transplant phases, especially in PT-Cy setting, while shingles (varicella zoster) are mostly seen beyond 6 months after transplant and mainly after discontinuation of prophylactic acyclovir (Noviello et al. 2023).

HSV1-related complications are more frequent in early weeks and also in patients under continuous immunosuppression for GVHD and may occur despite prophylactic aciclovir. Other infectious conditions of the skin include fungal infections (mainly due to dermatophytes and, less frequently, *Aspergillus* or *Mucor* species) and bacterial infections. Management of infections mostly consists of systemic antiviral, antifungal, or antibiotic treatment.

#### 54.1.5 Malignant Complications of the Skin

Post-transplant malignant conditions of the skin include skin cancer (basal cell carcinoma, squamous cell carcinoma, and melanoma) and relapse of underlying malignant disease. BCC and SCC are much more common and have a better prognosis than melanoma. The incidence of melanoma has been reported to be higher after allo-HCT with standardized incidence ratios ranging from 1.4 to 8.3 (Inamoto et al. 2015). Secondary cancers, risk factors, and management are discussed in detail in Chap. 47. Overall, the survival for these patients is comparable to those of patients with the same de novo cancer (Tichelli et al. 2019).

Patients should be counseled to perform self-examination of the skin and adjacent mucosa, use adequate sun protection, and avoid excessive sun exposure. Country-specific general population recommendations for screening for cancer should be adapted and modified taking increased risk of HCT survivors into consideration.

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### 54.2 Musculoskeletal Complications

#### 54.2.1 Introduction

Complications involving the muscles include myopathies, myositis, and cramps. Musculoskeletal complications are reported in 35% of long-term survivors 10 years after allogeneic transplantation (Syrjala et al. 2005).

### 54.2.2 Myopathy

The most frequent causes of myopathy early after transplant are corticosteroid therapy and inactivity. Patients report muscle weakness with no pain, and laboratory investigations show normal creatine kinase. The proximal lower limb muscles, particularly the quadriceps muscles, are most severely affected. The main risk factors include increasing dose and duration of corticosteroid therapy, older patient age, and the extent and duration of inactivity, particularly when intensive care is required. Patients should receive physiotherapy as soon as corticosteroid therapy is initiated and be advised to exercise on their own (Mohammed et al. 2019). Systemic corticosteroids should be tapered or avoided when possible.

### 54.2.3 Myasthenia Gravis

Though rare (<1%), it has been reported after allogeneic hematopoietic cell transplantation, mostly in the context of chronic GVHD. Patients present with fatigable weakness during or after tapering of immunosuppression (Grauer et al. 2010). Diagnosis includes detection of antibodies against acetylcholine in blood. Treatment consists of cholinesterase inhibitors and IS therapies for chronic GVHD.

### 54.2.4 Muscle Cramps

Muscle cramps are painful and often visible contractions lasting up to 30 min. Though rarely reported, they appear to be frequent in patients with chronic GVHD (Filipovich et al. 2005); an association with chronic IS may also be possible. Magnesium deficiency and side effects of medications (e.g., ganciclovir, valganciclovir) should always be ruled out. If magnesium replacement and discontinuation of suspected causative drugs do not bring relief, treatment with, for example, quinine or antiepileptic drugs may be considered.

### 54.2.5 Myositis

Myositis has been reported in up to 3% of patients after allo-HCT. Though frequently associated with other symptoms of chronic GVHD, it can also be the sole manifestation of GVHD (Openshaw et al. 2009). Patients often present with pressure-sensitive muscle pain and increased blood creatine kinase. Management is within GVHD treatment (Couriel et al. 2002).

### 54.2.6 Complications Involving the Bones and Joints

The most frequent complications involving the bones and joints are chronic GVHD, avascular osteonecrosis, and bone loss (osteopenia/osteoporosis). Chronic GVHD of the joints is discussed in Chap. 44.

#### 54.2.6.1 Osteoporosis/Osteopenia

Osteopenia (defined as a T-score between  $-1$  and  $-2.5$ ) and osteoporosis (defined as a T-score less than  $-2.5$ ) have been reported in about 25–50% of patients after allo-HCT and up to 60% in patients with severe chronic GVHD (Pirsl et al. 2016). Risk factors include protracted IS, older patient age, higher cumulative corticosteroid dose (Schulte and Beelen 2004; Savani et al. 2007; Stern et al. 2001; Yao et al. 2008; Petropoulou et al. 2010; Abou-Mourad et al. 2010), lower body weight, malnutrition, physical inactivity, female gender, higher average NIH organ score, as well as higher platelet counts in patients with severe chronic GVHD (Pirsl et al. 2016).

In accordance with these risk factors, decrease in bone mineral density occurs most rapidly within the first year after transplant. If osteopenia or osteoporosis is diagnosed, endocrine causes like hyperthyroidism, hyperparathyroidism, and hypogonadism need to be ruled out. Screening using dual energy X-ray absorptiometry (DEXA) is recommended 1 year after transplant and repeat measurements in patients with recognized defects (Majhail et al. 2012).

Measures to prevent bone loss include vitamin D supplementation in regions with high prevalence of vitamin D deficiency, adequate calcium intake preferable through diet, and regular weight-bearing physical exercise. Beyond the above preventive measures, specific treatment is recommended for patients with severe osteopenia or osteoporosis (Bhatia et al., 2017). Hormone replacement therapy should be considered in patients with hypogonadism. Reduction in the risk of fractures has been demonstrated for several agents and agent combinations (Barrionuevo et al. 2019). Patients should also be counseled to modify negative lifestyle factors (e.g., cease smoking) and take measures to prevent falls (e.g., physical exercise including balance training, correct visual disorders).

#### 54.2.6.2 Avascular Necrosis

Avascular necrosis (AVN) has been reported in up to 19% of adult patients and up to 29% of patients younger than 20 years (Torii et al. 2001; Patel et al. 2008). Risk factors include GVHD, steroid therapy, microvascular changes due to GVHD and/or its therapy, younger age at transplant, and TBI (Socié et al. 1997; French et al. 2008; Patel et al. 2008; Campbell et al. 2009; Jagasia et al. 2010). Patients usually present with joint pain, restricted to one or two affected joints. Though most joints can be affected, the hips are by far most frequently involved, bilateral in the majority of cases.

Screening for AVN is not recommended; however, high index of suspicion and prompt MRI are necessary in early symptomatic patients with risk factors, to enable early detection and intervention (Bhatia et al. 2017).

Pain relief and maintenance or restoration of patient mobility are the main aims or treatment. Discontinuation of corticosteroid and other IS therapies should be considered where possible. Drug therapy is limited to pain relief. The role of nonsurgical causative therapies, such as bisphosphonates, statins, and prostacyclin analogues, is still unclear. Pressure relief by means of surgical core decompression may relieve pain and slow down progression in early stages, whereby additional autologous bone marrow grafting further

improves long-term outcome (Hernigou et al. 2018). In patients with late-stage disease with femoral head collapse, joint-preserving strategies are not effective, and total hip arthroplasty is the recommended long-term treatment.

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# Cardiovascular Diseases and Metabolic Syndrome

# 55

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## 55.1 Introduction

Cardiovascular disease (CVD) remains the leading cause of death globally, with 17.9 million deaths each year, mainly for coronary heart disease and cerebrovascular disease. The World Health Organization (WHO) estimates that the largest increment in deaths has been for ischemic heart disease, rising by more than 2 million in 2000 to 8.9 million in 2019, representing 16% of all global deaths. It is predicted that by 2030, almost 23.6 million people will die from CVD (WHO 2023).

Moreover, there is an increased incidence of metabolic syndrome (MetS), with reported prevalence rates of 31–49% after transplant.

After HCT, either autologous or allogeneic, an increased incidence of CVD has been shown com-

pared with the normal population and sibling donors (Baker et al. 2007). Retrospective EBMT analyses have shown the cumulative incidence of a first cardiovascular event 15 years after HCT rises to 6%.

## 55.2 Cardiovascular Disease in HCT

### 55.2.1 Abdominal Obesity

Abdominal obesity measured by waist circumference represents fat accumulation (visceral adipose deposits) which independently confers cardiometabolic risk. Changes in waist circumferences are seen after HCT with, for example, corticosteroid use and with onset of sarcopenic obesity.

### 55.2.2 Dyslipidemia

Dyslipidemia is defined by elevated levels of total cholesterol, LDL-C, or triglycerides or low levels of HDL-C. Prevalence in general population is estimated at 25% in the USA (Baker et al. 2007) and in European countries. Evidence suggests allo-HCT recipients have significantly higher risk of new onset dyslipidemia (RR 2.1 95% CI 1.15–4.65) compared with auto-HCT (Tichelli et al. 2007a) with the prevalence post-HCT estimated to be 43–73% (Chow et al. 2014; Oudin et al. 2015). TBI significantly increases the risk of dyslipidemia.

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### 55.2.3 Hypertension (HTN)

Hypertension (HTN) in the general population is defined as systolic BP  $\geq 140$  mmHg or diastolic BP  $\geq 90$  mmHg but defined in context of MetS as systolic BP  $\geq 135$  mmHg or diastolic BP  $\geq 85$ . HTN in people following allo-HCT is 2.06 times (95% CI 1.39–3.04) more likely compared with sibling donors or auto-HCT (Baker et al. 2007).

### 55.2.4 Insulin Resistance or Diabetes Mellitus (IR/DM)

DM is characterized by hyperglycemia resulting from defects in insulin secretion (type 1) and insulin resistance (type 2), and it is defined as a fasting pGL  $\geq 7$  mmol/L, an HbA1C  $\geq 6.5\%$ , a 2-h plasma glucose  $\geq 11.1$  mmol/L during a glucose tolerance test (GTT), or a random glucose  $\geq 11.1$  mmol/L.

Both allo-HCT and auto-HCT recipients have been found to report DM more often than sibling donors (OR for allo-HCT, 3.65; 95% CI, 1.82–7.32; OR for auto-HCT, 2.03; 95% CI, 0.98–4.21) (Baker et al. 2007). High-dose corticosteroids (cumulative PRD dose of  $>0.25$  mg/kg/day) increase the likelihood of developing DM (RR, 3.6; 95% CI, 1.7–7.5) and for having persistent DM at 2 years post-HCT (RR, 4.1; 95% CI, 1.0–18.2) (Majhail et al. 2009a, b). TBI is also a well-evidenced risk factor (Hirabayashi et al. 2014). TBI was linked to 3.42 times greater risk of diabetes development.

## 55.3 Risk Factors for CVD in HCT Recipients

There are several well-defined risk factors which indicate increased susceptibility to post-transplant CVD (Table 55.1).

### 55.3.1 Contribution of Type of Transplant

The contribution of the type of transplant in the CVD developing is not well defined. In a previous EBMT study, the cumulative incidence for

**Table 55.1** Risk factors for cardiovascular disease

Patient’s baseline cardiovascular (CV) risk factors	Family history Female sex Smoking Hypertension Dyslipidemia Diabetes Obesity Sedentary habit Chronic kidney disease
Chemotherapy strategies used in the underlying disease	Anthracyclines (doxorubicin or equivalent $>250$ mg/m <sup>2</sup> ) Steroids Site-specific radiotherapy—Dose $>35$ Gy to a field including the heart or $>15$ – $35$ Gy + doxorubicin $>100$ mg/m <sup>2</sup> Total body irradiation Other cardiotoxic agents: cyclophosphamide, busulfan, melphalan, thiotepa
GVHD prophylaxis	Steroids Calcineurin inhibitors
GVHD	Grades II–IV aGvHD Any grade cGvHD
Other secondary late effects	Hypogonadism Premature menopause Hypothyroidism

Chow et al. (2014); Oudin et al. (2015); Lopez-Fernandez et al. (2021); Greenfield et al. (2021)

the first CV event was 7.5% at 15 years post-allo-HCT versus 2.3% post-auto-HCT (Tichelli et al. 2007a). However, a more recent cross-sectional multicenter study found no difference in auto- vs allo-HCT (Greenfield et al. 2021).

A higher incidence of early cardiac events (ECE), occurring within the first 100 days after HCT, has been reported in PTCy-based approaches. Older age, sequential conditioning regimen, and CY exposure before transplant are additional risk factors for ECE in this context (Duléry et al. 2021).

## 55.4 Metabolic Syndrome Definition

*Metabolic syndrome (MetS)* is a cluster of inter-related factors which increase the risk of cardiovascular disease, diabetes mellitus (DM), and all-cause mortality. MetS is defined as the presence of three out of five risk factors as follows:



- Abdominal obesity measured by waist circumference: with population- and country-specific definitions
- Triglycerides  $\geq 1.7$  mmol/L
- HDL-C (men)  $< 1.0$  mmol/L
- HDL-C (women)  $< 1.3$  mmol/L
- Blood pressure  $\geq 130/\geq 85$  mmHg
- Fasting glucose  $\geq 5.6$  mmol/L

The International Diabetes Federation (IDF) estimates 25% of the world's population has MetS. After HCT, there is an increased incidence of MetS, with reported prevalence rates of 31–49% (Majhail et al. 2009b; Oudin et al. 2015; Greenfield et al. 2018). More recently, Greenfield et al. did a cross-sectional, multicenter, noninterventional study of 453 adult HCT patients surviving a minimum of 2 years post-transplant (Greenfield et al. 2021). Overall prevalence of MetS was 37.5%, rising to 53% in patients above 50 years old. Of significance, higher occurrence of cardiovascular events was found in those patients with MetS than in those without MetS (26.7% versus 9%,  $p < 0.001$ ).

#### 55.4.1 The Immune System as a Mediator of Metabolic Syndrome

Extensive research is now recognizing obesity as a chronic inflammatory state which drives the development of insulin resistance and MetS. Body mass index and the degree of abdominal adiposity are positively correlated with serum inflammatory markers, including C-reactive protein, TNF- $\alpha$ , and IL-6. In addition, adipocytes are now known to serve critical endocrine and immune function. Within lean individuals, adipose tissue is predominantly populated by regulatory T cells (Tregs), Th-2 cells, and M2 macrophages, whereas, in obese individuals, adipose tissue contains Th 1, M1 macrophages, CD8+ T cells, B cells, and dendritic cells. In the setting of HCT, significant tissue injury, inflammation, and perturbations in immune cell number and function occur, and this appears to play a key role in the initiation and development of the process (Turcotte et al. 2016).

### 55.5 Preventative Practices in the HCT and Late-Effect Clinic: A Practical Approach

The fact that HCT survivors require close follow-up and clinical review provides an opportunity to deliver screening for late effects and other long-term consequences of treatments. Screening for cardiovascular risk factors, including MetS and CV events, can be integrated into a broader program of long-term and late-effect follow-up. If cardiovascular risk factors are detected, they can usually be referred back to primary care clinicians or facilitate the referral to the appropriate specialist department. Algorithms, such as the Framingham risk score, may be useful in estimating a person's projected risk of developing CVD in the general population. Although these CVD risk scores have not been validated in HCT survivors and may potentially underestimate the risk, they may be reasonable to use as an initial guideline. However, there should be direct referral for clinically urgent cardiovascular problems to relevant hospital specialists, and, ultimately, a close communication between the different clinicians involved in the care of the patient is indispensable.

Given the specialized complexity of HCT and its many complications, the HCT clinic and associated late-effect service can have a major role in coordinating care and facilitating communication between other relevant specialists. This aspect is underpinned by the FACT-JACIE standards which feature systematic provision for late-effect follow-up, including cardiovascular risk factors and complications (FACT-JACIE eighth edition).

For the HCT late-effect clinic, Table 55.2 has been published as a guide to facilitate screening in the EBMT-CIBMTR guidelines (DeFilipp et al. 2017). This is a consensus opinion, and there is no good evidence of the safety or clinical effectiveness of these recommendations in HCT patients. Based on the available evidence, it is important to screen for other factors in HCT patients, including (a) personal history, (b) family history, (c) type of transplant (allo or auto), (d) use of TBI, (e) history of acute or chronic GvHD, and (f) use of CNI (CSA, TAC) (DeFilipp et al. 2017).

**Table 55.2** Screening guidelines for metabolic syndrome and cardiovascular risk factors for adult and pediatric patients among the general population and HCT survivors (taken from DeFilipp et al. 2017)

	General adult population	Adult long-term HCT survivors (Majthail et al. 2012)	General pediatric population	Pediatric long-term HCT survivors (Pulsipher et al. 2012)
Weight, height, and BMI	Weight, height, and BMI assessment in all adults (no specific recommendation for screening interval)	No specific recommendations	Weight, height, and BMI assessment after 2 years of age (no specified screening interval)	Weight, height, and BMI assessment yearly
Dyslipidemia	For persons with increased risk for coronary heart disease, assessments should begin at age 20  The interval for screening should be shorter for people who have lipid levels close to those warranting therapy and longer intervals for those not at increased risk who have had repeatedly normal lipid levels	Lipid profile assessment every 5 years in males aged $\geq 35$ years and females aged $\geq 45$ years  Screening should start at age 20 for anyone at increased risk (smokers, DM, HTN, BMI $\geq 30$ kg/m <sup>2</sup> and family history of heart disease before age 50 for male relatives or before age 60 for female relatives)	Lipid panel between 9 and 11 years of age or earlier if family history	Lipid profile at least every 5 years; if abnormal, screen annually
Blood pressure	Blood pressure assessment every 3–5 years in adults aged 18–39 years with normal blood pressure ( $< 130/85$ mmHg) who do not have other risk factors  Blood pressure assessment annually in adults aged $\geq 40$ years and for those who are at increased risk for high blood pressure (blood pressure 130–139/85–89 mmHg, those who are overweight or obese, and African–Americans)	Blood pressure assessment at least every 2 years	Blood pressure assessment yearly after the age of 3 years, interpreted for age/sex/height	Blood pressure assessment at each visit and at least annually
Hyperglycemia	Screening for abnormal blood glucose (HbA1C, fasting plasma glucose, or oral glucose tolerance test) every 3 years in adults aged 40–70 years who are overweight or obese	Screening for type 2 DM every 3 years in adults aged $\geq 45$ years or in those with sustained higher blood pressure ( $> 135/80$ mmHg)	Fasting glucose every 2 years after the age of 10 years in overweight children with other risk factors	Fasting glucose at least every 5 years; if abnormal, screen annually

BMI body mass index, DM diabetes mellitus, HbA1C hemoglobin A1C, HCT hematopoietic cell transplantation, HTN hypertension

## 55.6 Future Directions: Implementation, Education, and Research

As survival after HCT gradually increases, there is also increased recognition of HCT-related CVD and its risk factors, including MetS. Most research has been cross-sectional and observational. There are progressively more information in the pathogenesis of Mets, but still more prospective research is needed both on defining the incidence above the normal aging population and on interventional strategies, targeting individual risk factors and/or components of the MetS.

New indications for HCT, such as systemic autoimmune disease, and alternative platforms, such as haplo-HCT or cord-HCT, require individualized assessment. Pharmacological, lifestyle, and rehabilitation interventions are common in the general population in respect to CVD. However, their impact in HCT recipients (both before and after HCT) needs to be defined in the context of the wide range of indications and age at which patients receive their HCT, along with the individual prognosis of each indication after successful HCT.

### Key Points

- Long-term survivors have an increased risk of premature MetS and CVD. The best approach is to screen all patients (i.e., both autologous and allogeneic HCT) according to international consensus guidelines (DeFilipp et al. 2017) and manage risk factors on an individual basis.
- The challenge of universal implementation of screening and management of late effects across various health services providing HCT will be facilitated by FACT-JACIE accreditation standards.
- Randomized controlled trials of interventional strategies and mechanistic studies of cardiovascular risk in HCT survivors are still needed.

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## 56.1 Endocrine Function

Endocrine dysfunction is frequent in long-term survivors after HCT, particularly in children with a global rate of 58.7% with at least one endocrine abnormality (Güemes et al. 2022). Underlying disease, pretransplant therapy, age at HCT, type of conditioning, total body irradiation, development of chronic GVHD, and its treatment can all contribute to the risk of endocrine dysfunction. Consideration of pretransplant risk factors and their interaction with HCT-related exposure should be considered to assess the risk of endocrine dysfunction. Since many long-term complications may not manifest for years or even decades after HCT, survivors need ongoing, life-long monitoring (Bhatia et al. 2017). Systematic follow-up is important to identify and treat endocrine defects before clinical impact, and this is

particularly important in children where growth and puberty are at risk.

### 56.1.1 Thyroid Dysfunction

#### 56.1.1.1 Background

Thyroid dysfunction is a well-recognized late complication after HCT. The most common abnormality of thyroid function after HCT is primary compensated hypothyroidism. Approximately one in four patients develops hypothyroidism after allogeneic HCT, with a greater incidence in females (Felicetti et al. 2023). This may not require treatment and commonly resolves. Overt hypothyroidism may be primary or less commonly central. Other thyroid disorders include autoimmune thyroid disease (thyroiditis, Graves' disease) and thyroid cancers (carcinomas or benign adenomas). In a single-center study of 791 patients followed up for 38 years, new cases of thyroid dysfunction continued for 28 years after HCT highlighting the need for indefinite follow-up (Sanders et al. 2009).

Risk factors for hypothyroidism post-HCT include the use of TBI with single dose being associated with a fivefold to sixfold higher risk than fractionated TBI. BU-based regimens are more likely to cause thyroid problems than those containing CY only, and patients with malignant disease (e.g., Hodgkin lymphoma) are more likely to get thyroid dysfunction than patients

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with non-malignant diseases (e.g., aplastic anemia). The age of the patient is also important with younger patients at higher risk (Cohen et al. 2007). In adults, recent data show that advanced age at transplantation is associated with higher risk for hypothyroidism (Ataca Atila et al. 2020). Pre-transplant TSH levels may predict the onset of post-HCT hypothyroidism (Felicetti et al. 2023). Thyroid dysfunction is more likely to occur in patients receiving prolonged immunosuppression for chronic GVHD (Savani et al. 2009). Furthermore, the risk of developing hypothyroidism is higher in patients treated with multiple allo-HCTs who have been transplanted for acute leukemia (Medinger et al. 2017). In relation to thyroid cancer, a retrospective study which included data on >68,000 patients showed that the relative risk (RR) of thyroid cancer was approximately threefold higher following HCT than in the general population. The RR was >20 if transplanted before the age of 10 years and close to 5 if transplanted between age 11 and 20 years. Female gender and GVHD were additional risk factors (Cohen et al. 2007).

#### 56.1.1.2 Prevention/Management

Continuous and long-term monitoring of thyroid function after HCT is essential for early diagnosis and to provide timely and appropriate treatment. Patients should have annual laboratory assessment with thyroid-stimulating hormone (TSH) and free thyroxine (FT4) with more frequent testing if there is clinical suspicion of thyroid dysfunction. Annual clinical examination should include palpation of the thyroid gland, and there should be a low threshold for arranging a thyroid ultrasound (Bhatia et al. 2017).

### 56.1.2 Hypoadrenalism

#### 56.1.2.1 Background

The main risk factor is the use of glucocorticoids which lead to central corticotrophin deficiency. Patients are at risk if they have received supra-physiological doses for 1 month or more, and topical, inhaled, intranasal, oral, and injectable forms all pose a risk (Gurnell et al. 2021). TBI

can also cause corticotrophin deficiency as can drugs. Diagnosis can be difficult as many patients will have few if any symptoms or they may present with nonspecific symptoms such as fatigue, weakness, nausea, weight loss, and hypotension. Some symptoms may mimic GVHD. Identifying patients who are at higher risk is helpful in guiding the diagnosis. Diagnosis requires paired morning cortisol and ACTH levels. If the results are inconclusive, then additional investigations should be arranged with an endocrinologist.

#### 56.1.2.2 Prevention/Management

When hypoadrenalism is confirmed, hydrocortisone should be given with additional doses to cover stresses such as illness, infection, or surgery. Subsequently, regular evaluation is required as it may be possible to reduce/stop medication (Cornillon et al. 2013).

### 56.1.3 Growth

#### 56.1.3.1 Background

Short stature is multifactorial after transplant. It is a recognized side effect of radiation to the hypothalamic-pituitary area given in childhood due to a reduction in growth hormone (GH) secretion. Radiation can also induce bone lesions. Pretransplant cranial radiation (e.g., patients with ALL) is also relevant, and single-dose TBI rather than fractionated radiation increases the risk further.

Additional contributory factors to short stature in these patients include underlying disease (e.g., Fanconi anemia), other hormone deficiencies (including thyroid and gonadal hormones), nutritional deficits, illness, steroids, and GVHD. Male sex and young age at time of transplant are additional risk factors.

#### 56.1.3.2 Prevention/Management

Children's growth velocity should be closely monitored with height and weight documented at each clinic visit. A possible increased risk of secondary malignancies has been described in patients receiving growth hormone (GH) replacement therapy after previous neoplasia; this has



raised concerns regarding the use of GH in the absence of sufficient long-term follow-up data. As a consequence of this, there are currently no clear guidelines for the use of GH in these patients. A pediatric endocrinologist should be involved if growth rate is abnormal based on bone age and pubertal stage (Chow et al. 2016; Lawitschka et al. 2019), and the use of GH therapy should be considered for children whose height standard deviation score is less than 2.

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## 56.2 Gonadal Dysfunction and Infertility

### 56.2.1 Background

Normal reproduction in both sexes requires germ cells and an intact hypothalamic-pituitary endocrine axis. In female patients, the uterus must be both receptive to implantation and capable of undergoing growth during pregnancy. Chemotherapy and radiation can lead to damage in all of these areas and compromise the likelihood of successful parenthood after HCT. Before starting any chemoradiotherapy regimen, the potential effects on the future fertility of the patient should be considered and discussed with the patient together with a discussion of fertility-preserving strategies and depending on the country may require a dedicated interview with a reproductive medicine specialized physician.

### 56.2.2 Gonadal Dysfunction in Women Following Chemoradiotherapy

Women are born with a finite number of eggs which can be fertilized for pregnancy or depleted over time as a result of physiological apoptosis or else menstruation. Chemoradiotherapy depletes further the number of follicles by (1) activating apoptotic pathways, (2) causing fibrosis of stromal blood vessels, and (3) activating resting (antral) follicles, leading to a “burnout” effect (Meirow and Nugent 2001; Kalich-Philosoph et al. 2013). The degree of ovarian damage is

related to the dose and type of chemotherapeutic agent used and baseline ovarian reserve which in turn is dependent on age and previous treatment. Manifestations of premature ovarian failure range from premature menopause to varying degrees of infertility. Alkylating agents have the highest age-adjusted odds ratio of ovarian failure (Meirow 2000). Even though some pregnancies and live births have been reported after either chemo-based or TBI-based myelo-ablative conditioning regimens, the risk of infertility after such MAC is very high. A combination of BU and CY is particularly gonadotoxic to females, but younger patients who receive CY only may have some gonadal function preserved, and pregnancies following CY are well described (Salooja et al. 2001).

Being transplanted in the peri-pubertal period with full-dose BU does represent a very high risk of infertility. Considering serum gonadotrophin level as a marker of ovarian failure, TREO seems to be less toxic than BU. However, we do not yet have evidence that pregnancy rates are higher in young female patients receiving TREO compared to those receiving BU (Faraci et al. 2019). TBI is also potentially sterilizing. The estimated median lethal dose of radiation for the human oocyte is less than 2 Gy (Wallace et al. 2003a). The effective sterilizing dose (ESD) decreases with increasing age, and while estimated as 18.4 Gy at 10 years of age, the ESD is approximately 14.3 Gy at 30 years of age and only 6 Gy in women over age 40 (Wallace et al. 2003b).

### 56.2.3 Gonadal Dysfunction in Men Following Chemoradiotherapy

In male patients, spermatogenesis is frequently impaired following chemoradiotherapy, but testosterone levels generally remain normal because of the relative resistance of testosterone producing Leydig cells to chemoradiotherapy. As a result, secondary sexual characteristics remain normal for male patients, and typically testosterone levels and luteinizing hormone (LH) levels are in the normal range. However, testosterone

levels have to be monitored on an annual or biannual basis since some male patients do experience hormonal deficiency, and this is associated with a reduction in quality of life. Spermatogonia are very sensitive to irradiation, and it takes approximately 2 years for sperm counts to recover to pre-irradiation levels after a single dose of 1 Gy (Meistrich and van Beek 1990). With higher doses, azoospermia persists longer or may be permanent. Following HCT conditioned with myeloablative doses of TBI, the majority of men will be azoospermic. Chemotherapy-only regimens are also associated with azoospermia but to a lesser degree (Rovo et al. 2013). Following BU, for example, approximately 50% will be azoospermic, while after CY alone recovery of spermatogenesis is more frequent (Mathiesen et al. 2020, 2021, 2022).

#### **56.2.4 Uterine Dysfunction in Women After Radiation**

Uterine development commences at puberty and is associated with an increase in both size and vascularity (Laursen et al. 1996). Exposure to radiation leads to reduced vascularity, fibrosis, and hormone-dependent endometrial insufficiency, which subsequently lead to adverse reproductive outcomes. Increased rates of infertility, miscarriage, preterm labor, intrauterine growth retardation, and low newborn birth weight have been described (Reulen et al. 2009), particularly if conception occurred within a year of radiotherapy (Fenig et al. 2001). Recently, uterine damage after BU has been reported (Courbiere et al. 2023).

#### **56.2.5 Prevention/Management of Gonadal Failure**

##### **56.2.5.1 Fertility Preservation in Males**

Sperm cryopreservation is an established fertility preservation option for postpubertal boys and men. Sperm can be used either for artificial

insemination or, if the quantity and/or quality of sperm are insufficient, for intracytoplasmic sperm injections for in vitro fertilization. There is a chance of sperm recovery with time particularly if the patient was under the age of 25 years at transplant, did not have TBI, and has no evidence of chronic GVHD (Rovo et al. 2013). These patients require reassessment at intervals to ascertain their fertility potential. Prior to transplant some patients who are unable to ejaculate will require interventions such as penile vibratory stimulation or else electroejaculation. If these fail, testicular sperm extraction (TESE) can be used to obtain samples for cryopreservation. (Halpern et al. 2020).

Testicular tissue cryopreservation in prepubertal male patient represents a feasible but experimental technique since hitherto there has been no demonstration of obtaining vital sperm from the testis sample in vitro or in vivo in humans. Some animal models including nonhuman primates are encouraging (Kanbar et al. 2022).

##### **56.2.5.2 Fertility Preservation Techniques in Females**

###### **Gonadotropin-Releasing Hormone Agonists (GnRHa)**

Despite success in animal models, the value of GnRHa to preserve ovarian function during chemotherapy in human subjects is uncertain. A Cochrane database review concluded that the use of GnRH agonists should be considered for ovarian protection in women of reproductive age who are receiving chemotherapy (Chen et al. 2011).

###### **Embryo and Oocyte Cryopreservation**

Embryo and oocyte cryopreservation are preferred methods of fertility preservation in women who require sterilizing treatment. The use of donor embryos/oocytes can also be discussed with the patient because they offer the possibility of pregnancy and parenthood albeit with a nongenetic child. Mature oocyte collection requires ovarian stimulation. These oocytes can then either be frozen or else fertilized

in vitro before freezing. These options are not open to all patients however. Ovarian stimulation takes a minimum of 2 weeks, and this delay is prohibitive for many patients with hematological malignancies. The requirement for a partner or donor sperm for embryo cryopreservation is another potential drawback; for some patients, sperm is not available, and for others, the involvement of a partner/sperm donor limits future reproductive autonomy as consent from the sperm provider must be given not only at the time of cryopreservation but also at the time of reimplantation.

### **Ovarian Tissue Cryopreservation (OTC)**

OTC is no longer considered experimental by the American Society for Reproductive Medicine Practice Committee (2019), but it remains unavailable to many patients. It is the only option open, however, to prepubertal patients or to women who cannot tolerate a significant delay in treatment due to disease severity or progression. Cortical fragments containing primordial follicles with immature oocytes can be obtained by laparoscopy and cryopreserved. Ideally, ovarian tissue should be obtained before the patient has been exposed to chemotherapy, but this is not always possible and is not an absolute requirement.

A major concern reimplanting cryopreserved ovarian tissue is the possibility of reseeding the tumor. The risk depends on the individual disease. Assessment by PCR of ovarian tissue taken from patients with leukemia (CML, AML, ALL), tested positive for disease in a number of cases and assessment of tissue from mice with severe combined immunodeficiency confirmed the leukemic potential of the tissue (Rosendahl et al. 2013). As a result, reintroduction of ovarian tissue from patients with leukemia would not currently be recommended. In the future, maturation in vitro of follicles from cryopreserved tissue may enable production of a viable disease-free alternative. In patients with lymphoma, histologically negative samples of ovarian tissue have been transplanted without initiating relapse, but in some cases the follow-up time was short.

### **56.2.5.3 Children and Adolescents**

Fertility preservation in children has been the subject of recent guidelines from the pediatric diseases working party of the EBMT (Dalle et al. 2017; Balduzzi et al. 2017). Extreme sensitivity is required, and parents have to be given complete information on the process, associated risks, and success rates. For prepubertal girls, OTC is currently the only potential fertility-sparing option. In peri-pubertal boys, it is sometimes possible to extract sperm using surgery/microdissection or electroejaculation under general anesthetic. In prepubertal males, the only option is testicular tissue cryopreservation; although work in animal models is encouraging, there have been no reports to date of reimplanted testicular tissue leading to human live births.

### **56.2.6 Management of Pregnancy After HCT**

Most patients or their partners who conceive after HCT have uncomplicated pregnancies. Chemoradiotherapy can potentially affect a variety of maternal organs relevant to a successful pregnancy outcome, for example, renal, cardiac, and pulmonary toxicity. Patients at risk should have an expert medical review early in pregnancy and may require regular specialist monitoring throughout and review by an anesthetist prior to delivery. Patients who have had TBI or pelvic irradiation have an increased risk of premature and small birth weight babies and may be at increased risk of miscarriage. In the absence of TBI, miscarriage rates are typically comparable to the background population, and no significant increase in congenital malformations or genetic abnormalities has so far been described when conception has taken place long after completion of therapy (Meirow and Schiff 2005; Green et al. 2009). Animal experiments suggest that most cytotoxic drugs are mutagenic and teratogenic to oocytes exposed during the maturation phase. In humans, this phase lasts approximately 6 months (Meirow and Schiff 2005), so there is a theoretical advantage to delaying conception for 6 months after completing gonadotoxic treatments.

## 56.3 Sexual Function

### 56.3.1 Background

Sexual dysfunction is one of the most frequently reported complications after HCT. It has a significant impact on quality of life, and it is important to patients, ranking among their top unmet needs (Bevans et al. 2017; Schover et al. 2014). In a study including 1742 survivors of transplant with a mean follow-up of 11.9 years, 40% of women and 27% of men had not been sexually active in the previous year, while 64% of women and 32% of men who were sexually active reported low sexual function (Syrjala et al. 2021).

Complications affect recipients of both allogeneic and autologous transplants and include decreased libido, dyspareunia ± vaginal dryness (females), and erectile and ejaculatory dysfunction (males) (Li et al. 2015). Allogeneic recipients have additional problems linked to acute or chronic GVHD (Wong et al. 2013). Sexuality is also affected, and this is multifactorial due to decreased self-confidence, stress, anxiety, and fear of recurrence, together with a change in body image (Yi and Syrjala 2009).

A number of medical conditions can impact on sexual health after HCT, for example, genital changes, hormone changes, vascular disease, or other chronic illness. The sexual well-being of the survivor is also determined by their relationship with their partner who may experience a decrease in sexual desire and anxiety about initiating sexual activity with their survivor partner (Langer et al. 2007).

### 56.3.2 Prevention/Management

It is important to identify relevant issues before problems with sexuality and intimacy become entrenched. Furthermore, discussion with a health-care professional (HCP) may be associated with a reduction in the development of sexual problems (El-Jawahri et al. 2018; Humphreys et al. 2007). Current guidelines endorse the importance of discussion including both transplant follow-up guidelines (Majhail et al. 2012)

and cancer survivorship guidelines from ASCO and NCCN (Carter et al. 2018; Denlinger et al. 2017). Transplant guidelines recommend regular discussion of sexual function at 6 months, 1 year, and annually thereafter while guidelines from NCCN and ASCO recommend that discussion is initiated during treatment planning. The patient experience, however, is that discussion happens infrequently (Gjaerde et al. 2023; Kim et al. 2020; Yoo et al. 2018), and this is mirrored by the perception of HCPs (Eeltink et al. 2018). Factors which have been proposed to facilitate discussion by HCP are (1) knowledge of sexual difficulties faced in this setting, (2) sufficient time for discussion, and (3) the use of a care plan to prompt discussion (Eeltink et al. 2018).

The following screening questions have been proposed (Syrjala et al. 2021):

1. Do you have any concerns regarding your sexual function, sexual activity, sexual relationship, or sex life? Yes/No
2. Are these concerns causing you distress, or would you like to discuss them? Yes/No.

If these two questions are affirmative, a more complete evaluation should be undertaken, and multimodal intervention is recommended (Syrjala et al. 2021). This approach is also endorsed by the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network in the USA (NCCN) for adult patients diagnosed with cancer (Carter et al. 2018; Denlinger et al. 2017). In transplant recipients, medical problems should first be identified and treated. For example, hormone deficiencies should be addressed, and some male patients benefit from the prescription of erectile dysfunction medication. Women with vaginal dryness may benefit from lubricants or topical estrogens, and those with GVHD may benefit from topical steroids (Tirri et al. 2015). Vaginal dilators or a low-dose estradiol vaginal ring may be helpful if vaginal stenosis has occurred. Subsequently, individual and/or couple counseling should be provided as it is recognized that psychological factors can play a large part in sexual dysfunction after transplant both for the

patient and for their partner. In transplant patients, a pilot study of multimodal intervention for patients with sexual dysfunction causing distress showed promising results leading to significant improvements in the following parameters: the number of patients who were sexually active, satisfaction with and interest in sex, erectile function, orgasm, and vaginal comfort (El-Jawahri et al. 2018).

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## **Part VII**

### **Prevention and Management of Relapse**

**Topic Leaders: Nicolaus Kröger  
and Peter Bader**



# Monitoring Measurable Residual Disease in ALL and AML

# 57

Peter Bader, Hermann Kreyenberg,  
and Gert Ossenkoppele

## 57.1 Monitoring MRD in ALL

Peter Bader and Hermann Kreyenberg

### 57.1.1 Introduction

In ALL evaluation of molecular treatment response, assessment of minimal residual disease, nowadays named measurable residual disease (MRD), is a substantial independent predictor of outcome, as proven by randomized studies (Conter et al. 2010; Gökbuget et al. 2012; Bassan and Spinelli 2015). Consequently, MRD is implemented in virtually all clinical protocols in order to supplement or to redefine multifactorial risk stratification with optional customized treatment intensity. The detection of leukemic

cells below the limit of classical cytomorphology is feasible by either disease-specific alterations of the immune phenotype or unique genetic features. Several competing and complementing MRD methods have been developed with preference application according to clinical protocols (Van der Velden et al. 2007; van Dongen et al. 2015).

### 57.1.2 MRD Assessment by IG/TCR Real-Time PCR

The discontinuous immune receptor genes provide the immune repertoire by somatic recombination of variable (V), diversification (D), and junction (J) elements, thus forming hypervariable CDR3 (complementarity-determining region 3) regions during lymphocyte maturation. Such rearrangements can serve as clonal index of leukemia blasts originating from lymphoid precursor stages. Additionally, due to a relaxed regulatory control, leukemia blasts can harbor incomplete rearrangements and cross-lineage rearrangements and tend to accumulate simultaneously multiple rearrangements. Quantitative real-time PCR using junction complementary allele-specific oligonucleotides (ASO) frequently reaches a detection limit of  $1E-05$  with a quantitative range of  $1E-04$ , is applicable to vast majority of cases, and has a high degree of standardization (Van der Velden et al. 2007).

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### 57.1.3 MRD Assessment by IG/TCR Digital PCR

One significant drawback of qPCR is its relatively high level of inaccuracy, susceptibility to inhibiting substances, and reliance on reference samples for MRD quantification. A novel technique, known as digital PCR and considered the third generation of PCR (Vogelstein and Kinzler 1999; Starza et al. 2022), has been developed. Although the digital PCR approach shares similarities with qPCR in terms of targets and primers, it can overcome these limitations. However, achieving precision in digital PCR relies on maintaining a well-balanced sample load in the reaction compartments (Huggett et al. 2013). The clinical usefulness of this method is still being verified (Della Starza et al. 2021).

### 57.1.4 MRD Assessment by Fusion Gene Transcript

Most frequent recurrent reciprocal translocations are in ALL t(9;22)(q34;q11) (*BCR-ABL1*), t(12;21)(p13;q23) (*ETV6-RUNX1*), and t(4;11)(q21;q22) (*MLL-AFF1*) with age stage-associated preponderance in adults, childhood, and infant ALL, respectively. Derived chimeric fusion transcripts are validated marker for MRD detection by real-time PCR with an achievable detection limit of 1E-06. The methodology has been standardized by the European Against Cancer (EAC) program (Gabert et al. 2003).

### 57.1.5 NGS (Next-Generation Sequencing)

High-throughput sequencing (HTS) of immune receptor genes by next-generation sequencing (NGS) is a novel option for MRD. This methodology provides comprehensive qualitative and quantitative information regarding clonal consistency of the diagnostic sample and shares one protocol for index determination and MRD assessment without the need of individual reagents. Potential subleukemic and new emerg-

ing leukemic clones also are covered. PCR steps during library construction can introduce bias effecting results internal controls and normalization calculations are necessary the generated data volume is high and data interpretation demand biostatistics expertise. Due to high sample capacity, NGS favors a centralized concept, and service is available to commercial providers by academic centers (Kotrova et al. 2015).

### 57.1.6 Flow Cytometry

MRD by multicomponent flow cytometry (MFC) distinguishes leukemia-associated immune phenotypes (LAIP) and regular cells. LAIP consists of cell lineage maturation stage-specific (backbone) markers in combination with illegitimate markers. The standard four- to six-color approaches have been developed simultaneously by several centers. Therefore, the applied marker panels depend on study protocol. The consistently achieved detection limit is 1E-04. Recently, increase of specificity and sensitivity was enabled by high-throughput procedures demanding eight- or ten-color equipment. Here, the options for targeted and visualized antigens allow simultaneous visualization of all developmental lymphocyte stages serving as background to distinguish leukemic cells. The EuroFlow Consortium validated available antibody panels and controls which can be applied in a standardized way, including automated gating with supportive software, data storage and comparison, accurate quantitative result, and option for IVD development. Similar to the NGS approach, the generated data volume is high, and data interpretation demands biostatistics expertise; nevertheless, the concept allows decentralized data acquisition (Pedreira et al. 2013).

### 57.1.7 Limitations of MRD Assessment

The determined level of MRD always is a result of complex interrelation of baseline characteristics of tumor and patient, time point of MRD

evaluation, therapeutic agents, course of clearance, and degree of therapy resistance. Several measurements therefore are mandatory. Adverse circumstances for MRD assessment are clonal selection and clonal evolution, since the associated index might be missed. Potentially impacted are leukemia with initial oligoclonality as observed in approximately 15% of B-ALL, and up to 1000 subclones have been reported (Wu et al. 2016). Phenotypic plasticity under treatment and massive lymphocyte regeneration can cause false negativity or positivity, a solvable problem by applying mentioned high-throughput methodologies. Achievable detection limit is correlated with cell count of sample, and aplastic samples are challenging. Finally, all methodologies use different sample preparations, and analyses refer to different units, a circumstance which interferes result comparison.

### 57.1.8 MRD in the Setting of HCT

As all adult patients with ALL who relapse after initial chemotherapy have an absolute indication for allo-HCT, pediatric patients are stratified into different treatment groups. Main prognostic determinants in these patients are the blast immune phenotype, time to relapse, and site of relapse. High-risk patients who experienced early isolated BM relapse, early relapse involving BM, and any BM relapse of T-lineage ALL have clear indications for HCT. Intermediate-risk patients experienced early or late combined BM relapse and a late isolated BM relapse of a B-cell precursor (BCP).

ALL and very early and early isolated extramedullary relapse of either BCP-ALL or T-ALL have indication for HCT if post-induction MRD exceeds a threshold of  $1E-03$  (Eckert et al. 2013).

During the past decades, it could be clearly shown by several studies that the level of MRD immediately prior to transplant does have a clear prognostic impact on post-HCT outcome (Knechtli et al. 1998). Retrospective studies in children with relapsed ALL revealed an important cutoff for post-HCT outcome. Patients who received transplantation with an MRD load of  $\geq 10$  to 4 leukemic cells had a by far inferior

prognosis than patients with lower MRD loads before transplant (Bader et al. 2009). Based on these findings, several studies are now underway investigating strategies to improve outcome in these ultrahigh-risk patients. Adaption of transplant approaches might allow successful transplantation (Leung et al. 2012).

Spinelli et al. showed that almost half of the patients with high levels of MRD before transplantation achieved molecular remission by day +100 (Spinelli et al. 2007). This finding indicates that MRD detection posttransplant provides additional value to the MRD assessment prior to transplantation. It could be demonstrated in prospective clinical studies that the close monitoring of MRD by different approaches allows the prediction of relapse and may therefore form the basis of different intervention strategies making use of leukemia-specific targeted therapy (Bader et al. 2015; Balduzzi et al. 2014). Future perspectives will focus on MRD-guided intervention to prevent overt relapse (Rettinger et al. 2017).

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## 57.2 MRD in AML

Gert Ossenkoppele

### 57.2.1 Introduction

Defining residual disease below the level of 5% leukemic cells has changed the landscape of risk classification (Ossenkoppele 2013). The measurable residual disease (MRD) approach establishes the presence of leukemia cells down to levels of  $1:10^3$  to  $1:10^6$  white blood cells, compared to  $1:20$  for morphology. The ELN 2022 recommendation response criteria includes apart from CR without measurable residual disease ( $CR_{MRD-}$ ) now also  $CR_{iMRD-}$  and  $CR_{hMRD-}$  and is defined as  $CR_{iMRD-}$  and  $CR_{hMRD-}$  with negativity for a genetic marker by RT-qPCR or CR with negativity by multicolor flow cytometry (MFC) (Döhner et al. 2022).

The reasons to apply MRD assessment in AML are (1) to provide a quantitative methodology to establish a deeper remission status; (2) to

better predict outcome and guide post-remission treatment; (3) to identify early relapse as a robust posttransplant surveillance, in order to enable early intervention; and (4) in the future to serve as a surrogate endpoint for survival to accelerate drug testing and approval (Ossenkoppele and Schuurhuis 2016).

The recent ELN MRD consensus document includes leads for standardized multiparameter flow cytometry-based MRD (MFC-MRD) and molecular MRD, MRD thresholds, and guidelines for clinical implications (Heuser et al. 2021).

## 57.2.2 Methods for MRD Detection

### 57.2.2.1 MRD Detection by PCR

Real-time quantitative PCR (RT-qPCR) allows MRD detection in cases with chimeric fusion genes generated by balanced chromosomal rearrangements (Grimwade and Freeman 2014). Other genetic alterations can also be used for MRD detection including mutated *NPM1*, *RUNX1-RUNX1T1*, *CBFBMYH11*, *PML-RARA*, *KMT2A-MLLT3*, *DEK-NUP214*, *BCR-ABL*, and *WT1*. Apart from *t(15;17)* and *RUNX1-RUNX1T1* and *CBFB-MYH11*, currently, *NPM1* is the best-validated molecular marker for MRD assessment. PCR assessment of MRD is in about 40–60% of patients in principle possible. The methodology has been standardized for several molecular markers for clinical implementation in the Europe Against Cancer (EAC) program (Gabert et al. 2003).

### 57.2.2.2 Immune MRD by Multicolor Flow Cytometry

The basic principle is to integrate diagnostic leukemia-associated immune phenotypes (LAIP) and different-from-normal (DfN) aberrant immunophenotype approaches to enable tracking of diagnostic and emergent leukemic clones (Heuser et al. 2021). These LAIPs consist of normally occurring markers, present in aberrant combinations in AML but in very low frequencies in normal and regenerating BM. The background levels of LAIP in normal and regenerating BM levels, in particular, although low, prevent specific

detection of aberrancies with sensitivities higher than 1:10,000.

If no diagnosis sample is present, one can make use of “different-from-normal” approach which uses a standard fixed antibody panel to recognize leukemic cells based on their difference with normal hematopoietic cells (Loken et al. 2012).

Currently, immune MRD aberrancies can be detected in over 90% of AML cases at diagnosis.

### 57.2.2.3 MRD Detection by NGS

NGS-based molecular MRD assessment targeted NGS-based MRD testing using specific mutations identified at diagnosis vs agnostic panel approaches are now mostly exploratively applied. Diagnostic AML samples are generally screened for mutations using a multigene panel (Jongen-Lavrencic et al. 2018).

Prognostic impact has been shown for selected mutations present at diagnosis and/or in complete remission (CR) samples (Ghannam et al. 2020; Dillon et al. 2023). Germline mutations (*ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *RUNX1*, and *TP53*) are noninformative as NGS-MRD markers (Godley 2021).

*DMT3A*, *TET2*, and *ASXL1* (DTA) mutations can be found in age-related clonal hematopoiesis and like germline mutations should not be used for MRD analysis as these mutations often persist during remission and do usually not represent the leukemic clone (Shlush 2018; Hasserjian et al. 2020).

Recently, three studies showed that *FLT3-ITD* is a highly prognostic biomarker in AML patient when measured after induction chemotherapy (Grob et al. 2023; Loo et al. 2022; Dillon et al. 2020). By a bioinformatic approach, *FLT3-ITDs* can be reliably detected with NGS (Blätte et al. 2019). These studies uniformly show that *FLT3-ITD* MRD  $\geq 0.01\%$  is clearly associated with outcome.

## 57.2.3 MRD in Clinical Studies

Despite a multitude of prognostic factors at diagnosis, the outcome of patients is still highly



variable and not individually predictable. On-treatment parameters in combination with prognostic factors present at diagnosis may be more useful.

The prognostic value of MRD in remission has been shown in patients treated with both intensive and more recently less-intensive treatment modalities (Terwijn et al. 2013a; Maiti et al. 2021; Freeman et al. 2013; Pratz et al. 2021). A recent systematic meta-analysis of 81 publications has convincingly shown the prognostic value of MRD for relapse and overall survival (Short et al. 2020). However, MRD is far from perfect, since relapses still occur in MRD-negative patients. Thus, a negative MRD test result may not indicate complete disease eradication but refers to disease below the MRD test threshold in the tested sample. Conversely, not all patients who are MRD-positive will relapse. Of note, Mol-MRD may remain detectable at low levels (CRM RD-LL) without prognostic significance and, therefore, are called negative operationally if the MRD values are below the threshold linked to prognosis (Dillon et al. 2020; Heuser et al. 2021). For instance, in CBF-AML and *NPM1* mutant AML, the transcripts may show persistent low-level expression after treatment, but this is not prognostic of relapse (Freeman et al. 2018).

Unfortunately, surrogacy for survival has not been proven yet (Hourigan et al. 2017; Ossenkoppele and Schuurhuis 2016; Walter et al. 2021).

#### 57.2.4 MRD in Relation to Transplant

Evidence is accumulating that the presence of MRD assessed by multicolor flow cytometry immediately prior to allogeneic HCT is a strong independent predictor of posttransplant outcomes in AML (Buckley et al. 2017; Walter et al. 2015). Araki et al. showed that in 359 adults, the 3-year relapse rate was 67% in MRD-positive patients, compared to 22% in MRD-negative patients, resulting in OS of 26% vs. 73%, respectively (Araki et al. 2016). This applies for the myeloablative as well as for the non-myeloablative transplant setting. The same was found in a large EBMT study (Gilleece et al. 2018).

Also, molecular MRD as measured by RT-PCR in *NPM1*-mutated AML has a significant impact on outcome after allo-HCT (Balsat et al. 2017).

Pretransplant MRD is prognostically also useful in CR2 (Gilleece et al. 2020). Importantly, it was demonstrated that the intensity of the conditioning regimen is dependent on MRD status. Myeloablative conditioning was only advantageous in the MRD+ setting (MRD+, RIC 34% vs MAC 59% 3 years OS; MRD-, RIC 61% vs MAC 60% 3 years OS) (Hourigan et al. 2020). We recently showed that MRD-guided therapy in intermediate-risk AML patients is a valuable strategy in reducing the number of allogeneic transplants without negatively affecting survival (Tettero et al. 2023).

Only sparse data is available for the posttransplant situation. Most available studies showed that the presence of posttransplant MRD had an adverse prognostic impact (Klyuchnikov et al. 2022; Zhou et al. 2016). In one study, this was irrespective of pretransplant MRD in patients with AML (Loke et al. 2023). Many trial groups certainly in Europe decide which post-remission treatment should be given based on MRD. Allo-HCT in the favorable group is usually not applied in the MRD-negative setting. For the intermediate group MRD- AML patients do not get an alloHCT in many centers and is only applied in CR2. The adverse-risk patient receives an allo-HCT independent of MRD status. The value should still be prospectively proven.

#### 57.2.5 Clinical Intervention Studies Posttransplant

There is a current much interest on intervention studies in the posttransplant setting based on MRD assessment. Preemptive therapy with azacitidine can prevent or substantially delay hematological relapse in MRD-positive patients with MDS or AML who are at high risk of relapse (Platzbecker et al. 2018). A number of groups summarized by Biederstadt et al. have explored administering DLI preemptively on detection of measurable residual disease (MRD) or mixed chimerism. Evidence for the effectiveness of this

strategy, although encouraging, comes from only a few, mostly single-center (Biederstädt and Rezvani 2023). Also, application of targeted therapy after transplant (e.g., FLT3 inhibitors) is currently under investigation.

It is clear that novel treatment strategies before, during, and after transplant are urgently needed to improve outcomes in AML. Thereby, depth of response prior to transplant, as measured by level of MRD, has emerged as one of the most important predictors of transplant outcome. Randomized trials are warranted to determine if MRD-guided preemptive therapy is associated with improved outcome.

Most importantly no clinical trial including transplantation trials should be performed without including MRD assessment.

### 57.2.6 Future Developments

New technologies are emerging to assess MRD. Standardization and harmonization are important and are currently further explored by the ELN MRD WP (Tettero et al. 2021). Quantifying leukemic stem cells seems a promising approach (Ngai et al. 2023; Terwijn et al. 2014; Zeijlemaker et al. 2016). MRD-guided studies like post-remission decisions, MRD conversion pretransplant, MRD-directed intensification of conditioning regimens, and posttransplant intervention should be encouraged.

#### Key Points

- MRD is now included in the definition of CR.
- MRD positivity is an independent predictor of relapse after chemotherapy in AML patients and a negative predictor for ALL patients.
- Pretransplant MRD positivity is highly indicative for relapse.
- MRD assessment should be implemented in every clinical trial.
- Prospective intervention studies guided by MRD are being performed.

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## Further Readings

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# Prevention and Treatment of Relapse by Drugs

# 58

Nicolaus Kröger and Nico Gagelmann

## 58.1 Introduction

Relapse has become the most frequent cause of treatment failure after HCT (Horowitz et al. 2018). Because outcome after relapse remains poor, major efforts are focused on prevention of relapse. Beside adoptive cell-based options, such as DLI and CAR T cells, the availability of novel effective pharmacological compounds has opened new avenues in clinical research to use those drugs early after HCT in order to prevent and treat relapse (Kroger et al. 2014). The optimal pharmacological compound should have a safe toxicity profile, an antitumor effect to the underlying disease, and an immune profile which can be used to booster the graft-versus-leukemia (GVL) effect and to reduce the risk of GVHD.

## 58.2 Tyrosine Kinase Inhibitors (TKI) Targeting BCR/ABL

Beside a direct antitumor effect, TKIs targeting BCR/ABL in BCR/ABL-positive acute lymphoblastic leukemia or chronic myeloid leukemia are considered to induce also immunomodulating effects by inducing effect on T-cell cytolytic function, reducing T-cell PD-1 expression, and

reducing myeloid-derived suppressor cells. TKIs targeting BCR/ABL such as *imatinib* induce more than 60% molecular remission in CML patients who relapsed after allograft. Smaller studies have investigated second-generation TKI successfully as maintenance therapy after allo-HCT for CML (Olavarria et al. 2007) and maintenance with TKIs after transplantation for blastic phase in CML seems to improve outcome (Niederwieser et al. 2021).

TKIs as maintenance therapy for Ph + ALL led to nonconclusive results. The CIBMTR did not find a difference in Ph + ALL patients who received posttransplant TKIs regarding relapse at 3 years, while in an EBMT study Ph + ALL patients who received TKIs posttransplant had lower relapse incidence and an improved LFS. In a small randomized study comparing TKI prophylactically or preemptive in Ph + ALL, no difference in survival was observed (Pfeifer et al. 2013). In a position statement, EBMT recommended in MRD-negative patients after allo-HCT either prophylactic or preemptive treatment (Giebel et al. 2016).

## 58.3 TKI Targeting FLT3-ITD

TKIs in the setting of FLT3-ITD-positive AML are of clinical relevance because a higher risk of relapse has been described for FLT3-ITD-positive patients who received allo-HCT CR1 (30% vs. 16%). Animal experiences had shown that

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*sorafenib* stimulated immunogenicity by induction of IL-15 which enhanced T-cell activation and GVL effect (Mathew et al. 2018a).

*Midostaurin* which is approved in the treatment of FLT3-positive AML has been tested in a phase II study as maintenance therapy in FLT3-ITD-positive patients with a low relapse rate at 12 months of only 9.2%, but a randomized trial could not find a survival benefit (Maziarz et al. 2018). In contrast, two prospective randomized trials compared sorafenib to placebo as maintenance therapy after allogeneic HCT and both studies showed significant benefit for sorafenib regarding event-free and overall survival (Burchert et al. 2020; Xuan et al. 2020). A meta-analysis suggested clear benefit of TKI post-allograft in FLT3-positive AML patients, favoring sorafenib (Gagelmann et al. 2021). Currently under investigation are randomized trials with other TKIs in FLT3-positive AML such as *quizartinib*, *gilteritinib*, and *crenolanib*.

For relapsed FLT3-ITD-positive patients, sorafenib can induce long-lasting CR, and retrospective data show better outcome of sorafenib plus DLI in comparison to DLI alone (Mathew et al. 2018a; Metzelder et al. 2012).

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## 58.4 Checkpoint Inhibitors

Checkpoint inhibitors blocking CTLA-4 and PD-1 are now widely used in solid tumors and also in hematological malignancies such as Hodgkin's disease (Ansell et al. 2015). Because of reversal of T-cell exhaustion by checkpoint inhibitors which may enhance a graft-versus-malignancy effect, this compound has also raised interest to be investigated after HCT. After auto-HCT PD-1 antibody, *pidilizumab* as maintenance therapy in DLBCL was well tolerated in a phase II study, and *nivolumab* has shown high response rate in patients with HL who relapsed after auto-HCT (Younes et al. 2016), while the combination of brentuximab vedotin plus nivolumab as consolidation strategy after auto-HCT was highly active for patients with high-risk relapsed or refractory classic HL (Herrera et al. 2023).

There is a concern about a higher risk of GVHD after checkpoint inhibition post-allograft, but *ipilimumab* did not induce high incidence of GVHD in phase I and phase II trials although the efficacy was limited with an overall response rate of less than 30% (Davids et al. 2016). PD-1 blockade investigated in a European trial was reported for 20 patients with HL who relapsed after allograft. The remission rate was high with 95% and 30% developed GVHD which was fatal in one patient. In a similar trial including 31 lymphoma patients who relapsed after allograft, the response rate was 77%, but 54% developed acute GVHD, and 8 patients died from GVHD-related complications (Haverkos et al. 2017).

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## 58.5 Hypomethylating Agents

Methylation has a crucial role in epigenetic regulation of gene expression and malignant cells using hypermethylation to switch off a variety of genes which are responsible for growth inhibition and apoptosis. DNA methyltransferase inhibitors such as *azacytidine* or *decitabine* are active in MDS and AML, and according to their toxicity profile, they can be used after allo-HCT. Beside their effect on gene modification for differentiation and cell growth, hypomethylating agents (HMA) lead also to an upregulation of HLA and tumor-associated antigen which may be targeted by donor T cells (Hambach et al. 2009; Goodyear et al. 2010). Furthermore, CD4 and CD8 T cells were strongly suppressed by HMA, while an increase of regulatory T cells has been described.

Azacytidine and decitabine either as single agent or in combination with DLI for relapsed patients have been reported, and up to 28% CR could be achieved including long-lasting remission (Schroeder et al. 2013). In a large EBMT study, an ORR of 25% with 15% CR and a 2-year OS of 12% have been reported for azacytidine after allo-HCT relapse in AML/MDS patients. Overall, the incidence of acute GVHD was low, and the addition of DLI did not improve response or OS. Smaller studies also reported efficacy of azacytidine to convert decreasing donor cell

chimerism into full donor cell chimerism (Platzbecker et al. 2012).

Treating patients with HMA prophylactically to prevent relapse has been tested in a randomized trial, but no benefit for EFS and OS could be observed (Oran et al. 2020), and currently oral azacytidine (CC-486) is investigated in prospective randomized clinical trials.

In contrast, G-CSF in combination with decitabine has significantly reduced relapse after allogeneic HCT in a randomized trial (Gao et al. 2020).

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## 58.6 Immunomodulating Drugs (IMiDs)

After auto-HCT, *thalidomide* has been tested alone and with glucocorticoids as maintenance to prevent relapse/progression. Most of these phase III trials demonstrated an improved PFS or EFS with variable improvement in OS, but due to toxicity, the drug has not become a standard care of treatment (Barlogie et al. 2008; Spencer et al. 2009). *Lenalidomide* is approved as maintenance therapy since a significant improvement in PFS has been shown in two randomized trials and improved OS on one randomized trial (McCarthy et al. 2012; Attal et al. 2012). A meta-analysis with data from three large studies (CALGB 100104, IFM-05-02, and GIMEMA RV-MM-PI-209) demonstrated an OS and a PFS benefit for lenalidomide maintenance. However, an increased risk of secondary primary malignancies was observed after lenalidomide maintenance therapy.

After allo-HCT, a stimulation of T cells has been shown for thalidomide, but second-generation IMiDs such as lenalidomide and *pomalidomide* induce an even more potent stimulation of T-cell-mediated immunity. IMiDs also stimulate the innate immune system including  $\gamma/\delta$ -T cells and NK T cells. Of note, it has been shown that thalidomide even when combined with DLI was not associated with increased risk for GVHD risk (Kroger et al. 2004). Because of the stronger T-cell stimulation, lenalidomide given early post-allo-HCT can cause severe

GVHD (Sockel et al. 2012), but starting with a low dose of only 5 mg and given the drug after discontinuation of IS reduces the risk of GVHD markedly (Wolschke et al. 2013).

Overall, IMiDs are potent agents for preventing relapse after auto-HCT, but their use post-allo-HCT remains to be defined primarily due to the increased risk of GvHD.

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## 58.7 Proteasome Inhibitors

Proteasome inhibitors are mainly used as induction therapy prior auto-HCT. Some studies investigated proteasome inhibitors as maintenance therapy after auto-HCT to reduce the risk of relapse. In a prospective study, *bortezomib* as maintenance therapy was superior to thalidomide particularly in patients with renal insufficiency and high-risk cytogenetics t(4;14) or del(17q) (Goldschmidt et al. 2018).

Bortezomib after allo-HCT was tested so far only in smaller studies with acceptable rates of GVHD (Caballero-Velazquez et al. 2013), and novel proteasome inhibitors such as *ixazomib* have been investigated as maintenance therapy after allografting in MM without significant benefits (Bashir et al. 2023).

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## 58.8 Monoclonal Antibodies

Most studies of maintenance therapy with MoAb have been conducted after auto-HCT. While maintenance therapy after autograft with anti-CD20 antibody *rituximab* failed to demonstrate an advantage for DLBCL with respect to RFS and OS (Gisselbrecht et al. 2012) for follicular lymphoma, an improved PFS but not an improvement in OS has been reported in a randomized study (Pettengell et al. 2013). An improved PFS and OS with rituximab as maintenance therapy have recently been shown for mantle cell lymphoma after auto-HCT (Le Gouill et al. 2017).

After allo-HCT for DLCL, rituximab maintenance therapy did not improve overall survival (Glass et al. 2014). Anti-CD30 antibody drugs conjugate *brentuximab vedotin* as maintenance

therapy after auto-HCT for HL did improve PFS but not OS (Moskowitz et al. 2015).

Anti-CD22-conjugated antibody *inotuzumab ozogamicin* has been approved for relapsed ALL and has shown also activity in patients with ALL who relapsed after HCT (Kantarjian et al. 2016), but the risk of SOS/VOD is about 11% and up to 22% for those who underwent allo-HCT after inotuzumab ozogamicin.

Bispecific antibodies such as CD19-directed CD3 T-cell-engaged *blinatumomab* are active in relapsed and refractory ALL and also in MRD-positive ALL and have been investigated successfully in combination with DLI after relapse post-allo-HCT (Ueda et al. 2016). Using blinatumomab as maintenance post-allogeneic HCT was found to be feasible, while its benefit may be dependent on the immune milieu at the time of treatment (Gaballa et al. 2022).

### 58.9 Histone Deacetylase Inhibition (HDACI)

Histone deacetylation is a crucial mechanism of epigenetic modulation, and HDACI promotes gene expression by unwinding of histone-bound DNA. Since HDACI reduces inflammatory cytokines and increases T-regulatory cells, the

drug was also used for GVHD prevention in a phase I/II study (Choi et al. 2014). *Panobinostat* was tested in two trials as maintenance therapy after allo-HCT in AML/MDS with or without (Bug et al. 2017) DLI resulting in an encouraging 1-year RFS of 66% in combination with DLI and 2-year RFS of 74% if used as single agent.

### 58.10 BCL-2 Inhibitors

*BCL-2 inhibitor (venetoclax)* has shown promising results in treatment of acute myeloid leukemia. Small phase II studies using *venetoclax plus decitabine* to prevent relapse have been reported, but until results from randomized phase III are available the drugs should be used only in clinical trials.

### 58.11 IDH1/IDH2 Inhibitors

IDH1 and IDH2 inhibitors ivosidenib and enasidenib have shown efficacy and favorable safety profile in the treatment of IDH1- or IDH2-positive acute leukemia and also in early phase I studies as maintenance post-allograft (Fathi et al. 2023).

Drug	Disease	Setting	Impact
Imatinib	CML	Prophylactic	Beneficial
	Ph + ALL		No benefit
Nilotinib	Ph + ALL	Prophylactic/preemptive	Unclear
Dasatinib	Ph + ALL	Prophylactic	Unclear
Sorafenib	FLT3-ITD AML	Maintenance	Beneficial
Midostaurin	FLT3-ITD AML	Maintenance	May be beneficial
Gilteritinib	FLT3-ITD AML	Maintenance	No benefit
Pidilizumab	DLBCL	Prophylactic	Unclear
Nivolumab	HL	Consolidation	Beneficial
Pembrolizumab	HL	Consolidation	Beneficial
Ipilimumab	Myeloid+lymphoid	Treatment	Unclear
Azacytidine (+DLI)	AML/MDS	Treatment	May be beneficial
		Prophylactic	No benefit
Decitabine (+DLI)	AML/MDS	Treatment	May be beneficial
Decitabine + GCSF	AML	Prophylactic	Beneficial
Ivosidenib	IDH1-mutated AML	Maintenance	Unclear
Enasidenib	IDH2-mutated AML	Maintenance	Unclear

Drug	Disease	Setting	Impact
IMiDs (Len, Thal, Pom)	Myeloma	Maintenance post-auto	Standard
		Post-allo	Unclear
PI (Bor, Car, Ixa)	Myeloma	Maintenance post-auto	May be beneficial in HR
		Post-allo	Unclear
Brentuximab vedotin	HL	Maintenance post-auto	Beneficial
		Post-allo	Unclear
Rituximab	DLBCL	Maintenance post-auto	No benefit
		Post-allo	
	MCL	Maintenance post-auto	Beneficial
		Post-allo	Unclear
Inotuzumab ozogamicin	ALL	Treatment	Unclear
Blinatumomab (+DLI)	ALL	Treatment	Beneficial
		Maintenance	May be beneficial
Venetoclax (+DLI/AZA)	AML	Prophylactic	Unclear

*AML* acute myeloid leukemia, *MDS* myelodysplastic syndromes, *ALL* acute lymphoblastic leukemia, *Ph+* Philadelphia chromosome-positive, *HL* Hodgkin lymphoma, *DLBCL* diffuse large B-cell lymphoma, *MCL* mantle cell lymphoma, *DLI* donor lymphocyte infusion, *Len* lenalidomide, *Thal* thalidomide, *Pom* pomalidomide, *Bor* bortezomib, *Car* carfilzomib, *Ixa* ixazomib, *AZA* azacytidine, *HR* high risk

### Key Points

- Outcome after relapse to allogeneic stem cells remains poor, and major efforts should focus on prevention of relapse.
- Beside adoptive cell-based options such as DLI, the availability of novel effective pharmacological compounds has opened new avenues in clinical research, mainly:
  - Tyrosine kinase inhibitors (TKI) targeting BCR/ABL
  - TKI targeting FLT3-ITD (sorafenib, midostaurin, quizartinib, gilteritinib, crenolanib)
  - Checkpoint inhibitors (pidilizumab, nivolumab, ipilimumab, pembrolizumab)
  - Hypomethylating agents (azacytidine, decitabine)
  - Immunomodulating drugs (thalidomide, lenalidomide, pomalidomide)
  - Proteasome inhibitors (bortezomib, ixazomib)
  - Antibodies (rituximab, brentuximab vedotin, inotuzumab ozogamicin, blinatumomab)

- Histone deacetylase inhibition (panobinostat)
- BCL-2 inhibitor (venetoclax)
- IDH1/IDH2 inhibitors (ivosidenib and enasidenib)
- The optimal pharmacological compound should have a safe toxicity profile, an antitumor effect to the underlying disease, and an immune profile which can be used to booster the GVL effect and to reduce the risk of GVHD.

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# Delayed Transfer of Immune Cells or the Art of Donor Lymphocyte Infusion (DLI) 2.0

# 59

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## 59.1 Biology of Donor Lymphocyte Infusion (DLI)

### 59.1.1 Diversity of Lymphocyte Subsets Used for DLI

In the context of an allogeneic hematopoietic cell transplantation (HCT), the interplay between host and donor immune cells is considered to be the primary mechanism responsible for graft-versus-leukemia (GVL) reactivity and also able to mediate graft-versus-host disease (GVHD) (Schmid et al. 2021). The tissue specificity of the immune response determines the balance between GVL and GVHD, as well as tropism of

GVHD. The main population for success and failure of HCT and DLIs originates from  $\alpha\beta$ T cells. Other subsets are also key modulators of efficacy. For example, NK cells most likely provide acute control of leukemia and of infections like CMV. However, NK cells become rapidly educated over time (Orr and Lanier 2010) and lose their antileukemia activity. Thus, donor transfer of NK cells is obsolete and needs additional, for example, genetic modification to engineer long-term efficacy (Laskowski et al. 2022; Liu et al. 2020). Other subsets, like  $\gamma\delta$ T cells, appear to have a more prolonged antileukemia effect (Handgretinger and Schilbach 2018; Sebestyen et al. 2020) and are also helpful in controlling CMV reactivation (Scheper et al. 2013; de Witte et al. 2018). However, also, donor  $\gamma\delta$ T cells can lose activity over time, and sustainable activity requires, outside the context of an HCT, most likely further modifications (Sebestyen et al. 2020; Li et al. 2023). NKT cells, like regulatory T cells, have been mainly reported to influence GVHD. While an increase in NKT cells in the graft associates with a reduced GVHD incidence (Malard et al. 2016), depletion of regulatory T cells in donor lymphocyte infusions (DLI) improves GVL effects, although it augments the risk of GVHD (Maury et al. 2010). Thus, lymphocyte infusions as part of the graft at the time of transplantation, or delayed as DLI, have multiple effector cells that need to be considered in terms of different alloreactive effects (for review see also (Schmid et al. 2021)).

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### 59.1.2 Naïve $\alpha\beta$ T Cell-Host Dendritic Cell (DC) Interaction as a Key Driver of Immune Responses

Since in the context of HLA-matched transplantation most alloreactive  $\alpha\beta$ T cells are present within the naïve repertoire of the donor, recipient-derived dendritic cells (DC) play an essential role in provoking the  $\alpha\beta$ T-cell immune response (Stenger et al. 2012). DCs are key players in provoking appropriate T-cell activation, and because DCs are derived from the hematopoietic system, an immune response of donor origin targeting DC from the recipient will likely result in an immune response against recipient hematopoietic cells, including the malignant population, and therefore give rise to GVL. The level of cross-reactivity against antigens broadly expressed on non-hematopoietic cells will determine the likelihood and severity of GVHD. DCs are present in the lymphohematopoietic system but also with relatively high frequencies in the target tissues of GVHD. At the time of transplant, all DCs are of recipient origin. When activated by danger signals provoked by tissue damage and pathogens, DCs will present endogenous antigens, as well as cross-present antigens derived from the non-hematopoietic tissues and pathogens. Therefore, in T-cell-replete HCT, it is difficult to dissect the GVL and GVHD effects (Boelens et al. 2018; Admiraal et al. 2017). Consequently, many current transplantation techniques deplete immune cells from the graft and administer DLIs at later time points as standard part of the transplantation regimen. Several T-cell depletion strategies are being used including a complete immune depletion by selection of CD34-positive stem cells or the use of specific antibodies including antithymocyte immunoglobulins (ATG) or alemtuzumab (Pasquini et al. 2012). Since after transplantation the recipient DC is gradually replaced by the donor DC, the magnitude of the immune response by infused donor T cells will gradually decrease allowing early DLIs for the majority of patients (e.g., from 100 days after transplantation) and an improved segregation of GVL and GVHD effects. Partial depletion of alloreactive T cells through post-

transplantation cyclophosphamide (PTCY) (Mielcarek et al. 2016) gives rise to a special situation. Since in this strategy an actively ongoing alloimmune response is abrogated, not only activated alloreactive donor T cells are being abrogated, but recipient DC may already have been attacked since these DCs are likely to have been involved in the initiation of this T-cell response. As a result, it is likely that many recipient DCs will be depleted at this phase. This DC depletion may allow earlier DLIs without causing severe GVHD, but vice versa may require higher doses to provoke an effective GVL reactivity. Similarly, following resolved GVHD which is associated with elimination of recipient DC, higher doses of DLI may also be required to induce an effective GVL response. In the case of haploidentical or partially mismatched transplantation, DLI may more readily provoke a profound immune response since in these cases the alloreactive T cells will also be present in the memory repertoire with a lower threshold of alloimmune activation not requiring professional recipient DC to be present, though little consensus in daily practice was reported (Santoro et al. 2023). Other more recent transplantation strategies consider the variety of immune cells. These novel strategies utilize either a selective depletion of  $\alpha\beta$ T cell (Locatelli et al. 2017; de Witte et al. 2023; Nijssen et al. 2023) or naïve subsets (Bleakley et al. 2015) to abrogate GVHD while maintaining early immune surveillance directed against infections as well as leukemia. Such strategies might require lower dosages for DLIs as compared PTCY depletion (de Witte et al. 2021a), as DCs of patients are not harmed during or after conditioning.

### 59.1.3 Diversity of Immune Repertoires and Potential Impact on Interventions

After HCT, the  $\alpha\beta$  and  $\gamma\delta$ TCR repertoire is reconstituted out of the graft of the donor, which contains in T-cell replete transplantations, between  $5 \times 10^7$  and  $1 \times 10^9$  T cells/kg (de Witte et al. 2021b). Of the T cells, the  $\gamma\delta$ T cells are the first

to reach normal numbers, followed by the CD8+  $\alpha\beta$ T cells and finally the CD4+  $\alpha\beta$ T cells which do not reach normal levels within the first year after HCT (de Koning et al. 2021). It is important to note that numerical reconstitution of the T cells does not mean that the diversity of the repertoire is already normalized, reflected by the clinical observations that patients are highly vulnerable to many infections for years after HCT. Repertoires for both  $\alpha\beta$  (van Heijst et al. 2013) and  $\gamma\delta$ T cells (Ravens et al. 2017) are stable over time in healthy individuals. Factors that influence the T-cell repertoire reconstitution after HCT include the source of the graft, occurrence of infectious challenges such as CMV and EBV, GVHD, and cellular interventions such as DLI. The repertoire of  $\alpha\beta$ T cells after HCT has been studied extensively in different HCT settings. Six months after HCT, the  $\alpha\beta$ TCR repertoire is still very restricted when compared to that of healthy individuals. A cord blood graft leads to a greater diversity of the  $\alpha\beta$ TCR repertoire at 6 and 12 months, compared to other graft sources (van Heijst et al. 2013). Even 2–5 years after HCT, the repertoire is still not as diverse as in healthy individuals (van Heijst et al. 2013; Kanakry et al. 2016). CMV reactivation shapes the repertoire in such way that a marked contraction of the diversity is observed (van Heijst et al. 2013; Kanakry et al. 2016; Suessmuth et al. 2015). GVHD has been associated with both an increased (van Heijst et al. 2013) and a decreased diversity (Yew et al. 2015).

However, we favor the hypothesis that selective GVL reactivity is associated with lower diversity, lower magnitude, and relatively tissue-specific recognition of hematopoiesis by alloreactive  $\alpha\beta$ T cells (van Bergen et al. 2017). Less is known about the diversity of the  $\gamma\delta$ TCR repertoire after HCT. The repertoire of the  $\gamma\delta$ T cells seems to be established quite early, at 30–60 days after HCT. CMV reactivation promotes a massive expansion of a few  $\gamma\delta$ T cells (Ravens et al. 2017). However, whether CMV reactivation is needed for repertoire focusing in  $\gamma\delta$ T cells is still under debate. Within this context, it is reasonable to argue that the administration of a DLI might in the future depend not only on the type of the dis-

ease or timing but also on the size of the  $\alpha\beta$  and  $\gamma\delta$ T-cell repertoire observed at a given time point as well as the existing pool of antigen-presenting cells such as DC, which depends again on the type of transplantation regimen (de Witte et al. 2021b).

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## 59.2 Recommendations for Prophylactic and Preemptive DLI as well as DLI After Relapse

### 59.2.1 General Considerations

Currently, neither the diversity of the TCR repertoire nor the infusion of subsets of lymphocytes is used to guide or fine-tune the intervention DLI in daily practice. To prevent relapse of the underlying disease, timing and dosing of non-manipulated DLI after HCT can be used to relatively skew the immune response toward GVL reactivity, as tissue damage after transplantation is gradually repaired and the donors' DCs steadily replace the recipients' DCs within the first 6 months after HCT. Therefore, the magnitude and diversity of the interplay between host and donor immune subsets will progressively diminish. This is evidenced by the clinical observation that when the interval between HCT and the infusion of DLI increases, the total number of  $\alpha\beta$ T cells that can be administered without induction of severe GVHD will increase from less than  $10^5$ /kg after 3 months to more than  $10^6$ /kg at 6 months (Table 59.1) (Yun and Waller 2013; van der Zouwen et al. 2023). Main prerequisite at the time of DLI is therefore also the absence of tissue damage and inflammatory circumstances, thus a lack of GVHD and uncontrolled infections.

### 59.2.2 Timing, Dosing, and Frequency of DLI

The following recommendations refer to the infusion of non-manipulated donor cells after no or in vivo T-cell-depleted transplantation from matched sibling or unrelated donors in patients

**Table 59.1** Timing and dosing of prophylactic and preemptive DLI. Level C evidence. A DLI can be repeated at on log higher 6–8 weeks after the first DLI, when, for example, MRD is still present and no GVHD is observed. GVHD as endpoint of repetitive DLIs for preemptive DLIs is in the era of MRD monitoring no longer recommended

	Timing of first dose	MRD	MUD	MMUD/Haplo	Number of DLI	References
Prophylactic	3 months (ex vivo TCD: any risk; no ex vivo TCD: high-risk disease)	$1 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	1–3 <sup>b</sup>	Schmid et al. (2012)
	6 months <sup>a</sup> (high-risk disease)	$1 \times 10^6/\text{kg}$	$1 \times 10^6/\text{kg}$	$5 \times 10^5/\text{kg}$		Tsirigotis et al. (2016)
Pre-emptive	3 months	$1\text{--}5 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	$1 \times 10^5/\text{kg}$	1–4 <sup>c</sup>	De Witte et al. (2021a, b)
	6 months <sup>a</sup>	$1\text{--}3 \times 10^6/\text{kg}$	$1 \times 10^6/\text{kg}$	$5 \times 10^5/\text{kg}$		
Relapse	After chemotherapy	$1 \times 10^7/\text{kg}$	$1 \times 10^7/\text{kg}$	$1 \times 10^6/\text{kg}$	1–4 <sup>d</sup>	Santoro et al. (2023) van der Zouwen et al. (2023)

Interval between DLI: at least 4–6 weeks

<sup>a</sup>Starting at 4 months, higher doses in the range employed at 6 months can be discussed

<sup>b</sup>Second and third dose: increase each DLI dose 0.5–1 log

<sup>c</sup>Number of DLI: MRD/chimerism guided, consider continuation when MRDneg/full chimerism; in absence of MRD marker: GvHD guided

<sup>d</sup>Second to fourth dose: increase each DLI dose 1 log, to a maximum dose of  $1 \times 10^8/\text{kg}$

with acute leukemia or MDS, which is the most frequently studied scenario. Further aspects, which may modify these recommendations, are discussed below. With respect to the indication of DLI for prevention of overt hematological relapse, two situations are distinguished. Furthermore, DLIs can be given within the context for overt relapses:

1. A *prophylactic DLI* is applied in patients with a high risk of relapse but at a stage when there is no evidence of the underlying disease. Usually, prophylactic DLI are given starting from day+90 or +100 after transplantation, provided that the patient is off immunosuppression and free of GVHD for about 1 month. CD3+ doses used for the first infusion depend on donor type and timing and vary between  $1 \times 10^5/\text{kg}$  patient and  $1 \times 10^6/\text{kg}$  (Table 59.1). In the absence of GvHD, most groups have given prophylactic DLIs as single-shot intervention, but also repetitive DLIs are reported
2. *DLIs are administered preemptively*, i.e., in case of persistent minimal residual disease (MRD) or when the first signs of relapse are observed, like MRD positivity or a decreasing donor chimerism. For persisting MRD, either the same initial cell dosages as for prophylactic DLI is used, followed by repetitive DLIs in 4–12 weeks' intervals, using an escalated dose schedule and increasing the cell dosages by fivefold to tenfold at each infusion.

(Table 59.1 (Tsirigotis et al. 2016; Jedlickova et al. 2016)). As with any maintenance therapy, the balance between relapse prevention and toxicity (i.e., GvHD) of prophylactic DLI needs to be acknowledged. Hence, treatment success after DLI has been defined as being alive without relapse and immunosuppression for GVHD by some investigators (Eefting et al. 2016). The optimal number of DLIs and timing between DLIs needed still needs to be defined and might depend on the initial transplantation regimen as discussed.

Alternatively, fivefold to tenfold higher initial cell doses are used in the preemptive situation as compared to prophylaxis. A total of three to four DLIs may be administered, and subsequent infusions are mostly taken from the same apheresis as the first but are frozen in the previously planned dosages. Occurrence of GVHD after DLI will result in no further DLI administration. For reappearance of MRD or mixed chimerism, obviously timing of DLI depends on the occurrence of these circumstances.

3. *For overt relapses*, a combination of DLI with chemotherapy is mandatory (Schmid et al. 2012), and cell doses used in that situation are usually one order of magnitude higher than in the prophylactic or preemptive situation ( $1 \times 10^7/\text{kg}$ ). In particular, in acute leukemia, DLI alone may not be the preferred strategy for treatment of relapse. Repetitive DLIs can be considered after overt relapses based, for example, on MRD positivity 6–8 weeks after DLI.

### 59.2.3 Factors that May Influence Timing, Dosing, and Frequency of DLI

Due to the great variabilities among different approaches and indications for DLI, a discussion of clinical outcome is beyond the scope of this summary. A comprehensive review has been performed recently (Schmid et al. 2021). Key factors with impact on clinical outcome are as follows:

1. *MRD*: Six weekly scheduled DLI with escalating doses until the first signs of GVHD as described above might no longer be necessary in the era of molecular disease monitoring, as increased numbers of DLI associate with an increased incidence of GVHD (Yan et al. 2017). An MRD-driven strategy with more time between DLIs (8–12 weeks) might still allow for control of the hematological malignancy while avoiding long-term side effects like acute or chronic GVHD (Schmid et al. 2022).
2. *Underlying disease*: The different underlying diseases might require different doses, considering their sensitivity to a DLI-mediated GVL effect. The relapse workshop of the National Cancer Institute has proposed an estimate of the sensitivity of different diseases to DLI (Alyea et al. 2010). Accordingly, sensitivity is regarded as high for CML, myelofibrosis, and low-grade NHL; as intermediate for AML, MDS, multiple myeloma, and Hodgkin's disease, and as low for ALL and DLBCL.
3. *Donor origin*: Dosage of DLI can under certain circumstances relate to the origin of the donor (Table 59.1). There is no consensus as to whether the dose between an unrelated and a related donor needs to differ. Similarly, cell doses in the haploidentical setting are unclear. More importantly and not well understood, but of greater impact, is most likely the processing of the DLI product with higher potency of freshly infused DLI when compared to frozen DLIs or DLIs used from the mobilized stem cell product due to different viabilities and compositions (Lemieux et al. 2016). However, a most recent meta-analysis showed no difference between a conventional and G-CSF-mobilized DLIs in the risk of all-cause mortality, though causes of mortality differed (Kirkham et al. 2022).
4. *Mechanism of immune escape*: Beyond, the increasing understanding of mechanisms behind escape from the allogeneic immune surveillance in patients with increasing or fully developed post-transplant relapse might be relevant. Among others, the loss of mismatched HLA after haploidentical HCT (Vago et al. 2009) and the downregulation of the HLA machinery by other mechanisms in the HLA identical setting (Toffalori et al. 2019) are thought to influence both indication for and dosing of DLI.
5. *Balance of host antigen-presenting cells and immune and graft-versus-host status*:  
As discussed in 59.1.3, different transplantation regimens can have different impact on the presence of host antigen-presenting cells, and additional immune repertoires present at

the time point of DLI (de Witte et al. 2021b). Therefore, dosage and number of repetitions of DLI need to be placed within the context of a defined transplantation regimen. This includes type of T-cell depletion and conditioning (e.g., ATG or PTCY) as both can act on the presence of host dendritic cells and therefore impact efficacy and toxicity.

6. *Combination with other drugs*: DLIs are used in many diseases in combination with specific drugs targeting molecular aberrations of the underlying malignancy and/or acting via immune-modulating activities. However, the early administration of lenalidomide after transplantation has been associated with a high incidence of GVHD (Kneppers et al. 2011), indicating that doses of DLI can also critically depend on the co-administration of drugs. Combinations with interferon- $\alpha$  and GM-CSF have also been reported as successful intervention to enhance the GVL effect (Dickinson et al. 2017). Other drugs currently explored are 5-azacytidine, HDAC inhibitors (Bug et al. 2017), and Flt3-inhibiting TKI (Mathew et al. 2018), and dosage as well as timing of combined DLIs might be guided by the experience from prophylactic and preemptive DLIs but need to be carefully monitored.

### 59.3 Conclusion

Based on these considerations, it is challenging to provide specific guidelines for dosing and timing of DLIs. A lack of consensus on how to precisely administer DLIs might generate, if carefully monitored, a unique opportunity to gain new insights into how DLIs need to be given. Strict institutional guidelines and rigorous reporting on details of DLI like processing, timing, dosing, intervals, and combination with immune-modulating drugs are therefore needed, and the new cellular therapy registry of EBMT is designed to allow for analysis of daily practice and its impact on clinical outcome in years to come.

#### Key Points

- Naïve  $\alpha\beta$ T-cell-host dendritic cell (DC) interaction is a key driver of immune responses.
- The balance of naïve  $\alpha\beta$ T cell and DC differs between different transplantation techniques after HCT and thus impacts most likely the dose of DLI that can be given at a certain time point after transplantation.
- Dosage of DLI depends also on timing after HCT as over time host DCs are replaced by donor DC.
- The underlying disease can determine efficacy as well as toxicity of a certain dose of DLI.

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# Cellular Therapy with Engineered T Cells, Efficacy, and Side Effects: Gene Editing/Gene Therapy

# 60

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## 60.1 Introduction

The cellular basis of cancer immune surveillance, already hypothesized in ancient times, was only proven with the advent of HCT. Indeed, the discovery of the nature of GVHD and its antileukemic effects (Weiden et al. 1979) was followed by the first successful attempts of adoptive immunotherapy using donor leukocytes (Kolb et al. 1990). To address the significant GVHD risk associated with allogeneic T cells, several

approaches of T-cell manipulation were developed and tested (Table 60.1). Some of these strategies rely on the genetic manipulation of T cells. First, suicide gene therapy approaches were established to promote GVL and immune reconstitution while controlling GVHD. More recently, strategies based on the genetic transfer of tumor-specific T-cell receptors (TCRs) or chimeric antigen receptors (CARs) were developed to improve antitumor efficiency of T cells. This chapter provides an overview of this vastly evolving area.

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**Table 60.1** T-cell-based cellular therapy approaches to increase GVL/GVI while taming GVHD

Strategy	Mechanism of action	References
Infusions of pathogen (i.e., CMV, EBV)-specific T cells	Isolation and infusion of T cells specific for opportunistic pathogens to control posttransplant infectious morbidity and mortality	Riddell et al. (1992), Rooney et al. (1995), Koehne et al. (2003)
Infusions of T cells depleted of alloreactive specificities	In vitro activation of host-reactive T cells followed by their depletion, infusion of remaining cells with the aim of promoting immune reconstitution with a reduced GVHD risk	André-Schmutz et al. (2002), Hartwig et al. (2008), Mielke et al. (2008)
Infusions of regulatory T cells	Isolation and/or expansion T-cell subsets with regulatory properties to promote immune reconstitution with a reduced GVHD risk	Groux et al. (1997), Chen et al. (2003), Trenado et al. (2004), Brunstein et al. (2011), Di Ianni et al. (2011), Bacchetta et al. (2014)
Infusion of T cells depleted of regulatory T cells	Infusion of T cells depleted of regulatory T cells to increase the antileukemic activity of DLI	Maury et al. (2010)
Infusions of leukemia-specific T cells	Isolation and infusion of T cells specific for leukemia-associated antigens to boost the GVL potency of DLI	Warren et al. (2010), Bornhäuser et al. (2011), Chapuis et al. (2013), Comoli et al. (2017)
Infusion of alpha-/beta-depleted T cells	Infusion of a graft in vitro depleted of conventional alpha/beta T cells, thus enriched of gamma-delta T cells, endowed with antitumor activity and a low GVHD potential	Lang et al. (2015), Airoidi et al. (2015), Maschan et al. (2016)
Infusion naïve-depleted T cells	Infusion of donor T-cell subsets in vitro depleted of naïve cells, with the aim of promoting immune reconstitution with a reduced GVHD risk	Bleakley (2015)
Infusions of CIK	Infusions of in vitro activated donor CIK cells to promote GVL and reduce the risk of GVHD	Introna (2007), Introna et al. (2010)
Suicide gene therapy	Donor lymphocytes are genetically engineered to express a suicide gene and then infused after HCT to promote GVT and immune reconstitution while selectively controlling GVHD with the prodrug-mediated activation of the suicide gene	Bonini et al. (1997), Ciceri et al. (2009), Fehse et al. (2004), Di Stasi et al. (2011), Zhan et al. (2013), Oliveira et al. (2015)
CAR/TCR T cells	Lymphocytes are genetically engineered to express a chimeric antigen receptor (CAR) or a T-cell receptor (TCR) that confers to T cells specificity for an antigen expressed by cancer cells	Kochenderfer et al. (2010), Porter et al. (2011), Brentjens et al. (2013), Morgan et al. (2006), Robbins et al. (2011)

CIK cytokine-induced killer

## 60.2 Suicide Gene Therapy

The transfer of a suicide gene into donor lymphocytes was designed and tested at preclinical and clinical level in the 1990s, with the aim of transferring the entire donor T-cell repertoire, inclusive of cancer and infectious specificities, to transplanted patients while enabling the selective elimination of the transferred lymphocytes in case of GVHD (Bonini et al. 1997). The first suicide gene, and to date the most extensively tested in clinical trials, is thymidine kinase of herpes simplex virus (HSV-TK). In dividing cells, HSV-TK expression confers selective sen-

sitivity to the antiviral drug ganciclovir. Upon retroviral gene transfer, HSV-TK is stably expressed by donor T lymphocytes not interfering with their functionality. However, when exposed to ganciclovir, highly proliferating HSV-TK-expressing T cells (TK cells) will die in a dose-dependent manner. Thus, if ganciclovir is administered during GVHD to patients treated with TK cells, activated and thus proliferating alloreactive TK cells will be eliminated. The HSV-TK/ganciclovir suicide system proved highly effective in controlling GVHD in several transplant settings, including haploidentical HCT (haplo-HCT). After T-cell-depleted haplo-

HCT, the infusion of TK cells promoted broad and rapid immune reconstitution, which, being associated with GVHD control, led to abrogation of late transplant-related mortality (Ciceri et al. 2009). Overall, clinical results obtained with TK cells led to their conditional approval by EMA in 2016, thus representing the first genetically engineered medicinal product approved for cancer patients in Europe. Although when infused after haplo-HCT TK cells could be detected for more than 14 years (Oliveira et al. 2015), their persistence might be limited when cells are infused to immunocompetent patients, due to the viral origin of HSV-TK and to its subsequent immunogenicity in humans. Alternative suicide genes were designed and tested in clinical trials. iCasp9, in particular, is an innovative suicide gene based on human components and thus with a reduced risk of immunogenicity, which was recently proposed and successfully tested in clinical trials (Di Stasi et al. 2011; Zhou et al. 2014). Overall, more than half of the patients who had received suicide gene-expressing donor T cells experienced a clinical benefit in terms of immune reconstitution and GVL. Of notice, all cases of GVHD were completely controlled by the suicide gene/prodrug systems.

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## 60.3 CAR-T Cells

### 60.3.1 CAR-T-Cell Clinical Efficacy

CARs are designer molecules comprised of several components: an extracellular antigen-binding domain, usually the variable light and heavy chains of a monoclonal antibody (scFv), a spacer and transmembrane region that anchors the receptor on the T-cell surface and provides the reach and flexibility necessary to bind to the target epitope, and an intracellular signaling module, most commonly CD3 zeta and one or more costimulatory domains that mediate T-cell activation after antigen binding, resulting in their profound proliferation and eventually selective tumor cell killing.

The first clinical development was the use of CARs specific for the B-lineage marker CD19.

Several groups demonstrated that CD19 CAR-T cells are able to induce durable complete remissions in patients with chemotherapy- and radiotherapy-refractory B-cell ALL, NHL, and CLL (Maude et al. 2014; Park et al. 2018; Turtle et al. 2017).

However, in a fraction of patients, resistance mechanisms to CD19 CAR-T-cell therapy have become apparent, including the development of leukemia cell variants that lost their CD19 antigen expression, particularly in ALL. Several mechanisms may contribute to the development of this phenotype including lymphoid-to-myeloid transdifferentiation, selection of preexisting CD19-low/CD19-negative leukemia clones, and emergence of clones that lost the specific epitope targeted by the CD19 CAR due to alternative splicing (Gardner et al. 2016; Sotillo et al. 2015; Ruella and June 2016). In ALL, CD19-low/CD19-negative leukemia cells may still express CD20, CD22, and/or CD123 that are being pursued as rescue antigens. A recent study highlighted the potential to re-induce remissions in patients that had relapsed with CD19-low/CD19-negative leukemia and subsequently received CD22 CAR-T cells (Fry et al. 2018). Unfortunately, CD22 itself is prone to internalization and downregulation, and indeed a significant proportion of patients experienced successive CD22-low/CD22-negative leukemia relapse. At present, combinatorial targeting of CD19 with either CD20, CD22, or CD123 is being explored, either through bi-specific CAR constructs with two scFvs *in cis* or through co-expression of two CAR constructs in the same T cells (Zah et al. 2016).

Since 2017, outcomes of CAR-T-cell clinical trials (Table 60.2) led to the approval by FDA and EMA of six CAR-T-cell therapeutic products, including four specific for CD19, approved for B-cell lymphoma and acute lymphoblastic leukemia (<https://doi.org/10.1056/NEJMoa1707447>, <https://doi.org/10.1056/NEJMoa1804980>, [https://doi.org/10.1016/S0140-6736\(20\)31366-0](https://doi.org/10.1016/S0140-6736(20)31366-0), <https://doi.org/10.1056/NEJMoa1914347>), and two directed to BCMA and used for multiple myeloma ([https://doi.org/10.1016/s0140-6736\(21\)00933-8](https://doi.org/10.1016/s0140-6736(21)00933-8), <https://doi.org/10.1056/NEJMoa2024850>). All



**Table 60.2** First clinical trials with CAR-T cells

Clinical application	Antigen	No. of patients	Clinical response	Toxicity	No. of T cells infused/kg	CAR design	Gene transfer vector	Safety technology	References
ALL	CD19	30	90% CR, 79% MRD	B-cell aplasia CRS Neurotoxicity	$0.76-20 \times 10^6/\text{kg}$ BW	FMC63 scFv CD8 alpha spacer 4-1BB costim	Lentivirus	None	Maude et al. (2014)
ALL	CD19	16	88% CR, 75% MRD	B-cell aplasia CRS Neurotoxicity	$3 \times 10^6/\text{kg}$ BW	SI25C1 scFv CD28 ECD spacer CD28 costim	$\gamma$ -Retrovirus	None	Davila et al. (2014)
ALL	CD19	29	93% CR, 86% MRD	B-cell aplasia CRS Neurotoxicity	$2 \times 10^5-2 \times 10^7/\text{kg}$ BW	FMC63 scFv IgG4 Hinge spacer 4-1BB costim	Lentivirus	EGFRt depletion marker	Turtle et al. (2016)
NHL/CLL	CD19	15	53% CR, 26% PR	B-cell aplasia CRS Neurotoxicity	$1-5 \times 10^6/\text{kg}$ BW	FMC63 scFv CD28 ECD spacer CD28 costim	$\gamma$ -Retrovirus	None	Kochenderfer et al. (2015)
NHL/CLL	CD19	32	50% CR, 72% ORR	B-cell aplasia CRS Neurotoxicity	$2 \times 10^5-2 \times 10^7/\text{kg}$ BW	FMC63 scFv IgG4 Hinge spacer 4-1BB costim	Lentivirus	EGFRt depletion marker	Turtle et al. (2016)
CLL	CD19	20	21% CR, 53% PR	B-cell aplasia CRS Neurotoxicity	$2 \times 10^5-2 \times 10^7/\text{kg}$ BW	FMC63 scFv IgG4 Hinge spacer 4-1BB costim	Lentivirus	EGFRt depletion marker	Turtle et al. (2017)
MM	CD19	10	1CR, 2PR	B-cell aplasia CRS (mild)	$1-5 \times 10^7$ (total)	FMC63 scFv CD8 alpha spacer 4-1BB costim	Lentivirus	None	Garfall et al. (2015)
MM	BCMA	12	1 CR, 1PR, 2 VGPR	Hematologic (cytopenia) CRS Neurotoxicity	$0.3-3 \times 10^6/\text{kg}$ BW	C11D5.3 scFv CD28 ECD spacer CD28 costim	$\gamma$ -Retrovirus	None	Ali et al. (2016)

ALL acute lymphoblastic leukemia, NHL non-Hodgkin lymphoma, CLL chronic lymphocytic leukemia, MM multiple myeloma, BCMA B-cell maturation antigen, CR complete remission, MRD minimal residual disease, PR partial remission, VGPR very good partial remission, CRS cytokine release syndrome, kg kilogram, BW body weight, scFv single-chain variable fragment, costim costimulatory domain, IgG immunoglobulin G, ECD extracellular domain, EGFRt, epidermal growth factor receptor

approved products are manufactured by retroviral ( $\gamma$ -retroviral or lentiviral) gene transfer and made headlines due to their considerable market price and the complex logistics behind this treatment. This involves harvesting the patient's T cells at a leukapheresis center, shipping to a centralized manufacturing facility to perform CAR gene transfer and T-cell expansion, and return shipment of the cryopreserved cell product. There is a recent increase in the use of exportable, GMP-certified manufacturing devices that are anticipated to provide on-site, point-of-care CAR-T-cell manufacture to reduce costs and wait time. Currently, approved cellular products are tested as earlier lines of treatment, and an intense effort is ongoing toward the identification of new CAR targets for further hematological malignancies, e.g., SLAMF7 (Gogishvili et al. 2017), CD44v6 (Casucci et al. 2013), CD38 (Mihara et al. 2009), CD33, CD70, CD123, CLL-1, and FLT3 (<https://doi.org/10.1111/ejh.14047>). Clinical progress of CAR-T cells for solid tumors has been lacking behind, since it meets—besides identification of suitable target antigens—several additional challenges, including but not limited to difficult accessibility, immunosuppressive tumor microenvironment, and high degrees of heterogeneity. Novel CAR constructs and treatment concepts are underway to address these difficulties (<https://doi.org/10.1038/s41586-023-05707-3>).

### 60.3.2 Side Effects and Their Management

Results from pioneering clinical studies investigating CAR-T cells in patients with hematological cancers highlight the frequent occurrence of severe adverse reactions, which in some cases were fatal. The most obvious toxicity by CAR-T cells is the elimination of lineage cells expressing the target antigen of choice. For example, profound and, in some cases, long-lasting B-cell aplasia was observed after the infusion of CD19 CAR-T cells in patients with ALL, NHL, and CLL (Maude et al. 2014; Park et al. 2018; Turtle et al. 2017). By analogy, BCMA CAR-T cells

are expected to induce plasma cell ablation in MM patients. The depletion of antibody-producing cells, or their precursors, in turn causes hypogammaglobulinemia, requiring constant supplementation with immunoglobulins. Besides these expected on-target/off-tumor effects, a new class of on-target/on-tumor adverse reactions is represented by the cytokine release syndrome (CRS) and by neurotoxicity. CRS is initiated by CAR-T-cell recognition of tumor cells, igniting the release of massive amounts of inflammatory cytokines, possibly by recruiting cells of the innate immunity. A master cytokine of the CRS is IL-6, as demonstrated by prompt and often complete response to the anti-IL-6 receptor monoclonal antibody tocilizumab. CRS symptoms range from high fever, headache, and myalgia to life-threatening cardiocirculatory and renal insufficiency. Clinical data reported so far utilize three slightly different systems for severity grading, which makes it difficult to draw meaningful comparisons in CRS liability between CAR-T-cell trials (Table 60.3). Nonetheless, there is a generalized consensus on the fact that severe CRS is more frequent in ALL compared to NHL and that high tumor burden is an important risk factor. Different from CRS, the pathophysiology of neurotoxicity by CAR-T cells remains an uncharted territory and decisively worthy of further research, given its highly dismal prognosis, as demonstrated by several cases of lethal cerebral edema (Berger et al. 2023). Initially thought to be caused by tumor recognition by CAR-T cells within the brain, neurotoxicity is now recognized to be independent from leukemic localization to the CNS. Moreover, unresponsiveness to tocilizumab suggests that excessive IL-6 signaling may not be sufficient to explain neurotoxicity and that additional pharmacological measures should be investigated. Finally, in a substantial proportion of patient, profound and in some cases very long-lasting leukopenia associated with high rates of infectious complications was reported after CD19 CAR-T therapy and referred to as CAR-HEMATOTOX (Rejeski et al. 2021).

**Table 60.3** CRS severity scoring systems

	Penn scale	CTCAE	Lee et al. (2014)
Grade 1	Mild reaction treated with antipyretics and/or antiemetics	Mild reaction, no treatment needed	Non-life-threatening reaction responsive to symptomatic treatment
Grade 2	Moderate reaction requiring hospitalization and i.v. therapy (no fluid resuscitation)	Moderate reaction responsive to symptomatic treatment within 24 h	Moderate reaction requiring oxygen <40%, fluid resuscitation or low-dose pressors. Any G2 organ toxicity
Grade 3	Severe reaction requiring high-flow oxygen or noninvasive lung ventilation, fluid resuscitation, or low-dose pressors	Prolonged reaction nonresponsive to symptomatic treatment	Severe reaction requiring oxygen >40%, high-dose pressors. Any G3 organ toxicity
Grade 4	Life-threatening reaction requiring high-dose pressors and/or mechanical ventilation	Life-threatening reaction, pressor, or ventilator requirement	Life-threatening reaction requiring mechanical ventilation. Any G4 organ toxicity

## 60.4 TCR Gene Transfer, TCR Gene Editing, and Future Perspectives

In contrast to CARs that only bind surface molecules, TCRs recognize small pieces (peptides) derived from any cellular protein and presented by MHC molecules. Since the vast majority of tumor-specific/tumor-associated antigens are expressed intracellularly, they will only be addressable by TCRs, but not CARs. Moreover, therapeutically relevant cancer driver mutations in most cases happen in intracellular proteins (e.g., signal transducers).

At the same time, the advantage of TCRs represents a major hurdle for broad clinical application: Any transgenic TCR only functions in the context of one specific HLA complex. Thus, in order to offer TCR-T-cell therapy to virtually all candidate patients, for each antigen, a whole set of active TCRs will have to be established for different HLA molecules.

The first TCR gene therapies were applied to melanoma patients (MART-1 antigen), but meanwhile many cancers have been addressed. Based on their almost complete absence in adult tissues, cancer/testis antigens (e.g., NY-ESO1) represent particularly promising targets. Many studies showed significant antitumor activity, but on-target and off-target activities were associated with severe side effects, including mortality (Morris and Stauss 2016).

Genome editing has been proposed to improve efficacy and decrease side effects of TCR gene

therapy. Editing might be used to knock out the endogenous TCR to increasing expression of the transgenic one and decreasing the mispairing risk between endogenous and transgenic TCR chains (potentially leading to autoreactive T cells) (Provasi et al. 2012), and is required for the development of allogeneic T-cell products (<https://doi.org/10.1126/scitranslmed.aaj2013>). The recent development of CRISPR/Cas9 and its application to T cells have significantly increased the efficiency of gene disruption and of homology-directed repair (HDR) while also permitting simultaneous multiple gene editing (<https://doi.org/10.1126/scitranslmed.abg8027>). TCR-edited T cells were proven safe in a pilot study on cancer patients (<https://doi.org/10.1126/science.aba7365>). Moreover, targeted integration in the TCR locus can improve long-term expression of transgenic TCRs (<https://doi.org/10.1038/s41551-019-0409-0>) or CARs (Eyquem et al. 2017). Finally, replacing nuclease activity with alternative enzymes, such as deaminases for nucleotide conversion, is further improving our toolbox for T-cell engineering (<https://doi.org/10.1056/NEJMoa2300709>).

In conclusion, T-cell therapies have become a promising novel anticancer weapon. Their broad application will require (1) identification of additional targets, (2) availability of TCRs against established targets for many HLA molecules, (3) implementation of innovative gene transfer and genome-editing biotechnological tools, and (4) improved methods for large-scale GMP production.

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Luca Vago and Francesco Dazzi

## 61.1 Introduction

It is widely accepted that the curative potential of allo-HCT for malignant diseases relies on the transfer of healthy donor immune cells capable of recognizing transplantation antigens on residual tumor cells (graft versus tumor (GvT)) and eliminating them. However, as extensively documented in solid cancers, if tumor eradication is incomplete, the prolonged immune pressure selectively allows immune-resistant subclones to survive (Schreiber et al. 2011). There is growing evidence that such an “immunoediting” also accounts for relapse after HCT. Malignant cells evade GvL either by reducing their immunogenicity and conveying inhibitory signals to the donor immune system (intrinsic evasion) or through the microenvironment (extrinsic evasion).

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## 61.2 Mechanisms of Immune Evasion

### 61.2.1 Mechanisms Intrinsic to the Malignant Clone

A remarkable example of tumor-intrinsic mechanism of immune evasion is the *genomic loss of the mismatched HLA haplotype* frequently documented in leukemia relapses after HCT from HLA haploidentical family donors (Vago et al. 2009). In this setting, donor T cells mount a vigorous alloreactive response against the incompatible HLA molecules, and this reaction is not only responsible for a significant risk of severe GvHD but also a major contributor to the GvT effect. Yet, this strong and selective immune pressure is easily overturned by tumor cells which, by losing the allogeneic HLA haplotype, find a mean to avoid recognition and re-emerge. “HLA loss” variants account for up to one third of relapses after HLA-haplo-HCT (Crucitti et al. 2015) and have been described also in the setting of HCT from partially HLA-incompatible URD (Waterhouse et al. 2011). The documentation of HLA loss at relapse has an important clinical impact, because IS withdrawal or administration of DLI would be much less effective against these disease variants (Tsirigotis et al. 2016).

More recent studies have documented alternative modalities through which leukemic cells can evade the GvT effect, including the epigenetic silencing of HLA class II molecules (Christopher

et al. 2018; Toffalori et al. 2019), and the overexpression of molecules capable of dampening immune responses such as programmed death ligand (PD-L)1 (Toffalori et al. 2019). This observation provides a rationale for the use of “checkpoint blockade” to restore immune control at relapse. Initial experience in patients with relapsed lymphoma or extramedullary leukemia with anti-PD1 and anti-cytotoxic T-lymphocyte-associated antigen (CTLA)-4 MOAb is very promising (Davids et al. 2016; Herbaux et al. 2017). However, the risks of triggering life-threatening GvHD represent a concern.

Another evidence that supports “leukemia immunoediting” is the occurrence of *isolated extramedullary relapses* after allo-HCT or even more frequently after DLI. These relapses may occur, but not necessarily, in immunological sanctuaries, including the CNS. Although to date the biological drivers of extramedullary relapses remain unknown, some studies have suggested a link with immune-related factors such as chronic GvHD (Solh et al. 2012; Harris et al. 2013).

### 61.2.2 Mechanisms Extrinsic to the Leukemic Cells

The alternative, but not mutually exclusive, strategy by which malignant cells enact evasion from immune cell recognition relies on hijacking the stem cell niches in which normal HSC self-renew and differentiate. By doing this, malignant cells create a *tumor microenvironment* (TME) that has profound consequences on disease progression and relapse. The initial studies conducted on solid tumors have shown that the TME consists of two major cellular populations that alone or in combination drive resistance to conventional therapies and suppress antitumor immune responses. The first group comprises a diverse and heterogeneous group of myeloid-derived cells which, according to a yet unresolved debate on their nomenclature, can be generally classified as tumor-associated monocytes/macrophages (TAM) and myeloid-derived suppressor cells (MDSC) (Bronte et al. 2016). The IS activity of these cells is mediated by factors that include

nitric oxide synthase-2 (NOS-2), arginase-1, heme oxygenase-1 (HO-1), interleukin (IL)-10, transforming growth factor (TGF)- $\beta$ , and prostaglandin E2 (PGE2). All these molecules also favor the recruitment of regulatory T cell (Tregs) that eventually contributes to the inhibition of antitumor CD8<sup>+</sup> T-cell and natural killer cell effector function (Ostuni et al. 2015). Although most of these mechanisms have been initially demonstrated in solid tumors, there is consistent evidence that they are also involved in hematological malignancies. High-risk AML can actually behave as MDSC by upregulating NOS and suppressing T-cell responses (Mussai et al. 2013). The presence of MDSC in AML has later been confirmed and also identified in multiple myeloma whereby they protect malignant cells through MUC1 oncoprotein (Bar-Natan et al. 2017; Pyzer et al. 2017).

The second cellular group consists of an equally heterogeneous population of mesenchymal origin, variously referred to as *mesenchymal stromal cells* (MSC) or *cancer-associated fibroblasts* (CAF) (Raffaghello and Dazzi 2015). Regardless of their developmental heterogeneity, they all play a similar role by protecting the malignant cells from cytotoxic agents and immune responses. In the bone marrow, MSC protect CML and AML cells from imatinib and Ara-C via the CXCR4-CXCL12 axis (Vianello et al. 2010). However, recent evidence suggests that arming chimeric antigen receptor (CAR) T cells with CXCR4, which results in significant improvement of chemotaxis toward recombinant the bone marrow niche, may enhance their therapeutic efficacy in acute myeloid leukemia (Biondi et al. 2023).

Much information has been provided about the *IS activity of MSC* that is exerted in a nonantigen-specific fashion (Jones et al. 2007). One of the primary direct mechanisms responsible for this involves the expression of indoleamine 2,3-dioxygenase-1 (IDO-1), which consumes the essential amino acid tryptophan. Additional IS mechanisms include the release of suppressive factors such as TGF- $\beta$ 1, hepatocyte growth factor, PGE2, soluble human leukocyte antigen G, and TNF- $\alpha$ -stimulated gene 6 protein

(TSG-6). However, more recent data have demonstrated the susceptibility of MSC to undergo apoptosis and as a consequence to recruit tissue-resident monocytes/macrophages in delivering a more sustainable IS effect (Cheung and Dazzi 2018).

Finally, the role of *Tregs* in generating immune resistance has been much discussed. While there is plenty of data indicating how these cells exert a very negative impact on the outcome of solid tumors, data in preclinical models of allogeneic HCT have suggested that *Tregs* may selectively inhibit GvHD without compromising GvL (Edinger et al. 2003). In contrast, clinical data suggest to consider *Treg* levels posttransplant with caution (Nadal et al. 2007). Most recent information from the analysis of *Treg* repertoire and transcriptome has shed light on their activity on GvHD (Lohmeyer et al. 2023).

#### Key Points

- Leukemia can counteract the beneficial graft-versus-leukemia effects posttransplant.
- This is effected either by changes in the tumor cells which make them evade immune recognition or by instructing different components of the microenvironment to deliver in situ immunosuppression.

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## Regulatory Aspects of ATMP Versus Minimally Manipulated Immune Cells

Christian Chabannon and Harry Dolstra

In 2023, three categories of therapeutic products obtained through the collection and subsequent engineering of hematopoietic cells exist and are valuable to patients treated for neoplastic diseases as well as a variety of non-neoplastic disorders: blood cell transfusions, stem and immune cell transplants, and cellular therapy medicinal products. The procurement and nature of various blood products and transfusion practices are described elsewhere in this handbook. In this chapter, we focus on hematopoietic cellular therapies as currently defined and managed in the FACT-JACIE International Standards for Hematopoietic Cellular Therapies (nowadays in version 8). Over the last two decades, major changes have occurred in the EU regulatory framework (as well as in other parts of the world, notably in the USA) that result in the coexistence of two categories of hematopoietic cellular therapies. Innovative and industry-manufactured somatic cell therapy or gene therapy medicinal

products have entered the field at an accelerating pace since the last edition of this handbook. Some of them are distributed worldwide on a large scale, and a few of these medicinal products already complete or compete with traditional hematopoietic cell transplantation practices. We here update the description of organizational consequences of this historical transition for academic facilities and the new opportunities as well as challenges these advances are bringing to patients and healthcare practitioners, including strong needs for educational initiatives.

Under current European regulations, hematopoietic cellular therapies fall under two categories: stem cell transplants and advanced therapy medicinal products (ATMPs). Routinely administered autologous and allogeneic hematopoietic cell transplants (HCT)—including subsequent peripheral blood allogeneic mononuclear cells (DLI)—undergo non-substantial manipulations following cell procurement and before being administered to the recipient. These therapeutic products that are in use for now more than 60 years and have cured hundreds of thousands of patients worldwide (Passweg et al. 2021) never received marketing approvals nor were classified as medicinal products. Nevertheless, lessons learned from the practice of hematopoietic cell transplantation have inspired some of the developments of modern hematopoietic advanced cellular therapies (Chabannon et al. 2018). Cell processing is performed in facilities termed tis-

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sue establishments (TEs) under the EU Tissues and Cells Directive,<sup>1</sup> which are authorized by national and/or regional competent authorities (CA). TEs usually operate on a relatively small scale, in compliance with Good Cell and Tissue Practices (GCTP), serving the clinical program(s) in their immediate vicinity (“point-of-care” (POC) cell processing activities), although some national or regional services may support a more extensive network of clinical programs. The combination of a clinical department(s) with a collection and a processing facility represents the core structure for a transplant program that applies for the JACIE accreditation (see Chap. 5). Such an organization leaves room for significant procedural and organizational variations, many driven by local or national factors, despite all attempts from the various professional associations to harmonize practices through surveys, the publication of guidelines and regularly revised standards for these therapies, e.g., the already mentioned FACT-JACIE International Standards for Hematopoietic Cellular Therapies.<sup>2</sup> The EU Commission recently released a proposal for a new EU Regulation on Substances of Human Origin (SoHO) that—when adopted—will replace the aforementioned Tissues and Cells Directives, as well as the Blood Directive.<sup>3</sup> This proposal is under review at the EU Parliament and should be voted and released in the upcoming months, after the review process is completed. One of the objectives of this new Regulation is to tackle some of the previously cited caveats of the current framework, including improved and equal access to cell and tissue transplantation while maintaining the principle of voluntary unpaid donation (VUD), decreasing dependency of European countries on other countries, harmonization of practices through the use of a set of standards published in the European Directorate for the Quality of Medicines &

HealthCare (EDQM) *Guide to the Quality and Safety of Tissues and Cells for Human Application* ((CD-P-TO) EECPAoOT 2022), the creation and maintenance of a directory of TEs, and improved organization for donor follow-up.

Additionally, the small size of the market for reagents and disposables used for cell collection and cell processing in an academic context and a POC organization makes it especially vulnerable to market withdrawal decisions. Manufacturers may decide against the necessary investments needed to upgrade industrial process and make them compliant with more stringent regulations. A recent example of such a situation is the current lack in Europe of medical devices approved for bone marrow collection, following the implementation of the (EU) 2017/745 Medical Devices Regulation and the restriction imposed by Regulation (EC) No. 1907/2006 REACH on the use of DEHP.<sup>4</sup>

ATMPs represent a category of medicinal products defined in EU Regulation 1394/2007.<sup>5</sup> ATMPs—known in the USA as human cells, tissues, and cellular and tissue-based products (HCT/Ps) regulated under Section 351 of the PHS Act and/or the FD&C Act<sup>6</sup>—are subdivided into four categories, of which two are relevant in the context of hematopoietic cellular therapies: somatic cell therapy medicinal products (SCTMP) and gene therapy medicinal products (GTMP). Examples of SCTMP include ex vivo expanded autologous or allogeneic stem cells (de Lima et al. 2012; Delaney et al. 2010), mesenchymal stem cells (Le Blanc et al. 2008), and allogeneic T lymphocytes depleted of alloreactive T cells (Andre-Schmutz et al. 2002). Examples of GTMP include autologous or allogeneic chimeric antigen receptor (CAR)-T cells (Schuster et al. 2017; Neelapu et al. 2017;

<sup>1</sup>[https://ec.europa.eu/health/blood\\_tissues\\_organisms/tissues\\_en](https://ec.europa.eu/health/blood_tissues_organisms/tissues_en). Accessed 10 July 2023.

<sup>2</sup>[www.jacie.org](http://www.jacie.org). Accessed 10 July 2023.

<sup>3</sup>[https://health.ec.europa.eu/blood-tissues-cells-and-organs/overview/proposal-regulation-substances-human-origin\\_en](https://health.ec.europa.eu/blood-tissues-cells-and-organs/overview/proposal-regulation-substances-human-origin_en). Accessed 10 July 2023.

<sup>4</sup>[https://www.europarl.europa.eu/doceo/document/E-9-2022-002133\\_EN.html](https://www.europarl.europa.eu/doceo/document/E-9-2022-002133_EN.html). Accessed 10 July 2023.

<sup>5</sup><http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:en:PDF>. Accessed 10 July 2023.

<sup>6</sup><https://www.fda.gov/biologicsbloodvaccines/tissueissueproducts/regulationoftissues/ucm150485.htm>. Accessed 10 July 2023.



Abramson et al. 2020; Berdeja et al. 2021; Munshi et al. 2021; Wang et al. 2020) and autologous CD34+ cells genetically engineered to express a miniglobin gene and designed to treat inherited  $\beta$ -globin disorders (Cavazzana-Calvo et al. 2010; Ribeil et al. 2017; Locatelli et al. 2022) or engineered to re-express fetal hemoglobin as a substitute to  $\beta$ -globin (Frangoul et al. 2021). The regulation was designed in part to foster the competitiveness of European pharmaceutical companies in this emerging field, but the number of ATMPs that have received a centralized marketing authorization has remained relatively low and with poor overall commercial success until the first two autologous CAR-T cells targeting CD19 were approved by EMA in the summer of 2018, approximately 1 year after these two products were approved by the FDA. In the HCT field, it was not until 2015 that an ATMP of interest reached the market with authorization given for Zalmoxis<sup>®</sup> (allogeneic T cells engineered to express a suicide gene) (Ciceri et al. 2009). Production, distribution, and administration of ATMPs imply a totally different organization than that used for HCT, with manufacturing at a central facility in compliance with good manufacturing practices (GMP) (Wang and Rivière 2016; Wang and Riviere 2017), a version of which was recently released by the European Medicines Agency (EMA) to specifically deal with manufacturing of ATMPs.<sup>7</sup> Since a majority of the ATMPs that progress to authorization or at least to clinical trials are manufactured from autologous mononuclear cells, starting material is currently procured by hospital- or blood bank-operated apheresis facilities creating a peculiar situation in which a product starts under one regulation, Tissues and Cells Directive and likely the SoHO Regulation in the near future, before transitioning to another, ATMP and Pharma Regulations, and where a hospital acts as a service provider to industry, an interaction that requires further definition of the respective responsibilities and liabilities.

<sup>7</sup>[https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017\\_11\\_22\\_guidelines\\_gmp\\_for\\_atmps.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf). Accessed 10 July 2023.

Publication of Regulation 1394/2007 created a situation in which cell- or tissue-based therapeutic products that were previously prepared and delivered through a POC organization similar to that for cell transplants are now classified as ATMPs. This had a limited impact in the field of hematopoietic cellular therapies, although some cell-based products such as allogeneic T cells with specific anti-CMV activity engineered through the IFN-gamma-catch technology (Feuchtinger et al. 2010) were affected. In recognizing that many potential ATMPs were used for limited numbers of patients and with no commercial motivation, Regulation 1394/2007 created the so-called hospital exemption (HE) under Article 28 exempting from authorization requirements those ATMPs manufactured in hospitals, universities, or start-up companies where the medicine is prescribed for individual patients under the care of a medical practitioner. This manufacture should occur on a non-routine basis according to specific quality standards (GMP) (Vives et al. 2015), and the ATMP should be used in a hospital and only within the same member state. National authorities oversee the approval of HE products which has resulted in significant variations between member states in how it is applied and which has led to criticism from both industry and academia that it is unclear and inconsistent. Recently, Spanish investigators have developed several CAR-T cells that are structurally and functionally comparable to approved commercial products (Ortiz-Maldonado et al. 2021; Oliver-Caldes et al. 2023), one of which, ARI-0001, has been conditionally approved by EMA (Trias et al. 2022). The Pharma Regulation is also undergoing revision at the EU Commission, and the HE is one of the key aspects that is examined in the context of public consultations with all interested stakeholders.

Access to ATMPs including cellular therapies is likely to be a particular challenge for patients, healthcare professionals, and national health systems due to the high costs of the medicinal products but also of apheresis sessions, inpatient and outpatient stays necessary to administer bridging chemotherapy when needed, lymphodepleting or conditioning regimen ahead of the infusion of

SCTMP or GTMP, and preventing or treating the many side effects (Hayden et al. 2022). Even access to decades-old HCT remains strongly associated with higher-income countries (Gratwohl et al. 2015). One potential effect of limited access is the so-called stem cell tourism whereby patients with sufficient financial resource travel to centers outside their own districts or countries in order to get access to these treatments, whether with unproven efficacy or safety or while not being fully informed to make this important decision. The International Society for Cellular Therapy (ISCT) leads the publication of patient advice and other documentation on this phenomenon.<sup>8</sup>

Academic facilities, including stem cell transplant practitioners at large, should strive to remain active players in the development of ATMPs. Academia remains very active in the early phases of clinical trials designed to evaluate innovative SCTMP and GTMP as potential complements, substitutes, or bridges to historical forms of hematopoietic cell transplants (Pearce et al. 2014). Many public institutions have invested significant resources to upgrade their processing facilities to GMP-compliant levels, thus allowing for small-scale POC manufacturing of investigational medicinal products to support phase I and possibly phase II studies, often with the potential that industry will take over in case that promising results warrant further development (de Wilde et al. 2016a). It is important to keep in mind that the development of a medicinal product goes well beyond compliance with GMP during the manufacturing step, but also involves the production of a broad array of preclinical data to support initiation of early clinical trials, and is thus strikingly different from process validation and optimization that are now routinely requested from TEs. Furthermore, academia has to become a proactive stakeholder in the regulatory area by engaging with the authorities, sharing their know-how, and voicing their opinion (de Wilde et al. 2016b). The GoCART Coalition that was recently co-founded by both the EBMT and the European

Hematology Association (EHA) in part serves this purpose and aims at facilitating patients' access to CAR-T cells and other cellular immunotherapies through the engagement of multiple stakeholders.<sup>9</sup>

The field is moving at a fast pace and is now well beyond the proof of concept that tissue-based or cell-based medicinal products can be manufactured by a "conventional" pharmaceutical company (Locke et al. 2017), although with continued reliance on critical contributions from academic facilities, e.g., basic science and provision of starting materials. Some of these innovative medicinal products have demonstrated remarkable clinical efficacy for severe or debilitating diseases although sometimes at the expense of equally remarkable toxicity. In 2022, barely 4 years after initial approvals supported with small single-arm international multicenter trials, positive results were published for two out of three large randomized international multicenter trials comparing autologous CAR-T cells targeting CD19 against the standard of care (SOC) including salvage chemotherapy completed with consolidation high-dose chemotherapy supported with autologous hematopoietic cell transplantation in chemo-sensitive patients (Bishop et al. 2022; Kamdar et al. 2022; Locke et al. 2022); superiority of CAR-T cells in the ZUMA-7 (Locke et al. 2022) and TRANSFORM (Kamdar et al. 2022) studies led to the approval of these treatments in second line for patients with refractory or early first relapse diffuse large B-cell lymphoma (DLBCL), heralding the end of a medical practice that arose approximately 30 years earlier (Philip et al. 1995). The rapid adoption of CAR-T cells in real-world practices (Passweg et al. 2020), with the first published reports from continental or national registries demonstrating comparable results with results from the original registration studies (Bachy et al. 2022; Pasquini et al. 2020), supports robust scientific and financial investments in the field, translating in a growing number of investigational cellular immunotherapies being evaluated (Saez-Ibanez et al. 2022). These developments still carry many

<sup>8</sup><http://www.celltherapysociety.org/page/UCT>. Accessed 21 Feb 2018.

<sup>9</sup><https://thegocartcoalition.com>. Accessed 10 July 2023.

regulatory and operational uncertainties, including the sustainability of multiple and parallel supply chains for different medicinal products, that create an increasing workload for academic facilities and place a growing financial burden on public and private healthcare payers. The availability of multiple types of hematopoietic cellular therapies at the same time when other categories of therapies also reach the market in large numbers contribute to the growing complexity in the evaluation of the medical value of these innovative and expensive therapies, in patients who have received and will receive multiple lines of treatments over many years of care (Chabannon et al. 2015). In this context, organization of long-term follow-up is challenging.<sup>10</sup> Academia through continental registries such as EBMT<sup>11</sup> will continue to play a key role with data and know-how that will be very useful not just for researchers but also for industry, healthcare regulators, and payers.

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<sup>10</sup>[http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fregulation%2Fgeneral%2Fgeneral\\_content\\_000658.jsp&mid=WC0b01ac0580961211](http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fregulation%2Fgeneral%2Fgeneral_content_000658.jsp&mid=WC0b01ac0580961211).

<sup>11</sup><https://www.ebmt.org/registry/car-t-data-collection-initiative>. Accessed 10 July 2023.

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## **Part VIII**

### **Specific Modalities of HCT and Management**

**Topic Leaders: Anna Sureda, Nicolaus Kröger  
and Andrew Gennery**



## 63.1 Introduction

The main indications for autoHCT are lymphoid malignancies (90%) with plasma cell disorders (MM and others) comprising 55% of all autoHCT (Passweg et al. 2021). Toxicity and mortality associated with autoHCT have been reduced significantly with the use of mobilized peripheral blood HSC, the extended use of cryotherapy associated with MEL, and the improvements in prophylactic antibiotic and antiemetic regimens. Besides this, outpatient parenteral antimicrobial treatment has been proven feasible and safe, thanks to modern CVC and infusion devices. All these advances have led to the development of outpatient autoHCT programs, and several studies have demonstrated their feasibility and safety (González et al. 2021).

There are various reasons for transferring the support of the neutropenic phase of autoHCT to the ambulatory setting, including patient preference, reduced exposure to hospital microorganisms, better use of hospital resources, and cost-saving issues (Martino et al. 2020). In this model, however, patients experience time-consuming daily travel to the outpatient clinic for blood tests and physician checkups. “Hospital at

home” is an alternative, designed to reduce hospital outpatient admissions by providing hospital equivalent care to patients in the home setting (Fernández Avilés et al. 2006; González-Barrera et al. 2023).

## 63.2 Ambulatory AutoHCT Models (Martino et al. 2020)

<b>Complete outpatient program</b>	
Conditioning regimen, HPC infusion, and management of the aplastic phase	Outpatient clinic
<b>Delayed admission</b>	
Conditioning regimen, HPC infusion, and management of the aplastic phase	Inpatient Early discharged (+1) and readmission (+5)
<b>Mixed inpatient-outpatient</b>	
Conditioning regimen and management of the aplastic phase	Outpatient clinics
HPC infusion	Inpatient
<b>Early discharge outpatient</b>	
Conditioning regimen and HPC infusion	Inpatient
Management of the aplastic phase	Outpatient clinics
<b>Early discharge at home</b>	
Conditioning regimen and HPC infusion	Inpatient
Management of the aplastic phase	At home

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### 63.3 Suggested Inclusion Criteria for Ambulatory AutoHCT

Patient	Age $\leq$ 65 years, recommended in newly created programs ECOG $\leq$ 2 No organ failure Recent documented infection with a proven secondary prophylaxis Absence of refractoriness to platelet transfusion Signed written informed consent
Transplant Center	Outpatient clinics available 24 h per day or bed reserved in the transplant unit Dedicated phone line 24 h $\times$ 365 days to allow patients or their caregivers to contact an expert physician of the transplant team
Disease	CR or PR before the autoHCT No symptomatic advanced disease
Caregiver	Availability of a suitable caregiver 24 h per day, 7 days a week
Home	Clean house Travel time from home to the hospital less than 60 min at rush hours

### 63.4 General Recommendations for At-Home AutoHCT

Dose of HPC and supportive care (such as management of nausea and vomiting, hydration, analgesic therapy) should not differ from recommended conventional autoHCT guidelines. Antimicrobial prophylaxis for outpatient autoHCT should not differ from that required for conventional procedure, although some authors have intensified it with intravenous antibiotics significantly reducing the incidence and severity of neutropenic fever (Rodríguez-Lobato et al. 2020a, b). Recently, the usefulness of G-CSF after HPC infusion has been questioned; indeed, some transplant groups have stopped using it, observing no changes in relevant transplant outcomes and avoiding potential adverse effects

including its potential association with engraftment syndrome (Grosso et al. 2022). Finally, some studies suggest that for MM patients, the addition of primary prophylaxis with corticosteroids after autoHCT may minimize the incidence of febrile neutropenia and engraftment syndrome, which would increase the safety of outpatient management of these patients without compromising outcomes (Mossad et al. 2005; Rodríguez-Lobato et al. 2020a, b).

### 63.5 Most Frequent Reasons for Readmission (Ordered by Frequency)

- Fever persisting after 2 days of broad-spectrum antibacterial therapy
- Severe oral mucositis or gastrointestinal toxicity (WHO grade III or IV)
- Severe sepsis with organic failure
- Request of the patient (psychological distress) and low compliance
- Loss of caregiver support

### 63.6 Treatment of Fever in At-Home Setting

The use of empiric antibacterial therapy must follow international accepted guidelines. If fever occurs, empiric broad-spectrum antibacterial therapy should be initiated within 1 h of the clinical evaluation and as soon as the fever workup was completed. IV antibiotics should be preferred and chosen in the light of clinical and laboratory findings. After at least 6-h monitoring, hemodynamically stable patients without relevant clinical problems may be followed at home. Table 63.1 shows the different empiric antibiotic therapy that could be used at home.

**Table 63.1** Empirical antibiotic therapy

Prophylaxis with quinolones	Empirical antibiotic therapy
No	Levofloxacin (PO or IV) Moxifloxacin PO Ciprofloxacin PO associated or not with amoxicillin/clavulanate Ciprofloxacin PO associated or not with linezolid PO
Yes	<ul style="list-style-type: none"> <li>– IV ceftriaxone or piperacillin/tazobactam<sup>a</sup> or meropenem<sup>b</sup> associated with teicoplanin IV<sup>c</sup> if intense oral mucositis</li> <li>– If there is a high suspicion of CVC infection, add teicoplanin and an anti-GNB such as amikacin IV, and evaluate the CVC withdrawal</li> <li>– If allergic to beta-lactam, quinolones PO/IV associated with teicoplanin IV and amikacin IV</li> </ul>

<sup>a</sup> Stable at room temperature so it can be administered at home by electronic intermittent infusion pump

<sup>b</sup> Accurate refrigeration to achieve adequate stability for home administration

<sup>c</sup> The first option at home would be teicoplanin once daily instead of vancomycin IV (twice a day). Other alternatives rarely necessary in the context of autoHCT are daptomycin IV or linezolid PO

### 63.7 Incidence of Readmission in Outpatient and At-Home AutoHCT

The incidence of readmissions is closely related to the experience of the group of professionals in outpatient or at-home management of complications and by the support infrastructure available in the hospital.

In patients with MM, usually conditioned with MEL, the lowest readmission rates have been reported (less than 20%) due to the low organic toxicity (Martino et al. 2020). They are clearly the best option when considering starting an outpatient or at-home autoHCT program.

In patients with NHL or HL usually conditioned with a more toxic regimen (BEAM or BEAC), there is a higher readmission rate, between 20 and 30% (Scortechini et al. 2014; Jaime-Pérez et al. 2021) and 80% (Faucher et al. 2012).

In the *Hospital Clinic* at-home autoHCT experience, the actualized readmission rate in MM and lymphoma patients is only of 2% and 1%, respectively (Rodríguez-Lobato et al. 2020a, b), thanks to the significant reduction of febrile neutropenia rate with the intensification of antibiotic prophylaxis (piperacillin/tazobactam in NHL and LH patients) and the addition of primary prophylaxis of engraftment syndrome with corticosteroids after autoHCT in MM patients, as well as the successful control of fever at home. The overall readmission rate in this experience is significantly lower (6.5%) in a series of 537 patients.

### 63.8 Quality of Life

Patients who receive autoHCT experienced various symptoms on different levels and at different frequencies after transplant. Patients and their caregivers need guidance and a planned and individualized education in the process to reduce their anxiety (Caliskan and Can 2022). Thus, Summers et al. (2000) reported significantly higher scores for emotional well-being and global QOL in outpatients, while Martino et al. (2017) indicated that the outpatient model neither improves nor impairs global patient QOL on the first 30 days after autoHCT. Usually, a good clinical outcome following autoHCT was associated with better QOL and greater satisfaction with care. In summary, the data published are limited and contradictory, so the QOL remains an area that requires further research.

### 63.9 Cost Data

The study of “real” costs of these ambulatory/domiciliary autoHCT programs is still to be carried out. In the absence of well-designed studies aimed at evaluating the “real” savings achieved with outpatient/at-home autoHCT programs, some authors cite direct savings between 10 and 50% (González et al. 2021), especially influenced by the release of hospital beds and low readmission rates.

## 63.10 At-Home AlloHCT

Some centers have published their data regarding ambulatory alloHCT using RIC/NMA and MAC regimens and have demonstrated safety, feasibility, and cost-effectiveness. After more than two decades of the first ambulatory alloHCT program, the experience in this modality is limited. However, in low- and middle-income countries, where the development of a traditional HCT unit is often unrealistic, ambulatory alloHCT emerges as an affordable, safe, and realistic option.

### Key Points

- At-home autoHCT is feasible and safe with a good selection of patients.
- MM is the best indication.
- There are no randomized studies that clearly indicate which model is better than another, which is the real impact of ambulatory autoHCT in the QOL of patients and their caregivers, as well as what is its real cost.

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# Role of Umbilical Cord Blood Transplantation

Jaime Sanz and Vanderson Rocha

## 64.1 Introduction

- Umbilical cord blood transplant (UCBT) from unrelated donors (UD) was developed to overcome the limitations of HLA-matched bone marrow (BM)/peripheral blood (PB) UDs and improve access to allogeneic transplant (allo-HCT) both by increasing the donor pool and fasten time to HCT.
- UCBT activity has sharply decreased in favor of partially HLA-matched related donor transplants (haplo-HCT) using posttransplant cyclophosphamide (Passweg et al. 2021). This procedure shares most of the advantages of UCBT. It has expanded its use worldwide due to its effectiveness, lower financial impact, and relative simplicity. Two randomized trials have shown superiority of haplo-HCT over UCBT (Fuchs et al. 2020; Sanz et al. 2020). However, it is still a clinical option for some patients lacking other alternatives, due to either the absence of relative donors at due time or the presence of donor-specific anti-

bodies. Whether or not it could be prioritized over haplo donors with negative characteristics like older age or gender mismatch should be the focus of future research.

- Recent advances in conditioning, graft manipulation, and supportive care have improved UCBT outcomes. These advances reflect in very encouraging outcomes after UCBT for adults and children with high-risk leukemias and some genetic diseases.

## 64.2 Potential Advantages and Disadvantages of UCBT

UCBT versus BMT/PBHCT	
Advantages	Disadvantages
<ul style="list-style-type: none"> <li>• Expanded access to transplant*               <ul style="list-style-type: none"> <li>– Higher availability of donor*</li> <li>– Faster search and shorter time to transplant*</li> <li>– Greater HLA disparity allowed with low incidence of GVHD*</li> </ul> </li> <li>• Lower risk of transmission of viral infections</li> <li>• More versatile transplant planning*</li> <li>• No risk of donor refusal</li> <li>• No risk to the donor</li> </ul>	<ul style="list-style-type: none"> <li>• Slower engraftment</li> <li>• Higher risk of non-immunological rejection (graft failure)</li> <li>• Remote possibility of transmission of a genetic disease*</li> <li>• Greater delay in immune reconstitution</li> <li>• No possibility of donor lymphocyte infusion*</li> </ul>
*Advantages shared with partially matched related (haploidentical) HCT	*Disadvantages not shared with haploidentical HCT

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Similar to UCBT, haploidentical HCT can also be used on an urgent basis and extends donor availability to the vast majority of patients. In addition, haploidentical HCT allows a DLI if necessary.

### 64.3 Indications

Except for some patients with severe bone marrow failure, such as aplastic anemia and paroxysmal nocturnal hemoglobinuria, UCBT in adults is performed almost exclusively in patients with malignant hematological diseases. However, UCBT in children has been used for many other nonmalignant diseases, including primary immunodeficiency diseases and inherited metabolic disorders.

The American Society for Blood and Marrow Transplantation (ASBMT) (Kanate et al. 2020) and the European Society for Blood and Marrow Transplantation (EBMT) (Snowden et al. 2022) have recently published their respective guidelines that include recommendations for transplant indications in children and adults. It should be

noted that the ASBMT did not differentiate recommendations for transplant indications based on donor or graft source and EBMT grouped the same indications for all HLA-mismatched alternative donors (UCB, haplo, and mismatched unrelated donors).

Allo-HCT indications should not defer according to donor or graft source but rather the expertise of the transplant center along with patient- and/or disease-related factors, and availability should determine the donor/graft source for the individual patient. More recent data shows that UCBT is probably a preferred donor choice for patients with very high-risk leukemias and some genetic and metabolic disorders in children; however, comparative studies with other alternative donors are lacking in the literature.

### 64.4 Approaches to Improve Outcomes After UCBT

Strategies have been developed aiming to shorten the time to engraftment, improve immune reconstitution, and decrease NRM.

Approaches to improve outcomes after UCBT	Expert point of view
1. Refining criteria for UCB unit selection	Selection of the most appropriate UCB unit is key to transplant success. Recent comprehensive consensus guidelines have been recently published (Politikos et al. 2020; Ruggeri 2019). Selection of double cord blood units including high-resolution HLA typing for adults have been recently published (Fatobene et al. 2020; Fatobene et al. 2023). See chapter of banking, processing, and procurement of cord blood cells
2. Optimization of conditioning regimens	Specific conditioning regimen can influence transplant outcomes. See Sect. 64.5
3. Strategies aiming to shorten the time to engraftment:	
(a) Double UCBT	In children, two randomized trials have demonstrated no benefit and increased risk of GVHD (Wagner et al. 2014; Michel et al. 2016) In adults, retrospective studies showed no advantage when single-unit with TNC dose $>2.5 \times 10^7/\text{kg}$ available (Scaradavou et al. 2013)
(b) Co-infusion with third-party cells	Has consistently demonstrated benefit to accelerate hematopoietic recovery. No proved benefit on NRM or survival (Sanz et al. 2017)
(c) Ex vivo expansion of UCB cells	Omidubicel, the first FDA-approved UCB ex vivo-expanded cellular therapy product, has demonstrated faster engraftment, fewer infections, and decreased NRM compared with unmanipulated UCB that was confirmed in long-term follow-up (Horwitz et al. 2021). Should be considered the standard of care. However, logistical issues and costs may hamper real-life use. Other compounds to expand CB cells such as UM171 are currently being explored in phase 2 clinical trials with encouraging results (Cohen et al. 2020)
4. Improvement of supportive measures	Supportive care to prevent or treat opportunistic infections until neutrophil and immune recovery has occurred is critical in UCBT. See Sect. 64.7

## 64.5 Conditioning Regimens

The selection of conditioning regimen for HCT, including UCBT, should take into account the risk of toxicity and the risk of graft failure and relapse in malignant diseases. In UCBT, given the relatively lower cell dose (T cells and CD34+ cells) and the use of HLA-mismatched grafts, graft failure is of particular concern, especially in adults. It usually combines agents with additive immunosuppressive properties. The choice of the conditioning regimen is of primary importance and can influence transplant outcomes. A comprehensive and exhaustive review of myeloablative and nonmyeloablative/reduced intensity conditioning regimens in the UCBT setting has been published (Cord blood transplantations

2017). The American Society for Transplantation and Cellular Therapy Cord Blood Special Interest Group has also recently published their recommendations (Metheny et al. 2021).

The use of in vivo T-cell depletion with ATG in the conditioning regimen has been used to enhance myeloid engraftment as well as to prevent GVHD. Its use has been associated with reduced rates of GVHD but also with increased mortality due to delayed T-cell recovery in hematologic malignancies and is generally not recommended in this setting. Some data suggest that safety of ATG can be improved by adjusting timing or dose with ATG pharmacokinetics (Admiraal et al. 2016) to avoid posttransplantation exposure.

Some conditioning regimens options of varying intensities to be considered are as follows:

<i>Myeloablative conditioning regimens (MAC)</i>	
<i>Chemotherapy-based</i>	
• TBF regimen (Sanz et al. 2012)	TT 10 mg/kg + BU IV 9.6 mg/kg + Flu 150 mg/m <sup>2</sup>
<i>TBI-based</i>	
• TBI-Flu-Cy regimen (Barker et al. 2015)	TBI 13.2 Gy + CY 120 mg/kg + Flu 75 mg/m <sup>2</sup>
TT thiotepa, BU busulfan, Flu fludarabine, TBI total body irradiation, CY cyclophosphamide	
<i>Intermediate intensity conditioning regimens (IIC)</i>	
• TBI-TT-flu-Cy regimen (Barker et al. 2020)	TT 10 mg/kg + CY 50 mg/kg + Flu 150 mg/m <sup>2</sup> + TBI 4 Gy
TT thiotepa, Flu fludarabine, CY cyclophosphamide, TBI total body irradiation, CY cyclophosphamide	
<i>Reduced intensity conditioning regimens (RIC)</i>	
• rTBI-Ffu-Cy regimen (Fuchs et al. 2020)	TBI 2 Gy + CY 50 mg/kg + Flu 200 mg/m <sup>2</sup>
TBI total body irradiation, CY cyclophosphamide, Flu fludarabine	

## 64.6 GVHD Prophylaxis

The most important advantage of UCB over unrelated donor grafts is the capability to tolerate HLA disparities and facilitate a low incidence of chronic GVHD. However, acute GVHD is still one of the most important contributors to morbidity and mortality. Different GVHD prophylaxis regimens have been explored with no evidence of benefit of any specific strategy. Methotrexate is generally not recommended to avoid myelotoxicity and delayed neutrophil recovery although it is widely used in Asia. The most frequently used regimen worldwide is the combination of calci-

neurin inhibitors for 6–9 months with mycophenolate mofetil for 2–6 months.

## 64.7 Supportive Care

The supportive measures described below are not intended to be recommendations but only to be taken into account and to consider their use in the context of each institution's own experience and epidemiology. The most common measures are described merely as a guide since they have a very variable level of evidence.

Prophylaxis, monitoring, and treatment options to be considered for infections.

<b>Supportive measures for bacterial infections</b>		
<b>Prophylaxis</b>	<b>Monitoring</b>	<b>Treatment</b>
Levofloxacin or ciprofloxacin	Surveillance cultures to detect colonization with multidrug-resistant gram-negative bacteria	Empirical antibacterial therapy according to institutional epidemiologic patterns
<b>Supportive measures for fungal infections</b>		
<b>Prophylaxis</b>	<b>Monitoring</b>	<b>Treatment</b>
Mold-covering azole	Galactomannan and beta-D-glucan assays <sup>a</sup>	Liposomal amphotericin B, azoles, and/or echinocandins (according to previous prophylaxis)
<b>Supportive measures for viral infections</b>		
<b>Prophylaxis</b>	<b>Monitoring</b> (quantitative PCR)	<b>Treatment</b>
CMV: letermovir	Weekly on days 0–100 and then as clinically indicated	Ganciclovir, valganciclovir, foscarnet
HHV-6: none	As clinically indicated	Ganciclovir, valganciclovir, foscarnet
Adenovirus: none	Weekly on days 0–100 and then as clinically indicated <sup>b</sup>	Cidofovir
EBV: none	Weekly on days 0–100 and then as clinically indicated	Preemptive rituximab
<b>Supportive measures for protozoal infections</b>		
<b>Prophylaxis</b>	<b>Monitoring</b>	<b>Treatment</b>
Pneumocystis: cotrimoxazol, pentamidine, or atovaquone	–	Cotrimoxazol, pentamidine, or atovaquone
Toxoplasmosis: cotrimoxazol, atovaquone, or pyrimethamine	–	Cotrimoxazol, atovaquone, or pyrimethamine

<sup>a</sup> Both have been included as microbiological criteria in the definitions of invasive fungal infections by the European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG)

<sup>b</sup> Specially in children

<sup>c</sup> Reduced intensity conditioning and ATG are risk factors for EBV-PTLD

## 64.8 Results

UCBT outcomes are still improving in more recent years, probably explained by better patient and CBU selection, improved conditioning, and supported care. Registry data also showed important center effect with superior survival obtained in experienced centers. Eurocord recently updated clinical results.

Multiple retrospective studies have demonstrated that UCBT offers similar long-term outcomes compared with the gold standard of HLA-matched unrelated donor transplants in patients with hematologic malignancies, both in children and adults (Eapen et al. 2007; Brunstein et al. 2010; Atsuta et al. 2012). Interestingly, UCBT seems to offer a potent antileukemic efficacy, through yet unknown mechanisms. A markedly reduced relapse rate after UCBT as compared to URD transplantation in patients transplanted with minimal residual disease has been reported and needs to be validated (Milano et al. 2016). Moreover recently, the same observation has been reported in children with very high-risk leukemia transplanted with single CB units. Two-year EFS was 80% for CR 2, 67% for high risk (primary refractory or relapsed leukemia), and 61% for patients transplanted in CR1 (Horgan et al. 2023). In the same direction, very impressive results have been reported in children with genetic and metabolic disorders. Two-year overall survival was 91% with a median follow-up time of 4 years (Martinez et al. 2023).

## 64.9 Adults

	2-year OS (%)
<i>Outcomes according to DRI</i>	
Low	55 ± 3
Intermediate	47 ± 1
High	27 ± 2
Very high	19 ± 3
<i>Disease-specific outcomes</i>	
Acute leukemia	37 ± 1
MDS/MPS	32 ± 2
Lymphoproliferative disorders	45 ± 2
Plasma cell disorder	37 ± 5

OS overall survival, DRI disease risk index, MDS myelodysplastic syndrome, MPS myeloproliferative syndrome

## 64.10 Children

	2-year OS (%)
<i>Malignant disorders</i>	
Acute leukemia	52 ± 1
MDS	55 ± 3
Lymphoproliferative disorders	55 ± 3
<i>Nonmalignant disorders</i>	
Inborn error of metabolism	70 ± 2
Hemoglobinopathies	68 ± 9
Primary immunodeficiency	68 ± 2
Histiocytic disorders	60 ± 4
Bone marrow failure syndrome	52 ± 3

OS overall survival, MDS myelodysplastic syndrome

## 64.11 Conclusions

UCBT activity has drastically decreased in favor of other donor/stem cell options, specially haplo-HCT that shares the main advantage of UCB: high and rapid availability. However, UCB remains a valuable source of stem cells for HCT in selected patients that lack other options or in highly specialized units with preference of UCB. Important improvements in the field have taken place with a beneficial impact on NRM. Strategies to enhance engraftment with UCB ex vivo expansion have shown impressive success, although its widespread use and benefit in real-life clinical practice remain to be seen. There is still a room for improvement; however, clinical research in the field will be challenging in the future.

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# Haploidentical HCT

# 65

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## Abbreviations

AML	Acute myeloid leukemia
ATG	Anti-thymocyte globulin
BM	Bone marrow
CNI	Calcineurin inhibitors
CSA	Cyclosporine A
DFS	Disease-free survival
EBMT	European Group for Blood and Marrow Transplantation
FK	Tacrolimus
G-PB	G-CSF mobilized PB
GvHD	Graft versus host disease
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen

HCT	Hemopoietic cell transplantation
MMF	Mycophenolate
MTX	Methotrexate
PB	Peripheral blood
PTCy	Post-transplant cyclophosphamide
TCD	T-cell depletion
Treg	Regulatory T cells
TRM	Transplant-related mortality

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## 65.1 Ex Vivo TCD Platforms

The physical removal of donor T cells from the graft has been pioneered on adult patients by the group of Perugia in the late 1990s (Aversa et al. 1998). The original concept was to prevent GvHD by infusing a high-dose CD34-enriched graft with a T cell content  $<1 \times 10^4/\text{kg}$  of recipient body weight.

### 65.1.1 Positive CD34 Selection

The first generation of ex vivo graft manipulation by antibody-based immunomagnetic methodology has been the positive selection of CD34+ cells using the CliniMACS® System. Using G-CSF  $\pm$  Plerixafor mobilized PBSC, CD34+ selected grafts provide a megadose of stem cells ( $>10^{10}/\text{Kg}$ ) along with a negligible dose of T cells. (Aversa et al. 1998; Ciceri et al. 2008; Reisner et al. 2011). To ensure engraftment, the

infusion of such a graft required the combination of fully myeloablative and immunoablative conditioning regimens including ATG, TBI, fludarabine, and thiotepa.

Despite the application of such intensive immunoablative regimens, 10–15% of transplanted patients required a second HCT due to primary graft failure or rejection. While this approach was very effective to prevent GvHD, patients given these low dose T cell grafts, and especially when receiving serotherapy as part of conditioning, were typically characterized by substantially delayed immune reconstitution resulting in high incidence of life-threatening opportunistic infections and leukemia relapse (Reisner et al. 2011; Perruccio et al. 2005; Ciceri et al. 2008). Therefore, adoptive cellular therapies were developed to enhance T cell immune reconstitution without concomitant risk for GvHD (Perruccio et al. 2005; Di Ianni et al. 2011; Ciceri et al. 2009). Donor T cells genetically modified to express HSV-thymidine kinase suicide gene (Zalmoxis®) have been registered by the European Medicines Agency as adjunctive therapeutic tool post haploidentical HCT. In the pediatric population, virus-specific T cells have shown promise (Leen et al. 2009; Feucht et al. 2015).

### 65.1.2 CD3/CD19 Negative Selection

To overcome some of the shortcomings of the CD34+ selection method, a second generation of ex vivo graft manipulation was developed using CD3/CD19 depletion by the same CliniMACS system. This approach keeps stem and progenitor cells untouched while retaining immune effector cells, in the cellular product (Bethge et al. 2006; Federmann et al. 2011, 2012). Compared to CD34+ positive selection, the CD3/CD19 depletion approach seems associated with more favorable CD4 T cell reconstitution, while the difference for other immune and clinical outcome parameters is less evident (Salzmann-Manrique et al. 2018).

### 65.1.3 TCR $\alpha/\beta$ and CD19 Depletion

To more selectively remove the GvHD causing T cells from the graft, CD3 depletion was replaced by depletion of TCR $\alpha/\beta$  expressing T cells with the aim to reduce the risk for GvHD while maintaining the potentially beneficial anti-leukemic and anti-pathogen gamma-delta T cells and NK cells in the graft (Li Pira et al. 2016). In the last decade, this method has been proven to be associated with positive outcomes in pediatric and adult patients with malignant as well as non-malignant diseases, including low rates of TRM, low rates of relapse, fast neutrophil and platelet recovery, and improved chronic GvHD-free/relapse-free survival, compared to unmanipulated mismatched unrelated HCT. These results indicate that the TCR $\alpha/\beta$  depletion approach can be considered an effective and safe option for patients who require a stem cell transplant but lack a fully matched donor (Bertaina et al. 2014; Locatelli et al. 2013; Lum et al. 2022; Merli et al. 2022; Tsilifis et al. 2022). Despite these advances, delayed reconstitution of TCR $\alpha/\beta$  T cells remains an issue and is associated with a high rate of viral reactivations (~50%) in the first 2–3 months. To overcome this window of immune deficiency, several adoptive GvHD-sparing cellular therapy approaches have been and are being explored in clinical trials including virus-specific T cells, suicide gene-modified DLI, T cell progenitors, and memory T cell infusions (Sect. 65.1.5).

### 65.1.4 Co-Infusion of Regulatory T Cells

The Perugia group as well as others recently implemented a new variation of ex vivo TCD, which includes the co-infusion of regulatory T cells, followed by mature T cells (Pierini et al. 2021): preferential migration of regulatory T cells to the lymph nodes, but not the bone marrow, prevents GvHD (in the lymph nodes) and allows, at the same time, a strong graft versus

leukemia (in the bone marrow). The result is an extremely low incidence of leukemia relapse (Pierini et al. 2021).

### 65.1.5 CD45RA+ Depletion

Mouse models have demonstrated the capacity of naïve T cells (CD45RA+) to cause severe GvHD, whereas memory T cells (CD45RA-RO+) induced mild or no GvHD (Anderson et al. 2003). In the last decade, few groups have implemented the use of CD45RA+ depletion targeting naïve T cells to reduce the risk of GvHD in mismatched HCST (Triplett et al. 2018; Gasior Kabat et al. 2021; Maschan et al. 2017).

## 65.2 Unmanipulated Haploidentical HCT

The number of unmanipulated HLA haploidentical transplants has been rapidly increasing over the past 15 years (Passweg et al. 2012), due to the successful prevention of two major problems: lethal GvHD and graft rejection. There are currently three main platforms to perform unmanipulated haplo-HCT:

ATG based → together with CSA, MMF, and MTX (Lu et al. 2006),

PTCy based → together with FK/CSA, MMF (Luznik et al. 2008), and

ATG + PTCy → together with other in vivo immunosuppressants (DeZern et al. 2017; Duléry et al. 2018)

### 65.2.1 Anti-Thymocyte Globulin (ATG) Based

Following the pioneering work of Dao Pei Lu (Lu et al. 2006), the ATG-based prophylaxis has been shown to allow significant engraftment and control of GvHD, in recipients of haplo-HCT, such that the outcome is comparable with recipients of HLA-matched grafts (Wang et al. 2013).

The Beijing protocol consists of a myeloablative conditioning regimen (ARA-C, BU 4 days and CY 2 days), together with rabbit ATG 2.5 mg/kg/day (Fig. 65.1). GvHD prophylaxis is based on 4 drugs (ATG, CSA, MTX, MMF) (Fig. 65.1). The stem cell source is a combination of G-CSF (G)-mobilized bone marrow (G-BM) and G-mobilized PB. A modification of the Beijing protocol has used unmanipulated G-BM alone (Ji et al. 2005) and included intensive GvHD prophylaxis with ATG, CSA, MTX, and MMF with the addition of basiliximab, an anti-CD25 antibody. The same GvHD prophylaxis has been reported by an Italian consortium (Di Bartolomeo et al. 2010), with a different conditioning regimen (thiotepa, busulfan, fludarabine) (TBF), originally described by Sanz and coworkers for cord blood transplants (Sanz et al. 2012).

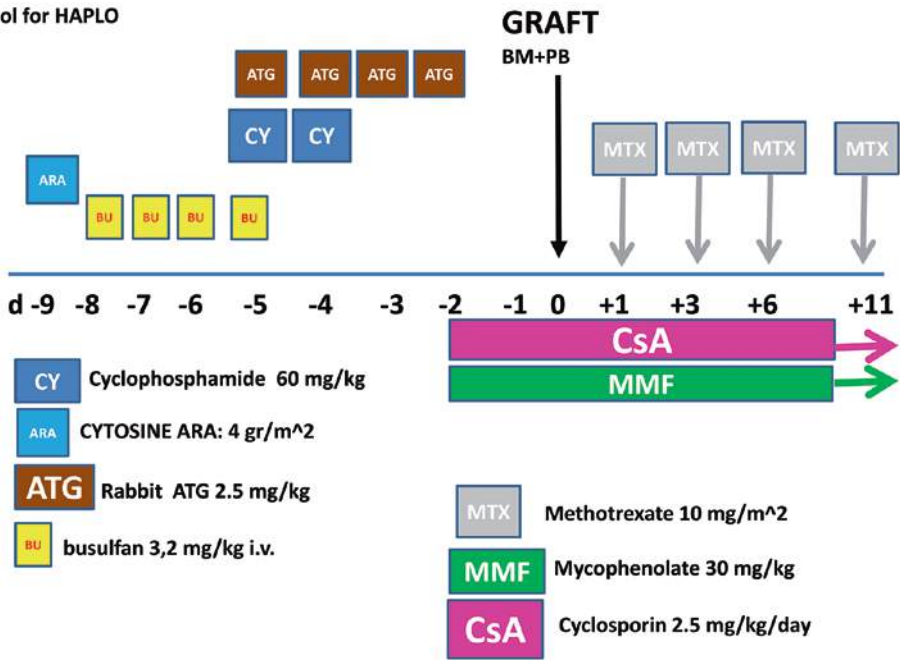
### 65.2.2 Post-transplant Cyclophosphamide (PT-Cy) Based

The use of PT-Cy on day +3 and +4 after an unmanipulated haplo-HCT has been pioneered by the Baltimore group (Fig. 65.2). It is based on the combination of FLU Cy and low dose TBI (2 Gy), followed by unmanipulated haplo BM; GvHD prophylaxis consists of high-dose CY on day +3 +4 (50 mg/kg) combined with a CNI and MMF (Fig. 65.2).

It is based on the idea that high-dose Cy (50 mg/kg) will kill alloreactive T cells proliferating on day +3 and +4 after the transplant, whereas stem cells would be protected because they are not proliferating and with a high concentration of aldehyde dehydrogenase. In 2008, the Baltimore group published their first clinical study and showed that PT-Cy was able to protect patients from GvHD after haplo-HCT (Luznik et al. 2008). not only GvHD could be prevented, but GvL seemed superior, at least in patients with HL.

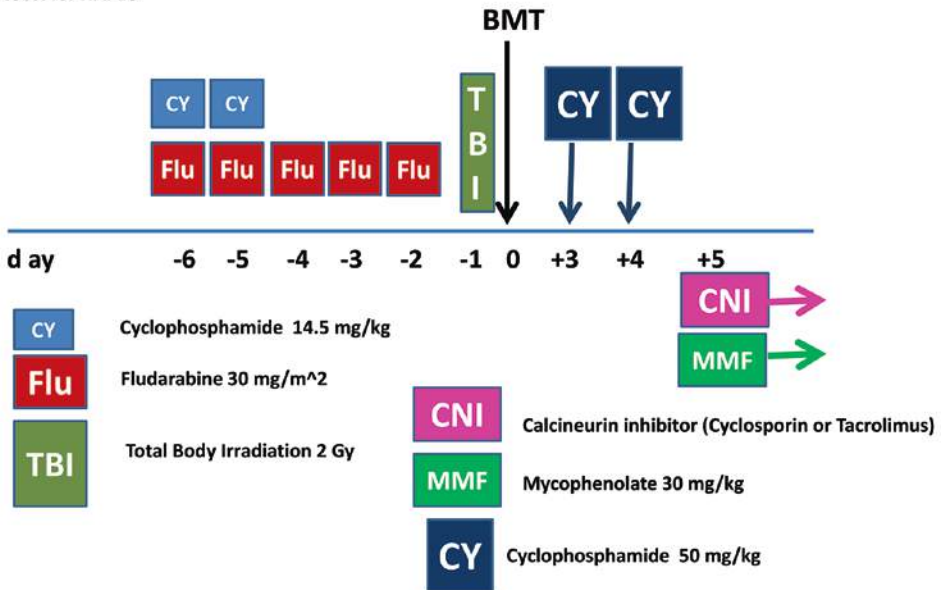
There have been numerous variations of the Baltimore protocol, with use of G-PB instead of BM (Bashey et al. 2017), rapamycin, instead of a CNI (Cieri et al. 2015), the use of a MAC regi-

**BEIJING protocol for HAPLO**



**Fig. 65.1** The Beijing protocol, first published in 2006 (Lu et al. 2006), used for patients with hematologic malignancies

**Baltimore protocol for HAPLO**



**Fig. 65.2** The Baltimore protocol with high-dose Cy post-transplant on days +3 +4

men instead of the NMA regimen of Baltimore (Raiola et al. 2013; Bashey et al. 2017), suggesting a certain degree of flexibility of PTCy-based

platforms. Engraftment is achieved in over 95% of patients, and there is good control of acute and chronic GvHD.

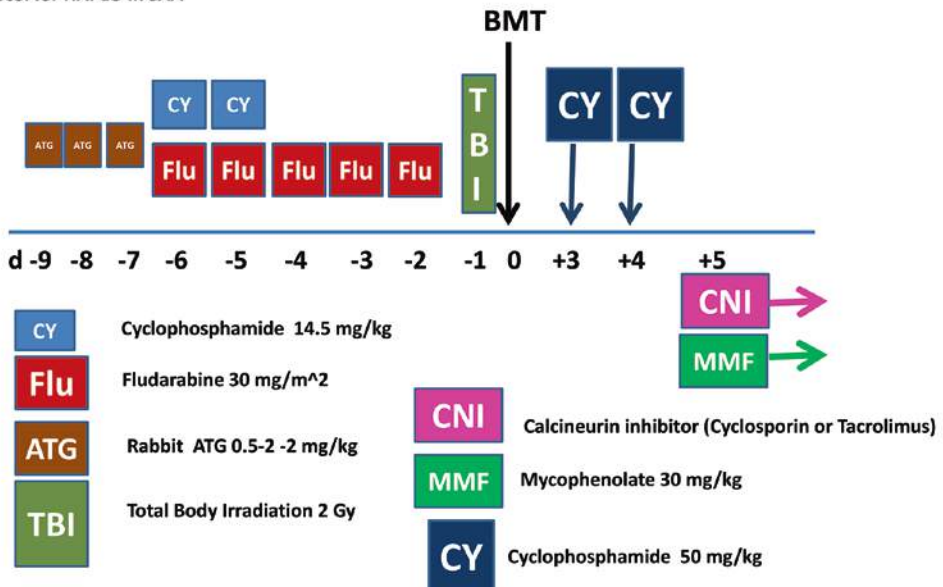
PTCy dose and timing. There are several open questions: the first is whether the dose of 50 mg/kg × 2 can be modified. The question is relevant since cardiac toxicity of PTCy 50 mg/kg × 2 has been reported (Duléry et al. 2021), with clinical consequences such as atrial fibrillation, heart failure, and death. A reduced dose of 40 mg/kg × 2 (Sugita et al. 2021) as well as 25 mg/kg × 2 (McAdams et al. 2021) has been reported as effective. It would be clearly very important to assess the minimal effective dose of PTCy, in order to reduce cardiac toxicity, accelerate neutrophil recovery, and maintain protection against GvHD. Another open question is the timing of PTCy. The Baltimore protocol calls for PTCy on days +3 +4 with CNI and MMF starting *after* PTCy (Fig. 65.2). One center has introduced a different timing with PT-Cy on days +3 and +5, together with CSA and MMF starting *before* PT-Cy (Raiola et al. 2013); the feasibility and effectiveness of this timing have been confirmed (Chiusolo et al. 2018). The EBMT has compared the two different options for PTCy: day +3 +4 vs. day +3 +5, in patients with AML (Ruggeri et al. 2020); patients receiving PTCy day +3 +5, had reduced incidence of relapse and improved

DFS. It should be said that this regimen is safe when using BM as a stem cell source, with acute GvHD III–IV rates of 3%; however, it is not known what the outcome would be with PB as a stem cell source, since CSA will protect some T cells from PT-Cy purging, and these may produce a beneficial GvL effect but also cause detrimental GvHD.

### 65.2.3 ATG + PT-Cy

Some centers are combining the two basic platforms (PT-Cy and ATG), and early results seem promising. The Baltimore group is using this combination for patients with aplastic anemia, in the attempt of avoiding GvHD completely (DeZern et al. 2017, 2020) (Fig. 65.3). The difference here is adding ATG before the conditioning regimen on days -9-8-7, and combining with PTCy, CNI, and MM (4 drug GvHD prophylaxis). Also, the group in Saint-Antoine, Paris, is using a combination of ATG 2.5 mg/kg and PT-Cy, CSA, and MMF for patients with acute leukemia undergoing a MAC haplo-HCT (Duléry et al. 2018).

De Zern Protocol for HAPLO in SAA



**Fig. 65.3** 4 drug GvHD prophylaxis (ATG, PTCY, CNI and MMF) for haplo transplants in SAA (De Zern et al 2020). TBI has recently been increased to 4 Gy

## 65.3 Other Relevant Aspects of Haplo-HCT

### 65.3.1 Choice of the Best Haploidentical Donor

The EBMT ALWP has established younger donor age and kinship, as a major determinant of outcome for leukemia patients grafted from haploidentical donor (Canaani et al. 2018). The Beijing group has confirmed younger age to be relevant, using their ATG-based platform together with a mismatch for non-inherited maternal antigen (NIMA) (Wang et al. 2014). One report has suggested that NK alloreactivity, together with HLA-DP disparity might identify an optimal haplo donor (Solomon et al. 2018). Other reports have denied an effect of increasing HLA disparity on major outcomes (Wang et al. 2014). There is currently no solid data to choose a haploidentical donor based on HLA disparities.

### 65.3.2 Comparison of ATG-Based Versus PT-Cy-Based Platforms

The EBMT Acute Leukemia Working Party has compared these two platforms (Ruggeri et al. 2017). In a Cox analysis, ATG-based haplo grafts had a higher risk of failure, in terms of LFS (RR 1.48,  $p = 0.03$ ), GvHD relapse-free survival (RR 1.45,  $p = 0.03$ ), and OS (HR 1.43,  $p = 0.06$ ): there was for all end points a very strong center effect ( $p < 0.001$ ), suggesting that a learning curve is required for optimal results in haplo-HCT.

### 65.3.3 Bone Marrow or Peripheral Blood

There are two large registry-based studies comparing BM versus PB for unmanipulated haplo-HCT: the EBMT study (Ruggeri et al. 2018) shows increased GvHD II–IV and III–IV with PB, same chronic GvHD, same relapse, and same 2-year OS (55% and 56%). The CIBMTR shows increased GvHD II–IV, but not III–IV with PB grafts, increased chronic GvHD, and reduced

relapse (Bashey et al. 2017): survival at 2 years also in this study is quite similar, 54% vs. 57%. So, it seems that one can use both stem cell sources, with some difference in the short term (more GvHD with PB) and perhaps some differences in the long term (cGvHD and relapse): at the end survival seems comparable.

#### Key Points

- Following the pioneering work of the Perugia group, HLA-haplotype mismatch family transplants using ex vivo T cell depletion is evolving, with the use of T cell subpopulations and the use of Treg Tcon.
- Unmanipulated haplo-HCT is rapidly increasing in numbers, worldwide, due to the introduction of post-transplant cyclophosphamide (PTCy).
- There are two platforms for unmanipulated haplo-HCT: ATG based or PTCy based.
- ATG can be combined with PTCy, CSA, and MMF, to further reduce GvHD: this is being tested especially in non-malignant disorders.
- One important question is how haplo-HCT compare with unrelated donor grafts, and to answer this question, randomized trials have been designed and are about to start.
- One should consider that HLA-haplotype mismatch transplants remain an alternative donor procedure and should be regarded as such complications, including blood stream infections, invasive fungal disease, viral infections, GvHD, and toxicity may occur with significant frequency and expose the patients to the risk of TRM. For this reason, HLA-haplotype mismatch grafts, whether TCD or unmanipulated, should be performed in centers with expertise in MUD or CB HCT and should follow clinical protocols.



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# Photopheresis in Adults and Pediatrics

# 66

Hildegard Greinix

## 66.1 Introduction

Extracorporeal photopheresis (ECP) is a leukapheresis-based treatment that has been used during the last decades by many clinicians. Based on the results of a prospective, multicenter, international clinical trial in patients with cutaneous T-cell lymphoma (CTCL), ECP was approved by the FDA as the first cellular immunotherapy for cancer in 1988 (Edelson et al. 1987). During the last decades, ECP has been investigated worldwide for the prevention and treatment of a variety of T-cell-mediated diseases including acute and chronic GVHD, solid organ and tissue transplantation, systemic sclerosis, systemic lupus erythematoses, and Crohn's disease (Knobler et al. 2020, 2021). Administering ECP to patients suffering from these diseases revealed promising results both in prospective and retrospective single and multicenter clinical studies. Despite its frequent use, the mode of action of ECP remains elusive but includes reduction of pro-inflammatory cytokines, induction of anti-inflammatory cytokines, and modulation of immune cell populations.

## 66.2 Technical Aspects

During ECP, the patient's blood is collected via an antecubital vein or a permanent catheter, and the white blood cells are separated from the red blood cells and plasma by centrifugation in a device that is specifically constructed for the procedure (Knobler et al. 2020, 2021; Schoonemann 2003). Collected mononuclear cells (MNCs) using either continuous or discontinuous cell separators are then exposed *ex vivo* to a photosensitizing agent, 8-methoxypsoralen (8-MOP), which is added directly to the buffy coat/plasma fraction followed by photoactivation with ultraviolet A (UV-A) irradiation and then reinfusion of the photoactivated product (Schoonemann 2003).

ECP has originally been developed as a single procedure that combines the separation of the MNCs from the whole blood with irradiation of the 8-MOP-treated leukapheresis products within a single machine ("closed system of ECP"). The "offline technique" (two-step method) of ECP treatment includes as the first step cell separation with a standard blood cell separator that can also be used for the collection of peripheral blood stem cells. The apheresis product is transferred into another disposable, 8-MOP is added, and irradiation is performed with a separate machine at a dosage of 2 J/cm<sup>2</sup>. After irradiation, transfusion of the treated cells is carried out manually by a standard transfusion set. Both ECP techniques have demonstrated clinical efficacy, but almost all clinical studies have been performed with the

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single ECP technique, and studies comparing both systems are almost completely lacking (Schoonemann 2003; Andreu et al. 1994; Brosig et al. 2016).

### 66.3 Results of ECP in Acute GVHD

Until recently, no consensus on the optimal choice of agents for salvage therapy of steroid-refractory acute GVHD has been reached, and treatment choices are based on the physician's experience, risk of toxicity and potential exacerbation of pre-existing comorbidity, interactions with other agents, and ease of use (Martin et al. 2012). During the last years, more and more HCT centers have administered ECP to patients with steroid refractory acute GVHD. Results of larger prospective studies on the use of ECP in this indication are shown in Table 66.1. The intensified schedule of ECP with two to three treatments per week on a weekly basis significantly improved response rates in patients with GI involvement and grade IV acute GVHD (Greinix et al. 2006).

In a systematic review of prospective studies including 6 studies with 103 patients given ECP for steroid-refractory acute GVHD, an overall

response rate (ORR) of 69% was achieved including ORR for skin, liver, and GI involvement of 84%, 55%, and 65%, respectively (Abu-Dalle et al. 2014). Compared to anticytokine treatment, administration of ECP for steroid-refractory acute GVHD not only achieved significantly higher ORR (66% vs. 32%) and CR (54% vs. 20%), but ECP was also an independent predictor of response and survival and was associated with significantly lower NRM and superior survival in steroid-refractory grade II acute GVHD (Jagasia et al. 2013). Compared to other IST, ECP has an excellent safety profile with limited toxicity concerns, no increased concerns for viral reactivations, and no documented interaction with other drugs (Martin et al. 2012).

In May 2022, the European Medical Agency (EMA) approved ruxolitinib for the treatment of patients aged 12 years and older with acute GVHD who have inadequate response to corticosteroids or other systemic therapies based on the results of the REACH2 study comparing ruxolitinib with best available therapy in patients with steroid-refractory acute GVHD (Zeiser et al. 2020). Therefore, ruxolitinib has been frequently used as second-line therapy during the last year. In a recently published single-center experience of combining ruxolitinib with ECP in 18 patients

**Table 66.1** Results of second-line treatment of acute GVHD using extracorporeal photopheresis

Author (year)	No. of patients	CR skin no. (%)	CR liver no. (%)	CR gut no. (%)	OS%
Salvaneschi et al. (2001)	9	6/9 (67)	1/3 (33)	3/5 (60)	67
Dall'Amico (2002)	14	10/14 (71)	4/7 (57)	6/10 (60)	57
Messina et al. (2003)	33	25/33 (76)	9/15 (60)	15/20 (75)	69 at 5 y
Greinix et al. (2006)	59	47/57 (82)	14/23 (61)	9/15 (60)	47 at 5 y
Garban et al. (2005)	12	8/12 (67)	0/2 (0)	2/5 (40)	4
Kanold et al. (2007)	12	9/10 (90)	5/9 (56)	5/6 (83)	75 at 8.5 m
Calore et al. (2008)	15	12/13 (92)		14/14 (100)	85 at 5 y
Perfetti et al. (2008)	23	15/23 (65)	3/11 (27)	8/20 (40)	48 at 37 m
Gonzalez-Vicent et al. (2008)	8	8/8 (100)	2/2 (100)	4/7 (57)	38
Perotti et al. (2010)	50	39/47 (83) (1)	16/24 (67) (1)	8/11 (73) (1)	64 at 1 y
Jagasia et al. (2013)	57	38/57 (67) (1)	38/57 (67) (1)	38/57 (67) (1)	59 at 2 y
Calore et al. (2015)	72	50/64 (78)	10/12 (84)	42/55 (76)	71 at 5 y
Niittyvuopio et al. (2018)	52	24/31 (77)	1/3 (33)	14/41 (34)	51 at 1 y
Worel et al. (2018)	99	71/88 (80)	18/29 (61)	26/35 (75)	50 at 5 y
Amat et al. (2021)	37	9/28 (32)	3/11 (27)	12/30 (40)	80 at 3 y

Abbreviations: *No* number, *CR* complete resolution, *OS* overall survival, *y* years, *m* months  
Results were provided as complete and partial resolution

with severe SR acute GVHD of lower GI tract (50% each overall grades III and IV) and other organ manifestations (skin  $n = 7$ , liver  $n = 6$ , and upper GI tract  $n = 2$ ), the majority of patients ( $n = 15$ , 83%) received ruxolitinib a median of 20 days before starting ECP (Modemann et al. 2020). The investigators observed a best ORR of 55% including a CR rate of 44% and an additional PR rate of 11%, respectively. The mean daily steroid dose was 130 mg at the diagnosis of SR acute GVHD and at the start of lead-in ruxolitinib (83% of patients) or ruxolitinib with ECP (17% of patients) treatment and could be tapered to less than 20 mg by day 21 and stopped after a median of 27 days. Thus, the feasibility of a rapid steroid taper and discontinuation of steroids with the combination of ruxolitinib and ECP could be demonstrated. Responding patients had a two-year OS of 70% with a median survival of 18 months. These promising results should be confirmed in prospective studies to increase the treatment options for patients with severe SR acute GVHD.

## 66.4 Results of ECP in Chronic GVHD

Many therapeutic options have been reported for salvage treatment of steroid-refractory chronic GVHD, and until recently, no single class of IS agent has been established as standard therapy (Wolff et al. 2011). In August 2017 ibrutinib and in September 2021 ruxolitinib were approved by the FDA for the treatment of patients with chronic GVHD after failure of one or more lines of systemic therapies.

ECP represents a frequently used therapeutic approach for the treatment of chronic GVHD patients failing corticosteroids (Table 66.2) (Knobler et al. 2020, 2021; Wolff et al. 2011; Greinix et al. 1998; Flowers et al. 2008; Jagasia et al. 2009; Greinix et al. 2011). Most of the clinical experience in ECP treatment of steroid-refractory chronic GVHD patients is based on retrospective analyses with consistently high response rates in up to 80% of patients with cutaneous manifestations and substantial improve-

**Table 66.2** Results of the use of extracorporeal photopheresis in chronic GVHD

Author (year)	No of patients	CR/PR skin (%)	CR/PR liver (%)	CR/PR oral (%)	ORR (%)
Greinix et al. (1998)	15	80	70	100	Na
Salvaneschi et al. (2001)	14	83	67	67	64
Messina et al. (2003)	44	56	60	–	57
Seaton et al. (2003)	28	48	32	21	36
Apisarnthanarax et al. (2003)	32	59	0	Na	56
Foss et al. (2005)	25	64	0	46	64
Rubegni et al. (2005)	32	81	77	92	69
Greinix et al. (2006)	47	93	84	95	83
Couriel et al. (2006)	71	57	71	78	61
Kanold et al. (2007)	15	75	82	86	50
Perseghin et al. (2007)	25	67	67	78	73
Flowers et al. (2008)	48	40	29	53	40
Jagasia et al. (2009)	43				65
Perotti et al. (2010)	23	96	100	80	69
Dignan et al. (2012)	82	92	Na	91	74
Greinix et al. (2011)	29	31	50	70	Na
Del Fante et al. (2016)	102	Na	Na	Na	81
Ussowicz et al. (2013)	13	67	89	86	69
Hautmann et al. (2013)	32	59	100	60	44
Dignan et al. (2014)	38	65	–	29	50
Sakellari et al. (2018)	88	83	53 <sup>a</sup>	Na	73
Gandelman et al. (2018)	77	55	Na	Na	62

Abbreviations: *No* number, *CR* complete resolution, *PR* partial resolution, *ORR* overall response rate, *na* not available

<sup>a</sup>Visceral involvement



ment in sclerodermatous skin involvement (Knobler et al. 2020, 2021; Wolff et al. 2011).

In a multicenter, randomized, controlled, prospective phase II study of ECP in 95 patients with steroid-refractory/dependent/intolerant chronic GVHD, significantly more patients in the ECP arm achieved a complete or partial response of cutaneous manifestations ( $p < 0.001$ ) as well as a 50% reduction in steroid dose and at least a 25% decrease in total skin score ( $p = 0.04$ ) by week 12 (Greinix et al. 1998). A steroid-sparing effect of ECP has also been reported by other investigators (Knobler et al. 2020, 2021; Wolff et al. 2011; Flowers et al. 2008; Jagasia et al. 2009).

In a systematic review of prospective studies on the use of ECP in patients with chronic GVHD, an ORR of 71% in cutaneous, 62% in GI, 58% in hepatic, 63% in oral mucosal, and 45% in musculoskeletal manifestations of chronic GVHD was reported (Abu-Dalle et al. 2014). The rate of IS discontinuation was 23%, and ECP was tolerated excellently. In another meta-analysis, high response rates in cutaneous and extracutaneous manifestations of chronic GVHD including 48% of responses in lung involvement were confirmed (Del Fante et al. 2016). The ECP schedule in chronic GVHD is empirical ranging from multiple treatments per week on a weekly basis to two treatments biweekly and in case of response prolongation of the treatment interval to 4–6 weeks, respectively. No clear association between ECP dose intensity and response has been reported. Higher response rates were achieved in steroid-refractory patients given ECP earlier in the course of their disease (Malik et al. 2014; Messina et al. 2003). Improvements in the quality of life and survival in ECP responders have been reported (Knobler et al. 2020, 2021; Wolff et al. 2011; Greinix et al. 1998; Malik et al. 2014; Messina et al. 2003).

In a retrospective analysis, 23 patients with SR chronic GVHD (57% NIH grade 3, 91% beyond second-line treatment, and 87% with more than one organ involved) received the combination of ruxolitinib at 5–10 mg bid and ECP with two treatments on consecutive days every

2–4 weeks (Maas-Bauer et al. 2021). Thirty-five percent of patients started ECP and ruxolitinib treatment simultaneously, whereas 30% started ECP first and the median time of ECP therapy prior to combination treatment was 3.25 (1–7) months. During ECP alone, the best response was PR in 43% (3/7) of patients and 57% (4/7) were nonresponders. Thirty-five percent of patients started ruxolitinib treatment first a median of 15 (1–29) months prior to combination treatment. The best ORR of ruxolitinib alone was PR in 62.5% (5/8), and 37.5% (3/8) did not respond. The best ORR of ECP combined with ruxolitinib was 74% (17/23) including 9% CR and 65% PR and a two-year OS of 75%. Thus, combinational treatment increased ORR in heavily pretreated patients with multiorgan involvement SR chronic GVHD and was able to improve the outcome of patients after inadequate responses to ECP or ruxolitinib monotherapy. Patients received a median of six months of combination therapy.

ECP is a safe and efficacious treatment for patients with chronic GVHD with steroid-sparing capacity. Transient hypotension during treatment and mild anemia and/or thrombocytopenia have been reported as side effects of ECP. Prospective clinical studies are warranted to assess the efficacy of ECP in well-defined cohorts of chronic GVHD patients treated earlier in the course of their disease. Recently, Jagasia and colleagues reported the first results of a randomized, controlled, multicenter study in NIH-defined moderate/severe chronic GVHD patients given ECP in the study arm in combination with standard of care IS (Jagasia et al. 2017). Besides an ORR of 74%, and thus, a promising efficacy ECP demonstrated to be safe and tolerated well.

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## 66.5 Conclusions

ECP has been used for over 30 years in the treatment of CTCL, acute and chronic GVHD, and solid organ transplant rejection. Multiple scientific organizations recommend its use due to ECP's efficacy and excellent safety profile

(Knobler et al. 2020, 2021). Due to the lack of interactions with other agents and the avoidance of general IS, ECP compares favorably with other IS strategies, supporting its increasingly frequent use as salvage therapy of steroid-refractory/dependent acute and chronic GVHD. Of note, the corticosteroid-sparing potential of ECP has been confirmed in numerous retrospective and prospective studies and translates into immediate clinical benefit for patients with GVHD as well as a reduction of transplant-associated morbidity and mortality.

No general recommendation can be made on the treatment schedule due to missing evidence. Ideally, ECP treatment should be initiated as early as possible after the indication is confirmed. Especially in patients with steroid-refractory acute GVHD, earlier treatment onset and an intensified weekly ECP schedule resulted in improved response rates and patients' outcomes. Prospective studies on the use of ECP as upfront treatment in GVHD as well as in combination with recently approved agents are warranted.

#### Key Points

- ECP is a safe and efficacious adjunct therapy of steroid-refractory acute and chronic GvHD.
- Results in upfront therapy of chronic GvHD are promising.

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# Overweight and Obese Patients

# 67

Claudia Langebrake

## 67.1 Introduction

“More obese people in the world than underweight” was the headline on BBC News on 1 April 2016. This statement is based on a study comparing the prevalence of body mass index (BMI) categories of more than 19.2 million adult participants in 186 of 200 countries. Comparing the age-standardized mean BMI by country in 1975 and 2014, there is a significant increase in both men (21.7 vs. 24.2 kg/m<sup>2</sup>) and women (22.1 vs. 24.4 kg/m<sup>2</sup>). According to the World Obesity Atlas, one billion people globally are estimated to be living with obesity by 2030.

Compared with people of healthy weight, those who are overweight or obese are at greater risk for many diseases, including diabetes, high blood pressure, cardiovascular disease, stroke, and at least 13 types of cancer, as well as having an elevated risk of death from all causes. Furthermore, obesity seems to be associated with a worse outcome after hematopoietic cell transplantation (HCT).

### 67.1.1 Definitions and Size Describers of Obesity

Classification of excess weight and obesity is usually based on BMI which is calculated using height and weight of an individual. According to the World Health Organization (WHO), adults are defined to be normal weight with a BMI of 18.5–24.9 kg/m<sup>2</sup>, overweight with 25–29.9 kg/m<sup>2</sup>, and obese with  $\geq 30$  kg/m<sup>2</sup>. However, one has to keep in mind that although BMI has been shown to correlate with subcutaneous fat (but not with percentage body fat), in individuals with greater muscle mass, women or the elderly, BMI might not be the best describer, as muscle mass is more dense than fat mass. In those people, percent body fat would better describe body composition, but the direct measurement is usually not readily available as it requires advanced technical equipment (e.g. hydrodensitometry, skin-fold measurement, bioelectrical impedance analysis, or dual-energy X-ray absorptiometry) (Hanley et al. 2010). As a consequence, indirect measures of body composition, such as BMI or ideal body weight (IBW), remain the standard, as they are easy to calculate.

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## 67.2 Influence of Excess Weight and Obesity on the Pharmacokinetics of Drugs

Obesity is associated with physiological changes that can alter the pharmacokinetic (PK) parameters of many drugs. Observed physiological changes in obese patients influencing the pharmacokinetic behavior of drugs and resulting consequences for drug dosing are summarized in Table 67.1.

Nevertheless, it has to be kept in mind that the effects of physiologic changes are usually drug-specific and that for the majority of drugs, both pharmacokinetic and clinical data in obese patients are sparse. Due to unusual distribution processes, the kinetics of drugs is difficult to predict in obese patients.

The impact of obesity on glomerular filtration rate (GFR) as well as on tubular secretion is not

completely understood. Discrepant results regarding GFR in obese as compared with normal-weight individuals might be explained by estimating GFR using serum creatinine, as no instrument has been validated for obesity. Especially, if using weight-based formulas like the widespread Cockcroft–Gault formula, estimated GFR (eGFR) will be overestimated if total body weight (TBW) is used but underestimated if ideal body weight is used. Therefore, it is reasonable to use adjusted ideal body weight (AIBW) for estimation in overweight or obese patients. However, the use of weight-independent formulas, such as MDRD (Modification of Diet in Renal Disease) or CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration), that has been shown to result in more reliable estimates (Bouquegneau et al. 2015), has limitations: as the eGFR is provided in mL/min/1.73 m<sup>2</sup>, the possibly incorrect calculation of body surface area (BSA) in the obese might negatively influence the results.

**Table 67.1** Overview of physiologic changes in obese individuals influencing pharmacokinetics of drugs

	Changes in obese patients	Consequences of drug dosing
Absorption	<ul style="list-style-type: none"> <li>• Increased gastrointestinal blood flow</li> <li>• Accelerated gastric emptying</li> <li>• Changes in the composition and thickness of the subcutaneous fat tissue</li> </ul>	<ul style="list-style-type: none"> <li>• Only little data on oral bioavailability</li> <li>• For a couple of drugs including cyclosporine A, midazolam, or propranolol, no differences in oral bioavailability have been observed</li> <li>• Unsuccessful intramuscular injections</li> <li>• Accidental intramuscular administration intended for subcutaneous injection</li> <li>• Paucity of information on the rate of absorption and bioavailability administered subcutaneously</li> </ul>
Distribution	<ul style="list-style-type: none"> <li>• Hydrophilic drugs: Vd is similar in normal-weight and obese patients</li> <li>• Moderate or high lipophilic drugs: significant differences in Vd</li> <li>• Tissue blood flow may be reduced</li> <li>• Obesity does not appear to have an impact on plasma protein binding</li> </ul>	<ul style="list-style-type: none"> <li>• Vd is important for the determination of a loading dose in order to achieve a rapid and adequate exposition</li> <li>• Vd changes are drug-specific (attributable to the physicochemical properties of the drug)</li> </ul>
Clearance (renal and hepatic)	<ul style="list-style-type: none"> <li>• Altered hepatic blood flow</li> <li>• TBW-proportional increase in phase II metabolism</li> <li>• GFR and renal perfusion are similar, but imprecision in GFR estimation (see text below)</li> <li>• Obese individuals exhibit higher absolute drug clearance</li> <li>• Clearance does not increase linearly with TBW</li> <li>• Clearance and lean body weight are linearly correlated</li> </ul>	<ul style="list-style-type: none"> <li>• Elimination of hydrophilic and extensively renally cleared drugs mainly depends on renal function</li> <li>• No apparent relationship between lipophilicity and clearance mechanism</li> <li>• Essential parameter to determine maintenance dose</li> <li>• Physicochemical attributes of drugs have little impact on clearance</li> </ul>

Abbreviations: *GFR* glomerular filtration rate, *TBW* total body weight, *Vd* volume of distribution



Taken together, there is only limited evidence-based information about drug clearance in obese patients due to restrictions of clinical trials and the lack of pharmacokinetic (PK) analyses. It is important to remember that there is no single-size descriptor for all drugs.

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## 67.3 Recommendations for Drug Dosing

Besides the above-described physicochemical attributes and PK properties, recommendations from the literature and plasma concentration monitoring are important to determine drug dosing in morbidly obese patients (Green and Duffull 2004; Han et al. 2007; Hanley et al. 2010).

### 67.3.1 Which Weight to Use for Calculation?

For some drugs, the use of adjusted ideal body weight (AIBW) resulted in similar drug exposure in obese as compared to normal-weight patients: This is, for example, true for aminoglycosides, acyclovir (Turner et al. 2016), or liposomal Amphotericin B. AIBW is calculated by adding 25–40% of the difference between total body weight (TBW) and IBW to the IBW. This method is also well examined using population PK models for busulfan (Nguyen et al. 2006). On the other hand, initial vancomycin dosing should be based on TBW with subsequent therapeutic drug monitoring. However, for many drugs, the optimal basis for dose calculation has still to be determined.

### 67.3.2 Antibiotics, Chemotherapy, and Other Drugs

The majority of dosing recommendations in obese patients exist for antimicrobial drugs. A comprehensive overview of current literature of antibiotic dosing was first published in 2017 and updated in 2022 (Meng et al. 2023).

For the dosing of chemotherapy—except high-dose regimens—the American Society of Clinical Oncology (ASCO) published the following main statements in 2012 that were updated in 2021 (Griggs et al. 2021):

- Dose should be selected according to body surface area (BSA) using actual body weight.
- Dose reductions of systemic antineoplastic therapies should be based on toxicity and comorbidities independent of the obesity status—there is no evidence that obese patients experience increased toxicity when actual weight is used for the calculation of chemotherapy.
- Full, approved doses of immunotherapy and targeted therapies should be offered to obese adults with cancer.

However, some limitations have to be kept in mind, as (1) there are no RCTs comparing actual body weight with other adjusted dosing approaches in obese patients, (2) recommendations are based on subgroup analyses of obese patients from RCTs or observational studies using actual versus adjusted weight calculation, and (3) there are no recommendations for HCT conditioning.

Some recommendations have also been published for the dosage of low-molecular-weight heparins. In the context of prophylaxis, for example, fixed-dose enoxaparin shows an inverse linear correlation between the AUC or anti-Xa activity and body weight between 50 and 150 kg, with the lowest levels in moderate-to-severe obese patients (Rocca et al. 2018). Therefore, an increased dose has been suggested for venous thromboembolism prophylaxis and weight-based dosing (without dose capping, but with anti-Xa monitoring in severe obesity) for antithrombotic therapy.

One case report described experiences of drug dosing in a morbidly obese patient undergoing allogeneic HCT (Langebrake et al. 2011). Here, it was observed that for hydrophilic and extensively renally cleared drugs, standard dosages for adult patients or dosing based on ideal body

weight can be used. For more lipophilic drugs like cyclosporine A or digitoxin, it could be shown that after achieving sufficient plasma levels using high initial doses, maintenance doses similar to those used in normal-weight patients are sufficient. Monitoring of plasma concentrations is highly recommended for drugs with a narrow therapeutic index.

### 67.3.3 Preparative Regimens Prior to HCT

In patients undergoing autologous or allogeneic HCT, specific features and purposes have to be taken into account. In autologous HCT, high-dose chemotherapy aims to reduce tumor burden, while in allogeneic HCT therapeutic effect is based on donor immune cells and myeloablation.

There is uncertainty as to appropriate practice in relation to dose adjustments of both conditioning chemotherapy and graft-versus-host disease (GvHD) prophylaxis in obese patients undergoing HCT, as there are insufficient data to determine optimal drug dosing in obese patients undergoing HCT. In Europe, the vast majority of transplant centers consider dose adjustments in conditioning chemotherapy, although with significant variation in the methods used to categorize obesity and the degree of modification (Smith et al. 2023). Most centers refer to the ASBMT recommendations published in 2014, although it had been concluded that “dose adjustments for obesity have been based empirically or extrapolated from published data in the nontransplantation patient population” and that “clear standards or dosing guidelines are unable to be made for the obese population because Level I and II evidence are unavailable at this time.” (Bubalo et al. 2014).

For conditioning agents with intermediate (e.g. busulfan, cyclophosphamide, etoposide, melphalan, thiotepea, and treosulfan) or high volume of distribution (e.g. carmustine, clofarabine, cytarabine, and fludarabine), pharmacokinetic considerations suggest that exposure is increased in overweight or obese patients compared to

normal-weight patients when the TBW is used for dose calculation.

In particular, for busulfan, there is good evidence from pharmacokinetic and clinical studies supporting the use of AIBW for dosing busulfan. Especially for children and myeloablative regimens, the implementation of therapeutic drug monitoring is recommended (Palmer et al. 2016).

For high-dose cyclophosphamide, data from both pharmacokinetic (Takahashi et al. 2022) and clinical studies (Bachanova et al. 2016) are available that support dosing based on AIBW.

The approach to use AIBW-based BSA for dose calculation of melphalan prior to autologous HCT in multiple myeloma patients has been shown to be non-inferior as compared to the non-obese population in terms of 3-year event-free survival (Shultes et al. 2017). On the other hand, for lymphoma patients receiving high-dose BEAM prior to autologous HCT, no requirement for weight-based dose adjustment has been proposed based on tolerability and outcome (Fair et al. 2017).

Even for antithymocyte globulin (ATG) that is characterized by a very low volume of distribution, TBW for dose calculation is recommended by ASBMT. However, from a pharmacokinetic point of view, it would be more reasonable to use ideal body weight, as ATG (like other protein-based drugs) has a volume distribution that is almost equal to the whole blood volume. Furthermore, it has been proposed, to rather base ATG dosing on absolute lymphocyte count, as this is the target of ATG (Kennedy et al. 2018).

Reports of obese patients undergoing HCT are challenging to interpret because of the heterogeneity of obesity definitions, underlying diseases, graft sources, and chemotherapy regimens employed. Compared with normal-weight patients, it appears that obese patients undergoing allogeneic HCT have a higher risk of non-relapse mortality and inferior survival, whereas those receiving autologous HCT appear to have equivalent outcomes. Another important limitation for the interpretation of published data is that there is no consistent standard for calculating chemotherapy dose in this group. Therefore, it is recommended that future studies utilize more

consistent and biologically relevant definitions of obesity and that the pharmacokinetics and pharmacodynamics of specific conditioning regimens should be studied (Weiss et al. 2013).

### Key Points

- Obesity is associated with a significant increase in morbidity (including metabolic diseases and cancer) and mortality.
- Indirect measures of body composition, like BMI or ideal body weight, remain the standard as they are easy to calculate.
- There is only limited evidence-based information about drug clearance in obese patients due to restrictions of clinical trials and the lack of pharmacokinetic analyses.
- Evidence for clear standards or dosing guidelines is currently not available as there are insufficient data to determine optimal drug dosing in obese patients undergoing HCT.
- Despite that, in clinical practice, about 80% of HCT centers routinely perform dose adjustment for obesity. However, the methods used for determining the weight for chemotherapy calculation are different among the transplant centers.

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## 68.1 Introduction

The hematological malignancies, which are the most common indications for auto- and allo-HCT (e.g., AML/MDS, NHL, MM, and others), are diagnosed at a median age greater than 65 years. Thus, if classical chronological age exclusion criteria were followed, a majority of patients with these malignancies would not be offered a HCT, despite it being their treatment of choice and in many cases their only curative option (Snowden et al. 2022). While elderly patients are more likely to face toxic effects from HCT, this risk must be considered and balanced against the poor outcome of transplant candidates with these malignancies who do not proceed to HCT (Dohner et al. 2022).

## 68.2 HCT Activity in Elderly Patients

Auto- and allo-HCT annual activity continues to steadily increase in Europe and worldwide with no signs of saturation (Gratwohl et al. 2015;

Passweg et al. 2023). Specifically, in elderly patients, HCT activity at EBMT centers has increased markedly in the past two decades. Auto-HCT activity in patients  $\geq 65$  years old increased from 3.4% (443 out of 13,163 autologous HCT) in 2000 to 9.8% (2444 out of 23,883 auto-HCT) in 2014 (Sánchez-Ortega et al. 2016). Allo-HCT activity in patients  $\geq 65$  years old increased from  $<1\%$  (37 out of 6413 allo-HCT) in 2000 to 6.7% (1057 out of 16,765 allogeneic HCT) in 2014 (Basak et al. 2016). Additional recent data demonstrated also the consistent increase in the use of auto-HCT in patients with MM, with the proportion of patients aged  $>65$  years increasing dramatically, from 7% in the 90 s and first twenty-first century decade to 30% in the 2010s (Swan et al. 2022). This applies also for allo-HCT for NHL as per the EBMT registry, and the proportion of recipients aged 51–70 years increased from 8% in 1991–1995 to 58% in 2011–2015 (Kyriakou et al. 2019). For myelofibrosis, prior to 2006 only 8.7% of myelofibrosis patients undergoing allo-HCT were  $> 60$  years, whereas it increased to 47% for recipients in the 2015–2018 cohort (McLornan et al. 2021).

In the USA, the number of auto-HCT and allo-HCT for the treatment of malignant diseases in patients aged  $\geq 65$  years continues to increase (D'Souza et al. 2020). In 2020, 27% of allo-HCT recipients were aged 65 years and older and 37% of auto-HCT recipients for lymphomas and MM

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were aged 65 years or older as compared to 4% for allo-HCT and 18% for auto-HCT in 2005. Moreover, trends in allo-HCT for AML in the last two decades show an increase in use in recipients aged 65 years and older (Auletta et al. 2021).

Improvements in supportive care, HSC mobilization, the use of RTC and RIC regimens, wider donor availability, including haploidentical for most patients, and the increase of newer therapeutic options improving remission status prior to HCT have contributed to the increase in HCT activity overall and, in particular, to the increase of HCT activity rates in elderly patients. With sustained improvement in these areas, and as the population ages, these numbers will only continue to increase.

### 68.3 HCT Outcomes in Elderly Patients

Compared to younger adults, elderly patients may have higher overall rates of transplant failure. Potential comorbidities, impaired health, and performance status could lead to higher transplant-related morbidity and mortality. In addition, malignancies in elderly patients often have more adverse disease features (e.g., higher-risk cytogenetics and molecular patterns in AML/MDS patients) and may have been treated less aggressively prior to HCT, which may potentially also increase the risk of disease relapse.

Historically, HCT outcome analysis in elderly patients has been limited by the fact that these patients are underrepresented in clinical trials and the majority of data come from relatively small series and subgroup analyses of small subsets of elderly patients in larger disease-specific studies including adults of all ages. More recently, HCT outcomes of elderly patients are being analyzed specifically and have reported feasibility and safety of autologous HCT in MM patients aged >65 years (Winn et al. 2015; Auner et al. 2015), in selected populations of elderly patients with R/R DLBCL (Chihara et al. 2014) and in R/R HL in patients aged ≥60 years (Martínez et al. 2017).

Prospective studies addressing the value of allogeneic HCT compared to non-transplant approaches are limited and generally restricted to patients aged <65 years. Interestingly, a recent study in a large series of AML patients aged 60 years and older suggests that age alone is not a barrier to successful HCT (Maakaron et al. 2022), and several large series in AML/MDS patients reported that NRM and OS were negatively affected by KPS <80–90% but not by chronological age (Heidenreich et al. 2017; McClune et al. 2010; Ringdén et al. 2019). Despite significantly poorer outcomes in older patients, additional trials have also not shown a significant impact of advanced age on major outcomes including NRM (Sorró et al. 2011; Chevallier et al. 2012).

The largest experience reported to date on auto- and allo-HCT outcomes in elderly patients comes from two EBMT studies including a total of 21,390 auto-HCT and 6046 allo-HCT in patients aged ≥65 years between 2000 and 2014 (Basak et al. 2016; Sánchez-Ortega et al. 2016). Patient numbers and key HCT outcomes overall and by age group are presented in Table 68.1.

These studies confirm the feasibility of auto- and allo-HCT in elderly patients, with acceptable NRM and OS at 1 and 3 years, respectively. Multivariate analyses in both studies showed that performance status (i.e., Karnofsky score) had a more significant independent impact on patient outcomes than chronological age. Thus, these data in a large cohort of elderly patients strongly suggest that age per se should not be an exclusion criterion to consider HCT in this population. Undoubtedly, this is presumably a highly selected

**Table 68.1** HCT outcomes in elderly patients: EBMT experience<sup>a</sup>

Type of HCT	All cases ≥65 years	Group I 65–69 years	Group II ≥70 years
Autologous, <i>n</i>	21,390	17,531	3859
– NRM year 1	4.9%	4.6%	5.9%
– OS year 1	87%	88%	83%
– OS year 3	67%	69%	61%
Allogeneic, <i>n</i>	6046	4914	1132
– NRM year 1	27%	26%	29%
– OS year 1	57%	57%	53%
– OS year 3	39%	40%	35%

*n*, number of cases.

<sup>a</sup>Basak et al. (2016) and Sánchez-Ortega et al. (2016)



fraction of elderly patients considered for auto- and allo-HCT. Nevertheless, this further endorses the need to assess comorbidity and frailty beyond the age in older HCT candidates to improve outcomes further.

## 68.4 Assessment of Elderly Candidates for HCT

In addition to the elements already discussed in Chap. 11 for younger patients, the evaluation and counseling of elderly patients as candidates for auto- and allo-HCT require the evaluation of additional health domains of interest in patients of advanced age. The following tables address general principles and considerations for the evaluation and counseling of these patients, discuss the issue of patient frailty beyond age and comorbidities, and describe the key elements of a multidimensional geriatric assessment in this population.

### 68.4.1 General Principles and Considerations for Elderly HCT Candidates

- HCT decision *should not be driven by chronological age* but by a broader multidimensional assessment, including fitness, comorbidities, physiologic reserve, and frailty.
- Elderly patients require *information and counseling in plain language* regarding the HCT process, donor sources, specific protocol, timeline, risks, benefits, and outcomes.
- They also need information regarding patients' *quality of life outcomes, caregivers, and psychosocial needs*, for which social workers and other support staff will be needed.
- A *multidisciplinary individualized assessment* is required to appropriately address the multidimensional nature of the evaluation of elderly patients.
- Fit older transplant candidates should follow the *same indications for auto- and allo-HCT as younger adults*.
- In the case of allo-HCT, particular consideration of *RIC and NMA regimens* is essential, and donor selection must take into account the *age of the donor*, as donor older age may associate with impaired outcomes.
- Outcome analysis in elderly patients may require the use of *clinically relevant composite endpoints* that, beyond survival, incorporate quality of life, good overall mental and physical condition, and freedom from severe complications.

### 68.4.2 Frailty in Elderly HCT Candidates

- Frailty is a term used to describe a multidimensional syndrome of loss of physiologic reserves (energy, physical ability, cognition, and health) that leads to vulnerability.
- The ability to measure frailty in elderly patients is useful clinically.
- Although it appears to be a valid construct to assess elderly patients, how exactly to define it remains unclear. There is a large abundance of possible scales to measure frailty, which likely reflects uncertainty about the term and its components.
- A. Hedge et al. have recently reported on frailty as the missing piece of pre-HCT assessment (Hegde and Murthy 2018). Data show that the prevalence of frailty prior to HCT in patients aged  $\geq 50$  years is higher than in the general geriatric population at around 25%. Importantly, age has no effect on the prevalence of frailty.
- Frailty is associated with poorer OS even after adjusting for age and HCT-CI and may be associated as well with an increased incidence of disease relapse (Muffy et al. 2014; Hegde and Murthy 2018).

## 68.4.3 Geriatric Assessment for Elderly HCT Candidates

### 68.4.3.1 General Concept

- The geriatric assessment is a multidimensional, multidisciplinary assessment designed to evaluate an older person's functional ability, physical health, cognition, mental health, and socio-environmental circumstances (Artz 2016).
- The goal of geriatric assessment in this context would be to capture vulnerability pre-HCT to help deciding on patient suitability for the procedure as well as to individualize post-HCT support strategies to prevent complications and reduce transplant-associated morbidity and mortality (Artz 2016; Jayani et al. 2020).

### 68.4.3.2 Elements Involved in Elderly HCT Candidates

- Ensure appropriate performance status (Karnofsky score  $\geq 80$ ).
- Rule out significant comorbidities by the HCT-CI (Sorrer et al. 2005), as their prevalence increases with age.
- Assess the modified EBMT (Armand et al. 2014) and the revised PAM scores (Au et al. 2015), as global prognostic models that incorporate both NRM and disease factors.
- Measure functional status by self-reported function and performance-based testing (ability to perform tasks necessary to live independently in the community [i.e., shopping, food preparation, housekeeping, telephone, laundry, transportation and driving, manage finances and medication, number of times a patient can rise from the chair (i.e., timed up and go), gait speed, 6-min walk test, hand grip strength, or provocative cardiopulmonary testing], polypharmacy requirements).
- Cognitive function: if necessary, perform neuropsychological testing and/or consult geriatrics (Oli et al. 2020).
- Psychosocial evaluation (assessment of social support, availability of a caregiver, financial matters, psychological disturbances, etc.).
- Nutritional status and weight loss.

- Biomarkers to characterize physiologic age (serum C-reactive protein, ferritin, serum albumin, or protein biomarkers panels in development).

### 68.4.3.3 Scales

- No standard geriatric assessment scales have been validated for HCT.
- Most scales available for geriatric assessment in cancer patients are complex and time-consuming, which limits its use in daily practice.
- The Geriatric Assessment in Hematology (GAH) scale is a brief, comprehensive geriatric assessment scale designed and validated for older patients diagnosed with hematological malignancies (MDS, AML, MM, and CLL) (Bonanad et al. 2015).
- The GAH scale has been shown to be responsive to clinical changes in patient's health status (Cruz-Jentoft et al. 2017) and may also help discriminate patients who will benefit most from intensive treatments from those requiring an adapted approach (de la Rubia et al. 2023).
- The GAH scale includes 30 items grouped into 8 predefined dimensions (number of drugs, gait speed, mood, activities of daily living, subjective health status, nutrition, mental status, and comorbidities) and requires a relatively short period of time to be administered in routine clinical practice (10–12 min).
- Thus, the GAH scale could be an interesting tool to assess elderly patients with hematological malignancies who are being considered for transplantation. However, it still needs to be validated in the setting of HCT.

#### Key Points

- HCT activity in elderly patients has increased markedly in the past two decades and is predicted to continue to increase as the population ages, with a sustained improvement in HCT methodology and care.

- Auto- and allo-HCT in elderly patients is feasible and has acceptable outcomes.
- Age should not be an exclusion criterion per se to consider elderly patients for HCT.
- Assessing comorbidity is essential in older HCT candidates, but adjusting only for comorbidity may not identify frail patients vulnerable to adverse outcomes.
- Frailty is a multidimensional syndrome of loss of physiologic reserves (energy, physical ability, cognition, and health) that leads to vulnerability, is higher in HCT recipients than the general geriatric population, and associates with poorer HCT outcome.
- Geriatric assessment is a multidimensional, multidisciplinary assessment designed to evaluate an older person's functional ability, physical health, cognition, mental health, and socio-environmental circumstances.
- The goal of geriatric assessment in HCT would be to capture vulnerability pre-HCT to help deciding on patient suitability for the procedure and to adapt post-HCT support strategies to improve outcomes.
- Currently, there are no standard geriatric assessment scales validated for HCT. The GAH scale has been described and validated in elderly patients with hematological malignancies, is relatively simple to apply in clinical practice, and may be a candidate scale for elderly HCT candidates, validation pending.

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# Feasibility and Experiences of HCT in Resource-Constrained Settings

# 69

Alok Srivastava, Fernando Barroso Duarte,  
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## 69.1 Introduction

Even though hematopoietic cell transplant (HCT) has been in vogue for more than six decades as a curative therapy for many hematological disorders, with increasing trends in all parts of the world (Fig. 69.1), the density of HCT services is low and access to existing services remain an unmet need in the resource-constrained settings (RCSs) of low- and middle-income countries

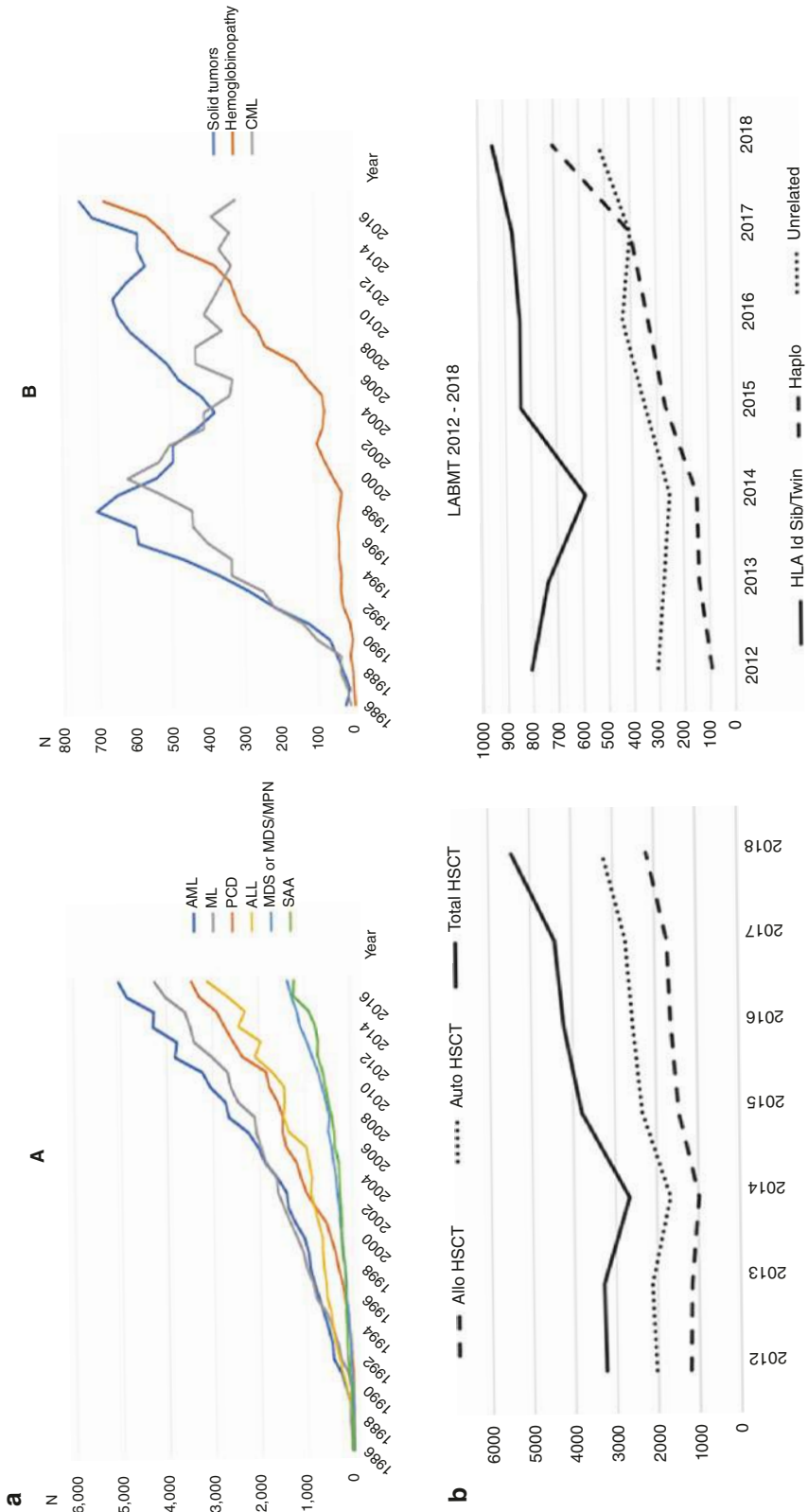
(LMICs) (Niederwieser et al. 2022). The silver lining though is that within these limitations, there are several examples of high-quality HCT services functioning in several countries, some with full national/international accreditation showing that even in those circumstances this is possible (Damodar et al. 2021). The goal of this chapter will be to discuss how good HCT services may be established and sustained in RCSs (Pasquini et al. 2019; Faulkner et al. 2021).

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**Fig. 69.** Trends in hematopoietic cell transplantation: **a.** APBMT; **b.** LABMT



**Table 69.1** Stages of development of a HCT program

	Stage I	Stage II	Stage III
Types of transplantation performed	•Autologous	Stage I +	Stage II +
	•HLA-matched sib donors	•All MSD transplants including MMSD	•Haploidentical
		•Autologous with cryopreserved products	•And/or MUD. MMUD
			•And/or UCB
			•And/or T-cell depleted
Number of HCT	5 Auto-HCT/year	10 Auto-HCT/year	>10 Auto-HCT/year
	3–5 Allo-HCT/year	5–10 Allo-HCT/year	>10 Allo-HCT/year

*MMSD* indicates mismatched sibling donor, *MSD* matched sibling donor, *MMUD* mismatched unrelated donor, *MUD* matched unrelated donor, *UCB* umbilical cord blood, *auto-HCT* autologous hematopoietic cell transplant, *allo-HCT* allogeneic hematopoietic cell transplant

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## 69.2 Establishing HCT Centers: Personnel/Infrastructure/Types of HCTs

All HCT centers even within RCS will ultimately need to offer the full range of HCT services for pediatric and adult patients depending on the indications for the patients being served. However, the most critical issue in determining the focus and direction of any new HCT service is the training and experience of the lead transplant physician and the access to various support infrastructure, as exemplified in a recent report from Bangladesh and Nepal (Mahfuz et al. 2021; Poudyal et al. 2022). While it would be desirable for HCT services to be offered in government-funded public hospitals, this has not been a successful model in many RCS where paradoxically HCT rates are higher when services are offered in private hospitals as reported from Latin America (Jaimovich et al. 2021). Autologous HCT in the context of malignancies is often the starting point particularly if cryopreservation facilities are available with the exception of multiple myeloma where the very short conditioning does not require this. However, starting with allogeneic HCT particularly for nonmalignant conditions, such as bone marrow failure syndromes, major hemoglobin, and immune deficiency disorders, is also not uncommon with very good results expected in children particularly with matched related donors (MRDs) but increasingly also with

alternate donors. There is no clear recommendation on this aspect of starting an HCT service except that the lead physician and the team should be adequately trained for the services they wish to start. A suggested approach is shown in Table 69.1. It is of course very common for such physicians to seek real-time “consultative” help from experienced physicians at other more established centers.

## 69.3 Patient Selection and Indications for HCT

Selecting patients for HCT can pose challenges for centers starting their programs in RCS. On the one side is the need of the individual patient, adult or pediatric, who presents with a disease that could be cured by HCT, and on the other side is the capability and capacity of the HCT center that may wish to prioritize certain indications. The choice finally depends on the transplant physician and the team at that center, their experience and training as well as institutional priorities. It would be advisable to start with standard risk younger patients with matched related donors—the actual indication will depend on the region of the world and practice pattern at that center. Given the paucity of HCT services in RCS, it should be recognized that many centers offer services to both adult and pediatric patients (Chandy 2008; Benakli et al. 2020). Physicians trained at

such HCT centers may well be capable of managing HCTs in a wide range of patients and indications. The presence of trained pediatricians in the transplant team is of course necessary whenever possible. It is also important that transplant physicians be aware of the evolving indications for HCT and the possible outcomes with different donors. The expected outcomes in each of these situations are critical for counseling patients and their families as well as planning resources for the HCT (Snowden et al. 2022).

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### 69.4 Donor Selection: Alternative Donors—Graft Manipulations

Access to locally available reliable HLA typing services has been a challenge in preventing allogeneic transplants in many parts of the world. With a molecular approach to HLA typing, more services are accessible. This then allows for appropriate donor selection. Once this is available, matched related donors (MRDs) become the first option to start a service with. High-resolution typing might yet be desirable in regions with high consanguinity or close ethnicity (Agarwal et al. 2017), and it also allows the initiation of unrelated donor searches. As experience and resources advance and the logistics of international transportation of grafts improve, matched unrelated donors (MUDs) are also being increasingly used. However, with increasing experience around the world with haplo-identical donor (HID) HCTs, these types of donor transplants can be offered early within HCT services, depending on the training and experience of the physician involved as well as the team along with access to diagnostic and consultation services (Niederwieser et al. 2022). An important aspect of any allogeneic HCT service is to be able to carry out basic graft manipulation at least for red cell depletion when needed for blood group mismatched donors. Familiarity with manual centrifugation or precipitation-based techniques is needed if automated instrument platforms are not available for the same (Sawa et al. 2023).

### 69.5 Harvest of Graft: CD34+ HSC Dose Calculation and Cryopreservation Issues

With the increasing use of peripheral blood stem cells (PBSCs) for allogeneic grafts, collection of such grafts on one of the several apheresis platforms is quite easily established in most HCT centers. However, the use of PBSC grafts may be associated with an increased risk of graft versus host disease (GVHD), particularly chronic, and it is important that transplant physicians should be familiar with bone marrow harvesting. As this requires general anesthesia and access to an operating room facility, those aspects also need to be considered. The assessment of the quality of the graft even in terms of the CD34+ cell count can be a limitation in many centers. Arrangements to outsource this evaluation are often possible. Another limitation encountered is related to both capability and capacity to cryopreserve grafts mainly for autologous HCTs. Though reports of grafts being used after storage at 4–8°C for up to 6 days exist, (Bekadja et al. 2021) having appropriate control rate freezing equipment is desirable for optimal graft storage and transplantation outcomes.

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### 69.6 Conditioning Regimen

Conditioning is a critical aspect of any HCT protocol, and the possibilities are many between myeloablative and reduced intensity options with various myelotoxic and immunosuppressive drugs with or without total body or more limited irradiation fields (Ma et al. 2020). It is very important that the transplant physician should be familiar with these possibilities and understand the principles of using these options. The final choice depends on what drugs and whether facilities for radiation therapy are locally available as well as the personal experience of the physician concerned. The good thing is that nearly for all indications of HCT various chemotherapy-based conditioning is possible with good outcomes (Rehman et al. 2023). Regimen-related toxicities

can be a major cause of morbidity and mortality in HCT, and this should be borne in mind when choosing conditioning protocols.

### 69.7 Supportive Care

While disease-related and individual patient-related variables are a major determinant of outcomes of HCT, what matters most next in determining outcome is the supportive care provided during HCT. There are two important aspects to this—first, the availability of appropriate therapeutic products (Table 69.2) and next the knowledge and training of the entire team to uti-

lize them. Standardization of protocols for the management of different aspects of HCT goes a long way toward improving outcomes (Snowden et al. 2020). No amount of effort is too much in enhancing supportive care at the nursing, consultation services, and relevant diagnostic investigations in any HCT program.

- *Blood product support:* Access to the full range of blood bank products such as packed red cells, platelet concentrates, and fresh frozen plasma is absolutely essential to run a transplant program (Warner et al. 2019). Blood product irradiation is required for immunosuppressed patients to avoid

**Table 69.2** Medicines considered essential for HCT programs

	Required (score 1 and 2) with votes above 70%	Preferred (score 3 and 4) with votes above 70%	Ideal (score >4) with votes above 70%
Stage I program	Mobilization: –Chemotherapy and filgrastim for autologous PBSCT mobilization –Filgrastim for autologous PBSCT mobilization –Filgrastim for allogeneic PBSCT mobilization Conditioning: –Cyclophosphamide –Cytarabine –Melphalan –Fludarabine Seizures prophylaxis: –Benzodiazepine GVHD Prophylaxis/Treatment: –Cyclosporine –Methotrexate –Methylprednisolone –Prednisone –Dexamethasone Antimicrobials: –Piperacillin/Tazobactam –Cefepime –Ciprofloxacin –Vancomycin –Fluconazole –Acyclovir –Ganciclovir –Bactrim Supportive: –IV narcotics –5-HT3 antagonists	Mobilization: none Conditioning: –Oral busulfan –Intravenous busulfan –Carboplatin –Carmustine –Etoposide Seizures prophylaxis: –Phenytoin –Levetiracetam GVHD Prophylaxis/Treatment: –Tacrolimus –Mycophenolate –Rituximab Antimicrobials: –Carbapenem –Levofloxacin –Tigecycline –Amphotericin B (liposomal) –Echinocandin –Voriconazole –Posaconazole –Valganciclovir –Foscarnet –Valacyclovir –Pentamidine Supportive: –Phenothiazines –Neurokinin antagonists –Ursodiol –IVIG –TPN –PCA	Mobilization: –Plerixafor for autologous PBSCT mobilization Conditioning: –Intravenous busulfan with TDM Seizures prophylaxis: none GVHD Prophylaxis/Treatment: –Antithymocyte globulin rabbit –Budesonide –Sirolimus –Etanercept –Alemtuzumab –Basiliximab –Extracorporeal photopheresis –Infliximab Antimicrobials: –Amphotericin B (conventional) –Isavuconazonium –Cidofovir –Letermovir Supportive: –Defibrotide –Olanzapine

TDM indicates therapeutic drug monitoring

Adapted from Transplantation and Cellular Therapy. 2021 Mar;27(3):267 e1–5

transfusion-associated GVHD. When dedicated blood irradiators are not available, a clinical Co60 teletherapy unit can also be used to irradiate both red cell and platelet products (Goes et al. 2006). Where there is a lack of access to large blood banks that can supply products ad lib, having an apheresis unit within the transfusion service in the institution and using screened relatives of patients and other volunteers is a practical way to manage this critical requirement particularly for platelet transfusions.

- *Immunosuppressants*: Access to relevant immunosuppressant drugs is absolutely essential for any HCT program (Penack et al. 2020). Commonly used drugs such as calcineurin inhibitors (cyclosporin A and tacrolimus), mycophenolate mofetil, and sirolimus along with methylprednisolone form the basis for the management of immune complications of HCT most of the time. Posttransplant cyclophosphamide is a good option at low costs which is not only useful in HID transplant but may also be good for MRD transplants (Sawa et al. 2023). A wide range of other drugs including biologicals may also be needed in a small proportion of patients. Measurement of drug levels is important in ensuring efficacy (Table 69.1).
- *Antibiotics*: Given the extent of antimicrobial resistance, particularly in RCS, availability of and access to a wide range of broad-spectrum antibiotics is absolutely essential for initiating HCT services. Therapeutic drug monitoring is desirable but not critical in most situations. However, knowledge of local patterns of antimicrobial resistance is very important for making antibiotic protocols (Table 69.1).
- *Diagnostics*: Intense laboratory monitoring is the pillar of supportive care in HCT. Access to reliable round-the-clock laboratory services for blood counts, hemostasis tests, and common biochemical assessments are indispensable for establishing a successful HCT program (Aljurf et al. 2019). A range of microbiological assessments including bacterial and fungal cultures and antibiotic sensitiv-

ity assays are also essential. Viral infection monitoring, particularly for CMV in the context of GVHD, is needed. Specialized radiological evaluation is also frequently needed through experienced radiologists for ultrasound, CT scan, and MRI-based assessments.

- *Consultation services*: In any ongoing HCT program, multisystem complications resulting from regimen-related toxicities, infections, or graft versus host disease are not uncommon. These require suitable consultation services to be available on call. Most commonly used services are gastroenterology for endoscopies, nephrology for renal insufficiency, infectious diseases, and dermatology.

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## 69.8 Graft Versus Host Disease (GVHD) Management

- *Acute*: This is one area where compromises are best avoided and standard protocols are followed depending on the type of donor and graft. Fortunately, most of the commonly used prophylactic drugs are widely available and not too expensive. Access to second-line drugs for steroid-resistant GVHD can be a challenge in some RCS and specific plans, and protocols should be in place to address these situations (Penack et al. 2020). The success of haplo-identical HCTs with posttransplant cyclophosphamide has made this an increasingly used option. This may also be useful in other donor HCTs and could make GVHD prophylaxis more cost-effective (Rimando et al. 2023).
- *Chronic*: Extensive chronic GVHD can be one of the most difficult complications to manage in RCS because of the need for prolonged expensive immunosuppressive therapy and other associated complications (Saleem et al. 2019). In the absence of adequate resources to support such care, this is a major cause of major morbidity and mortality. There are no specific low-cost protocols, and general principles of the management of chronic GVHD also need to apply in RCS.

## 69.9 Long-term Follow-Up

All HCT centers must aspire to have full long-term follow-up on all patients. An important aspect of achieving this goal is to provide adequate information and counseling to the patient and their families regarding the importance and significance of such follow-up visits (Hashmi et al. 2018). Streamlining the assessments, a caring attitude of the team toward the individual patient and offering relevant advice during such visits enhances compliance. However, given the paucity of HCT centers in RCS, patients often travel very long distances to have the HCT done and going back frequently for follow-up can be challenging. A hub and spoke model should be considered in those situations by establishing partnership with patients and local physicians for a shared care plan.

## 69.10 Socioeconomic Issues

The major challenge that restricts access to HCT in RCS is the cost of HCT (Aljurf et al. 2019). What contributes heavily to costs are the drugs and disposables. These costs are variable depending on where they are manufactured. It is important therefore that a facilitatory regulatory environment be created for such life-saving drugs and disposables to be made available and locally relevant prices. Human resource costs are often modest and usually in a structure that the healthcare system has learnt to accommodate. The low density of HCT services in most RCS around the world are related to two major reasons—cost and lack of adequately trained personnel who know how to innovate and establish such services within the local context. Both these need to be addressed (Aljurf et al. 2019).

## 69.11 Conclusion

Creating greater awareness among healthcare professionals, comprehensive training programs and establishing appropriate infrastructure along

with access to the required drugs and disposable combined with modifications and innovations in management protocols which are practical in those conditions are all needed to make HCT more widely available and accessible all over the world.

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## **Part IX**

### **Indications and Results**

**Topic Leaders: Selim Corbacioglu, Nicolaus Kröger, Anna Sureda, Raffaella Greco and Arnon Nagler**



## AML in Adults

# 70

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### 70.1 AML in CR1

Jurjen Versluis and Jan J. Cornelissen

#### 70.1.1 Definition, Subtypes

AML is a malignancy of hematopoietic immature precursors (myeloblasts) that accumulate in the BM at the expense of their normal counterparts. AML is increasingly being recognized as a heterogeneous malignancy based on distinct disease biology and underlying cytogenetic and molecular profiles. Although a blast count of  $\geq 20\%$  in the BM or PB is still considered to diagnose AML, the 2022 World Health Organization

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(WHO) classification and International Consensus Classification (ICC) considered a lower threshold to allow for disease-defining genetic abnormalities and to acknowledge the biologic continuum between MDS and AML (Khoury et al. 2022; Arber et al. 2022). A less strict definition of blast cutoff in the updated WHO classification and ICC emphasizes a comprehensive assessment of morphology, cytogenetic, and molecular genetic analyses to classify MDS and/or AML.

The ELN recommendations have also been updated in 2022, which stratified patients into three risk groups, including favorable risk, intermediate risk, and adverse risk, based on pretreatment cytogenetic abnormalities and gene mutations (Dohner et al. 2022). The ELN 2022 further recognizes the underlying disease biology in its risk classification by adding a secondary type, MDS-associated mutations to the adverse-risk category (Dohner et al. 2022).

#### 70.1.2 Clinical Presentation

The median age at diagnosis is approximately 70 years, and the annual age-standardized incidence rate varies between 3 and 4 cases per 100,000. Patients with AML typically present with symptoms such as fatigue, bruising, or infections, whereas lymphadenopathy and/or hepatosplenomegaly may be found by physical examination. Analysis of blood work often

reveals thrombocytopenia, anemia, and/or neutropenia. In some patients, a serious bleeding diathesis can occur, particularly in the early phase of treatment, because the leukemic blasts are able to activate the coagulation cascade and cause hyperfibrinolysis.

### 70.1.3 First-Line Treatment

Achieving and maintaining a first CR is crucial in AML patients, but treatment may largely fail because of relapse from CR rather than primary induction failure or treatment-related mortality (TRM). The standard intensive AML induction treatment has consisted of 7–10 days of the anti-metabolite cytosine arabinoside (Ara-C) and 3 days of an anthracycline (i.e., daunorubicin or idarubicin) since the 1980s. CR rates with standard induction estimate between 70 and 90%. Favorable-risk patients have relatively good outcomes with overall survival rates of approximately 60–70%, whereas outcomes for patients with intermediate-risk and particularly adverse-risk AML remain unsatisfactory.

Targeted therapies have been introduced in AML, of which the kinase inhibitor midostaurin added to intensive induction and consolidation chemotherapy has been approved for first-line treatment of AML patients with mutated *FLT3* based on the survival benefit observed in the RATIFY trial (Stone et al. 2017). Other promising targeted therapies against *FLT3* (e.g., gilteritinib and quizartinib) or *IDH1/2* mutations (e.g., ivosidenib and enasidenib) are currently being investigated in first-line intensive induction treatment of which results are awaited. Alternative induction strategies might consist of gemtuzumab-ozogamicin, a humanized anti-CD33 antibody-drug conjugate or CPX-351, a liposomal formulation of cytarabine and daunorubicin, for specific subgroups of patients. CPX-351 has been shown to improve overall outcome compared with standard intensive induction chemotherapy in patients aged 60–75 years with therapy-related or secondary-type AML, including MDS-related cytogenetic abnormalities (Lancet et al. 2018).

Treatment options have increased considerably for AML patients deemed not eligible for intensive treatment to induce the first CR. Hypomethylating agents, including azacitidine and decitabine, may be a treatment option in mono or combination therapy. Venetoclax, a BCL2 inhibitor, combined with azacitidine increased response rates, which were rapid and durable, and improved survival and quality of life compared with azacitidine alone (DiNardo et al. 2020). As a result, azacitidine and venetoclax treatment has been established as the new standard of care for first-line treatment of older or unfit AML patients. Alternatively, for patients with mutated *IDH1*, ivosidenib combined with azacitidine was associated with higher response rates and superior survival compared with azacitidine alone in a randomized phase III trial (Montesinos et al. 2022). These targeted therapies have different toxicity profiles compared with conventional chemotherapy and need specific monitoring of drug–drug interactions and adverse events such as cardiac QTc assessment, differentiation syndrome, and prolonged cytopenias.

Once a remission is being obtained, post-remission treatment decisions are directed by the ability of pretreatment features such as those incorporated in the ELN risk classification to predict outcome. However, probably more prognostically important than the pretreatment features is response to treatment and especially the presence, in hematological remission, of measurable residual disease (MRD) as assessed by flow cytometry or targeted quantitative PCR for specific markers.

## 70.1.4 HCT and AML Risk Categories

### 70.1.4.1 AML Risk Categories

Previously, conventional cytogenetics and mutations in *NPM1*, *FLT3*-ITD, *CEBPA*, *RUNX1*, *ASXL1*, and *TP53* genes were included in the ELN 2017 risk classification of AML patients (Dohner et al. 2017). The current ELN 2022 risk classification has added mutations in *BCOR*,

*EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2*, which are being recognized as MDS-related mutations with an adverse outcome (Table 70.1) (Dohner et al. 2022). Other important changes include (1) the *FLT3*-ITD allelic ratio not being considered in the classification; (2) only in-frame mutations in the b-zip region of *CEBPA* are now considered as favorable risk, irrespective of being mono- or bi-allelic; (3) *NPM1* mutations are not classified as favorable if adverse-risk cytogenetics are present; (4) recurring cytogenetic abnormalities including t(3q26;v) or t(8;16)(p11.2;13.3) are being included in the adverse-risk group; (5) hyperdiploid karyotypes are not considered complex; and

(6) only *TP53* mutations at a variant allele fraction of  $\geq 10\%$  are considered adverse risk.

Similar to the previous risk classification, the ELN 2022 AML risk classification is advocated to be used for risk-stratifying AML and to a risk-adapted treatment approach for patients with AML. Such a risk-adapted treatment approach for patients with AML depends not only on the risk of relapse of the underlying AML but also on the risk of TRM associated with the applied post-remission treatment. The application of MRD, detected by either multiparametric flow cytometry or quantitative PCR for specific molecular markers may further improve AML risk classifications. MRD may be detected at time points

**Table 70.1** Risk-adapted post-remission treatment for patients with AML in the first CR<sup>a</sup>

AML risk classification <sup>b</sup>	MRD status <sup>c</sup>	Preferred post-remission treatment
<i>Favorable</i>		
t(8;21)(q22;q22.1); <i>RUNX1-RUNX1T1</i>	Negative	Chemotherapy/auto-HCT
Inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>		
Mutated <i>NPM1</i> without <i>FLT3</i> -ITD (without adverse-risk cytogenetic abnormalities)	Positive	Allo-HCT <sup>b</sup> (unless excessive TRM can be predicted)
bZIP in-frame mutated <i>CEBPA</i>		
<i>Intermediate</i>		
Mutated <i>NPM1</i> with <i>FLT3</i> -ITD (without adverse-risk cytogenetic abnormalities)	Negative	Consider allo-HCT <sup>b</sup> (if acceptable risk of TRM; alternative, chemo/auto-HCT)
Wild-type <i>NPM1</i> without <i>FLT3</i> -ITD (without adverse-risk genetic lesions)		
t(9;11)(p21.3;q23.3); <i>MLL3-KMT2A</i>	Positive	Allo-HCT <sup>c</sup>
Cytogenetic and/or molecular abnormalities not classified as favorable or adverse		
<i>Adverse</i>		
t(6;9)(p23;q34.1); <i>DEK-NUP214</i>	Negative	Allo-HCT <sup>c</sup>
t(v;11q23.3); <i>KMT2A</i> rearranged		
t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i>	Positive	Allo-HCT <sup>c</sup>
t(8;16)(p11.2;p13.3); <i>KAT6A-CREBBP</i>		
Inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); <i>GATA2</i> , <i>MECOM(EV11)</i>		
t(3q26.2;v)/ <i>MECOM(EV11)</i> -rearranged		
-5 or del(5q); -7; -17/abn(17p)		
Complex karyotype (excluding hyperdiploid), monosomal karyotype		
Mutated <i>ASXL1</i> , <i>BCOR</i> , <i>EZH2</i> , <i>RUNX1</i> , <i>SF3B1</i> , <i>SRSF2</i> , <i>STAG2</i> , <i>U2AF1</i> , and/or <i>ZRSR2</i>		
Mutated <i>TP53</i> with VAF $\geq 10\%$		

<sup>a</sup>Adapted from (Cornelissen et al. 2012b), Table 4

<sup>b</sup>Allo-HCT using HLA-identical sibling or 10/10 MUD donors

<sup>c</sup>Adapted from (Dohner et al. 2022), Table 6

<sup>d</sup>Detected with multiparametric flow cytometry or with for qPCR specific markers

<sup>e</sup>Allo-HCT using HLA-identical sibling, MUD, MMUD umbilical cord blood, or haploidentical donors

early after induction treatment to assess the remission status of the AML but also after post-remission treatment to detect imminent relapse. Consequently, MRD negativity has been introduced as an end point in patients with a hematological CR (Dohner et al. 2017).

#### **70.1.4.2 Transplantation Risk Categories**

The risk-adapted approach of patients with AML in the first CR should also include the assessment of TRM for each individual patient. TRM may be attributed to GVHD, infectious complications, organ toxicity, and other causes (Penack et al. 2020). A number of parameters may relate to allo-HCT-related mortality, including the procedure (e.g., conditioning regimen and application of TCD), donor characteristics (e.g., HLA-matching), and recipient features (e.g., age and comorbidity). The risk of mortality may be quantified by composite risk scores, which have been established to predict TRM and overall outcome.

Two generally approved transplant risks were developed and validated, including the EBMT risk score (Gratwohl et al. 1998) and the hematopoietic cell transplantation-comorbidity index (HCT-CI) (Sorrer et al. 2005). The EBMT risk score is based on patient and transplantation characteristics, which was developed in CML patients and subsequently validated in other patient groups including AML (Gratwohl et al. 2009). The HCT-CI consists of 17 comorbidities that contribute to a cumulative score, which has been extensively validated and continuously being refined, including age, disease status, or biomarkers (Sorrer et al. 2007, 2014). A machine-based learning model was developed by the EBMT-acute leukemia working party (ALWP) based on 10 variables, which was highly predictive of mortality at 100 days and 2 years (Shouval et al. 2015). Other groups have also developed predictive models for TRM modifying the weights of the EBMT risk score and the HCT-CI (Barba et al. 2010), whereas others combined transplant-related parameters and patient characteristics or used biomarkers (Parimon et al. 2006; Barba et al. 2014; Luft et al. 2017).

Developments in allo-HCT care have improved outcomes over the last decades, attributable to for example the introduction of reduced intensity conditioning, improved supportive care and infectious prophylaxis, and better GVHD prophylaxis or GVHD treatment. Consequently, TRM has been strongly reduced and the use of allo-HCT as post-remission treatment for older or less fit patients with comorbidities has increased (Snowden et al. 2022). Several groups have reported less predictive power of the established TRM risk models in the current era of allo-HCT. The EBMT-ALWP has developed updated scoring systems applicable to the setting of RIC in older patients with AML and PTCY as GVHD prophylaxis which both had increased predictive power (Versluis et al. 2015; Hermans et al. 2023). Similar to updating AML risk classifications, the prediction of TRM also needs continued refinement and reassessment in specific patient groups and novel treatments.

#### **70.1.5 HCT in First-Line AML Treatment: A Risk-Adapted Approach**

AML risk classifications are being used for tailoring patients' optimal post-remission treatment, which include allo-HCT, high-dose chemotherapy followed by auto-HCT, and continued chemotherapy. Allo-HCT is the most optimal post-remission treatment for the prevention of relapse due to a potent GVL effect, which has been demonstrated to be exerted irrespective of underlying AML cytogenetic subcategories and MRD status (Cornelissen et al. 2012a; Versluis et al. 2017a). However, absolute estimates of relapse incidence differ and reflect molecular or cytogenetic differences resulting in resistance of the AML. Although the GVL effect of allo-HCT is unequivocally present in patients with AML in the first CR, concurrent TRM compromises overall outcome, especially in AML patients with a relatively low incidence of relapse. Thus, a risk-adapted approach of post-remission treatment for patients with AML in the first CR should include an assessment of the TRM risk in addition to leu-

kemia characteristics and MRD (Cornelissen et al. 2012b; Cornelissen and Blaise 2016). Table 70.1 summarizes a risk-adapted approach based on the ELN 2022 AML risk classification, MRD status, and the risk for TRM. The risk for TRM should be preferably assessed with dedicated scores for specific subgroups of patients.

Allo-HCT is generally not indicated in patients with a favorable AML risk profile; for those patients auto-HCT or continued chemotherapy may be preferred (Cornelissen et al. 2012b; Cornelissen and Blaise 2016; Dohner et al. 2022). However, favorable-risk patients with MRD are considered high risk for relapse and preferably receive an allo-HCT in the first CR, unless excessive TRM is predicted even with RIC.

Results of allo-HCT compared with non-allo-HCT post-remission therapies have yielded contradicting results in intermediate-risk patients, especially taking molecular markers into account (Koreth et al. 2009; Stelljes et al. 2014; Versluis et al. 2017b). A risk-adapted AML trial which allocated patients with MRD negative, intermediate-risk AML to auto-HCT, whereas intermediate-risk patients with MRD positivity received allo-HCT, showed similar outcomes for auto-HCT and allo-HCT (Venditti et al. 2019). Consequently, assessment of MRD status is strongly advocated for post-remission treatment decisions in patients with an intermediate-risk AML. Allo-HCT may be applied in patients with intermediate-risk AML with MRD after induction chemotherapy, except for patients with a high risk for TRM. Allo-HCT might also be considered for patients with intermediate risk, MRD negative AML, but auto-HCT and chemotherapy are alternative treatment options, particularly when the predicted risk for TRM is high.

Adverse-risk patients with MRD should be transplanted with an allograft as soon as a hematological CR is obtained. Adverse-risk patients without MRD still have a significant risk of relapse and are also candidates for allo-HCT, although patients with a very high risk for TRM may be considered for non-allo-HCT approaches.

## 70.2 Allo-HCT in Advanced AML

Charles Craddock

### 70.2.1 Introduction

Allo-HCT plays an increasingly important role in the management of AML in adults (Dohner et al. 2022). The advent of RIC regimens coupled with increased donor availability has dramatically increased the number of patients in whom allo-HCT can be contemplated. At the same molecular characterization at diagnosis coupled with MRD, quantitation after induction chemotherapy has considerably improved our ability to predict relapse risk in patients treated with intensive chemotherapy alone refining our ability to identify patients with the potential to benefit from allogeneic transplantation (Loke et al. 2020). Consequently, allo-HCT is now a pivotally important personalized component of the treatment algorithm in adults with AML in CR1. At the same time, there is an emerging recognition of the curative potential of allografting in patients with advanced-phase AML, particularly patients with primary refractory (PREF) AML (Dohner et al. 2022). It is therefore extremely important that all fit adults with newly diagnosed AML are tissue typed at presentation and, unless there are compelling reasons to believe allo-HCT will not be included in the patients' treatment algorithm, a donor search is commenced in a timely manner. Encouraging results have been achieved using both matched sibling and volunteer unrelated donors and more recently transplantation using haploidentical donors (in conjunction with post-transplant cyclophosphamide GVHD prophylaxis) and cord blood units with a high nucleated cell dose. To date, there is no compelling data concerning whether there is an optimal stem cell source and prospective studies are required to address this important point. In both patients transplanted in CR1 and those with advanced phase disease, there has been a substantial reduction in transplant-related mortality (TRM) over the decades, but the risk of disease relapse post-



transplant remains stubbornly high and now represents the major cause of treatment failure in patients allografted for AML. There remains, therefore, an urgent requirement to develop novel strategies with the potential to reduce the risk of disease recurrence in all patients allografted for AML.

### 70.2.2 The Role of Allo-HCT in the Management of Primary Refractory AML

Ten to forty percent of adults with newly diagnosed AML have PREF AML defined as a failure to achieve a morphological CR after two courses of intensive induction chemotherapy, including at least one cycle of intermediate dose cytarabine (Ferguson et al. 2016). Factors determining refractoriness to induction chemotherapy include adverse disease biology and patient age. Patients with PREF AML have genuinely chemo-refractory disease with long-term survival rates <10% if treated with further chemotherapy, and allo-HCT represents the only treatment modality with the potential to deliver long-term disease-free survival. Evidence that allo-HCT can deliver long-term survival in a significant proportion of patients with PREF AML has been accumulating over the last decade and represents an important advance in the management of this sizeable patient population for whom no other effective therapy exists (Craddock et al. 2011; Todisco et al. 2017; Brissot et al. 2017; Boyiadzis et al. 2023). Nonetheless, outcomes in patients allografted for PREF AML remain unsatisfactory, and both TRM and disease relapse continue to represent significant barriers to long-term survival. There is also a lack of clarity concerning which patients with PREF AML are the most likely to benefit from transplantation. Outcome is superior in patients who proceed swiftly to transplant after no more than two courses of intensive chemotherapy, and relapse appears to be lower in those with a lower burden of disease at the time of transplantation. It is therefore important

to consider transplant as a potentially curative treatment modality in fit patients with PREF AML, providing a suitable donor can be identified rapidly and the predicted TRM is acceptable. However, further studies both on the impact of disease biology and evidence of chemo-responsiveness on outcome after allo-HCT are required if we are to be able to identify with greater clarity both which patients with PREF AML are likely to benefit from allo-HCT and equally for whom this further intensification in treatment is unlikely to deliver long-term survival. At present, accumulating data identifies very poor outcomes for patients with high-risk molecular features such as the presence of a *TP53* mutation or *inv(3)(q21.3q26.2)* and on the basis of current knowledge it is probably reasonable to reserve allogeneic transplantation in this patient group for patients who have achieved a morphological CR (Loke et al. 2022; Daver et al. 2022; Badar et al. 2023).

The optimal conditioning regimen in patients with PREF AML remains a matter of conjecture, but it is recommended that a myeloablative regimen is utilized in patients under the age of 50 years unless there are compelling reasons to the contrary. In older adults, there is no evidence that any particular RIC regimen is to be favored. Although encouraging results using a FLAMSA sequential regimen were reported in patients with PREF AML, it is relevant to note that a recent randomized trial failed to demonstrate any benefit of such a regimen compared with the standard RIC regimen FB2 in adults with AML in CR1 or CR2, although insufficient patients with PREF AML were randomized to address whether this regimen has particular activity in this clinical setting (Craddock et al. 2021). As with all patients allografted for high-risk AML, it is important to assess response to transplant by performing MRD studies on a bone marrow aspirate 30–45 days posttransplant, since this may guide the implementation of an early immunosuppression taper or use of prophylactic/pre-emptive donor lymphocyte infusion (DLI) (Loke et al. 2023).

### 70.3 Practical Issues for Allo-HCT in AML

Arnon Nagler

AML is the most frequent indication in Europe for allogeneic HCT. As per the latest publication by Passweg et al., 7123 allo-HCT has been performed in Europe in 2021 for AML (4266 for de novo AML in the first CR, 1775 for de novo AML not in CR, and 1082 for AML-therapy-related or myelodysplasia-related changes) accounting for 38% of the allogeneic transplant activity in Europe (Passweg et al. 2021). In sharp contrast to the lymphatic malignancies, the allogeneic activity in AML has not been decreased as CAR-T cell treatment for AML is still in its infancy (Passweg et al. 2021). As in many of the AML patients with an indication for allo-HCT, transplant is currently the only curative procedure and in those achieving CR after first induction results are better. It is therefore recommended to perform HLA typing of the patient and the potential family donors upfront once the AML is diagnosed and to start a search (Carreras et al. 2019). In recent years, continuous novel developments in the field of allo-HCT and mainly the reduced intensity/reduced toxicity conditioning and haploidentical donor allo-HCT with post-transplant cyclophosphamide (PTCY) have significantly reduced transplant-related toxicities and mortality combined with the improved supportive care (Slavin et al. 1998; Kasamon et al. 2015; Shimoni et al. 2005). Consequently, the median age of the AML patients that are undergoing transplantations is increasing with many of the transplanted patients being over 60 years or even 65 years of age. Many of these patients are receiving the allo-HCT following upfront induction with low-intensity Venetoclax-based protocols which further reduced toxicities (Dohner et al. 2022; Keith et al. 2022). Achieving measurable residual disease (MRD) negativity, pre-allo-HCT correlates with outcomes and becomes a major goal in the treatment paradigm for acute leukemias and other hematological malignancies as well (Keith et al. 2022; Abhishek et al. 2021; Bazinet et al. 2023). Most of the allo-

HCTs that are being performed nowadays are from unrelated including mismatched unrelated and haploidentical donors and with mobilized PBSC (Nagler and Mohty 2022; Arslan and Al Malki 2022; Anasetti et al. 2012; Raghunandan 2022). Patients with a high risk of relapse post-allo-HCT including those with high-risk AML, secondary poor prognostic mutations, or secondary AML should be considered for maintenance or preemptive therapy post-allo-HCT in an attempt to reduce the relapse rates (Eshrak et al. 2023). Finally, PTCY-based regimens are becoming the preferred mode of GVHD prophylaxis including for patients with AML undergoing HLA-matched related, unrelated, and haploidentical allo-HCT, and it may change other aspects of allo-HCT including the intensity of the conditioning as well as the risk of relapse post-transplantation (Bolanos-Meade et al. 2023).

### 70.4 Acute Promyelocytic Leukemia

Miguel Á. Sanz

#### 70.4.1 Concept and Incidence

APL is a subtype of AML with peculiar clinical and morphological characteristics, which presents a specific genetic alteration, the t(15; 17), with its corresponding molecular counterpart, the rearrangement *PML-RARA*, which confer a particular sensitivity to all-*trans* retinoic acid (ATRA) and arsenic trioxide (ATO). It also highlights the presence of a hemorrhagic diathesis associated with a peculiar coagulopathy, which causes a high incidence of hemorrhagic complications at presentation and early during the induction treatment.

APL accounts for 5–15% of AML, with a median age of around 40 years and similar distribution by sex. About 10% occur after the use of cytotoxic drugs (especially topoisomerase II inhibitors) or radiation.

## 70.4.2 Diagnosis

### 70.4.2.1 Morphology, Immunophenotyping, and Other Features

M3 typical (hypergranular) 75–80%	M3 variant (microgranular) 20–25%
<i>Morphology</i>	
<ul style="list-style-type: none"> <li>• Cytoplasm with dense granulation. Frequent Auer rods</li> <li>• Reniform or bilobed nucleus</li> </ul>	<ul style="list-style-type: none"> <li>• Cytoplasm with fine granulation or hypogranular. Less frequent Auer rods</li> <li>• Reniform nucleus, bi- or multilobed</li> </ul>
<i>Immunophenotyping</i>	
HLA-DR <sup>-</sup> /CD34 <sup>-</sup> /CD33 <sup>+</sup> a/CD13 <sup>+</sup> b/CD15 <sup>-/+</sup>	HLA-DR <sup>±</sup> /CD34 <sup>±</sup> /CD33 <sup>+</sup> a/CD9 <sup>+</sup> /CD2 <sup>±</sup> /CD13 <sup>+</sup> b/CD56 <sup>±</sup>
<i>Other associated features</i>	
<ul style="list-style-type: none"> <li>• Most frequently, low WBC counts</li> <li>• Less frequently, BCR3 isoform</li> </ul>	<ul style="list-style-type: none"> <li>• Most frequently, high WBC counts</li> <li>• Most frequently, BCR3 isoform</li> </ul>

<sup>a</sup>Intense and homogeneous expression

<sup>b</sup>Heterogeneous expression

### 70.4.2.2 Genetic Diagnosis

<i>Conventional cytogenetics</i> $t(15;17)(q22;q21)$
<i>Pros</i>
<ul style="list-style-type: none"> <li>– Very specific</li> <li>– Detects additional anomalies in 30% (+8 the most frequent)</li> </ul>
<i>Cons</i>
<ul style="list-style-type: none"> <li>– Low sensitivity (80%)</li> <li>– Inadequate, bad metaphases or normal karyotype (false-negative) in 20%</li> </ul>
<i>FISH PML-RARA</i>
<i>Pros</i>
<ul style="list-style-type: none"> <li>– Very specific and rapid</li> </ul>
<i>Cons</i>
<ul style="list-style-type: none"> <li>– Not very sensitive and does not provide information about the isoform</li> </ul>
<i>RT-PCR</i>
<i>Pros</i>
<ul style="list-style-type: none"> <li>– Very specific, rapid, and sensitive</li> <li>– Identifies the isoform, which allows MRD monitoring</li> </ul>
<i>Cons</i>
<ul style="list-style-type: none"> <li>– Occasional artifacts and contaminations</li> </ul>
<i>Immunostaining with anti-PML antibody (PG-M3)</i>
<i>Pros</i>
<ul style="list-style-type: none"> <li>– Very specific, rapid, and cheap</li> <li>– Characteristic microspeckled pattern by indirect immunofluorescence</li> </ul>
<i>Cons</i>
<ul style="list-style-type: none"> <li>– Does not provide information about the isoform</li> </ul>

### 70.4.2.3 Other Rearrangements of the RARA Gene on Chromosome 17

Chromosomal abnormality	RARA rearrangement
• $t(11;17)(q23;q21)$	• <i>ZBTB16 (PLZF)/RARA</i> (ATRA-resistant)
• $t(17;17)(q21;q21)$	• <i>STAT5b/RARA</i> (ATRA-resistant)
• $t(11;17)(q23;q21)$	• <i>STAT5b/RARA</i> (ATRA-resistant)
• $t(5;17)(q35;q21)$	• <i>KMT2a/RARA</i> (ATRA sensitivity unknown)
• $t(11;17)(q13;q21)$	• <i>NPM1/RARA</i> (ATRA sensitivity unknown)
• $t(17;17)(q21;q24)$	• <i>NUMA1/RARA</i> (ATRA sensitivity unknown)
• $t(X;17)(p11;q21)$	• <i>PRKARIA/RARA</i> (ATRA sensitive)
• $t(4;17)(q12;q21)$	• <i>BCOR/RARA</i> (ATRA sensitive in two cases)
• $t(2;17)(q32;q21)$	• <i>FIP1L1/RARA</i> (ATRA sensitivity unknown)
• $t(3;17)(q26;q21)$	• <i>NABP1 (OBFC2A)/RARA</i> (ATRA sensitive in one case)
• $t(7;17)(q11;q21)$	• <i>TBLR1/RARA</i> (insensitive to ATRA)
• $t(1;17)(q42;q21)$	• <i>GTF2I/RARA</i> (ATRA sensitive)
• $t(3;17)(q26;q21)$	• <i>IRF2BP2/RARA</i> (ATRA sensitive)
• $der(17)$	• <i>FNDC3B/RARA</i> (ATRA sensitive)
• $ins(15;17)(q22;q21;q25)$	• <i>STAT3/RARA</i> (ATRA sensitivity unknown)
• $t(1;17)(q3;q21)$	• <i>PML/ADAMTS17/RARA</i> (ATRA sensitivity unknown)
	• <i>THRAP3/RARA</i> (ATRA-resistant)

### 70.4.3 First-Line Treatment

The ELN recommendations in 2009 acknowledged the promising outcomes reported in several non-randomized studies using ATRA plus ATO, with or without minimal use of chemotherapy, but the standard of care remained the combination of ATRA plus anthracycline-based chemotherapy (Sanz et al. 2009). However, recent findings have led to modify this recommendation in the updated ELN guidelines (Sanz et al. 2019).

The long-term results of a non-randomized study (Abaza et al. 2017) and two randomized clinical trials (Lo-Coco et al. 2013; Burnett et al. 2015), comparing the efficacy and safety of ATRA plus ATO versus the standard ATRA plus chemotherapy approach, strongly support the former combination as the new standard of care for low-to-intermediate-risk APL patients with WBC counts lower than  $10 \times 10^9/L$  at presentation. Nevertheless, in countries where chemotherapy is more affordable than ATO, the classical combination of ATRA and chemotherapy remains an acceptable option. For high-risk patients, however, there are two valid options, either ATRA plus chemotherapy or ATRA plus ATO with a certain amount of cytoreductive chemotherapy, at least during the induction phase.

HCT is never indicated in patients in CR1, except for the small fraction of patients with persistent RQ-PCR positivity of *PML-RARA* after consolidation (<1%), given the poor prognosis associated with this subset. HCT is also indicated in APL patients who relapse and achieve second or subsequent CR.

### 70.4.4 Salvage Therapy

Apart from patients with MRD positivity at the end of consolidation (molecular persistence), there is a consensus that patients experiencing molecular or hematological relapse later on require immediate additional treatment, including HCT. The goal of salvage treatment is to achieve molecular remission as a bridge to HCT. In cases where ATRA plus chemotherapy was the frontline therapy, salvage treatment with

ATRA plus ATO is recommended. Conversely, if frontline therapy involves ATRA plus ATO, ATRA plus chemotherapy is the preferred option.

The use of gemtuzumab ozogamicin may also be considered in both situations, but always as a bridge to HCT. Various studies (Yanada et al. 2013; Holter Chakrabarty et al. 2014; Lengfelder et al. 2015) suggest that auto-HCT should be considered the first choice for eligible patients achieving a second molecular remission. Patients who are unsuitable for HCT or have a very prolonged CR1 can be managed with some maintenance therapy, which would be chosen considering previous treatments and clinical condition.

Allo-HCT should be reserved for patients with a high risk of relapse and low risk of TRM, but also as a second option for patients who relapse after an auto-HCT.

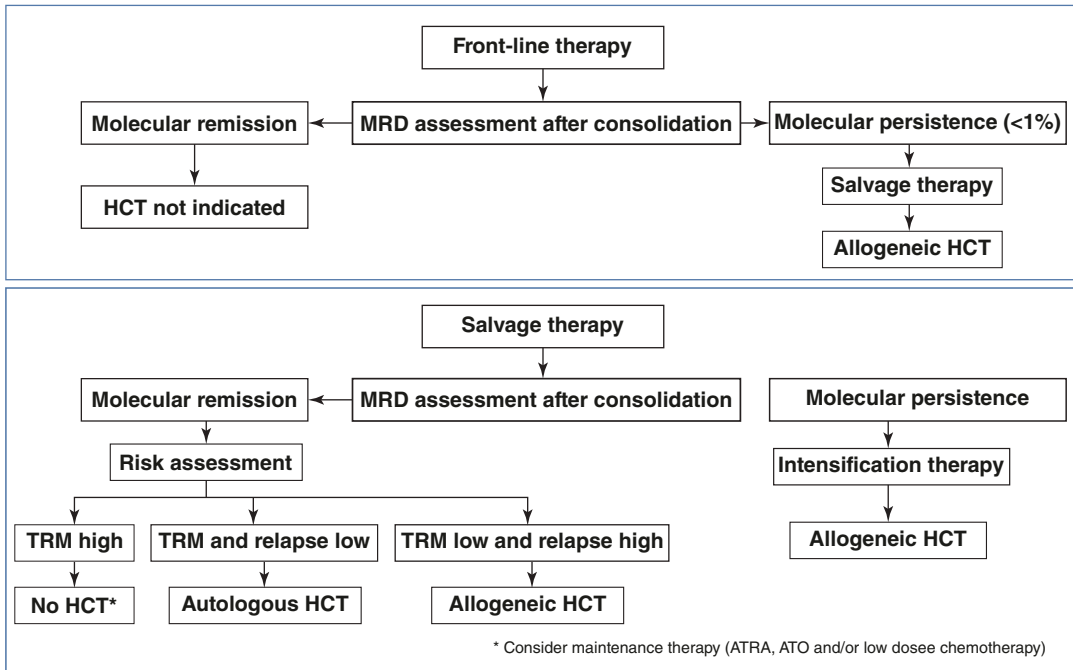
### 70.4.5 Indications of HCT

HCT is never indicated in patients in CR1, with the exception of those who fail to achieve molecular remission at the end of consolidation (<1%). For a comprehensive overview of HCT indications and other recommendations for patients who require HCT, please refer to Table 70.2 and the algorithm depicted in the following figure.

### 70.4.6 Main Series Reported on HCT in APL

There are no randomized trials to evaluate the efficacy and safety of the different modalities of HCT in refractory/relapsed APL. The data come mostly from retrospective studies comparing historical cohorts from registries (Table 70.3). Registry studies of both the EBMT (Sanz et al. 2021) and the CIBMTR (Holter Chakrabarty et al. 2014) showed superiority in overall survival for autologous compared to allogeneic HCT, although there were no significant differences in the relapse rate. Other non-comparative JALSG studies have also recently reported the remarkable efficacy of autologous HCT after ATO salvage therapy (Yanada et al. 2013, 2017).

**Table 70.2** Indications of HCT in patients with APL



	Auto-HCT	Allo-HCT
Not indicated	CR1 in molecular remission	CR1 in molecular remission
Indicated	≥CR2, but in molecular remission	<ul style="list-style-type: none"> <li>– ≥CR2 with <i>PML-RARA</i> (+) after salvage therapy</li> <li>– ≥CR2 if an auto-HCT has failed previously</li> <li>– ≥CR2 in patients with a high risk of relapse and low risk of TRM</li> </ul>
Salvage therapy as a bridge to HCT	Attempt to achieve molecular remission with ATRA plus ATO in patient who relapsed after ATRA plus chemotherapy as front-line therapy, whereas ATRA plus chemotherapy is the option when patients relapse after ATRA plus ATO	Attempt to achieve molecular remission with ATRA plus ATO in patient who relapsed after ATRA plus chemotherapy as front-line therapy, whereas ATRA plus chemotherapy is the option when patients relapse after ATRA plus ATO
Conditioning regimen	Either for use in AML, preferably containing HDAC (e.g., BEA (Gondo et al. 1997): BU/VP/Ara-C)	Either for use in AML
Cell source	Mobilized peripheral blood	Mobilized peripheral blood
Indication of CNS prophylaxis	ITT with MTX, hydrocortisone, and Ara-C, especially in those who presented relapse in CNS	ITT with MTX, hydrocortisone, and Ara-C, especially in those who presented relapse in CNS
Maintenance therapy post-HCT	Not proven, but conceivable that ATO + ATRA may be effective	Not proven, but conceivable that ATO + ATRA may be effective
Molecular monitoring	Recommended by RQ-PCR at least every 3 months for 2–3 years	Recommended by RQ-PCR at least every 3 months for 2–3 years

**Table 70.3** Main series reported on HCT in APL

Reference	Study period – salvage therapy	No. of patients	Overall survival, %	Relapse rate, %
Sanz et al. (2007)	1993–2003 ATRA+CHT	Auto = 195 Alo = 137	Auto = 51 <sup>a</sup> Alo = 59 P = ND	Auto = 37 Alo = 17 P = ND
De Botton et al. (2005)	1992–2001 ATRA+CHT	Auto = 50 Alo = 23	Auto = 60 Alo = 52 P = 0.04	Auto = 79 <sup>b</sup> Alo = 92 P = 0.2
Kohno et al. (2008)	1999–2004 Miscellaneous	Auto = 15 Alo = 13	Auto = 76 Alo = 46 P = 0.2	Auto = 79 <sup>b</sup> Alo = 92 NS
Lengfelder et al. (2015)	2003–2011 ATO-based	Auto = 60 Alo = 33	Auto = 77 Alo = 79 NS	Auto = 37 Alo = 39 NS
Pemmaraju et al. (2013)	1980–2010 Miscellaneous	Auto = 10 Alo = 17	Auto = 86 Alo = 49 NS	Auto = 30 Alo = 18 NS
Fujita et al. (2013)	1997–2002 ATRA+CHT	Auto = 6 Alo = 21	Auto = 83 Alo = 76 NS	Auto = 58 <sup>c</sup> Alo = 10 P = 0.007
Alimoghaddam et al. (2011)	1989–2011 ATO-based	Auto = 11 Alo = 29	Auto = 47 Alo = 76 NS	NA
Holter Chakrabarty et al. (2014)	1995–2006 NA	Auto = 62 Alo = 232	Auto = 75 Alo = 54 P = 0.002	Auto = 30 Alo = 18 P = 0.4
Sanz et al. (2021)	2004–2018 NA	Auto = 341 Alo = 228	Auto = 82 Alo = 64 P = 0.001	Auto = 23 Alo = 28 P = 0.3

ATRA all trans-retinoic, ATO arsenic trioxide, QT quimioterapia, NA not available, NS no significant

<sup>a</sup> Leukemia-free survival

<sup>b</sup> Disease-free survival

<sup>c</sup> Crude relapse rate

### Key Points

- The application of allo-HCT for patients with AML in first CR should be based on a risk-adapted strategy assessing both the risk of TRM and the risk of the AML.
- Allo-HCT remains a potentially curative treatment modality in fit patients with primary refractory AML providing a suitable donor can be identified rapidly and the predicted TRM is acceptable.
- Allo-HCT in acute promyelocytic leukemia is only indicated in specific cases (eg,  $\geq$ CR2 not in molecular remission or after previous auto-HCT).

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# Acute Myeloid Leukaemia in Children

# 71

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## 71.1 Introduction

Whilst the outcome of frontline AML (including PR) has improved, treatment of R/R AML remains a challenge. Despite the majority of children (>90%) achieving CR, the relapse risk (RR) in CR1 remains high at 20–30%, albeit varying by risk group. Relapse remains the most common cause of death.

HCT reduces the RR in CR1 in all risk groups when compared to chemotherapy as consolidation treatment. Historically, this reduction in RR has not always translated into an improvement in OS due to the treatment-related mortality (TRM). However in the current era of a relatively low TRM, the balance shifts in favour of HCT for

children at high risk of relapse. When considering the benefits of transplantation in children, it is important to acknowledge the potential for associated late effects, particularly infertility. HCT offers children with R/R AML their only real chance of long-term survival, which may rely on augmenting the graft versus leukaemia (GVL) effect. Post-HCT strategies such as the employment of FLT3 inhibitors (sorafenib and midostaurin) for *FLT3/ITD*-mutated AML, donor lymphocyte infusion (DLI), adoptive T-cells, or NK-cell immunotherapy may reduce the risk of further relapse post-transplant. A number of CAR T-cells are in development and may offer hope for the future.

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## 71.2 Prognostic Factors and Indications

### 71.2.1 First Complete Remission

Consolidation therapy with allogeneic HCT in CR1 of paediatric AML has been shown consistently to reduce the RR through a GVL effect, which is stronger in AML than ALL. Recognising the importance of the GVL effect in preventing relapse has led to the adoption of techniques to augment the GVL effect such as the use of cord blood stem cells, omission of serotherapy and early withdrawal of immunosuppression.

Improvements in HLA typing and expansion of the donor pool through the use of cord blood

stem cells and haploidentical donors have made HCT an option for the majority of patients. All patients with AML should be tissue-typed at presentation in order to optimise the chance of early transplant should this be indicated.

The criteria for transplanting patients has evolved from transplanting patients irrespective of the risk group, to transplanting all patients other than those with good-risk (GR) cytogenetics (about 70–80% of all patients), to the current practice of restricting transplant to those with poor-risk (PR) cytogenetics (about 30% of all patients), who are at the highest risk of relapse and most likely to benefit from HCT in CR1 in an era of low TRM.

There is no international agreement on the definition of PR disease. Most groups define PR disease which may benefit from HCT in CR1 through a combination of PR cytogenetics/molecular abnormalities, which are currently considered to be the strongest indicator of outcome, and the persistence of MRD after chemotherapy course 1 or 2. There is evolving consensus on which cytogenetics/molecular aberration constitutes high-risk disease, although some national groups place more value on MRD.

There is consensus that failure to achieve CR carries a poor outcome. There is no advantage for HCT in CR1 for patients with GR disease, that is, those with  $t(8;21)(q22;q22)/RUNX1::RUNX1T1$ ,  $inv.(16)(p13;q22)/CBFB::MYH11$ ,  $NPM1$  mutation or biallelic  $CEBPA$  bZIP in-frame insertion/deletion mutation. The benefit of HCT in CR1 for patients with intermediate-risk (IR) cytogenetics is less clear, and these may be the patients without PR cytogenetics but with a suboptimal early response to chemotherapy in whom MRD assessment can identify those at high risk of relapse who may benefit from transplant. There is no role for HCT in CR1 of acute promyelocytic leukaemia (APL) or Down syndrome myeloid leukaemia (ML-DS). HCT for patients with Fanconi anaemia and MDS/AML and those with JMML are discussed elsewhere.

### 71.2.1.1 Cytogenetics

Cytogenetic/molecular abnormalities are strong prognostic indicators of outcome in AML and are

used to risk-stratify treatment. There is consensus that patients with GR cytogenetics have favourable outcomes with chemotherapy alone and, with the exception of rare cases with a suboptimal response to induction therapy, should not proceed to transplant. The approach to patients with IR cytogenetics is largely based on response to chemotherapy as assessed by MRD. HCT is generally recommended for patients with PR risk cytogenetic/molecular abnormalities. There is increasing consensus on which cytogenetic/molecular abnormalities are PR and those most commonly considered indicative of being at high risk of relapse include:

- $inv.(3)(q21q26)/t(3;3)(q21;q26)/abn(3q26)$  (MECOM rearrangements),
- $-5/del(5q)$
- $-7$
- $t(6,9)(p23;q34)/DEK::NUP214$ ,
- $t(9;22)(q34;q11)/BCR::ABL1$ ,
- 12p abnormalities
- $t(6,11)(q27;q23)/KMT2A::MLLT4$ ,
- $t(4;11)(q21;q23)/KMT2A::AFF1$ ,
- $t(10;11)(p11 \sim 14;q23)/KMT2A::MLLT10$  and other  $KMT2A$  partners located on 10p (e.g.,  $AB11$ ),
- All  $NUP98$  fusions,  $t(5;11)(q35;p15.5)/NUP98::NSD1$ ,  $NUP98::KDM5A$ ,
- $t(7;12)(q36;p13)/MNX1::ETV6$ ,
- $inv.(16)(p13.3;q24.3)/CBFA2T3::GLIS2$ ,
- $FLT3$ -ITD without  $NPM1$  mutation or  $CEBPA$  in-frame bZIP mutation or CBF-AML (NB:  $CEBPA$  double mutation with  $FLT3$ -ITD is intermediate risk).
- Complex karyotype – no consensus.

It is difficult to risk-stratify rare fusions, but  $FUS::ERG$  and  $PICALM::MLLT10$  have been reported in the literature as poor risk. Not all groups consider a complex karyotype (3 or more abnormalities) to be poor risk. The translocation  $t(11;19)(q23;p13.3)/KMT2A::MLLT1$  is variably classified as intermediate or high risk.  $KMT2A$  ( $MLL$ ) rearrangements with the exception of  $t(4;11)(q21;q23)/KMT2A::AFF1$ ,  $t(6;11)(q27;q23)/KMT2A::MLLT4$  and  $t(10;11p11-p14;q23)/KMT2A::MLLT10/KMT2A::AB11$  are



generally considered intermediate risk, but in rare incidences intermediate risk *KMT2A* abnormalities may co-occur with poor-risk features (e.g., *FLT3-ITD*, monosomy 7, 12p abnormalities, etc.), which may alter the risk. *FLT3-ITD* is considered as poor risk but often co-occurs with other poor-risk cytogenetic abnormalities, when the risk may be augmented. The absence of GR cytogenetic abnormalities and the allelic ratio (>0.4) may influence the risk and approach to treatment. An increasing number of clinically relevant fusions are becoming recognised, a number of which are cytogenetically cryptic and require more comprehensive diagnostic assessment. Currently, PR cytogenetics comprise about 30–35% of all AML in children.

AML is a heterogeneous disease, and not all PR cytogenetic groups will benefit to the same degree from HCT in CR1. The European Society for Blood and Marrow Transplantation (EBMT)/Paediatric Diseases Working Party (PDWP) recently reported a review of children ≤18 years of age at HCT reported to the European Society for Blood and Marrow Transplantation (EBMT) registry, who received their first allogeneic HCT for AML in CR1 between 2005 and 2020 and who had an evaluable diagnostic karyotype. The aim was to establish whether PR cytogenetic abnormalities at diagnosis remain predictive of OS after HCT. Patients were subgrouped as (a) monosomy 7/del(7q) or monosomy 5/del(5q) (24%), (b) 11q23 abnormalities excluding t(9;11)(p12;q23)/*KMT2A::MLL3* (37%), (c) complex or monosomal karyotype (24%), or (d) “other” (15%). A complex karyotype was defined as three or more structural abnormalities, and a monosomal karyotype as a monosomy with one or more structural abnormalities, excluding WHO-designated recurring translocations or inversions (2017 ELN recommendations). The “other” subgroup included t(6;9)(p23;q34)/*DEK::NUP214*, t(3;5)(q25;q34)/*NPM1::MLF1*, t(9;22)(q34;q11)/*BCR::ABL1*, t(8;16)(p11;p13)/*MYST3::CREBBP*, inv.(3)(q21q26) or t(3;3)(q21;q26)/*RPN1::MECOM*, t(16;21)(p11;q22)/*FUS::ERG*, abn(11)(p15) and del(12p)/abnormality of 12(p13). The review included 744 children (median age at HCT:

8.6 years [0.3–18 years]). Median follow-up after HCT was 4.4 years. Eighty-six per cent of 346 evaluable patients were MRD-negative pre-HCT and 97% of patients received myeloablative conditioning (MAC). The OS and leukaemia-free survival (LFS) for the entire cohort were 76% and 70%, respectively, at 2 years. In a multivariate model, 11q23 (hazard ratio (HR) = 0.59,  $P = 0.01$ ) and “other” PR cytogenetic abnormalities (HR = 0.49,  $P < 0.01$ ) were associated with significantly better OS compared to monosomy 7/del(7q) or 5/del(5q) (HR1). The “other” PR cytogenetic abnormalities category was also associated with a lower risk of disease relapse after HCT (HR = 0.4,  $P = 0.01$ ). Receipt of an unrelated donor was associated with a lower relapse incidence (RI) (HR = 0.58,  $P = 0.03$ ).

### 71.2.1.2 Measurable Residual Disease Assessment (See Chap. 57)

Whilst genetic risk is the most important prognostic factor in AML, MRD is an independent post-remission prognostic factor important for risk stratification and treatment decision (Schuurhuis et al. 2018). Detection can be done by multiparametric flow cytometry (MFC), reverse-transcription quantitative PCR (RT-qPCR), digital droplet PCR and next-generation sequencing (NGS), all of which have different sensitivities and specificities. The European Leukaemia Net (ELN)-MRD Working Party has produced guidelines for standardisation and harmonisation which includes recommendations for the use of MRD prognostically, selection of the methodology, appropriate time points for assessment, MRD thresholds and definition of response (Heuser et al. 2021).

MFC is most commonly employed in paediatric AML either by measuring leukaemia aberrant immunophenotype (LAIP), a “different from normal” (DfN) phenotype or a combination of both methodologies. MRD positivity by MFC at early time points is strongly predictive of outcome. It is sensitive to a level of 0.1–0.01% and with an appropriate panel about 90% of children will have an informative LAIP by MFC.

Currently, the commonest threshold level for MFC is 0.1%, and the time point for post-course

is 1 or 2. However, the ability to define absolute risk remains limited with nearly a quarter of patients with flow MRD  $\leq 0.1\%$  post-course 1 relapsing and a similar proportion of patients with flow MRD  $>0.1\%$  remaining relapse-free (Sievers et al. 2003; Paietta 2018). More recently, a systematic review of the prognostic value of MRD in paediatric AML was reported. Thirteen studies were included and in all studies, MRD positivity during treatment was associated with worse clinical outcome. However, MRD negativity during treatment is associated with significantly better clinical outcome but does not exclude the possibility of relapse, whilst positivity early during treatment does not exclude cure (Seegerink et al. 2021).

Molecular MRD assessment by RT-qPCR generally has a sensitivity level of 0.1–0.001% and is applicable in approximately 80% of children who have a target for RT-qPCR such as a fusion gene or NPM1 mutation. Not only can molecular MRD be used to guide treatment but can also be used sequentially for the early detection and pre-emptive treatment of relapse. Further work is required to define molecular MRD thresholds at early time points that are predictive of relapse in the paediatric setting, bearing in mind that these may vary by molecular subtype. However, serially rising transcript levels (i.e., MRD relapse) are strongly predictive of haematological relapse, and some consider this an indication for pre-emptive therapy. Digital PCR and next-generation sequencing have some potential advantages for MRD monitoring over existing molecular techniques but currently remain in the research arena. Similarly, leukaemia stem cell monitoring may add prognostic information to current flow cytometry-based assays (Schuurhuis et al. 2018).

Preliminary data from adult studies and AAML 03P1 (Loken et al. 2012) suggest a limited discriminatory value for flow MRD in patients with PR cytogenetics. Observation from COG AAML03P1 and AIEOP 2002/01 that patients considered MRD negative at the end of treatment, but with previously documented MRD post-course 1, remain at high risk of relapse and poor outcome, suggesting that intervention

beyond clearance of MRD with chemotherapy alone is required for improved outcome (Buldini et al. 2017; Loken et al. 2012).

In patients with GR cytogenetics, stable or falling transcript levels may be an indication for further chemotherapy rather than HCT. It is recognised that some patients with GR cytogenetics may have disease which is detectable molecularly after course 1, but patients may still obtain a durable CR with chemotherapy alone. Karlsson et al. reported a small study of 15 children treated on NOPHO-AML 2004 which compared results by RT-qPCR and MFC. Eight children had a *RUNX1::RUNX1T1*, one *CBFB::MYH11* and six *KMT2A::MLLT3*. Ten of 22 samples were discordant with a cutoff for positivity of  $\geq 0.1\%$ . The majority (9/10) were MRD-positive with RT-qPCR but MRD-negative with MFC. This was shown to be due to the presence of fusion transcripts in mature cells as well as in CD34-expressing cells. Measurement of RT-qPCR suggests slower response kinetics than indicated by MFC, and the authors suggest that the prognostic impact of early measurement with RT-qPCR remains to be determined (Karlsson et al. 2022).

In the NOPHO-AML 2004 trial, MRD detected by MFC was performed on day 15 and before consolidation and was evaluable in 101 patients. Using a 0.1% MRD cutoff level, MRD-negative and MRD-positive patients at the start of consolidation therapy had a 5-year EFS of  $57 \pm 7\%$  and  $11 \pm 7\%$ , respectively ( $P < 0.001$ ) and an OS of  $78 \pm 6\%$  and  $28 \pm 11\%$  ( $P < 0.001$ ). Patients who were MRD-positive before consolidation had a cumulative incidence of relapse (CIR) of  $82 \pm 9.3\%$  compared to  $38.2 \pm 6\%$  in those who were MRD-negative at this time point. In multivariate analysis, only MRD correlated significantly with survival and MRD before consolidation therapy was the strongest independent prognostic factor for EFS and OS (Tierens et al. 2016).

HCT has been shown to reduce the negative impact of a poor early treatment response. Thirty one of 267 (12%) children treated on NOPHO-AML 20024 were defined as poor responders (15% blasts morphologically after course 1 or 5% blasts after course 2). These patients had

time-intensive chemotherapy followed by HCT in 25 of 31 with a donor. The 3-year probability of survival for these high-risk patients was 70%. Patients classified as intermediate risk (defined as 5–14.9% blasts after course 1) had a significantly inferior EFS compared to high-risk patients. Both groups had time-intensive chemotherapy, but only high-risk patients proceeded to HCT (Wareham et al. 2013; Abrahamsson et al. 2011).

The St Jude's group showed that flow MRD positivity >0.1% after course 1 was associated with a 3-year EFS of 43% compared to 74% in those who were MRD-negative (Rubnitz et al. 2010). Likewise on the COG AAML03P1 protocol, of 188 patients who achieved morphological CR after course 1 or 2, 46 (24%) had detectable flow MRD > 0.1%. Those with and without MRD > 0.1% at the end of course 2 had a 3-year-RR of 67% and 30% and a relapse-free survival (RFS) of 29% and 65%, respectively (Loken et al. 2012).

Compared to patients with PR cytogenetics, the benefit of HCT in CR1 for patients with IR cytogenetics is less clear.

A recent I-BFMSG study evaluated the benefit of HCT in CR 1 in a heterogeneous cohort of patients with *KMT2A-r* (rearranged) AML including high- and intermediate-risk rearrangements based on the fusion partner. Irrespective of risk group, MRD negativity post-course 2 was associated with superior EFS and a trend towards lower CIR. However, within the intermediate-risk group, the CIR was similar for MRD-negative and MRD-positive patients post-course 2. The authors recognised the limitations of a retrospective study in which the transplant rate was low (21%) by today's standards, the limitations of MFC MRD in the era studied, and that the study was not powered to assess the effect of HCT in CR1 (van Weelderen et al. 2023).

### 71.2.2 Relapsed Disease

Patients with relapsed AML across all cytogenetic risk groups have a dismal prognosis with chemotherapy alone, and it is generally accepted that they should proceed to transplant in CR2.

The first international I-BFM AML Relapsed Study reported the chance of achieving a second CR after relapse to be dependent on the length of CR1: CR1 < 1 year versus CR >1 year is 50% versus 75% with an overall CR rate of 60% and OS for CR1 < 1 year 26% versus 45% CR1 > 1 year,  $p < 0.001$  (Kaspers et al. 2013). Cytogenetics are strong prognostic indicators in relapse as in de novo disease with patients with CBF leukaemias fairing the best: CBF leukaemias versus others—OS 67% versus 31%,  $p < 0.001$ . Other significant prognostic indicators were no HCT in CR1 and speed of response to reinduction.

A retrospective analysis of two large international study groups (COG and BFM) has updated these outcomes. The OS at 5 years was 42% (BFM) and 35% (COG). Initial high-risk features and short time to relapse predicted poor outcomes. OS for all patients who received a HCT was  $54\% \pm 4\%$ . In the BFM cohort, 82% of patients proceeded to HCT which represented an increase compared to previous trials and an improvement in outcomes was observed (Rasche et al. 2021).

The AML-SCT BFM 0227 trial showed similar outcomes. Patients transplanted in CR2 had a 4-year EFS of 46% with a RR of 27% (Sauer et al. 2020).

Outcomes in multiply relapsed AML are poor. An AML-BFM study group reported a 5-year probability of OS of  $31 \pm 9\%$  following HCT ( $n = 25$ ) in patients transplanted after a second relapse. Twenty-one of 25 (88%) had a previous HCT. Early second relapse was associated with a dismal outcome and dependent on a favourable response to further treatment. Although survival at the second relapse is poor, it is possible (Rasche et al. 2021). Novel therapies may have a role in multiple relapsed AML to achieve remission prior to HCT.

### 71.2.3 Refractory Disease

Residual disease/MRD positivity pre-HCT increases the risk of relapse post-HCT, but the susceptibility of AML to GVL does not preclude

transplant. MRD status just prior to HCT is an important prognostic indicator. A small study reported a 5-year OS of 80.4% for children with <0.01% MRD ( $n = 27$ ), 66.7% for those with 0.01–5% MRD ( $n = 9$ ) and 58.3% for those with >5% MRD (Leung et al. 2012). In a retrospective study from the UK of 44 paediatric patients transplanted for refractory AML, 68% achieved CR following HCT with a 5-year LFS of 43% and RR of 32% (O'Hare et al. 2017). Outcomes in patients with primary refractory disease ( $n = 23$ ) were equivalent to those with R/R AML ( $n = 21$ ). Blast percentage  $\leq 30\%$  in the bone marrow (BM) pre-HCT, myeloablative conditioning and acute GVHD were favourable prognostic features. Stratification according to age  $\geq 10$  years and > 30% blasts in BM pre-HCT enabled prognostication, i.e., patients with neither or one of these risk factors had a LFS of 53% whereas those with both factors had a LFS of 10%. It should be noted, however, that these data were in patients who proceeded to HCT and there is thus significant selection bias. Recent data suggest particularly impressive outcomes with unrelated cord blood transplant in patients with refractory disease with a 61% EFS in patients with primary refractory disease ( $n = 29$ ) and 45% in those with R/R disease ( $n = 23$ ) (Horgan et al. 2023). Overall these data suggest that a significant subset of patients with refractory disease are curable by HCT.

### 71.3 Conditioning Regimens

No advantage has been shown for total body irradiation (TBI) in AML, and chemotherapy-only regimens should be used to limit the burden of morbidity associated with endocrine dysfunction. Adult data from the CIBMTR demonstrated improved NRM, OS and DFS in patients with AML transplanted using IV BU with therapeutic drug monitoring (TDM) compared with TBI (Copelan et al. 2013). Myeloablative conditioning (MAC) regimens are most commonly used, but a number of reduced toxicity conditioning (RTC) regimens are being tested. There is no proven “best” chemotherapy conditioning regimen, though MAC regimens with BU and CY

with TDM of BU levels are currently the standard of care.

However, the AUC to be targeted has been systemically evaluated for the combination of BU with CY only. When BU was combined with agents other than CY, relationships between BU exposure and clinical outcomes have been shown to be altered (McCune et al. 2000). For example, the use of BU TDM varied considerably with BU/FLU (Parmar et al. 2013; Ayala et al. 2015), Bu/clofarabine (Andersson et al. 2011; Kebriaei et al. 2012) and BU/CY/etoposide (Zhang et al. 2012; Chen et al. 2015). A retrospective EBMT study of Bu, Cy and Melphalan (Mel) (enhanced MAC) in paediatric AML in CR1 suggested improved RR and LFS compared with Bu-Cy, but the majority of patients receiving Bu-Cy on this study did not undergo TDM (Lucchini et al. 2017). The prospectively conducted and centrally monitored BFM trial AML SCT-BFM 2007 used BU/CY/MEL (enhanced MAC) for children with a matched family or unrelated donor. BU dosing was weight-adapted only. Four-year EFS and OS were 61% and 70%, respectively, for the complete cohort. CIR was 22%. TRM was 15% and correlated with age reaching 9% (SE 3%) in children younger than 12 years and 31% (SE 9%) in older children and adolescents. Of note, children under the age of 12 years, who were transplanted in CR1, achieved an exceptional OS of 94%, an EFS of 84% and a TRM of 6% at four years. Due to unacceptable TRM rates between 20 and 30%, the trial was halted for teenagers, and this regimen should be avoided or used with caution in this age group (Sauer et al. 2020). There is an increasing body of experience with BU/FLU-based MAC, which is well tolerated, but no published randomised comparisons are available to determine the relative anti-leukaemic activity of BU/FLU versus BU/CY (Harris et al. 2018). The recently completed MyeChild01 trial is expected to give answers to this unresolved question.

Replacing BU with Treosulfan (TREG) to reduce toxicity whilst maintaining efficacy is being tested and given in combination with CY (TREG/CY) or with FLU and Thiotepea (FTT). The choice of conditioning regimen is a balance between efficacy and toxicity. Comorbidity, pretreatment with drugs that may contribute to toxicity, i.e., gemtu-

zumab and VOD/SOS, age and HLA disparity may influence the choice of conditioning regimen. Comorbidity or heavy previous treatment may indicate a reduced intensity conditioning (RIC) regimen with BU/FLU or FLU/MEL. Targeted BU levels will differ between MAC, RTC and RIC. Patient toxicities may suggest avoidance of specific agents. Newer regimens which include clofarabine (CLO) are being tested. A prospectively randomised study comparing CLO/FLU/BU and BU/CY/MEL is currently being conducted by the NOPHO-DBH consortium.

An important concept has been introduced by the FLAMSA-RIC approach (Schmid et al. 2006). It incorporates prophylactic DLI after a rapid CSA taper to compensate for the potentially decreased anti-leukaemic potency of RIC chemotherapy. The AML SCT BFM 2007 trial for children with refractory disease reported an EFS and OS of 53% and 57%, respectively, at 4 years for children with primary refractory de novo AML (Sauer et al. 2020). The trial delivered sequential conditioning for R/R AML immediately followed by allo-HCT. The prospectively randomised phase III ASAP trial has very recently substantiated this concept in adults and produced comparable LFS for conventional intensive induction treatment prior to transplant (Stelljes et al. 2022).

## 71.4 Donor Selection Hierarchy and Stem Cell Source

### 71.4.1 Allogeneic HCT

The choice of donor for allo-HCT is based on HLA compatibility and CMV status. Outcomes are similar for MSD and MUD. The degree of mismatch which is acceptable depends on the risk of relapse and CR status. MMUD or cords and haplo-HCT are generally reserved for HR disease or early relapses.

Patients and their siblings should be tissue-typed at diagnosis. In the absence of a HLA MFD, an UD and a CBU, a search should be initiated as soon as possible after induction course 1 for patients with IR or PR cytogenetics. Donors should be selected using the selection hierarchy

of the national group. Medium-/high-resolution typing is required for adult URD (HLA A, B, C, HLA-DRB1, HLA-DQB1 and HLA-DPB1) and unrelated cords (HLA A, B, C and DR loci). The risk of relapse does not direct the need for transplantation, but the HLA discrepancy which is acceptable. MFD or well-MUD should be identified for CR1 patients, whilst mismatched donors and cords or haplo-HCT should be reserved for high-risk disease, CR2, or refractory disease. For MFD/UD, BM is the preferred stem cell source, but the use of peripheral blood stem cell (PBSC) is permissible. The use of PBSC from mismatched donors should be avoided wherever possible.

In the UK, serotherapy is only given to patients transplanted from unrelated donors, 9/10 mismatched family donors, or 5/8 matched cord blood units, but not to patients receiving grafts from matched family donors or 6–8/8 unrelated cord blood units (Table 71.1).

The optimal stem cell source for HCT in AML remains the subject of intense debate. A retrospective study comparing the outcomes of MSD, MUD and CB HCT in paediatric AML showed no difference in RR or LFS but improved chronic GVHD-free/LFS with unrelated CB HCT (Keating et al. 2019). Another recent retrospective analysis comparing outcomes of T-replete

**Table 71.1** Donor and source for HCT: the hierarchy of the UK

Choice	Family donor	Unrelated donor	Unrelated cord
1st	MFD (BM, PBSC and CB)		
2nd		10/10 MUD; 9/10 1DQ MMUD	8/8 MUCB (total nucleated cell (TNC >3 × 10 <sup>7</sup> /kg)
3rd	9/10 MMFD	9/10 (other) MMUD	5–7/8 MMUCB (TNC >3 × 10 <sup>7</sup> /kg) <sup>a</sup>

<sup>a</sup>For unrelated cord blood, a single cord is used if the cryopreserved TNC dose is >3 × 10<sup>7</sup>/kg. If <3 × 10<sup>7</sup>/kg, a double-cord transplant is preferred

*MFD* matched family donor, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *MUCB* matched unrelated cord blood, *MMFD* mismatched family donor, *MMUCB* mismatched unrelated cord blood



unrelated CB HCT ( $n = 112$ ) with other stem cell sources ( $n = 255$ ) suggests a significantly improved EFS (HR 0.57) with CB HCT in multivariate analysis (Horgan et al. 2023). Similar to what has previously been described in adults (Milano et al. 2016), this benefit was particularly seen in patients who were flow MRD-positive ( $>0.1\%$ ) pre-HCT where a striking reduction in relapse was observed in the CB cohort compared to other stem cell sources (36% vs. 66% HR 0.46), suggesting this is mediated through enhanced GVL effects. Interestingly, while aGVHD was common after T-replete CB HCT, the incidence of chronic GVHD was reduced (HR 0.28).

Bertaina et al have compared the outcomes of TCR $\alpha\beta$ -depleted haploidentical transplant with MUD and MMUD HCT in a multicentre, retrospective analysis in paediatric patients with acute leukaemia, including 105 patients with AML. They report a lower incidence of acute and chronic GVHD and similar LFS between the three cohorts but an improved chronic GVHD-free/RFS in those with haploidentical or MUD donors compared to MMUDs. However, only a minority of these patients had AML, so we would caution extrapolating this data to this disease where the GVL effect is significant (Bertaina et al. 2018). In contrast to the adult setting, data on haploidentical HCT with post-transplant cyclophosphamide (PTC) are limited in the paediatric setting, and to date it is not possible to comment on which approach to haploidentical HCT is preferable. Ultimately, prospective randomised studies are needed to truly determine the optimal stem cell source for this indication.

## 71.5 GVHD Prophylaxis

All patients should receive IS with CSA. Most, but not all, groups add short-course methotrexate (MTX) for all patients. Patients receiving grafts from a MMD or those in whom the stem cell source is PBSC or unrelated CB should receive prophylaxis in addition to CSA with either MMF or short-course MTX.

## 71.6 Prevention of Relapse Post-Transplant

### 71.6.1 Withdrawal of Immunosuppression

Withdrawal of immunosuppression in order to optimise the GVL effect is frequently used pre-emptively in patients at high risk of relapse or in those who develop mixed chimerism (MC) or MRD. In adult AML, increased exposure to CSA was associated with increased relapse and decreased survival (Craddock et al. 2010), supporting early withdrawal of IS where possible. In the absence of GVHD, MMF can be stopped at day 28 post-transplant, and CSA tailed over 4–6 weeks from day 60 (MFD), day 100 (MUD), or earlier if mixed chimerism is detected in the whole blood.

### 71.6.2 Donor Lymphocyte Infusion (DLI)

The evidence of benefit for DLI is weak. Rettinger et al. (2017) investigated the use of pre-emptive immunotherapy with reduction of IS and low-dose DLI in patients with paediatric AML developing MC after HCT for AML: 6/13 patients with MC who received immunotherapy remained in long-term CR, whereas all 7 patients with MC who did not receive immunotherapy relapsed. Based on these limited data, our practice is to use pre-emptive immunotherapy in patients with confirmed MC (defined as  $>1\%$  autologous cells in the whole blood on two occasions 1 week apart) without active aGVHD  $>$ Grade 1 or cGVHD in the first-year post-transplant. If patients are still receiving IS, this should be discontinued and chimerism reassessed a month later. In patients already off IS, chimerism should be reassessed a month off IS. If MC persists, DLI should be given to recipients of MFD or MUD. DLI is not recommended in the context of 9/10 mismatched donor HCT. The DLI cell dose administered is dependent on the donor source and the timing post-transplant. In the future, the use of pre-emptive



DLI is likely to be based on the detection of flow or molecular MRD in the bone marrow in the absence of GVHD.

### 71.6.3 Targeted Maintenance Therapy

Several options have emerged as post-HCT maintenance strategies to sustain the remission achieved by HCT. These include hypomethylating agents (HMAs) (Decitabine and Azacitidine), tyrosine kinase inhibitors (TKIs) (for FLT3-mutated AMLs) and Venetoclax.

- (a) Hypomethylating agents (Decitabine and Azacitidine): HMAs act on the DNA methyltransferases (DNMTs), reduce epigenetic dysregulation and promote tumour suppression. Their utility and good tolerance as frontline treatment of AML in elderly and frail patients prompted their use as oral prophylactic agents post-allogeneic HCT to prevent relapse in adults. A pilot trial in HR adult AML (RICAZA trial) employing Azacitidine as relapse prophylaxis in high-risk adult AML ( $n = 37$ ) demonstrated a 1-year/2-year-RFS of 57%/49%, respectively, with a beneficial impact of positive CD8+ T-cell response (HR, 0.30; 95% CI, 0.10–0.85;  $p = 0.02$ ). In this trial, patients with stable engraftment commenced treatment with Azacitidine on day +42 at a dose of 36 mg/m<sup>2</sup> subcutaneously (reduced to 24 mg/m<sup>2</sup> in dose-limiting toxicity) for 5 days, every 4 weeks for 12 months after HCT (Craddock et al. 2016).

Because the demethylating effect of Decitabine was noted to be cyclin-dependent, it was explored as a combination therapy with recombinant human G-CSF (100 mcg/m<sup>2</sup> from D0–5) in a prospective phase II RCT (Gao et al. 2020) in adults ( $n = 152$ ) and children ( $n = 52$ ). The dose of Decitabine (5 mg/m<sup>2</sup> from D1–5) was lowered to minimise its myelosuppressive side effects. Patients randomised to Decitabine + G-CSF (adults = 75; children = 25) were noted to have a lower

CIR at 2 years (15% versus 38.3%; HR, 0.32;  $p < 0.01$ ). G-CSF was tolerated well without a relative increase in cGVHD. In a more recent study, Booth et al. employed a combination of Azacitidine (36 mg/m<sup>2</sup>/day for 5 days, starting on D + 60, repeated every 4 weeks) and DLI as prophylaxis post-allogeneic HCT in 17 patients with IR/HR-AML and reported a 2-year-LFS of 88.2% (61.5% in pre-intervention historical cohort ( $n = 39$ )) (Booth et al. 2023).

- (b) FLT3 inhibitors (FLT3i): The role of FLT3i was first identified in adult AMLs in both frontline and post-HCT approaches. TKI maintenance therapy for *FLT3-ITD* positive AML has prospectively been shown to improve the outcome significantly. With a median follow-up of 41.8 months, the SORMAIN study reported a HR for relapse or death of 0.39 in the sorafenib group versus placebo. Twenty-four-month RFS probability was 53.3% with placebo versus 85.0% with sorafenib (Burchert et al. 2020). Mechanistic studies suggest that the combination with DLI might even be more potent (Mathew et al. 2018). A retrospective paediatric study (Tarlock et al. 2015) demonstrated the feasibility and tolerability of sorafenib (median dose 150 mg/m<sup>2</sup>; started at a median interval of 100 days post-HCT, for a median duration of 12 months post-HCT), and all patients who received sorafenib for MRD positivity immediately prior to transplant or with the emergence of MRD post-HCT are alive and remain in complete remission at a median of 48 months post-HCT. This benefit was explored further in a randomised, multi-centre COG AAML 1031 trial, in a cohort of 72 children with high allelic ratio-FLT3-mutated AML (Pollard et al. 2022). While the outcome analysis indicated an improved EFS with a HR of 3.03 (95% CI: 1.31–7.04) for all patients exposed to sorafenib, results focused on outcomes of maintenance therapy post-HCT ( $n = 46$ ) are not available.

Targeted inhibition of the FLT3 ligand by sorafenib has been shown to be associated

with deeper and more durable remission in patients relapsing after prior HCT (Metzelder et al. 2012), reflecting an anti-leukaemic synergism between sorafenib and allo-immune effects exerted by the stem cell graft. It is important to consider the dose-limiting effects of sorafenib, particularly those of cardiotoxicity, palmoplantar dysesthesia and myelosuppression.

TKI resistance and off-target toxicities associated with first-generation TKI led to their replacement with second-generation

TKIs, but their role in paediatric AML remains unclear given the paucity of data. In a small retrospective case series ( $n = 8$ ), the use of Gilteritinib (a more potent and selective FLT3 inhibitor) was associated with a 1-year-OS of 70% (McCall et al. 2021). Of note, 4/8 (50%) received Gilteritinib as maintenance post-HCT, and all were alive. Newer second-generation TKIs (including Gilteritinib and Quizartinib) are being tested in early-phase prospective paediatric trials (see Table 71.2).

**Table 71.2** Summary of novel emerging treatments in paediatric AML

Newer treatment	Target/mechanism of action	Paediatric AML target population	Current stage	Ref/trial ID
<b>Antibody-drug conjugates</b>				
Gemtuzumab ozogamicin	CD33	Frontline treatment of all AMLs	Component of the recently concluded MyeChild 01 trial and standard of care in a phase III COG AAML1831	MyeChild01: NCT02724163; AAML1831: NCT04293562
IMGN632	CD123	Relapsed/refractory AMLs	Phase I/II	NCT05320380
Flotetuzumab	CD123-CD3 DART	Relapsed/refractory AMLs	Phase I	NCT04158739
STRO-002 <sup>a</sup>	CBAF2-GLIS2	AML with 'RAM phenotype'	Tested on compassionate basis; awaiting trials	Meshinchi et al. (Blood 2022); NCT03748186 (Approved in ovarian/endometrial cancers)
Tagraxofusp	CD123	Relapsed/refractory CD123 expressing haematological cancers	Phase I	NCT05476770
<b>FLT3 inhibitors</b>				
Sorafenib <sup>^</sup>	1st gen (type II) TKI	Frontline treatment and also as post-HCT maintenance in FLT3 + ve with high allelic ratio	Phase III RCT	Pollard et al. (2022), NCT01371981
		FLT3-mutated acute monocytic leukaemia	Phase III (in combination with chemotherapy)	NCT05313958
Midostaurin	1st gen (type I) TKI	Frontline treatment of FLT3-mutated AML	Phase I/II (in combination with chemotherapy)	NCT03591510
Quizartinib	2nd gen (type II) TKI	Relapsed/refractory FLT3-mutated AML	Phase I/II (in combination and as maintenance monotherapy)	NCT03793478
Gilteritinib (ASP2215)	2nd gen (type I) TKI	Relapsed/refractory FLT3-mutated AML	Phase I/II (in combination with chemotherapy)	NCT04240002
		Frontline treatment of all AMLs	Phase III (in combination with chemotherapy; COG AAML1831)	NCT04293562
Crenolanib <sup>-</sup>	2nd gen (type I) TKI	Relapsed/refractory FLT3-mutated AML	Phase I (in combination with Sorafenib)	Inaba et al. (2022), NCT02270788

**Table 71.2** (continued)

Newer treatment	Target/mechanism of action	Paediatric AML target population	Current stage	Ref/trial ID
<b>KMT2A fusion inhibitors</b>				
Pinometostat <sup>b</sup>	DOT1L	Relapsed/refractory KMT2A (r) leukaemias	Phase I (published)	Shukla et al. (2016)
Revumenib <sup>c</sup>	Menin inhibitor	Relapsed/refractory KMT2A (r) AML (adults + children)	Phase I/II (in combination with chemotherapy)	Issa et al. (2023)
		Frontline treatment of AML > 12 years with KMT2A (r)	Phase I (in combination with decitabine/cedazuridine and venetoclax)	NCT05360160
<b>CAR T-cells</b>				
Autologous lentiviral CAR-T	CD33	Relapsed/refractory AMLs (CD33 + ve)	Phase I (costimulatory domains: 41BB, CD3 $\zeta$ , HER1t)	Tambaro et al. (2021), NCT03126864
		Relapsed/refractory AMLs (CD33 + ve)	Phase I/II	NCT03971799
Autologous lentiviral CAR-T	CD123	Relapsed/refractory AMLs	Phase I	NCT04678336
Autologous retroviral CAR-T	CLL-1	Relapsed/refractory AMLs (>30% CLL-1 blasts on flow)	Phase I (costimulatory domain: CD28)	NCT04219163
CAR T-cells	CD38	Relapsed/refractory AMLs (CD38 + ve)	Phase I/II	NCT04351022
Autologous CART (with a suicide gene)	CD44v6	Relapsed/refractory AMLs (CD44v6 + ve)	Phase I/II	NCT04097301

Abbreviations: AML acute myeloid leukaemia, DART dual-affinity retargeting, TKI tyrosine kinase inhibitor, FLT3 Fms-like tyrosine kinase 3, CART chimeric antigen receptor–T-cell, COG Children’s Oncology Group, RCT randomised controlled trial

<sup>a</sup>Used on compassionate basis in 16 patients with CBAF2-GLIS2 fusion (monotherapy in 10) with 6 (38%) achieving MRD-negative CR (Meshinchi et al. 2022)

<sup>b</sup>Pinometostat at a R2PD of 70 mg/m<sup>2</sup> showed transient reductions in peripheral/BMblasts were detected in nearly 40% of pts. ( $n = 7/18$ ); however, no objective responses were observed

<sup>c</sup>Revumenib associated with CR/CRi of 30% (18/60 evaluable patients) with undetectable flow MRD negativity in 78% (14/18)

<sup>d</sup>Results described in the text (Pollard et al. 2022); ~Crenolanib (and sorafenib combination was tolerable without dose-limiting toxicities, and three complete remissions (one with incomplete count recovery), and one partial remission were observed in 8 evaluable children

(c) Venetoclax: Venetoclax is a highly selective and potent inhibitor of BCL2, which is an apoptosis-regulating protein, upregulated in haematological malignancies. Data on its use as prophylaxis against relapse post-HCT are limited. In a phase I dose-escalation study (Karol et al. 2020) in R/R paediatric AML patients ( $n = 20$ ), CR/CRi and partial remission was observed in 70% and 10%, respectively. The recommended phase 2 dose (R2D) in this early-phase trial was 360 mg/m<sup>2</sup>, when

used in combination with either Cytarabine or Idarubicin. More recently, in a larger cohort of high-risk paediatric AML patients ( $n = 43$ ; PR cytogenetics in 35(85%), prior history of BMT in 17 (37%) treated on a venetoclax-based regimen, a CR/CRi and partial remission of 40%/5%, respectively, was reported for the overall cohort and CR/CRi and partial remission of 29%/6%, respectively, in patients with previous BMT. None of the patients received venetoclax monotherapy (Trabal et al. 2023).

Whilst there definitely seems to be a benefit in combining  $\geq 2$  agents (i.e., HMA + venetoclax, venetoclax+FLT3i, DLI + HMA), determining the best possible combination, the optimal dose, duration of therapy and positioning of these therapies in the treatment algorithm (either as post-HCT maintenance, definitive therapy for first relapse or as a bridge to subsequent HCT) will best be answered by prospective trials. It is likely that in the future, maintenance therapy post-HCT with agents targeting specific high-risk genetic abnormalities will play an increasing role.

## 71.7 Role of the Second HCT

For selected patients who relapse late ( $>1$  year) post-first HCT and respond to reinduction chemotherapy, the second HCT may be curative with survival rates of 24–35% reported (Yaniv et al. 2018). However, for the entire relapse population, the prognosis is grim. Two large groups have shown similar dismal outcomes following the second HCT in children relapsing after the first HCT: (Uden et al. 2020) ( $n = 122$ ; 4-year-OS/NRM/CIR = 31%/22%/45%); ( $n = 251$ ; for patients in remission, the 5-year-OS/NRM/CIR = 31%/29%/46%). Remission status impacted survival in the former study and graft/donor choice (better survival in BM grafts and MFD) in the latter. In a smaller cohort of 46 children relapsing after the first HCT, Taga et al. demonstrated a 5-year-OS of 41.7% after the second HCT, with an inter-HCT interval of  $>24$  months conferring a better outcome (63% vs. 27%;  $p = 0.01$ ). In a multicentre national analysis of mismatched T-replete CB for R/R AML (Horgan et al. 2023), an impressive 2-year-EFS of 69% was noted in a cohort of patients with a previous history of HCT ( $n = 24$ ).

These data demonstrate an urgent need to improve outcomes for this group of children and young adults. Interestingly, CR has been seen in cutaneous (but not BM) relapse of AML post-transplant with the checkpoint inhibitor Ipilimumab (Davids et al. 2016).

Treatment options for patients who relapse early after transplant remain limited, and at present, for the majority of such patients, we recommend symptom care or enrolment in a clinical trial. Antibody–drug conjugates, bispecific T-cell-engaging antibodies and CAR T-cells are under development and offer hope for the near future.

### Key Points

- There is increasing evidence that patients with cytogenetic or molecular high-risk features may benefit from HCT in CR1. About 30% of children fall into this risk group. A TRM below 10% should be achievable.
- A MFD or MUD is considered the optimal donor, and BM is the preferred stem cell source. A mismatched donor may be considered appropriate for patients with poorly responding disease.
- Children who achieve CR2 after first relapse have a bleak prognosis without HCT.
- MAC, TBI-free, conditioning is recommended for patients transplanted in CR1 and CR2, and to date the standard regimen had been BU/CY.
- Novel conditioning regimens incorporating TREO or CLO are being explored, and these need to be compared with BU/CY in prospective, randomised studies.
- Relapse after HCT in CR1 is associated with a very poor outcome and is not curable without the second HCT. TRM for the second HCT exceeds 30% which might favour the use of a RIC in this setting. The prevention of relapse remains the major challenge.
- Several novel treatment options, including CAR T-cells, have emerged in the last decade, but their position in the treatment algorithm needs clarity.

## Abbreviations

ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
APL	Acute promyelocytic leukaemia
BFM	Berlin Frankfurt Munster
BM	Bone marrow
CAR	Chimeric antigen receptor
COG	Children's oncology group
CR	Complete remission
CSA	Cyclosporin A
DART	Dual affinity re-targeting
DLI	Donor lymphocyte infusion
EFS	Event-free survival
FLT3	Fms-like tyrosine kinase 3
G-CSF	Granulocyte colony-stimulating factor
GR	Good risk
GVHD	Graft versus host disease
GVL	Graft versus leukaemia
HLA	Human leucocyte antigen
HMA	Hypomethylating agents
HR	Hazard ratio
HCT	Haematopoietic cell transplant
IR	Intermediate risk
ITD	Internal tandem duplication
KMT2A	Lysine methyltransferase 2A
LAIP	Leukaemia aberrant immunophenotype
LFS	Leukaemia-free survival
MAC	Myeloablative conditioning
MC	Mixed chimerism
MDS	Myelodysplastic syndrome
MFC	Multiparametric flow cytometry
ML-DS	Myeloid leukaemia of down syndrome
MLL	Mixed lineage leukaemia
MMF	Mycophenolate mofetil
MMUD	Mismatched unrelated donor
MRD	Measurable residual disease
OS	Overall survival
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PDWP	Paediatric Diseases Working Party
PR	Poor risk
R/R	Relapsed/refractory
RCT	Randomised controlled trial

TKI	Tyrosine kinase inhibitor
TRM	Transplant-related mortality
VOD/SOS	Venoocclusive disease/sinusoidal obstruction syndrome

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# Acute Lymphoblastic Leukemia in Adults

# 72

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and Sebastian Giebel

## 72.1 Definition and Epidemiology

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites. While 80% of ALL occurs in children, it represents a much less curable disease in adults. The incidence of ALL is bimodal, with the first peak occurring in childhood and a second peak occurring around 50 years. The estimated overall incidence of ALL and lymphoblastic lymphoma in Europe is 1.28 per 100,000 individuals annually, with significant age-related variations (0.53 at 45–54 years, ~1.0 at 55–74 years, and 1.45 at 75–99 years) (Terwilliger and Abdul-Hay 2017).

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## 72.2 Diagnosis

Typical but non-specific clinical manifestations of patients with ALL are constitutional symptoms, bleeding, infections, and/or bone pain, with less than 10% of individuals having symptomatic CNS involvement at diagnosis, more common in T-cell disease. Mediastinal mass with wheezing and stridor can be a presenting feature of T-lineage ALL. A comprehensive work-up is necessary to allow a precise differential diagnosis, an accurate stratification and to establish an optimal monitoring of minimal residual disease (MRD). It includes blood and marrow morphology, flow cytometry, karyotype, and molecular genetics. Bone marrow infiltration >20% is used as cutoff point to distinguish ALL from LBL although it is arbitrary.

## 72.3 Classification

In 2022, the updated WHO classification and International Consensus Classification were published (Alaggio et al. 2022; Arber et al. 2022). Both distinguish B- and T-cell precursor neoplasms, including variety of genetically defined subtypes. Among B-ALL/LBL, Philadelphia-positive (Ph+) subtype characterized by the presence of t(9;22)(q34.1;q11.2) and *BCR::ABL1* fusion gene is the most frequent one accounting for 20–30% of adults. Its frequency increases with age.

## 72.4 Risk Factors

Clinical risk factors include age and white blood cell count at the time of diagnosis. Increasing age portends a worse prognosis. Patients over 60 years have particularly poor outcomes, with less than 20% long-term survival (Geyer et al. 2017). In most studies, the cut point for high-risk ALL has been  $30 \times 10^9/L$  for B-cell precursor ALL and  $100 \times 10^9/L$  for T-cell precursor ALL, respectively.

Immunophenotyping allows for evaluation of the maturation status of leukemic cells and has been historically used for risk stratification. Among T-ALL/LBL, the early T-cell precursor ALL defined by reduced expression of T-cell markers (CD1a, CD8, and CD5), and aberrant expression of myeloid or stem cell markers is associated with high-risk of treatment failure (Chiaretti et al. 2014). With the application of modern treatment protocols, the prognostic value of other phenotypically defined subtypes subtypes is unclear.

Several genetically defined subtypes are associated with poor prognosis. In adults, the most frequent one is  $t(v;11q23.3)$  associated with KMT2A rearrangements. The prognostic relevance of low-hypodiploidy and complex karyotype (five or more chromosomal aberrations) in ALL remains controversial among different study groups. Ph + ALL was historically defined as very high risk subtype. In modern era, treatment results for Ph + and Ph- ALL are comparable. Among older patients ineligible for allo-HCT, the presence of *BCR::ABL1* may even be associated with favorable outcomes due to sensibility to tyrosine kinase inhibitors. In a significant proportion of Ph-ALL, gene expression profile resembles that of *BCR::ABL1+* ALL. This subtype,

named Ph-like ALL, is associated with unfavorable prognosis. Its identification, however, is not routinely available in many countries.

Response to initial therapy is a strong predictor of the overall outcome. This includes the need for more than one cycle of induction to achieve CR as well as inadequate response at the level of MRD. MRD  $>10^{-3}$  of bone marrow cells after induction and/or  $>10^{-4}$  during/after consolidation is considered a high-risk feature (Giebel et al. 2019). An increase of MRD level above  $10^{-3}$  after initial response represents a very high risk of relapse (Brüggemann et al. 2010). MRD is evaluable using either multi-channel flow cytometry or the real-time quantitative polymerase chain reaction (RQ-PCR). Aberrant phenotypes are identified on the basis of different combinations and/or asynchronous expression and/or variable intensity staining of several antigens. PCR targets are transcripts of fusion genes associated with chromosomal abnormalities (e.g., *BCR::ABL1*) or rearranged immunoglobulin or T-cell receptor sequences (TCR  $\beta$ ,  $\gamma$ ,  $\delta$ , IgH, and IgK-Kde) unique to each patient with ALL. Innovative, more sensitive methods include next-generation flow cytometry, digital drop PCR, and next-generation sequencing. They require further standardization before wide implementation in clinical practice.

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## 72.5 Prognostic Factors Used to Indicate Allo-HCT in CR1

Indications for allo-HCT in CR1 are restricted to patients with high-risk ALL. However, criteria used for stratification in particular study groups vary strongly (Giebel et al. 2019).

Factors considered by all study groups	Factors considered by majority of study groups	Factors considered by some study groups
Inadequate response during/ after consolidation: • MRD $>10^{-4}$ /detectable at any level	Inadequate response to induction I: • No hematological CR • MRD $>10^{-3}$ after induction	Initial CNS involvement
Age (various cut points)	High initial WBC: • $>30 \times 10^9/L$ in B-ALL • $>100 \times 10^9/L$ in T-ALL	Adverse immunophenotype: • Early T-precursor • Mature T • Pro-B
<i>BCR:ABLI</i>	Other genetic factors: • KMT2A rearrangements • Hypodiploidy • Complex karyotype	

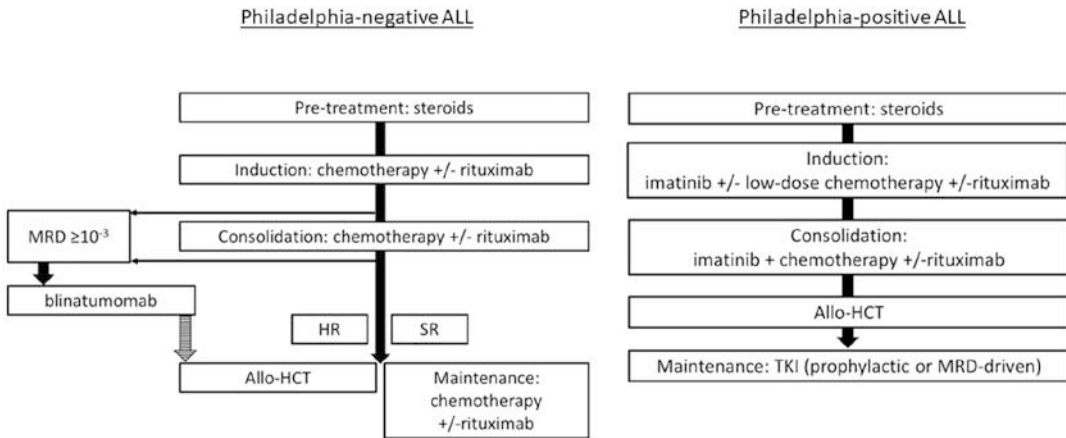
## 72.6 First-Line Treatment

The first-line chemotherapy usually consists of prephase, induction, treatment intensification/consolidation, and either long-term maintenance or allo-HCT, with CNS prophylaxis given at intervals throughout therapy (Fig. 72.1). The goal of induction therapy is to achieve CR remission and to restore normal hematopoiesis.

In Ph-ALL intensive chemotherapy inspired by pediatric protocols is recommended. The backbone of induction therapy typically includes VCR, DEX, and an anthracycline with or without L-asparaginase and CY. CR rates are in a range of 90–95% for younger adults and 70–90% for older individuals. For consolidation therapy, most study groups recommend six to eight courses, two to four of which contain high-dose MTX, Ara-C, and L-asparaginase, and one to two represent reinduction blocks. Postremission consolidation is most often followed by allo-HCT or long-term maintenance with daily oral mercaptopurine and weekly MTX for 2 years or longer, sometimes with periodic applications of, e.g., VCR, PRD, or other drugs. The addition of RTX to the induction and consolidation therapy for patients with B-ALL and CD20 expression has significantly improved the outcome in these subgroups (Maury et al. 2016). In case of MRD persistence or recurrence, a

bispecific anti-CD3/CD19 antibody, blinatumomab, may be administered with high rate of MRD responses. Results of recent studies indicate that also patients with MRD-negativity may benefit from blinatumomab administered in sequence with consolidation chemotherapy. In older individuals, inotuzumab ozagamicin, an anti-CD22 immunotoxin, may be alternative to upfront conventional chemotherapy, although it has not yet been approved for this indication. The addition of nelarabine to first-line treatment of T-ALL has not been shown to influence outcome in adults.

In Ph + ALL TKIs are the most important treatment component. Imatinib (IM) is the only drug approved for first-line therapy. During induction, it may be administered in combination with low-dose chemotherapy (VCR, DEX) allowing for 90–100% CR. More intensive chemotherapy does not increase the CR rate (Chalandon et al. 2015). During consolidation, IM used to be combined with high doses of MTX as CNS prophylaxis and other chemotherapeutic agents. All patients should be considered for allo-HCT in CR1, followed by TKI maintenance (Giebel et al. 2016). New regimens, including up-front use of dasatinib or ponatinib in sequence with blinatumomab, increase the chance of molecular CR and may reduce the role of allo-HCT in future.



**Fig. 72.1** Therapeutic algorithm for patients with newly diagnosed Ph- and Ph + ALL. Additional agents (e.g., binatumomab consolidation for MRD neg. patients) might

become available in the near future. HR, high risk; SR, standard risk; MRD, measurable residual disease

## 72.7 Second-Line Treatment

Approximately 5–10% of all adults with ALL are refractory to induction therapy while among those achieving CR, 30–50% experience relapse. Conventional standard chemotherapy regimens for adults with relapsed or refractory B-cell ALL are associated with rates of CR of 31–44% when they are the first salvage therapy administered after an early relapse and 18–25% when they are the second salvage therapy (Gokbuget et al. 2016). Results of randomized trials demonstrated that CR rates increased to 80% using inotuzumab and 44% using blinatumomab, which translated into prolonged survival compared to standard chemotherapy.

Any treatment regimen for relapsed/refractory ALL should be considered a “bridge” to allo-HCT in order to increase a chance of cure. The use of inotuzumab ozogamycin is associated with increased risk of VOD, especially after more than two treatment courses, in case of preexisting liver impairment and using double alkylators for conditioning. Consequently, the allo-HCT should be scheduled ideally within 6–8 weeks after the start of salvage therapy.

Anti-CD19 CAR T-cells are a new option for patients with relapsed/refractory B-ALL. Tisagenlecleucel is approved for chil-

dren and younger adults up to 25 y.o. with 80–90% CR rate and approximately 50% RFS (Maude et al. 2018). Brexucaptogene autoleucel in Europe is approved for patients  $\geq 26$  years old and allows for approximately 71% CR with median OS exceeding 2 years (Shah et al. 2021). The need for subsequent allo-HCT is a matter of debate. Given the high relapse rates reported so far after CAR-T cell therapy and the limited treatment options for patients failing CAR-T cell therapy, a subsequent allo-HCT as consolidation therapy should be considered in patients who have not had a previous HCT although more data are needed.

Treatment options for patients with relapse/refractory T-ALL are limited. Standard chemotherapy regimens such as FLAG (FLU, Ara-C, and G-CSF)  $\pm$  idarubicin result in only 30–40% response rates with 6 months median OS in responders. Nelarabine as monotherapy or in combination with other chemotherapeutic agents is a reasonable alternative option. Anti-CD7 or anti-CD5 CAR T-cells are being investigated with promising early results (Pan et al. 2021). Bortezomib-based strategies may be evaluated as an effective and well-tolerated treatment option for adult patients with relapsed/refractory ALL, as a bridge to immunotherapy or allo-HCT (Nachmias et al. 2018). Moreover, daratumumab



has started to be used in advanced ALL without other therapeutic options (Cerrano et al. 2022). In any case, allo-HCT should be considered to consolidate response.

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## 72.8 Autologous HCT

### 72.8.1 Indication

Auto-HCT is not considered a standard therapy for adult ALL. Optional for patients with MRD-negative ALL, not eligible for allo-HCT.

### 72.8.2 Conditioning

Fractionated TBI (e.g.,  $6 \times 2$  Gy) in combination with CY and/or VP.

### 72.8.3 Results

In some trials, patients excluded from allo-HCT were randomly assigned between chemotherapy and auto-HCT. In the largest study, chemotherapy proved superior, while a marginal superiority of auto-HCT was ascertained in high-risk patients in another one. In a European retrospective analysis on auto-HCT, a cohort of patients who were MRD negative had a significantly better survival compared to those being MRD positive. Results of another retrospective study comparing auto- and allo-HCT for adults with Philadelphia-positive ALL in first complete molecular remission showed similar survival rates for both groups (higher rate of relapse after auto-HCT and higher rates of death in remission after allo-HCT). Finally, comparable results after auto-HCT and RIC-allo-HCT have been reported for patients >55 years old.

It remains a matter of debate if the MRD-negative patients in these retrospective trials would have shown similar results with conventional chemotherapy. The value of high-dose therapy, particularly in ALL patients being early

MRD negative after induction therapy, has to be evaluated in prospective trials.

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## 72.9 Allogeneic HCT

### 72.9.1 Indication

Standard therapy for patients with high-risk ALL in CR1 (see Sect. 72.5) and standard therapy for patients with subsequent remission after induction failure or relapsed ALL (Giebel et al. 2019). Optional for patients with standard-risk ALL in CR1 and unexpected treatment-related toxicities (e.g., prolonged severe cytopenia), which preclude continuation of conventional therapy. Optional for patients with refractory/active ALL (Pavlu et al. 2017).

### 72.9.2 Conditioning

For fit patients <45 years and no relevant comorbidities, preferably fractionated TBI (cumulative dose of 12–13 Gy) in combination with CY or VP (Marks et al. 2006); alternative BU (preferable IV BU targeted plasma-drug level monitoring) in combination with CY although this may be associated with higher relapse rates (Kebriaei). For patients aged 45 years and older, dose-adapted/dose-reduced conditioning should be considered. So far, no standard regimen has been established. Reasonable options are TBI-based therapies (e.g., 8 Gy TBI in combination with FLU or CY) and MEL-, BU-, or TREO-based conditioning regimes.

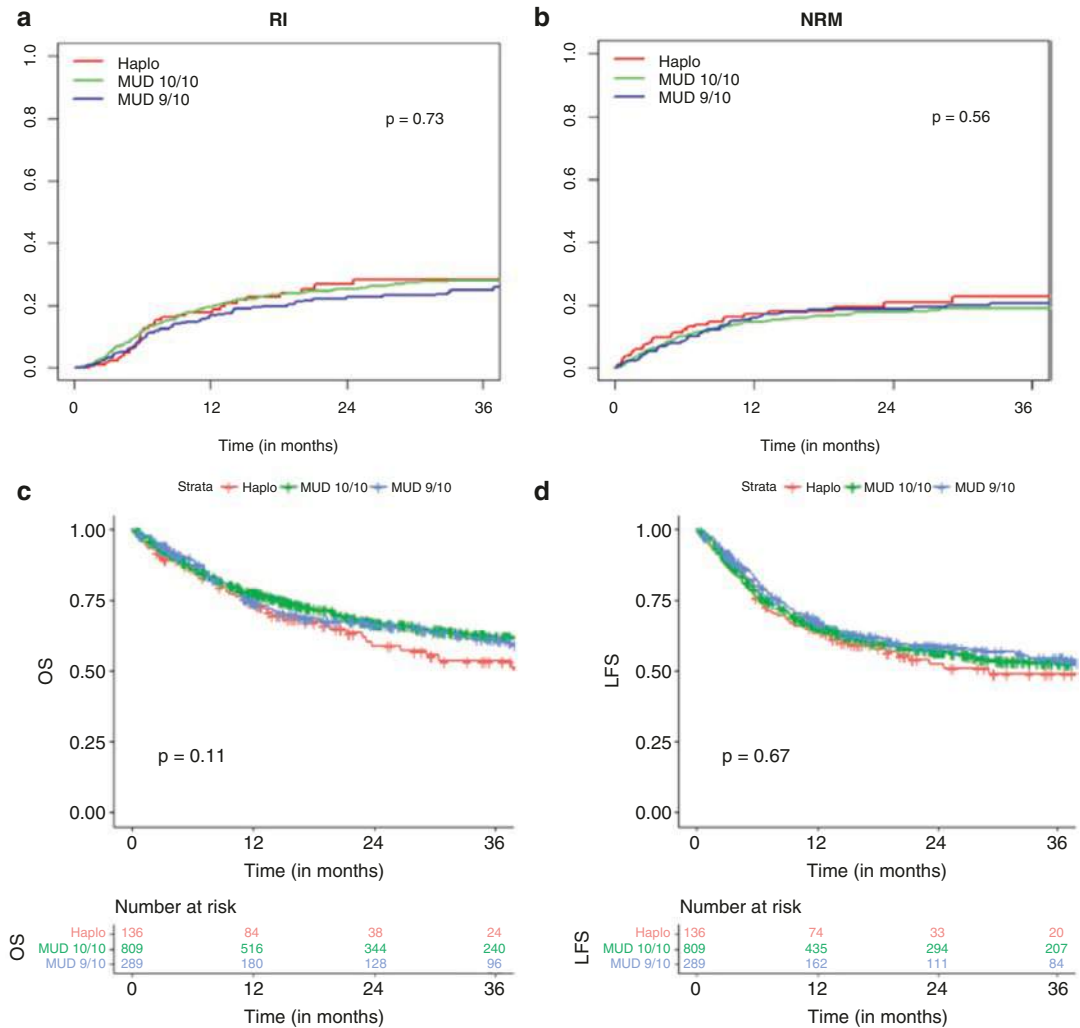
Especially patients transplanted beyond first remission are at risk for severe transplant-related toxicities with cumulative incidence of death in remission exceeding 30% and more. Consequently, dose-reduced conditioning regimes should be discussed in patients being in a MRD-negative subsequent remission after treatment with novel antibody-based salvage therapies. Moreover, conditioning therapies associated with significant toxicities (e.g., SOS/

VOD for patients treated with inotuzumab ozogamicin) must be avoided.

### 72.9.3 Donor

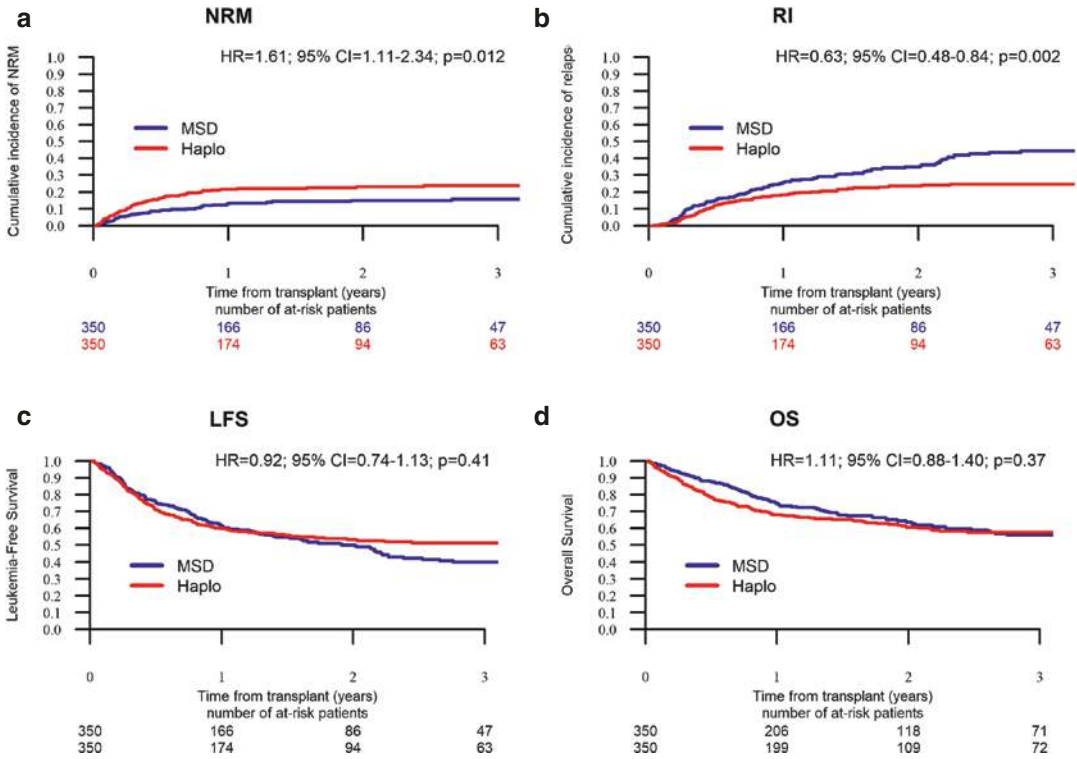
MSD, HLA-MUD (at least matched for HLA-A, HLA-B, HLA-C, and DR), HLA-MMUD, hap-

loidentical donor. Results of a retrospective, EBMT registry-based analyses showed comparable results for all donor types (Shem-Tov et al. 2020; Nagler et al. 2021) (Figs. 72.2 and 72.3).



**Fig. 72.2** Outcome of matched unrelated donor, mismatched unrelated donor, and haploidentical donor—HCT performed between 2007–2016 for adults with ALL

in CR1. (a) Relapse incidence (RI), (b) non-relapse mortality (NRM), (c) leukemia-free survival (LFS), (d) overall survival (OS) (Shem-Tov et al. 2020)



**Fig. 72.3** Outcome of *matched sibling donor and haploidentical*—hematopoietic cell transplantation for adults with acute lymphoblastic leukemia (ALL) in first or second complete remission (CR1). Changes over time in the

period 1993–2012. (a) non-relapse mortality (NRM), (b) relapse incidence (RI), (c) leukemia-free survival (LFS), (d) overall survival (OS) (Nagler et al. 2021)

## 72.10 Therapeutic Algorithm Recommended by the Authors

### 72.10.1 Stem Cell Source

Most likely no relevant difference with regard to GvHD between BM and PBSC as transplant source from an unrelated donor when ATG is part of the conditioning. Faster engraftment and low risk of graft failure with PBSC. For T-replete haploidentical HCT using post-transplant CY, BM may be associated with improved OS and GRFS (Nagler 2020).

### 72.10.2 GvHD Prophylaxis

CSA + MTX or CSA + MMF are standard options. ATG should be considered in all patients receiving an allograft from an unrelated donor although in registry-based analyses it was associated with increased risk of relapse for both Ph and Ph + ALL. Using post-transplant CY instead of ATG is recommended for haplo-HCT, using T cell replete allografts. It is also an option for allo-HCT from MMUD and MUD.

### 72.10.2.1 Maintenance

For patients with Ph + ALL, maintenance with TKI after allo-HCT should be applied as a prophylactic or preemptive therapy. The choice of TKI should take into account previous TK muta-

tion analyses. At least in patients with B-ALL and positive findings for MRD after allo-HCT, preemptive therapies with antibodies/antibody-drug conjugates or CAR-T cells are valuable options to be evaluated in prospective trials.

#### Key Points

Allo-HCT indication	– CR1: Ph + ALL, high-risk Ph- ALL <sup>a</sup> – >CR1: all patients with no contraindication for allogeneic HCT	
Donor	MSD > (MUD = MMUD = Haplo)	
Conditioning	– <45 years: TBI/CY; TBI/VP; TBI/CY/VEP, IV BU/CY. TBI probably associated with lower relapse rates, TBI dose for patients <45 years: Cumulative 12–13 Gy – >44 years (or < 45 + contraindication for MAC) FLU/IV BU; FLU/MEL; FLU/TBI 8 Gy; FLU/TREO; TBF <sup>b</sup>	
Source of SC	PB/BM	
GvHD prophylaxis	CSA + MTX or CSA + MMF (ATG in MUD, MMUD, MSD); post-transplant CY in haplo (consider in MMUD, MUD)	
Maintenance	TKI in case of Ph + ALL (prophylactic or preemptive)	
TRM	CR1 (age 18–55 years)	MSD: 11–24% (2 years) MUD: 19–23% (3 years)
	CR1 (age > 60 years)	MSD: Approx. 23% (3 years) MUD: Approx. 24% (3 years)
	CR2	MSD/MUD/MMUD/haplo: 28–35% (2 years)
REL	CR1 (age 18–55 years)	MSD: 23–32% (2 years) MUD: 14–21% (2 years)
	CR1 (age > 60 years)	MSD: Approx. 47% (3 years) MUD: Approx. 35% (3 years)
	CR2	MSD/MUD/MMUD/haplo: 33–38% (2 years)
OS	CR1 (age 18–55 years)	MSD: 60–76% (2 years) MUD: 62–70% (2 years)
	CR1 (age > 60 years)	MSD: Approx. 39% (3 years) MUD: Approx. 46% (3 years)
	CR2	MSD/MUD/MMUD/haplo: 38–47% (2 years)

<sup>a</sup> Definition of “high risk” differs between study groups; most important risk factors: persisting MRD after two or more courses of therapy, high initial leukocyte count, high-risk cytogenetic

<sup>b</sup> For patients treated with inotuzumab ozogamicin, avoid regimens associated with SOS/VODS

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# Acute Lymphoblastic Leukaemia in Children and Adolescents

# 73

Christina Peters, Franco Locatelli, and Peter Bader

## 73.1 Introduction

Acute lymphoblastic leukaemia (ALL) is the most common paediatric cancer; approximately 60% of ALL cases occur in children and adolescents younger than 20 years (Inaba and Pui 2021). Allogeneic haematopoietic cell transplantation (HCT) became the most commonly applied cellular immunotherapy and the standard of care for children with ALL who are either at high risk for relapse or had previously experienced a disease recurrence. HCT represents a successful therapeutic option and a relevant proportion of patients achieved long-term survival (Balduzzi et al. 2019). The most fre-

quent cause of treatment failure is relapse after allogeneic HCT. The risk of post-transplant relapse is influenced by several factors, including remission status at transplantation, conditioning regimen and donor type (Peters, et al. 2021). Strategies aimed at reducing the risk of treatment failure have included a reduction of minimal residual disease (MRD) pre-transplant (Bader et al. 2019), the substitution of toxic chemotherapy before transplantation with bispecific antibodies (Locatelli et al. 2021), replacement of HCT with chimeric antigen receptor (CAR) T-cell therapy (Maude et al. 2018a, b), improved transplant strategies for specific groups, including infants (Pieters et al. 2019), adolescents and young adults (AYA) (Diesch-Furlanetto et al. 2021) and innovative prophylaxis and treatments for acute and chronic graft-vs.-host disease (GvHD). Furthermore, therapeutic drug monitoring with dose adjustment of some drugs, including busulfan (Diesch-Furlanetto et al. 2021) and novel radiation techniques might enable a more individualised approach (Hoeben et al. 2021).

To offer the patients the best available treatment options, a close collaboration between international study groups and transplant consortia is necessary. As an example, this cooperation was realized within the treatment consortia for childhood leukaemia (e.g. IBFM-SG, IntReALL, NOPHO, UKALL, AIEOP, FRALLE and others) and the paediatric transplant community (e.g. EBMT-PD WG, IBFM-SC SCT,

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GETMON and GITMO). ALL trial groups assess outcome according to their chemotherapy protocols and stratify patients into standard, intermediate and high relapse risk groups. In contrast to adults, only high-risk patients are eligible for allo-HCT in first complete remission (CR).

### 73.2 Prognostic Factors and Indications for HCT

HCT indications have to be defined prospectively and must be re-evaluated and regularly revised at intervals dependent on modifications and improvements in non-transplant approaches for both front-line and relapse protocols. Some risk factors such as recurrent molecular lesions predicting a poor outcome conveying a dismal prognosis in childhood ALL can be identified at diagnosis (Moorman 2016; O'Connor et al. 2018). In addition, the response to induction therapy measured by MRD levels has a strong prognostic value and now defines many indications for HCT (Bader et al. 2009; Conter et al. 2010; Schrappe et al. 2011; Eckert et al. 2013; Berry et al. 2017).

#### 73.2.1 Indications: CR1

Only patients with high-risk cytogenetic features or unsatisfactory response to chemotherapy are eligible for HCT in first remission. In contrast to earlier recommendations, for these patients an MSD and an MUD and for the highest relapse category also mismatched donors (MMD) are an option (Truong et al. 2021). In the ongoing AIEOP-BFM 2017-trial, the very-high-risk subgroup with an indication for HCT in first CR is defined by

1. The presence of TCF3-HLF gene fusion,
2. KMT2A-AFF1 gene fusion,
3. Hypodiploidy defined as <44 chromosomes,
4. IKZF1<sup>plus</sup> deletions and medium-risk/high-risk MRD levels at the end of consolidation,
5. PCR-MRD HR, and
6. T-ALL with PPR and/or FCM-MRD d15 HR and/or IF.

Patients with MRD negativity at EOI are excluded from a HCT indication. MMD-HCTs are nowadays reserved for a PCR-MRD  $TP2 \geq 5 \times 10^{-3}$ , all TCF3-HLF fused leukaemias and those with induction failure (Table 73.1).

**Table 73.1** Indications for allogeneic HCT in CR1 according to AIEOP-BFM ALL 2009-trial

		PCR-MRD results <sup>a</sup>				
		MRD-SR	MRD-MR <sup>b</sup>	MRD-HR		No MRD result
	MRD TP2 $\geq 10^{-3}$ to $<10^{-2}$			MRD TP2 $\geq 10^{-2}$		
Criteria hierarchical	No CR d33	No <sup>c</sup>	MMD	MMD	MMD	MMD
	t(4;11) <sup>d</sup>	No	MD	MD	MMD	MD
	Hypodiploidy <44 chromosomes <sup>e</sup>	No	MD	MD	MMD	MD
	PPR + T-ALL	No	No	MD	MMD	MD
	None of the above features <sup>f</sup>	No	No	MD	MMD	No

PPR Prednisone Poor Response on day 8, NRd33 No Remission on day 33 MRD Minimal Residual Disease, no Allo HCT not indicated, MD Permitted donor: HLA-matched sibling or non-sibling donor, MMD Permitted donor: HLA-matched or HLA-mismatched donor

<sup>a</sup>FCM-MRD results have no impact on the allo-HCT indication

<sup>b</sup>Including MRD-MR SER (MRD TP1  $\geq 10^{-3}$  and TP2  $10^{-4-5}$ )

<sup>c</sup>Non-remission in patients with this rare constellation should be due to extramedullary disease. Allo-HCT indication in these cases should be discussed with the national study coordinator

<sup>d</sup>Independent of prednisone response

<sup>e</sup>The finding of exactly 44 chromosomes qualifies for HR treatment but has no impact on allo-HCT indication

<sup>f</sup>Including patients with 44 chromosomes

**Table 73.2** Indication for HCT according to IntReALL SR 2010 and HR protocol criteria

Relapse risk group	Phenotype	Time of relapse	Site of relapse	MRD-status	Donor type
Very high	T-ALL	Any time	I-BM, C-BM, I-EM		MSD, MD, MMD
	Non-T-ALL	Very early	I-BM, C-BM, I-EM		
		Early	I-BM, C-BM	PR, ND	
High	Non-T-ALL	Late	I-BM, C-BM	PR, ND	MSD, MD
		Early	C-BM	GR	
			I-EM		
		Late	I-BM	PR, ND	
			C-BM	ND	

*I-BM* isolated bone marrow, *C-BM* combined bone marrow and extramedullary site, *I-EM* isolated extramedullary, *MRD*: *GR* good response as defined by the specific chemotherapy-protocol, *PR* poor response, *ND* not detectable

### 73.2.2 Indications: CR2 and Later

All patients with relapse of T-ALL and patients with B-ALL who relapse during frontline therapy or within 6 months from treatment discontinuation (very early and early relapse) have a dismal prognosis when treated with conventional chemotherapy. Allo-HCT from any donor type is the contemporary standard post-remission consolidation therapy (Table 73.2a).

In multiple relapsed patients, if they achieve a third or higher remission, allo-HCT should be considered in all patients, provided that their condition allows such a procedure. Patient not in morphological remission should not be transplanted except in extraordinary experimental situations (e.g. well-designed clinical trial with clear scientific questions).

## 73.3 Donor Selection and Stem Cell Source

OS and incidence of NRM in patients transplanted from a MSD as well as from a > 9/10 MUD have steadily improved over time, and today the results for these outcomes do not differ according to the type of donor. However, it has been shown that in children HCT from an HLA-identical sibling results in a faster myeloid engraftment, prompter immunoreconstitution and less severe infections and should remain the preferred option (Peters et al. 2015a, b).

Since less than 25% of patients have a MSD, HCT from an alternative donors is most frequently applied. Several groups have demonstrated that an HCT from a MUD, identified by HLA high-resolution typing, has a similar outcome as a MSD-HCT (Zhang et al. 2012; Fagioli et al. 2013; Burke et al. 2015) Notably, Dalle et al. recently reported the 3-year consolidated results of 612 patients aged 4–21 years, treated at >100 pediatric centers in 26 countries following HCT from either MSD ( $n = 186$ , 30%) or MUD (defined as 9 or 10/10 4-digit molecular HLA compatibility,  $n = 426$ , 70%) following 12Gy TBI-VP16 for ALL in CR1. GvHD-relapse-secondary malignancy-free survival (GRFS) was significantly better in MUD vs. MSD ( $62 \pm 3$  vs.  $\pm 51 \pm 5\%$ ,  $P = 0.04$ ) (Dalle et al. 2022).

Several methods were developed to overcome the HLA barriers in those patients who lack an HLA compatible donor. Currently, it is not clear which of the following, HLA-mismatched CB, TCD (alpha-beta depleted, CD34+ selected or CD3/CD19 depleted) haplo-identical grafts or PT-CY approaches, will result in the best outcome (Lang and Handgretinger 2008; Smith et al. 2009; Ruggeri et al. 2014; Locatelli et al. 2017; Rocha, et al. 2021) (Tables 73.3 and 73.4). Very promising results have been recently confirmed in children with ALL receiving a graft from an  $\alpha/\beta$  T cell depleted HLA-haploidentical relative, with a very low risk of NRM and of both acute and chronic GvHD (Merli et al. 2022).

**Table 73.4** Donor hierarchy—further selection criteria

Variable/ order	Priority
<i>CMV-status</i>	
Patient CMV IgG positive	
1	Donor CMV IgG positive
2	Donor CMV IgG negative
Patient CMV IgG negative	
1	Donor CMV IgG negative
2	Donor CMV IgG positive
<i>Gender</i>	
(Locatelli et al. 2022)	
1	Male or female (preferentially not Allo-immunized by prior pregnancy) donor
Male patient	
1	Male donor
2	Female (preferentially not Allo-immunized by prior pregnancy) donor
<i>Age</i>	
1	Younger donor if body weight enables sufficient SC harvest
2	Older donor
<i>Stem cell source</i>	
HCT from MSD or MD	
1	Bone marrow
2	PPBSC (CAVE: Adjust GvHD-prophylaxis for matched siblings)
2	Cord blood with sufficient cell number (>3 × 10 <sup>7</sup> NC/kg)
HCT from MMD: Possible options	
	BM, 8/10 matches, unmanipulated
	PBSC, haploidentical, CD3/CD19 depleted, α/β depleted
	CB, sufficient stem cell dose
	PBSC, haploidentical, CD34+ selection
	PT-CY

**Table 73.3** Matching criteria according to HLA typing/matching and stem cell source for children and AYAs with ALL

	HLA-genotypically matched sibling, or 10/10 allelic match (if parental haplotypes unknown)	BM, PBSC
MSD	6/6 or 8/8, 5/6 or 7/8 <sup>a</sup>	CB
MD	9/10 or 10/10 allelic matched related or unrelated	BM, PBSC
MD	5–6/6 unrelated or 6–7–8/8 unrelated	CB
MMD	Less than 9/10 matched	BM, PBSC
MMD	Less than 5/6 or 6/8 UCB	CB

MSD matched sibling donor, MD matched donor, MMD mismatched donor

<sup>a</sup>4 digits high-resolution typing recommended also for CB matching definition

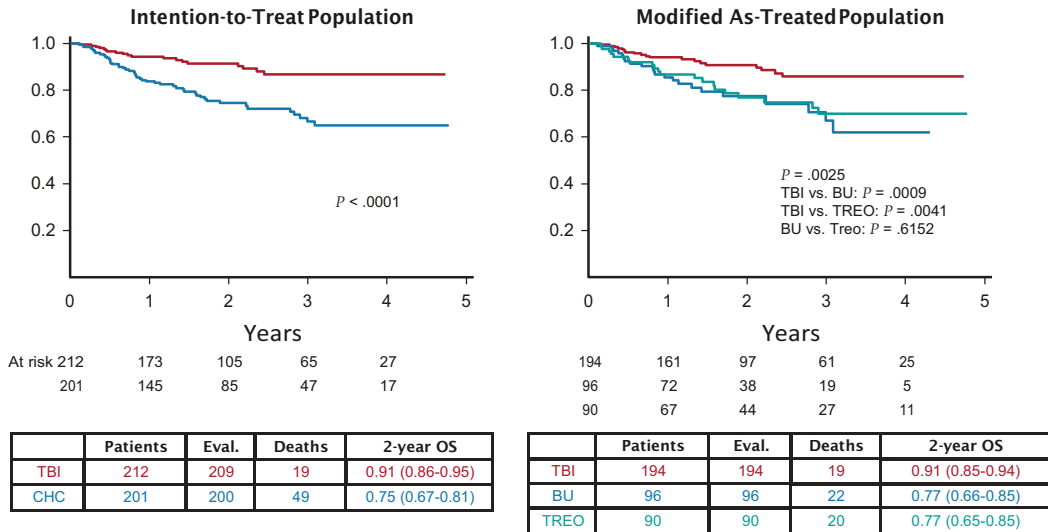
Other factors to be considered in the choice of the donor are detailed in Tables 73.3 and 73.4. In particular, the donor/recipient CMV serology plays a relevant role in the post-transplant outcome as well as the donor age.

### 73.4 Conditioning Regimen

A myeloablative conditioning regimen is the treatment of choice for children and AYA with ALL. The FORUM trial demonstrated the significant superiority of the TBI/etoposide scheme in a randomized, prospective, multicenter, phase III trial. At a median age of 4.5 years, patients over 4 years of age with high-risk ALL had a 20% better OS and EFS and a lower NRM compared to patients who received a chemotherapy-based conditioning regimen.(Locatelli et al. 2022). In addition, the risk of leukaemia recurrence was much lower in patients given TBI as part of the conditioning regimen in comparison to those prepared with a chemo-therapy-based myeloablation. The same study showed that a myeloablative regimen containing treosulfan, thiotepea and fludarabine was associated with an outcome comparable to that of patients treated with busulfan, thiotepea and fludarabine (Peters, et al. 2021) (Fig. 73.1).

Whether a myeloablative conditioning with treosulfan will have a more benign adverse event profile and a higher chance for fertility preservation remains to be proven (Wachowiak et al. 2011; Boztug et al. 2015; Lee et al. 2015; Faraci et al. 2019).

Whenever possible, the interval between the end of the last chemotherapy and the start of the conditioning regimen should be 3–6 weeks to reduce the risk of NRM. If infection or toxicity requires a delay in conditioning, patients receive risk-adjusted chemotherapy or immunotherapy to bridge the time to transplant.The most successful approach to allow a reduction of MRD before transplantation is the use of blinatumomab. The continuous infusion of the bispecific antibody enables not only a deeper remission but also to spare chemotherapy-associated organ toxicity which results in better pre-transplant performance status (Locatelli et al. 2000a, b).



Primary end point: Overall survival. BU, busulfan; CHC, chemo-conditioning; CIR, cumulative incidence of relapse; EFS, event-free survival; OS, overall survival; TBI, total body irradiation; TREO, treosulfan; TRM, treatment-related mortality.

**Fig. 73.1** Outcome of the randomized, prospective, multicentre phase III FORUM-study: (© 2020 by American Society of Clinical Oncology)

### 73.5 GVHD Prophylaxis

Children transplanted with bone marrow from MSD might benefit from an augmented GVL effect if only cyclosporine A is administered as GVHD prophylaxis (Locatelli et al. 2000a, b; Peters et al. 2010). However, careful monitoring and rapid intervention are crucial to prevent the development of severe GVHD. After HCT from non-sibling donors, a combination of a calcineurin inhibitor with short-course MTX and ATG is given in most patients (Peters et al. 2015a, b; Veys et al. 2012a, b; Balduzzi et al. 2019).

### 73.6 Children below 4 Years of Age

Both relapse and NRM contribute to treatment failure in infants and young children with high-risk ALL undergoing HCT. The optimal chemotherapeutic approach able to improve event-free survival (EFS) and to reduce the risk of NRM is not yet defined (Peters et al. 2022).

#### 73.6.1 Mixed Chimerism (MC) and MRD

*Mixed chimerism (MC) and MRD* strongly predict risk for relapse in children (Bader et al. 2015).

Preemptive immunotherapy, e.g. withdrawal of IS or DLI guided by chimerism and MRD monitoring, may prevent impending relapse. However, the dynamic of leukaemic reappearance hampers the final success of these methods. Therefore, new post-transplant intervention strategies with less risk for severe complications like bi-specific antibodies or CAR-T-cell interventions may expedite the control of impending relapse (Handgretinger et al. 2011; Maude et al. 2018a, b).

#### 73.6.2 Children with Ph + ALL

*Children with Ph + ALL* should receive TKIs post-transplant for relapse prevention. Whether the prophylactic approach (all Ph + patients will

receive TKIs) or a preemptive therapy (only patients with a persistence/reappearance of BCR/ABL fusion transcript) is more effective has yet to be demonstrated (Schultz et al. 2010; Bernt and Hunger 2014). Both strategies are currently under investigation.

### 73.6.2.1 The Amended EsPhALL Recommendation

Administration of imatinib prophylaxis post HCT when more than 50,000 platelets are reached is recommended with a duration of 365 days after HCT.

### 73.6.2.2 TKI According to MRD Result

Administration of imatinib post HCT for all MRD-positive patients until two negative results are achieved. FACS- and PCR-MRD analyses are accepted.

#### Key Points

- Only children and adolescents with very high or high relapse risk should be candidates for allo-HCT in CR1 and CR2. The assessment of relapse risk is largely influenced by the presence of recurrent molecular and cytogenetic abnormalities, response to chemotherapy, assessed through MRD evaluation and in relapsed patients – by time and site of relapse.
- MRD levels pre- and post-HCT are powerful predictors for outcome after HCT.
- Patients who are not in morphological remission before conditioning should not undergo allogeneic HCT except in extraordinary situations.
- Fractionated TBI/VP16-MAC is recommended for all children above the age of 4 with ALL. If this is not possible, a myeloablative chemo-conditioning is an option.

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# Myelodysplastic Neoplasms/ Syndromes (MDS)

# 74

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## 74.1 Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterized by peripheral cytopenias and dysplastic features in blood and bone marrow. The natural history of these diseases varies from an indolent course, over a number of years, to a more rapid transition into secondary acute myeloid leukemia (AML). MDS is mainly diagnosed in elderly patients, with an annual incidence of 4.9/100,000 person/year, increasing up to 20–50 cases after the age of 60. In 2022, two classifications for MDS/AML have been published, an update of the WHO classification and the International Consensus Classification (ICC) (Khoury et al. 2022; Arber et al. 2022). As compared to the previous WHO 2016 classification, these 2 new proposals for MDS classification include genetic classification and do not consider marrow blast percentage as the single criteria to define MDS or AML. Both classifications recognize *SF3B1*

mutated MDS and *TP53* biallelic mutated MDS as specific entities. Notably, several cases of MDS will now be defined as MDS/AML when they will show 10–19% BM blasts, recognizing the diagnostic continuum between these two nosologic entities.

Due to the variable course the disease may take, a number of different *risk-scoring systems* have been developed. The initial one is the International Prognostic Scoring System (IPSS) (P. Greenberg et al. 1997) revised in 2012 to account also for the degree of cytopenias (P. L. Greenberg et al. 2012) (Table 74.1). The importance of transfusion dependency is included in a WHO classification-based prognostic scoring system (WPSS) (Della Porta et al. 2015).

The role of somatic mutations has been explored recently, highlighting their prognostic impact, now taken into account in the most recent classifications. For instance, *SF3B1* mutations are commonly associated with refractory anemia with ringed sideroblasts and expected survival of >10 years. Poor prognostic genomic alterations, such as *TP53* mutations, occur mainly in patients with HR-MDS (especially those causing biallelic inactivation) and confer a higher risk of transition to AML (Bernard Elsa et al. 2022). The urge to incorporate such molecular information into MDS prognostication led to the development of the molecular IPSS (IPSS-M), currently being validated in different variety of MDS settings (Bernard Elsa et al. 2022; Sauta et al. 2023;

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**Table 74.1** “Revised IPSS”

Points	0	0.5	1	1.5	2	3	4
Marrow blast	<3		3–4		5–10	>10	
Cytogenetic <sup>a</sup>	Very good		Good		Intermediate	Poor	Very poor
Cytopenia <sup>b</sup>	No	Mild	Moderate	Severe anemia			
Risk	<i>Very low</i>	<i>Low</i>	<i>Intermediate</i>	<i>High</i>	<i>Very high</i>		
Number of points	≤ 1.5	2–3	4–4.5	5–6	>6		
Median OS (years)	8.8	5.3	3	1.6	0.8		
Median time to 25% AML transformation in years		10.8	3.2	1.4	0.73		

<sup>a</sup>Cytogenetics: *very good*, -Y, del(11q); *good*, normal, del(5q), del(12p), del(20q), double including del(5q); *intermediate*, del(7q), -8, -19, i(17q), any other single or double independent clones; *poor*, -7, inv.(3)/t(3q)/del(3q), double including -7/del(7q), complex, 3 abnormalities; *very poor*, complex, >3 abnormalities

<sup>b</sup>Cytopenia, *mild cytopenia*, platelet count <100 × 10<sup>9</sup>/L or neutrophil count <0.8 × 10<sup>9</sup>/L; *moderate cytopenia*, hemoglobin <10 g/dL but >8 g/dL, platelet count <50 × 10<sup>9</sup>/L; *severe anemia*, hemoglobin <8 g/dL

Kewan et al. 2023). The IPSS-M can be calculated using <https://mds-risk-model.com>.

In the setting of allo-HCT, both somatic mutations and cytogenetic characteristics conserve their prognostic impacts after transplantation, and this aspect will be discussed further hereafter.

In the 3 prospective trails based on donor availability (Robin et al. 2015; Kröger et al. 2021; Nakamura et al. 2021) higher risk patient benefit more from allogeneic stem cell transplantation than from conventional therapy. Higher risk categories are patients (HR-MDS) classified intermediate-2 or high according to the classical IPSS, as compared to intermediate-1 and low risk patients (LR-MDS). Survivals of these higher risk patients may correspond to high and very high risk with the IPSS-M, but this needs to be confirmed. Especially LR-MDS are very heterogeneous with expected median survival between 3 and 10 years. As a result, intensive treatment strategies are predominantly applied in patients with HR-MDS, whereas LR-MDS tend to be treated conservatively (Robin et al. 2015; Kröger et al. 2021; Nakamura et al. 2021).

Allo-HCT is increasingly performed in MDS. Data from the EBMT registry show that 2591 MDS patients were transplanted in 2019, increasing from 946 patients in 2006. This has been the consequence of an expanded access to HCT also in older patients (>60 years), represent-

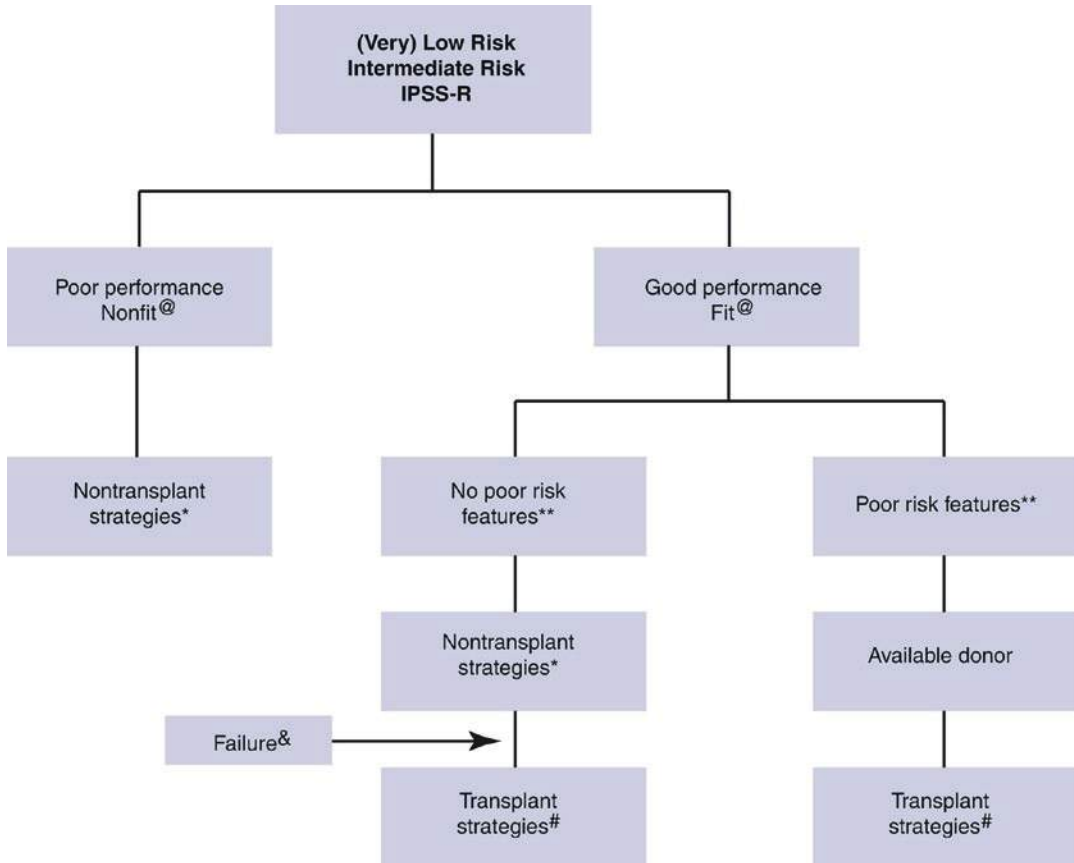
ing 52% of all transplants in 2019 vs. 24% in 2006, as well as the recourse to more MURD (43% vs. 34%, respectively). The use of HLA mismatched related donor, mainly haplo-identical donor, has indeed grown from 4.5% in 2006 to 20.5% in 2019.

## 74.2 Indication of HCT in MDS and Timing to Transplant

HCT is an established procedure for MDS leading to potential long-term survival. The indications for HCT may change following the introduction of new treatment strategies, and the HCT approach itself has consistently evolved over time. NRM should always be balanced against the benefits associated with HCT. Prospective trials based on donor availability showed a gain in life expectancy in HR-MDS patients who have a donor (Robin et al. 2015; Kröger et al. 2021; Nakamura et al. 2021). Retrospective studies showed that LR-MDS patients do not benefit from upfront HCT (Cutler et al. 2004; Koreth et al. 2013). International expert panel has confirmed the indication of HCT in HR-MDS and in LR-MDS when they have or acquire specific poor prognostic features, including genetic alterations, failure to respond to usual treatment, life-threat-

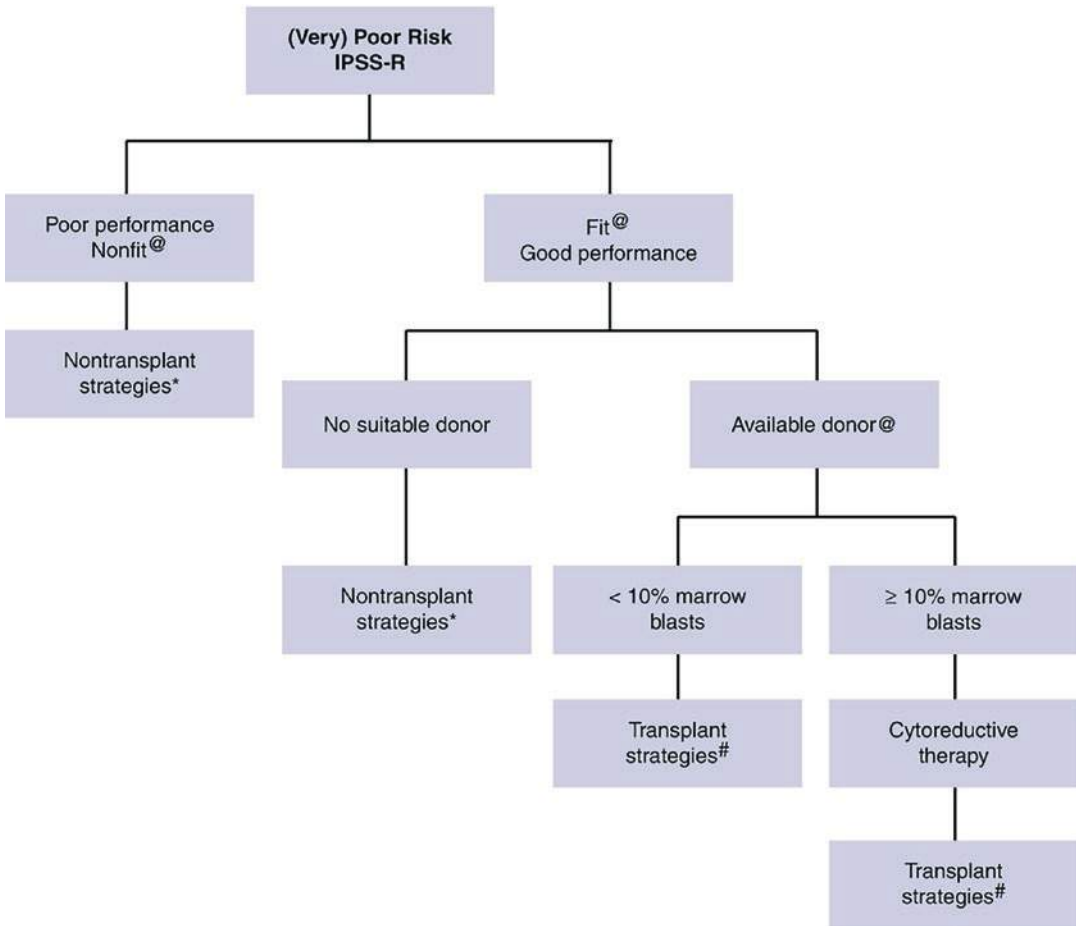
ening cytopenias, and high transfusion burden (de Witte et al. 2017; DeFilipp et al. 2023). Figures 74.1 and 74.2 summarize transplant indications in MDS patients. Currently, efforts to incorporate molecular scores to guide decision for HCT are under investigations, including

the use of the new IPSS-M emphasizing that not only disease-specific but also transplant-related characteristic must be considered in the prognosis of MDS patients undergoing HCT (Sauta et al. 2023; Gurnari et al. 2023).



**Fig. 74.1** Therapeutic flow chart for adult MDS patients with (very) low-risk or intermediate-risk IPSS-R scores @ indicates nonfit (patients with multiple comorbidities and/or poor performance) or fit (patients with no comorbidities and good performance status). \* indicates nontransplant strategies according to most recent versions published by international MDS expert groups, including ELN and NCCN. & indicates failure of nontransplant strategies. \*\* indicates poor-risk features (defined as poor-risk cytogenetic characteristics, persistent blast

increase [ $>50\%$  or with  $>15\%$  BM blasts], life-threatening cytopenias, high transfusion burden  $>2$  units per months for 6 months; molecular testing should be considered, in case of absence of poor-risk cytogenetic characteristics or persistent blast increase). # indicates transplant strategies (all forms of HCT, for details of the donor selection, type of conditioning, and post-transplant strategies, see text; no upper age limit if patients are fit, without serious comorbidity, and with good Karnofsky status)



**Fig. 74.2** Therapeutic flow chart for adult MDS patients with poor IPSS-R scores. @ indicates nonfit (patients with multiple comorbidities and/or poor performance) or fit (patients with no comorbidities and good performance status). \* indicates nontransplant strategies according to most recent versions published by international MDS expert groups, including ELN and NCCN. & indicates failure of nontransplant strategies. \*\* indicates poor-risk features (defined as poor-risk cytogenetic characteristics, persistent blast increase [ $>50\%$  or with  $>15\%$  BM blasts],

life-threatening cytopenias, high transfusion burden  $>2$  units per months for 6 months; molecular testing should be considered, in case of absence of poor-risk cytogenetic characteristics or persistent blast increase). # indicates transplant strategies (all forms of HCT, for details of the donor selection, type of conditioning, and post transplant strategies, see text; no upper age limit if patients are fit, without serious comorbidity, and with good Karnofsky status)

### 74.3 Treatment Prior to HCT

Pregraft therapy is still a matter of debate. Upfront transplantation, hypomethylating agents alone or in combination, or chemotherapy may be all viable options in HR-MDS, although more studies are needed to determine the precise allocation of these therapy. The use of pregraft ther-

apy may prevent transformation into AML in cases whereby the transplantation cannot be performed in a timely fashion.

International guidelines generally recommend with a low level of evidence that patients with more than 10% marrow blast should receive cytoreductive treatment (de Witte et al. 2017; DeFilipp et al. 2023). Refractoriness to pregraft treatment



is generally associated with poor outcomes (Potter et al. 2016). As shown by the BMT-AZA prospective study, azacitidine bridge in HR-MDS is feasible before HCT (Voso et al. 2017).

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#### 74.4 Preparative Regimen

The use of RIC regimens for HCT has raised considerable interest. Multiple centers have developed novel RIC regimens that have reduced NRM and morbidity and subsequently expanded the curative potential of HCT to older individuals who have historically not been considered to be HCT candidates. An EBMT prospective study including 120 patients and comparing the use of RIC (FLU/BU) and MAC (CY/BU) in patients with MDS or secondary AML (Kröger et al. 2017) failed to show any impact of the regimen intensity with regards to OS and RFS. The BMT-CTN performed a prospective study on 272 patients with MDS or AML who were randomized between RIC and MAC. In the MDS subgroup, there was no difference in OS, despite a higher relapse rate after RIC (Scott et al. 2017). Recently, the CIBMTR performed a registry analysis based on a Disease Risk Index (DRI) in 4387 adults with MDS and AML (aged 40–65 years). While in low/intermediate DRI cases, RIC associated with lower DFS rates (HR, 1.19;  $P = 0.001$ ), in high/very high DRI, DFS was similar regardless of conditioning intensity (Bejanyan et al. 2021). A phase 3 trial comparing RIC and sequential regimen has also reported similar outcomes in both arms (Craddock et al. 2021).

Nowadays, the conditioning regimen is still based on the fitness of the patients, using MAC only in eligible, fit and generally younger patients.

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#### 74.5 Post-HCT Outcomes

As aforementioned, post-HCT outcomes depend both on disease- and transplant-specific risk factors. Poor and very poor risk cytogenetics, includ-

ing monosomal karyotypes, and HR-MDS are associated with poorer outcomes. Age and hematopoietic cell transplant-comorbidity index (HCT-CI) are patient-specific risk factors. An EBMT study defined a transplant-specific risk score including age, donor type, performance status, cytogenetic category, recipient's cytomegalovirus status, percentage of blasts, and platelet count, which outperformed previous scoring systems specifically predicting post-HCT outcomes (Gagelmann et al. 2019). Somatic mutations, i.e., biallelic *TP53* inactivation, *ASXL1*, *RUNX1*, and *RAS* pathways mutations, have also been reported to be prognostic independent players (Lindsley et al. 2017; Sauta et al. 2023).

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#### 74.6 Alternative Donors and Donor Choice

While MUD and HLA-matched sibling donor are both suitable options in MDS patients (de Witte et al. 2017), as well as in other diseases, the recourse to haplo-identical donor have progressively increased. Conversely, unrelated cord blood units are less used, due to the limited number of cells and the slow hematological and immunological recovery. In recent analyses of the EBMT registry, transplantation from an haplo-identical donor showed promising results, albeit without reaching the outcomes of HLA-matched unrelated or related donors, due to an excess risk of NRM (Raj et al. 2022; Robin et al. 2017, 2019). A specific issue is the impact of donor age on post-transplant outcomes, suggesting the use of younger donors (Kröger et al. 2013; Murthy et al. 2022). This is particularly relevant in MDS, where both recipients and related donors are typically old.

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#### 74.7 Post-HCT Treatment

MDS patients with relapse after HCT become often refractory to treatment, or are not fit enough to be treated. The main risk factors for treatment response are time from transplantation

to relapse and percentage of marrow blast with better prognosis in patients who have only a molecular relapse. An EBMT study involving 181 MDS patients treated with AZA for post-HCT relapse confirmed that lower blast counts upon relapse and a time gap of >6 months after HCT were both good prognostic factors (Craddock et al. 2016). In this study, the addition of DLI did not modify outcomes. Another EBMT study exploring the use of cellular therapy after relapse (DLI or second transplant) showed that a second allo-HCT performed in CR may rescue some patients, especially those with no prior history of GVHD, and for whom a new donor is available (Schmid et al. 2018). In a French SFGM-TC study of 147 MDS patients relapsing after transplant (Guièze et al. 2016), only those receiving DLI or second HCT were able to achieve long-term survival (32% versus 6% for chemotherapy alone).

Besides DLI, other strategies involve preventive or preemptive treatment after transplantation to avoid morphological relapse (maintenance). These strategies based on the underlying risk or monitoring of minimal residual disease may help in patients who present a high risk of post-transplant relapse, namely cases with biallelic *TP53* inactivation or complex cytogenetics (DeFilipp et al. 2023). Although relapse remains the most common cause of transplant failure, particularly in patients with high-risk features, the preemptive use of AZA or DLI may be effective in improving historically poor outcomes. Preventive post-transplant treatment testing and HMA early after transplantation have also been reported in small prospective studies and a recent systematic review and meta-analysis showed a better survival outcomes in patients receiving preventively HMA after HCT (Kungwankiatichai et al. 2022). However, the phase 3 trial randomizing 5-azacytidine to observation has not concluded to a benefit for the interventional arm (Oran et al. 2020). Trials using novel oral azacitidine formulations and targeted agents (venetoclax, enasidenib, and eprenetapopt) alone or in combinations are expected.

### Key Points

- Allo-HCT is the treatment of choice for all patients with HR-MDS who are fit enough to be considered for transplantation.
- In HR-MDS, delayed HCT is associated with reduced chances of prolonged relapse-free survival. Conversely, patients with LR-MDS may benefit from deferred HCT upon disease progression.
- Allo-HCT outcomes have improved progressively in recent years, mainly due to a gradual reduction in non-relapse mortality. Reduced-intensity conditioning (RIC) regimens have extended the use of allo-HCT to older patients, including those entering their eighth decade.
- The use of alternative donors has broadened the chance to recourse to HCT in MDS with satisfying long-term outcomes.
- A number of questions remain to be solved by prospective studies, such as the role of molecular markers in the HCT decision algorithm and the post-transplant maintenance strategies, in line of the availability of new targeted treatments.

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# Allogeneic Hematopoietic Cell Transplantation in Pediatric MDS Including Refractory Cytopenia of Childhood and in Juvenile Myelomonocytic Leukemia

Charlotte M. Niemeyer and Brigitte Strahm

## 75.1 Introduction

Most pediatric patients with MDS or juvenile myelomonocytic leukemia (JMML) can be cured by allogeneic HCT. Since MDS and JMML are associated with heterogeneous germline genetic conditions and/or somatic oncogenic mutations, specific attention to their subtypes is crucial.

## 75.2 Pediatric MDS Including Refractory Cytopenia

### 75.2.1 Classification

Pediatric MDS can be associated with germline predisposition, related to cytotoxic or immunomodulatory therapy, or occurs as de novo disease. The term primary MDS summarizes de novo MDS and MDS in germline predisposition other than the classical inherited BMF disorders. MDS can present as MDS-EB with 2–19% blasts in PB and/or 5–19% blasts in BM. Occasionally, disease with 20–30% blasts lacks clinical features of acute leukemia and behaves more like MDS than AML. In approximately 80% of MDS cases, the

BM is hypocellular. Patients with a hypocellular BM and normal blast count may present with a pattern of refractory cytopenia of childhood (RCC), a well-recognized type of BM failure in children with persistent cytopenia and evidence of dysplasia (Arber et al. 2022).

### 75.2.2 Germline Predisposition and Monosomy 7

GATA2 deficiency and SAMD9/SAMD9L syndrome emerged as the most frequent hereditary cause of primary pediatric MDS with a prevalence of 8% and 7%, respectively (Sahoo et al. 2021). Half of all cases of primary MDS with monosomy 7 arise from germline *SAMD9/9L* or *GATA2* mutations. In SAMD9/SAMD9L syndrome, somatic genetic rescue events are frequent, and spontaneous loss of monosomy 7 with hematologic recovery can occur in children <5 years of age. Germline mutations in *RUNX1*, *ETV6*, *ANKRD26* and *ERCC6L2* may also give rise to MDS in young individuals.

Considerations for HCT in predisposition syndromes listed above are generally no different from those for HCT in wildtype conditions. Retrospective analyses of EWOG-MDS indicated similar outcome of SAMD9/9L syndrome, GATA2 deficiency syndrome and wildtype when stratified according to blast count, karyotype and BM cellularity (Sahoo et al. 2021; Bortnick et al. 2021). The acute post-transplant course may,

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however, be complicated by syndrome-related comorbidities (Ahmed et al. 2019). In addition, potential family donors need to be evaluated for presence of the respective underlying predisposition.

### 75.2.3 MDS-EB with *UBTF-TD*, Role of Cyto-reductive Therapy Prior to HCT

Tandem duplication in upstream binding transcription factor (*UBTF*) are noted in close to a third of patient with “de novo” MDS-EB (Erlacher et al. 2022). *UBTF-TD* is associated with normal karyotype or trisomy 8, poor response to AML-type chemotherapy, and inferior outcome after HCT compared to wildtype.

While the role of intensive chemotherapy prior to HCT remains unknown, regimens like venetoclax and hypomethylating agents may bridge patients with increasing blast count to HCT (Masetti et al. 2023). In germline conditions with immunodeficiency (like *GATA2* and *SAMD9/SAMD9L* syndrome), intensive chemotherapy prior to HCT is to be avoided whenever possible.

### 75.2.4 HCT in Primary MDS with Normal Blast Count

MDS with monosomy 7 is at high risk of progression, and patients should be transplanted as soon as possible. For RCC with monosomy 7, del(7q) or  $\geq 2$  aberrations, MAC is recommended. EWOG-MDS currently advocates a TREO-based regimen, which results in prompt initial engraftment with a low incidence of secondary graft failure and an OS of approx. 90% (see <https://ewog-mds.org>). Historical data with BU/CY demonstrate an OS of 75% with NRM being the major cause of treatment failure.

In the absence of monosomy 7, RCC patients with mild cytopenia (not transfusion dependent for RBC and/or platelets, absolute neutrophil count  $> 1 \times 10^9/L$ ) may have a stable course of

disease and therefore qualify for a watch-and-observe strategy. For patients with more pronounced cytopenia treatment is stratified according to cellularity. In normo- or hypercellular BM a MAC regimen like that described for monosomy 7 can be utilized irrespective of karyotype. In hypocellular BM and normal karyotype, HCT with RIC is the treatment of choice. HCT with a preparative regimen of TT/FLU resulted in an OS of 94% and EFS of 88% (Strahm et al. 2017). However, approx. 10% of patients experience primary and secondary graft failure requiring a stem cell boost and/or second HCT. Thus, EWOG-MDS currently recommends a preparative regimen of TREO/FLU resulting in an improved rate of engraftment (see <https://ewog-mds.org>). With a very low risk of disease recurrence, GVHD should be avoided, and BM is the preferred stem cell source combined with an effective GVHD prophylaxis.

### 75.2.5 HCT in Primary MDS-EB

In a large cohort, allo-HCT with full MAC consisting of the combination of BU/CY/MEL resulted in a probability of OS at 5 years of 63%, with TRM and relapse contributing equally to treatment failure (Strahm et al. 2011). Outcome for patients, who received a graft from an MSD or a UD matched for 9/10 or 10/10 HLA-loci is superimposable. Because patients  $\geq 12$  years of age had a high risk of NRM, EWOG-MDS recommends an intensified GVHD prophylaxis (CSA/MTX) for older patients transplanted from an MSD (see <https://ewog-mds.org>). Presence of a structurally complex karyotype is strongly associated with poor prognosis.

### 75.2.6 HCT in Therapy-Related MDS

In this heterogeneous patient population, OS in the presence of a structural complex karyotype and/or *TP53* mutation is  $\leq 20\%$ , for all other karyotypes/molecular subgroups approx. 50% (Kornemann et al. 2023).

## 75.3 Juvenile Myelomonocytic Leukemia

### 75.3.1 Clinical Features and Genetic Subtypes

JMML is a unique clonal hematopoietic disorder of early childhood. Splenomegaly, leukocytosis, monocytosis, and myeloid and/or erythroid precursors on PB smear are noted in close to all cases (Niemeyer and Flotho 2019). The pathobiology is characterized by constitutive activation of the RAS signal transduction pathway. Canonical RAS pathway mutations in the *PTPN11*, *NRAS*, *KRAS*, *NFI*, *CBL* genes are present in leukemic cells of more than 95% of patients and define genetically and clinically distinct subtypes.

### 75.3.2 Risk Factors and Indication of HCT

Risk factors for poor outcome are age  $\geq 2$  years, hemoglobin F  $\geq 15\%$ , DNA hypermethylation (cross-continental molecular classifier in high, intermediate, low methylation) and presence of secondary mutations (e.g., *SETBP1*, *JAK3*, other *RAS* pathway genes). Risk factors are strongly correlated and allow risk allocation within genetic subtypes.

### 75.3.3 Therapy Prior to HCT

For approximately 80% of patients with JMML early alloHCT is the therapy of choice (Table 75.1). In Europe, standard therapy prior to

**Table 75.1** Characteristics of genetic subtypes

Genetic subtype		
<i>PTPN11</i> (41%)	Presentation	Non-syndromic
	Mutation	Heterozygous, somatic gain of function mutation, broad overlap with Noonan syndrome with transient myeloproliferative disease → analysis of non-hematopoietic cells (hair bulbs, buccal swab, fibroblasts) required
	Risk profile HCT	Most often high Indicated
<i>NFI</i> (12%)	Presentation	$\geq 6$ café au lait spots not always present; higher platelet count, higher blast % and more often $>5$ years of age compared to other subtypes
	Mutation	<i>NFI</i> germline mutation with biallelic inactivation in hematopoietic cells
	Risk profile HCT	Often high Indicated
<i>NRAS</i> (16%)	Presentation	Non-syndromic
	Mutation	Heterozygous somatic <i>NRAS</i> mutations at codons 12, 13, rarely 61
	Risk profile HCT	very heterogeneous; in some low-risk patients spontaneous clinical regression in the absence of therapy Indicated for high-risk patients
<i>KRAS</i> (14%)	Presentation	Non-syndromic, median age 0.9 years
	Molecular	heterozygous, somatic mutations involving codon 12, 13, rarely 61; monosomy 7 more frequent than in other subtypes
	Risk profile HCT	Most often low or intermediate (by methylation class) Indicated for most patients; in some children sustained clinical and molecular CR following azacitidine therapy
<i>CBL</i> (12%)	Presentation	$\pm$ Noonan-like phenotype, occasional patient with grossly enlarged spleen, risk of vasculopathy (moyamoya, opticus arteritis, and major arteries)
	Mutation	<i>CBL</i> germline mutation with biallelic inactivation; occasionally heterozygous mutation only
	Risk profile HCT	Generally low Rarely required, may be indicated for patients with vasculopathy

(continued)

HCT is azacitidine (FDA approved) with best responses in low- and intermediate-risk patients (Niemeyer et al. 2021). 6-MP and low dose ara-C are effective as well. Patients with very aggressive disease (platelet transfusion dependent, pulmonary insufficiency) may benefit from high-dose Ara-C with FLU. MEK-inhibition (e.g., trametenib) alone may not result in significant responses (Stieglitz et al. 2021). Splenectomy prior to HCT improves neither engraftment nor outcome following HCT and is generally not recommended.

### 75.3.4 HCT and Outcome

MAC with BU/CY/MEL is standard conditioning for HCT in JMML from MSD and MUD (Locatelli 2005; Dvorak et al. 2018). Recommended stem cell source is BM. TRM varies among genetic subgroups (*PTPN11*-mut. 5%, *NFI*-mut. 27%). Relapse is the most important cause of failure. DFS and RI for patients (all genetic subtypes) with HbF  $\leq$  15% is 71% and 14%, respectively, and for HbF > 42% and 49%, respectively (EWOG-MDS unpublished). In high-risk disease, early tapering of immunosuppressive therapy in the absence of grade II-IV acute GVHD and steroid treatment is recommended (start at day +40, discontinuation between day +60 and +90). For patients with *PTPN11*-mutated JMML and a high risk of relapse, post engraftment therapy with azacitidine and DLI is currently being piloted. For children lacking a HLA-compatible relative, UBCT (Locatelli and Strahm 2018) or haploidentical HCT with T-cell depletion (i.e., TCRalpha/beta/CD19 or PTCY) a potential option.

#### Key Points

- Outcome of HCT in MDS associated with GATA2 deficiency or SAMD9/SAMD9L syndrome is similar to that of wildtype when stratified according to blast count, karyotype, and BM cellularity.

- MDS-EB and MDS with monosomy 7 require an MAC, whereas hypocellular RCC without monosomy 7, del(7q) or  $\geq$ 2 aberration can successfully be transplanted with a less intensive regimen.
- Tandem duplications in *UBTF* are noted in approximately one-third of pediatric patients with de novo MDS-EB. They are associated with poor response to intensive AML-type therapy and inferior outcome following MAC HCT.
- Molecular alterations in *PTPN11*, *NRAS*, *KRAS*, *NFI*, or *CBL* define clinically distinct JMML subtypes. Azacitidine is FDA approved for newly diagnosed JMML.
- Approx. 80% of children with JMML require HCT for cure. Standard preparative regimen is BU/CY/MEL.
- In JMML, risk factors for relapse following HCT (age > 2 years, HbF > 15%, presence of subclonal mutations, and high DNA methylation pattern) are closely related.

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# Myelodysplastic/ Myeloproliferative Neoplasms

# 76

Francesco Onida and Yves Chalandon

## 76.1 Definition, Epidemiology, Diagnosis, and Classification

The myelodysplastic-myeloproliferative neoplasms (MDS/MPNs) are a heterogeneous group of hematologic malignancies characterized by dysplastic and myeloproliferative clinical, laboratory, and morphological overlapping features, both in marrow and in blood. The MDS/MPN category, recently updated by the last revision to the WHO classification of myeloid and histiocytic/dendritic neoplasms (Khoury et al. 2022), includes chronic myelomonocytic leukemia (CMML), MDS/MPN with neutrophilia (previously called atypical CML), MDS/MPN with SF3B1 mutation (in its absence with ringed sideroblasts) and thrombocytosis (MDS/MPN-SF3B1-T), as well as MDS/MPN not otherwise specified (MDS/MPN-NOS) (Table 76.1).

MDS/MPN are typically diagnosed in elderly age with CMML being definitely the most frequent subtype (incidence of around 0.3–0.4 case/100,000 inhabitants per year, median age 74–79 years) (Benzarti et al. 2019). Being very uncommon, data concerning the incidence of MDS/MPN with neutrophilia, MDS/MPN-SF3B1-T, and MDS/MPN-NOS are currently unknown.

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**Table 76.1** Classification and diagnostic criteria of MDS/MPNs

Disease	Blood findings	Bone marrow findings
Chronic myelomonocytic leukemia (CMML): Myelodysplastic CMML (MD-CMML) Myeloproliferative CMML (MP-CMML)	Monocytes $\geq 0.5 \times 10^9/L^a$ , and relative ( $\geq 10\%$ ) <20% blasts MD-CMML: $< 13 \times 10^9/L$ WBC MP-CMML: $\geq 13 \times 10^9/L$ WBC	<20% blasts <sup>b</sup> Not meeting the criteria of chronic myeloid leukemia or other myeloid neoplasms. Not meeting diagnostic criteria of myeloid/lymphoid neoplasms with tyrosine kinase fusions. Dysplasia in one or more myeloid lineages <sup>c</sup>
Myelodysplastic/myeloproliferative Neoplasms with neutrophilia (previously called atypical CML)	Leukocytosis due to increased numbers of neutrophils with IMC, $\geq 10\%$ of WBC Basophils <2%, Monocytes <10% <20% blasts Dysgranulopoiesis	<20% blasts Dysgranulopoiesis Hypercellularity, with granulocytic proliferation and granulocytic dysplasia, $\pm$ dysplasia in the erythroid and megakaryocytic lineages No evidence of BCR/ABL1, PDGFRA, PDGFRB, or FGFR1 rearrangement or PCM1-JAK2
MDS/MPN with SF3B1 mutation and thrombocytosis	Anemia, $\geq 15\%$ ring sideroblasts, platelet count $\geq 450 \times 10^9/L$ <1% blasts	Erythroid lineage dysplasia with or without multilineage dysplasia, <5% blasts The presence of a SF3B1 mutation or, in its absence, no history of recent cytotoxic or growth factor therapy that could explain the MD/MP features and presence of $\geq 15\%$ ring sideroblast Then called MDD/MPN with ring sideroblasts and Thrombocytosis
MDS/MPN not otherwise specified (MDS/MPN-NOS) <sup>d</sup>	<20% blasts	<20% blasts

IMC immature myeloid cells (promyelocytes, myelocytes, and metamyelocytes)

<sup>a</sup>If blood monocytes  $\geq 0.5$  but  $< 1.0 \times 10^9/L$ , acquired clonal cytogenetic or molecular abnormality is an additional required criteria for CMML diagnosis. Abnormal partitioning of peripheral blood monocyte subsets is introduced as a new supporting criterion

<sup>b</sup>Including blasts equivalent: myeloblasts, monoblasts, and promonocytes

<sup>c</sup>Morphologic dysplasia should be present in  $\geq 10\%$  of cells of a haematopoietic lineage in the bone marrow

<sup>d</sup>Myeloid neoplasms with mixed proliferative and dysplastic features that do not meet the criteria for CMML, MDS/MPN with neutrophilia, or MDS/MPN-with SF3B1 mutation and thrombocytosis are classified as MDS/MPN-NOS

## 76.2 Risk Factors and Prognostic Index

The clinical course of MDS/MPN varies from an indolent course over several years for a minor fraction of patients with CMML and MDS/MPN-SF3B1-T to a more rapid progression with dismal prognosis and frequent transformation into secondary acute myeloid leukemia in the preponderance of patients with CMML and in the vast majority of patients with MDS/MPN with neutrophilia and MDS/MPN-NOS, for whom allo-HCT still represents the only curative option (Onida and Beran 2008; Onida 2017; Patnaik and Tefferi 2022, 2023).

CMML is highly heterogeneous, with clinical and hematological characteristics varying from mainly myelodysplastic to predominantly myeloproliferative which has led both the last version of the WHO classification (Khoury et al. 2022) and the International Consensus Classification (Arber et al. 2022) to retain the distinction of MD-CMML and MP-CMML variants, on the base of total WBC count  $<$  or  $\geq 13 \times 10^9/L$ , respectively (Bennett et al. 1994). Based on marrow and peripheral blood blast percentage, there are now only two disease subtypes (CMML-1, and CMML-2), associated to a corresponding decreasing life expectations (Khoury et al. 2022; Arber et al. 2022). Over the latest



**Table 76.2** Prognostic systems in MDS/MPN

MDS/MPN	Prognostic model	Variables included [score]	Risk groups	Median OS (months)
CMML	GFM (Itzykson et al. 2013)	Age > 65 years [2] WBC >15 × 10 <sup>9</sup> /L [3] PLT <100 × 10 <sup>9</sup> /L [2] Anemia (F < 10 g/dL, M < 11 g/dL) [2] ASXL1 mutation [2]	Low (score ≤ 4) Intermediate (score = 5–7) High (score ≥ 8)	Not reached 38.5 14.4
	CPSS (Such et al. 2013)	WBC ≥13 × 10 <sup>9</sup> /L [1] WHO CMML-2 subtype [1] CMML-specific CG <sup>a</sup> inter/high [1/2] Transfusal dependency [1]	Low (score = 0) Intermediate-1 (score = 1) Intermediate-2 (score = 2–3) High (score = 4–5)	72 31 13 5
	MMM (Patnaik et al. 2014)	Hb <10 g/dL [2] AMC >10 × 10 <sup>9</sup> /L [2] Circulating IMC >0% [2] PLT <100 × 10 <sup>9</sup> /L [1.5] ASXL-1 Mut (frameshift/nonsense) [1.5]	Low (score = 0) Intermediate-1 (score = 1.5–2) Intermediate-2 (score = 2.5–4.5) High (score ≥ 5)	97 59 31 16
	CPSS-Mol (Elena et al. 2016)	WBC ≥13 × 10 <sup>9</sup> /L [1] BM blasts ≥5% [1] Genetic risk group <sup>b</sup> [score 0–3] Transfusal dependency [1]	Low (score = 0) Intermediate-1 (score = 1) Intermediate-2 (score = 2–3) High (score ≥ 4)	Not reached 64 37 18
MDS/MPN with neutrophilia	MDACC (Onida et al. 2002)	Age > 65 years [1] Hb ≤10 g/dL [1] WBC >50 × 10 <sup>9</sup> /L [1]	Low (score = 0–1) High (score = 2–3)	38 9
MDS/MPN-SF3B1-T	Mayo (Patnaik et al. 2016)	Hb <10 g/dL [1] CG abnormalities [2] ASXL-1 mutation [1] SETBP1 mutation [1]	Low (score = 0) Intermediate (score = 1) High (score ≥ 2)	80 42 11

<sup>a</sup>CMML-specific cytogenetic risk classification, low, normal, and isolated –Y; intermediate, other abnormalities; and high, trisomy 8, complex karyotype (≥3 abnormalities), and abnormalities of chromosome 7

<sup>b</sup>Genetic risk group, CMML-specific cytogenetic risk classification + ASXL1/NRAS/SETBP1 mutation (score = 1)/RUNX1 mutation (score = 2)

years, a number of disease-specific prognostic systems have been developed in CMML in order to allow the best treatment strategy allocation (Onida 2017). The most recent ones are listed in Table 76.2.

MDS/MPN with neutrophilia, previously called atypical CML, is a rare hematologic malignancy with an overall dismal prognosis (median 24 months). Age, hemoglobin level, and leukocyte count have been identified as variables with independent prognostic significance, allowing the stratification of two groups with significantly different life expectations. Likewise, for MDS/

MPN-SF3B1-T, three risk categories of patients were recently differentiated by a Mayo Clinic prognostic model including molecular investigations (Table 76.2).

MDS/MPN-NOS is the most heterogeneous and the least well-characterized entity, with no currently recognized specific molecular findings. Some description of the biological and clinical characteristics have been reported in two series (DiNardo et al. 2014; Wang et al. 2014), with median survival of 12.4 and 21.8 months, respectively, and possible association of thrombocytosis with a more favorable outcome. More recently, a

retrospective analysis including 88 patients from the Mayo Clinic and 47 from the Moffitt Cancer Center reported median OS of 26 and 33 months, respectively (Mangaonkar et al. 2020).

## 76.3 Pretransplantation Treatment

For this rare group of diseases, there are few prospective studies on therapy, most being either retrospective analyses or case reports, making it difficult to give recommendations. Similarly to MDS, in high-risk patients with MDS/MPN and low blast count (<10%), upfront transplantation is the most frequently preferred strategy. In general, because apart from allo-HCT, no therapy has been shown to modify the disease course, pretransplantation treatments point toward symptom control rather than the achievement of disease remission (Odenike et al. 2015).

### 76.3.1 CMML

In general, treatment strategies in patients with CMML with symptomatic or progressive disease are based on the dysplastic versus proliferative features and the percentage of marrow blasts (Onida et al. 2013; Itzykson et al. 2018). In the presence of rising leukocytosis and/or organ infiltration (mostly splenomegaly) with low marrow blast percentage, hydroxyurea (HU) remains the drug of choice. Patients showing high blast percentages may be bridged to transplant through AML-like induction chemotherapy or by means of hypomethylating agents (HMAs), with a reported 40–50% overall response rate (Patnaik and How 2022). New treatment strategies based on the combination of HMAs with other agents (e.g., lenalidomide, venetoclax) or innovative modalities including targeted therapies (e.g., JAK2 inhibitors, MEK1/2 inhibitors, IDH1/2 inhibitors) or receptor agonists/antagonists immunotherapy approaches currently under experimental phase (Renneville et al. 2021) may further increase the response rate leading to an overall improvement of post-transplantation outcomes.

### 76.3.2 MDS/MPN with Neutrophilia (Previously known as Atypical CML)

Due to its absolute rarity in patients having no age or comorbidity barrier to allo-HCT, no consensus subsists on to whether any pretransplant treatment may have an impact on post-transplantation outcome and what kind of therapy should be best used. Control of leukocytosis is generally achieved with cytoreductive agents such as HU or IFN- $\alpha$  immunomodulation. Chemotherapy induction treatment is preferred when facing high blast count in advanced disease phases or in patients showing AML transformation.

Some efficacy of decitabine (Tong et al. 2015) and of ruxolitinib single agent (Dao et al. 2020) has also been reported, whereas a phase II trial of AZA and ruxolitinib in combination in a series of 35 MDS/MPN patients showed promising activity, with an overall response rate of 57% according to the 2015 international consortium response criteria for MDS/MPN (Savona et al. 2015), even though median survival of the few aCML included patients ( $n = 4$ ) was only 8 months (Assi et al. 2018). According to recently reported data, ASXL1, SETBP1, and ETNK1 are the most frequently mutated genes, with a total of 43.2%, 29.7, and 16.2%, respectively (Fontana et al. Hemasphere 2020), whereas JAK2 mutation is rare (0–7%), and CSF3R mutations are only occasionally observed (Sun et al. 2023). Even though in future these findings may influence therapeutic approaches by means of evolving targeted therapies, currently allo-HCT remains the only treatment strategy with established curative potential in eligible patients (Dao et al. 2017; Patnaik and Tefferi 2023).

### 76.3.3 MDS/MPN-SF3B1-T

MDS/MPN-SF3B1-T generally represents the disease entity associated with the best prognosis among overlap syndromes, with a median survival of about 6 years (Kuendgen et al. 2021). Guidelines for disease management are not formally recognized, and treatment strategies are generally extrapolated from low-risk MDS and MPN, with adjusted individual management

depending on presenting problems (Patnaik and Tefferi 2017). While lenalidomide has been occasionally reported to reduce transfusion need, anti-platelet, and cytoreductive treatments are often required due to the high risk of thrombosis (Kuendgen et al. 2021). Similar to lower risk MDS and MPN patients, allo-HCT is reserved for patients with refractory cytopenias, high-risk cytogenetics and/or molecular genetics, or progressive disease (Patnaik et al. 2016). Based on the different gene mutations possibly involved (*SF3B1* in ~85%, *JAK2V617F* in ~50%, *TET2* in ~25%, *ASXL1* in ~20%, *DNMT3A* in ~15%, and *SETBP1* in ~10%), attentiveness in targeted therapy is developing.

### 76.3.4 MDS/MPN-NOS

MDS/MPN-NOS is a very rare and heterogeneous disease entity, with no consensus on which therapy (if any) should be given for patients candidate to allo-HCT. Allogeneic HCT should be encouraged in eligible patients with TP53 mutations (Patnaik and Tefferi 2023). Augmented leukocyte proliferation is generally managed by means of cytoreductive agents such as HU or through immunomodulation with IFN $\alpha$ , while HMAs as well as lenalidomide may represent an option in case of prevailing cytopenias. JAK inhibitors are also potential therapeutic options, either alone or in combination with HMAs (Assi et al. 2018). When patients are progressing to AML transformation, induction chemotherapy should be used as a bridge to allo-HCT.

## 76.4 Autologous HCT

Because the harvesting of polyclonal hematopoietic progenitor cells is not feasible through the currently available treatment options, autologous HCT is currently not a recommended strategy in MDS/MPN.

## 76.5 Allogeneic HCT

Currently still representing the only curative strategy, the role of allo-HCT in adult MDS/MPN patients remains controversial mainly due to the lack of prospective studies, being therefore generally considered a possible treatment option for eligible patients with high-risk diseases.

In *CMML* benefits and risks of allo-HCT have been analyzed retrospectively in various series, with different characteristics at transplant and much variable outcomes described (Table 76.3). Recent recommendations from an international expert panel agreed to limit indication for allo-HCT in *CMML* patients classified in the intermediate-2 and high-risk CPSS categories (de Witte et al. 2017), representing the preferred treatment modality for younger patients with acceptable comorbidity index (Patnaik et al. 2015; Patnaik and Tefferi 2022). This indication was recently reinforced by the results of a study comparing transplant vs no transplant strategy in a large series of *CMML* patients from an international collaborative analysis (Robin et al. 2022).

As *MDS/MPN with neutrophilia* is extremely rare in people younger than 65 years, outcome after allo-HCT has been described only in small single-institution series. A 5-years OS and RFS of 51% and 36%, respectively, were recently reported by the EBMT-CMWP in a retrospective analysis of 42 patients transplanted between 1997 and 2006. With an RR of 40%, a better OS was recognized in young patients with low EBMT risk score (Onida et al. 2017).

In *MDS/MPN-SF3B1-T* allo-HCT is generally not indicated, being reserved for patients developing refractory cytopenias or accelerated/blastic transformation (Sharma et al. 2017), whereas eligible patients with *MDS/MPN-NOS* should always be considerate as potential candidate for allo-HCT due to the general dismal prognosis (Mangaonkar et al. 2020).

**Table 76.3** Summary of selected studies on allo-HCT in CMML

Author (year)	Pt N.	Median age (range)	Disease type/stage	Donor type	Conditioning (MAC vs RIC)	TRM/relapse rate	Survival outcome
Kröger et al. (2002)	50	44 (19–61)	CMML-1 = 28 CMML-2 = 17 Missing = 5	MRD = 43 MUD = 7	MAC = 50 RIC = 0	TRM = 52% RR = 28%	OS (5y) = 21% DFS (5y) = 18%
Eissa et al. (2011)	85	51 (1–69)	CMML-1 = 57 CMML-2 = 26	MRD = 38 MUD = 47	MAC = 58 RIC = 27	TRM (10y) = 35% RR (10y) = 27%	OS (10y) = 40% DFS (10y) = 40%
Park et al. (2013)	73	53 (27–66)	CMML-1 = 24 CMML-2 = 26 Missing = 23	MRD = 41 MUD = 32	MAC = 30 RIC = 43	TRM = 35% RR = 35%	OS (3y) = 32% DFS (3y) = 29%
Symeonidis et al. (2015)	513	53 (18–75)	CMML-1 = 87 CMML-2 = 32 s-AML = 95 Missing = 299	MRD = 285 MUD = 228	MAC = 249 RIC = 226	TRM (4y) = 41% RR (4y) = 32%	OS (4y) = 33% DFS (4y) = 27%
Kongtim et al. (2016)	83	57 (18–78)	CMML-1/2 = 47 sAML = 36	MRD = 30 MUD = 47 MMR = 6	MAC = 64 RIC = 19	TRM (3y) = 31% RR (3y) 33%	OS (3y) = 35% DFS (3y) = 34%
Liu et al. (2017)	209	57 (23–74)	CMML-1 = 140 CMML-2 = 52 Missing = 17	MRD = 73 MUD = 127 MMUD = 9	MAC = 105 RIC = 99 Missing = 5	TRM (5y) = 28% RR (5y) = 52%	OS (5y) = 30% DFS (5y) = 20%
Ironaga et al. (2018)	159	54 (16–75)	Not reported	MRD = 51 MUD (BM) = 66 Cord = 30 MMR = 12 MDR = 29	MAC = 92 RIC = 67	TRM = 28% RR = 39%	OS (3y) = 33%
Pophali et al. (2020)	70	58 (18–73)	CP 66% BT 34%	MUD = 32 mMUD/haplo = 9	MAC = 32 RIC = 38	TRM = 28% RR = 27%	OS (5y) = 51% (CP) OS (5y) = 19% (BT)
Rovó et al. (2023)	1466	60 (IQR 54–65)	CMML-1 = 52% CMML-2 = 48% Missing = 68%	MRD = 28% MUD/mMUD = 64% Haplo = 8%	MAC = 36% RIC = 64%	TRM = 32% RR = 37%	OS (5y) = 37% PFS (5y) = 31%

## 76.6 Source of HSC

No impact of HSC source on the transplant outcome has been observed in the largest CMML series reported by the EBMT-CMWP (Symeonidis et al. 2015). This was in contrast to the CIBTMR study, in which the survival was statistically better with PBMC than with BM, with no clear explanation outside the small proportion of BM transplants (16%) (Liu et al. 2017). The source of stem cell is therefore left open, but PBSC may potentially be preferred to decrease the risk of graft failure and the relapse risk, particularly with the use of RIC.

For MDS/MPN with neutrophilia, MDS/MPN-SF3B1-T, and MDS/MN-NOS, data are too scarce to make clear recommendations.

## 76.7 Conditioning and GvHD Prophylaxis

In MDS/MPN patients, the choice of conditioning regimen depends on many different conditions, the major ones being comorbidities, patient age, disease phase at transplant, type of donor, and HSC source. In the two largest retrospective series of CMML patients (Symeonidis et al. 2015; Liu et al. 2017), MAC and RIC were almost equal in proportion, with no outcome difference. Likewise, in the largest reported series of MDS/MPN with neutrophilia patients, conditioning intensity had no impact on the outcome (MAC were used in 76%). Noteworthy, an improved outcome following a combined fractionated 6–8 Gray TBI/FLU-conditioning regimen was reported in advanced CMML (Radujkovic et al. 2017).

In general, for young patients (<60 years), with a HCT-CI (Sorrer et al. 2005) less than 3, MAC regimens such as BU-CY, TT/BU/FLU (TBF), TT/TREO/FLU (TTF), or the reduced-toxicity FLU/BUx4 (FB4) may be advisable, particularly in the proliferative variant of CMML and in other MDS/MPN with predominant proliferative features, whereas a RIC regimen such as BU/FLU, TREO10/FLU, or reduced TBF/TTF may be preferred for patients with older age or

comorbidities and for patients undergoing transplant with disease remission following pretransplant treatment.

## 76.8 Maintenance/Post Transplant Strategies

As disease recurrence represents the major cause of transplant failure in MDS/MPN, there is a growing interest toward post transplant strategies, although few data are currently available in this particular setting.

Indirect evidence of a graft versus CMML by a reduced incidence of relapse in patients with chronic GvHD has been recently reported (Itonaga et al. 2018). Some effect of DLI has also been reported in patients with relapsing CMML and low disease burden.

With more molecular markers potentially available, cell therapy-based interventions may be planned on the base of residual or increasing MRD.

Potential interest both as preemptive and as maintenance strategy derive from the use of post transplant HMAs, alone or in combination with DLI, as reported in AML and MDS.

The use of lenalidomide and checkpoint inhibitors, but also JAK2 or PARP inhibitors, alone or even in combination, together with post transplant targeted therapies represents areas of growing interest under development.

### Key Points

- MDS/MPNs include heterogeneous hematologic malignancies with mostly dismal prognosis (except low-risk CMML and MDS/MPN-SF3B1-T), for which allogeneic HCT still represents the only curative treatment option.
- In the absence of major comorbidities, allo-HCT is recommended for patients with high-risk CMML according to the CPSS or CPSS-Mol, MDS/MPN with neutrophilia, and MDS/MPN-NOS up to 75 years of age.

- For young patients with a HCT-CI less than 3, MAC regimens may be advisable, particularly in the proliferative variant of CMML and in other MDS/MPN with predominant proliferative features, whereas a RIC regimen may be preferred for patients with older age or comorbidities.
- Although there is no evidence from dedicated trial, in patients with CMML-2 or other MDS/MPN with marrow blast higher than 10%, a pretransplant treatment based on HMAs or AML-like induction chemotherapy may be discussed.

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## 77.1 Primary and Post ET/PV Myelofibrosis

### 77.1.1 Definition and Risk Scores

Polycythemia vera (PV) and essential thrombocythemia (ET) have a favorable outcome without need for allo-HCT unless the disease has progressed to post-ET/PV myelofibrosis or secondary AML (Lussana et al. 2014).

Primary myelofibrosis (PMF) or post-ET/PV myelofibrosis is one of the “Philadelphia-negative” myeloproliferative neoplasms (MPN) with the worst overall survival of approximately 6 years. Allo-HCT can cure a substantial number of patients but is still not universally applicable due to toxicity, which may lead to therapy-related morbidity and mortality (McLornan et al. 2021).

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## 77.1.2 Transplant Results in Myelofibrosis

In the late 1980s and the early 1990s, the feasibility of allo-HCT for myelofibrosis was highlighted in several small case series. One retrospective, multicenter study described outcomes from MAC in relatively young patients (median age 42 years) with a NRM of 27% and a 9% incidence of graft failure. The OS and PFS was 47% and 39% at 5 years (Guardiola et al. 1999). A single-center study from Seattle included 104 patients most of whom received allo-HCT after MAC, and NRM at 5 years of 34% and OS at 7 years of 61% were reported (Deeg et al. 2003).

The evidence of graft-versus-myelofibrosis effect was documented by responses to DLI after failure of allo-HCT (Byrne et al. 2000). RIC for myelofibrosis was investigated in two prospective studies. The prospective EBMT study reported results of 103 patients who received a BU/FLU-based RIC followed by related or unrelated HCT. The median age was 55 years, and the NRM at 1 year was 16%. Cumulative incidence of relapse was 22% at 3 years. PFS and OS at 5 years were 51% and 67%, respectively. Advanced age and HLA-mismatched donor were independent predictive factors for reduced survival (Kroger et al. 2009). A recent update of the study after a median follow-up of 60 months showed an 8-year OS of 65% with stable plateau. Five-year DFS was 40%, and 5-year cumulative incidence of relapse/progression was 28% with a 3-year NRM of 21%.

The Myeloproliferative Disorders Research Consortium performed also a prospective phase II trial including 66 patients with primary or post-ET/PV myelofibrosis investigating a reduced conditioning regimen with MEL/FLU. With a median follow-up of 25 months, OS was 75% in the sibling group and only 32% in the unrelated (URD) group due to a higher NRM in the URD group (59% vs. 22%) (Rondelli et al. 2014). Other studies using RIC or MAC confirmed the curative effect of allo-HCT irrespective of the intensity of the conditioning (summarized in

Kröger et al. 2015a). Transplantation outcomes in accelerated phase are similar to chronic phase while outcome in blastic phase is worse (Gagelmann et al. 2022a, b; Orti et al. 2023).

### 77.1.3 Disease-Specific Risk Factors

Patients with PMF or post-ET/PV myelofibrosis have a median survival of approximately 6 years, but survival varies from less than 2 to more than 15 years. Risk scores (see Table 77.1) such as

**Table 77.1** Prognosis risk scores and transplant specific (MTSS) score for myelofibrosis

Score	Adverse factors (puntos)	Risk group and median SRV
IPSS	Age > 65 years (1 p) Constitutional symptoms (1 p) Hb <100 g/L (1 p) Leucocytes >25 × 10 <sup>9</sup> /L (1 p) Blasts in PB ≥1% (1 p)	Low (0 p), 11.3 years Intermediate-1 (1 p), 7.9 years Intermediate-2 (2 p), 4 years High (3–5 p), 2.3 years
DIPSS	Age > 65 years (1 p) Constitutional symptoms (1 p) Hb <100 g/L (2 p) Leucocytes >25 × 10 <sup>9</sup> /L (1 p) Blasts in PB ≥1% (1 p)	Low (0 p), not reached Intermediate-1 (1–2 p); 14.2 years Intermediate-2 (3–4 p), 4 years High (5–6 p), 1.5 years
DIPSS plus	DIPSS Int-1 (1 p) DIPSS Int-2 (2 p) DIPSS high (3 p) Platelets <100 × 10 <sup>9</sup> /L (1 p) Transfusion requirement (1 p) Unfavorable karyotype <sup>a</sup> (1 p)	Low (0 p), 15.4 years Intermediate-1 (1 p), 6.5 years Intermediate-2 (2–3 p), 2.9 years High (4–6 p), 1.3 years
MIPSS70	Leucocytes >25 × 10 <sup>9</sup> /L (2 p) Hb <100 g/L (1 p) Constitutional symptoms (1 p) BM fibrosis grade ≥ 2 (1 p) Blasts in PB ≥ 2% (1 p) CALR typ1 (1 p) Platelets <100 × 10 <sup>9</sup> /L (2 p) HMR (1 p) HMR ≥2 (2p)	Low (0–1 p) 27.7 years Intermediate (2–4 p) 7.1 years High (≥5 p) 2.3 years
MTSS	Platelets <150 × 10 <sup>9</sup> /L (1 p) Leucocytes >25 × 10 <sup>9</sup> /L (1 p) Karnofsky <90% (1 p) Age ≥ 57 years	Low (score 0–2) OS 90% (5 years) NRM 10% Intermediate (score 3–4) OS 77% (5 years), NRM 22%
	NonCALR/MPL mutation (2 p) ASXL-1 (1 p) Mismatch unrelated donor (2 p)	High (score 5) OS 50% (5 years) NRM 36% Very high (≥6) OS 34% (5 years) NRM 57

DIPSS: <https://qxmd.com/calculate/dipss-prognosis-in-myelofibrosis>

DIPSS-plus: <https://qxmd.com/calculate/dipss-plus-score-for-prognosis-in-myelofibrosis>

HMR Highmolecular risk: IDH1/2,SRSF2,ASXL-1, or EZH2

<sup>a</sup>+8; -7/7q-; -5/5q-; i17q; 12p-; rearrangement 11q23

IPSS (Cervantes et al. 2009), dynamic IPSS (DIPSS) (Passamonti et al. 2010), or DIPSS plus (Gangat et al. 2011) are currently used in clinical practice to determine the prognosis of patients with PMF. More recently, molecular markers have been introduced into the PMF specific risk score (MIPSS70) (Guglielmelli et al. 2018), and a specific MYSEC score for post-ET/PV myelofibrosis has been proposed (Passamonti et al. 2017; Kroger et al. 2015a). The EBMT/ELN consensus paper recommended allo-HCT for patients less than 70 years with an estimated median survival of less than 5 years. This would include patients with IPSS or DIPSS intermediate-2 and high risk and is based on a comparison between transplanted and non-transplanted patients in the pre-ruxolitinib era (Kroger et al. 2015a, b). Patients with intermediate-1 risk can be considered for allo-HCT if other high-risk features such as *ASXL1* mutation, more than 2% peripheral blasts, refractory transfusion-dependent anemia, or adverse cytogenetics according to DIPSS plus are present (Kroger et al. 2015a).

#### 77.1.4 Transplant-Specific Risk Factors

In most of the transplant studies, alternative donors were associated with a worse outcome independent of disease-specific risk factors. CBT resulted in a high risk of graft failure (Robin et al. 2014). Haplo-identical donor with PT-CY as GVHD prophylaxis is currently under investigation, but more recent EBMT data reported a 5-year survival of only 38% (Raj et al. 2016).

The intensity of the conditioning regimen has not been investigated within prospective studies, but retrospective comparisons of MAC and RIC preparative regimens resulted in similar outcome. (McLornan et al. 2019; Gagelmann et al. 2022a, b) Because of the reduced toxicity and a generally older age of patients with myelofibrosis, RIC regimens are currently used more frequently and account for about two-thirds of allotransplants for myelofibrosis reported to the EBMT registry. Recently, the transplant-specific risk score (MTSS) including molecular genetics, platelet,

and WBC count as well as Karnofsky index, age, and stem cell donor can predict outcome after allo-HCT (Gagelmann et al. 2019a, b).

#### 77.1.5 Patient-Specific Risk Factors

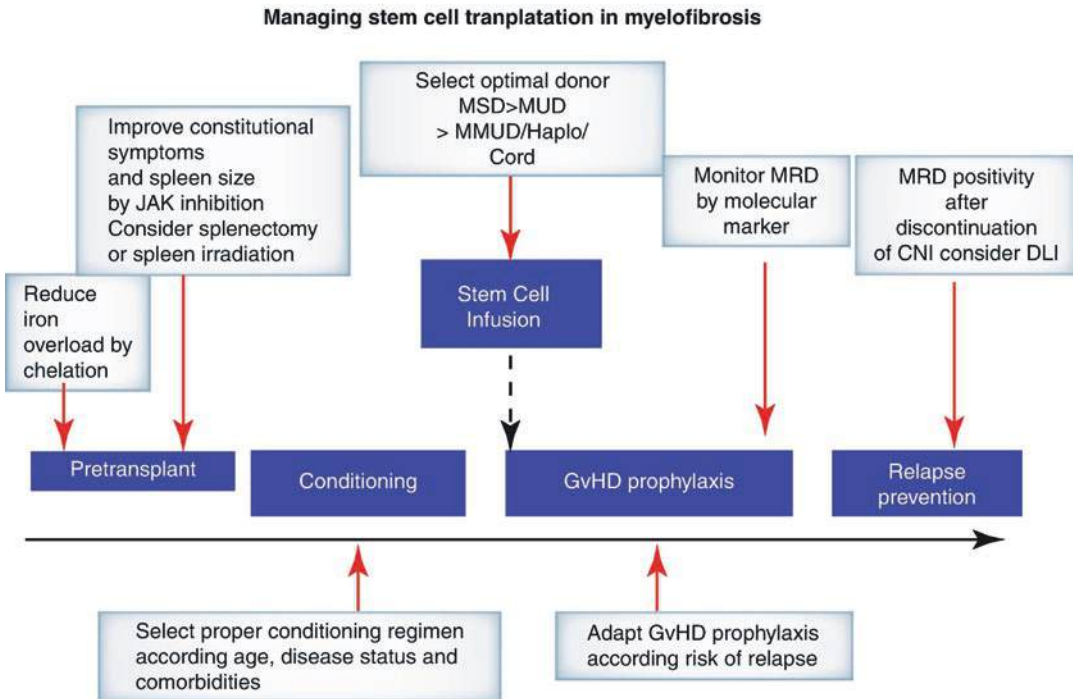
Age is a significant patient-specific risk factor for outcome after allo-HCT (Scott et al. 2012; Kroger et al. 2015a). Besides age, comorbidities and geriatric assessments (see Chap. 11) also impact on outcome after allo-HCT but have not been studied specifically in myelofibrosis patients to date.

#### 77.1.6 Role of Splenectomy and JAK Inhibition

Splenomegaly is a hallmark of myelofibrosis and may have an impact on engraftment and graft function after HCT. Splenectomy is an option to reduce spleen size prior to transplantation, (Polverelli et al. 2021). Splenic irradiation to reduce spleen size has been reported successfully in single cases prior to conditioning. Since ruxolitinib is approved for myelofibrosis, the drug can be used prior to transplantation to improve constitutional symptoms and to reduce spleen size. The European LeukemiaNet and the European Society for Blood and Marrow Transplantation recommend the use of ruxolitinib at least 2 months prior to HCT and a careful weaning prior to conditioning to avoid the rebound phenomenon. More recent data suggests better outcomes after HCT if patients received transplant after responding to ruxolitinib rather than postponing the transplant until ruxolitinib failure (Kröger et al. 2021).

#### 77.1.7 Impact of Molecular Remission and Posttransplant Adoptive Immunotherapy

About 90% of myelofibrosis patients harbor one of the driver mutations *JAK2V617F*, calreticulin (*CALR*), or *MPL* which are used to monitor MRD



**Fig. 77.1** *MSD* matched sibling donor, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *MRD* minimal residual disease, *CNI* calcineurin inhibitor, *GVHD* graft-versus-host disease

in PB by highly sensitive qPCR or digital PCR to determine molecular remission (Wolschke et al. 2017). In a retrospective single center experience, no achievement of molecular remission on day 180 post-allograft was associated with a significantly higher incidence of a subsequent clinical relapse. Due to a graft-versus-myelofibrosis effect, donor lymphocyte infusion has been successfully applied in patients with molecular or hematological relapse to induce a molecular remission (Kröger et al. 2009; Gagelmann et al. 2023) (Fig. 77.1).

Furthermore, BM fibrosis is another hallmark of the disease, with rapid regression after allo-HCT suggesting that fibrogenesis is a highly dynamic process. Systematic investigations have shown that about 60% of the patients have a complete or nearly CR of BM fibrosis on day+100, and the percentage of patients increased to 90% at day+180. Notably, those patients with a rapid resolution of BM fibrosis had the best long-term outcome (Kröger et al. 2007).

#### Key Points

- Primary or post-ET/PV myelofibrosis can only be cured by allo-HCT which can induce molecular remission and resolution of bone marrow fibrosis.
- Indication for allo-HCT is recommended for patients younger than 70 years and a median survival expectation of less than 5 years such as risk score intermediate or high risk according to DIPPS or intermediate-1 risk with additional risk factors and taking into account risk profile according to the transplant specific risk score (MTSS).
- Splenectomy or splenic irradiation prior to transplant can be considered in patients with extensive spleen size and or response to JAK inhibitor treatment prior to transplantation.
- Major risk factors for worse outcome are advanced age and use of a HLA-mismatched donor.



## 77.2 Chronic Myeloid Leukemia

Yves Chalandon

### 77.2.1 Definition, Epidemiology, Diagnosis, and Classification

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of the HSC. CML was the first leukemia described and the first to be characterized by a consistent chromosomal aberration, the 22q- or “Philadelphia” (Ph) chromosome, later identified as a reciprocal translocation, t(9;22), encoding the BCR-ABL oncoprotein.

CML is the most common of the myeloproliferative disorders. The incidence is 0.4–1.75 per 100,000 population per year, and it increases with age (Höglund et al. 2015). The disease can occur at any age, but the median age at presentation ranges between 45 and 55 years. There is a slight male predominance, with a male to female ratio of 1.3:1.

CML present initially as an indolent or chronic phase (CP), easily controlled with treatment. The natural history continues with a bi- or triphasic stage, becoming more aggressive through accelerated phase (AP) and then blast crisis (BC) or directly from CP to BC.

### 77.2.2 Risk Factors and Prognostic Index

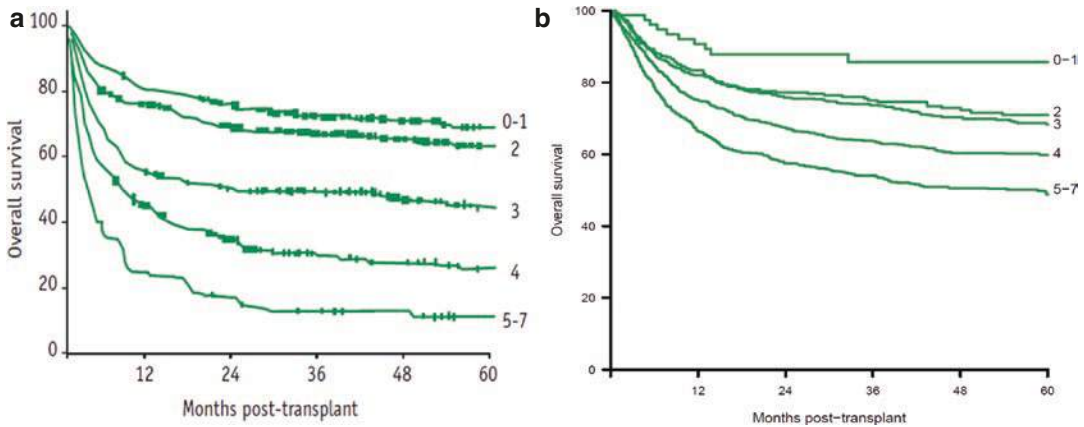
Several multivariable-derived prognostic models and staging have been proposed to help define individual prognosis and allow assigning patients to different strategies of therapy based on risks. The most commonly used are the Sokal and Hasford one (Sokal et al. 1984; Hasford et al. 1998) and more recently the ELST one (Pfirrmann et al. 2016).

The benefit of allo-HCT is that it can provide cure, but the clear disadvantage is its association with considerable morbidity and mortality, which typically occur early post procedure. Outcome can be improved by better selection of those most

likely to benefit. In this context, the EBMT developed a risk score for patients with CML, based on five variables: donor type, disease phase, recipient age, donor/recipient gender combination, and interval from diagnosis to transplant, which together results in a score of 0–7 (see risk factors in Chap. 11) (Gratwohl et al. 1998).

Results of transplant are now highly predictable based on these five factors. It is worth remembering that the EBMT or “Gratwohl” score was developed in the mid-1990s and was based on 3142 patients transplanted between 1989 and 1996 (Fig. 77.2a). With overall improvements in supportive care, it would be reasonable to expect that a similar analysis performed on patients transplanted more recently would demonstrate improved results across all-risk scores. However, the analysis is complicated by the change in approach to management of CML. During the period of the original analysis, allo-HCT was the treatment of choice for all patients. Since 2000 allo-HCT has been replaced by tyrosine kinase inhibitors (TKI) as frontline therapy, and hence the reasons for patients coming to transplant are not always clear from registry data. Although this should be compensated by the use of factors such as age at transplant, disease phase, and time from diagnosis to transplant, some caution should be exercised in the interpretation of more recent results. Having said this, the analysis has been repeated recently for 3185 patients transplanted from 2011 to 2021 and confirmed improved outcome of 5-year OS across all-risk scores by 11–41% (Fig. 77.2b) but with this improvement there is also a decrease between the different risk groups, particularly 2–3 which could nowadays be regrouped together. Although these pretransplant factors are known to affect outcome in all diseases, it is worth focusing specifically on the impact of disease phase in CML, in particular because one of the few problems of TKI therapy is that within the cohort of patients receiving transplants, the proportion transplanted in advanced phases as opposed to first CP has increased over time (Table 77.2).

Data provided by Mrs. Linda Koster on behalf of the EBMT CMWP.



**Fig. 77.2** (a) OS of CML patients after allo-HCT according to EBMT risk score. Original curves published in 3142 patients transplanted between 1989 and 1996.

Modified from (Gratwohl et al. 1998). (b) OS curves supplied by Mrs. Linda Koster for the EBMT CMWP and based on 3497 patients transplanted from 2011 to 2021

**Table 77.2** Change in proportion of patients transplanted in each disease phase from 2011 to 2021

Year of transplant	1st CP	AP	≥2CP	BC
	Number (% total)	Number (% total)	Number (% total)	Number (% total)
2011	154 (46)	50 (15)	84 (25)	46 (14)
2012	128 (45)	34 (12)	75 (27)	44 (16)
2013	137 (44)	42 (13)	77 (25)	55 (18)
2014	140 (43)	49 (15)	75 (23)	60 (19)
2015	141 (44)	43 (14)	75 (23)	62 (19)
2016	115 (41)	31 (11)	73 (26)	62 (22)
2017	99 (39)	29 (11)	76 (30)	51 (20)
2018	130 (48)	31 (11)	55 (20)	56 (21)
2019	127 (43)	40 (14)	69 (23)	59 (20)
2020	108 (44)	26 (10)	59 (24)	55 (22)
2021	96 (37)	33 (12)	47 (18)	87 (33)

Allografts for CML were initially restricted to patients in AP, and improvements in survival came only when transplant was performed in the CP. Data of 138 patients with CML transplanted between 1978 and 1982 and reported to the IBMTR showed 3-year survivals of 63%, 36%, and 12% for patients transplanted in the CP, AP, and BC, respectively. The probability of relapse for those transplanted in CP was 7% (Speck et al. 1984). The effect of disease phase on the outcome of transplantation has not changed over the years. To optimize the effect of allo-HCT for a patient who has progressed to blast crisis, a second CP should be achieved using TKI and/or con-

ventional combination chemotherapy, but to further improve the results, physicians who are taking care of CML patients should follow them closely and send them to transplant as soon as there is molecular progression after all line of TKIs given prior to being in a more advanced phase (AP, BC, ≥CP2) (Chalandon et al. 2023).

### 77.2.3 Pretransplantation Treatment

Early descriptions of therapy included radiotherapy, introduced at the beginning of the twentieth century and later oral chemotherapy, in particular BU and hydroxycarbamide. These approaches could control the signs and symptoms of CML in chronic phase but could not prevent its inevitable transformation into a rapidly fatal chemoresistant blastic disease. The first treatment that eradicated the Ph-positive clone and induced cure was BMT, initially described in syngeneic twins and soon followed by procedures involving HLA-matched siblings and later URD. Transplantation, once the treatment of choice for this disease, has been relegated to second-, third-, and even fourth-line treatment in parallel with the development of the TKI. As more potent TKI move to first-line therapy, patients destined to respond poorly to these drugs are identified earlier, and transplant will possibly return to use as an earlier line strategy.

## 77.2.4 Autologous HCT

Autologous HCT for CML started about at the same time as allo-HCT in the late 1970s early 1980s in Europe with the goal to set up the clock to early phase with high-dose therapy followed by reinfusion of autologous HSC. However, following the introduction of targeted therapy with TKI, the number of auto-HCT in Europe has decreased rapidly, with only 0–1 per year between 2017 and 2022 as per the EBMT registry data. Auto-HCT is currently not a recommended strategy in CML; however, it should be mentioned that due to the lack of randomized studies, the potential role of autologous HCT for CML remains unknown.

## 77.2.5 Allogeneic HCT

### 77.2.5.1 Indication

Although the introduction of TKI in the early 2000s dramatically changed the therapeutic strategy for CML, allo-HCT has still a place, offering a very long-term PFS. This is particularly true as the leukemic quiescent stem cells are not dependent on BCR-ABL signaling for survival, and therefore those cells are not targeted by TKIs leading to a proportion of patients who will relapse or will have resistant disease despite TKI treatment. With extended follow-up, it appears that some 60% of patients can achieve excellent long-term disease control on imatinib, and a small proportion may even be able to stop treatment without experiencing disease recurrence. Approximately half of this group will achieve or regain remission on one of the second-generation TKI (2ndGTKI), bosutinib, dasatinib, and nilotinib, or third-generation TKI (3rdGTKI) ponatinib or with the new class inhibitor of the myristoyl pocket of ABL (STAMP inhibitor), asciminib which are the only one that are effective against the T315I mutation.

The efficacy of 2ndGTKI has led to their use as first-line therapy, and phase III studies suggest that approximately 80% of patients will achieve complete cytogenetic remissions within the first year, compared to 65% on imatinib. Based on

these results, dasatinib and nilotinib have both been licensed for use in newly diagnosed patients. However, allo-HCT remains the therapy of choice for advanced phase CML as well as for those with CP who failed to respond, develop TKI-resistant mutations, and lose an established response and/or are intolerant of the drug.

The time to proceed to transplant remains controversial. This is particularly true for the substantial number of patients being started on 2ndGTKI as first-line therapy, who, in case of resistance, progression, or relapse, may be rescued with either another 2ndGTKI, 3rdGTKI, or asciminib, and then the question to proceed to transplant immediately or wait for another progression and third- or fourth-line therapy rescue before to have allo-HCT is a matter of debate. This is less true for those who are failing third- or fourth-line therapy or who have a T315I mutation for whom allo-HCT is recommended.

A number of national and international study groups are now reporting that long-term response to imatinib and 2ndGTKI can be predicted by the rate of fall of BCR-ABL transcript levels (as measured by RQ-PCR at 3 and 6 months). It is therefore possible to identify the patient destined for transplant within the first year of diagnosis while still in CP and return to a more measured approach to transplant. Recently, the CMWP of the EBMT analyzed the data of patients transplanted for CML in the 3rdGTKI era that showed that the number of TKI given prior to allo-HCT seems not to impact on the outcome; however, the stage of the disease as well as the performance status of patients did have an important impact (Chalandon et al. 2023). It is therefore very important to try to keep patients in first CP and avoid progression, even for those rescued to second or more CP after having progressed to advanced phase, as the results after transplantation are worse in this category. Allo-HCT is also recommended for patients with BC after debulking with second or 3rdGTKI plus induction chemotherapy. For AP CML patients, this should be individualized, but the search for a donor and referral to a transplant center should be done rapidly, and transplant should be initiated after obtaining a new response to TKI for those pro-

gressing from CP to AP under therapy as their outcome is not good without allo-HCT.

#### 77.2.5.2 Source of SC

About two-thirds of the transplantation done nowadays for CML use PBSC as source of HSC (Chalandon et al. 2023); this is close to what is seen in other hematological malignancies (Holtick et al. 2014). It appears that there is no difference in general outcome depending on the stem cell source, although BM seems to have a decrease incidence on chronic GvHD and its severity. The source of stem is therefore left open, but PBSC may potentially be preferred to decrease the risk of graft failure and the relapse risk in more advanced disease, particularly with the use of RIC.

#### 77.2.5.3 Conditioning and GvHD Prophylaxis

For CML patients, the best conditioning regimen as well as the best GvHD prophylaxis remains to be determined. Regarding the MAC, CY combined either with BU or TBI is still the one that has shown the best overall long-term survival (Copelan 2006). RIC that has been introduced later to offer transplantation to older patients or with more comorbidities did not show improved outcome over MAC, particularly in relation with a higher incidence of relapse with RIC (Kebriaei et al. 2007; Chalandon et al. 2023). Therefore, for elderly patients or those with comorbidities, RIC (FLU with BU or MEL) will be the choice, and for the others, particularly with advanced phases in order to control better the disease, MAC should be proposed.

For GvHD prophylaxis, CSA combined with short course MTX seems also to remain the standard for allo-HCT for CML (Copelan 2006). In order to reduce the incidence and severity of GvHD, TCD was introduced in the 1980s; however, there was an increase of relapse rate (Apperley et al. 1986). This led to many groups abandoning the use of TCD in sibling allografts for CML and often also in URD procedures. Others continued with its use and have reported good outcomes in sibling transplants, particularly following the introduction of DLI. In a small

series of 23 CML patients with a median age of 36 years (range 18–58 years) transplanted with sibling donors and MAC between 1998 and 2016 at the University Hospital of Geneva using partial TCD with Campath-1H (alemtuzumab), the 15-year OS and LFS was 95% using the strategy of escalating doses DLI for early molecular relapses with a low incidence of acute and chronic GvHD (Chalandon, unpublished data). More recently, the advent of cyclophosphamide post-transplantation has offered new possibilities of GvHD prophylaxis allowing allo-HCT with haplo-identical donors or mismatched unrelated donors with outcomes similar to the one of matched unrelated donors (Gagelmann et al. 2019a, b). This is also the case for CML allo-HCT and very recently the CMWP of the EBMT did report on haplo-identical donor for CML giving similar OS but a slightly lower RFS as compared to MUD, but not to MRD (Onida et al. submitted for publication). The same holds true when using post-CY for MUD or MMUD as compared to standard GvHD prophylaxis calcineurin inhibitors and methotrexate, but in that case there were not differences within the 3 cohort either in OS, RFS, RI, or NRM (Ortí et al. 2023).

#### 77.2.5.4 Post Transplant Strategies

After allo-HCT, rising or persistently high levels of BCR-ABL1 mRNA can be detected prior to cytogenetic or hematological relapse. Low or falling BCR-ABL1 transcript levels are associated with continuous remission, while high or rising transcript levels predict relapse. Therefore, monitoring BCR-ABL1 post-allo-HCT for CML is of utmost importance, even in the long term, due to relapses that have occurred up to more than 15 years post-HCT.

Many CML patients will remain RQ-PCR positive during the first 3 months after allo-HCT, especially in the era of RIC or using TCD. In patients who are at least 4 months post-allo-HCT, one working definition of molecular relapse is one of the following:

- (a) BCR-ABL/ABL1 ratio higher than 0.02% in three samples a minimum of 4 weeks apart.

- (b) Clearly rising BCR-ABL/ABL1 ratio in three samples a minimum of 4 weeks apart with the last two higher than 0.02%.
- (c) BCR-ABL/ABL1 ratio higher than 0.05% in two samples a minimum of 4 weeks apart (Kaeda et al. 2006).

Administration of DLI can re-induce remission in 60–90% of patients with CML transplanted in, and relapsing in CP. The use of escalating doses in case of persistent disease reduces the risk of GvHD (Mackinnon et al. 1995). An EBMT study showed 69% 5-year survival in 328 patients who received DLI for relapsed CML. DLI-related mortality was 11%, and disease-related mortality was 20%. Some form of GvHD was observed in 38% of patients. Risk factors for developing GvHD after DLI were T-cell dose at first DLI, time interval from transplant to DLI and donor type. In a time-dependent multivariate analysis, GvHD after DLI was associated with a 2.3-fold increase in risk of death as compared with patients without GvHD (Chalandon et al. 2010).

With the advent of TKI, the CML post transplant interventions are more complex but give more opportunities to rescue patients. It is possible to combine DLI and TKI for relapsing patients; however, the best order (TKI first, DLI first, or both combined) has not yet been defined. The CMWP of the EBMT reported 431 patients with CML relapses post-allo-HCT who received TKI either alone (55%) or in combination with DLI (14.5% before, 4.4% at the same time, and 26% after TKI). Only 42% of the patient obtained either a complete molecular (17.7%), cytogenetic (4.4%) or hematological (20.2%) remission with a 5-year OS of 60% and of 47% for RFS (Chalandon et al. 2017). This rather low response rate may be in relation with the fact that 235 patients were transplanted for advanced phases (AP, BC, or > CP1).

Maintenance with TKI post allo-HCT has been evaluated in a registry study of the CIBMTR which suggested that patients who received TKI maintenance did not have a better outcome as compared to the one who did not with a 5-year

leukemia-free survival of 42% vs 44%, respectively,  $p = 0.65$  (DeFilipp et al. 2020).

### Key Points

- With the advent of targeted therapy, allo-HCT use has decreased; however, it is of importance to monitor closely patients who are under TKI and avoid that they progress to advanced phase (AP or BC) as the outcome after transplant is better for CP1 as compared to all other conditions.
- Allo-HCT should be considered for patient with BC after their return to CP, for those progressing from CP to AP and for the one in CP after failure of third-line or fourth-line therapy or with T315I mutation.

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# Acquired Bone Marrow Failure: Severe Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria

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## 78.1 Definition and Epidemiology

Severe aplastic anemia (SAA) is an autoimmune disorder (AID) due to the attack of autoreactive cytotoxic T lymphocytes to the hematopoietic component of the bone marrow. The triggering antigen is so far unknown. The incidence of SAA is about 2–3/million in Europe and the United States and threefold higher in East Asia, with two age peaks of incidence (in young adults and in the elderly) (Young and Kaufman 2008).

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Paroxysmal nocturnal hemoglobinuria (PNH) is another bone marrow failure syndrome (BMFS), which is often closely associated with SAA. PNH results from the clonal expansion of hematopoietic stem cells that have somatic mutations in the X-linked gene *PIG-A* (Takeda et al. 1993). *PIG-A* mutations cause an early block in the synthesis of glycosylphosphatidylinositol (GPI) anchors, which tether many proteins to the cell surface. Intravascular hemolysis is a prominent feature of PNH and is the consequence of the absence of the GPI-linked complement regulatory protein CD55 & CD59 (Motoyama et al. 1992). PNH includes hemolytic anemia but also thrombophilia in addition to bone marrow failure; thrombophilia represents the major cause of death in PNH patients (de Latour et al. 2008).

## 78.2 Diagnosis and Indication for Treatment for SAA

SAA is usually diagnosed in the setting of pancytopenia and a hypocellular BM. Diseases such as myelodysplasia, myelofibrosis, hypocellular acute leukemia, and inherited BMF (Fanconi's anemia or Telomere Biology Diseases) need to be excluded. In this respect, wide scale genetic testing (targeted NGS panels or whole genome/exome sequencing) may help to identify undiagnosed constitutional bone marrow failure syndromes that may have a dismal outcome if transplanted with classical SAA conditioning

regimens (McReynolds et al. 2022). Also, immunological screen was shown to detect immune deficiency and immune dysregulation disorder among a proportion of pediatric patients with apparently acquired AA (Miano et al. 2021) and should therefore be part of the diagnostic workup.

Cytogenetic abnormalities can be found in up to 10% of immune mediated SAA such as del(20q), +8, del13q and -Y (outside MDS defining cytogenetic abnormalities (Khoury et al. 2022; Hosokawa et al. 2012)). Somatic mutations might also be found in acquired aplastic anemia at diagnosis or during follow-up but are not informative if isolated and with low variant allele frequency (Peffault de Latour et al. 2022).

There is a close relationship between PNH and acquired SAA with a concomitant diagnosis in 40% of cases. SAA is diagnosed when marrow hematopoietic cellularity is <30%, and two of three of the following criteria are met: absolute neutrophil count  $<0.5 \times 10^9/L$ , absolute reticulocyte count  $<60 \times 10^9/L$ , and platelet count  $<20 \times 10^9/L$  (Camitta 1988).

Treatment requires careful planning and may be prolonged. A watch and wait strategy is often used initially if there is milder pancytopenia (e.g., moderate AA). Conversely, in case of transfusion requirement or if the criteria for SAA are met, treatment should begin with no delay. Prior to treatment the patient should be stable clinically with control of bleeding and infections. Once the diagnosis is confirmed, and the disease severity is assessed, family HLA-typing and matched unrelated donor search should be done in the work-up phase. In the absence of signs and symptoms of intravascular hemolysis, patient's treatment algorithm is directed towards SAA despite the presence of PNH clones.

## 78.3 Treatment of SAA

### 78.3.1 First-Line Treatment for SAA

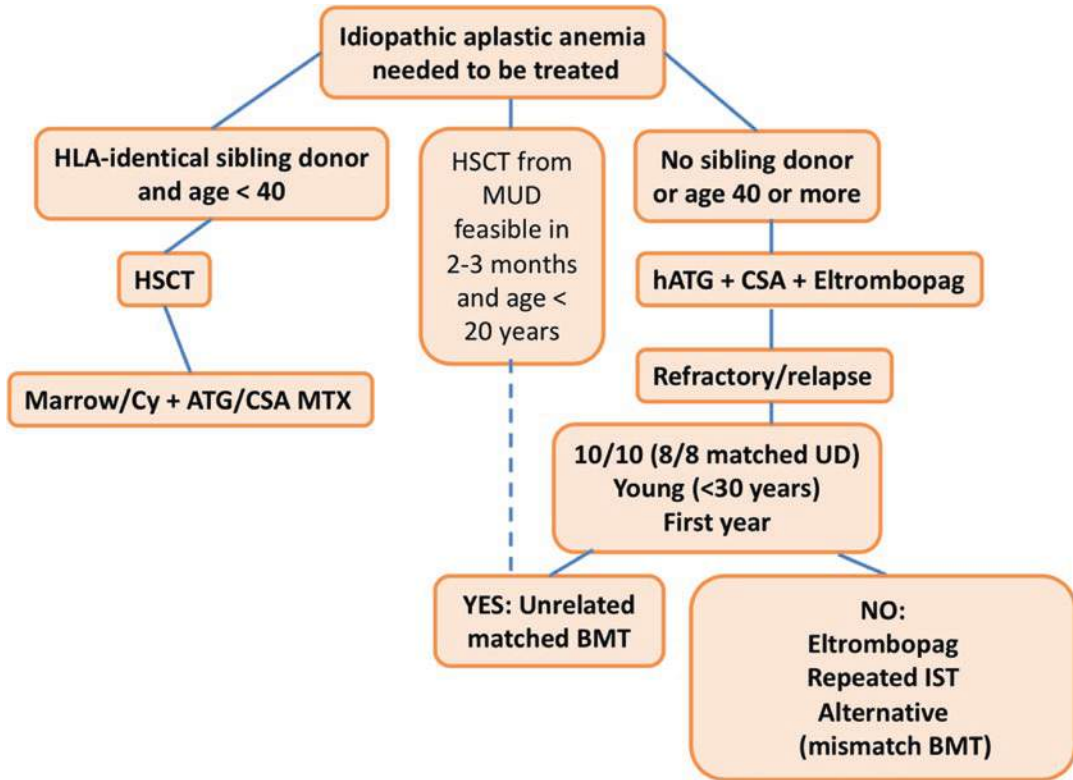
#### 78.3.1.1 Upfront Matched Sibling (MSD) Transplantation

The choice of first-line treatment depends on the age of the patient and the availability of an HLA

matched sibling donor (MSD) (Fig. 78.1). The standard first-line treatments for a newly diagnosed patient with SAA are HCT from an HLA-identical sibling donor or immunosuppressive therapy (IST) using a combination of horse ATG and CSA (ATG + CSA). Early bone marrow HCT after a conditioning regimen with CY, ATG, and GVHD prophylaxis combining CSA and MTX promotes excellent engraftment (95%) and OS (90% at 2 years) (Bacigalupo et al. 2010; Peffault de Latour 2016). This approach enabled also a very good long-term outcome with a rather limited number of late effects like avascular necrosis, endocrine dysfunctions, and very rarely secondary malignancy (Konopacki et al. 2012). However, toxicity related to transplantation as well as increased risk of GvHD is still a problem for patients older than 40 years of age and for those with high comorbidity index (Marsh et al. 2011). Different studies have shown that best results are seen when ATG is included in the preparative regimen to prevent both GVHD and graft failure (due to the underlying immune-mediated pathophysiology of SAA) (Bacigalupo et al. 2010; Peffault de Latour 2016); low-dose TBI (200 cGy) may also help to reduce the risk of graft failure in older patients (Bacigalupo et al. 2010). The importance of a T-cell directed serotherapy in this setting was confirmed using alternative T-cell depleting agents as alemtuzumab (Marsh et al. 2011; Dufour et al. 2015a, b).

#### 78.3.1.2 Standard IST in Case a Sibling Donor Is Not Available

For these categories first-line immunosuppression is recommended. Many efforts to improve results of the standard treatment with horse ATG and CSA have failed since 40 years (Scheinberg and Young 2012). Excellent results obtained with eltrombopag in monotherapy in refractory patients prompted American colleagues from the NIH to test if the addition of eltrombopag to standard IST as the first treatment for SAA would have increased the rate of CR and improved the long-term outcome. In the best cohort (eltrombopag associated to ATG and CSA from day 1), complete and overall response rates at 6 months



**Fig. 78.1** Treatment algorithm of SAA in 2023

were 58% and 94%, respectively. After a median follow-up of 2 years, survival rate is 97% (Townsley et al. 2017). Rates of relapse and clonal evolution were similar to historical experience. The severe aplastic anemia working party of the EBMT ran an open-label, multicenter, randomized, phase 3 trial, to compare the efficacy and safety of horse ATG plus cyclosporine with or without eltrombopag as front-line therapy in previously untreated patients with severe aplastic anemia. The primary endpoint was reached with higher complete response at month 3 in the experimental arm. At 6 months, the overall response rate (the percentage of patients who had a complete or partial response) was 41% (ATG + CSA) and 68% (ATG + CSA + EPAG). The response was quicker with EPAG and more patients were transfusion independent. The safety was similar between both groups. The addition of eltrombopag to standard immunosuppressive therapy thus

improved the rate, rapidity, and strength of hematologic response among previously untreated patients with severe aplastic anemia and should be considered as the new standard of care in those patients (Peffault de Latour et al. 2022).

### 78.3.1.3 Upfront Matched Unrelated Donor (MUD) Transplantation

Although pediatric patients respond better to IST, the long-term risks of relapse, CSA dependence, and clonal evolution are high (Dufour et al. 2015a, b; Tichelli et al. 2020). UK investigators reported an excellent estimated 5-year FFS of 95% in 44 consecutive children who received a 10-antigen (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1) MUD HCT; 40 of these children had previously failed IST. HCT conditioning was with FLU, CY, and campath (FCC) (Samarasinghe and Webb 2012). Because of those excellent results, upfront MUD HCT

became an attractive first-line option in children. Between 2005 and 2014, a UK cohort of 29 consecutive children with idiopathic SAA received UD HCTs (including five patients with 1 Ag mismatched transplants) as first-line therapy after conditioning with FCC. Results were excellent, with OS and EFS of 96% and 92%, respectively, low GVHD rates, and only one death (from idiopathic pneumonia). This cohort was then compared with historical matched controls who had received (1) first-line MRD HCT, (2) first-line IST with horse ATG + CSA, and (3) MUD HCT post-IST failure as second-line therapy. Outcomes for the up-front unrelated cohort were similar to MRD HCT and superior to IST and UD HCT post-IST failure (Dufour et al. 2015a, b). Similar results were observed in another pediatric study (Choi et al. 2017). The SAAWP of the EBMT recently published positive results using this approach (Petit et al. 2022). A Recent North American comparative feasibility study showed that upfront MUD HCT is feasible in children with SAA and, although numbers were small, that the outcome is likely to be superior than that with ATG + CSA (Pulsipher et al. 2020).

At the moment, if a 10/10 MUD is available and the transplant appears feasible within 2–3 months since diagnosis, this type of HCT has become a reasonable frontline option for young patients in many centers.

### 78.3.2 Second-Line Treatment for SAA

The choice of second-line treatment is also driven by age, comorbidities, and presence of a matched related (MRD) or unrelated donor (MUD):

- In older patients with a MRD and confirmed refractory SAA, HCT should be considered in the absence of significant comorbidities.
- In younger patients with SAA refractory to or relapsed after IST, if a MUD is available, HCT is recommended. Results of MUD HCT have improved to such an extent that OS of idiopathic SAA are not statistically inferior to MRD transplants (Dufour et al. 2015a, b).

This improvement has been largely attributed to better donor selection through allele matching, progress in supportive care, prophylaxis of GVHD, incorporation of FLU in conditioning regimens, and the addition of low-dose TBI. Some factors were found to positively affect OS after MUD HCT including age  $\leq 30$  years, transplant within the first year after diagnosis (Devillier et al. 2016), use of BM vs PB, and CMV status (Dufour et al. 2015a, b).

- In retrospective trials of refractory SAA, monotherapy with eltrombopag, an oral thrombopoietin-receptor agonist, induced an overall response of 40% with trilineage responses in some cases (Olmes et al. 2012; Desmond et al. 2014) and might help even if the patients were already exposed to eltrombopag in front line. Androgens might also be useful and have been very recently revisited (Pagliuca et al. 2023).

### 78.3.3 Emerging Strategies for SAA: Alternative Donor Transplantation in SAA

Alternative HCTs (MMURD, CB, and haplo-family donors) are possible for individuals with no suitable MUD. Alternative HCTs may be curative, but the risks of graft rejection, infectious complications, and GVHD are higher than those for MRD or MUD HCT. Patient age, comorbidities, and alternative HCT specificities are thus important issues in the decision-making process. Age and comorbidities are the first barriers to this type of procedure. Most of the larger retrospective cohorts (>50 patients) tend to mainly include pediatric patients. In historic studies, long-term OS of about 60% (Yagasaki et al. 2011; Peffault de Latour et al. 2011; Horan et al. 2012) compared to 5-year OS seen in refractory patients receiving only supportive care (Valdez et al. 2011). However, more recently, tremendous progress has been done. In pediatric patients refractory to IST, good results have been published using cord blood as source of stem cells (Peffault de Latour et al. 2018). The use of



haplo-identical transplantation in patients with aplastic anemia has also improved drastically using T-cell replete grafts with the administration of post-transplantation cyclophosphamide. The Baltimore group recently published a confirmatory study on the use of PTCy in refractory/relapsed young patients (median age 24.9 years) with aplastic anemia in the context of haplo-identical donor (DeZern et al. 2022): (1) despite HLA barriers, cure rates for patients with acquired AA following HLA-haploidentical BMT using a non-myeloablative conditioning regimen and PTCy exceed 80% overall survival with low rates of GVHD and eventually low TRM, (2) the results were drastically improved by added anti-thymocyte globuline (2.5 mg/kg total dose) to the “classical” Baltimore protocol (Luznik et al. 2008), and (3) the recommended source of stem cells is bone marrow (over peripheral blood stem cells) due to the low incidence of GvHD with bone marrow grafts and the amount should be higher than ( $>2.5 \times 10^8$  nucleated marrow cells per kg of recipient ideal bodyweight). Promising results were also reported on behalf of the Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplantation group on 36 patients (median age 42 years) transplanted between 2010 and 2017 with 1-year overall survival about 78% that peaks to 93% for those who received Baltimore-like protocol (Prata et al. 2020). More recently, a 3-year OS of 92% with 7% grade II–IV Ac GvHD and 4% cGvHD were achieved with the same protocol in 27 SAA patients transplanted front-line from haploidentical donors. In the subset of patients treated with higher TBI dose (400 cGy), OS and GRFS were 100% (DeZern et al. 2023). These results prompted our US colleagues to propose this approach upfront (first line) in young patients.

### 78.3.4 Supportive Care in SAA patients

The therapy of SAA is based not only on definitive modalities such as IST and HCT; indeed,

even in case of excellent responses, hematological recovery is not expected to occur for 3–6 months after IST, and patients remain at high risk of disease-associated complications for a long time. They include obvious consequences of pancytopenia, such as bleeding and infections. Thus, prompt and effective strategies of supportive care are essential to prevent (or treat) such unavoidable complications. The highest attention needs to be focused towards prevention and treatment of infectious complications; indeed, it has been shown that most of the improved outcome of SAA patients (which includes also patients who do not respond to IST) is due to the availability of more effective anti-infectious treatments (especially against fungal infections) (Valdez et al. 2011).

## 78.4 Treatment of PNH

Clinical presentation of PNH is extremely heterogeneous, including a variable combination of bone marrow failure, hemolytic anemia, and thromboembolism (de Latour et al. 2008). These clinical manifestations may change during the disease course of each individual patient so that the treatment of PNH should target the specific clinical presentation.

The treatment of marrow failure in PNH parallels that of SAA, and it has been described above; indeed, the presence of a PNH clone does not change the treatment algorithm of SAA. Even in case of clinically meaningful clones that may account for concomitant hemolysis, the management of AA (either severe or moderate) should always be taken into account, even when a concomitant anticomplement treatment has been given or may be indicated (Pagliuca et al. 2018; Griffin et al. 2018). Transplantation in PNH should not be performed in case of thrombosis occurrence because of the high risk of toxicity. It might be of help in patients with hemolytic PNH when complement inhibition is not available and is part of the treatment in case of aplastic anemia (Peffault de Latour et al. 2012). Given the paucity of systematic studies, open questions remain in



the field of HCT for PNH, starting with the best conditioning regimen (Marotta et al. 2014). Patients transplanted for a marrow failure, SAA-like regimens represent the best option, although busulphan-based myeloablative conditioning regimens have been used for PNH patients with normocellular/hypercellular marrow (Peiffault de Latour et al. 2012; Raiola et al. 2000), as an alternative to RIC (Takahashi et al. 2004). Since PNH, as SAA, is a nonmalignant disease and GvL is not needed for preventing disease recurrence, GVHD should be spared and thus strategies to prevent GVHD must be preferred.

Nevertheless, nowadays the room for HCT in PNH seems limited, since the treatment of complement-mediated hemolytic anemia and of thromboembolic PNH is based on complement inhibition through anti-C5 monoclonal antibodies. Eculizumab, the first in class anti-C5, has proven to be effective in inhibiting intravascular hemolysis of PNH, leading to hemoglobin stabilization and transfusion independency in about half of patients (Hillmen et al. 2006; Hillmen et al. 2007; Brodsky et al. 2008). This dramatic effect on intravascular hemolysis, eventually resulting in improved quality of life, is also associated with a significant reduction of the risk of thromboembolic complications (Hillmen et al. 2007). Notably, eculizumab treatment leads to a significant improvement of overall survival of PNH patients (Kelly et al. 2011; Loschi et al. 2016). The development of second-generation complement inhibitor began by the optimization of anti-C5 therapy, using long-acting monoclonal antibodies, ravulizumab. Ravulizumab is a derivative of eculizumab, with four amino acid substitutions, results in extended half-life. Given intravenously with eight-week dosing intervals, it was investigated in two large phase 3 studies enrolling untreated (Lee et al. 2019) or eculizumab-treated PNH patients (Kulasekararaj et al. 2019), respectively. Ravulizumab was shown to be non-inferior to eculizumab in terms of LDH change or normalization, transfusion avoidance, breakthrough hemolysis, hemoglobin stabilization and patient-reported outcomes and is now available on the market.

### 78.4.1 Emerging Strategies for PNH

The development of novel anti-complement agents exploits a new strategy of inhibition, which targets the early phases of complement activation aiming to address extravascular hemolysis (Risitano et al. 2009). The targets are (1) C3 with a pegylated peptide (pegcetacoplan), which block the access of the C3 to the convertases; (2) Factor D (FD) with danicopan, an oral complement FD inhibitor blocking the cleavage of complement Factor B and therefore the formation of functional C3 (and C5) convertase; (3) Factor B with iptacopan, an oral selective small molecule inhibitor of complement Factor B, which prevents its cleavage and therefore the formation of functional C3 (and C5) convertase.

The safety and efficacy of pegcetacoplan (anti-C3) were investigated in a phase III, open-label randomized study enrolling adult PNH patients with hemoglobin <10.5 g/dL on eculizumab therapy. At week 16, pegcetacoplan was superior to eculizumab in terms of hemoglobin change from baseline, with an adjusted mean treatment difference of 3.84 g/dL ( $p < 0.0001$ ). Transfusion avoidance was higher (85.4%, versus 15.4% in the pegcetacoplan and eculizumab arms, respectively), while noninferiority was demonstrated for the change in absolute reticulocyte count, but not for LDH. The safety profile was acceptable (Hillmen et al. 2021). These data have since been confirmed in the long-term analysis (48 weeks) (de Latour et al. 2022). The first-in-class factor D inhibitor, danicopan, is developed at the moment as an add-on treatment, in patients who respond poorly to eculizumab in a phase 3 randomized study (NCT04469465). Last but not least, factor B inhibitor iptacopan, an oral agent, which was investigated in an open-label phase two study enrolling 10 PNH patients with signs of active hemolysis on eculizumab treatment (Risitano et al. 2021). Eight out of the 10 patients achieved full normalization of hemoglobin level, with the mean hemoglobin level increasing from  $9.77 \pm 10.5$  at baseline to  $12.63 \pm 1.85$  g/dL at 13 weeks ( $p < 0.001$ ). Iptacopan is now being

investigated in two large phase three multicenter trials enrolling PNH patients with suboptimal hematological response to standard-of-care anti-C5 treatment (NCT04558918) and in patients naive to complement inhibition (NCT04820530).

### Key Points

- SAA is usually diagnosed in the setting of pancytopenia and a hypocellular BM when other diseases, especially inherited BMF such as Fanconi's anemia, telomere diseases, and immune deficiency/dysregulation have been excluded.
- The preferred treatment of SAA is HCT from HLA-identical sibling donor. Transplantation from a MUD may be considered for patients without a sibling donor after failure of IS therapy or up front in younger  $\leq 20$  years if feasible in 2–3 months since diagnosis.
- The association of ATG + CSA + Eltrombopag is now the new standard of care of patients with severe or very severe aplastic anemia who are not eligible for HCT.
- Alternative donor HCT, especially haplo-identical BMT improved tremendously in the recent years and is already considered as front-line treatment in pediatric patients by US authors.

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# Fanconi Anemia and Other Hereditary Bone Marrow Failure Syndromes

# 79

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## 79.1 Introduction

Inherited bone marrow failure syndromes (IBMFS) are a heterogeneous group of rare blood disorders due to hematopoiesis impairment, with different clinical presentations and pathogenic mechanisms. Some patients present with congenital malformations, may progress through clonal evolution (myelodysplasia, MDS and acute leukemia, AL) and are at risk for solid tumors at early ages. With the rapid evolution of diagnostic accuracy, the number of identified genetic defects rises annually. HCT is a curative treatment for these congenital disorders. However, it should be

well understood that it will only correct the hematopoietic deficiency and will not cure the congenital malformations or reduce the risk of solid tumors. Moreover, HCT per se may increase this risk additionally. Consequently, the decision to transplant a patient should be taken by a multi-disciplinary team. HCT must be performed at specialized centers owing to patient susceptibilities to toxicity and the need for specific management during and after the procedure. The general recommendations for management of IBMFS are included in the key points at the end of the chapter.

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## 79.2 Fanconi Anemia

### 79.2.1 Pathogenesis and Principal Clinical Features

Fanconi anemia (FA) is the most common IBMFS with an estimated incidence of 1/200,000. FA is a disorder of DNA damage repair, leading to increased chromosomal breakage in diagnostic assays. At least twenty-three underlying genes have been identified. The presentation is variable with somatic abnormalities in 70% of patients, marrow failure, and a highly increased risk of malignancies (MDS, leukemia, head and neck cancer, gynecological cancers) at an early age. The diagnosis can be delayed into adulthood, e.g., in cases of (familial) pancytopenia, myelodysplasia, head, and neck cancer at young age or unexpected severe toxicity of treatment. FA patients are highly susceptible to alkylating agent-based chemotherapy and irradiation-induced damage such as mucositis and bowel toxicity.

### 79.2.2 Indications of HCT

Indications for transplant include marrow failure (transfusion dependency or severe neutropenia) and myelodysplasia/leukemia. Since transplantation implies exposure to chemo/radiotherapy and since the outcome is dependent on age at transplant, the decision to proceed to transplant should be individualized and discussed with experts in the field trying to intercept the most appropriate time.

### 79.2.3 Specific Considerations for Conditioning Regimen

Conditioning regimens should be adapted for FA patients, with reduced doses of many cytotoxic drugs and irradiation. Current conditioning regimens generally contain FLU (cumulative dose 150 mg/m<sup>2</sup>), in combination with reduced doses of CY (up to 50 mg/kg cumulative) and/or low-dose TBI (100–300 cGy) in the case of unrelated

donors. For adults no clear recommendations can be given at this point. Patients with MDS with excess of blasts, overt AML, or BRCA2/FANCD1 patients may benefit from pre-HCT cytoreduction with fludarabine and cytarabine; however, it is very important to secure a donor before starting chemotherapy given the risk of prolonged chemotherapy-associated aplasia.

### 79.2.4 Results

Current results of transplant for marrow failure in children with FA show survival rates depending on donor type ranging from 50% to more than 90%. Factors influencing outcome include age at transplant, MSD availability, and the use of FLU in the conditioning regimen. An EBMT study of the period 2000–2009 reported 78% and 65% OS at 5-year post-transplant for MSD and MUD, respectively.

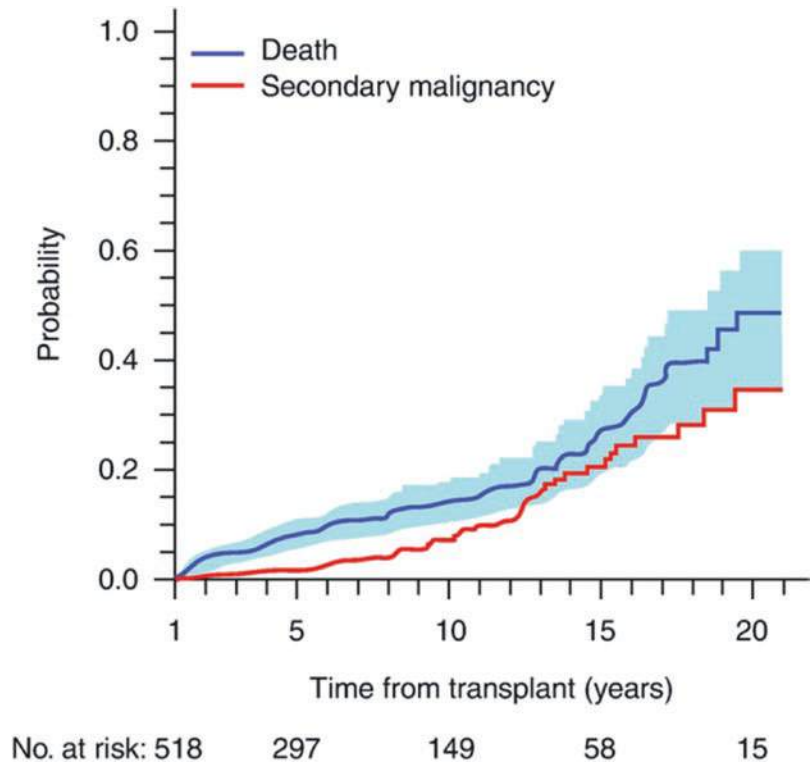
Eight hundred and thirteen patients under 18 years from the EBMT data registry were reported at ASH 2022. Ninety one percent of patients received a first HCT for marrow failure whereas 9% for either MDS or AML. Eighty three percent received FLU-based conditioning regimen, 87% any type of serotherapy, and 88% did not receive any irradiation. 5y-OS was 83%, statistically better for those transplanted from either MSD or MUD compared to those transplanted from MMUD or haploidentical donor. Being transplanted for MDS/AML dramatically worsened OS 40% vs. 85% for those transplanted for AA. Clonal disease and adult age at transplant remain a challenge. Both TRM and relapse of malignant disease contribute to these generally poor results.

### 79.2.5 Recommendations for Long-Term Follow-Up

After transplant, the risk of secondary malignancies is high (Fig. 79.1). Patients should be carefully monitored for head-neck malignancies (regular ear, nose, mouth, and throat specialist checks for early signs of malignant transforma-



**Fig. 79.1** Fanconi's anemia: probability of death and secondary malignancies post transplant. The EBMT experience. Peffault de Latour R. *Blood* 2013; 122: 4279–86



tion, and women undergo gynecological check-ups and screening for breast cancer with ultrasound and MRI) and other manifestations of FA (e.g., endocrinopathies such as thyroid dysfunction, diabetes, growth problems, and early menopause). As more FA patients survive into adulthood, specialist multidisciplinary teams taking care of these complex patients are needed.

Due to the increased risk of both head and neck and perineal secondary cancers, vaccination against human papilloma virus is strongly recommended.

is frequently associated with bone marrow failure and organ involvement (pulmonary fibrosis, liver, neurological and gastrointestinal abnormalities, ocular impairment, and cancer predisposition). The causative mechanism of the disease is abnormal telomere shortening due to a defect in one of the genes encoding for the telomerase-shelterin complex. Bone marrow failure is the main cause of death, although pulmonary fibrosis, liver cirrhosis, and cancer significantly contribute to morbidity and mortality. Androgens have shown some effect on hematopoiesis and lung function.

## 79.3 Telomere Disorders

### 79.3.1 Pathogenesis and Principal Clinical Features

Telomere disorders are multisystemic disorders. The classic example being dyskeratosis congenita (DC), characterized by the triad of nail dystrophy, skin pigment alteration, and oral leukoplakia,

### 79.3.2 Indications for Transplant

HCT is the only curative option for bone marrow failure in DC. Organ dysfunction is not corrected by HCT and limits its indication by negatively affecting outcome. HCT is not indicated as a pre-emptive measure but is recommended in cases of progressive marrow failure without significant organ dysfunction.

### 79.3.3 Specific Considerations for Donor Selection and Conditioning Regimen

MSD are the donors of choice when familial genetic study is available and demonstrated the donor is not affected; MUD and mismatched related donors are associated with inferior outcomes. Given tissue “fragility,” a reduced intensity combination containing fludarabine would be preferable to a myeloablative regimen. A thorough evaluation of organ status is recommended prior to transplant.

### 79.3.4 Results

Five- and 10-year survival is 57% and 23%, respectively. Age > 20 years at HCT, HCT before 2000 and alternative donor transplant were poor prognostic markers. Patients transplanted after 2000 had improved early survival to 70% at 5-year post transplant. However, medium- and long-time follow-up still remain poor. One of the unsolved caveats is the lack of a clear genotype–phenotype association. In fact, it is possible that some telomeroopathies are associated with a good outcome after HCT, while others are associated with poor survival, regardless of the type of transplantation.

### 79.3.5 Specific Recommendations for Long-Term Follow-Up

Since HCT may increase the risk of secondary malignancies in these patients, long-term follow-up is mandatory.

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## 79.4 Severe Congenital Neutropenia and Shwachman-Diamond Syndrome

### 79.4.1 Pathogenesis and Principal Clinical Features

The term “severe congenital neutropenia” (SCN) covers a group of inherited disorders character-

ized by a persistent absolute neutrophil count (ANC) below  $0.5 \times 10^9/L$  and early onset of severe infections. To date, more than 30 distinct genes have been associated with SCN, which may manifest as an isolated disorder or associated with various extra-hematologic features. The most common form (60%) of genetic neutropenia, at least in the Western registries, is due to mutations in the *ELANE* gene. Shwachman Diamond syndrome (SDS) caused by a biallelic pathogenic variants in the *SBDS* gene is the most common form of neutropenia associated with extra-hematologic features (exocrine pancreas deficiency, metaphyseal dysplasia, mental retardation, cardiomyopathy, and immune dysfunction).

The underlying pathogenic mechanism of most genetic SCN is accelerated apoptosis of promyelocytes causing blockage of neutrophil maturation. In SDS, the defect in the SBDS protein causes abnormal ribosomal assembly and inadequate maintenance of the stromal microenvironment.

The SCN clinical phenotype consists of predisposition to severe infections. The use of granulocyte colony-stimulating factor (G-CSF) improved the prognosis of the disease, which had been lethal in almost 50% of cases. The aim of treatment is to maintain protective neutrophil values (between  $1.0$  and  $5.0 \times 10^9/L$ ) that are usually achieved with G-CSF doses of 3–5  $\mu\text{g}/\text{kg}/\text{day}$ . Patients requiring G-CSF between 10 and 15  $\mu\text{g}/\text{kg}/\text{day}$  are defined as “poor responders,” whereas those requiring >20  $\mu\text{g}/\text{kg}/\text{day}$  are considered “non-responders.”

Another feature of SCN and SDS is its tendency to transform into MDS/AML. The overall cumulative incidence of MDS/AML is 10.8% and 22% after 15 and 20 years of G-CSF treatment according to the French and International Severe Chronic Neutropenia Registries, respectively. In SDS, the cumulative incidence is between 18% and 36% at 20 and 30 years.

### 79.4.2 Indications for Transplant

The definitive cure of the hematologic defect is HCT. For SCN, established indications are

patients with absent or poor response to G-CSF and MDS/AML. However, given the long-term dependency to G-CSF, HCT for children <10 years old with a healthy MSD may be considered. For SDS, the indications for transplant are worsening cytopenias with increased transfusion dependence and transformation into MDS/AML. However, given the very poor prognosis of SDS patients with leukemia, HCT should be performed before leukemia is established in patients with unfavorable cytogenetics or MDS.

### 79.4.3 Specific Considerations for Conditioning Regimen

A MAC regimen is considered appropriate in SCN, while a RIC is recommended in SDS owing to possible secondary organ dysfunction (e.g., heart disease). In patients with MDS/AML, the safety of a chemotherapy-based myeloablative conditioning should be evaluated case by case. Cytoreductive chemotherapy before HCT is an option, although its efficacy is in debate.

### 79.4.4 Results

For SCN patients, an EBMT study reported a 5-year post transplant OS of 82% with TRM of 17% in the largest cohort (136 patients) described. Better results were obtained in patients under 10 years of age, in those transplanted after 2000 and in cases of MSD transplants. For SDS in another study from the EBMT the OS at 5-year post transplant was 63.3%, and the TRM was 19.8%.

## 79.5 Diamond-Blackfan Anemia

### 79.5.1 Pathogenesis and Principal Clinical Features

Diamond-Blackfan anemia (DBA) is a rare IBMFS mainly caused by heterozygous mutations in ribosomal genes. No genetic aberration is identified in approximately 30% of patients. Patients usually present with transfusion-

dependent macrocytic anemia at birth or in early infancy. Mild neutropenia and progressive thrombocytopenia have been observed in the course of the disease. Despite various possible physical abnormalities (short stature, abnormal thumbs, cleft palate, heart defects, and urogenital malformations), the nonhematologic phenotype is usually rather subtle in around 50% of patients. Patients with DBA are at increased risk of developing hematologic (AML/MDS) and nonhematologic malignancies (osteosarcoma, colon cancer).

After the first year of life, at least two trials of steroid therapy are recommended; around 60% of the patients are responders. Patients <12 months of age or those who are steroid nonresponders are treated with red blood cell (RBC) transfusions. Iron chelation is essential to prevent organ damage from iron overload. About 20% of patients become transfusion independent with no further treatment (spontaneous remission).

### 79.5.2 Indications for Transplant

HCT is the only cure for hematologic manifestations. Indications are nonresponse to steroids, steroid dependency at a dose  $\geq 0.3$  mg/kg/day, transfusion dependency, alloimmunization to RBC, progressive pancytopenia, or MDS/AML. Published data indicate that HCT should be performed before the age of 10 years; however, an earlier time point might be preferable to avoid iron overload. Indications must be evaluated taking into account the alternative approach with RBC transfusions combined with rigorous iron chelation.

### 79.5.3 Specific Considerations for Donor Selection and Conditioning Regimen

HCT from a MSD including cord blood has resulted in OS >80% and is recommended for all indications. Sibling donors should be carefully assessed to rule out silent carrier status. Recent reports have described no differences in terms of survival between sibling and MUD transplants,

although GVHD was higher in MUD transplants. By contrast, data supporting HCT from mismatched donors as standard procedure are scarce.

The majority of transplants reported were performed with myeloablative conditioning. Based on available data, a standard regimen including FLU and BU or TREO is recommended. ATG/ATLG has been used in multiply transfused patients transplanted from MSD and MUD with excellent engraftment rates.

### 79.5.4 Results

In children aged <10 years outcomes are comparable for HCT performed using MSD and MUD. A study of DBA patients registered in Germany and France reported a cGFS of 90% for patients <10 years and of 78% for those aged 10–18 years. Lower OS and higher TRM have been reported in patients with higher ferritin levels as a marker of iron overload.

### 79.5.5 Specific Recommendations for Long-Term Follow-Up

Long-term care for patients being transplanted for DBA should focus on the management of iron overload. Depending on its extent, phlebotomies, and/or iron chelation therapy might be indicated. Furthermore, patients and physicians should be aware of the increased risk of malignancies (especially osteosarcoma and colon cancer).

## 79.6 Congenital Amegakaryocytic Thrombocytopenia

### 79.6.1 Pathogenesis and Principal Clinical Features

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare IBMFS caused by mutations in the gene coding for the thrombopoietin receptor *MPL*. Patients usually present with thrombocytopenia at birth or within the first year of life. Most patients develop hypocellular bone marrow and

progress to pancytopenia early in the course of disease. Clonal evolution with acquired chromosomal aberrations and the development of myelodysplastic syndromes are very rare events. Characteristic nonhematologic manifestations of the disease have not been described.

### 79.6.2 Indications for Transplant

HCT is the only curative treatment and should be offered to all patients with transfusion-dependent thrombocytopenia, pancytopenia, or clonal evolution.

### 79.6.3 Specific Considerations for Donor Selection and Conditioning Regimen

HCT from an MSD is the preferred option, and successful transplants from heterozygous-related donors have been reported. HCT from MUD ( $\geq 9/10$ ) is an acceptable alternative. Successful HCT from mismatched family donors or mismatched unrelated cord blood donors have been reported: these should preferentially be performed in clinical trials or experienced centers.

In view of a considerable graft failure rate, a MAC with FLU in combination with TREO or BU is preferred. There have been reports of successful engraftment after a RIC, and this might be considered in cases with severely hypocellular bone marrow in the absence of clonal aberrations and alloimmunization to platelet transfusions.

### 79.6.4 Results

Five-year OS was 77% with TRM of 12.6% in a retrospective EBMT study. However, this series included HCT performed over a period of 26 years with a variety of donors, regimens, and stem cell sources, with no difference in outcome.

These results were updated for ASH annual meeting in 2022 and demonstrated a 6-year OS of 85.6% with a transplant-related mortality of

8.0%. GVHD/graft failure-free survival was 65.7% at 6 years. 6-year OS was significantly lower after cord blood (37.5%) than after BM (95%) or PB (88.9%) ( $p < 0.001$ ).

### Key Points

Patient	Evaluate carefully hematologic and extra-hematologic manifestations of the disease prior to transplant
Donor	The best donor is MSD; however, it is mandatory to test the genetic defect in the donor since some IBMFS may present different clinical and hematologic expression in members of the same family Consider MUD in case of no appropriate MSD Mismatched related and UD and unrelated CB only in experienced centers and preferentially in clinical trials
Source of stem cells	BM is the best source of stem cells Matched related CB is a good option PB is associated with higher risk of cGVHD and should be avoided
Cell dose	It is important for graft failure prevention: NC > $3 \times 10^8$ /kg recipient bw for BM NC > $3 \times 10^7$ /kg recipient bw for related CB NC > $4 \times 10^7$ /kg recipient bw for unrelated CB
Conditioning regimen	MAC or RIC depending on the type of IBMF Irradiation should be avoided owing to the known risk of cancer Patients with Fanconi's anemia and dyskeratosis congenita must receive a RIC
GVHD prophylaxis	GVHD must be avoided Include two immunosuppressive drugs Serotherapy for UD transplants
Long-term follow-up	It is mandatory owing to high risk of secondary malignancies, extra-hematologic manifestations, and iron overload Patients should be followed-up by a multidisciplinary team

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# Hemoglobinopathies (Sickle Cell Disease and Thalassemia)

# 80

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## 80.1 HCT for Sickle Cell Disease

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### 80.1.1 Definition and Epidemiology

Sickle cell disease (SCD) is the most common inherited hemoglobinopathy worldwide. It is caused by a single-nucleotide substitution that leads to a propensity toward hemoglobin polymerization and red blood cell sickling. SCD is

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characterized by anemia, ongoing hemolysis, and acute and chronic vaso-occlusive complications affecting multiple organs. SCD affects mostly patients of African origin, but with a high prevalence also in certain parts of Turkey and the Arabic countries. The substantial migration in particular over the last decade led to a prominent rise of the prevalence in all European countries.

The implementation of newborn screening, penicillin prophylaxis, vaccination programs, narcotics, chronic transfusions, hydroxyurea, and the early detection of cerebral vasculopathy with transcranial doppler have basically abrogated the SCD-related mortality in childhood (Brandow and Liem 2022) but had little impact on the overall survival from SCD. Novel, disease-modifying treatment options such as Voxelotor, L-Glutamine, and Crizanlizumab, focusing on stabilization of the oxygenated hemoglobin state, reducing anti-sickling or cellular adhesion, or activating pyruvate, intend to effectively control the disease (de la Fuente et al. 2020) (Table 80.1), but the impact on the SCD-related chronic vasculopathy is unpredictable and the Phase III trial on Crizalinizumab failed to reach the primary endpoint.

### 80.1.2 Allo-HCT with an HLA Identical Sibling

Allogeneic HCT remains the only curative therapy for SCD. The goal when performing HCT is to replace the patient's marrow with donor stem

**Table 80.1** Current and future disease modifying therapies for sickle cell disease; modified from de la Fuente et al. (2020)

	Indication	Stage of development	Proposed mechanism of action
Hydroxycarbamide (hydroxyurea)	Prevention of vaso-occlusive crisis, acute chest syndrome, and primary stroke; also recommended for treatment of severe anemia	Standard care	Enhances fetal hemoglobin production, increases mean corpuscular volume, and reduces white blood cells and reticulocytes; might also be a nitric oxide donor
Chronic blood transfusion	Treatment and prevention of acute and chronic complications of sickle cell disease	Standard care	Suppresses endogenous erythropoiesis, decreases the concentration of red blood cells containing hemoglobin S, and increases hemoglobin concentration
Glutamine	Reduction in vaso-occlusive crisis	Approved in USA (2017, for SCD pts. $\geq 5$ years old) but not approved in Europe	Raises nicotinamide adenine dinucleotide redox ratio and prevents oxidative damage within hemoglobin S-containing erythrocytes
Voxelotor	Treatment of sickle cell disease has been shown to increase hemoglobin concentration and reduce biomarkers of hemolysis.	Approved for SCD pts. $\geq 12$ years old in the USA (2019) and Europe (2022)	Synthetic allosteric modifier of hemoglobin; inhibits polymerization of hemoglobin S
Crizanlizumab	Reduction in vaso-occlusive crisis	Approved for SCD pts. $\geq 16$ years old in USA (2019) and Europe (2020) Phase III trial failed primary endpoint (2023)	Humanized monoclonal antibody, blocks P-selectin-mediated multicellular adhesion

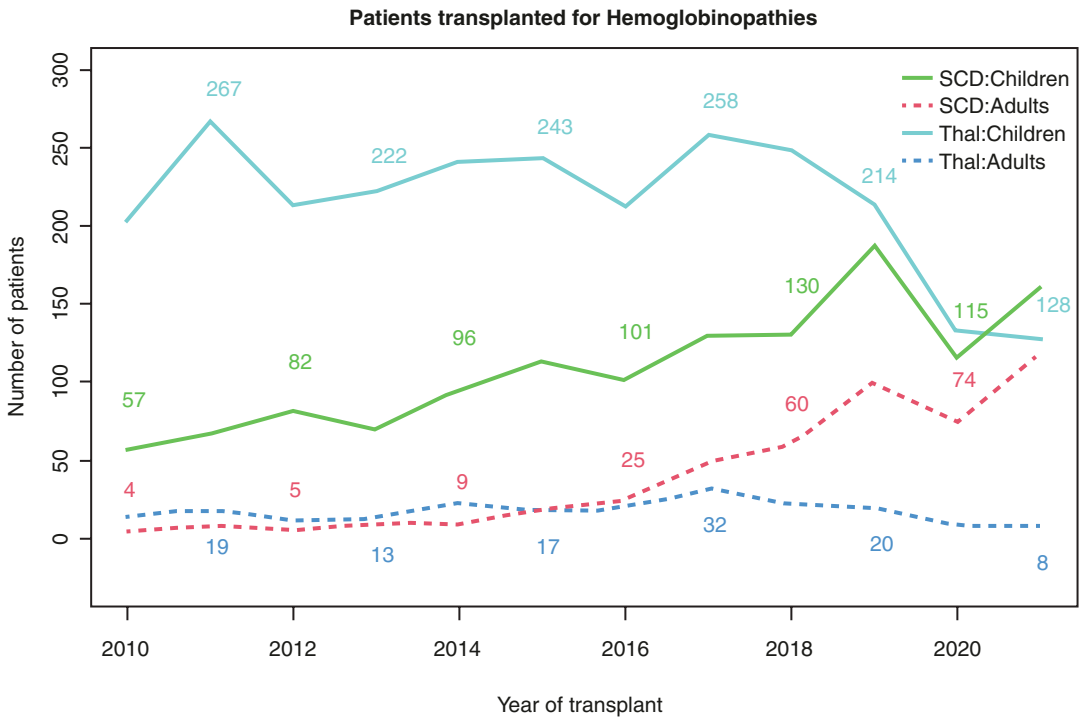
cells prior to major organ dysfunction and irreversible damage.

Several barriers prevent widespread application of HCT including lack of a suitable donor, lack of information, and limited understanding of HCT. Moreover, HCT encompasses a certain risk of early- and late-onset regimen-related toxicities, such as rejection, GVHD, and treatment-related mortality which impedes the decision for patients/caregivers to prefer HCT over non-curative treatment options, especially since hemoglobinopathies are so called non-malignant diseases. Nevertheless, the transplantation frequency for hemoglobinopathies in Europe has been increasing in the last decade (Fig. 80.1). According to the registry of the EBMT, the frequency of transplantations in adult SCD patients has risen significantly within the last decade. Most remarkable is the 2-yr overall survival of these patients that exceeds that of adult TDT patients, who have

been transplanted for a substantially longer time period (Table 80.2).

The first successful HLA identical HCT was performed in a patient affected by both SCD and AML in 1984. After that, many groups have described series of patients transplanted from an HLA identical sibling with an OS that varies between 91% and 100% and EFS that varies between 73 and 100% (Bernaudin et al. 2007). An international survey including 1000 HLA identical transplants, performed between 1986 and 2013 and reported to EBMT, Eurocord, and the CIBMTR, showed a 5-year EFS and OS of 91.4% and 92.9%, respectively. Graft failure was observed in 23 patients (Gluckman et al. 2017).

Age at transplantation is an important predictor of survival. Patients younger than 5 years have an excellent OS and EFS (Cappelli et al. 2019; Bhalla et al. 2023); therefore, HLA-identical sibling HCT should be proposed early



**Fig. 80.1** Transplant activities in the EBMT registries for hemoglobinopathies in the last decade

**Table 80.2** Two-years overall survival in the EBMT registries for hemoglobinopathies in the last decade

	2 y OS
SCD: Children	95.6 (94.1–96.6)
SCD: Adults	93.5 (90.7–95.5)
Thal: Children	92.1 (90.8–93.2)
Thal: Adults	84.4 (78.1–89.0)

in life, before complications occur. It must be noticed that at a cutoff of 15 years, the incidence of cGVHD in matched sibling donors can rise beyond 20% (Cappelli et al. 2019).

Other studies have shown that different to most other diseases a fully matched unrelated donor yields different EFS, OS, and also the incidence of cGVHD is substantially higher compared to an age matched sibling donor, again with a similar cutoff for worse outcome at age  $\geq 13$  years (Eapen et al. 2019).

Patient's age should be also be taken into account for the choice of the best donor selection and treatment strategy according to HCT-related complications (de la Fuente et al. 2020) (Fig. 80.2).

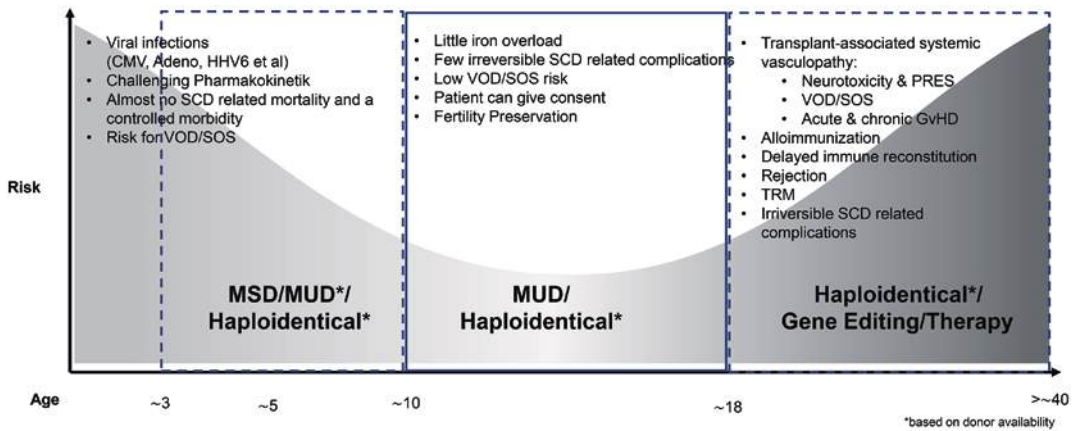
Family-directed cord blood banking could represent a useful stem cell resource for families with a child affected by SCD (Rafii et al. 2017).

A recent survey evaluating changes on experienced health and personal life goals demonstrated that transplantation has a positive impact on physical, mental, and social health in adult SCD patients (Dovern et al. 2023).

There is an increasing body of evidence that reversibility affecting almost all organs, including cerebral perfusion (Bernaudin 2020) and osteonecrosis (Hernigou et al. 1997), is possible. Pivotal part of any pretransplant consultation is a thorough assessment of SCD-related organ damage, followed by a discussion of the expectations to avoid disappointment and frustration.

### 80.1.3 Conditioning Regimen

To date, a myeloablative conditioning (MAC) regimen is the gold standard for HLA identical sibling HCTs (EFS: 73–96%, OS: 91–100%)



**Fig. 80.2** Age related risk factors for allogeneic HCT in SCD patients and recommendations for donor selection/treatment strategy. From Josu de la Fuente et al.

(2020)HCT hematopoietic cell transplantation. GVHD graft-versus-host disease. \*Based on donor availability

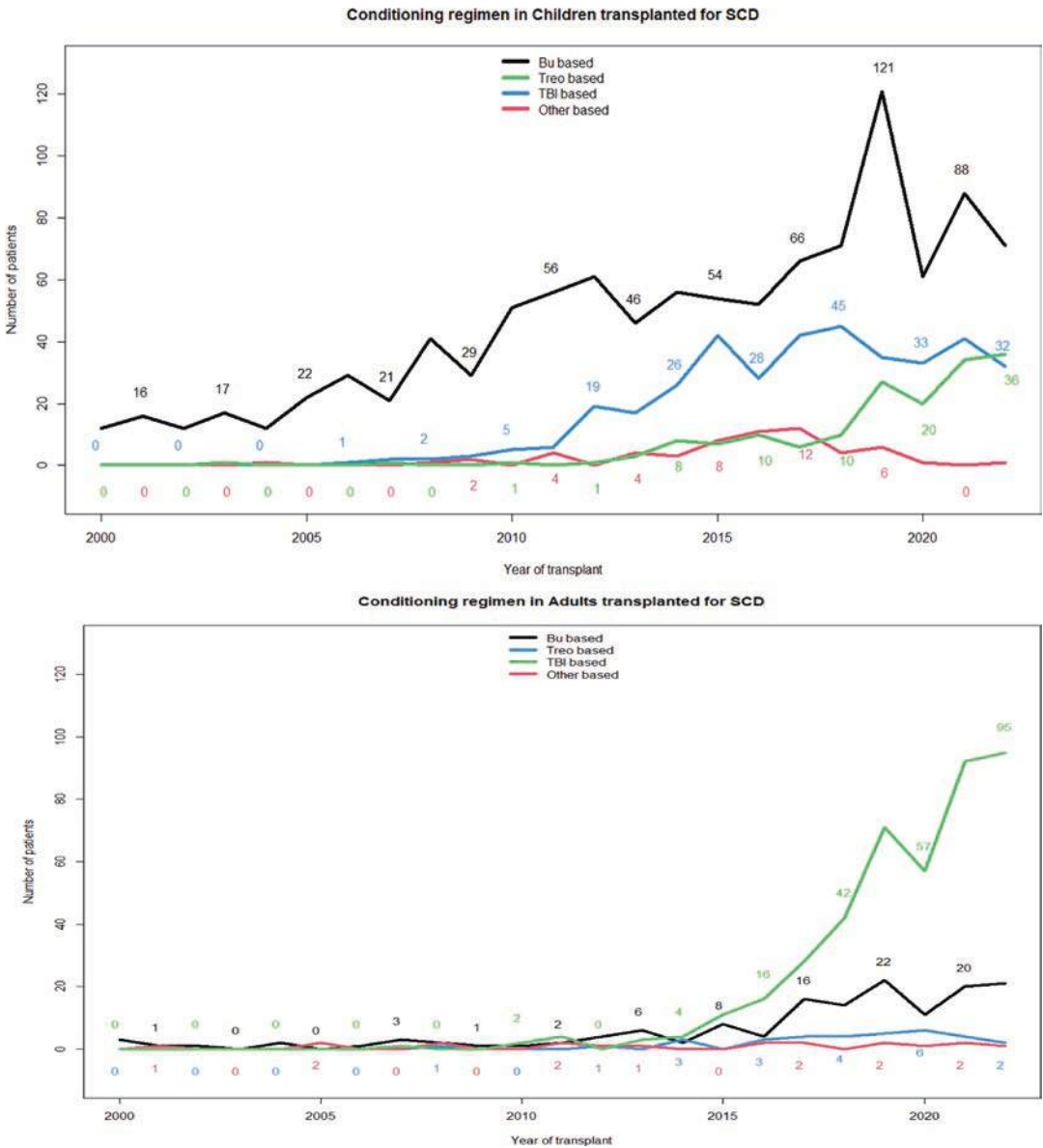
despite the risk of long-term transplant-related toxicity, especially when patients are already suffering from organ damage due to their SCD status (Bhalla et al. 2023). Historically, a BU-based conditioning regimen including cyclophosphamide or FLU has been used but with high toxicities and GvHD risk; therefore, in order to lower the GvHD risk, the addition of ATG is recommended. In recent years, fludarabin, treosulfan, and thiotepa (FTT) emerged as a frequently used contemporaneous MAC conditioning in SCD in children and adults. The reason is that FTT is well tolerated with the advantage of a limited endothelial toxicity and less blood–brain barrier passage (EBMT registry data) (Foell et al. 2020) and a higher tendency for a preserved fertility (Faraci et al. 2019) (Fig. 80.3).

A reduced intensity conditioning (RIC) regimen has been explored to decrease toxicity allowing a stable mixed chimerism. The aim of a tailored conditioning regimen in children is to preserve fertility, whereas in adults, it is to reduce toxicity in severely compromised patients due to their underlying disease. Recently, encouraging outcomes and low early- and long-term toxicity have been observed with FLU-based RIC regimens or after a chemotherapy-free regimen (ALEM-TBI 300 cGy; DFS = 92% and OS = 100%) (Bhalla et al. 2023; de la Fuente et al. 2020).

Despite MAC regimens, a mixture of both donor and recipient hematopoietic cells (mixed donor chimerism) can be consistently observed in approximately 10–20% of transplanted children. Interestingly, this mixed chimeric state with the presence of both recipient and donor blood cells is sufficient to direct bone marrow to preferentially produce donor-type hemoglobin (rather than abnormal hemoglobin of the recipient), reverse the red blood cell SCD phenotype, and minimize the risk of GvHD, confirming the therapeutic efficacy of mixed chimerism for hemoglobinopathies. In cases of a mixed chimerism, split chimerism analyses are pivotal for a proper understanding of the hematopoietic compartments, where a full myeloid chimerism encourages a watch-and-wait strategy. This approach avoids unnecessary and dangerous interventions to achieve a full donor chimerism, which is not mandatory in case of a non-malignant myeloid disease.

#### 80.1.4 Alternative Donors

Finding a potential matched unrelated donor (MUD) is based on the ethnic and racial background of the recipient; for SCD patients the probability for an 8/8 HLA MUD or CB donor is less than 18%. Nevertheless, some small series of



**Fig. 80.3** Conditioning regimens in Children and Adults transplanted in EBMT centers

patients using unrelated donors have been published, but for now relapse rate and GvHD risk remain high (Gluckman et al. 2020). Whereas in many diseases, outcome does not differ much between MSD and MUD, a recent retrospective multicenter, cohort study in SCD found significant differences in pivotal outcome parameters such as OS and GVHD in SCD. As in MSD HCT, the threshold for worse outcome is again adolescent age ( $\geq 13$  years) (Eapen et al. 2019).

The development of both in vivo and ex vivo T-cell depletion strategies has facilitated the emergence of haploidentical donor HCT as a solution with universal availability of donors. Initial attempts resulted in a high rate of graft failure (43%); therefore, different conditioning regimens have been developed. A nonmyeloablative conditioning regimen including ATG, fludarabine, thiotepa, cyclophosphamide, and low-dose total body irradiation, followed by

post-transplantation cyclophosphamide showed an excellent OS with a low graft failure and a low mortality rate (de la Fuente 2019).

A single-center experience with TCR $\alpha\beta$ -depleted and CD19-depleted grafts conditioned with FTT and ATG showed rapid engraftment and a low incidence of GvHD with OS and DFS comparable to MSD HCT (Foell et al. 2019). This approach is currently evaluated in a prospective stratified trial (NCT04201210). Taken together, these results show that outcomes of haploidentical HCT are increasingly similar to MSD HCT and seem feasible, safe, and effective, thus offering a curative option for the majority of SCD patients with no matched donor.

### 80.1.5 Gene therapy and Gene editing

To date, several gene therapy or gene editing clinical trials have been conducted or are ongoing [Home - ClinicalTrials.gov](#).

Lentiviral vector based approaches carrying a modified beta globin or gamma globin gene are under investigation for safety and efficacy. In 2021, lentiviral vectors trials were temporarily suspended due to the observation of three cases of leukemia after gene therapy with Lentiglobin (Bluebird). Lentiviral vector BB305 was not shown responsible of the clonal transformation. In order to predict the risk of leukemia or other secondary tumors in SCD patients after transplantation or after gene therapy, it seems very important to determine if some genetic factors, such as clonal hematopoiesis of indeterminate potential (CHIP) are present. Evidence supporting this hypothesis would support the screening of sickle cell patients for pre-leukemic genetic factors before HCT, gene therapy or gene editing.

B-cell lymphoma/leukemia 11A (BCL11A), a transcription factor that represses  $\gamma$ -globin expression, has been identified as a target to restore high expression of fetal hemoglobin (HbF). New strategies of gene therapy infusing

autologous CD34+ cells transduced with the BCH-BB694 lentiviral vector, which encodes a short hairpin RNA targeting in the erythroid lineage BCL11A mRNA embedded in a microRNA, lead to a robust and stable HbF induction, with marked clinical improvements (Esrick et al. 2021).

The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 nuclease system is another strategy used targeting the erythroid-specific enhancer region of BCL11A. Using this strategy, the CLIMB 121 SCD study is ongoing and preliminary results are very encouraging. Most advanced is a (CRISPR)-Cas9-based approach using nonviral, ex vivo editing of the erythroid-specific enhancer region of *BCL11A* in CD34+ cells to reduce erythroid-specific expression of BCL11A (NCT03745287) (Frangoul et al. 2021).

CD34+ cells collection after G-CSF stimulation is now contraindicated in SCD patient for severe complications, whereas plerixafor (CXCR4 chemokine receptor antagonist) alone or in combination with GRO- $\beta$  (CXCR2 agonist) has been used successfully (Frangoul et al. 2021; Leonard et al. 2022).

### 80.1.6 Conclusion

Sickle cell disease is increasingly recognized as a health-care priority with several novel disease modifying drugs being licensed in recent years and excellent outcomes after HLA-matched sibling donor transplantation, especially if performed at young age. Improved conditioning regimens, and novel GvHD prophylaxis have contributed to an increased use and acceptability of HCT for SCD. Moreover, for patients lacking an HLA-matched sibling donor, outcomes of haploidentical HCT are improving. Innovative gene therapy and gene editing clinical trials are ongoing and show promising results. The future challenges are to stratify patients according to the disease risk, to revise transplantation indications, and to define the best therapeutic approach for each patient.



## 80.2 Thalassemia

Miguel R. Abboud and Josu de la Fuente

### 80.2.1 Introduction

The outcome of transfusion-dependent thalassemia (TDT) has improved dramatically over the past two decades due to improvements in supportive care, and iron chelation therapy with magnetic resonance-based tissue iron monitoring (Taher et al. 2021). Life expectancy for TDT patients currently exceeds 40 years (Vitran et al. 2017), but despite these advances it remains below that of the general population (Kountouris et al. 2021; Jobanputra et al. 2020). Furthermore, patients have a significant burden of morbidity and quality of life is significantly affected starting from childhood (Jobanputra et al. 2020; Shah et al. 2021). Recently, luspatercept was approved and was shown to decrease transfusion requirements in patients with thalassemia. The ultimate impact of this drug remains to be determined (Sheth et al. 2023).

Despite the recent approval of a gene addition therapy for some patients with TDT (see below), matched family donor (MFD) allo-HCT is currently considered the only curative standard therapeutic approach for this disorder (Oikonomopoulou and Goussetis 2021). HCT, though associated with significant risks results in life-long transfusion independence, allows reversal of tissue iron load with possible resolution of iron accumulation complications and improved quality of life (Caocci et al. 2011a, b; La Nasa et al. 2013; Mulas et al. 2022).

### 80.2.2 Best Transplant Candidates and Conditioning

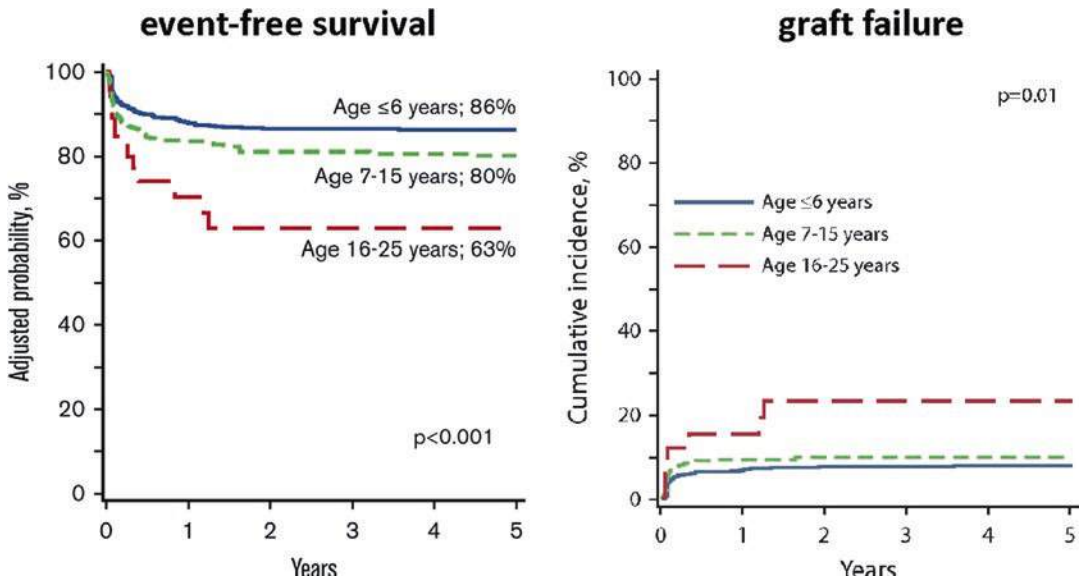
In the late 1990s, the Pesaro group proposed a risk classification for pediatric patients undergoing MFD HCT for TDT (Lucarelli et al. 1998). The classification depended on three risk factors (Table 80.3) and was validated in the pediatric

**Table 80.3** Pesaro classifications for risk assessment prior to HCT for TM (Lucarelli et al. 1998)

Risk factor	Class 1	Class 2 (min. 1, max. 2)	Class 3
Inadequate chelation	×	×/✓	✓
Hepatomegaly >2 cm	×	×/✓	✓
Portal fibrosis	×	×/✓	✓

population; however, it did not predict risk in adult patients (Angelucci et al. 2017). The Pesaro classification is applicable in the setting of best medical care. In developing countries, where medical care might not be optimal, a very-high-risk group was identified in Pesaro class 3 patients if liver size is >5 cm below the costal margin and if the patient age is >7 years (Mathews et al. 2007). The introduction of fludarabine-based conditioning regimens has abrogated the risks for all but the most severely iron loaded, including the class 3 patients (Bernardo et al. 2008, 2012). The EBMT has recently identified age of 14 years as the oldest age for best outcome in HCT for TDT (Baronciani et al. 2016). Age as a risk factor was corroborated by more recent data from CIBMTR with optimal outcomes up to 7 years of age (Li et al. 2019) (Fig. 80.4). A recent retrospective analysis of the Turkish experience (1469 patients treated in 25 centres) further supports the need to incorporate HCT in the treatment algorithm at a young age (Yisilipek et al. 2022).

Accurate assessment of iron content in the liver and heart is crucial before proceeding to transplant. Serum ferritin level might not reflect accurately the severity of iron overload and does not provide information regarding the stage of fibrosis. Liver biopsy is the gold standard; however, it carries the risks of the invasive procedure and transient elastography (FibroScan) has been shown to be reliable noninvasive methods to predict liver fibrosis secondary to iron overload in adults (Hamidieh et al. 2014, 2015). In addition, center experience is a significant factor in outcomes, and hence, it is important HCT for TDT occurs in designated centers with experience (Yisilipek et al. 2022). Transplantation allows reversal of end-organ damage (Mureto et al.



**Fig. 80.4** Age is the major determinant in event-free survival and graft failure in TDT (Li et al. 2019)

2002); thus, development of new predictive indices that allow the identification of risks associated with the procedure and the patients most likely to benefit from it in different settings should be targets of future research (Kulkarni et al. 2022).

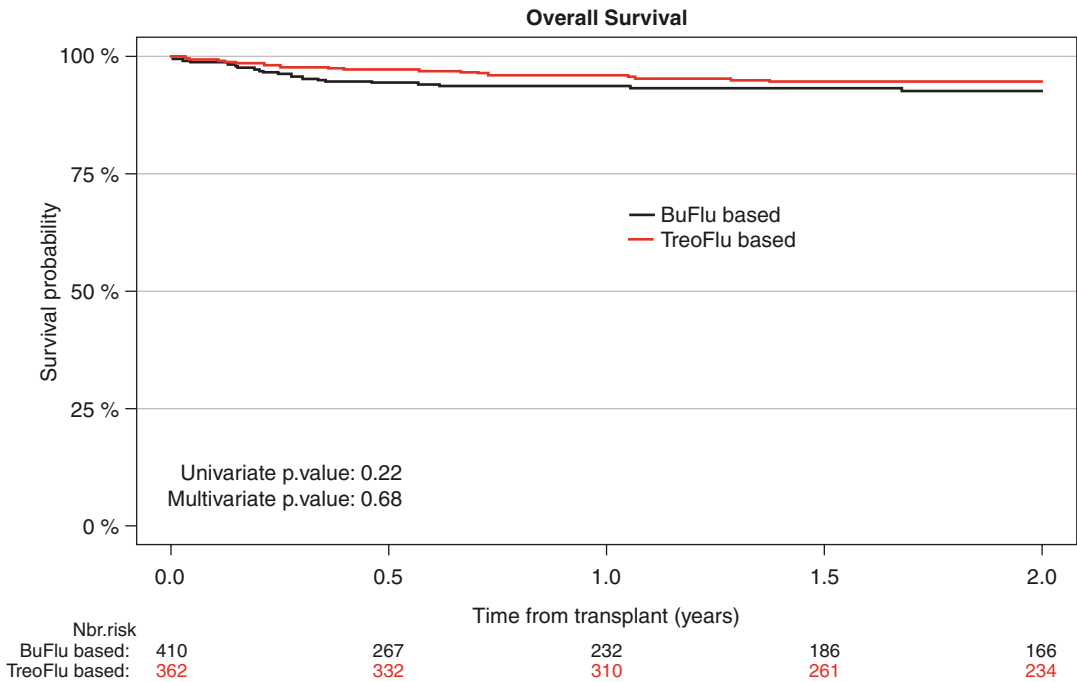
### 80.2.3 Conditioning Regimens

Myeloablative busulfan (BU) and cyclophosphamide (CY) were the standard conditioning regimen for HCT for TDT due to the increased marrow activity and the allo-sensitization in heavily transfused patients (Lucarelli et al. 1990). However, this regimen was associated with hepatic and cardiac toxicity due to the iron overload and the effects of BU and CY, respectively. Younger patients have a greatest risk of rejection, which can be abrogated with the addition of thiotepa 10 mg/kg to the conditioning regimen (Lucarelli and Gaziev 2008; Chiesa et al. 2010). Additional interventions that can minimize the risk of graft failure are the pretransplantation suppression of endogenous hemopoiesis with hypertransfusions, the use of hydroxycarbamide 30 mg/kg and azathioprine 3 mg/kg pre-

transplantation, and the use of ATG/ATLG (Sodani et al. 2004; Shen et al. 2008; Cappelli et al. 2009).

BU/CY-based conditioning regimens have been progressively superseded by reduced toxicity fludarabine-based myeloablative conditioning regimens, increasingly with treosulfan use (Bernardo et al. 2008, 2012). It was recently demonstrated that there is no overall difference in outcomes between busulfan and treosulfan in fludarabine-based conditioning regimens (Fig. 80.5), though there is a trend toward less toxicity but higher graft failure with treosulfan (Lüftinger et al. 2022). Initially, serotherapy was used only in the setting of unrelated donors but since the identification of the favorable effect of ATG on engraftment (Bernaudin et al. 2007; Cappelli et al. 2009), its use is now widely adopted. Concerning other regimens, there is limited evidence, needing further corroboration, that non-myeloablative approaches may also be feasible in TDT, which would significantly expand HCT in adults (Shin et al. 2020).

The incidence of GVHD is a major determinant of post-transplant morbidity, mortality, and quality of life (Caocci et al. 2011a, b). Thus, new approaches to GVHD prevention are being evalu-



**Fig. 80.5** Effect of treosulfan compared with busulfan-based regimens on OS (Lüftinger 2022)

ated, Abatacept added to routine GVHD prophylaxis in the context of fludarabine reduced toxicity myeloablative conditioning for hemoglobinopathies reduced the incidence of day +100 severe acute GVHD without impacting engraftment or survival in thalassaemia (Khandelwal et al. 2021). This agent is also protective against the development of chronic GvHD through extended dosing in the context of haploidentical transplantation with post transplantation cyclophosphamide (PTCy) for transfusion-dependent thalassaemia (Jaiswal et al. 2020).

Defibrotide has been used successfully to prevent SOS/VOD in patients with TDT undergoing HCT with conditioning regimen containing IV BU (Cappelli et al. 2009). The use of this agent is, however, restricted to the treatment of SOS in many countries. Basing busulfan dosing on BU pharmacokinetics was associated with better engraftment and less toxicity (Gaziev et al. 2010); however, these studies are available in a limited number of institutions worldwide.

## 80.2.4 Alternative Donors

### 80.2.4.1 Matched Unrelated Donors (MUD)

Advances in high-resolution typing, supportive care, and the availability of fludarabine-based conditioning regimens containing both busulfan and treosulfan have enabled the consideration of matched unrelated transplantation as a suitable alternative (Bernardo et al. 2012; Li et al. 2012) with outcomes not significantly different to related donors. Equivalent outcomes have been validated in real-world data from CIBMTR (Li et al. 2019) and the recent retrospective analysis of the Turkish experience found identical hazards ratio for GvHD-free thalassaemia-free survival for related and unrelated donors (Yesilipek et al. 2022). Hence, matched unrelated transplantation should be considered a standard option alongside related transplantation notwithstanding the limitations of non-Caucasian overall donor availability (Gragert et al. 2014).

#### 80.2.4.2 Unrelated Umbilical Cord

The use of unrelated umbilical cord (UCB) as a source of stem cells for HCT in TDT is hampered by the high incidence of graft failure due to the low stem cell dose. The graft failure rate could be as high as 57% (Ruggeri et al. 2011). This could be partially overcome by the use of double UCB units. The 5-year overall and thalassemia-free survival rates were 88.3% and 73.9%, respectively, when using two units instead of one if no single units included more than  $25 \times 10^6$  total nucleated cells/kg of recipient weight. Other strategies to overcome the main barrier of low cell dose include co-transplantation of third-party mesenchymal stromal or TCD haploidentical cells (Kwon et al. 2014).

#### 80.2.4.3 Haploidentical HCT

Due to the low probability of finding a MUD in some ethnicities and the previously mentioned issues with umbilical-cord transplant, new strategies were employed to develop an effective and safe haploidentical transplant procedure for TDT patients. Historically, the use of mismatched donors has been associated with increased mortality and reduced long-term engraftment (Li et al. 2019; Lüftinger et al. 2022). Ex vivo T cell depletion (TCD) with CD34+ selection techniques were associated with high rate of infections and increased risk of graft failure due to allo-sensitization and hyperactive marrow (Gaziev et al. 2000). However, these challenges are being addressed with novel TCR $\alpha\beta$  CD19+ depleted TCD techniques (Bertaina et al. 2017; Merli et al. 2022) and PTCy (de la Fuente et al. 2019; Jaiswal et al. 2020; Anurathapan et al. 2020), with results approaching the outcomes of both related and unrelated transplantation.

#### 80.2.5 Stem Cell Source

The ideal source of stem cells remains bone marrow, and this is associated with lower rates of acute and chronic GVHD, therefore associated with normalization of quality of life (Caocci et al. 2011a, b). Nonetheless, recently good outcomes have been reported with adequate GVHD pro-

phylaxis and peripheral blood stem cells (PBSC) to obtain sufficient stem cells for recipients with high body weight (Yesilipek et al. 2022).

#### 80.2.6 Mixed Chimerism

Mixed chimerism is common and often results in stable chimerism or complete engraftment and patients remain transfusion independent (Andreani et al. 2000). However, this situation needs to be stabilized because it can lead to poor graft function and clonal evolution (Gassas et al. 2021). Mixed chimerism is usually driven by mixed T cell chimerism. This is best addressed by enhancement of immunosuppression, usually by maintaining or adding mycophenolate mofetil as a second immunosuppressive agent which maximizes the donor fraction (Mehta et al. 2023). An alternative approach is to use DLI though it carries a risk of GVHD and its efficacy can be limited (Frugnoli et al. 2010; Karasu et al. 2012; Chen et al. 2020). Pretransplantation immunosuppression (PTIS) can minimize mixed chimerism and graft failure in higher risk transplants like haploidentical and reduced intensity transplantation (Anurathapan et al. 2020; Jaiswal et al. 2020).

#### 80.2.7 Post-Transplant Iron Chelation and Follow Up

Iron overload remains a problem after HCT, and most investigators rely on phlebotomy to decrease excessive iron stores. In a recent phase II, multicenter, single-arm trial, deferasirox at a dose of 20 mg/kg/day, starting after a minimum of 6 months of transplant, and continued for 1 year, was safe and associated with decreased burden of iron overload after transplant (serum ferritin, liver, and cardiac iron content by MRI) (Yesilipek et al. 2018). Patients with thalassemia who are cured by stem cell transplants experience significantly improved quality of life; however, they require lifelong follow-up for complications that may have preceded the transplant as well as transplant-related issues.

## 80.2.8 Gene Therapy

The advent of gene addition and gene editing strategies will inevitably impact the indication for stem cell transplantation in thalassemia. To date no malignancies have been reported in patients with thalassemia who received gene therapy after myeloablative busulfan. Although, the initial lentiviral gene addition strategy proved more successful in patients with non- $\beta^0/\beta^0$  genotypes (Thompson et al. 2018; Locatelli et al. 2022a, b) and children (Markt et al. 2019), improvements in manufacturing and provision have led to high rates of long-term to freedom from transfusion with improvement of dyserythropoiesis and iron overload in all patients (Thompson et al. 2021; Magrin et al. 2022). Recently, excellent results were published utilizing a CRISPR-Cas9-based approach using nonviral, ex vivo editing of the erythroid-specific enhancer region of BCL11A in CD34<sup>+</sup> cells to reduce erythroid-specific expression of BCL11A and increase the expression of fetal hemoglobin. Most patients became transfusion independent with a few having decreased transfusion burden (NCT03745287) (Frangoul et al. 2021). This technique was effective in both  $\beta^0/\beta^0$  and non  $\beta^0/\beta^0$  genotypes. It is important to establish the patients for whom its priority is indicated, particularly in the early phase of use outside experimental trials conducted in highly selected centers (Baronciani et al. 2021).

### Key Points

- HLA identical sibling HCT is an established treatment option for SCD and TDT.
- Unrelated transplantation offers equivalent outcomes to sibling transplantation in TDT.
- HCT should be performed as early as possible.
- Haplo identical HCT, gene therapy, and gene editing trials for SCD and TDT are ongoing with promising results.
- Mixed chimerism is common and its management is key to optimal outcomes.

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### 81.1 Which Patients Are Candidates for Autologous Hematopoietic Cell Transplantation?

The concept of high-dose therapy (HDT) followed by autologous hematopoietic cell transplantation (AHCT) remains the standard for treating newly diagnosed multiple myeloma in young and in select, fit, elderly patients. The introduction of ImiDs and proteasome inhibitors

administered before and/or after HDT/AHCT gave way to the groundbreaking achievement of stringent complete response (sCR), immunophenotypic CR, and molecular CR, in addition to significantly increased CR and CR plus very good partial response rate (VGPR; Table 81.1). In randomized studies, age of participants is limited to 65 years to avoid selection bias and limit toxicities and withdrawal from studies. However, this does not mean that AAHCT is not feasible in older patients. A study whereby the median age of patients was 72 years old concluded that elderly multiple myeloma patients should not be excluded from transplantation displaying good results with melphalan 140 mg/m<sup>2</sup>. Currently, in many centers, fit patients up to age 70, and even 75 years old, receive AHCT.

Renal impairment, per se, is not a contraindication to receiving HDT/AHCT with the recommendation for the use of 140 mg/m<sup>2</sup> of Melphalan. Nonetheless, it is a prompt reason to consider lower doses of therapy, as patients with renal impairment are more likely to suffer from HDM toxicities. Dialysis-dependent patients were more likely to develop toxicities and complications such as mucositis and infections, but had PFS and OS comparable to matched patients with normal renal function. Interestingly, a proportion of patients were able to attain dialysis-independence after transplantation.

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**Table 81.1** Current risk stratification and response criteria

<b>Risk stratification</b>
<b>ISS:</b> Stage I (beta2-m < 3.5 mg/L and albumin >3.3 g/dL), Stage III (beta2-m > 5.5 mg/L) and Stage II (all remaining cases).
<b>1st revision (R-ISS):</b> I: ISS I, standard-risk cytogenetics <sup>a</sup> and normal LDH, III: ISS III, plus high-risk cytogenetics or high LDH and II: all remaining cases.
<b>2nd revision (R2-ISS):</b> points assigned for ISS-III (1.5), ISS-II (1), del(17p) (1), high LDH (1), 1q + (0.5) I: 0 points II: 0.5–1 points III: 1.5–2.5 points IV: 3–5 points
<b>Response criteria</b>
<b>MRD:</b> bone marrow aspirate, analyze CD38, CD138, and CD45 in combination with CD19, CD56, CD27, CD81, and CD117 by FCM or sensitivity level $\leq 10^{-5}$ and $10^{-6}$ nucleated cells using next-generation sequencing
<b>CR:</b> negative serum and urine immunofixation, less than 5% BMPCs and no soft-tissue plasmacytomas
<b>Stringent CR:</b> as above plus normal free light-chain ratio and absence of clonal plasma cells.
<b>VGPR:</b> 90% or more decrease in the serum M-protein and urine M-protein <100 mg/24 h.
<b>PR:</b> 50% or more decrease in the serum M-protein, 90% or more decrease in urine M-protein or to <200 mg/24 h plus 50% or more decrease in soft-tissue plasmacytomas.
<b>PD:</b> $\geq 1$ of the following: increase in $\geq 25\%$ from nadir in serum M-protein (absolute increase of at least 0.5 g/dL), urine M-protein (absolute increase $\geq 200$ mg/24 h), BMPC (absolute increase $\geq 10\%$ ), soft-tissue plasmacytomas, development of new bone lesions, soft-tissue plasmacytomas, or hypercalcemia

MRD measurable residual disease, CR complete response, VGPR very good partial response, PR partial response, PD progressive disease, BMPC bone marrow plasma cells, LDH lactate dehydrogenase, FCM flow cytometry<sup>a</sup>FISH identifying the following poor cytogenetic findings: t(4;14), t(14;16), and/or del 17p

## 81.2 What Is the Optimal Induction Treatment Prior to AHCT?

The role of induction is to decrease tumor burden, thus deepening the response rate and increasing the likelihood of engraftment, while retaining the maximum possible tolerability and minimum

possible toxicity on normal hematopoietic cells. Multiple trials have proven the superiority of induction regimens containing one or two novel agents (thalidomide or bortezomib) over the historical VAD chemotherapy regimen in increasing CR, CR plus near-complete response (n-CR), or VGPR rates pre- and post-AHCT. Trials that compared two-drug (TD: thalidomide-dexamethasone or VD: bortezomib-dexamethasone) to three-drug induction (VTD: bortezomib, thalidomide, dexamethasone) have proven supremacy of the latter combination. VTD was also proven superior to bortezomib, cyclophosphamide and dexamethasone (VCD) thus highlighting the synergistic effect of combining an IMiD with bortezomib and dexamethasone. Furthermore, the use of 6 cycles of VTD (instead of 3–4 cycles) was associated with deeper responses. This is to be weighed against increased side effects, specifically neuropathy, upon administering 6 cycles instead of 3–4.

Similarly, the three-drug regimen bortezomib, lenalidomide, and dexamethasone (VRD) resulted in significantly increased PFS, response duration and OS resulting in the IFM introducing VRD as induction. In addition, the PETHEMA/GEM trial investigated induction with VRD-GEM with full dose lenalidomide from days 1 to 21, demonstrating an ORR of 85% post induction and 58% of patients achieving MRD-negativity post consolidation (Table 81.2).

Daratumumab (DARA), an anti-CD38+ monoclonal antibody, has been evaluated in patients with refractory disease. The Cassiopeia phase III trial and the Griffin phase II trial compared DARA-VTD to VTD and DARA-VRD to VRD respectively demonstrating positive results of adding daratumumab. Daratumumab plus cyclophosphamide, bortezomib, and dexamethasone (Dara-CyBorD) during induction was investigated in the phase II Lyra trial. Recent updates of the trial demonstrate activity and tolerability of Dara-CyBorD irrespective of high-risk cytogenetics with 12-month PFS and OS rates 87% and 99%, respectively. Finally, daratumumab is also being combined with carfilzomib, lenalidomide and dexamethasone (KRD) in a phase Ib trial whereby the combined regimen yielded 100%

**Table 81.2** Selected prospective studies of AHCT vs no AHCT in newly diagnosed myeloma

Study groups	IFM 2009	BMTCTN 0702	Forte	Determination	Cardamon	EMN02/HO95
	LenBorDex + AHCT vs LenBorDex 8 cycles All with 1y Len maintenance	Tandem AHCT vs AHCT + LenBorDex vs AHCT All with Len maintenance until progression	CarLenDex + AHCT vs CarCyDex + AHCT vs CarLenDex 12 cycles Maintenance with CarLen or Len	LenBorDex vs LenBorDex + AHCT + LenBorDex Both groups with Len until disease progression	CarCyDex + AHCT vs CarCyDex 4 cycles All with Car maintenance 18 cycles	AHCT (single or tandem per center policy) vs VMP All with Len maintenance
Location	France	US	Italy	International (mainly USA)	UK	Europe
Follow-up, months	44	NR	51	76	40	60
Results						
PFS	Median: 50 months vs 36 months ( $P < 0.001$ )	38-months: 59% vs 58% vs 54%	4y: 69% vs 51% vs 56%	46 vs 68 months ( $P < 0.001$ )	2y: 75% vs 68%	Median: 57 months vs 42 months ( $P < 0.001$ ) 5y: 54% tandem AHCT vs 45% single AHCT
OS	3y: 81% vs 82%	6y: 73% vs 75% vs 76%	4y: 86% vs 76% vs 85%	5y: 79% vs 81%	2y: 94% vs 91%	5y: 80% tandem AHCT vs 73% single AHCT vs 72%

PFS progression-free survival, OS overall survival, y year, AHCT autologous hematopoietic cell transplantation, Len lenalidomide, Dex dexamethasone, Bor bortezomib, Car carfilzomib



ORR, 91%  $\geq$  VGPR and 43%  $\geq$  CR, with no negative impact on stem cell harvesting while retaining consistency of the DARA-KRD safety profile. Isatuximab is another anti-CD38 monoclonal antibody in investigation as part of the quadruplets combinations previously mentioned and there is a phase 3 GMMG-HD7 trial ongoing comparing Isa-RVd versus RVd before and after HDT-AHCT. Preliminary results showed a significantly higher undetectable MRD rate in the Isa-RVd arm (50.1%) versus RVd alone (35.6%) after 3  $\times$  6-week cycles.

MRD negativity, defined as the absence of disease within  $10^5$  bone marrow cells, has been examined due to its important prognostic value at different stages of the transplantation process. The final analysis of the IFM2009 prospective trial demonstrated the significance of MRD negativity whereby patients achieving MRD negativity after induction with VRD had a similar OS irrespective of whether they received an AHCT or not. Similar results were reported in the phase 3 Determination study, with a similar design to the IFM2009 but maintenance with lenalidomide was given until progression disease. The lack of benefit in OS was equally observed and, in this study, only 29% of the patients in the control arm received AHCT at relapse (89% in the IFM2009 trial). In addition, MRD negativity proved in both studies a more powerful predictor of outcome than cytogenetics whereby patients with high-risk cytogenetics who achieved MRD negativity had better outcomes than patients with standard-risk cytogenetics who did not. Thus, MRD could potentially become essential in stratifying patients during maintenance and consolidation randomization and when deciding on maintenance duration.

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### 81.3 What Is the Optimal Conditioning Regimen Prior to AHCT?

The current accepted standard for HDT is intravenous high-dose melphalan (200 mg/m<sup>2</sup>). Previous trials attempting to replace this with oral and intravenous busulfan have failed, due to increased toxicity and lack of superiority.

Similarly, studies failed to show a significant benefit for combining bortezomib and high-dose melphalan. The role of bendamustine added to melphalan is being explored and not well established yet. As such, HDM remains the standard conditioning regimen prior to AHCT awaiting results of clinical trials of other conditioning regimens (if any).

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### 81.4 What Is the Impact of Consolidation Therapy After AHCT?

The second randomization in the EMN02/HO95 trial compared the aftermaths of receiving 2 cycles of VRD consolidation followed by lenalidomide versus lenalidomide maintenance alone, demonstrating the significant advantage VRD consolidation inferred in prolonging PFS. Moreover, PFS was prolonged in most of the predefined groups in the study including ISS I and II, low-risk cytogenetics, irrespective of whether patients received VMP (bortezomib, melphalan and prednisone) or transplantation prior to consolidation. Nonetheless, VRD consolidation failed to improve PFS in patients with high-risk cytogenetics ((del(17p) and/or t(4;14) and/or t(14;16)). This confirms the benefit of VRD consolidation followed by lenalidomide maintenance in younger, newly diagnosed multiple myeloma patients with low-risk disease. Along the same line of the EMN02/HO95 trial, the StaMINA phase III trial randomized patients to compare HDM/AHCT plus VRD consolidation plus lenalidomide maintenance, versus tandem HDM/AHCT plus lenalidomide maintenance, versus single HDM/AHCT plus lenalidomide maintenance. It concluded that the addition of VRD consolidation or a tandem AHCT was not superior to standard AHCT followed by lenalidomide in upfront treatment of newly diagnosed multiple myeloma. With the currently available data, the role of post-transplant consolidation remains controversial. European guidelines do not make any formal recommendation about consolidation, but the most recent phase 3 trials that incorporate the

anti-CD38 monoclonal antibodies as part of the induction with either VTD or VRD do include 2 cycles of consolidation after 4 induction cycles followed by AHCT. A practical possibility not evaluated in prospective trials would be to consider the consolidation as optional depending of the depth of response achieved after AHCT, and it would not be necessary for those patients already in CR and undetectable MRD.

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### **81.5 What Is the Impact of Maintenance Therapy After AHCT?**

AHCT is not curative, and progressions and relapses are common even if CR is attained post-transplant. Maintenance therapy is thus added and is expected to be gentle with the safest profile post AHCT, but unlike consolidation, it is administered long-term to deepen the response, prevent progression, and prolong OS. Thalidomide maintenance demonstrated benefit in terms of response rates but not OS and was repeatedly associated with peripheral neuropathy, fatigue, and other side effects, all of which resulted in patient-reported decreased quality of life despite prolonged duration of disease control. In contrast, lenalidomide maintenance has been shown to be well tolerated and to dramatically improve PFS and OS. A meta-analysis of 3 randomized trials, CALGB, IFM, and GIMEMA, that compared lenalidomide maintenance to placebo or observation, has demonstrated a significantly improved PFS in all subgroups of patients regardless of age, myeloma severity and staging, and induction regimen (52.8 versus 23.5 months), even though patients who had received lenalidomide in induction, or had achieved a deeper response post-transplant, were more likely to benefit from lenalidomide. OS was also significantly improved in the lenalidomide arm, except in women older than 60 years with poor cytogenetics. Overall, the addition of lenalidomide reduced the chance of death by a substantial 25%, thus increasing median survival by approximately 2.4 years. As demonstrated in previous studies, an increased incidence of second primary malignancies, albeit

modest, was associated with lenalidomide, though the time to death due to a second primary malignancy did not differ between the two groups. Such results propose lenalidomide as a standard maintenance drug in transplant-eligible patients. Recent updates of the Myeloma XI trial's results were in concordance with the meta-analysis.

So far in previous trials, lenalidomide has been given in low doses until progression or adverse events develop, and this practice is currently approved by both, FDA and EMA. Given that 30% of cases with premature termination of lenalidomide were attributed to toxicities and second primary malignancies, the question that remains is regarding the optimal duration of treatment with lenalidomide for safety and cost.

Finally, bortezomib was also tested as part of maintenance, either alone or in combination with IMiDs, demonstrating improved PFS, but not OS. Nonetheless, bortezomib poses an obstacle due to its subcutaneous administration. The first oral PI, ixazomib, has been evaluated as maintenance in comparison with placebo in a phase 3 trial and although the trial met its primary endpoint, the magnitude of the benefit is limited with a difference in PFS of only 5 months (26.5 vs 21.3) and not approved in this setting. Monoclonal antibodies anti-CD38 are being evaluated as maintenance, in majority of the trials in combination with lenalidomide, and there are at least two phase 3 clinical trials comparing lenalidomide with lenalidomide plus daratumumab and one comparing lenalidomide with lenalidomide plus isatuximab. Results are awaited to confirm the superiority of such combination.

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### **81.6 What Is the Value of Single Versus Tandem AHCT?**

The concept of tandem AHCT came about in an era where conventional chemotherapy was the only available drug. Previous randomized trials had demonstrated improved outcomes with tandem transplantation in terms of PFS and OS even in patients who had not achieved a VGPR after

the first transplant. An alternative treatment approach, total therapy 3 (TT3), including induction, tandem AHCT, consolidation, and maintenance, has allowed one of the best results to be achieved (CR/nCR rate of 83%, 2-year PFS of 84%, and 2-year OS of 86%).

In the modern era, the impact of tandem transplantation was evaluated in the EMN02/HO95 and StaMINA trials. The EMN02/HO95 trial explored the result of tandem versus single transplantation in newly diagnosed multiple myeloma patients. Tandem transplantation was shown to improve the depth of the response by 25% with more than 50% of the patients achieving at least a CR. PFS and OS were significantly improved after a second transplant, with approximately 30% reduction in the risk of death and progression. Updated results of the EMN02/HO95 confirmed the improved 3-year PFS from 63 months after one AHCT to 73.1 after two AHCTs. Importantly, the positive effect of tandem AHCT was seen in high-risk groups, in which randomization to receive double AHCT was found to be an independent predictor of PFS. The analysis thus concluded that double frontline AHCT was superior to single AHCT in terms of PFS and OS in all patients, including poor prognosis subgroups, indicating that the latter were the most likely to benefit. On the other hand, the StaMINA trial failed to show superiority of tandem versus single transplant in the era of novel agents. It is noteworthy that more than 30% of patients randomized to tandem transplant did not receive the second transplant.

Overall, with the currently available data, a second AHCT may be beneficial in high-risk patients including patients with high-risk cytogenetics and R-ISS 3 category of disease.

The next challenge is to evaluate the necessity of HDT/AHCT when a monoclonal antibody such as daratumumab or isatuximab is added to a powerful induction regimen combining an IMiD and a PI, and whether this strategy can cure a fraction of patients. This strategy is currently being evaluated in at least 2 different phase 3 clinical trials (MIDAS and MASTER-2). The impending challenge remains whether or not transplantation will be later substituted by less intensive novel agent combinations.

## 81.7 What Is the Role of AHCT as Salvage Therapy?

Salvage therapy is defined as AHCT given to a patient with signs of disease progression after an earlier AHCT. By the BSBMT/UKMF Myeloma X trial, salvage AHCT with 200 mg/m<sup>2</sup> melphalan was superior to cyclophosphamide 400 mg/m<sup>2</sup> weekly for 12 weeks upon relapse and reinduction with VAD. The time to disease progression (19 versus 11 months) and OS (67 vs. 52 months) were significantly in favor of salvage AHCT. As such, AHCT can be considered for salvage in fit patients if the interval between the first AHCT and relapse is relatively very long (e.g. at least 2–3 years). The randomized GMMG phase III trial ReLApSE compared salvage AHCT and lenalidomide maintenance versus. Lenalidomide/dexamethasone for relapsed multiple myeloma and although the incorporation of salvage AHCT into relapse treatment with Rd was feasible in 71% of patients it did not significantly prolong PFS and OS on ITT analysis. Overall, and except in a few highly selected patients, the role of AHCT in the relapsed setting is currently questionable.

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## 81.8 What Is the Role of Allogeneic Hematopoietic Cell Transplantation in Multiple Myeloma?

Allogeneic transplantation may result in durable remissions in subsets of myeloma patients. However, a long follow-up is required to observe a potential survival benefit after passing the initial risk of transplant-related mortality. Trials that employed the biologic assignment based on the availability of a HLA-matched sibling donor reported conflicting results in newly diagnosed myeloma. Two trials reported better PFS and OS with an “allo-approach”. However, a meta-analysis showed that the benefits of allografting are offset by high rates of treatment-related mortality. Currently, due to the lack of robust clinical data, there is considerable uncertainty, in the era

**Table 81.3** Results of the key CAR-T cell studies in multiple myeloma

Product	N	Lines of therapy, median	High-risk cytogenetics/EMD	ORR	PFS, median in months	CRS/ICANS (grades 3–4)
Ide-cel	128	6	35%/39%	73%	9	5%/3%
	386	3	42%/24%	71%	13	4%/3%
Cilta-cel	97	6	24%/13%	98%	35	4%/9%
	419	2	59%/21%	85%	NR	1%/3%
ARI0002h	30	4	33%/20%	100%	15	0%/0%

of novel agents and consolidation/maintenance strategies concerning the subset of patients who could most benefit from an allograft and its timing. Novel strategies could not be designed without the combination of new drugs that may enhance graft-versus-myeloma effects to allow long-term disease control and prolong OS, in particular in patients with high-risk disease. Enhancement of the graft-versus-myeloma effect after allografts through the immunomodulatory properties of novel agents may be a key factor to improve clinical outcomes. There is evidence that a synergy between the donor immune system and immunomodulatory drugs may prolong response. Though optimal timing of an allograft and dosage of new drugs remain to be determined, in the absence of other alternatives, younger patients with high-risk features, suboptimal response, and/or early relapse may be considered by some investigators for an allograft, preferably in the context of a clinical trial.

### 81.9 The Emerging Role CAR-T Cells in Multiple Myeloma

Chimeric antigen receptor (CAR-) T cell therapy represents a new and promising option for relapsed/refractory myeloma patients. Two commercial products, both targeting the myeloma-specific antigen B cell maturation antigen (BCMA), have been approved: idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel). The indications for both products are patients with >3 previous lines of therapy, including an IMiD, proteasome inhibitors, and anti-CD38 monoclonal antibodies. Subsequent phase 3 results have been reported. The KarMMa-3 study enrolled participants with 2–4 previous

lines, randomizing ide-cel or standard-of-care chemotherapy regimens. The median PFS was 13 months in the ide-cel group versus 4 months. The CARTITUDE-4 trial included patients with lenalidomide-refractory MM and 1–3 previous lines. The median PFS was not reached for the cilta-cel group versus 12 months. Remarkably, these therapies also induced deep responses in high-risk patients, including those with extramedullary disease, highlighting its potential to revolutionize treatment algorithms for these patients.

Because of the financial burden of such therapies, academic CAR-T products are emerging. ARI0002h (an academic product from Spain) was administered in a fractionated manner with a booster dose after 3 months for patients after >1 previous lines of therapy including a proteasome inhibitor, an IMiD, and an anti-CD38 antibody. This induced deep and sustained responses, with a low toxicity. Other efforts from Israel achieved comparable results, underscoring the potential for point-of-care CAR-T cell therapy that may increase accessibility without loss of efficacy (Table 81.3).

### 81.10 Conclusion and Future Perspectives

More than thirty years after its introduction, HDT/AHCT remains the standard of care for patients with newly diagnosed multiple myeloma. Despite the advent of novel agents, AHCT remains a very common treatment modality, especially in Europe and is included in all ongoing and proposed trials. The latter is yet to be challenged by many novel agents (including earlier use of CAR T-cells) which are continuously

explored. When dealing with unfavorable cytogenetics and poor prognostic factors, tandem transplantation appears to be an encouraging strategy. Whereas the use of post-transplant consolidation is controversial, lenalidomide maintenance prolongs PFS and OS and can be considered as a standard of care. One important endpoint to be measured in studies is MRD negativity in standard or high-risk disease, since it is a significant predictor of PFS, OS, and potential cure in a fraction of patients.

### Key Points

- AHCT is the preferred treatment approach (standard of care) in young and fit myeloma patients.
- Prior to AHCT, patients should receive at least a triplet-based induction regimen (ideally a quadruplet including an anti-CD38 antibody) aiming to achieve a deep response.
- High-dose MEL 200 mg/m<sup>2</sup> is the standard conditioning for AHCT in myeloma.
- Patients should receive some form of post AHCT therapy (maintenance therapy preceded eventually by consolidation).
- Double AHCT can be considered for high-risk myeloma (e.g., patients with a del17p cytogenetic abnormality).
- The role of allogeneic-HCT (briefly discussed in this chapter) is highly controversial in myeloma and should be performed as part of a clinical trial.
- In the mid-term, the role of AHCT will be challenged by the advent of CAR-T cells.

### Further Reading

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# Systemic Light Chain Amyloidosis

# 82

Monique Minnema and Stefan Schönland

## 82.1 Definition and Epidemiology

Systemic light chain (AL) amyloidosis is a protein misfolding and deposition disorder with an incidence of 5–10 persons per million per year. Clonal plasma cells or rarely B cells produce immunoglobulin light chains with the potential to misfold. These light chains are deposited as extracellular amyloid fibrils in peripheral tissues and cause morbidity and mortality. Organs most frequently involved are the heart, kidney, liver, autonomic and peripheral nervous system, gastrointestinal tract, and soft tissue.

## 82.2 Diagnosis

AL amyloidosis should be suspected in any patient with a monoclonal gammopathy and a compatible clinical syndrome such as heart failure with a preserved ejection fraction, nephrotic range proteinuria, unexplained weight loss, peripheral neuropathy, a bleeding diathesis, or carpal tunnel syndrome. Gammopathy work-up

should include a serum-free light chain (FLC) assay, immunofixation of serum and urine, bone marrow cytology, flow cytometry, histology and iFISH, and a full-body scan to exclude bone lesions due to symptomatic multiple myeloma (MM). Amyloidosis is diagnosed by histopathology with Congo red staining and the typical apple-green birefringence under polarized light. Screening biopsies such as abdominal fat, salivary gland, upper GI tract, or bone marrow as well as symptomatically involved organs can be utilized (Palladini et al. 2020). The amyloid subtype (i.e., AL, ATTR, AA, or another even more rare subtype) has to be further confirmed by immunohistochemistry, immune electron microscopy, or laser microdissection and mass spectrometry. Besides the more common systemic AL amyloidosis, this type of amyloidosis can also occur as a local presentation with a very good prognosis, for example, in the pharynx or bladder, and requires another treatment approach which will not be discussed in this chapter.

## 82.3 Classification

AL amyloidosis can be classified by the origin of the underlying bone marrow disease: a clonal plasma cell or more rarely lymphoid dyscrasia, which is often an IgM related AL amyloidosis. Plasma cell dyscrasias can further be divided into monoclonal gammopathy, smoldering MM, and symptomatic MM among other things depending

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on the size of the clone. Each of those can lead to AL amyloidosis, but the clone is usually small (Palladini et al. 2020).

## 82.4 Risk Factors and Prognostic Scores

The underlying bone marrow disease as well as organ damage-related biomarkers can be utilized to stratify patients into risk groups. A bone marrow plasma cell infiltration above 10% and a high difference between involved and uninvolved serum-free light chain (dFLC) are negative prognostic factors for overall survival. Comparable to MM genetic aberrations can be detected on iFISH in plasma cell dyscrasias and be utilized to predict response to specific treatments (e.g., in patients with translocation (11;14) HDM/HCT is more effective) (Palladini et al. 2020).

Commonly used is the cardiac biomarker based classification from the Mayo clinic with the European modification. This staging system uses the biomarkers NT-ProBNP and cardiac troponins (cTnI, cTnT) measured at diagnosis. This classification system with stages I, II, IIIa, and IIIb is strongly related with survival and also provides guidance for the intensity of treatment that patients can tolerate.

For patients with renal involvement, total proteinuria/24 h and estimated glomerular filtration rate (eGFR) can anticipate the risk for terminal renal failure. The depth of response is also a significant prognostic factor as patients achieving an amyloidosis VGPR (dFLC below 40 mg/L) or CR after treatment have a significantly better outcome (Palladini et al. 2020).

## 82.5 First-Line Treatment

Risk-adapted treatment is strongly preferred and many patients are fragile and do not tolerate standard used dosing regimens (see Table 82.1). Three categories are defined with low-risk patients, transplant eligible, being a minority ( $\leq 20\%$ ). High-risk patients are defined

**Table 82.1** First-line treatment options according to risk status

Risk status	Treatment
Low risk Stage I	<ul style="list-style-type: none"> <li>(± induction treatment) Mel (200 mg/m<sup>2</sup>) + autoHCT</li> <li>Daratumumab CyBorD or CyBorD</li> </ul>
Intermediate risk Stages II–IIIa	<ul style="list-style-type: none"> <li>Daratumumab CyBorD or CyBorD</li> <li>Bortezomib-Mel-dex</li> </ul> Significant neuropathy: Mel-dex or Lenalidomide-dex
High risk Stage IIIb	<ul style="list-style-type: none"> <li>Dose reduced therapies like Daratumumab monotherapy or modified CyBorD</li> </ul>

*CyBorD* cyclophosphamide, bortezomib, dexamethasone. *Mel* melphalan

by Stage IIIb and/or having NYHA class III or IV heart disease. Other factors to consider are age, performance status, eGFR, neuropathy and systolic blood pressure. Frequent assessments of hematological response during treatment are needed, and the goal is to achieve a CR or VGPR as a deep hematologic response is closely related to survival. Patients having a hematologic response may gradually achieve an organ response.

In 2021, the EMA approved the first-line treatment of Daratumumab-Cyclophosphamide-Bortezomib-Dexamethasone with Daratumumab maintenance for patients with cardiac stages I, II, IIIa after the publication of the Andromeda study (Kastritis et al. 2021). This study demonstrated a better deep hematological response as well as better major organ deterioration and hematologic progression free survival in the daratumumab-treated patients. At 6 months, more cardiac and renal responses occurred in the daratumumab group than in the control group (41.5% vs. 22.2% and 53.0% vs. 23.9%, respectively). This regimen is the current preferred treatment regimen for most patients, but excludes cardiac IIIb patients (Wechalekar et al. 2023). Transplant eligible patients can still receive AutoHCT, especially when the hematological response is less than a VGPR, because the long-term data of transplant are excellent with a low relapse rate in patients in CR (Sanchorawala et al. 2022).

## 82.6 Second-Line Treatment

There is no positive randomized trial data to guide treatment at relapse. Patients with a good duration of response who tolerate initial treatment well may be retreated with the same initial regimen. Patients with a short response are best treated with an alternative agent combination using agents to which the patient has not been exposed, autoHCT or in a clinical trial tailored to the individual patient in terms of their age, comorbidities, extent of organ involvement, and the patient's wishes. Lenalidomide and pomalidomide can be considered in relapsed disease although data on durability of response are limited (Basset et al. 2021). Toxicity with lenalidomide is a significant issue, and it is recommended to start at a dose of 15 mg daily, with further dose reduction based on glomerular filtration rate (GFR) (Basset et al. 2021). Patients with t(11;14) have a very high chance for reaching a VGPR or CR with venetoclax (Premkumar et al. 2021) with a reasonable toxicity making this treatment very appealing.

## 82.7 Autologous HCT

### 82.7.1 Indication

Eligibility criteria for autoHCT are variable depending on the transplanting center. However, the usual eligibility criteria include age  $\leq 70$  years, performance status 0–2, NYHA class I or II, absence of significant clinical cardiac involvement (NT pro BNP  $<5000$  ng/L, left

ejection fraction  $\geq 45$  to 50%), absence of severe orthostatic hypotension (i.e., systolic blood pressure  $\geq 90$  mm Hg), and eGFR  $>40$  mL/min. Induction therapy before stem cell mobilization can be given, especially in patients who fulfill (smoldering) myeloma definition criteria, i.e.,  $\geq 10\%$  bone marrow plasma cell infiltration.

The correct selection of patients is extremely important since the mortality associated with autoHCT in AL amyloidosis can be unacceptable high if not done properly. Since the selection criteria also include the cardiac biomarkers, treatment-related mortality has dropped from around 20 to 2%; also see Table 82.2.

### 82.7.2 Recommended

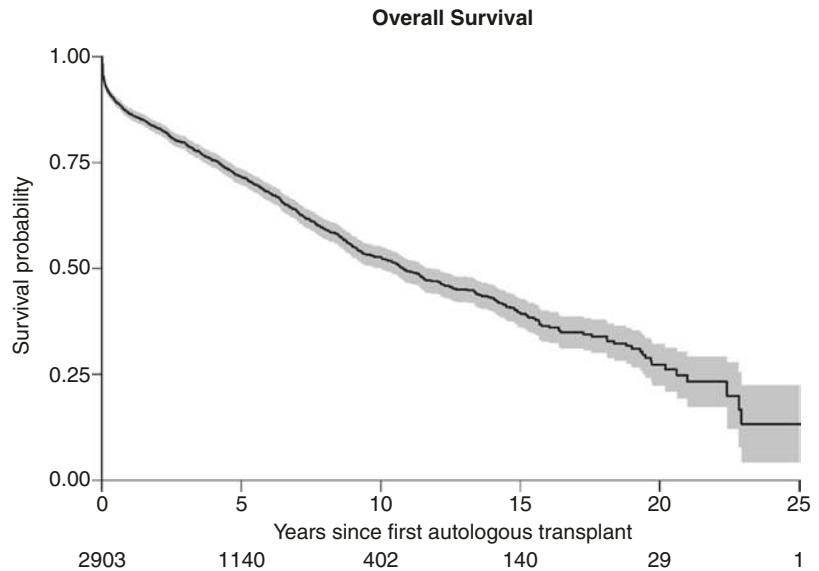
Stem cell mobilization and leucapheresis can be associated with unusual morbidity, and a syndrome of hypoxia and hypotension has been described both during mobilization with G-CSF and during the leucapheresis procedure itself, probably as a result of a capillary leak syndrome triggered by G-CSF. Therefore, use of reduced doses of G-CSF (such as 10  $\mu\text{g}/\text{kg}$  per day for 4–5 days) is recommended. In low-burden disease (i.e., plasma cells  $<10\%$ ), the use of CY mobilization chemotherapy does not seem to be necessary.

Conditioning regimens are based on high-dose MEL. The usual MEL dose is 200 mg/m<sup>2</sup>, since lower dose melphalan is associated with decreased hematological response and PFS and therefore other treatment non-transplant options may be more suitable (Cibeira et al. 2011).

**Table 82.2** Summary of the outcome of patients with systemic AL amyloidosis undergoing autologous stem cell transplantation, according to the more recent publications

Source	Type	No. of patients	Overall response rate (CR) (%)	TRM (%)	Overall survival (%)
Landau et al. (2017)	Retrospective	143	CR 43% at 12 months (83 pts. only)	5%	Median 10.4 years
D'Souza et al. (2015)	Retrospective	1536	ORR 71% CR 37% (2007–2012 cohort)	5% (2007–2012 cohort)	5 years 77% (2007–2012 cohort)
Minnema et al. (2019)	Prospective	50	ORR 86% CR 46%	0%	5 years 86%
Abdallah et al. (2020)	Retrospective	651			13 vs. 11.4 years, early vs. deferred ASCT

**Fig. 82.1** AutoHCT EBMT data (patients treated from 1993 until 2021), with a median follow-up of 46 months for patients alive



### 82.7.3 Results

Figure 82.1 shows OS of patients included in the EBMT database treated with autoHCT with a median survival longer than 10 years. In Table 82.2, the results from other publications are summarized. The use of induction therapy before HCT has been more frequently applied and seems to demonstrate better hematologic responses than HCT alone (Gustine et al. 2022).

## 82.8 Cellular Therapies

The largest retrospective analysis on allo-HCT for AL amyloidosis was performed by the EBMT in 2006 (Schönland et al. 2006). Nineteen patients were analyzed. Seven patients received MAC, and eight RIC. 40% of patients died of TRM. Long-term survival and sustained CR were achieved in seven patients and were associated with chronic GVHD in the majority of them. DLI has been successfully performed in a few patients with AL amyloidosis, thereby demonstrating a potent “graft-versus-plasma cell-dyscrasia” effect. The EBMT initiated a noninterventional prospective study (NIS) for patients with AL amyloidosis undergoing allo-HCT. Preliminary results have been presented at the EBMT meeting

in 2016 with improved overall survival of more than 70% after 5 years. Allo-HCT after RIC can be discussed as a treatment option for relapse after auto-HCT in patients <60 years with preserved organ functions and a HLA-identical donor. It might be a curative treatment for highly selected patients.

To date, there are only 9 cases published (some only as abstract) by centers in Spain and Israel who have been treated with academic BCMA CAR T-cells. Efficacy and tolerability have been very good. All patients achieved CR of the underlying clonal bone marrow disorder and organ responses could be observed (Oliver-Caldes et al. 2021; Kfir-Erenfeld et al. 2022). Clinical trials are on the way to apply this treatment to a larger cohort of AL amyloidosis patients.

#### Major Key Points

- AL amyloidosis therapy is directed against the underlying plasma cell or B cell clone.
- Deep hematological response is the goal of therapy and improves survival.
- Intensity of chemotherapy has to be risk adapted.

- High-dose chemotherapy with auto-HCT is still a good choice for low-risk patients treated in experienced centers.
- Results from cellular therapies are encouraging and will play an important role in the future.

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# Poems Syndrome and Disease Produced by Other Monoclonal IGs

# 83

Gordon Cook and Montserrat Rovira

## 83.1 POEMS Syndrome

### 83.1.1 Introduction

POEMS syndrome (acronym of: *polyradiculoneuropathy, organomegaly, endocrinopathies, monoclonal protein and dermatopathy/skin*) is a rare multisystemic disease due to an underlying plasma cell neoplasm. The pathogenesis of the syndrome is not well understood. Other names of the POEMS syndrome that are less frequently used are osteosclerotic myeloma, Takatsuki syndrome, or Crow-Fukase syndrome.

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### 83.1.2 Clinical and Laboratory Manifestations

• Male predominance • Age (maximum incidence): 50–60 years

Polyneuropathy	Typically demyelinating. Peripheral, ascending, symmetrical, and affecting both sensation and motor function. It is the dominant characteristic
Organomegaly	Hepatomegaly (50%), splenomegaly, or lymphadenopathy
Endocrinopathy	Present in 84%: gonadal, thyroid, pituitary, parathyroid, pancreatic, adrenal (in order of frequency, and many times multiple)
Monoclonal protein	Almost always $\lambda$ light chain. Usually Ig A or IgG and $\leq 3$ g/dL. Bone marrow smear: $<5$ – $10\%$ plasma cells
Skin changes	Hyperpigmentation, hypertrichosis, glomeruloid hemangiomas, white nails, plethora, acrocyanosis, flushing
Other important manifestations	<ul style="list-style-type: none"> <li>– Sclerotic bone lesions<sup>a</sup> (95%)</li> <li>– Castleman disease (in 11–30%)</li> <li>– Papilledema (in one-third of patients)</li> <li>– Extravascular volume overload</li> <li>– Thrombocytosis (in 54%)</li> <li>– VEGF<sup>b</sup> elevation</li> </ul>

<sup>a</sup>Radiology and CT/PET can be useful

<sup>b</sup>VEGF *Vascular endothelial growth factor* is the cytokine that correlates best with disease activity. The helpful cut-off for plasma and serum VEGF levels for diagnosis are  $\geq 200$  pg/mL (specificity 95%, sensitivity 68%) and  $\geq 1920$  pg/mL (specificity 98%, sensitivity 73%), respectively



### 83.1.3 Diagnosis

Not all the features within the acronym are required to make the diagnosis. There are other relevant features not included in the POEMS acronym also important: PEST (*p*apilledema, *e*xtravascular volume overload, *s*clerotic bone lesions, *t*hrombocytosis/erythrocytosis), elevated VEGF levels, abnormal pulmonary function tests, and a predisposition to thrombosis. There is a Castleman variant of POEMS syndrome that may be associated with a clonal plasma cell disorder. When Castleman disease variant of POEMS syndrome occurs without evidence of plasma cell disorder, then this entity should be considered separately.

#### 83.1.3.1 Criteria for the Diagnosis of POEMS Syndrome

The diagnosis of POEMS syndrome is confirmed when:

- Both mandatory major criteria +.
- another of the other 3 major criteria +,
- at least one of the minor criteria.

##### Mandatory major criteria

Polyneuropathy  
Clonal plasma cell dyscrasia (monoclonal immunoglobulin)

##### Other major criteria

Castleman disease  
Sclerotic bone lesions  
VEGF elevated

##### Minor criteria

Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy)  
Extravascular volume overload (edema, pleural effusion, or ascites)  
Endocrinopathy<sup>a</sup>  
Skin changes  
Papilledema  
Thrombocytosis/erythrocytosis<sup>b</sup>

##### Other symptoms and signs

Digital clubbing  
Weight loss  
Hyperhidrosis  
Low vitamin B<sub>12</sub> values  
Diarrhoea  
Pulmonary hypertension/restrictive lung disease  
Thrombosis

Adapted from Dispenzieri A, Am J Hematol 2017

<sup>a</sup>Hypogonadism is the most frequent and because of the high prevalence of diabetes mellitus and thyroid abnor-

malities, these two last abnormalities alone are not sufficient to meet this minor criterion

<sup>b</sup>Anemia and/or thrombocytopenia are rare, unless associated with Castleman disease

### 83.1.4 Prognosis

- Chronic course, median survival of nearly 14 years, rarely progression to multiple myeloma.
- The number of POEMS features does not affect survival.
- Risk factors associated to better survival → albumin > 3.2 g/dL, achievement of a complete hematological response and younger age. Lower VEGF levels, better response to treatment.
- Risk factors associated to shorter survival → clubbing, extravascular volume overload, respiratory symptoms, papilledema, and coexisting Castleman disease.
- Thrombocytosis and high bone marrow infiltration are associated with risk of cerebrovascular accidents.
- Patients candidates for radiation therapy have a better overall survival.

### 83.1.5 Standard treatment

Need to differentiate between POEMS with chronic inflammatory demyelinating polyneuropathy and CANOMAD (chronic ataxic neuropathy with ophthalmoplegia, M-protein, cold agglutinins, and disialosyl antibodies).

- **In case of an isolated bone lesion** (or multiple, but localized):  
Radiotherapy (e.g., 35–50 Gy) to affected site(s) → improve the symptoms of POEMS syndrome and can be curative
- **Rest of patients (disseminated disease):**
  - → Lenalidomide + dexamethasone is mainstay of frontline therapy

- → Alternatives include melphalan + dexamethasone, thalidomide + dexamethasone, bortezomib + dexamethasone (these last two agents are of limited use due to the intrinsic risk of peripheral neuropathy), cyclophosphamide ± dexamethasone
- → Plasmapheresis, IgIV, IFN-α, tamoxifen, trans-retinoic acid, bevacizumab (anti-VEGF agent), argatroban, and strontium-89 (mostly single case reports)
- → Attention to supportive care is mandatory (physical therapy, orthotics, etc.)
- → Autologous HCT (see Sect. 83.1.6)

**83.1.5.1 Response Criteria**

Monitoring the response to treatment in POEMS syndrome is a challenge. Patients must be followed carefully comparing the deficits to baseline. VEGF is an imperfect marker due to discordances between disease activity and response. The size of monoclonal protein is typically small making standard multiple myeloma response criteria inapplicable. Patients can present clinical benefit without M-protein response therefore a clinical scoring system which can focus on organ-specific response would be useful clinically. So, response criteria for POEMS syndrome could be done as follows: hematological response using a modified amyloid response criteria; VEGF response; CT/PET response; and a simplified organ response (polyneuropathy assessment, pulmonary function tests, and extravascular overload).

**83.1.6 Autologous Hematopoietic Cell Transplantation (Autologous HCT)**

Background	<ul style="list-style-type: none"> <li>- In multiple myeloma → autologous HCT → high rate and depth of responses</li> <li>- In amyloidosis, a disease with similarities to POEMS syndrome with “low tumor” burden → autologous HCT → long remissions obtained</li> </ul>
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Indication	POEMS syndrome with disseminated disease: <ul style="list-style-type: none"> <li>• Good general condition</li> <li>• Either upfront or after suitable induction therapy (lenalidomide + dexamethasone), though mostly after induction to control symptoms prior to autologous HCT</li> </ul>
Conditioning	Melphalan 140–200 mg/m <sup>2</sup>
Stem cell source <sup>a,b</sup>	PBSC, mobilization with G-CSF ± Cy (1.5–3 g/m <sup>2</sup> ). Mobilize early if lenalidomide+dexamethasone used.
Morbidity	High rate of engraftment syndrome (up to 50%) (see Chap. 20), important to recognize and treat promptly with prednisone. In these cases, higher than expected transfusion need and delayed engraftment. No organ toxicities as observed in amyloidosis
Mortality <sup>c</sup>	As in other autologous HCT, recently reported 3.3% 1-year NRM <sup>d</sup>
Response <sup>e,d</sup>	Hematological CR can be achieved by 3 months post-autologous HCT, but neurologic response is usually delayed to 6–9 months, but full recovery can take up to 2–3 years. PET/CT evidence of response can take upto 12 months

<sup>a</sup>Mobilization failure is described, for this reason, if there is no response after 3 courses of induction, proceed to mobilization

<sup>b</sup>The incidence of engraftment syndrome can be reduced if mobilization is done with cyclophosphamide + G-CSF.

<sup>c</sup>Cook G, Hematologica 2017

<sup>d</sup>In the largest series, 3-y PFS 84% and OS 94%, and 5-y PFS 74% and OS 89%

**83.2 Monoclonal Ig Deposition Disease**

**83.2.1 Introduction**

Monoclonal Ig deposition is a clonal plasma cell discrasia in which light-chain and/or heavy-chain subunits of Igs form nonfibrillar deposits in various tissues, causing organ dysfunction. Light-chain deposition disease is the most common of these entities.

### 83.2.2 Clinical Manifestation/ Laboratory

Kidney	Always affected. Immunofluorescence shows deposition of light chains along glomerular and tubular basement membranes → nodular glomerulosclerosis. Deposits are nonfibrillar, almost always composed K chain and do not stain with Congo red dye → nephrotic syndrome, hypertension, and rapidly progressing renal insufficiency
Heart and liver	Less frequent affected. Restrictive cardiopathy, myocardial infarction, cholestatic jaundice, hepatic failure
Monoclonal gammopathy	Electrophoresis, immunofixation of serum and/or urine, serum-free light chain measurement

### 83.2.3 Diagnosis

Biopsy of the affected organ (almost always kidney).

### 83.2.4 Treatment

- Controversial, not standard due to the low incidence. Conventional chemotherapy commonly used for MM is unsatisfactory.
  - Melphalan + Prednisone
  - VAD
  - Thalidomide ± DXM, bortezomib + DXM
  - Autologous HCT (see Sect. 83.2.5)

### 83.2.5 Autologous Hematopoietic Cell Transplantation (Autologous HCT)

Background	– As in POEMS syndrome (see Sect. 83.1.6)
Indication	– Patients in good general condition and with basic requirements for autologous HCT – Patients not responding to previous MM-like treatment

Conditioning	Melphalan 140–200 mg/m <sup>2</sup>
Stem cell source	PB, mobilization with G-CSF ± Cy (3 g/m <sup>2</sup> )
Morbidity	Some patients require hemodialysis (HD) before and during the procedure. In that case, melphalan should be administered after HD
Mortality	As in other autologous HCT
Response	In the few cases reported: <ul style="list-style-type: none"> <li>– Hematological responses are described secondary to the control of the monoclonal gammopathy.</li> <li>– It can improve renal function. In selected cases, kidney transplantation could be an option if the patient achieve a CR and remain in HD.</li> </ul>

### Further Reading

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## 84.1 Definition and Epidemiology

Indolent lymphomas (iNHL) are mature small B-cell neoplasms that include but are not limited to follicular lymphoma (FL), marginal zone lymphoma (MZL) and lymphoplasmocytic lymphoma (LPL) as defined by the WHO and ICC classifications (Alaggio et al. 2022; Campo et al. 2022). This heterogenous group accounts for 1/3 of all malignant lymphoma with FL being the most common subtype (Al-Hamadani et al. 2015). iNHL are characterized by repeated relapses, with autologous (auto) and allogeneic (allo) HCT being the only curative options. The largest studies on HCT for iNHL included a majority of FL patients (Montoto et al. 2013), thus these strategies are detailed here specifically for FL unless otherwise specified and can be extrapolated for the other subtypes. The roles of both forms of HCT are evolving and have recently been challenged as T-cell engaging therapies emerge. The current indications for auto-HCT and allo-HCT are reviewed below.

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## 84.2 Diagnosis

The diagnosis of iNHL relies on the pathological findings on a surgical/excisional biopsy. The staging and assessment of response rely on, ideally, positron emission tomography (PET) scan or computed tomography (CT) scan.

## 84.3 Prognosis

The most frequently used prognostic score is the FLIPI (Dreyling et al. 2021). Patients that relapse within 2 years of the first-line therapy (Casulo et al. 2015) and those with high-grade transformation at relapse (Sarkozy et al. 2016) have been shown to have poor survival, and these patients should be considered for a HCT procedure once adequate disease control has been obtained. Patients with a high FLIPI score at relapse and those with multiple relapses may also have a poor prognosis, and these patients may also be considered for transplant options. It is important, however, to carefully counsel the patients regarding both the transplant and non-transplant therapies that are currently available of which there are many (Dreyling et al. 2021; Kuruvilla 2016).

## 84.4 First Line Treatment

First-line therapy for patients with advanced stage iNHL in need of treatment is to administer chemoimmunotherapy followed by maintenance with the chosen monoclonal antibody (Rituximab or Obinutuzumab) (Dreyling et al. 2021). There is no place for auto or allo HCT in first-line therapy.

## 84.5 Treatment at First Relapse and Beyond

At relapse, standard of care options include chemo-immunotherapy, Rituximab in combination with lenalidomide and consolidation of the response with autologous or allogeneic HCT (Dreyling et al. 2021; Ghione et al. 2023). Inclusion in clinical studies should always be considered. Ongoing clinical trials will clarify the role of CAR-T cells (Jacobson et al. 2022; Fowler et al. 2022; Morschhauser et al. 2023) and bispecific antibodies (Budde et al. 2022) in the therapeutic algorithm of iNHL.

## 84.6 Autologous HCT

### 84.6.1 Indication

Auto-HCT should be considered for iNHL patients with relapsed disease responding to second (or subsequent) line reinduction therapy.

### 84.6.2 Recommendations

There is a wide variety of different conditioning regimens that may be employed for auto-HCT in FL but a paucity of randomised trials comparing the efficacy and toxicity of the different regimens. The BEAM (BCNU, Etoposide, Ara-C, Melphalan) regimen has become one of the most widely used conditioning regimens for patients with lymphoma. A number of investigators have looked to improve upon BEAM by including Rituximab (R) and dexamethasone, or substitut-

ing BCNU with bendamustine which could be even effective but less toxic (Lachance et al. 2023; Visani et al. 2014).

The EBMT-LYM-1 study prospectively examined the role of purging with R pre-HCT in R-naïve patients with relapsed FL (Pettengell et al. 2013). In this study, no benefit could be demonstrated for in vivo purging. The same study demonstrated that R maintenance post-HCT resulted in a prolonged PFS. However, it is unclear how to extrapolate these findings to R-exposed patients.

- BEAM is amongst the most frequently used conditioning regimen but other regimens can be used.
- There is no proven role for purging strategies.

### 84.6.3 Expectable Results

Auto-HCT achieves a 10-year PFS of approximately 50% and may be curative in a proportion of patients.

The CUP trial is the only randomised study comparing consolidation with an auto-HCT (using purged or unpurged stem cells) with no further therapy in the relapse setting (Schouten et al. 2003) and included 140 patients with relapsed FL. The 2-year PFS for the chemotherapy alone arm was 26% compared with 58% and 55% for those receiving HCT with either purged or unpurged stem cells, respectively. In addition, there was an OS advantage in favour of the two transplant arms (Schouten et al. 2003). A number of other studies have also reported long-term follow-up of auto-HCT in relapsed FL and describe a 10-year PFS ranging between 31 and 50% (Kornacker et al. 2009; Montoto et al. 2007; Pettengell et al. 2021). Taken together, these results demonstrate that between 25 and 50% of patients experience prolonged PFS following an auto-HCT for relapsed FL, suggesting that this is a curative procedure for a proportion of patients.

It is important to recognise both the acute and long-term toxicities associated with auto-HCT. Whilst early TRM may be relatively low in younger patients, there is evidence that for



patients over the age of 60, the TRM may be in excess of 10% (Sánchez-Ortega et al. 2016). Given that the median age of patients with relapsing FL is 69 an auto-HCT might be associated with a significant TRM for some patients. An additional concern is the late risk of developing secondary malignancies including MDS/AML. In a prospective randomised study, patients undergoing an auto-HCT for FL had a significantly higher rate of both solid malignancies and MDS/AML compared to patients not receiving HCT (Gyan et al. 2009). Notably, the conditioning regimen used in this study was total body irradiation, which possibly increases the risk of secondary myeloid malignancies. (Baker et al. 2019) Evaluation of the BM for clonal haematopoiesis and cytogenetic abnormalities may enable the identification of patients at a greater risk of developing MDS/AML following an auto-HCT. For these patients, alternative relapse therapies may be more suitable.

## 84.7 Allogeneic HCT

In comparison with auto-HCT, allo-HCT offers the provision of a graft uncontaminated by lymphoma cells or exposed to mutagenic agents and the development of an allogeneic GVL effect. The curative potential of allo-HCT for indolent lymphoma is established (Peniket et al. 2003; van Besien et al. 2003).

### 84.7.1 Indication

Allo-HCT should only be considered in selected patients with relapsed disease.

Numerous factors have to be taken into consideration when selecting patients for an allo-HCT procedure. Patient-related factors such as age, comorbidities, performance status, organ function, the HCT comorbidity index (HCT-CI) (Sorrer et al. 2005) and patients' personal views will determine if a patient is fit to undergo a transplant and what the likely TRM rate will be. Allo-HCT should only be considered in patients where the lymphoma is considered to consider-

ably shorten survival. Relapses after three lines of treatment and especially after auto-HCT are reasonable indications for allo-HCT.

### 84.7.2 Recommendations

- Matched sibling, matched unrelated, haplo-identical and cord blood stem cell sources may be considered.
- Reduced intensity conditioning regimens are most appropriate for older patients with significant comorbidities. Young fit patients may be considered for more intensive regimens.
- There is no preferred GVHD prophylaxis. T-cell depletion may be employed but should be combined with chimerism directed donor lymphocyte infusions.
- There is no indication for maintenance therapy post allo-HCT for iNHL.

It is unclear whether a reduced intensity conditioning (RIC) or a myeloablative conditioning (MAC) allo-HCT offers superior outcomes in FL. A retrospective registry study demonstrated that the two approaches to allo-HCT resulted in similar outcomes in the sibling donor setting (3-year OS for the MAC and RIC were 71% and 62% ( $P = 0.15$ ), respectively) (Hari et al. 2008). However, the EBMT reported that in the unrelated donor setting, RIC allo-HCT was associated with a lower NRM and significantly longer PFS and OS when compared with MAC allo-HCT (Avivi et al. 2009). The median age at relapse of FL is 69, and therefore the majority of patients that may be considered for an allo-HCT will be considered too old for MAC regimens, and many authorities therefore recommend a RIC allo-HCT for FL.

The outcomes of both matched sibling donor (MSD) and MUD allo-HCT in FL are broadly similar. A recent large retrospective study conducted by the EBMT and the CIBMTR demonstrated that the PFS and OS following MSD and MUD were similar (Sureda et al. 2018). For patients lacking a MSD or MUD, either a cord blood or haploidentical family donor may be considered. The feasibility of umbilical cord blood

(Rodrigues et al. 2009; Brunstein et al. 2009) and haplo-HCT (with PT-CY) (Dietrich et al. 2016) in NHL (including FL) has been reported.

T-cell depletion of the graft is a well-established method to reduce the incidence of GVHD post-transplant but runs the risk of eliminating allo-reactive T cells that will mediate the GVL effect and consequently result in a higher relapse rate. The risk of relapse may be offset by employing donor lymphocyte infusion (DLI), and with this approach, the 4 years PFS and relapse risk were 76% and 24%, respectively, and the incidence of GVHD was low (Thomson et al. 2010), suggesting that this approach may also be an option for allo-HCT in iNHL.

### 84.7.3 Expectable Results

Early studies employed MAC regimens and demonstrated that cure could be achieved in a significant proportion of patients. In retrospective studies comparing allo- with auto-HCT, MAC allo-HCT was associated with a lower relapse rate but a higher TRM and consequently a similar OS. In an attempt to reduce the toxicity of allo-HCT, RIC allo-HCT has been developed (Robinson et al. 2002). A number of groups have demonstrated the safety and efficacy of RIC allo-HCT and demonstrated that this type of transplant may be employed in older patients with significant comorbidities and in those patients who have undergone a prior auto-HCT.

The largest series of patients undergoing a RIC allo-HCT after the failure of a auto-HCT was reported by the EBMT. The NRM at 2 years was significant (27%), but the 5-year PFS and OS were 48% and 51%, respectively (Robinson et al. 2016). The duration of response following the allo-HCT was also significantly longer than after the auto-HCT illustrating the potential of the allogeneic GVL effect in this disease. This data demonstrates that a RIC allo-HCT can act as an effective salvage strategy in this setting although the toxicity was significant. There is also a risk that patients may fail to respond to reinduction therapy, and therefore would not be eligible for an allo-HCT.

## 84.8 The Place of Auto- and Allo-HCT in the Therapeutic Algorithm of iNHL

- Only patients with (a) early relapse or (b) high-grade transformation after first-line therapy or (c) multiple relapses should be considered for HCT consolidation.
- Auto-HCT may cure some patients and is associated with lower toxicity compared to allo-HCT.
- RIC allo-HCT is a curative option for patients that relapse after an auto-HCT.
- Most European authorities recommend auto-HCT as the first transplant of choice and reserve allo-HCT for patients relapsing after an auto-HCT. However, some EBMT centres propose allo-HCT as first transplant in selected cases of relapsed iNHL (Robinson et al. 2013).

## 84.9 Latest (Selected) EBMT Results

### 84.9.1 Follicular Lymphoma

	EBMT <i>n</i> = 726 (Robinson et al. 2013)	Allo	EMBT/ CIBMTR <i>n</i> = 1567 (Sureda et al. 2018)
Auto			
TRM (1 y)	3%	TRM (5 y)	19%
REL (5 y)	47%	REL (5 y)	29%
OS (5 y)	72%	OS (5 y)	61%
PFS (5y)	48%	PFS (5 y)	52%

### 84.9.2 Marginal Zone Lymphoma

No specific reports on allogeneic HCT due to small numbers.

	EBMT <i>n</i> = 199 (Avivi et al. 2018)
Auto	
TRM (5 y)	9%
REL (5 y)	38%
OS (5 y)	73%
EFS (5 y)	53%
Second malignancies (5 y)	6%

### 84.9.3 Waldenstrom's Macroglobulinemia

No specific reports on allogeneic HCT due to small numbers.

Auto	EBMT <i>n</i> = 158 (Kyriakou et al. 2010)
TRM (1 y)	3.8%
REL (5 y)	52%
OS (5 y)	69%
PFS (5 y)	40%
Second malignancies (5 y)	8.4%

### 84.10 Conclusions

Both auto- and allo-HCT have an established role in the treatment of relapsed iNHL, and both forms of transplant can deliver curative therapy to patients with otherwise poor prognosis disease. Patient selection for transplant therapy is critical, and a current understanding of the rapidly evolving field of alternative non-transplant lymphoma therapies is mandatory. The treatment paradigm for iNHL evolves rapidly as novel agents are incorporated into clinical practice, and the place of these agents relative to transplantation is likely to change soon.

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## 85.1 Introduction

CLL is a rare indication for HCT since it usually follows an indolent course which does not require treatment in the majority of patients. In case of treatment indication, numerous pathway inhibitor therapy regimens are available, essentially based on Bruton's-Tyrosine-Kinase inhibitors (BTKi) or the BCL2 inhibitor venetoclax with or without CD20 antibodies. The high efficacy and the non-cross-resistance of the available chemotherapy-free treatment options offers chances for long-term survival although they cannot provide cure. Only a small minority of patients will follow an unfavorable course on pharmacological treatment and may benefit from cellular immunotherapy.

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## 85.2 Principles of Treatment for CLL

Since CLL is not curable with standard therapy, the diagnosis of CLL does not justify immediate start of treatment. Criteria that trigger treatment are symptomatic disease, progressive anemia or thrombocytopenia, a lymphocyte doubling time of less than 6 months, constitutional symptoms, massive splenomegaly or lymphadenopathy, and Richter transformation. Labeled first-line regimens comprise (1) covalent BTKi (Acalabrutinib, Zanubrutinib, Ibrutinib) with or without CD20 antibody loading phase; (2) Venetoclax with the CD20 antibody obinutuzumab; and (3) the combination of ibrutinib and venetoclax. Whereas (2) and (3) are administered for a fixed duration (12–15 months), (1) is given permanently until intolerance or CLL resistance. With all of these options, high response rates (>80%) and long-lasting responses (median duration of response beyond 5 years) can be achieved (Sharman et al. 2022; Al-Sawaf et al. 2023). Patients with CLL harboring a cytogenetic deletion 17p or with a *TP53* mutation detected by DNA-sequencing (combined in this manuscript as *TP53* abnormalities) as well as those with a complex karyotype may follow a less favorable course (Al-Sawaf et al. 2023; Huber et al. 2023; Furstenau et al. 2023).

If first-line therapy fails and a secondary treatment indication emerges, the general therapeutic principle is a class switch (i.e., 1 L BTKi - > 2 L



Venetoclax and vice versa; 1 L fixed duration with 2 L permanent covalent BTKi). Patients failing on both classes of pathway inhibitors have a poor outlook (Mato et al. 2020). It is unclear if the recent advent of noncovalent BTKi can improve the adverse prognosis of these “double refractory” patients (Mato et al. 2023).

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### 85.3 Allogeneic HCT for CLL

Accordingly, double refractory patients as well as those having failed one pathway inhibitor class in the presence of *TP53* abnormalities and/or complex karyotype should be considered for alloHCT (Dreger 2021). Patients having received a BTKi/Venetoclax fixed-duration combination as first-line therapy must not be considered as double refractory if treatment failure occurs after discontinuation of the BTKi. Regardless of pathway inhibitor exposure transplant-eligible patients with a history of Richter’s transformation have an indication of alloHCT.

Available evidence strongly suggests that alloHCT is currently the only therapy with curative potential in CLL (van Gelder et al. 2017; Krämer et al. 2017). AlloHCT can provide long-term disease control even in patients with an unfavorable biological and clinical risk profile, and those having failed pathway inhibitors (Schetelig et al. 2008, 2021; Roeker et al. 2020). The timing of alloHCT should be individually discussed with the patients by taking into consideration the risk of complications after alloHCT and the chances of pharmacological treatment options remaining. Standard risk scores like the HCT-CI or the EBMT-risk score can be used to assess the risk of nonrelapse mortality of an individual patient.

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### 85.4 Remission Induction Prior to Start of the Conditioning Regimen

Large prospective and retrospective studies uniformly show that the results of alloHCT deteriorate if the disease is not controlled at the time of

transplant. Thus, alloHCT should be performed in remission of CLL. Apart from experimental approaches, different options exist for remission induction and bridging to alloHCT, basically relying on covalent BTKi, venetoclax, CD20 antibodies, and combinations of these compounds. Non-covalent BTKi may add to the bridging armamentarium as soon as they are labeled (Mato et al. 2023). In addition, the PI3 kinase inhibitor idelalisib can be helpful for bridging albeit its efficacy in double refractory patients is limited (Schetelig et al. 2021)

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### 85.5 Conditioning Regimens

The crucial therapeutic principle of alloHCT in CLL is graft-versus-leukaemia (GVL) activity. Evidence for this comes from the observation that even some patients with refractory disease benefit from alloHCT, a reduced relapse risk in the presence of cGVHD, and the efficacy of immune modulation for the eradication of minimal residual disease (Krämer et al. 2017).

Accordingly, long-term disease control can be achieved with a broad range of conditioning regimens. Current evidence does not allow the definition of one standard conditioning regimen for CLL. The most convincing data supporting alloHCT in CLL come from studies of reduced-intensity conditioning (Krämer et al. 2017; Roeker et al. 2020; Schetelig et al. 2017). The choice of conditioning intensity may vary according to the individual situation. In the presence of comorbidity and chemosensitive disease RIC appear to be more appropriate, whereas high-intensity regimens might be preferable in younger patients with good performance status but poorly controlled disease.

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### 85.6 Outcome After Allogeneic Transplantation for CLL

In a large registry cohort from the pre-pathway inhibitor era, estimated event-free survival, overall survival, and non-relapse mortality

(NRM) 10 years after alloHCT were 28% (95% confidence interval (CI), 25–31), 35% (95% CI, 32–38), and 40% (95% CI, 37–42), respectively (van Gelder et al. 2017). Patients who passed the 5-year landmark event-free ( $N = 394$ ) had a 79% probability (95% CI, 73–85) of surviving the subsequent 5 years without an event. Relapse and NRM contributed equally to late treatment failure. Higher age, lower performance status, unrelated donor type, and unfavorable sex-mismatch have an adverse impact on 2-year NRM. Despite the risks of NRM and relapse/progression the prospect of long-term disease-free survival on average in almost one out of three patients remains an argument to consider allo-HCT especially for younger patients with high-risk CLL. Moreover, NRM appears to be decreased in CLL transplants performed in the pathway inhibitor era (Roeker et al. 2020; Kim et al. 2020; Tournilhac et al. 2021).

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### 85.7 Post-Transplant Minimal Residual Disease Monitoring and Immune Intervention in CLL

In CLL, sensitive MRD quantification (i.e., 1 cell in  $10^4$  or less) can be obtained by PCR- or flow cytometry-based assays. The decline of the MRD level is often delayed and is closely related to immuno-reconstitution after transplantation. GVL-induced MRD negativity after alloHCT is sustained in the majority of patients and is highly predictive of freedom from relapse. MRD monitoring is a valid instrument for the guidance of pre-emptive immune interventions directed at disease eradication after allo-HCT, such as the tapering of immunosuppression and the use of DLI. The published evidence suggests that CLL is sensitive to timely preemptive immune intervention by modulation of systemic immunosuppression (Krämer et al. 2017).

### 85.8 Autologous HCT and CAR-T Cell Therapy

Whereas autoHCT does not play a role in current CLL management algorithms except for selected patients with Richter transformation, there is accumulating evidence that CD19-directed CAR-T cells can provide sustained disease control at least in a minority of patients with advanced CLL (Siddiqi et al. 2023). However, as long as there is no labelled CAR-T product available, CAR-T treatment of patients with CLL should be performed only in clinical trials.

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### 85.9 Summary and Perspectives

AlloHCT from related or unrelated donors can induce long-term disease-free survival in patients with high-risk CLL. It is a standard treatment option for patients with high-risk CLL who have failed two pathway inhibitor classes, or who are refractory to one class in the presence of TP53 aberrations and/or a complex karyotype. Generally, alloHCT should be considered before the disease has advanced to a status of complete refractoriness. At the same time, alloHCT should not be recommended for patients who face a higher short-term risk of mortality after transplantation compared to conventional therapy. In the absence of randomized controlled comparisons of these treatment strategies, the outcome of an individual patient has to be predicted based on published data. This requires careful individual assessment of the risk of alloHCT versus continuation of conventional treatment. Patients should be referred to a transplant center once their disease proved refractory to at least one pathway inhibitor in order to get consultation with an expert in the field. Finally, all approved drugs for CLL can also be used for the treatment of post-transplant relapse and further improvements of donor selection, patient care and prevention of complications can be expected, thus, overall outcome after transplantation will continue to improve.

**Key Points: AlloHCT in CLL**

Indications for alloHCT	<ul style="list-style-type: none"> <li>• CLL after failure of 2 pathway inhibitor classes</li> <li>• CLL with TP53 aberration and/or complex karyotype after failure of one pathway inhibitor</li> <li>• History of Richter's transformation</li> </ul>
Remission induction prior to start of conditioning	Patient who receive alloHCT in remission have a lower risk of relapse. The most promising option for remission induction should be chosen
Donor selection and graft source and GVHD-prophylaxis	No disease-specific criteria have to be considered for the selection of the donor, graft source, or GVHD-prophylaxis
Conditioning	There is no advantage of using a myeloablative regimen over reduced-intensity conditioning
MRD monitoring	MRD-driven immune modulation after alloHCT may improve the likelihood of sustained CLL eradication
Risk factors for non-relapse mortality	<ul style="list-style-type: none"> <li>• Advanced age</li> <li>• Poor performance status and/or high HCT-CI score</li> <li>• Partially matched as compared to matched donor HCT</li> <li>• Unfavourable donor patients sex constellation</li> </ul>
Relapse after alloHCT	Relapse after alloHCT may be treated successfully. To current knowledge, the history of alloHCT does not restrict pharmacological treatment options for patients with relapsed CLL

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## 86.1 Definition and Epidemiology

The most recent WHO classification of tumors of hematopoietic and lymphoid tissues (Alaggio et al. 2022) classifies large B cell lymphomas (LBCL) into diffuse large B cell lymphoma (DLBCL) NOS with distinct morphological and molecular (germinal center B-cell, activated B-cell) subtypes, high-grade B-cell lymphomas (with MYC and BCL2 rearrangements or NOS subtype), and other less frequent subtypes.

With some important exceptions, diagnostic work-up and treatment are identical in all LBCL subtypes. It is beyond the scope of this article to fully describe the exceptions; we mention the most important differences but otherwise focus on transplantation and cell therapy for patients with relapsed/refractory (R/R) LBCL.

DLBCL is the most frequent lymphoma subtype and accounts for approximately one third of newly diagnosed lymphoma cases worldwide. In

Europe, the 10-year prevalence of DLBCL is estimated at 43.3 per 100,000 per year (Smith et al. 2015). The disease is slightly more frequent in men than in women; it mostly is a disease of the elderly (median patient age beyond 60 years) but can occur also in children and adolescents.

## 86.2 Diagnosis

The diagnosis is made according to the WHO classification from a sufficiently large surgical specimen or excisional lymph node biopsy; needle biopsies are not recommended. Beyond morphological evaluation by an experienced pathologist, determination of the immunophenotype of the malignant cells (positivity of malignant cells for CD19 and CD20 must be documented because of its therapeutic consequences) and determination of the cell of origin by adequate molecular methods are required (Alaggio et al. 2022).

## 86.3 Classification

The LBCL comprise the morphological and molecular subtypes of DLBCL NOS, high-grade B-cell lymphomas, and other LBCL (see Paragraph 1).

The WHO classification describes 14 other lymphomas of large B cells. Among these, primary large B-cell lymphoma of immune

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privileged sites (CNS, testicle, and vitreoretina), lymphomatoid granulomatosis, primary mediastinal large B-cell lymphoma (PMBCL), and plasmablastic lymphoma not only show significant differences in pathogenesis and clinical manifestation but in most centers are treated different from classical DLBCL. In order to fulfill all WHO requirements, the cell of origin (GCB or ABC-subtype by IHC or gene expression profiling) and the presence/absence of distinct chromosomal translocations (BCL2 and MYC by FISH testing or IHC) must be determined.

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## 86.4 Risk Factors

The International Prognostic Index (IPI) remains the most important tool in order to estimate the prognosis of patients with LBCL (Ziepert et al. 2010). The IPI takes into account five factors (age, stage, LDH, performance status, and number of extranodal sites involved). Patients within the low (IPI 0, 1), low-intermediate (IPI 2), high-intermediate (IPI 3), and high-risk group (IPI 4, 5) can expect 3-year overall survival of 91.4%, 80.9%, 65.1%, and 59.0%, respectively, if treated with R-CHOP or one of its variants. Other clinical risk factor models (R-IPI; NCCN-IPI) (Sehn et al. 2007; Zhou et al. 2014) have been proposed, but advantages over the original IPI seem limited.

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## 86.5 First-Line Treatment

First-line treatment of patients with LBCL generally consists of RTX at standard dose (375 mg/sqm) in combination with CHOP (CY, DOXO, VCR, and PRD). Six cycles of R-CHOP are generally used. Patients with early disease (IPI 0 and 1) have been treated with abbreviated chemotherapy and involved-field radiotherapy (RT). Some studies do not support a role for RT in such patients (Lamy et al. 2018). In patients with IPI

2–5, radiotherapy to bulky and extranodal disease is regularly recommended after R-CHOP in some but not in the majority of countries. Recently, prospective randomized trial has confirmed the benefit of adding polatuzumab to R-CHP in patients with IPI  $\geq 2$  in terms of progression-free survival (Tilly et al. 2022).

Several studies failed to demonstrate an advantage of consolidation with auto-HCT over conventional chemotherapy; therefore, it is generally not recommended as part of first-line therapy in LBCL.

Patients with primary large B-cell lymphoma of immunized privileged sites must receive chemotherapy penetrating into the CNS. More aggressive chemotherapies (CHOEP, DA-EPOCH, or ACVBP) in combination with RTX with or without RT are recommended in patients with PMBCL or plasmablastic lymphoma. Also, more intensive therapy has been recommended for patients with MYC and BCL2 rearrangements based mainly on retrospective studies (Oki et al. 2014).

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## 86.6 Second-Line Treatment

The principles of management of relapsed and refractory (R/R) LBCL are shown in Table 86.1. CAR T cells have become the standard of care for patients with refractory disease or early relapse (within 12 months from the end of first-line therapy). Auto-HCT is currently considered an option for patients with R/R LBCL especially in late relapse. All chemotherapy-based salvage regimens cause hematologic toxicity in many cases necessitating RBC and platelet transfusions. Mucositis, gastrointestinal toxicities, neutropenic fever, and infections are reported in a significant proportion of patients. Nephrotoxicity, hepatotoxicity, and other nonhematologic toxicities are also observed. Failure to mobilize hematopoietic stem cells in 10–20% of cases occurs with all salvage regimens. Efficacy of different salvage options is shown in Table 86.2.



**Table 86.1** Management of relapsed or refractory LBCL

- *New biopsy*: Highly recommended in all patients with R/R LBCL. Core biopsies acceptable
- *Radiological evaluation*: PET/CT recommended for evaluation of treatment outcome
- *Salvage therapy followed by auto-HCT* is currently considered the standard of care for patients with R/R DLBCL with late relapse (>12 months from the end of first-line therapy)
- *CAR T cells* is considered standard of care for patients with refractory disease or early relapse (within 12 months from the end of first-line therapy)
- *Allo-HCT* could be considered a potential treatment option in younger patients without comorbidities especially in patients with R/R after CAR T cells and with chemosensitive disease
- *Selection of salvage therapy*: Randomized studies failed to show significant differences in terms of efficacy or toxicity with different salvage regimens. Therefore, salvage strategies should take into account individual patient characteristics (age and comorbidities) considering potential cumulative hematologic and nonhematologic toxicity and the possibility of harvesting stem cells. Cardiac, pulmonary, renal, and liver function should be evaluated prior to treatment
- The objective of salvage chemotherapy is to induce a complete or partial response indicating that the tumor remains chemosensitive, this having a major impact on outcome after transplantation. PET negativity after salvage therapy is a surrogate marker of chemosensitivity and predicts patient outcome after auto-HCT

**Table 86.2** Response to salvage regimens

Regimens compared in prospective randomized trials	
R-DHAP (Gisselbrecht et al. 2010)	Dexamethasone, cytarabine, cisplatin ORR 62.8% (44.1%) <sup>a</sup> , CR 28% (14.3%) <sup>a</sup>
R-GDP (Crump et al. 2014)	Gemcitabine, dexamethasone, cisplatin ORR 45.1%, CR 13.5% after 2 cycles
R-ICE (Gisselbrecht et al. 2010)	Ifosfamide, carboplatin, etoposide ORR 63.5%, CR 24%
Addition of new drugs (lenalidomide, ibrutinib, brentuximab vedotin, polatuzumab, other) to RTX-chemo in order to improve response rates of salvage regimens is not recommended outside clinical trials	

<sup>a</sup>Percentages in brackets from Crump et al. (2014)

## 86.7 Autologous HCT

Auto-HCT is still considered the standard of care for patients with late relapse (>12 months from the end of first-line therapy) in PR or CR after salvage therapy and could be an option treatment for patients with early relapse or primary refractory disease when CAR T cells are not available. EBMT indications (Snowden et al. 2022) for auto-HCT in LBCL are shown in Table 86.3. Auto-HCT is generally not recommended as part of first-line therapy in DLBCL; however, it could be considered in those patients with high-grade B cell lymphomas treated with R-CHOP like regimens and in LBCL with CNS involvement at diagnosis with chemosensitive disease. We discourage auto-HCT for patients with refractory disease not responding to salvage therapy.

### 86.7.1 HSC Source

PBSC is used in >90% of auto-HCT.

### 86.7.2 Consolidation (High-Dose Therapy)

Consolidation (high-dose therapy) should eliminate malignant cells with minimal impact on organ systems other than hematopoiesis. The choice of the preparative regimen varies and is based on institutional experience rather than evi-

**Table 86.3** Indications for auto-HCT in LBCL

Disease status	Recommendations
First complete remission	Clinical option Level of evidence I
Chemosensitive early relapse, ≥CR2	Clinical option Level of evidence I
Chemosensitive late relapse, ≥CR2	Standard of care Level of evidence II
Refractory disease	Generally not recommended Level of evidence I

dence. The BEAM regimen typically consisting of BCNU (300 mg/m<sup>2</sup> × 1, day-6), VP (200 mg/m<sup>2</sup>, days -5 to -2), Ara-C (200 mg/m<sup>2</sup> bid, days -5 to -2), and MEL (140 mg/kg/day ×1, days -1) is the preferred regimen in EBMT centers.

Acute toxicities of BEAM include severe mucositis, nausea and vomiting, diarrhea, hepatotoxicity, nephrotoxicity, and noninfective pulmonary complications. Late toxicities include pulmonary complications such as chronic interstitial fibrosis and decrease in lung diffusion capacity (21%), infection (30%), metabolic syndrome (17%), cardiovascular complications (12%), secondary tumors (20%), and other toxicities (20%). The most frequent cause of NRM is subsequent malignancy (12-fold increased risk compared with the general population). Late death is also attributed to cardiac toxicity (2%), pulmonary complications (2%), and other treatment-related toxicities (15%).

Other high-dose regimens have been used sometimes because of shortage of MEL or BCNU. Some publications suggest that the BEAC (CY) and TEAM (TT) regimens show efficacy and toxicity similar to BEAM in most if not all lymphoma subtypes (Robinson et al. 2018).

### 86.7.3 Prognostic Factors

Adverse prognostic factors for auto-HCT identified in prospective studies include early relapse within 12 months of induction therapy (Crump et al. 2017), secondary age-adjusted IPI, poor performance status, and involvement of two or more extranodal sites at relapse.

### 86.7.4 Results of Auto-HCT

	NRM	OS at 3 years	EFS at 3 years
Gisselbrecht et al. (2012) <sup>a</sup>	1.4%	51% (DHAP)	35% (DHAP)
Crump et al. (2014) <sup>a</sup>	NR	39% (both arms)	26% (both arms)

<sup>a</sup>Results from prospective randomized studies. Differences in OS and EFS may be explained by differing patient characteristics and study design

### 86.7.5 Consolidation Treatment After Auto-HCT

There are no data and no recommendation for consolidative therapy after auto-HCT for LBCL. In the CORAL study (Gisselbrecht et al. 2012), RTX maintenance did not improve outcome.

### 86.7.6 Tandem Transplantation

No data from the RTX era are available.

### 86.7.7 Relapse After Auto-HCT

CAR T cells therapy is considered the standard treatment for these patients. Results of CAR T cells after failure of auto-HCT are reported below. Data on the use of new drugs such as bispecific antibodies are promising but currently in clinical trials (Thieblemont et al. 2023; Dickinson et al. 2022).

## 86.8 CART Cells

Recently, anti-CD19 CAR T cells, axicabtagene ciloleucel and lisocabtagene maraleucel, show significant improvement in PFS and a strong trend in OS in two phase III clinical studies in high-risk R/R LBCL compared with salvage therapy followed by auto-HCT (Locke et al. 2022; Kamdar et al. 2021). For this reason, CAR T cells have been recently considered the best option for patients with refractory disease or early relapse.

CAR T cells have been considered the standard of care for patients failing an autograft (grade of evidence I) (Schuster et al. 2019; Neelapu et al. 2017). Real-world evidence in third line use has been recently published confirming the efficacy and safety of these approach. These studies support higher efficacy and also a higher toxicity of axi-cel compared to tisa-cel (Bachy et al. 2022; Bethge et al. 2022).

There are some advantages of CAR T cells comparing with HCT; chemosensitivity is not needed and patients that are not candidates for HCT because of age or comorbidities could be rescued with this strategy (Sehgal et al. 2022).

## 86.9 Allogeneic HCT

Allo-HCT is still considered a curative treatment option for patients with LBCL who relapse or progress after CAR T cells (Mussetti et al. 2023). Survival and NRM for allo-HCT in LBCL are summarized in Table 85.9.6.

The only prospective randomized clinical trial reported so far including patients with R/R T and B aggressive lymphomas who underwent allo-HCT with MAC conditioning confirmed 3 year-PFS and OS 25% and 26%, respectively (Glass et al. 2014). Haploidentical allo-HCT with post-transplant cyclophosphamide (PT-CY) as GVHD prophylaxis has been associated with a lower chronic GVHD compared with matched sibling receiving calcineurin inhibitor-based prophylaxis and unrelated donor (URD) with or without T-cell depletion (Dreger et al. 2019). These data show that allo-HCT should be still considered a curative option for patients relapsing after CAR T cells.

### 86.9.1 Stem Cell Source

PBSC is the preferred stem cell source for allo-HCT. The use of haploidentical donors has somewhat increased the use of BM in some of the series.

### 86.9.2 Donor Selection

In recent years, there has been a significant increase in the use of haploidentical donors for allo-HCT after the introduction of PT-CY. Retrospective analyses from EBMT and CIBMTR (Kanate et al. 2016) suggest that allo-HCT from HLA-identical family and URD or from haploidentical donors give comparable results. However, no prospective clinical trials

comparing haploidentical donors versus HLA-identical siblings and MUD have been published so far.

### 86.9.3 Conditioning

RIC regimens reduce NRM after transplantation in many indications but also tend to increase RI after transplantation. Because no prospective clinical trials demonstrating the superiority of one conditioning regimen over another have been reported, the question if RIC or MAC should be preferred cannot generally be answered. Aggressive disease not completely responding to salvage therapy and high tumor are situations where MAC should be considered.

### 86.9.4 Prognostic Factors

The most important adverse prognostic factor that impacts long-term outcome of patients being treated with allo-HCT is disease status before the treatment. However, unlike the situation with auto-HCT, also patients not perfectly responding to salvage therapy, e.g., patients with minor response or stable disease, may benefit from allo-HCT.

### 86.9.5 The Use of Allo-HCT in the Era of New Drugs and CAR T Cells

Allo-HCT is still a curative therapeutic strategy for fit patients with chemosensitive disease, especially relapsing after CAR T cells or whenever CAR T cells are not available.

### 86.9.6 Results of Allo-HCT

	NRM (%)	OS at 3 years (%)	PFS at 3 years (%)
Glass et al. (2014)	35	26	25
Fenske et al. (2016)	30	31	37
Bento et al. (2021)	30	44	38

### 86.9.7 Disease Relapse After Allo-HCT

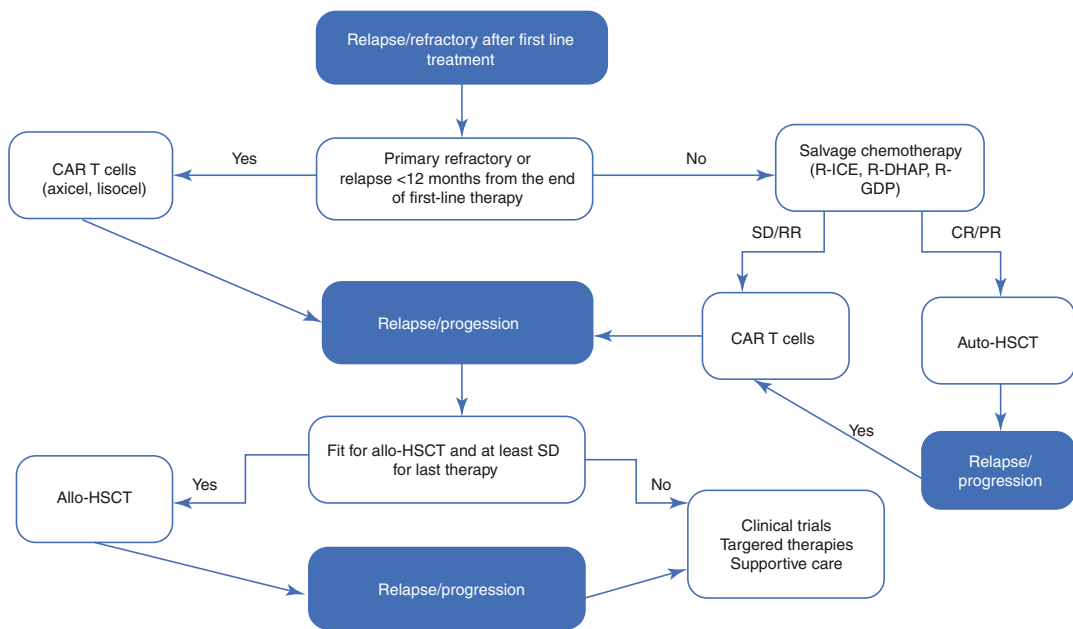
Patients relapsing after allo-HCT generally have a poor prognosis. Clinical trials should be actively considered including bispecific antibodies or other targeted therapies such as polatuzumab or lenalidomide. Unfortunately, palliative care is a reality in many cases.

### 86.9.8 Therapeutic Algorithm Recommended by the Authors (See Fig. 86.1)

See Fig. 86.1.

#### Key Points

- Auto-HCT is generally not recommended as part of first-line therapy in LBCL. It is still the standard of care for those LBCL patients with chemosensitive first late relapse.
- CAR T cells are considered standard of care for patients with refractory disease, early relapse (within 12 months from the end of first-line therapy) or patients relapsing after auto-HCT.
- Allo-HCT is the only curative treatment option for fit patients with relapse disease after CART cells. Conditioning should be guided by the individual clinical situation.



**Fig. 86.1** HCT and CAR T cells in R/R LBCL: treatment decision

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## 87.1 Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma, which is characterized by the chromosomal translocation  $t(11;14)(q13;q32)$  and overexpression of cyclin D1 in the vast majority of cases. Most patients present with advanced stage disease often with extra-nodal dissemination. High Ki67 proliferation index (Hoster et al. 2016), blastoid histologic variant (Bernard et al. 2001), and TP53 mutations represent high-risk features at diagnosis (Eskelund et al. 2017).

Front-line treatment of MCL has improved considerably, but success with chemotherapy-based treatments of relapsed and refractory (R/R) MCL has been limited (Dreyling et al. 2017). A paradigm shift from chemoimmunotherapy to targeted- and cellular therapies, such as chimeric antigen receptor (CAR) T-cell treatment, is currently changing the dismal prognosis of R/R MCL.

## 87.2 Autologous HCT

During recent years, the prognosis of patients with MCL has improved considerably, and the refinement of dose-intensified approaches such as auto-

HCT has contributed significantly to this development. A prospective randomized trial by the European Mantle Cell Lymphoma Network (EMCLN) has demonstrated the superiority of auto-HCT consolidation over interferon maintenance (Dreyling et al. 2005) in the pre-RTX era. The introduction of RTX and the addition of high-dose cytarabine (HD-ARAC) to the induction treatment before auto-HCT have further improved PFS and OS of MCL patients (Cheah et al. 2016; Geisler et al. 2008). The benefit of HD-ARAC before auto-HCT could be confirmed in prospective clinical trial (Hermine et al. 2016). The French study group conducted a prospective randomized phase III trial (LyMa trial) that investigated RTX maintenance versus observation after auto-HCT in previously untreated MCL patients. Final results of the LyMa trial confirmed the superiority of RTX maintenance with regard to PFS and OS (Le Gouill et al. 2017). The beneficial effect of RTX maintenance was observed in both PET-positive and PET-negative patients after induction treatment prior to auto-HCT (Mei et al. 2017). This finding implies that the benefit of RTX maintenance after auto-HCT is present for low- and high-risk MCL patients (Dietrich et al. 2014b, Graf et al. 2015).

Very recently, first results of the TRIANGLE study revealed that the addition of Ibrutinib to HD-ARAC induction followed by RTX and ibrutinib maintenance further improved first-line treatment of MCL (Dreyling et al. 2022). Ibrutinib with RTX- and HD-ARAC-based induction treatment can therefore be considered as the new

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first-line standard treatment for young and fit MCL patients. A second experimental arm of the TRIANGLE study investigated ibrutinib and HD-ARAC induction treatment without auto-HCT consolidation. So far, it can be concluded that ibrutinib and HD-ARAC without auto-HCT is significantly better than HD-ARAC followed by auto-HCT. If the auto-HCT after ibrutinib and HD-ARAC induction can be omitted needs to be shown with longer follow-up of the TRIANGLE study. In MIPI low-risk patients, however, it seems very unlikely that a consolidating auto-HCT further improves outcome. It seems also unlikely that *TP53*-mutated MCLs benefit from high-dose chemotherapy and auto-HCT. Auto-HCT in these two selected groups of MCL patients should therefore be critically discussed.

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### 87.3 Chimeric Antigen Receptor T-Cell Therapy

Although a significant proportion of patients with MCL enjoy long-term disease control after intensive first-line therapies, relapse remains the main cause of treatment failure. The prognosis of patients with MCL recurrence after auto-HCT appears to be extremely poor, especially if occurring early after transplant (Dietrich et al. 2011, 2014a). A proportion of almost 40% of MCL patients relapsing after auto-HCT were reported to suffer from chemotherapy-refractory disease (Dietrich et al. 2014a) with a high prevalence of clonal *TP53* mutations (Halldorsdottir et al. 2011).

The advance of autologous, CD19-directed CART-cell therapies marked a significant improvement for the treatment of R/R MCL. In patients with prior BTKi exposure, single infusion of Brexucabtagene autoleucel (brexu-cel) resulted in 91% response rate (68% CR) and median PFS of 28.2 month after three years of follow-up (Wang et al. 2023a). Real-world data from the Europe and United states confirmed the excellent outcome with significant proportion of long-term remissions (Iacoboni et al. 2022; Wang et al. 2023b).

Grade 3 or higher cytokine release syndromes (CRS) and neurologic events occurred in 15%

and 31% of patients, respectively. Patients below and above the age of 65 years had similar outcomes after treatment with brexu-cel (Wang et al. 2023a), suggesting that CAR T-cell treatment can be applied in MCL patients who are not eligible for autologous or allogeneic HCT. Frail patients (ECOG  $\geq 2$ ), however, are more frequently affected by higher grade neurologic toxicities (ICANS) (Wang et al. 2023b). Although much better tolerable than allogeneic HCT cumulative 1-year nonrelapse mortality Brexucabtagene can reach 9.1% in patients with MCL.

Due to its better safety profile, CAR T-cell therapy can be considered as the new standard for MCL relapse after ibrutinib treatment. As a consequence, most MCL patients receive CAR T-cell therapy in third line or later, but there is interest in using CAR T-cell therapy in earlier lines. The CARMAN study conducted the European Mantle Cell Lymphoma Network (EMCLN) will investigate the efficacy of CAR T-cells in first line for high-risk patients.

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### 87.4 Allogeneic HCT

In a large EBMT registry study, which investigated the outcome of MCL patients after first-line auto-HCT failure, 24% of all MCL patients received a rescue consolidation HCT. Only a minority of 2% received a second auto-HCT of whom only one patient experienced a long-term survival. These limited results do not justify a rescue auto-HCT as reasonable salvage strategy in this situation. In contrast, the majority of patients who received a second HCT underwent allo-HCT, and a significant proportion of them achieved a durable remission, translating into a 3-year OS of 43% (Dietrich et al. 2014a). Other registry studies and single-center experiences report similar results (Cook et al. 2010; Tam et al. 2009; Le Gouill et al. 2012).

Long-term efficacy of RIC allo-HCT was recently demonstrated in a large cohort of MCL patients (Robinson et al. 2018). The cumulative incidence of relapse was 40% at 5 years, and OS was 40% at 5 years. Patients who developed a chronic GVHD and/or patients who did not receive an in vivo TCD with CAMPATH had a

significantly lower relapse rate, suggesting the existence of a graft versus MCL effect. Despite long-term remissions after allo-HCT, chemorefractory disease (Robinson et al. 2018) or early relapse after first-line auto-HCT (Dietrich et al. 2014a) significantly reduced the long-term survival of MCL patients after allo-HCT. CAR-T-cell therapy seems to provide better outcome in this setting, although direct comparison is pending (Iacoboni et al. 2022; Wang et al. 2023a). It is important to note that there is a small group of relapsed MCL patients who survived longer than 5 years even without allo-HCT, suggesting a rather indolent disease course in a subset of patients (Dietrich et al. 2014a). Such patients with a low percentage of Ki67-positive tumor cells might not benefit from an allo-HCT.

#### Key Points

- Ibrutinib improves disease-free survival in addition to intensive induction- and RTX maintenance therapy.
- Auto-HCT in first remission should be questioned in low-risk patients and patients with *TP53* mutations not responding to chemotherapy.
- CD19-directed CAR T-cell therapy achieves high remission rates in relapsed and R/R MCL.
- Early treatment with CAR T-cells is currently evaluated by EMCLN in the CARMAN trial.
- While curative potential of CART-cell therapy in MCL remains unclear, allo-HCT should be evaluated for MCL patients relapsing after CAR T-cell therapy.

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## Other B- and T-Aggressive Lymphomas and Lymphomas Associated with HIV

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### 88.1 Burkitt Lymphoma

#### 88.1.1 Definition and Epidemiology

BL accounts for around 2% of all adult NHL with a higher incidence in patients with immunodeficiency and in patients who have HIV infection. There is an endemic pediatric subtype in equatorial Africa, which is strongly associated with EBV. The clinical course of BL usually is highly aggressive with a Ki67 expression of nearly 100% requiring prompt institution of therapy.

#### 88.1.2 Diagnosis

A tissue biopsy/cytology sample is mandatory for the diagnosis. The translocation of *MYC* with the immunoglobulin heavy-chain loci (*IgH*), 80%

or less frequently, with kappa or lambda light-chains, is the molecular hallmark of BL. Diagnosis requires a combination of morphology, immunophenotype, and genetic analysis.

#### 88.1.3 Prognostic Factors

Several studies have identified prognostic factors for poor outcome. The Burkitt lymphoma International Prognostic Index (BL-IPI) identified four variables (age  $\geq 40$  years, performance status  $\geq 2$ , serum LDH  $> 3 \times$  upper limit of normal, and CNS involvement) with independent prognostic value for PFS and OS (Olszewski et al. 2021).

#### 88.1.4 First Line Treatment

The optimal first-line therapy in BL has not been defined yet. To achieve a fast and stable remission, an intensive regimen combining several compounds is used in most centers. Combinations of rituximab, DOX, alkylators, vincristine, and VP with direct therapy to prevent CNS disease are highly active. Frequently used regimens are the HOVON R-CODOX-M/R-IVAC (RTX, CY, vincristine, DOX, MTX, IFO, Ara-C, VP) (Mead et al. 2002), R-HyperCVAD (RTX, hyperfractionated CY, vincristine, DOX, dexamethasone) (Thomas et al. 1999), and the GMALL-B-ALL-protocol (RTX, MTX, Ara-C, CY, VP, IFO)

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(Hoelzer et al. 2014). With these protocols, a significant portion of patients have a chance to get cured with first-line therapy. For patients not feasible for intensive treatment, dose-adjusted EPOCH-R (rituximab, VP, vincristine, CY, and DOX) may be an option (Roschewski et al. 2020).

### 88.1.5 Autologous HCT

There are several studies exploring the role of auto HCT in first remission. In a prospective trial, the HOVON group treated 27 patients with two cycles of intensive induction followed by BEAM-conditioned auto HCT for those patients achieving at least a PR (van Imhoff et al. 2005). The 5-year EFS and OS was 73% and 81%, respectively. In a retrospective analysis of 117 patients receiving auto HCT as part of first-line therapy between 1984 and 1994, patients in CR at time of transplant had a 3-year OS of 72% (Sweetenham et al. 1996). In the relapse situation, patients who were chemotherapy sensitive had a 3-year OS of 37% following auto HCT compared to just 7% for those who were chemotherapy resistant (Sweetenham et al. 1996). In summary, auto HCT in BL is feasible, but there is no documented advantage compared to standard combination chemotherapy for patients responding sufficient to first line. Auto HCT may be used to optimize remission in patients with insufficient response or as bridging to allo HCT. In the relapse setting, given the intensive regimens usually used as first line, the difficulty lies in achieving a response good enough to proceed to auto HCT and to collect SC, hence auto HCT is rarely used in BL.

### 88.1.6 Allogeneic HCT

Indicated in	CR $\geq$ 2
Donor	MRS > MUD > mMUD
Conditioning	RIC > MAC
TRM (1 year)	30%
OS (5 years)	30–40%
DFS	35%

Adapted from Peniket et al. (2003)

The kinetics of BL with rapidly progressive disease may not allow for the 4–6 week waiting

period during screening, apheresis, and manufacturing necessary for CAR T-cell therapy.

## 88.2 Lymphoblastic Lymphoma

### 88.2.1 Definition and Epidemiology

LBL is an aggressive neoplasm of precursor B cells (B-LBL) or T cells (T-LBL) with features of acute leukemia. It accounts for approximately 2% of all NHL. In adults, around 90% of all LBL are T-LBL.

### 88.2.2 Diagnosis

The diagnosis is based on an LN biopsy. T-LBL is usually TdT positive with variable expression of other T-cell markers (CD7 and CD3 are often positive).

### 88.2.3 Prognostic Factors

At this time, no convincing prognostic model for these patients is available. Several studies tried to identify risk factors, the following may be associated with an unfavorable outcome: elevated LDH, BM, or CNS involvement, and stage IV disease. There is also an association with lymphotropic viral infections, e.g., HTLV-1. The role of MRD in LBL has not been defined yet, but as we learned from acute leukemias and other lymphomas, persisted MRD positivity might be a predictor of poor outcome.

### 88.2.4 First-Line Treatment

Standard approaches for patients with LBL are adapted to ALL protocols. These regimens contain multiple drugs, such as corticosteroids, CY, MTX, vincristine, Ara-C, thioguanine, L-asparaginase, VP, nitrosoureas, and anthracyclines. Intrathecal CNS prophylaxis is mandatory. With these protocols, a CR rate more than 80% and a significant chance of cure has been reported [overview in Intermesoli et al. (2022)].

### 88.2.5 Autologous Transplantation

Since most centers prefer allo HCT over auto HCT because of GVL effect, there are only very few studies evaluating the role of auto HCT in LBL. In CR1, the use of auto HCT as a consolidation may improve relapse-free survival, but has no effect on OS (Sweetenham et al. 2001). In another study in 128 patients with LBL receiving auto HCT, RR at 5 years was 56% (Levine et al. 2003). No documented role in more advanced disease >CR1 is reported either. In conclusion, data for auto HCT in LBL are too scarce to come to firm conclusions.

### 88.2.6 Allogeneic Transplantation

There is also no established role for allo HCT in patients with LBL. Compared to auto HCT, allo HCT is associated with a higher TRM but lower RR. In 76 patients receiving allo HCT, 5-year RR was 34% (Levine et al. 2003). In this retrospective study, there was no significant difference in OS at 1 year and 5 years between auto HCT and allo HCT. In the relapse setting, bridging with nelarabine might be an option (Candoni et al. 2020). In general, the indication for allo HCT should be based on risk factors, remission, and MRD.

## 88.3 Peripheral T Cell Lymphomas

### 88.3.1 Definition and Epidemiology

PTCLs are a very heterogenous group of lymphomas originating from the T cell lineage. They account for approximately 10–15% of all NHL. Because of this low incidence, large randomized studies are difficult to perform.

### 88.3.2 Diagnosis

The diagnosis, as in any NHL, should be based on a LN biopsy. The differential diagnosis

between PTCL and other types of T-NHL is crucial for the outcomes, and in some specific cases, the treatments are very different.

### 88.3.3 Risk Factors

The IPI is the most commonly used prognostic tool in PTCL. The following factors are associated with worse outcome: age >60 years, ECOG >1, elevated LDH, stages III–IV, and extranodal involvement >1 site. Another prognostic tool is the PIT score, which is calculated by four parameters as age, ECOG, LDH level, and BM involvement. In anaplastic large cell lymphoma (ALCL), the tumors are categorized in ALK+ or ALK– with better prognosis for ALK+ lymphomas.

### 88.3.4 First-Line Treatment

The primary goal of first-line treatment is to get a deep and continuing remission. Standard regimens are anthracycline-containing combinations like CHOP or CHOEP, achieving a 3-year EFS of 50–70% and a 3-year OS of 75–80% (Schmitz et al. 2010). In CD30-positive PTCL, the addition of brentuximab to chemotherapy improves PFS and OS (Horwitz et al. 2022). In the relapse situation, the overall prognosis of PTCL is dismal, and the optimal treatment for these patients has not been defined yet. Relapse patients not able to receive intensive treatment including HCT may be offered single-agent therapy, e.g., gemcitabine, or in case of CD30 expression brentuximab vedotin.

### 88.3.5 Autologous Transplantation

Indicated in	CR1 (IPI >1); CR ≥2
Conditioning	BEAC, BEAM, LEAM, CBV
TRM (1 year)	5–7%
REL (3 years)	40–50%
OS (5 years)	70% (CR1) 50% (CR ≥2)
PFS (5 years)	50% (CR1) 20–35% (CR ≥2)

Adapted from Kyriakou et al. (2008), d'Amore et al. (2012), Wilhelm et al. (2016) and Kewalramani et al. (2006)

### 88.3.6 Allogeneic Transplantation

Indicated in	CR $\geq$ 2, relapse post auto HCT
Donor	MRS > MUD > mMUD
Conditioning	RIC > MAC
GVHD prophylaxis	CsA, CsA-MTX, CsA-MMF
Graft failure	<10%
TRM (1 year)	20–25%
REL (3 years)	20–40%
OS (3 years)	40–60%
PFS (3 years)	30–50%

Adapted from Kewalramani et al. (2006), Schmitz et al. (2014, 2018) and Dodero et al. (2012)

## 88.4 Cutaneous T Cell Lymphomas

### 88.4.1 Definition and Epidemiology

Cutaneous T cell lymphomas (CTCLs) are a spectrum of lymphoid malignancies characterized by clonal expansion of T-cells primarily involving the skin, which may extend to other organs in advanced forms. CTCLs are relatively rare, with an incidence of approximately six patients per million people (Dobos et al. 2020). The commonest forms (70%) are mycosis fungoides (MF) and Sezary syndrome (SS). Although the exact risk factors for CTCLs remain unclear, an interplay between intrinsic and environmental factors exists.

### 88.4.2 Diagnosis and Staging

Diagnosis of MF/SS can be challenging and requires clinical data, histopathological findings, and molecular testing. Staging is based on a TNMB classification revised by the ISCL and EORTC (Olsen et al. 2007). Patients with early-stage disease, including skin patches and plaques (stages I–IIA), have an indolent clinical course with excellent long-term survival. Patients with advanced-stage disease, including erythroderma, tumors, or significant blood, nodal or visceral involvement (stages IIB–IV), have poor prognosis with a median survival that does not go beyond 2–5 years.

### 88.4.3 Therapeutic Management

The initial management of CTCLs is directed to control symptoms and improve quality of life, particularly in patients with early-stage disease. Some of these patients can have an expectant period, but the vast majority will start with skin-directed therapies, such as topical steroids, total skin electron beam therapy, ultraviolet B phototherapy, PUVA, and others (Quaglino et al. 2021). Patients with refractory early-stage or advanced-stage disease have multiple therapeutic options, albeit most with low response rates and duration (Giordano and Pagano 2022). Some novel targeted therapies such as brentuximab vedotin and mogamulizumab have emerged with promising results. However, allogeneic HCT remains the only potential curative option.

### 88.4.4 Allogeneic HCT

EBMT has led international efforts to examine the role of allogeneic HCT in patients with CTCLs (Duarte et al. 2008, 2010, 2014; Domingo-Domenech et al. 2021). Overall, high relapse rate remains the major cause of failure and needs to be improved with better strategies before and after transplant. Nevertheless, with a long follow-up in survivors of more than 6 years, we have shown that allogeneic HCT can rescue over one third of patients with advanced-stage and refractory CTCLs, and it remains a clinical option for patients with advanced-stage CTCLs (Snowden et al. 2022). Also, over time there is a trend to offer allogeneic HCT earlier in the course of the disease, including older patients as well as the use of more unrelated donors.

## 88.5 HIV-Associated Lymphomas

### 88.5.1 Definition and Epidemiology

Patients infected with HIV have an increased risk of developing NHLs as compared to the general population. The most frequent subtypes are DLBCL and BL which both are AIDS-defining

illnesses, and HL, which is one of the non-AIDS-defining malignancies.

### 88.5.2 Risk Factors

Factors that determine prognosis in patients with HIV-related lymphoma are the same as in the general population. Additionally, a low CD4+ T-cell count and an uncontrolled HIV viral load are independent risk factors for HIV-lymphomas.

### 88.5.3 First-Line Therapy

The availability of combination antiretroviral therapy (cART), along with the better management of opportunistic infections, allows HIV-infected patients with lymphoma to receive the same treatment approaches as HIV-uninfected patients. The consequent use of cART during therapy is of major importance for successful treatment. The indication for HCT has to be discussed in the relapse situation.

### 88.5.4 Autologous Transplantation

Indicated in	CR $\geq 2$ ; same indications as in general population with the same type of lymphoma
Conditioning	BEAC, BEAM, LEAM, CBV
TRM (1 year)	5–7%
REL (3 years)	30–40%
OS (5 years)	50–60%
PFS (5 years)	50–60%

Adapted from Hübel et al. (2019), Diez-Martin et al. (2009) and Balsalobre et al. (2009)

### 88.5.5 Allogeneic Transplantation

Experience on the use of allo HCT in patients with lymphoma and HIV infection is limited and no definitive recommendation can be given at this time. There are some case reports or small retro-

spective analysis showing that allo HCT in HIV positive patients using MRD, MUD, or cord blood is feasible, but application of cART and viroimmunological reconstitution is a matter of debate. In a report of five HIV-positive patients who underwent allo HCT with various hematologic malignancies, there was no TRM or major infections (Mulanovich et al. 2016). HIV virus load remained undetectable with continuous cART. Three patients relapsed 6, 7, and 13 months after transplant, and two were alive and well after 42 and 55 months.

There are a few case reports available showing that the application of CAR-T-cell therapy is feasible in HIV-related lymphomas.

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# Classical Hodgkin's Lymphoma

# 89

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## 89.1 Definition and Epidemiology

HL is a malignancy arising from germinal centre or post-germinal centre B cells. The cancer cells form a minority of the tumour and are surrounded by a reactive inflammatory milieu comprising lymphocytes, eosinophils, neutrophils, histiocytes and plasma cells. These malignant cells can be pathognomonic, multinucleate giant cells or large mononuclear cells and, together, are referred to as Hodgkin and Reed–Sternberg (HRS) cells.

HL accounts for approximately 10% of cases of newly diagnosed lymphoma. The incidence of HL in Europe is 2.2 per 100,000 per year with a mortality rate of 0.7 cases/100,000 a year. The disease is more frequent in men than in women, and peaks in incidence are noted in young adults

and in people older than 60 years. Incidence has remained mostly unchanged during the past two decades.

## 89.2 Diagnosis

Pathological diagnosis should be made according to the WHO classification from a sufficiently large surgical specimen or excisional lymph node biopsy to provide enough material for fresh frozen and formalin-fixed samples (Eichenauer et al. 2014).

## 89.3 Classification

HL is classified as either classical (cHL, defined by the presence of HRS cells) or nodular lymphocyte-predominant (NLPHL). The immunophenotype of the malignant cells in cHL and NLPHL differs significantly and helps to establish the diagnosis. Four subtypes of cHL exist (nodular sclerosis, mixed cellularity, rich in lymphocytes and lymphocyte depleted), which differ in presentation, sites of involvement, epidemiology and association with EBV. Management, however, is broadly similar in all subtypes. NLPHL has a distinct clinical course, and it only represents less than 5% of the cases of HL. While in the recent fifth edition of the World Health Organization Classification of Haematolymphoid Tumours NLPHL remains an essentially

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unchanged diagnostic entity, in the 2022 International Consensus Classification of Mature Lymphoid Neoplasms, NLPHL is now renamed nodular lymphocyte predominant B cell lymphoma (NLPBL) in recognition of the distinct pathologic, biologic and clinical differences (Alaggio et al. 2022; Campo et al. 2022).

## 89.4 Risk Factors

The outlook for patients with early-stage disease (stages I–IIA) is excellent, with OS exceeding 90% in many trials. In advanced-stage disease (IIB, III–IV), OS is 75–90%. Risk factors for patients with early-stage disease are size of mediastinal mass, age, erythrocyte sedimentation rate, number of nodal areas, B symptoms and mixed cellularity or lymphocyte-depleted histology. Different risk stratification systems combining these factors are defined by the EORTC, GHSG, NCCN and National Cancer Institute of Canada and are currently used in clinical practice. Risk factors for advanced stages consist of albumin <4 g/dL, haemoglobin <10.5 g/dL, male, age ≥45 years, stage IV disease, leucocytosis ≥15 × 10<sup>9</sup>/L and lymphocytopenia (lymphocyte count less than 8% of white blood cell count and/or lymphocyte count less than 0.6 × 10<sup>9</sup>/L) (International Prognosis Score, 1 point per factor) (Eichenauer et al. 2014).

## 89.5 First-Line Treatment

The treatment of patients with cHL is primarily guided by the clinical stage and prognostic factors of disease. Patients with early-stage disease are usually treated with a combination of chemotherapy (ABVD) plus RTx. The amount of chemotherapy and dose of radiation differ for patients with favourable and unfavourable prognosis of disease. Chemotherapy (ABVD, escalated BEACOPP or Stanford V) was the main treatment for patients with advanced stage, and RTx may be used for selected patients as consolidation (Eichenauer et al. 2014). PET-CT scan adapted therapy is increasingly used allowing treatment

de-escalation to decrease toxicity or escalation in case of insufficient tumour control (Johnson et al. 2016; Casasnovas et al. 2019). Data from the Echelon-1 study demonstrated improved overall survival in first-line therapy of advanced HL when replacing bleomycine in ABVD with brentuximab vedotin (Ansell et al. 2022).

## 89.6 Second-Line Treatment Before Auto-HCT

The principles of management of relapse or refractory cHL are shown in Table 89.1 (von Tresckow and Moskowitz 2016). All

**Table 89.1** Principles of management of relapse or refractory cHL

### *New biopsy*

- Mandatory if relapse is ≥12 months after the end of primary treatment in order to exclude alternative diagnoses. Highly recommended for patients with suspected relapse <12 months
- If apparent primary refractory disease, histological confirmation of HL is only recommended if progression is suspected within new sites of disease. Biopsy may not be mandatory in patients with clear radiological progression in sites of primary disease during treatment

### *Radiological evaluation*

- A whole-body CT scan with contrast dye injection and a PET are recommended for further comparison

### *Therapy*

- Salvage therapy followed by high-dose chemotherapy and auto-HCT is currently considered the standard of care for relapsed cHL patients
- No study has compared effectiveness of different salvage regimens
- Salvage strategy should be tailored on an individual basis taking into account the initial therapy given, the risk of adding cumulative non-haematologic toxicity and the possibility of harvesting stem cells
- Cardiac and pulmonary function should be evaluated prior to treatment
- If indicated, reproductive counselling should be proposed prior to treatment
- Objective of salvage chemotherapy: to produce a response (tumour remains chemosensitive), which has a major impact on post-auto-HCT outcome. Achievement of PET negativity defines chemosensitivity and should be the goal of salvage chemotherapy

chemotherapy-based salvage regimens are associated with haematologic toxicity. Infection and neutropenic fever are reported in 10–24% of cases. Nephrotoxicity, hepatotoxicity, mucositis and gastrointestinal toxicity are observed in <10%. Haematopoietic stem cell mobilization appears adequate with all regimens. Efficacy of different salvage options is shown in Table 89.2.

**Table 89.2** Salvage regimens

<i>Conventional chemotherapy</i>	
DHAP	ORR 89%, CR 21%
ESHAP	ORR 67%, CR 50%
ICE	ORR 88%, CR 67%
Gemcitabine-containing regimens	ORR 81%, CR 54%
• IGEV	ORR 70%, CR 19%
• GVD	ORR 62%, CR 1%
• GDP	ORR 83%, CR 73%
• BeGEV	
<i>No chemotherapy strategies</i>	
Brentuximab vedotin (BV)	Currently approved after failure of at least two prior multiagent chemotherapy regimens in patients who are not auto-HCT candidates. ORR 50%, CR 12%
Pembrolizumab	Currently approved for the treatment of patients with refractory cHL or who have relapsed after three or more prior lines of therapy. ORR 69%, CR 22%
<i>New drugs in association with chemotherapy<sup>a,b</sup></i>	
<i>Sequential strategies</i>	
• BV followed by ICE	77%
• Nivolumab followed by ICE	86%
<i>Combination strategies</i>	
• BV plus bendamustine	74%
• BV plus ESHAP	70%
• BV plus ICE	69%
• BV plus DHAP	90%
• BV plus nivolumab	62%
• Pembrolizumab plus GVD	95%
• Pembrolizumab plus ICE	86%

<sup>a</sup>These combinations are not currently approved for this indication

<sup>b</sup>PET-negative response rate

## 89.7 Autologous HCT

Auto-HCT is currently considered the standard treatment for relapsed/refractory (R/R) cHL patients. Two landmark randomized clinical trials, the British National Lymphoma Investigation (BNLI) in 1993 and the joint German Hodgkin Study Group (GHSBG)/EBMT HD-R1 trial in 2002, compared high-dose chemotherapy followed by auto-HCT versus chemotherapy and showed significant a benefit of auto-HCT in terms of EFS and FFTR in front of conventional salvage chemotherapy; however, there was no significant OS benefit. EBMT current indications for autologous HCT in HL are shown in Table 89.3 (Snowden et al. 2022).

### 89.7.1 Stem Cell Source and Conditioning Regimen

Haematopoietic stem cells from mobilized PB are the preferred stem cell source for auto-HCT.

Although the choice of preparative regimen varies and is typically based on institutional experience, BEAM is the preferred option. Standard BEAM consists of BCNU (300 mg/m<sup>2</sup> × 1, day -6), VP (200 mg/m<sup>2</sup>, days -5 to -2), Ara-C (200 mg/m<sup>2</sup> bid, days -5 to -2) and MEL (140 mg/kg/day × 1, days -1). The CY, BCNU and VP (CBV) regimen is also commonly used in North America. The use of TBI-based regimens is not recommended due to the higher risk of developing secondary malignancies.

**Table 89.3** EBMT current indications for autologous HCT in cHL (Snowden et al. 2022)

Disease status	Recommendations
First complete remission	Generally not recommended Level of evidence I
Sensitive relapse/no previous auto HCT	Standard of care Level of evidence I
Sensitive relapse/previous auto HCT	Clinical option Level of evidence III
Refractory disease	Clinical option Level of evidence III

Late toxicities of BEAM include pulmonary complications (chronic interstitial fibrosis and decrease in lung diffusing capacity, 21%), infection (30%), metabolic syndrome (17%), cardiovascular complications (12%), secondary tumours (20%) and other toxicities (20%). The most frequent cause of NRM is subsequent malignancy (12-fold increased risk compared with the general population).

### 89.7.2 Prognostic Factors

Adverse prognostic factors for post-auto-HCT outcome consistent across many reported trials included primary induction failure, initial remission duration of <3 months, relapse within 12 months of induction therapy, extranodal disease, B symptoms, advanced stage at relapse, resistance to salvage chemotherapy and persistent disease at the time of transplant.

### 89.7.3 Results of Auto-HCT

Disease status pre-auto-HCT	NRM (%)	OS at 5 years (%)	PFS at 5 years (%)
Chemosensitive disease	0–18	75	50
Primary refractory disease	0–18	30–36	15–38

### 89.7.4 Consolidation Treatment After Auto-HCT

Brentuximab vedotin (BV) is currently the only drug approved for consolidation treatment after auto-HCT in patients at risk of relapse or progression. This approval was obtained after the results of the phase III AETHERA trial. In this multicentre randomized trial, 329 patients with relapsed or refractory HL were allocated to either consolidation therapy of up to 16 cycles of BV or placebo after auto-HCT. PFS was significantly longer in patients in the BV group (median PFS 43 months vs. 24 months,  $P = 0.0013$ ). When

patients were grouped by the number of risk factors, a higher number led to more notable benefits in the consolidation arm (Moskowitz et al. 2015).

## 89.8 Tandem Auto-HCT

Several groups have explored a tandem transplant approach to improve post-transplant outcomes of patients with poor risk factors. These studies showed that tandem auto-HCT is feasible and associated with a NRM of 0–5%, 5-year OS of 54–84% and 5-year PFS of 49–55% (Smith et al. 2018). According to these results, risk-adapted tandem auto-HCT can be considered an option for poor-risk patients, but integration of PET findings and new drugs such as BV and checkpoint inhibitors may help to refine the need for a second auto-HCT and possibly improve outcomes of these patients.

## 89.9 Disease Relapse After Auto-HCT

Patients relapsing following auto-HCT used to have an overall poor prognosis. Early relapse, stage IV, bulky disease, poor performance status and age  $\geq 50$  years at auto-HCT failure have been identified as predictors of poor outcome (Jethava et al. 2017; Kallam and Armitage 2018; Lapo and Blum 2016). Recently, a large retrospective analysis from the EBMT that included 1781 adult patients with relapsed cHL after auto HCT over a period of 12 years, noted a significant improvement in the 4-year OS after relapse, which increased from 32% in patients who relapsed between 2006 and 2008 to 63% in those who relapsed between 2015 and 2017 (Bazarbachi et al. 2022). In this study, survival increased with the length of time between auto-HCT and relapse, whereas increasing age, poor performance status, bulky disease, extranodal disease and presence of B-symptoms at relapse were associated with a worse OS (Bazarbachi et al. 2022). Therapeutic options are very heterogeneous (Table 89.4) (Martínez et al. 2013; Hahn et al. 2013).

**Table 89.4** Therapeutic options after auto-HCT relapse

Brentuximab vedotin (Chen et al. 2016)	Currently approved for the treatment of cHL relapsed after auto-HCT ORR 75%, CR 34% PFS 5.6 months
Nivolumab	Currently approved for the treatment of cHL relapsed after auto-HCT and BV ORR 69%, CR 16% 1-year OS 92%, median PFS 12–18 months
Pembrolizumab	Currently approved for the treatment of cHL relapsed after auto-HCT ORR 69%, CR 22% PFS 72% at 6 months
Gemcitabine-based chemotherapy	ORR 69–86%, EFS 10%
Bendamustine	ORR 53–78%, CR 29–33%
Lenalidomide	ORR 19%
Histone deacetylase inhibitors	ORR 4–74%, CR 0–4% 1-year OS 78%
Everolimus	ORR 47%
Tislelizumab	ORR 82%, CR 63%
CAR-T cells	ORR 39–100%, CR 50%
Second auto-HCT	NRM 15%, 5-year OS and PFS 30%
Allogeneic transplantation	See Sect. 89.10

## 89.10 Allogeneic HCT

Allo-HCT is still considered a curative treatment strategy for patients with cHL who relapse or progress after auto-HCT (Peggs et al. 2008). Our knowledge on the curative capacity of allo-HCT relies on the results of several retrospective analyses, some of them registry-based, phase II prospective clinical trials (Sureda et al. 2012) that included low number of patients and retrospective analyses that in a donor-versus-no-donor strategy demonstrate that allo-HCT offers a significant benefit in terms of both PFS and OS. EBMT current indications for allo-HCT in cHL are shown in Table 89.5.

### 89.10.1 Stem Cell Source, Type of Donor and Conditioning Regimen

HSC from mobilized PB are the preferred stem cell source for allo-HCT. The use of haploidentical donors has increased the use of BM in some of the series. Later studies have demonstrated no significant differences in terms of GVHD incidence with the use of PB in this setting.

In recent years, there has been a significant increase in the use of haploidentical donors with the introduction of the PT-CY approach. The interesting results observed with this type of transplant have already decreased the use of MUD and MRD in the EBMT reporting centres (Gayoso et al. 2016). Retrospectively, registry-based studies from both EBMT and CIBMTR indicate that outcomes of PT-CY-based haplo-HCT are similar to those of MRD and MUD; cumulative incidence of GVHD seems to be lower with the haploidentical approach and translates into a better PFS-cGVHD in some of the series (Martínez et al. 2017).

More than 50% of the patients with HL treated with allo-HCT receive a RIC protocol. RIC regimens have demonstrated to significantly reduce NRM after transplantation but also to increase RI after transplant (Sureda et al. 2008). There are no formal prospective clinical trials demonstrating the superiority of a given conditioning protocol in front of the others. Retrospective analysis indicates that low-dose TBI-containing regimens are associated with a higher RI and lower survival than non-TBI-containing protocols. A more recent retrospective analysis from the EBMT registry showed that with modern transplant practices, the NRM associated with MAC for HL has strongly decreased, resulting in a trend towards better EFS compared with RIC transplants (Genadieva-Stavrik et al. 2016). Therefore, an MAC could be an option to be considered on a case-by-case basis.

**Table 89.5** EBMT current indications for allogeneic HCT in cHL (Snowden et al. 2022)

Disease risk	MSD	MUD	Alternative donors <sup>a</sup>
First remission	GNR	GNR	GNR
	Level of evidence III	Level of evidence III	Level of evidence III
Chemosensitive relapse, previous auto-HCT: no	Developmental	Developmental	GNR
	Level of evidence III	Level of evidence III	Level of evidence III
Chemosensitive relapse, previous auto-HCT: yes	Standard	Standard	Standard
	Level of evidence II	Level of evidence II	Level of evidence II
Refractory disease	Developmental	Developmental	Developmental
	Level of evidence II	Level of evidence II	Level of evidence III

<sup>a</sup>MMUD, haploidentical donors, CB. GNR generally not recommended

### 89.10.2 Prognostic Factors

The most important adverse prognostic factor associated with long-term outcome after allo-HCT is the disease status before transplant. However, the impact of a PET-negative CR before the procedure is not as straightforward as in the auto-HCT setting.

### 89.10.3 The Use of Allo-HCT in the Era of New Drugs

The role and positioning of allo-HCT in patient's relapsing/progressing after auto-HCT are less clear with the introduction of new drugs. Numbers of allo-HCT for this indication seem to have decreased over the last 2 years.

BV has been used as a bridge to allo-HCT. There is no evidence of a need of a washout period between the last dose of BV and day 0 of HCT. The number of BV cycles being given before allo-HCT is usually between four and six. The use of BV before transplant does not modify post-transplant-related toxicities and might improve results by improving performance status and disease status before allo-HCT. It might also allow more patients to successfully go through the transplant and potentially reduce the incidence of chronic GVHD (Bazarbachi et al. 2018).

Checkpoint inhibitors (nivolumab, pembrolizumab) before allo-HCT seem very effective

with promising survival results (Dada 2018). However, follow-up is still too short, and it has been suggested that their use could be associated with increase in transplant-related toxicity (acute GVHD, SOS/VOD, post-transplant hyperacute febrile syndrome). It is generally recommended to hold checkpoint inhibitors for at least 6 weeks before allo-HCT and to use PTCY for GVHD prophylaxis.

The final decision of whether to allograft a patient that relapses after auto-HCT might rely on the risk profile of the underlying disease as well as the transplant-related risk.

### 89.10.4 Results of Allo-HCT

Disease status pre-allo-HCT	NRM (%)	OS at 3 years (%)	PFS at 3 years (%)
Chemosensitive disease	15–20	60–70	40–50
Chemorefractory disease	20–30	40–50	20–30

### 89.10.5 Disease Relapse After Allo-HCT

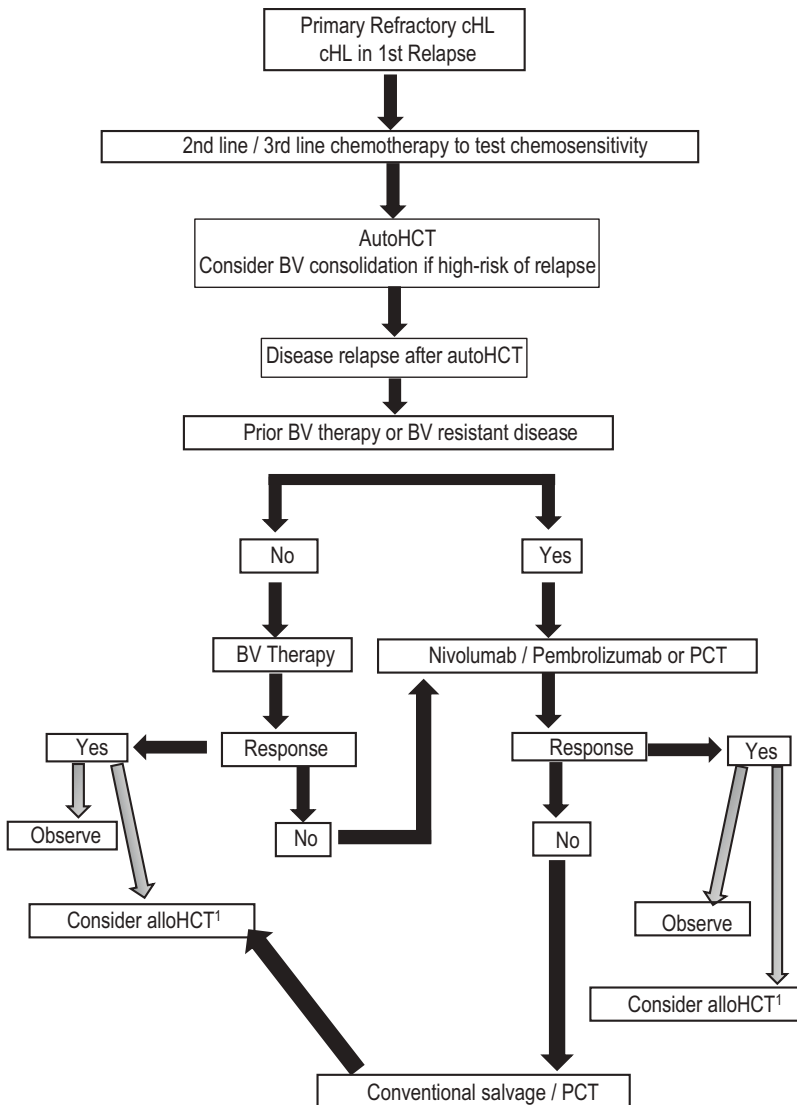
Disease relapse carries out a dim prognosis. Therapeutic options are variable and heterogeneous (Table 89.6), and in some cases, palliative care is the only feasible one.



**Table 89.6** Therapeutic options after allo-HCT relapse

DLI alone	ORR 33–54%
DLI + brentuximab vedotin	ORR 69% (CR 54%/PR 15%), PFS 5.5 months
DLI + bendamustine	ORR 55% (CR 16%/39%), PFS 6 months
Brentuximab vedotin (Gopal et al. 2012)	ORR 50–69% CR 31–38%/PR 37% Median PFS 7–8 months
Nivolumab (Herbaux et al. 2017)	ORR 77–95% CR 42–55%/PR 40–52% 1-year PFS 58%

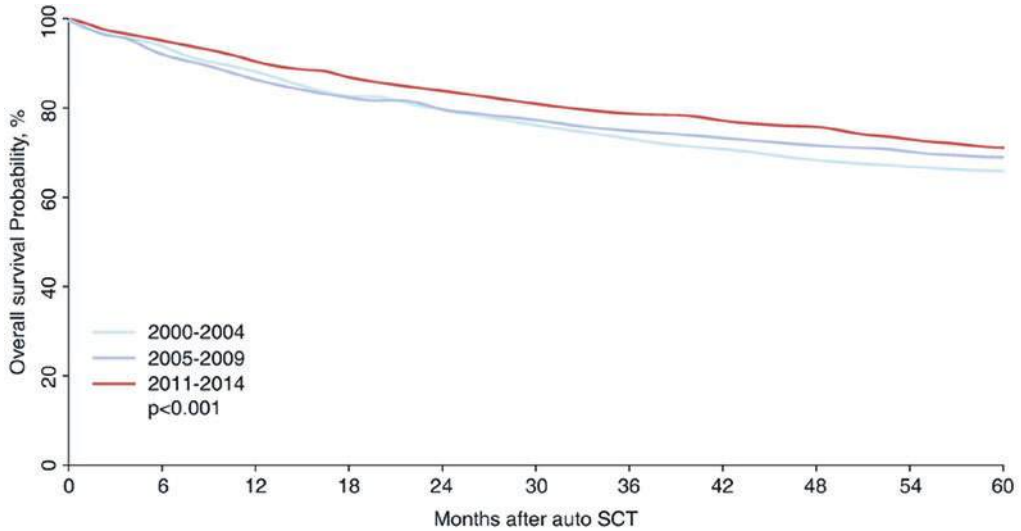
**89.11 Therapeutic Algorithm Recommended by the Authors (Modified from Yethava et al.)**



*PCT* prospective clinical trials. <sup>1</sup>In young and fit patients with responding disease and an adequate donor available. Grey arrows. Both options can eventually be considered

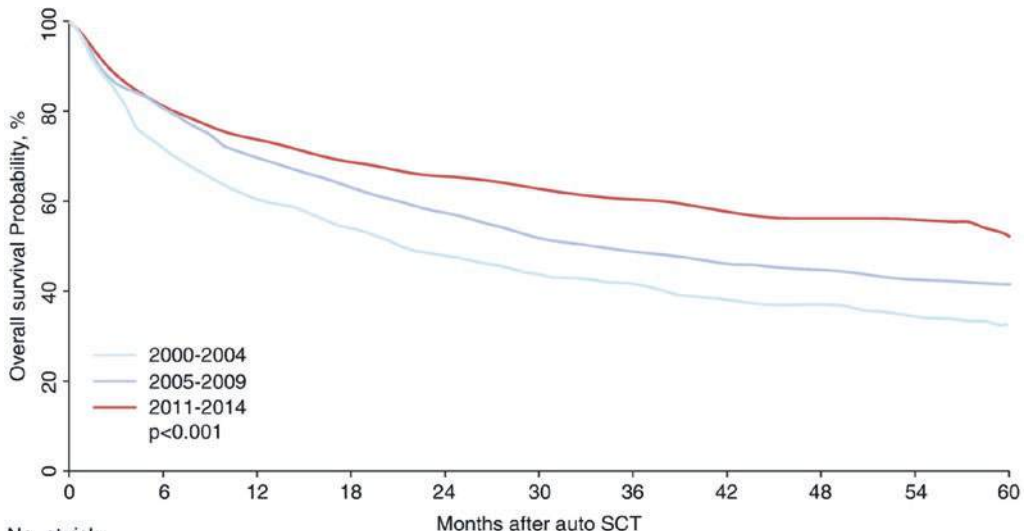
acceptable after a careful balance of adverse prognostic factors of the patient/transplant-related comorbidities/ careful discussion with the patient

### 89.12 Long-Term Outcomes of Auto-HCT and Allo-HCT in Patients with Relapsed/Refractory cHL (EBMT Database, with Permission)



No. at risk	0	6	12	18	24	30	36	42	48	54	60
2000-2004	1699	1469	1322	1217	1118	1047	960	914	857	816	
2005-2009	2560	2189	1912	1756	1631	1507	1386	1291	1211	1124	
2011-2014	2571	2095	1819	1603	1382	1161	933	763	570	418	

OS of auto-HCT in relapsed/refractory cHL over time



No. at risk	0	6	12	18	24	30	36	42	48	54	60
2000-2004	242	196	175	153	136	127	115	105	94	87	
2005-2009	550	447	386	344	297	281	260	238	224	211	
2011-2014	729	631	550	477	417	343	271	211	162	104	

OS of allo-HCT in relapsed/refractory cHL over time

### Key Points

- Auto-HCT is still the standard of care for those patients with primary refractory/chemosensitive first relapse. Results of auto-HCT might improve in the future with better selection of patients, improved results of salvage strategies and consolidation treatment in those patients with high risk of relapse after auto-HCT.
- Allo-HCT is the only curative treatment options for those patients relapsing after auto-HCT. The use of allo-HCT is being modified by the introduction of haploidentical donors as well as targeted therapies in this setting.

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# Inborn Errors of Immunity

# 90

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## 90.1 Introduction

Inborn errors of immunity (IEI) arise from genetic defects that lead to abnormalities in immune cell development or function with a wide spectrum in severity and clinical manifestations. These include infectious, autoimmune, autoinflammatory, hematological, and malignant presentations. Allo-HCT provides a life-saving and curative treatment modality in many but not all of these disorders by replacing the defective hematopoietic cell lineage(s) by those from healthy allogeneic donors. Other management

options including enzyme replacement therapy, gene transfer into autologous hematopoietic stem cells, and targeted therapies (see below) may provide an alternative or bridging approach to HCT in specific IEI.

## 90.2 Diseases

IEI may be broadly categorized into severe combined immunodeficiencies (SCID) and non-SCID. To further subdivide non-SCID, the phenotypic classification as suggested by the International Union of Immunological Societies (IUIS) Inborn Errors of Immunity Committee can be used, which encompasses >500 genetic causes of IEI (Table 90.1).

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**Table 90.1** Phenotypic classification of IEI as suggested by the International Union of Immunological Societies (IUIS) Inborn Errors of Immunity Committee (Bousfiha et al. 2022)

1.	Immunodeficiencies affecting cellular and humoral immunity (CID)
2.	CID with associated or syndromic features
3.	Predominantly antibody deficiencies
4.	Diseases of immune dysregulation
5.	Congenital defects of phagocyte number, function, or both
6.	Defects in intrinsic and innate immunity
7.	Auto-inflammatory disorders
8.	Complement deficiencies
9.	Bone marrow failures
10.	Phenocopies of IEI

Updated guidelines for HCT of IEI together with detailed protocols have been published by the EBMT Inborn Errors Working Party (EBMT IEWP) in 2021 (Lankester et al. 2021) and can be accessed at <https://www.ebmt.org/sites/default/files/2021-07/EBMT%20ESID%20IEWP%20Guidelines%20for%20HCT%20for%20inborn%20errors%20of%20immunity.pdf>

These guidelines include detailed recommendations for the use of six conditioning regimens with varying degrees of intensity depending on type of IEI, patient condition, and age.

### 90.3 SCID

The overall frequency of SCID was for a long time estimated to be 1 in 50,000–100,000 live births. However, in recent years, newborn screening programs making use of the T-cell receptor excision circles (TREC) technology have demonstrated that the frequency may actually be two- or more-fold higher with clear geographical and ethnic differences (Kwan et al. 2014; Rechavi et al. 2017; Speckmann et al. 2023).

The immunological phenotypes of SCID are shown in Table 90.2 representing monogenic inherited defects in T-, B-, and NK-cell differentiation leading to the absence or inactivity of corresponding mature cells.

In the absence of newborn screening programs or positive family history, most patients present within the first 3–6 months with severe, recurrent, or opportunistic, often life-threatening, infections, the most common being *Pneumocystis jiroveci pneumonia*. Other common symptoms include diarrhea, dermatitis, and failure to thrive. Survival in SCID patients depends on expeditious T-cell reconstitution, and in the absence of successful HCT, or autologous stem cell gene therapy, most children usually die during the first year of life. As many as 50% of SCID patients are engrafted with maternal T-cells but in most instances these cells do not initiate GvHD. Transfusion-associated GvHD, on the other hand, is frequently lethal in SCID, and any patient with a possible diagnosis of SCID must receive irradiated blood products. Bacille Calmette–Guérin (BCG) vaccination can

**Table 90.2** Gene defects typically associated with specific SCID phenotypes

T-B + NK–	T-B + NK+	T-B – NK–	T-B – NK+
<i>IL2RG</i> (SCID-X1)	<i>IL7R</i>	<i>ADA</i>	<i>LIG4</i>
<i>JAK3</i>	<i>CD3D</i>	<i>AK2</i> (reticular dysgenesis)	<i>RAG1</i>
	<i>CD3E</i>	<i>RAC2</i>	<i>RAG2</i>
	<i>CD247</i> (CD3ζ)		<i>DCLRE1C</i> (Artemis def.)
	<i>CORO1A</i> <i>ITPKB</i> <i>LAT</i>		<i>NHEJ1</i> (Cernunnos XLF)
	<i>PTPRC</i> ( <i>CD45</i> def.) <i>SLP76</i> Thymic defects* <i>FOXN1</i> <i>PAX1</i> Complete DiGeorge syndrome <i>CHARGE</i> syndrome		<i>PRKDC</i> (DNA-PKcs def.)

Adapted from Bousfiha et al. 2022. \*: these may present with a SCID phenotype or at newborn screening, but HCT may be contraindicated or not the primary treatment choice

give rise to disseminated BCG-osis, and rotavirus vaccine can cause persistent enteritis in SCID patients, and all live vaccines should be avoided if there is any suspicion, a positive NBS result, or a positive family history of severe T cell deficiency. With the ongoing introduction of NBS in Europe, the presentation of SCID patient is about to change to mostly healthy-appearing newborns without signs of active infection. However, not all peri- and early postnatal infections like, i.e., CMV will be avoidable in infants identified by NBS (Speckmann et al. 2023; Thakar et al. 2023).

#### 90.3.1 General Principles in Allo-HCT for SCID

The Stem Cell Transplant for primary Immune Deficiencies in Europe (SCETIDE) registry has collected data on SCID transplants comprising 50 years of HCT experience, and a number of



important publications have documented the outcomes and important risk factors (Fischer et al. 1990; Antoine et al. 2003; Gennery et al. 2010; Lankester et al. 2022). Recently, studies from the North American group have reported similar findings (Pai et al. 2014; Heimall et al. 2017; Thakar et al. 2023). The major factors positively influencing outcome reported in these studies include:

1. Absence of infections at HCT
2. Age at transplant <3.5 months or diagnosis via NBS
3. Use of MFD and MUD
4. Use of myeloablative conditioning
5. Recovery of naïve CD4 T lymphocytes  $>0.5 \times 10^3/\mu\text{L}$  at +1 year after HCT

### 90.3.2 Donor, Graft Source, and Conditioning in HCT for SCID

Overall survival for MD (related or unrelated) HCT in SCID has improved to over 90% (Lankester et al. 2022; Thakar et al. 2023). Virtually all infants have a haploidentical parental donor, and this is an alternative option, as the donor is readily available. HLA disparity necessitates rigorous *in vitro* or *in vivo* TCD in order to reduce to risk of GvHD. Most centers now employ either TCR alpha/beta depletion (Balashov et al. 2015; Shah et al. 2018) or PT-CY (Neven et al. 2019). Although promising survival rates have been reported, longer follow-up in a larger cohort of patients is required to determine the position of these approaches. CD34+ selection of the graft also remains an option in selected cases. CB can be a suitable alternative stem cell source (Fernandes et al. 2012). Theoretical advantages for using CB stem cells include rapid availability, less risk of GvHD compared to adult URD, no medical risk to the donor, and a greater proliferative life span which might be particularly important in such young recipients. There are some specific disadvantages including sometimes slower engraftment, lack of viral-specific cytotoxic T-cells, and lack of availability of the donor for a boost HCT.

Although HLA-genoidentical sibling donor BM may be infused into SCID recipients without any conditioning or GvHD prophylaxis, usually only T-cells of donor origin will engraft, and myeloid and often B-cells will remain of recipient origin. Patients with insufficient myeloid engraftment and/or poor or declining naïve T-cell compartments may experience severe complications that require a second, conditioned HCT (Riller et al. 2023). Therefore, and if tolerable for the patient, conditioning is recommended for all SCID patients in order to achieve optimal clinical and immunological outcomes (Lankester et al. 2022).

Individualized approaches making use of therapeutic drug monitoring provide novel and less toxic options to improve HCT outcome in these vulnerable young infants, while antibody-based conditioning approaches that may limit systemic toxicity are currently being developed.

### 90.3.3 Omenn's Syndrome

Omenn's syndrome (OS) is characterized by SCID typically associated with the triad of erythroderma, hepatosplenomegaly, and lymphadenopathy. There is a marked eosinophilia and a variable number of autologous, activated, and oligoclonal T lymphocytes, which infiltrate target organs and are generally poorly responsive to mitogens. Whereas outcomes in HCT for OS were traditionally inferior compared to classical SCID, results have improved in recent years (Gennery et al. 2010; Heimall et al. 2017; Thakar et al. 2023). The overall mortality in these studies was lower than previously reported and was due to early recognition of OS and rapid initiation of treatment with topical/systemic immune suppression with steroids, cyclosporin A, and T-cell directed serotherapy to control immune dysreactivity before proceeding to HCT.

## 90.4 Non-SCID IEI

**The landscape of HCT in non-SCID IEI has dramatically changed over the last decade**

- New genetic causes of IEI are being described in accelerating frequency thanks to next-

generation sequencing techniques (Bousfiha et al. 2022). Functional consequences of genetic variants in the same gene can be broad, from complete or partial loss to gain of function resulting in different clinical phenotypes. Somatic variants may also occur, mimicking disease caused by germline variants.

- The concept of “pure” immunodeficiencies with predisposition to infections has been abandoned with many newly described auto-immune, auto-inflammatory conditions, or syndromal disorders with immunodeficiency. Many of these diseases can be cured by HCT, while in syndromal disorders only the hematopoietic portion of the disease can be corrected, which may nevertheless be indicated and result in not just increased survival but also quality of life in selected patients.
- HCT outcomes have further improved with around 90% OS and low GVHD rates after MSD or MUD HCT in many non-SCID IEI, like, i.e., CGD and WAS (Chiesa et al. 2020; Albert et al. 2022b).
- In general, donor availability has become less of a factor in the decision-making process to go for HCT. Current outcomes of MFD and MUD are highly similar, while results with mismatched/haplo-identical HCT are gradually approaching those obtained with MD especially when performed in experienced centers (Shah et al. 2018; Neven et al. 2019; Kurzay et al. 2019).
- The importance of DFS and GRFS as compared to OS is increasingly appreciated and addressed in medium- to long-term outcome studies, also in comparison to non-HCT approaches (Speckmann et al. 2017; Barzaghi et al. 2018; Albert et al. 2022b).
- More IEI patients are discovered with very mild or atypical phenotypes of well-known IEI, and these often hypomorphic genetic variants are especially challenging with respect to timely recognition and management (Notarangelo et al. 2016; Schuetz et al. 2023). Especially in these “milder” cases, often recognized in adolescence and adulthood, quality

of life is increasingly a factor in HCT decision making (Cole et al. 2013; Cheminant et al. 2023).

- Adolescents and young adults with IEI are increasingly appreciated as candidates for HCT, and outcomes are encouragingly good (Albert et al. 2018; Fox et al. 2018), with survival determined by the underlying IEI entity, pre HCT comorbidities, and organ damage rather than age at HCT or donor type (Albert et al. 2022a).

The consequence of these developments has been that many more patients with IEI are today considered for, referred for, and counselled about HCT.

#### 90.4.1 Indication

One of the most challenging aspects of transplant for non-SCID IEI is the question of whether a high-intensity, curative therapy with HCT is indicated. For some IEI (like FHLH), the immediate HCT indication is clear, while for many other IEI this will be less evident because of the high interindividual variability of the clinical phenotype. In diseases where long-term prognosis is known to be poor, even presymptomatic patients can be subjected to HCT (like i.e. X-CGD). A genetic diagnosis is increasingly made in IEI patients and may support the decision to proceed to HCT. Similarly, a positive family history with severe disease manifestations or a known poor long-term prognosis of the disease may justify early preemptive HCT. Still, given the highly variable and frequently unpredictable disease course in IEI, a genetic diagnosis alone without clinical manifestations is in many IEI an insufficient reason to perform HCT. In all IEI patients, the decision to proceed with HCT should always include interdisciplinary discussions with patients and their families, also addressing issues like fertility/family-planning, school/work, other psychosocial factors, and quality of life.

### 90.4.2 Conditioning (Table 90.3)

In IEI, it is the goal is to establish sufficient long-term donor chimerism in the affected cell lineage, while reducing short- and long-term toxicity to a minimum. The required degree of donor chimerism for effective and sustainable disease correction varies depending on the type of IEI and has not yet been established for all entities. Accordingly, the graft source, serotherapy, and type of conditioning regimen should be chosen depending on many factors, including IEI entity, age of patient, donor type, active infections, comorbidities, organ damage, and experience of the center.

Conventional MAC preparation with BU-/CY-based regimens has historically been associated with significant treatment-related toxicity and TRM. The IEWP of EBMT had begun in 2005 to publish detailed recommendations for conditioning of IEI as discussed above (Lankester et al. 2021). These recommendations include:

1. Replacement of CY with FLU, as the combination of BU/FLU with pharmacokinetic targeting of BU AUC appears to be better tolerated in these patients.
2. The option to replace BU with a structural analogue, TREO, which is similarly immunosuppressive but causes less hepatic SOS/VOD (Slatter et al. 2018).
3. Addition of a submyeloablative dosing regimen for BU/FLU (Güngör et al. 2014).
4. Establishing RIC to achieve stable engraftment of immunocompetent donor cells with

reduced procedure-related morbidity and mortality (Veys 2010).

5. An alkylator-low regimen for radiosensitive disorders (Slack et al. 2018).

### 90.5 HCT for Radiosensitive IEI

Patients with radiosensitive IEI such as Nijmegen-breakage syndrome, DNA ligase 4 deficiency, Cernunnos deficiency, or DNA-PKcs deficiency including those with a SCID phenotype are increasingly being identified and considered for HCT. As many of the conditioning regimens are particularly damaging to DNA, less toxic regimens are required to successfully treat these patients (Slack et al. 2018). No definitive studies are available, but low-dose FLU/CY regimens like the one suggested by the EBMT IEWP guidelines have been proven effective and safe (Lankester et al. 2021).

### 90.6 Alternative Therapies

Alternative treatments to HCT have been developed for specific IEI over the last three decades.

#### 90.6.1 Enzyme Replacement Therapy (ERT) for Adenosine Deaminase Deficiency (ADA-SCID)

Enzyme replacement in ADA deficiency with PEG-ADA is administered weekly or twice weekly by IM injection and leads to rapid metabolic correction which is followed by cellular and humoral immune reconstitution. The extent of immune recovery is variable, and a significant number (~50%) remain on Ig replacement. Over a longer time period, patients show a decline in T-cell numbers and remain lymphopenic. Long-term follow-up shows that patients may remain clinically well, but clinical problems can arise and a number of cases of EBV-related lymphoma have been reported (Chan et al. 2005). Given the

**Table 90.3** Conditioning regimens recommended by IEWP (Lankester et al. 2021)

Regimen	
A	BU AUC 85–95 mg × h/L, FLU 160 mg/m <sup>2</sup>
B	TREO 30–42 g/m <sup>2</sup> , FLU 150–160 mg/m <sup>2</sup> , TT 8–10 mg/kg
C	BU AUC 60–70 mg × h/L, FLU 160–180 mg/m <sup>2</sup>
D	TREO 30–42 g/m <sup>2</sup> , FLU 150–160 mg/m <sup>2</sup>
E	FLU 150–160 mg/m <sup>2</sup> , MEL 140 mg/m <sup>2</sup>
F	FLU 150 mg/m <sup>2</sup> , CY 20–40 mg/kg

improved outcomes of HCT and GT in recent times, ERT is predominantly considered as a bridge to stem cell-based curative therapy.

### 90.6.2 Gene Therapy for Specific IEI

Autologous stem cell gene therapy (GT) via vector-mediated transfer of healthy copies of an affected gene into autologous CD34<sup>+</sup> cells has progressed from a highly experimental therapy to the first licensed gene therapy for an IEI (ADA-SCID) within the last two decades. One of the major conceptual advantages of GT is the elimination of the inherent risk of GVHD associated with allogeneic HCT procedures.

Clinical trials performed with gamma retroviral vectors for ADA-SCID, X-linked SCID (SCID-X1), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome (WAS) demonstrated that gene therapy can be an effective treatment option in patients lacking an HLA-identical donor (Hacein-Bey-Abina et al. 2002; Boztug et al. 2010; Stein et al. 2010; Aiuti et al. 2009). However, a high rate of insertional mutagenesis was observed in trials for SCID-X1, WAS, and CGD (Ott et al. 2006; Hacein-Bey-Abina et al. 2003; Braun et al. 2014). This has prompted the development of safer vectors based on self-inactivating retroviral or lentiviral vectors. Currently, a number of trials are ongoing or concluded for a number of IEI. All share the concept of submyeloablative conditioning followed by the infusion of autologous stem/progenitor cells transduced with the wildtype gene. Promising results were published, especially for ADA-SCID (Kohn et al. 2021; Cicalese et al. 2016), WAS (Ferrua et al. 2019) and SCID-X1 (Mamcarz et al. 2019). It is expected that gene editing approaches as an alternative for gene addition technologies will be developed for stem cell based GT in the next few years and may potentially also be employed to correct mature cells in diseases like, i.e., CD40 ligand deficiency, CTLA4, and IPEX.

In theory, autologous stem cell gene therapy offers the appealing prospect of avoiding allo-

immune reactions such as GVHD or rejection and a lower conditioning-related toxicity compared to allo-HCT. But its exact role in treatment algorithms still needs to be defined in the absence of comparative studies. Also, logistic, regulatory, and economic hurdles still have to be overcome before its widespread application in the treatment of IEI. Nevertheless, it has widened the therapeutic repertoire for patients with some IEI. The rapid evolution of novel gene correction approaches has the potential to lead to even safer and more effective treatment options.

### 90.6.3 Targeted Therapies

The unravelling of new genetic IEI entities, especially those caused by gain-of-function (GOF) variants and their pathophysiology, has for the first time opened the possibility to treat these diseases with highly specific, often small molecule inhibitors, some of which are already approved for other indications. These include but are not limited to abatacept for CTLA4 haploinsufficiency, ruxolitinib and other JAK-inhibitors for STAT1 GOF, leniolisib for APDS, etanercept for ADA2 deficiency, and IL-1-targeted therapies (anakinra, riloncept, and canakinumab) for auto-inflammatory recurrent fever syndromes (Ochs and Petroni 2018; Ombrello et al. 2019; Rao et al. 2023). We can expect that more substances (like, i.e., IL-18BP for XIAP) may be licensed or repurposed for the treatment of IEI in the future. At this point in time, the exact role of these agents in the treatment algorithm of IEI is unclear. Ideally, they could make HCT unnecessary for some patients. On the other hand, concerns about long-term toxicity, infection risk, and lymphoma risk exist. In any case, in some patients with excessive autoimmunity and/or inflammation, these therapies can be viewed as an ideal bridge to HCT and considered as a remission induction strategy to control the underlying IEI, because they have the potential to bring the patient into the best possible clinical condition for HCT.

### Key Points

- IEI require a tailored approach to HCT management, and disease-specific transplant protocols have been developed for these diseases, including stem cell-based GT.
- Preceding comorbidity, particularly infectious complications at HCT and concurrent end-organ damage, adversely affects outcome. For many diseases, HCT at an early age is recommended.
- Alternative therapies—often as a bridge to transplant—are increasingly available to improve patient outcomes.

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# Inborn Errors of Metabolism and Osteopetrosis

# 91

Robert Wynn and Ansgar Schulz

## 91.1 Inborn Errors of Metabolism

### 91.1.1 Definition and Epidemiology

Inborn errors of metabolism (IEM) comprise a large group of inherited diseases. They are individually rare. HCT is indicated only in a small number of diseases.

It can be curative in certain lysosomal disorders, peroxisomal disorders or certain disorders of mitochondrial function. This review will be limited to the commoner indications reported in HCT registries and which together account for most of transplanted IEM.

### 91.1.2 Diagnosis

Timely diagnosis is imperative in IEM since in all such diseases, HCT is better at preventing disease progression than reversing established disease manifestations. Indeed, HCT might be contraindicated in those patients presenting with more advanced disease.

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This is true for all lysosomal storage diseases and for X-ALD.

### Diagnosis is made in three ways

- Through early clinical recognition of disease manifestations
- Through screening of pre-symptomatic individuals within a known affected kindred
- Population screening for disease, such as in the neonatal period, with new-born screening

In X-ALD, transplant is indicated for the prevention of progression of cerebral inflammation, which manifests with a high interpersonal variability, even within an affected kindred. Progress of inflammation is detected with serial cerebral MRI scans (at 6 or 12 monthly intervals) in boys, that are genetically affected but currently well.

### 91.1.3 Classification (See Table 91.1)

### 91.1.4 Risk Factors

Patient performance score at transplant predicts transplant outcome. Patients with an adverse performance score at transplant also have an inferior long-term survival as the transplant fails in advanced disease to prevent disease progression.

**Table 91.1** Classification of inborn errors of metabolism

IEM	
Hurler syndrome, MPSIH	Hurler syndrome. This is the most severe phenotype of iduronidase deficiency, a lysosomal storage disorder (LSD), which results in the accumulation of glycosaminoglycans, heparan and dermatan sulfate. There is progressive multiorgan dysfunction including psychomotor retardation, severe skeletal disease, life-threatening cardiopulmonary complications, and premature death, HCT prevents early death and attenuates the multisystem disease manifestations as the deficient enzyme is donated by engrafted donor leucocytes to host tissues (“cross-correction”)
X-linked adrenoleukodystrophy (ALD)	In this X-linked disorder, there is accumulation of very long-chain fatty acids in the brain and adrenal glands arising from their defective metabolism by a peroxisomal, membrane protein encoded by the ABCD1 gene Clinical manifestations in genetically affected boys are highly variable, even within a kindred. The principle role of HCT is to prevent progression of early cerebral ALD, an inflammatory demyelinating disease of childhood that is seen in about 40% of genetically affected individuals HCT does <i>not</i> influence other illness such as adrenal insufficiency or the later myeloneuropathy of the spinal cord
Metachromatic leukodystrophy (MLD)	This is an autosomal recessive LSD, and there is accumulation of sulfatides, a myelin component, due to deficiency of the arylsulfatase A enzyme. There is demyelination in the central and peripheral nervous systems, and clinical manifestations are related to residual enzyme activity. In the late infantile disease, the commonest and most severe phenotype, there is progressive neurological dysfunction and early death usually by the age of 4 years HCT is ineffective in preventing progression of early presenting disease, although it may have a greater impact on later, attenuated disease especially when applied early in the course of the illness. HSC GT is recently available for presymptomatic late infantile disease, and corrects disease by delivery of supra-physiological enzyme by leukocytes, derived from gene-modified stem cells

### 91.1.5 Prognostic Index

Not available.

### 91.1.6 First-Line Treatment (Summary)

#### Multimodality therapies are usual in IEM.

- Residual disease manifestations will require management beyond the HCT episode. This will include orthopaedics, ENT, and speech therapy in lysosomal storage disorders (LSDs), as well as family and educational support.
- Pharmacological enzyme replacement therapy (ERT) is used in MPSI but does not correct neurological disease as it does not cross the blood–brain barrier, and alloantibody formation might limit its use in somatic disease. It is used to improve pre-HCT performance, but it has *not* been shown to influence transplant outcomes.

### 91.1.7 Second-Line Treatment (Summary)

See Sect. 91.1.6., above.

### 91.1.8 Autologous HCT

Gene-modified auto-HCT approaches have been shown to improve outcomes in late infantile MLD as the graft delivers more enzyme than possible in a conventional HCT. This treatment has been recently licensed by the EMA for therapy in several European countries.

Similar approaches are under investigation for other lysosomal disorders including MPSIIIA, MPSII, Gaucher (particularly where there is neurological involvement, GD3), and MPSIH. It is likely that this approach will greatly change the landscape of therapeutic approaches in metabolic disease in the coming years.

Similar approaches have been successful in X-ALD. The mechanism of action is different, since there is no supraphysiological production of enzyme. Of course, autologous HCT is also safer. Consecutively, no immune suppression is needed with a lower risk of infections and no risk of GVHD.

### 91.1.9 Allogeneic HCT in MPSIH (Hurler), MLD, and X-ALD (See Table 91.2)

**Table 91.2** Main characteristics of allo-HCT for MPSIH (Hurler), MLD, and X-ALD

<i>Indicated in</i>	
MPSIH (Hurler) is a standard indication for HCT	
In MLD, HCT is usually reserved for later (attenuated) forms of the disease, namely, juvenile and adult forms (note HCT GT is licensed and standard of care in the more severe forms of disease, manifesting earlier)	
In X-ALD, HCT is indicated in early cerebral inflammatory disease. Ordinarily, a genetically affected individual has serial (6 monthly or annual) MRI scans from early childhood, and HCT is carried out when there are early MRI changes of demyelination (the MRI changes are scored as a Loes score)	
<i>Contraindications</i>	
Where MPSIH is diagnosed late, the opportunity for HCT to meaningfully alter the natural history of the disease might be lost. No predetermined and simple rules can be applied. HCT is usually not offered to a child presenting beyond the age of 30 months, but careful multidisciplinary assessment is required. Not transplanting means the child will die of somatic disease manifestations, and the decision not to transplant is not easy to make	
Late infantile MLD is not usually considered for allogeneic HCT. Note that such disease—if diagnosed in a timely fashion—has been shown to be markedly improved using an autologous, ex vivo HSC gene therapy approach	
Advanced cerebral X-ALD is considered a contraindication for HCT. Disease will progress through transplant. The MRI scan-derived Loes score might predict those who will benefit most from HCT	
<i>Donor</i>	
In LSD, non-carrier MFD > MUD > carrier MFD	
In LSD, UCB is frequently preferred to BM, since the post-HCT chimerism is higher in such recipients, and the interval between referral and HCT is likely shortest (primary rejection might be higher using UCB)	
PB is rarely used as a donor cell source	
In X-ALD, MFD > MUD	
Haplo-HCT is rarely indicated in IEM	
<i>Conditioning: standard</i>	
Engraftment is difficult in IEM. Generally reduced intensity conditioning and ex vivo TCD are associated with high rates of graft loss	
MSD/MFD: IV BU (MAC AUC)/FLU (160 mg/m <sup>2</sup> )	
MUD: IV BU (MAC AUC)/FLU (160 mg/m <sup>2</sup> )	
AUTO (HCT GT): IV BU (MAC AUC). No immune suppression is necessary	
<i>Conditioning: reduced toxicity</i>	
Occasionally reduced toxicity conditioning might be employed	
In somatic IEM, such as Wolman or attenuated MPS: TREO (42 g/m <sup>2</sup> )/FLU (160 mg/m <sup>2</sup> )/TT (10 mg/kg)	
<i>Source of SC</i>	
UCB often preferred in LSD (higher chimerism, and better enzyme delivery therefore)	
BM rather than PB in MUD donors	
No ex vivo TCD as this is shown to contribute to graft loss	
<i>GvHD prophylaxis</i>	
MSD/MFD: ATG/Campath, CSA + MMF	
MUD: ATG/Campath, CSA + MMF	
Unleated CB: Proximal ATG (−9 to −6), CSA + MMF or CSA + PRD (note rituximab is often added to the preparative regimen in CB transplant to reduce post-transplant autoimmune cytopenia and to reduce primary graft rejection)	
<i>TRM in MPSIH</i>	<i>OS in MPSIH</i>
MSD: <5%	Engrafted survival of >80% and
MUD: <10%	Overall survival of 90%

MAC AUC doses adjusted to achieve MAC AUC, MSD match sibling donor, MFD match family donor, MUD much unrelated donor, LSD lysosomal storage disorder, EIM inborn errors of metabolism

## 91.2 Osteopetrosis

### 91.2.1 Definition and Epidemiology

Osteopetrosis (OP) is a generic name for a variety of rare monogenetic diseases characterized by sclerosis of the skeleton. At least nine variants are known with different modes of inheritance and severity, which cumulatively have an incidence ~1:100,000. The disease originates from reduced or complete lack of osteoclast function and, as a consequence, impairment of bone resorption.

### 91.2.2 Diagnosis

In addition to the obligate increased bone density of all bones (X-ray), a combination of symptoms can be found in *classical infantile osteopetrosis* after birth. These symptoms include characteristic changes of the head (macrocephalus, frontal bossing, choanal stenosis), vision impairment (due to narrowed foramina), hematological insufficiency (thrombocytopenia, anemia, leukocytosis), hepatosplenomegaly (due to extramedullary hematopoiesis), and hypocalcemia (with secondary hyperparathyroidism). Cave: OP is a genetic and phenotypical heterogenous disease with *atypical presentations* (incomplete and/or delayed onset of symptoms). In these cases, an intensive work-up including spine biopsy and cranial MRI is recommended.

### 91.2.3 Classification

Osteopetrosis	
Infantile “malignant” autosomal recessive OP (ARO)	Clinical symptoms in infancy, death without HCT usually in the first decade of life, biallelic mutations in <i>TCIRG1</i> , <i>CLCN7</i> , <i>SNX10</i> , <i>TNFRSF11A/RANK</i> , and <i>FERMT3/KINDLIN-3</i> ; HCT indicated, if excluded: <ul style="list-style-type: none"> <li>– “Neurodegenerative OP” (all <i>OSTM1</i> and about half of <i>CLCN7</i> cases)</li> <li>– “Extrinsic osteoclast defects” (<i>TNFSF11/RANKL</i> cases)</li> </ul>

Osteopetrosis	
Intermediate osteopetrosis	Clinical symptoms in the first decade, HCT may be indicated in severe forms with hematological insufficiency and (imminent) visual impairment after individual assessment Specific from: CA2 deficiency (renal tubular acidosis with cerebral calcifications): HCT may be considered after individual assessment in cases with hematological insufficiency and (imminent) visual impairment if neurological and renal problems are controlled
Benign osteopetrosis (ADO)	M. Albers Schoenberg (monoallelic <i>CLCN7</i> mutations): HCT usually not indicated; HCT may be considered after individual assessment in clinical severe cases

### 91.2.4 Risk Factors

There is an increased risk of pulmonary hypertension (pre and post HCT) and SOS/VOD (post BMT). The risk of non-engraftment and rejection increases with severity of disease and age.

### 91.2.5 Prognostic Index

Not available.

### 91.2.6 First-Line Treatment (Summary)

Symptomatic, steroids may be beneficial to improve hematological symptoms.

### 91.2.7 Second-Line Treatment (Summary)

Not available.

### 91.2.8 Autologous HCT

Clinical trials for gene-modified auto-HCT for *TCIRG1* defects in preparation.

### 91.2.9 Allogeneic HCT (See Table 91.3)

**Table 91.3** Main characteristics of allo-HCT for osteopetrosis

Indicated in	Infantile osteopetrosis: clinical symptoms and exclusion of neurodegenerative and extrinsic osteoclast defect
Contraindications	Neurodegenerative osteopetrosis: symptoms (non-hypocalcemic convulsions/EEC changes, severe progredient developmental delay) and/or biallelic mutations in <i>OSTM1</i> and <i>CLCN7</i> ; cave: only about half of <i>CLCN7</i> mutations cause neurodegeneration Osteopetrosis not intrinsic to defects in differentiation or function in osteoclasts: <i>TNFSF11/RANKL</i>
Donor	MFD > MUD > haplo (cord blood not recommended)
Conditioning: standard	MSD/MFD: IV BU (MAC AUC)/FLU (160 mg/m <sup>2</sup> ) MUD: IV BU (MAC AUC)/FLU (160 mg/m <sup>2</sup> ) Haplo: IV BU (MAC AUC)/FLU (160 mg/m <sup>2</sup> )
Conditioning: reduced toxicity	MSD/MFD: TREO/FLU (150 mg/m <sup>2</sup> )/TT (8–10 mg/kg) MUD: TREO/FLU (150 mg/m <sup>2</sup> )/TT (8–10 mg/kg)
Conditioning: post cy protocol	In patients with MMUD or haplo donors, an adapted PT-CY protocol should be used (see ESID guidelines. Lankester et al. 2021)
Source of SC	Matched donors, PT-CY protocol: T replete BM > PB
GvHD prophylaxis	MSD/MFD: CSA + MMF (consider ATG or Campath in MFD) MUD: CSA + MMF + ATG (or Campath) Haplo—T replete: Campath, PT-CY, TAC (or CSA) + MMF
TRM	MSD/MUD: 10–20% Haplo: ~20–30%
OS	MSD: ~90% MUD: ~80% Haplo/MMUD: ~70%

MAC AUC doses adjusted to achieve MAC AUC, MSD match sibling donor, MFD match family donor, MUD much unrelated donor, MMUD mismatch unrelated donor

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# Autoimmune Disease

# 92

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## 92.1 Introduction

Autoimmune diseases (ADs) are a heterogeneous group of diseases affecting 8–10% of the Western population, which constitute a heavy burden to society and are often debilitating and disabling for affected individuals. ADs are defined as an impairment of the immune system resulting in the loss of immune tolerance against self-tissues, by the existence of autoreactive T and B cells and by a complex mechanism of multifactorial aetiol-

ogy, across genetics and environmental factors (Alexander and Greco 2022). Autoimmunity is also linked to autoinflammation, having common features as the activation against self, with subsequent systemic inflammation (Chap. 93).

Current therapeutic strategies for AD are based on systemic immunosuppression (IS) or targeted biologic disease-modifying therapies (DMTs), which ameliorate symptoms and halt progression in the vast majority of patients but usually require continuous administration and

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may be associated with long-term side effects and substantial costs. Although biologic DMT has improved specificity and efficacy in the treatment landscape, cure remains elusive, and many patients still suffer from progressive disability with shortened life expectancy and comorbidity.

Initially supported by preclinical animal models and ‘serendipitous’ case reports, auto-HCT has grown as a promising and feasible treatment option for severe treatment-resistant patients, especially in diseases for which effective therapies are lacking. The annual number of patients treated with autologous HCT is constantly increasing (Alexander and Greco 2022), and AD are among the fastest growing disease category reported to the EBMT for autologous HCT (Passweg et al. 2021). Allo-HCT has also been undertaken, although caution related to its intrinsic risks has precluded widespread application.

The EBMT Autoimmune Diseases Working Party (ADWP) was established in 1997 by Alois Gratwohl, Alberto Marmont, Alan Tyndall and Athanasios Fassas, who developed the registry covering AD indications and produced early guidelines based on consensus opinion (Tyndall and Gratwohl 1997).

Subsequently, the ADWP built productively on these initial achievements, generating studies from the growing registry and developing relationships with other specialist societies, culminating with successful publication of three randomised controlled trials (van Laar et al. 2014; Hawkey et al. 2015; Mancardi et al. 2015), along with updated guidelines for clinical practice (Snowden et al. 2012), immune monitoring and biobanking (Alexander et al. 2015; Cencioni et al. 2022).

With increasing evidence, the guidelines have become more disease specific. A successful inter-

national collaboration involving also non-European experts resulted in the EBMT guidelines for auto-HCT in systemic sclerosis (Farge et al. 2017b). A further collaborative review with the European Crohn’s and Colitis Organisation (ECCO) has included recommendations for patient selection, transplant technique and follow-up of HCT in patients with Crohn’s disease (Snowden et al. 2018). EBMT guidelines for MS and other neurological diseases were published in 2020 (Sharrack et al. 2020). Guidelines for best practices were also provided during the COVID-19 pandemic (Greco et al. 2021, 2023).

The current state of the EBMT database in relation to various ADs is summarised in Tables 92.1 and 92.2. At the time of writing, over 4000 patients receiving HCT for an AD have been reported to the EBMT, the largest international database, with activity reported to other registries adding substantially to the worldwide numbers.

Based on this reported activity, this chapter will cover the main neurological, rheumatological and gastroenterological indications for auto-HCT, along with reference to the rare AD indications and allogeneic HCT. More detailed literature can be sourced from recently published reviews (Achini-Gutzwiller et al. 2022; Alexander and Greco 2022; Snowden et al. 2017).

**Table 92.1** Overview of data reported to the EBMT database (March 2023)

Patients	4055
Transplant procedures	4140
Centres/countries	323/44
Autografts/allografts	3854 (93%)/286 (7%)
Median age at autografts/allografts (years)	38 (3–76), 11 (<1–64)
Male/female	40/60%

**Table 92.2** Distribution of diagnosis in the EBMT database (March 2023)

<i>Multiple sclerosis (MS)</i>	2132	<i>Haematological diseases</i>	161
<i>Connective tissue diseases</i>	1028	Immune thrombocytopenia purpura (ITP)	42
Systemic sclerosis	835	Haemolytic anaemia	36
Systemic lupus erythemathosus	126	Evans syndrome	24
Polymyalgia/dermatomyositis	18	Other	60
Sjögren's syndrome	6	<i>Vasculitis</i>	67
Antiphospholipid syndrome	6	Granulomatosis with polyangiitis (GPA)	12
Other	37	Behcet's disease	18
<i>Arthritis</i>	209	Takayasu's arteritis	3
Rheumatoid arthritis	82	<i>Polyarteritis nodosa (PAN)</i>	4
Juvenile chronic arthritis (JIA):		<i>Eosinophilic granulomatosis with polyangiitis (EGPA) (EGPA)</i>	2
• Systemic JIA	74	Other	28
• Other JIA	19	<i>Other neurological diseases</i>	146
• Articular JIA	22	Chronic inflammatory demyelinating polyneuropathy (CIDP)	65
Psoriatic arthritis	3	Neuromyelitis optica	29
Other	9	Myasthenia gravis	11
<i>Inflammatory bowel diseases</i>	283	Other	41
Crohn's disease	233	<i>Insulin dependent diabetes mellitus (IDDM)</i>	20
Coeliac disease	18	<i>Other</i>	94
Other	32		

## 92.2 Mechanisms of Action

HCT represented an opportunity to gain insights into the aetiology and pathogenesis of AD, characterised by aberrant activation of the immune system with failure of the immune regulation to maintain adapted tolerance. HCT exerts its therapeutic effect through various mechanisms, including the immunosuppressive conditioning regimen able to eradicate the autoreactive immunologic memory, and the regeneration and renewal of the immune system, leading to the re-induction of immune tolerance to rewire aberrant immune response toward self-antigens (Cencioni et al. 2022; Lima-Junior et al. 2021). The effect of HCT on immunologic renewal has been the focus of many mechanistic studies. They collectively demonstrated that many of the age- or autoimmune-mediated immune disturbances may improve or even resolve. For example, immune reconstitution studies indicated a predominant repopulation of CD27-IgD+ naïve B cells (Alexander et al. 2009; Arruda et al. 2018), a stable output of recent thymic emigrants (RTE) (Farge et al. 2017a; Muraro et al. 2005), leading to a re-diversification of the receptor repertoire,

and recurrence of both regulatory B and T cells in various disease settings (Alexander et al. 2013; Lima-Junior et al. 2021). Allo-HCT has been less well explored, although there is an element of immune replacement and evidence for a 'graft-versus-autoimmune effect' (Greco et al. 2019). Further, areas for exploration include the impact of the microbiome on autoimmunity and the impact of HCT (Alexander et al. 2021).

## 92.3 HCT for Multiple Sclerosis (MS)

Since the first case report of using auto-HCT as a treatment for MS was published in 1995 (Fassas et al. 1997), the EBMT registry has now accumulated over 2000 patients (Table 92.2). This treatment was initially used in patients with advanced and progressive disease as a rescue therapy with limited efficacy. More recently, its use in patients with active relapsing MS has been associated with prolonged clinical and MRI responses and, in some cases, significant improvement in disability to a degree rarely seen with disease-modifying therapies (DMT) (Muraro et al. 2017b;

Tolf et al. 2019). The bulk of the data has been provided by observational cohort studies in which patients failing to respond to standard DMT were treated with an auto-HCT. Burman et al. identified the four most rigorously conducted cohort studies in which a total of 188 relapsing MS patients received auto-HCT (Burman et al. 2018). In these studies, PFS was observed in 70–91% of patients at 5 years (where progression is defined as a deterioration of at least 0.5–1 points from baseline in the Expanded Disability Status Scale (EDSS). Furthermore, no evidence of disease activity (NEDA), defined as the absence of clinical relapses, disability progression and MRI disease activity, was observed in 70–92% of patients at 2 years post-transplantation (Boffa et al. 2021; Muraro et al. 2017a). Such results were compared with DMT efficacy data reported in registration studies (Muraro et al. 2017a). Higher rates of NEDA were achieved with auto-HCT than with any other DMT, including those that are considered to have high efficacy, although these studies included different patient populations. Only two randomised trials of auto-HCT have been reported in the literature. The first was a phase II trial which showed auto-HCT to be more effective than mitoxantrone on MRI disease activity (Mancardi et al. 2015). The second was a phase III randomised controlled trial which showed that auto-HCT resulted in prolonged time to disability progression compared to DMTs. Improvement in the EDSS score (3.4–2.4 vs 3.31–3.98), reduction in relapse rate (1 vs 39) and reduced disability progression (6% vs 60%) were seen in the aHCT group vs controls, respectively. There were no significant side effects and no treatment related mortality in the aHCT arm (Burt et al. 2019). Three phase III randomised controlled trials, aimed to compare auto-HCT to DMTs are currently recruiting (BEAT-MS: Identifier: NCT04047628, RAM-MS Identifier: NCT03477500, STAR-MS: EudraCT Identifier: 2019-001549-42) and others are expected to be started (Sharrack et al. 2022). The conditioning regimens used in MS vary between treatment centres. The balance of efficacy and acceptable safety profile of ‘intermediate intensity’ regi-

mens, i.e. either the specific ‘BEAM + ATG’ regimen or the more generic regimen of CY 200 mg/kg combined with ATG, led to their recommended use in the current EBMT ADWP guidelines (Sharrack et al. 2020). Single-centre data suggest that ‘high-intensity’ regimens, incorporating Busulfan and ex-vivo T-cell depletion, have higher rates of PFS but potentially greater toxicity, including TRM (Atkins et al. 2016). Retrospective registry data suggest that graft purging has no added benefit to the transplant outcome (Sharrack et al. 2020).

Currently, auto-HCT is recommended as a standard of care for patients with active relapsing MS failing DMTs and as a treatment option for patients with progressive MS with evidence of disease activity or patients with aggressive MS as a first-line treatment. The best results are seen in patients who are not older than 45 years, have had the illness for less than 10 years, are not very disabled (EDSS  $\leq 6$ ) and have active disease with evidence of enhancement on their MRI. The ADWP has recently provided guidelines for the management of HCT in MS and other immune-mediated neurological ADs (Sharrack et al. 2020), and current clinical trials are summarised in a recent review (Sharrack et al. 2022). Updated interspecialty guidelines have been finalised following a joint EBMT-ECTRIMS workshop (Muraro et al. unpublished).

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## 92.4 HCT for Systemic Sclerosis (SSc)

Over the past 25 years, results from large European prospective observational studies and analysis of the European Scleroderma Trials and Research Group (EUSTAR) registry data base (Elhai et al. 2017) confirmed that SSc patients benefit only marginally from standard immunosuppressive drugs, including CY, with a progressive increase in SSc-specific mortality, predominantly related to cardiac (31%) and pulmonary causes (18%). Since three successive randomised trials, namely, ASSIST [American Scleroderma Stem cell versus Immune Suppression Trial, (Burt et al. 2011)], ASTIS

[Autologous Stem cell Transplantation International Scleroderma trial, (van Laar et al. 2014)] and SCOT [Scleroderma: Cyclophosphamide Or Transplantation, (Sullivan et al. 2018)], have now demonstrated that auto-HCT is superior to CY for early rapidly progressive SSc in terms of long-term survival as well as improvement of lung function and skin fibrosis, AHCT for early severe dcSSc patients is now recommended with a grade I level of evidence by the EBMT (Snowden et al. 2022), and by the American Rheumatism Association (ACR) and The European League Against Rheumatism (Kowal-Bielecka et al. 2017) and the American Society for Transplantation and Cellular Therapy (Majhail et al. 2015), with increased transplant activity reported to the EBMT registry and worldwide over the last decade.

Of note, ASSIST and ASTIS utilised a non-myeloablative regimen of cyclophosphamide (200 mg/kg total dose) and rabbit antithymocyte globulin (rATG), and the main difference between the two studies was that ASSIST mobilised stem cells with 2 g/m<sup>2</sup> CY and infused unmanipulated peripheral blood stem cells (PBSC), while ASTIS mobilised stem cells with 4 g/m<sup>2</sup> CY and infused CD34+ selected PBSC. The SCOT trial used no CY with only G-CSF for mobilisation and a lower dose of CY (120 mg/kg total dose) for conditioning plus TBI (800 cGy/4 fractions over 2 days/200 cGy to lungs and kidneys with shielding), which induces myeloablation.

In addition, patient selection was shown to directly affect transplant outcomes (Henes et al. 2021) with specific concern for cardiac involvement, undetected by echocardiography alone, becoming clinically overt during the transplant procedure, under the stress of fluid overload, CY and ATG administration and sepsis. Current guidelines recommend auto-HCT for SSc patients, according to the 2013 ACR/EULAR classification criteria (van den Hoogen et al. 2013), aged 18–65 years (certain paediatric indications may be considered on a case-by-case basis), with disease duration less than 2 years and a clinical phenotype of diffuse SSc, a modified Rodnan score (mRSS) greater than 20, a sedimentation rate >25 mm or an elevated C-reactive

protein >5 mg/L or a haemoglobin level less than 11 g/dL not explained by causes other than the evolvability of SSc; or less than 5 years SSc with a) a modified Rodnan skin score  $\geq 15$  plus severe organ involvement in respiratory, cardiovascular or renal systems or b) a mRSS <14 (limited skin involvement), in case of coexisting severe progressive lung involvement (alteration of FVC and/or TLC  $\geq 10\%$  and/or DLCO  $\geq 15\%$  compared to an initial value obtained 12  $\pm$  6 months previously). Treatment should be performed in JACIE-accredited centres where combined expertise from SSc disease specialists and dedicated transplant teams can assess and follow patients before, during and after the procedure according to Good Clinical Practices (Snowden et al. 2017). Since toxicity and efficacy arise from individual patient selection and the conditioning regimen, different chemotherapies may account for subtle differences in results, and further studies are warranted to analyse the use of attenuated conditioning regimens according to cardiac function and risk for renal crises (Burt et al. 2021).

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## 92.5 HCT for Systemic Lupus Erythematosus (SLE)

Despite the era of DMTs, a considerable number of patients with SLE do not achieve clinical remission, with an accumulating risk of organ failure and increased mortality. For these patients, autologous HCT has been applied since 1996 with more than 300 cases reported worldwide (Alexander and Greco 2022). The two largest experiences on auto-HCT for SLE come from the EBMT data registry ( $n = 53$ ; mean follow-up, 25 months) and from a single-centre trial performed at the Northwestern University ( $n = 50$ ; mean follow-up, 29 months), both demonstrating a probability of 5-year DFS of 50% despite discontinuation of chronic immunosuppression. Subsequently, a follow-up study from the EBMT registry reported the outcome of auto-HCT in SLE with various regimens between 2001 and 2008 ( $n = 28$ ; median follow-up, 38 months; range, 1–110 months) (Alchi et al. 2013).



Although PFS in this study was only 29% at 5 years, TRM had gradually improved. In addition, this study indicated that CD34-selection was associated with a significantly reduced risk for relapse. More recently, Richard Burt reported a remission rate of 92% at 1 year, 81% at 2 years, 71% at 3 years, and 62% at 4 and 5 years post-HCT, respectively, for 26 patients receiving a CYC/ATG/RTX conditioning (Burt et al. 2018). Long-term outcomes (Burt et al. 2021) are available from two independent Chinese reports, both with 10-year follow-up, demonstrating remarkable clinical responses with PFS of 86% and 68%, respectively, while TRM across both studies was only 2%.

Summarising the results from the most relevant 15 studies in the field covering 339 patients with a median follow-up of 5 years, the median remission rate achieved was 65% with a median mortality of 8%, with a gradual decline of TRM from 12% in the first EBMT registry survey in 2004 to <5% in most recent reports between 2017 and 2019 (Burt et al. 2021). Overall, responding patients are usually free of clinical symptoms and may regain seronegativity for antinuclear antibodies, which is rarely seen under conventional therapies. Early use of HCT also has the potential to protect against organ failure and toxicity-related morbidity and improve quality of life (Burt et al. 2018). The relapse rate is higher in patients receiving unmanipulated stem cell grafts and conditioning regimens without serotherapy (Alchi et al. 2013). Current evidence and expert consensus suggest HCT in SLE as ‘clinical option’ in patients with active disease (BILAG category A), state despite chronic immunosuppression with or without B-cell-targeted therapies (Illei et al. 2011; Snowden et al. 2012, 2022).

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## 92.6 HCT for Crohn’s Disease (CD)

Despite the major recent progress in the treatment of CD, based around corticosteroids, IS (thiopurines, MTX) and DMTs (Ab targeting TNF $\alpha$ ,  $\alpha$ 4 $\beta$ 7 integrin or IL-12/IL-23), some patients fail all available therapies. In many

cases, surgery may be an option but may lead to short bowel syndrome or to a permanent stoma, which may be unacceptable to patients. With this background, in the past few years, auto-HCT has emerged as a promising therapy in a subset of patients in whom the disease is refractory to all available therapies, with progressive tissue damage and potentially reduced life expectancy.

Auto-HCT has been investigated in several studies with encouraging responses, some prolonged, although a progressive incidence of relapse with long-term follow-up is recognised (Burt et al. 2010; Lopez-Garcia et al. 2017). Furthermore, patients regain response to anti-TNF therapy, although they had been refractory to this drug class prior to HCT.

In Europe, the EBMT-sponsored ASTIC trial in patients with refractory CD produced apparently negative results as few patients after auto-HCT met the stringent primary composite endpoint of clinical remission for 3 months (Hawkey et al. 2015). However, it should not be assumed that this single trial provides the definitive answer to the benefit of auto-HCT in CD, as the number of patients included was limited and encouraging long-term follow-up of ASTIC trial patients has since been reported (Lindsay et al. 2017). Encouraging results have also been reported by an EBMT retrospective analysis of 82 treatment-resistant patients who were not in the ASTIC trial. In this difficult-to-treat group of patients, around a quarter maintained remission without further medical therapy, and long-term disease control was maintained with the reintroduction of salvage therapies in the majority of patients who relapsed. TRM occurred in one patient (Brierley et al. 2018).

The EBMT ADWP and ECCO (European Crohn’s and Colitis Organisation) have published a joint position paper of auto-HCT in CD, which recommends auto-HCT ideally in the context of a multicentre clinical trial but on an individual basis may currently be considered for patients with active CD refractory to IS and biological treatments or unacceptable risks of surgical management (Snowden et al. 2018). These recommendations include conditioning regimens using

CY/ATG, which has been used in the majority of HCT for CD with an acceptable and predictable balance between safety and efficacy in these challenging CD cases. A cautionary note should be taken from a recent randomised controlled trial using FLU, CY and ATG-based conditioning regimens, which was stopped early due to unexpected severe toxicity, including thrombotic microangiopathy (Lindsay 2021).

The next steps in this field focus on minimising the risk of infectious complications in this setting. In this sense, CY-free mobilisation seems to be safe and effective (Giordano et al. 2022) and other modifications will be key to further development and deliverability of autologous HCT in patients with severe CD refractory to modern DMTs.

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## 92.7 Allogeneic HCT for Autoimmune Diseases

Autoimmune diseases have also been treated with allo-HCT from MRD, URD and CB sources. In the last 25 years, the EBMT registry has collated 286 cases. Because of the higher procedural risks and potential long-term impact on quality of life from late effects, allo-HCT has been largely restricted to life-threatening AD in paediatric practice with the most common indications in immune cytopenia followed by arthritis. In a recent summary of cases from the registry (Greco et al. 2019), 128 patients undergoing allo-HCT were treated between 1997 and 2014. The median age was 12.7 years (range 0.2–62.2). The incidence of grades II–IV aGvHD was 20.8% at 100 days. The cumulative incidence of cGvHD was 27.8% at 5 years. Non-relapse mortality (NRM) was 12.7% at 100 days. OS and PFS were 70.2 and 59.4% at 5 years, respectively. By multivariate analysis, age <18 years, males and more recent year of transplant were found to be significantly associated with improved PFS. Reduced conditioning intensity was associated with a lower NRM. In a subgroup of 64 patients with detailed information, a complete clinical response was obtained in 67% of patients at 1 year.

Moreover, allo-HCT may represent a potential treatment option for the novel entity called VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) (Bruno et al. 2023).

In summary, experience in allo-HCT is limited, but long-term data are supportive of basic biological differences in the responses of AD to allo- and auto-HCT in terms of curability. However, due to related NRM risks, the application of allo-HCT remained limited and developmental in AD. With time, allo-HCT in AD has become safer and activity continues, particularly in the paediatric field. Moreover, improved patient selection, reduced toxicity conditioning regimens and new strategies for GvHD prophylaxis (i.e. PT-CY) are currently under investigation, generating interest in extending the application to non-malignant disorders. In addition, there has been increasing recognition that the nature of the genetic component of some AD means that allo-HCT is the only realistic approach for long-term disease control. Thus, there is renewed interest in this area, particularly where there is overlap with autoinflammatory and immunodeficiency diseases, particularly in the paediatric setting as summarised in a recent EBMT review (Achini-Gutzwiller et al. 2022).

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## 92.8 Other Indications

A variety of other ADs have been treated (Table 92.2). Haematological immune cytopenias have been treated with a mixture of auto-HCT and allo-HCT. Type 1 diabetes in early ‘honeymoon’ phase has been the subject of clinical trials, with some ability to prevent or reduce insulin requirements. Otherwise, there is a mixture of rarer neurological, rheumatological and gastroenterological indications for which the registry is essential for developing an evidence base. Cautious recommendations for these rare indications are provided in the EBMT ADWP guidelines (Snowden et al. 2012; Greco et al. 2021) and recently updated EBMT recommendations on haematopoietic cellular therapy indications (Snowden et al. 2022).

## 92.9 Conclusions and Future Directions

With accumulating evidence and improved outcomes, along with the recognition that modern biological and other therapies are not universally effective, ADs have become the fastest-growing indication for HCT (Alexander and Greco 2022; Passweg et al. 2021). Initially applied as salvage therapy in patients with poor prognosis, HCT has emerged as a promising treatment option for AD patients earlier in the treatment algorithm. This is the result of successful large phase II and randomised controlled phase III trials and updated guidelines for patient selection and transplant techniques in SSc, MS and CD, respectively (Farge et al. 2017b; Sharrack et al. 2020; Snowden et al. 2012).

In 2023, the major indications for HCT in AD are MS, SSc and CD (Alexander and Greco 2022) for which significant subsets of patients still show an unsatisfactory response to both conventional and targeted biologic therapies.

Moving forward, further efforts are needed to drive HCT into routine clinical care. It is recommended that patients should be treated in experienced and JACIE-accredited transplant centres in a multidisciplinary setting. A future goal is to optimise the conditioning regimens according to disease-specific requirements and to outbalance the intensity to maintain outcomes while minimising toxicity and TRM risk. Moreover, positive preliminary experiences have been recently reported with chimeric antigen receptors (CAR) T cells, paving the way for the use of innovative cell therapy approaches in AD field (Chap. 93).

In addition, comprehensive data reporting, harmonisation and exploitation of existing biobanking infrastructure (Alexander et al. 2015), education at individual centre and network level, including ADWP educational activities (Burt et al. 2021) and health economic evaluations along with evidence-based recommendations will establish the future place of HCT in the treatment algorithms for various autoimmune and inflammatory diseases.

### Key Points

- With accumulating evidence, including randomised controlled trials, AD has become one of the fastest-growing indications for autologous HCT.
- Major indications for HCT in AD include multiple sclerosis (MS) and systemic sclerosis (SSc), where auto-HCT is now featuring in treatment algorithms.
- Although HCT for ADs is predominantly autologous, there is renewed interest in allo-HCT, particularly in ADs with autoinflammatory and immunodeficiency components.
- It is recommended that all patients should be treated in experienced and JACIE-accredited transplant centres with close multidisciplinary collaboration and reporting of data to the EBMT registry.
- Immune reconstitution studies are providing insights into the mechanisms of immune reset following HCT and disease processes underlying various AD.

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# CART Cells and Other Cell Therapies (ie MSC, Tregs) in Autoimmune Diseases

# 93

Raffaella Greco and Dominique Farge

## 93.1 Introduction

Auto-immune diseases (AD) are heterogeneous conditions, characterized by polyclonal activation of the immune system with a defect of B or T lymphocyte selection and altered lymphocytic reactions to auto-antigens components (Burnet 1959a, b), although it is rare to identify a single antigenic epitope. The native immune system and its tissue environment play an important role to determine if exposure to a given antigen will induce an immune response or tolerance or anergy. The role of the genes coding for the major histocompatibility system molecules, but also of many other genes, is important in the regulation of the immune response, although this does not explain all the observed phenomena during loss of tolerance (Matzinger 1994; Rioux and Abbas 2005).

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Over the years, a better understanding of the genetic background of ADs led to revise the traditional pathogenesis of autoimmune diseases. The presence of self-directed tissue inflammation as a component of each type of AD occurs independently of T or B cells abnormalities. Most of the classical AD are polygenic diseases which result from a combination of auto-inflammatory and autoimmune mechanisms (Rioux and Abbas 2005; McGonagle and McDermott 2006) with a predominant autoimmune component background [systemic lupus erythematosus (SLE), Type 1 diabetes (T1D), autoimmune thyroiditis], whereas other polygenic AD have a predominant auto-inflammatory component [Crohn's disease (CD) for instance]. More exceptionally, ADs are set by mutations associated with monogenic autoimmune diseases [Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) with *FOXP3* mutation] or on the other hand of the spectrum appear as monogenic auto-inflammatory diseases [Familial Mediterranean Fever with *MEFV*/pyrin, or TNF receptor-associated periodic fever syndrome (TRAPS) with *TNFRSF1A/TNFR1*]. Autoimmunity is used to cause by impairment of adaptive immunity, whereas autoinflammatory was originally defined as a consequence of unregulated innate immunity. Despite the contrast in the system primarily tangled, they share a lot of similarities, both in pathogenesis and clinical presentations. Therefore, the optimal treatment of an AD should be discussed considering

this specific pathological continuum between autoimmunity and auto-inflammation, which variably interacts in the ultimate phenotypic expression (Alexander and Greco 2022).

In this context, restoration of the immune tolerance with consequent resolution of the inflammatory response against self-antigens is one of the treatment goals to provide durable remissions.

Different types of cellular therapies, which include mesenchymal stem cells (MSC), regulatory T cells (Tregs) and chimeric antigen receptors (CAR) T cell therapies, have been developed to restore immunologic self-tolerance or foster tissue regeneration in AD patients.

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## 93.2 Mesenchymal Stem Cells (MSC)

MSC-based therapies (Burt et al. 2021) theoretically appear as ideal tools to target the respective auto-inflammatory and autoimmune components of AD.

MSC were first identified in the bone marrow (MSC- bone marrow [BM])-stem cell niche 45 years ago (Friedenstein 1976) and since extensively characterized as main support of haematopoiesis. These multipotent progenitor cells can be harvested and cultured *in vitro* from many other sources, mostly from adipose tissue-MSC(AT)-, umbilical cord (MSC- umbilical cord [UC]) or Wharton's jelly MSC (WJ) for therapeutic applications. *In vitro* expanded MSC were defined a minima in 2006 by the International Society of Cellular Therapy (ISCT) MSC committee as: (1) a plastic-adherent polyclonal population with fibroblast-like morphology, (2) positive for CD73, CD90 and CD105 markers (in >95% MSC), (3) negative for haematopoietic and endothelial markers and (4) able to differentiate *in vitro* into osteoblasts, adipocytes and chondroblasts (Dominici et al. 2006). MSC have *in vitro* and *in vivo* immune-modulatory and immunosuppressive effects on both the innate and adaptive immunity and also pro-angiogenic and

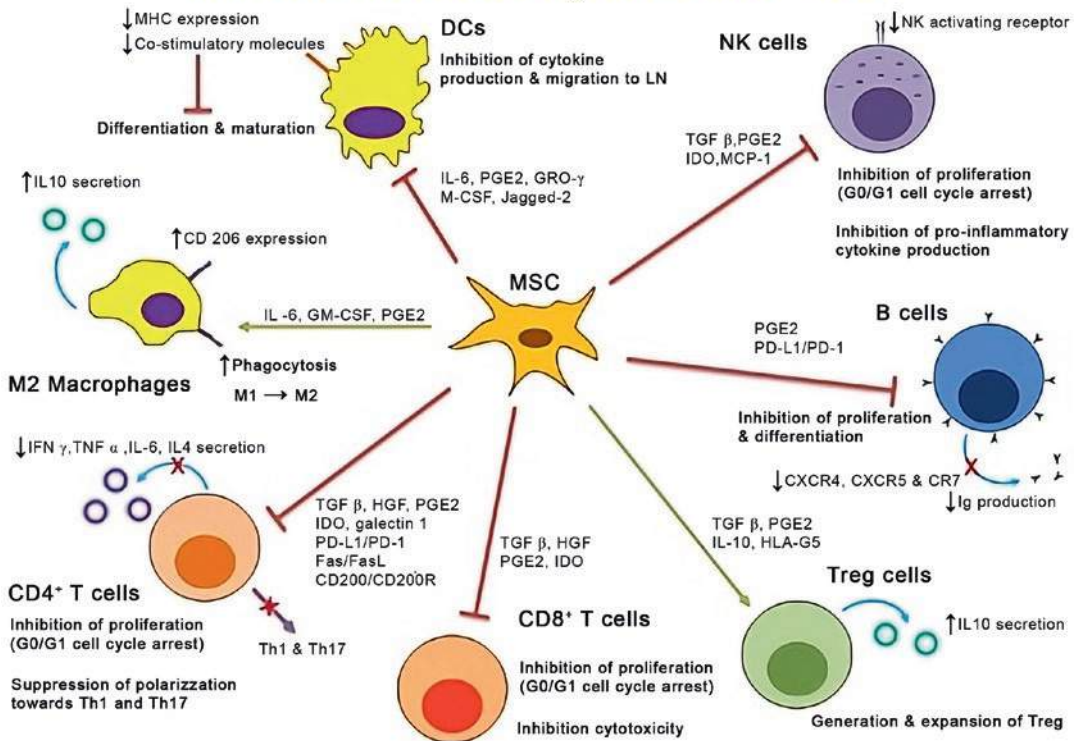
anti-fibrotic properties, all supporting their therapeutic use in several AD.

While MSC from various tissue sources share many biological features, they differ in terms of proliferation potential, multilineage capacities, overall transcriptional profile and functionality. Importantly, numerous other parameters modulate MSC functional properties, including autologous or allogeneic donor sources, each step of production processes (culture conditions, priming, scale of expansion, cryopreservation) and the recipients characteristics and inflammatory environment, which account for high heterogeneity amongst the various MSC products used for clinical application (Barrett et al. 2019; Fernandez-Santos et al. 2022; Menard and Tarte 2013).

MSC have long been considered as immunoprivileged, due to low level of MHC class I molecules and lack of MHC class II and several co-stimulatory molecules expression at basal state on their cell surface. However, MSC exposure to inflammatory environment increases the MHC class I and induces MHC class II molecules expression. The use of unmatched allogeneic MSC infusion may induce anti-HLA class I antibodies, with potential clinical consequences which are still under study (Farge et al. 2021, 2022; Menard and Tarte 2013).

MSCs interact with the humoral and the cell-mediated immune responses (Menard and Tarte 2013), including B-, T-, NK, and dendritic-cell inhibition, decrease in pro-inflammatory cytokine production, and blocking neutrophil recruitment (Fig. 93.1). They may act by cell to cell contact, but their primary mechanism of action is paracrine, through the secretion of enzymes (Han et al. 2022), with a central role for IDO/iNOS, and various growth factors, cytokines, hormones (e.g., VEGF, PDGF, ANG-1, IL-11, PGE2, TSG-6, SDF-1, HGF, IGF-1), which are not constitutively expressed by the MSCs, but induced by the inflammatory stimuli (e.g. IFN- $\gamma$   $\pm$  TNF- $\alpha$ , IL-1 $\alpha$  or IL-1 $\beta$ ) (MSC priming *in vitro* or at site of local tissue), and all contribute to tissue regeneration. Other MSC mechanism of actions, demonstrated on *in vivo* models, include: (a) mitochondrial transfer from MSC to T cells that was shown to

## Immunomodulatory Effects of MSC



**Fig. 93.1** Immunomodulatory effects of mesenchymal stem cells (MSC) in autoimmune diseases. Proinflammatory microenvironment in autoimmune disease patients shift MSC towards an immunomodulatory state, able to target multiple cells from the innate (inhibition of dendritic [DC] and natural killer [NK] cells, and polarization of M1–M2 macrophages) and acquired (inhi-

bition of B, T-CD4+ and T-CD8+ cells, and activation of regulatory T [Treg] cells) immune responses, notably through the production of metabolic enzymes (e.g.: Indoleamine 2,3 dioxygenase [IDO]) and cytokines (e.g.: transforming growth factor [TGF]- $\beta$ , interleukins [IL]-6 and IL-10, prostaglandin E2 [PGE-2])

trigger transcriptomic, metabolic, and functional reprogramming and favour Treg differentiation and (b) efferocytosis, the process by which apoptotic cells are engulfed, leading macrophages towards IDO expression and MSC towards PGE2 and IL-10 upregulation, which also accounts for their immunosuppressive activity (Galipeau and Sensebe 2018; Galleu et al. 2017; Pang et al. 2021; Zhang et al. 2019). MSC also produce extracellular vesicles (EVs), namely exosomes, microvesicles and apoptotic bodies, which are small membrane vesicles (44–100 nm diameter), with evident immunosuppressive and immunomodulatory activity in vitro and in ani-

mal models, for which clinical applications are currently under study in several AD.

The importance to assess MSC functional properties has been underlined since 2016 (Galipeau et al. 2016). The use of standardized functional markers of MSC potency and release potency assays, which must be defined to conduct advanced clinical studies, is of utmost importance and required to seek potential registration of the product. The preferred matrix assays to evaluate MSC immunosuppressive and immune-modulatory capacities include the use of: (a) quantitative RNA analysis of selected gene product, (b) flow cytometry analysis of function-

ally relevant surface markers, and (c) protein-based assay of secretome. In addition, multiparametric immune-monitoring tools have to be set up to characterize the patient immunological status and the various immune cell subsets before and after MSC treatment, to identify responders and thereby optimize clinical trials design.

### 93.2.1 MSC Clinical Applications for AD

MSCs produced from various sources [MSC(BM), MSC(UC)- and MSC(AT)] have been studied in around 1700 clinical trials worldwide for many conditions and their safety repeatedly demonstrated and summarized in a 2020 meta-analysis of 55 clinical trials in 12 countries using MSCs in 2700 recipients with various diseases (Thompson et al. 2020).

Initially tested in the pre-clinical model of multiple sclerosis (MS), the rationale for using MSCs in AD was subsequently obtained in many preclinical models and provide effective experimental support for their clinical application in patients with rheumatoid arthritis (RA), SLE and CD, which are characterized by a predominant auto-inflammatory component, but also in other AD, such as Sjogren disease, systemic sclerosis (SSc), type I and II diabetes or autism.

Numerous phase I–II trials and few completed or still ongoing phase III trials worldwide were developed first in China and Asia, then in US and Europe, to analyse the safety and efficacy of either autologous or allogeneic MSCs, from various tissue sources (UC, B, AT) using single or repeated iv, sc or local injections in neurological, rheumatological or gastrointestinal AD.

After 20 years of clinical research, since the first report (Le Blanc et al. 2004) on clinical efficacy of MSC(M) in a child with refractory aGvHD and more than 1360 clinical trials for MSC registered on Clinicaltrials.gov as of April 2023, in the field of AD per se only one MSC

product allogeneic MSC(AT) for the treatment of Crohn's fistula (Alofisel/Takeda) has been approved since 2018 in Europe, Japan and Canada. The first successful autologous MSC(AT) injection for CD rectovaginal fistula was performed in 2003. Several phase I and II studies for the treatment of anoperineal fistulas in CD reported 46–90% efficacy until the ADMIRE phase III randomized, double-blind, international multicentre trial (RCT) demonstrated the superiority of MSC(AT) over placebo in 212 patients with CD and one or more complex anoperineal fistulas (Garcia-Olmo et al. 2022). For the other auto-immune diseases, several phase I–II studies reported safety results and promising efficacy signals in progressive MS, RA, SLE, SSc still require large RCT to demonstrate MSC efficacy (Burt et al. 2021; Farge et al. 2022; Loisel et al. 2023; Petrou et al. 2020; Wen et al. 2019). Progress in the field has been hampered by large heterogeneity when considering each AD type and patient selection, MSC type, origin and production process, route of delivery and number of injections, clinical trial design and national regulations.

Although the number of clinical trials registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov) using MSC-EVs for therapeutic purposes increases in the last 10 years, few results are available (Burt et al. 2021).

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### 93.3 Regulatory T Cells (Tregs)

Regulatory T cells (Tregs) are a specialized subset of CD4<sup>+</sup> T lymphocytes endowed with immune-suppressive functions. Tregs are positively selected in the thymus and emigrate to the periphery. They represent a very heterogeneous population, distributed in secondary lymphoid organs and tissues. Tregs contribute to maintain the immune tolerance and to prevent autoimmunity. Tregs constitutionally express high levels of the interleukin-2 receptor alpha (CD25) and are highly enriched in the fraction of CD4<sup>+</sup>CD25<sup>bright</sup> cells. In addition, they are classically identified as

CD127<sup>low</sup> and Forkhead-box-P3 (FoxP3)<sup>+</sup> cells. The FoxP3 transcription factor has been identified as the master regulator, which is essential for Treg development (Ikegawa and Matsuoka 2021). Aberrant Treg plasticity, quantitative and functional deficiencies of Treg impair immune homeostasis and importantly contribute to AD (Selck and Dominguez-Villar 2021).

Considering their properties, Tregs represent the ideal candidate for immunotherapy in AD setting. To this end, several strategies (Fig. 93.2) have been developed to enhance the Treg response (Doglio et al. 2022; Xue et al. 2022).

The first approach relies on the induction of Tregs directly *in vivo*, by enhancing their activity and/or persistence. Several drugs employed for the treatment of autoimmune diseases act directly or indirectly on Treg numbers and/or functionality. In this context, the role of rapamycin/sirolimus in increasing the number of Tregs through the inhibition of the mTOR pathway is well established (Greco et al. 2021; Peng et al. 2020). Moreover, an indirect boost of Treg expansion may be induced by CY, as used after allogeneic haematopoietic cell transplantation (HCT) for the prevention of GvHD (Cieri et al. 2015; Fletcher et al. 2023).

Other interventions that increase the number of polyclonal endogenous Treg cells *in vivo* involve low-dose interleukin-2 (IL-2), mutant

IL-2, IL2/Anti-IL-2 Ab complexes. In contrast, applications of antigen-based treatments could lead to the enhancement of antigen-specific Treg subsets (Ikegawa and Matsuoka 2021).

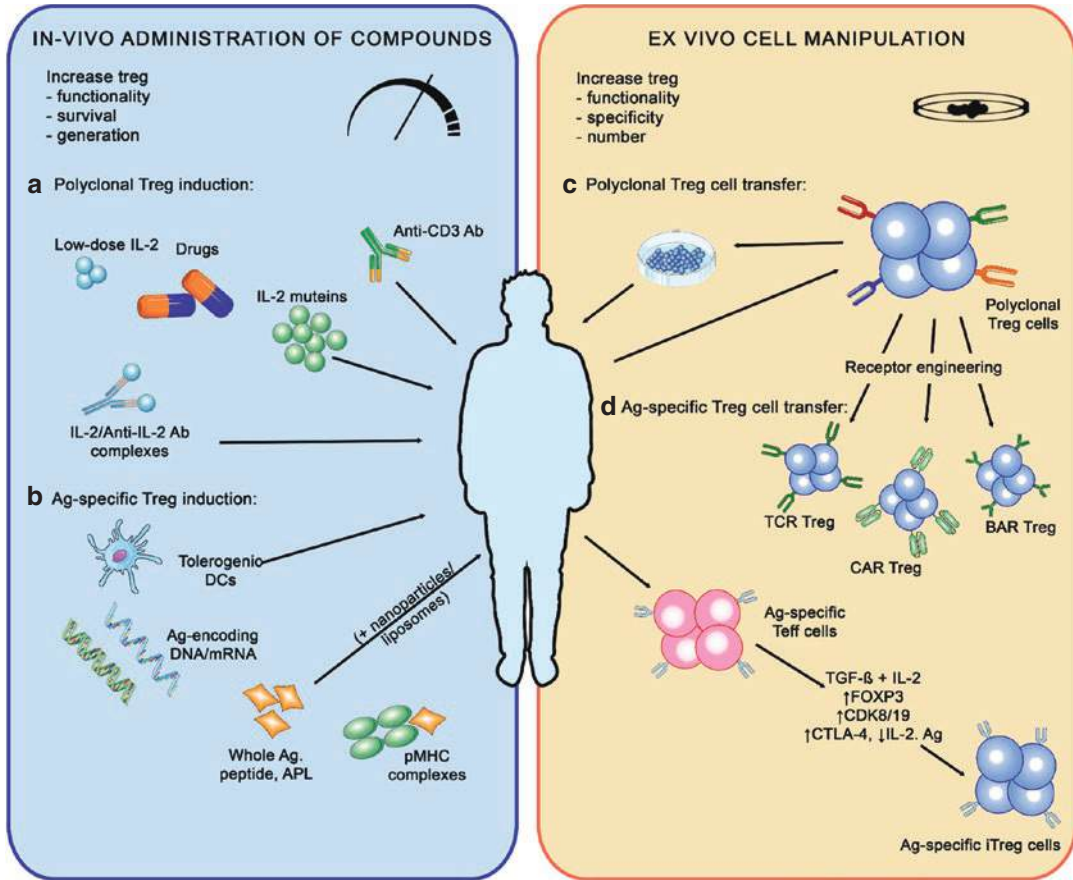
Moreover, after autologous HCT, the reset of Treg compartment is essential to obtain long-term remission in ADs (Baraut et al. 2014; Cencioni et al. 2022; Doglio et al. 2022).

A second approach relies on adoptive Treg cell therapies, through the optimal isolation and *in vitro* expansion of Treg cells.

Data have shown the feasibility and safety of adoptive polyclonal Treg transfer, with variable efficacy, explained by the low level of Treg persistence *in vivo* and a limited number of Ag-specific cells in the final cell product (Doglio et al. 2022; Eggenhuizen et al. 2020). Challenges in *in vitro* Treg expansion and long-term persistence, as well as difficulties in the identification of specific antigens, have significantly slowed their clinical application (Xue et al. 2022).

However, antigen-specific Tregs can be generated *in vitro* by genetic insertion of synthetic receptors, including engineered T cell receptors (TCR), B cell antibody receptors (BAR) or CAR (Scott 2021; Selck and Dominguez-Villar 2021). In the context of autoimmunity, CARs may be effective in redirecting the antigen specificity of Tregs, boosting their suppressive capacities. CAR-Tregs proved very effective in controlling





**Fig. 93.2** Regulatory T cells (Treg)-based approaches in autoimmune diseases (AD). Polyclonal and antigen-specific Treg cell-based therapies are the 2 main strategies: the administration of immunomodulatory agents that enhance the number and/or function of Treg cells in vivo (**a, b**), and the adoptive transfer of in vitro expanded Treg cells (**c, d**). Interventions that increase polyclonal endogenous Treg cells in vivo involve drugs (ie sirolimus), low-dose interleukin-2 (IL-2), mutant IL-2, IL2/Anti-IL-2 Ab complexes as well as selective depletion of effector T (Teff) cells by Anti-CD3 Ab (**a**). Applications of antigen-based treatments could lead to the enhancement of antigen-specific Treg subsets (**b**). Adoptive Treg cell therapies rely on the optimal isolation and expansion of Treg cells in vitro. One option is based on expanded polyclonal

Treg cell populations (**c**). Moreover, antigen-specific Treg cells can be generated in vitro (**d**) by genetic insertion of synthetic receptors (engineered T cell receptors [TCR], chimeric antigen receptors [CAR] or B cell antibody receptors [BAR]) or by transformation of antigen-specific Teff cells into induced Treg (iTreg) cells via stimulation in the presence of transforming growth factor beta (TGF- $\beta$ ) and IL-2, transgenic FOXP3 overexpression, blockade of cyclin-dependent kinase 8 (CDK8) and CDK19 signaling, or a combination of cytotoxic T lymphocyte antigen 4 (CTLA-4) overexpression, IL-2 ablation and antigenic stimulation. Ag antigen, DCs dendritic cells, APL altered peptide ligands, pMHC peptide-major histocompatibility complex. [Adapted from Selck et al. and Doglio et al. (Doglio et al. 2022; Selck and Dominguez-Villar 2021)]

inflammatory conditions in pre-clinical studies (Doglio et al. 2022).

### 93.4 Chimeric Antigen Receptor T Cells (CART)

Chimeric antigen receptors (CAR) are chimeric molecules capable of redirecting the specificity of engineered cells against target antigens, while simultaneously boosting their activation (Freitag et al. 2020).

CARs are composed of three major components: an extracellular domain, a linker peptide and an intracellular part. The extracellular domain accounts for the recognition of the antigen. The intracellular portion mediates the transduction of the signal upon antigen binding. The most frequently used intracellular portions are represented by the zeta-chain of CD3 (CD3z) and the intracellular portion of CD28, 4-1BB and OX-40, each molecule being involved in the co-stimulation and activation of T lymphocytes (Doglio et al. 2022). According to the composition of the intracellular part, different generations of CARs can be distinguished: (a) the first generation composed only by the CD3z, (b) the second generation with two different domains with CD28-CD3z and 4-1BB-CD3z, which represent the two most frequent combinations in use and (c) a third generation with three domains, generally obtained by adding OX-40 to a second generation CAR (Sadelain et al. 2017).

Conventional T cells (Tconvs) expressing CARs revealed impressive clinical results in patients affected by haematological malignancies, since their cytotoxic action could be specifically redirected against transformed cells (Sterner and Sterner 2021).

Additionally, CAR-T cells (CART) may be employed in the field of autoimmunity, thanks to their ability of conferring new antigen-specificities and to simultaneously boost cell activation (Alexander and Greco 2022).

B cells and their effectors such as antibodies and cytokines impact the pathophysiology of ADs (Lee et al. 2021), thus attracting innovative B cell depletion therapies. Compared to monoclonal antibodies, CART have the advantage of a broader depletion of autoreactive B cells, (Doglio et al. 2022), especially those maintained in inflamed tissues and access to lymphoid organ (i.e. lymph node and spleen).

Recent clinical experiences (Table 93.1) suggest the efficacy of CD19-CAR Tconvs in SLE, showing resolution of nephritis and other disease-related symptoms in five patients (Mackensen et al. 2022; Mougiakakos et al. 2021). This effect is associated with a profound B-cell depletion, followed by B-cell repopulation over time, after a median observation of 110 days post-infusion. After 3 months, circulating CARTs were still detectable. All patients received autologous CD19-directed CART, without developing relevant toxicities, and only mild cytokine-release syndrome was reported in these patients. All patients had a normalization of serum double-stranded DNA antibodies and complement levels.

Other preliminary experiences with CD19-targeted CART have been reported in a case of refractory antisynthetase syndrome (Muller et al. 2023) and a patient with severe SSc (Bergmann et al. 2023).

CART therapy that targets B-cell maturation antigen (BCMA) has been explored in 12 patients with relapsed/refractory neuromyelitis optica spectrum disorder (NMOSD) (Qin et al. 2023). Only a mild cytokine release syndrome was reported. No relapse has been observed at a

**Table 93.1** Clinical application of chimeric antigen receptor (CAR) T cells in autoimmune diseases (AD)

AD	CAR-T cell	Lymphodepletion	CRS/ICANS	Other toxicities	Disease response	Follow up
Mougiakakos et al. (2021)	CD19 CAR, 4-1BB co-stimulatory domain	Flu 25 mg/m <sup>2</sup> /day i.v. on days -5, -4, -3 and Cy 1000 mg/m <sup>2</sup> /day i.v. on day -3	None	None	Clinical remission (proteinuria, SLEDAI), serologic remission (dsDNA Ab; C3/C4)	44 days
Mackensen et al. (2022)	CD19 CAR, 4-1BB co-stimulatory domain	Flu 25 mg/m <sup>2</sup> /day i.v. on days -5, -4, -3 and Cy 1000 mg/m <sup>2</sup> /day i.v. on day -3	CRS grade 1 (3/5); no ICANS	No infections	Resolution of nephritis and disease-related symptoms, serologic remission (5/5)	8 months
Müller et al. (2023)	CD19 CAR lentiviral vector	Flu 25 mg/m <sup>2</sup> /day i.v. on days -5, -4, -3 and Cy 1000 mg/m <sup>2</sup> /day i.v. on day -3	CRS grade 1, transient CRS-related symptoms (increased creatinine kinase and myalgia)	Decreased Ig levels	Improvement in muscle strength and regained muscle endurance, decreased creatinine kinase and myoglobin, serologic remission, resolution of myositis at MRI	200 days
Bergmann et al. (2023)	CD19 CAR lentiviral vector	Flu 12.5 mg/m <sup>2</sup> ; days -5, -4 and -3 and Cy 500 mg/m <sup>2</sup> , day -3 (50% dose-reduced due to renal impairment)	CRS grade 1; no ICANS	None	Improvement of heart, joint and skin manifestations, serologic remission, stable pulmonary fibrosis	6 months
Qin et al. (2023)	BCMA CAR	Flu 30 mg/m <sup>2</sup> /day i.v. and Cy 500 mg/m <sup>2</sup> /day i.v. on days -4, -3, -2	CRS grade 1-2 (12/12); no ICANS, no other neurologic toxicities	Common haematotoxicity; 58% infections (no grade 4); 25% CMV	Drug-free and serologic remission (11/12), improvement in disabilities (12/12)	5.5 months

Ab autoantibodies, AD autoimmune diseases, CAR chimeric antigen receptor, CMV cytomegalovirus, Cy cyclophosphamide, CRS cytokine-release syndrome, dsDNA double-stranded DNA, Flu fludarabine, ICANS immune effector cell-associated neurotoxicity syndrome, Ig immunoglobulin, MRI magnetic resonance imaging, NMO/S optic spectrum disorder, SLE systemic lupus erythematosus, SLEDAI systemic lupus erythematosus disease activity index score, SSc severe systemic sclerosis

median follow-up of 5.5 months in 11 patients, paralleled by a reduction of autoantibodies.

Overall, initial experiences suggest a rapid response of AD to CART therapy, although extended follow-up is needed to determine long-term efficacy.

### 93.5 Conclusions and Future Directions

New insights are emerging in the complexity and power of innovative cellular therapies. MSC, a heterogeneous population of stromal cells with high regenerative capacity that can be isolated, cultured, and expanded *ex vivo* represent a promising source for cellular therapy approaches in AD due to their immunosuppressive, angiogenic and anti-fibrotic properties. MSCs plasticity and their regenerative and immunomodulatory properties are known to be driven by microenvironmental factors. MSC of different tissue origin have been investigated for treating several indications. Progresses in the field have been hampered by large heterogeneity of MSC products and sources. Promising results have been obtained in SSc, SLE and access to the market in CD.

Tregs, a specialized subset of T lymphocytes with immune suppressive capacities and dysfunctional in AD constitute the ideal candidate for adoptive cell therapy in AD. Despite safe, polyclonal Tregs mediated suboptimal/controversial responses in clinical trials, mainly due to low amount of disease relevant antigen-specific cells. CARs can redirect the T cell antigen specificity, aiming at restoring the immune tolerance.

CAR-Tregs were proven very effective in controlling inflammatory conditions in AD pre-clinical studies Current available clinical data reveal that CD19 conventional CART effectively deplete B cells in patients with SLE, leading to impressive drug-free remission in patients refractory to standard therapies. The clinical effect of CART appears to be associated with abrogation of autoimmunity and persists even after B cell reconstitution. Longer follow-up is warranted after these preliminary promising experiences with various CART approaches in a variety of

AD (antisynthetase syndrome, SSc, NMOSD). These findings show that the generation and administration of CART in AD is feasible and safe.

Future studies are warranted to further elucidate the mechanism of action of these cellular-based therapies, while shedding light on the underlying pathogenesis of AD.

#### Key Points

- AD are heterogeneous conditions characterized by aberrant activation of the immune system with failure of the immune regulation to maintain adapted tolerance.
- The optimal treatment of AD should be discussed, in light of this pathological continuum between autoimmunity and auto-inflammation, which variably interacts in each AD phenotypic expression.
- MSC based therapies appear as ideal tools to target the respective auto-inflammatory and autoimmune components of AD. Progresses in the field have been hampered by large heterogeneity.
- Tregs constitute the ideal candidate for adoptive cell therapy in AD, aiming at restoring the immune tolerance. Despite safe, polyclonal Tregs mediated controversial results in clinical trials. CAR-Tregs proved very effective in controlling inflammatory conditions in pre-clinical studies.
- CART approach is feasible, tolerable and highly effective in severe/refractory SLE and other AD (antisynthetase syndrome, SSc, NMOSD) in preliminary clinical reports. Longer follow-up is warranted.

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## 94.1 Introduction

In the absence of randomized prospective trials, the EBMT registry remains an important source to survey indications, outcome and clinical risk factors in patients with solid tumours treated by auto- and allo-HCT. At the end of 2022, the EBMT registry included 65,586 HCT for solid tumours in 47,221 patients, with a slight prevalence in adults compared with children (58% vs. 42%). Auto-HCT represented 97% of the total HCT, whereas allo-HCT was used in 3% of the procedures. Multiple transplants were performed in 1/3 of the cases (Table 94.1; Figs. 94.1 and 94.2) compare activity and indications between adults and children.

**Table 94.1** EBMT registry on HCT for solid tumours (data updated on February 2023)

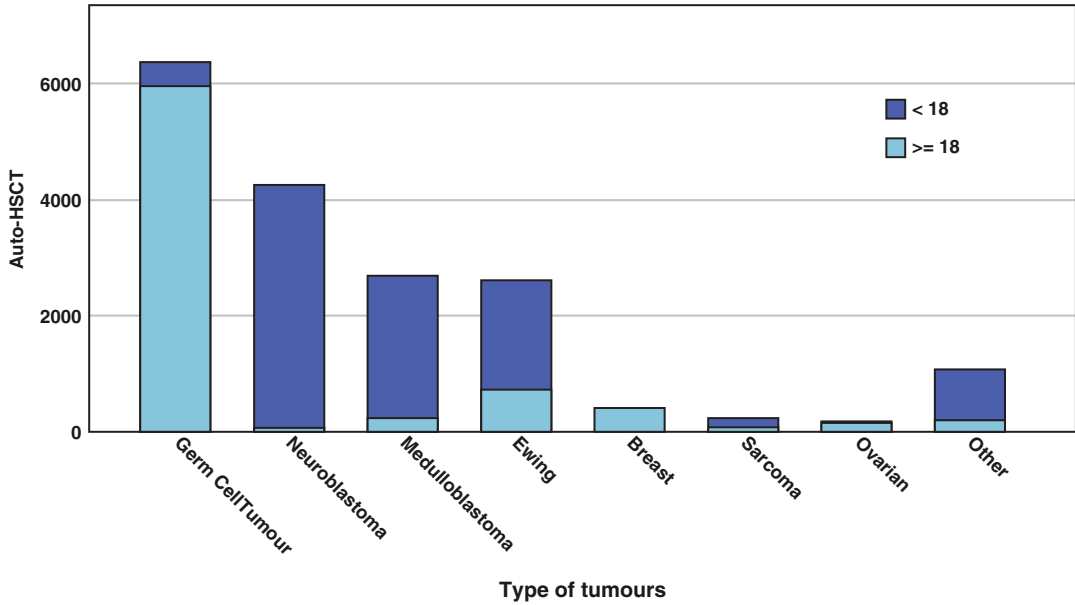
<i>Solid tumour registry</i>	65,0586	
<i>Patients</i>	47,221	
<i>Adults/pediatric (%)</i>	58/42	
<i>Male/female (%)</i>	48/52	
<i>Auto/allo (%)</i>	97/3	
<i>Nb of HSCT</i>	Auto (n = 63,592)	Allo (n = 1981)
<i>First HSCT</i>	45,997	1221
<i>Second HSCT</i>	12,124	577
<i>Third HSCT</i>	4470	134
<i>Fourth HSCT</i>	746	34
<i>≥Fifth HSCT</i>	210	1
<i>Median follow up (year &lt; 2020)</i>	3.49 (<1–39)	2.98 (<1–37)

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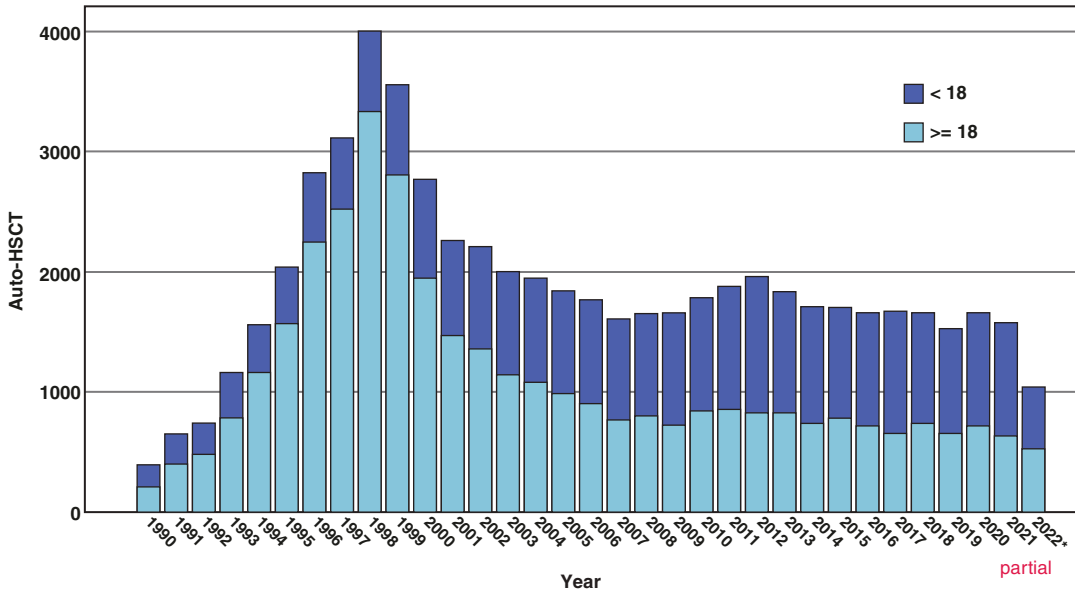
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**Fig. 94.1** EBMT registry on auto-HCT for solid tumours in the period 2012–2022 ( $n = 17,850$ ). Adults vs. paediatric HCT is reported (February 2023)



**Fig. 94.2** EBMT registry showing numbers of auto-HCT for solid tumour indication over the years: 1990/2022 in both adults and children (data of 2022 are partial)

## 94.2 Solid Tumours in Children and Adolescents

Ruth Ladenstein

Regarding the respective solid tumour entities relevant in the paediatric and adolescent age groups this year's report informs on recent advances in the literature. In brief, to date the evidence for high-dose-therapy (HDT)/HCT from prospective randomized trials is still limited to high-risk neuroblastoma hrNBL and Ewing sarcoma (Matthay et al. 2009; Whelan et al. 2018).

### 94.2.1 Recall on General Lessons Based on EBMT Data

Transplant-related mortality. TRM markedly decreased over time and is related with the HDT regimen (i.e., elimination of TBI) and most importantly use of peripheral stem cells. TRM rates associated to auto-HCT dropped to under 5% after 1992 and is since 2012 only 1%.

Total body irradiation. TBI showed no advantage in any of the solid tumour indications and should thus be avoided in children with solid tumours in view of late effects.

Remission status. First-line high-risk patients perform significantly better than after relapse. Response to induction treatments prior to HDT/HCT is critical in all indications. In brief, good response to first-line treatment (CR/VGPR/PR) in high-risk patients in and sensitive relapse (SR) are good indications, while patients with stable disease or minor response (<50%) (SD/MR) should only be considered for well-defined phase I/phase II trials. Patients with no response (NR) or tumour progression or refractory disease (RR) have a very short life expectancy even after HDT/HCT and thus should not be considered.

Age plays a crucial role as outcome predictor. Adolescent age is generally associated with inferior outcome. While age <10 years is a favourable factor in sarcomas (Ewing sarcoma and rhabdo-

myosarcoma), NBL has an earlier cut-off at 5 years. Patients with NBL  $\leq 18$  months at diagnosis need biological profiling and are only eligible with high-risk biological features, in particular MYCN amplification (Canete et al. 2009; Ladenstein et al. 2023).

Double HCT approaches. The EBMT data on tandem or repetitive HDT/HCT approaches show no advantage over single HDT/HCT. However, the elective selection of particularly poor-prognosis patients in phase II settings is a likely bias. A randomized trial in NBL emerged with superiority for the tandem strategy for hrNBL in front-line patients (Park et al. 2016).

Busulfan–melphalan. This HDT combination is the only one in the EBMT database resulting in significantly improved survival rates in NBL and Ewing tumours.

Allo-HCT. No advantage for allo-HCT can be detected in the EBMT data for any paediatric solid tumour indication. The potential bias of negative selection of particular poor-prognosis patients' needs to be considered.

The EBMT data showed a benefit from salvage HDT/HCT in responding patients relapsing after 12 months from diagnosis and without a previous HDT.

### 94.2.2 Neuroblastoma (NBL)

High-risk neuroblastoma (hrNBL) is defined in the front-line setting by widespread disease >18 months, but includes any stage and age in the presence of MYCN oncogene amplification (Cohn et al. 2009; Moroz et al. 2011; Canete et al. 2009; Ladenstein et al. 2023). In addition, genetic alterations of ALK (clonal mutations and amplifications) are independent predictors of poorer survival in hrNBL (Bellini et al. 2021; Goldsmith et al. 2023) ALK inhibitors are increasingly integrated in upfront treatments, mostly the third-generation ALK inhibitor lorlatinib, and contribute to improved response rates prior to HCT. Standard treatment approaches

include multicycle, intense induction where recently combinations with immunotherapy are evaluated in randomised trials, local control via mostly extensive surgery to the primary tumour site enhanced by radiotherapy including local and, in some collaborative groups, also metastatic sites delivered after HDT/auto-HCT. Immunotherapy for maintenance with respective ch14.18 antibodies is meanwhile considered standard (Park et al. 2016; Yu et al. 2010; Ladenstein et al. 2017, 2018).

#### 94.2.2.1 Autologous HCT in NBL

Although three randomized trials and a meta-analysis have demonstrated that HDT with aHCT improves EFS in high-risk NBL (Pritchard et al. 2005; Berthold et al. 2005; Matthay et al. 2009; Yalçın et al. 2013), there is no consensus which HDT regimen is superior. Whilst EBMT data and the HR-NB11/SIOPEN trial support Busulphan and Melphalan as HDT regimen (Ladenstein et al. 2008, 2017), the randomized results of the ANBL0532 COG found superiority for double HDT/HCT [first HDT, CY and TT; second HDT, CBP, VP and MEL (with reduced doses of single HDT CEM) vs. single HDT (CEM)/auto-HCT (Park et al. 2019) with Anti-GD2 antibody-based immunotherapy being beneficial for both arms (Yu et al. 2010)]. The value of a tandem approach including Busulphan and Melphalan is currently explored within the randomized HRNBL2/SIOPEN trial.

A recent analysis (Ladenstein et al. 2023) on MYCN amplified NBL in infants and toddlers found markedly improved outcomes within the HR-NB11/SIOPEN trial for infants and toddlers.

Targeted therapies, in particular iodine-131-metaiodobenzylguanidine (mIBG) therapy with and without chemotherapy or radiosensitisers like vorinostat and/or HDT followed by HCT, have generated increasing interest (Lee et al. 2017; Johnson et al. 2011; Ferry et al. 2018; Kraal et al. 2017, 2019; DuBois et al. 2021). Further HDT tandem approaches with or without mIBG are currently explored by the COG and SIOPEN in randomized and by other groups in non-randomized trials (Lee et al. 2017).

#### 94.2.2.2 Allogeneic HCT in NBL

The role of allo-HCT remains controversial. Allo-HCT as immunotherapy received special attention after introduction of RIC and NMA transplants. Some reports highlight a graft-versus-tumour (GvT) effect with adopted allo-HCT approaches, while early EBMT data showed no benefit with classical allo-HCT (Ladenstein et al. 1994). The CIBMTR conducted a retrospective review showing some benefit for allo-HCT in patients without prior auto HCT (Hale et al. 2013). The Japan Children's Cancer Group (JCCG) (Hara et al. 2022) reported recently improved outcomes with allo-HCT.

Haplo-HCT in very high-risk refractory/relapsed (R/R) patients is capable to induce long-term remission (Illhardt et al. 2018). Graft-versus-NBL effects can be elicited by transplantation of haploidentical hematopoietic cells (haplo-HCT) exploiting cytotoxic functions of natural killer cells and their activation by the anti-GD2 antibody dinutuximab beta (DB) (Flaad et al. 2023). Immunotherapy after haplo-HCT is feasible, with low risk of inducing GvHD and resulted in markedly improved long-term survival likely attributable to increased anti-NBL activity by donor-derived effector cells.

#### 94.2.2.3 Cell Therapy in NBL

The success of anti-GD2 therapy has proven that immunotherapy can be effective in NBL. Adoptive transfer of chimeric antigen receptor (CAR) T cells has the potential to build on this success. In early phase clinical trials, CART for NBL has proven to be safe and feasible, but significant barriers to efficacy remain. One such approach is adoptive transfer of CART cells, which combine the specificity of an antibody with the cytolytic capacity of T cells in an MHC independent manner. Persisting hurdles to date include lack of T cell persistence and potency, difficulty in target identification, and an immunosuppressive tumour microenvironment. Outcomes so far have been encouraging but modest, with only a fraction of patients achieving measurable responses and very few patients demonstrating long-term persistence of CART cells (Yang et al. 2017; Yeku

et al. 2017; Pinto et al. 2018; Richards et al. 2018; Heczey et al. 2017). Immunotherapy with CART cells that target the disialoganglioside GD2 expressed on tumour cells, i.e., third-generation GD2-CART cells expressing the inducible caspase 9 suicide gene (GD2-CART01) may be a new therapeutic opportunity in R/R advanced high-risk neuroblastoma (Del Bufalo et al. 2023).

#### 94.2.2.4 Recommended Indications NBL-HCT

Standard indications include first-line hrNBL >18 months at diagnosis with widespread metastatic disease or those of any age with MYCN amplified tumours with INSS stages 2–4. Any responding metastatic relapse in patients >18 months and any MYCN amplified tumour without prior HDT/HCT are suitable indications. Any other indication is reserved for well-designed experimental phase I/phase II trials. Children <18 months need to be evaluated for a high-risk biological risk profile prior to being considered for HDT/auto-HCT (Canete et al. 2009; Ladenstein et al. 2023; Moroz et al. 2011; Cohn et al. 2009). Immunotherapy-related approaches including recent haploidentical HCT and third generations CART cell approaches hold promise for patients advanced relapsed and refractory disease.

#### 94.2.3 Ewing Sarcomas (EWS)

Ewing sarcomas (EWS) are solid tumours of the bone and soft tissue that usually affect children, adolescents and young adults. An incidence rate of 4.5 per million a year is reported, with a peak incidence of 11 per million at the age of 12 years. Metastatic disease is detected in about 20–30% and is typically found in the lungs, bone, bone marrow or a combination of these. Presence of metastatic disease at diagnosis (primary metastatic disease) is the most important adverse prognostic factor and is associated with a 5-year survival lower than 30%. The hypothesis is that HDC regimens may overcome the resistance to standard multidrug chemotherapy and improve survival rates. Despite more intensive chemother-

apy, 30–40% of young people with Ewing sarcoma will have recurrence of the disease. Less than 30% of young people with a recurrence of EWS are alive at 24 months, and less than 10% are alive at 48 months (Haveman et al. 2021).

##### 94.2.3.1 Autologous HCT in EWS

For primary disseminated multifocal EWS (Ladenstein et al. 2010) receiving BU/MEL HDT/auto-HCT after VIDE induction (Euro- EWING 99 study group), an increased risk at diagnosis was observed for patients  $\geq 14$  years (HR = 1.6), with a primary tumour volume >200 mL (HR = 1.8), more than one bone metastatic site (HR = 2.0), BM metastases (HR = 1.6) and additional lung metastases (HR = 1.5), carry. A score based on these factors identified patients with an EFS rate of 50% for scores  $\leq 3$  (82 patients), 25% for a score >3 and  $\leq 5$  (102 patients) and 10% for score  $\geq 5$  (70 patients;  $p < 0.0001$ ). In Ewing2008R3, the effect of treosulfan and melphalan (TreoMel) HDT/HCT was investigated in patients with disseminated EWS with metastases to bone and/or other sites, excluding patients with only pulmonary metastases (Koch et al. 2022). Additionally, TreoMel-HDT was of no benefit for the entire cohort of patients, whereas TreoMel-HDT may be of benefit for children age <14 years.

When using standard methodological procedures as expected by Cochrane no evidence from RCTs or CCTs to determine the efficacy of HDC with AHCT compared to conventional chemotherapy was found for patients with primary metastases to locations other than the lungs (Haveman et al. 2021).

In EWS patients with poor histologic response ( $\geq 10\%$  viable cells) after VIDE induction (6 courses) or large tumour volume at diagnosis ( $\geq 200$  mL), the risk of event was significantly decreased by BU/MEL compared to VAI with better EFS and OS. For this group of patients, BU/MEL is now a standard of care (Euro- EWING 99 study group (Whelan et al. 2018).

In contrast, the R2Pulm trial of the Euro- EWING 99 study group and EWING 2008 randomised busulfan–melphalan HDT/HCT (BuMel) in comparison with standard chemo-



therapy with whole lung irradiation (WLI) in EWS presenting with pulmonary and/or pleural metastases (Dirksen et al. 2019). There was a clear benefit from BuMel compared with conventional VAI plus WLI which in addition was associated with less toxicity.

A retrospective study addressed the important question of compatibility of BU/MEL and whole lung irradiation (WLI) and found WLI at recommended doses and time interval after BU/MEL feasible (Abate et al. 2021).

A recent analysis on R/R EWS patients underpinned a role of HDT/HCT in 196 patients with 64 patients receiving HDT, 98 standard non-HDT chemotherapy and 34 no systemic therapy (Windsor et al. 2022).

However, a survey on patients with a first recurrence of EWS in children, adolescents, and young adults found no informative data from randomized controlled trials (RCTs) or (historical) controlled clinical trials (CCTs), so no conclusions may be drawn (Haveman et al. 2021).

#### 94.2.3.2 Allogeneic HCT in EWS

Tandem HDT and allo-HCT were part of the EICESS92 and Meta-EICESS protocols yielding long-term DFS in patients with advanced EWS (Burdach and Jürgens 2002).

Patients suffering a relapse generally have a poor prognosis with conventional chemotherapy. The role of HDT/auto-HCT still awaits clarification in randomized controlled studies (Tenneti et al. 2018; Ferrari et al. 2015). A GvT or improved survival following allo-HCT effect was highlighted in some reports, but a retrospective review (Thiel et al. 2011) could not identify benefits with either RIC or MAC or with either HLA-matched or HLA-mismatched grafts. Haplo-HCT for consolidation after conventional therapy seems to be of interest for some, but not for most patients with high-risk paediatric sarcomas (Eichholz et al. 2023; Sano et al. 2022).

#### 94.2.3.3 Recommended Indications in EWS

BU/MEL HDT for patients with a poor histological response after induction and/or a tumour volume  $\geq 200$  mL is now standard of care. Patients

with primary metastatic disease at sites other than the lungs and a low-risk score may be considered good candidates in the absence of a controlled trial. Any metastatic relapse without prior HDT may be considered for controlled phase II HDT protocols. Adoptive immunotherapy is an evolving field and subject to experimental early trials.

### 94.2.4 Soft Tissue Sarcoma (STS)

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma (STS) in childhood. Whereas more than 90% of patients with localized low-risk RMS can be cured, metastatic RMS have a dismal outcome, with survival rates of less than 30%.

#### 94.2.4.1 Recommended Indications

Currently there is no evidence-based standard indication for HDT/HCT in STS.

### 94.2.5 Osteosarcoma (OS)

Even in responding high-risk patients treated with HDT/HCT in first or second remission, the length of remission is short, and relapse occurs early after HDT. High-risk features include poor histological response or non-response of the primary tumour at the time of definitive surgery, inoperable, axial tumours (large volume), primary dissemination or relapse other than isolated, late lung metastases.

#### 94.2.5.1 Recommended Indications in OS

There is no standard indication for HCT based on published results or EBMT data.

### 94.2.6 Retinoblastoma (RB)

Retinoblastoma (RB) is the most frequent intraocular malignancy in children, with the incidence rate of 1 per 17,000–24,000 live births. Delayed time to diagnosis may lead to the development of

locally advanced or metastatic disease associated with poor survival, especially when CNS metastasis is present. Chemotherapy drugs that may be useful for these patients include platinum-containing agents (carboplatin and cisplatin), etoposide, vincristine, cisplatin, cyclophosphamide and anthracyclines.

#### 94.2.6.1 Recommended Indications in RB

Advocated HDT/auto-HCT approaches are CARBOPEC (CBP, VP, CY) (Jaradat et al. 2012), but for CNS-positive patients, TT or BU was introduced (Dunkel et al. 2010). Other groups used combinations including MEL and CBDCA and/or VP for metastatic RB and reported promising survival results for patients without CNS involvement. Future trials should take the following high-risk factors into consideration: involvement of the cut end or subarachnoidal space of the optic nerve after enucleation, orbital involvement and distant metastatic disease and CNS disease.

#### 94.2.7 Wilms' Tumour (WT)

Wilms tumour (WT) treatment regimens are curative for more than 80% of patients, but those with relapsed or refractory disease continue to have poor outcomes. HDT-autologous aHCT rescue is often utilized although outcomes remain variable. So far attempts to conduct a randomized trial comparing maintenance chemotherapy with consolidation versus HDT/auto-HCT have failed in this indication.

##### 94.2.7.1 Recommended Indications in WT

Experience of the SIOP, GPOH, NWTs, MRC and respective national groups over the last 20 years found the probability of cure of 30% at best in the presence of adverse prognostic factors. High-risk factors are unfavourable histology and metastatic disease (Presson et al. 2010; Delafoy et al. 2022; Groenendijk et al. 2022) and are after relapse again unfavourable histology and one of the following criteria: extra-pulmonary

relapse or abdominal relapse after radiation, stage IV, more than two drugs in the first-line regimen or relapse within 1 year. HDT is indicated if a response to second-line treatment is achieved.

#### 94.2.8 Recommended Indications in Brain Tumours

Patients with high-risk medulloblastoma (primary metastases/relapse) of any age older than 3 years are eligible for HDT/HCT in combination with radiation, while in infants HDT/HCT is used with the aim of reducing (volumes and doses) or avoiding radiation.

Metastatic PNETs at diagnosis or with additional high-risk features such as incomplete resection or young age (younger than 3 or 5 years) as well as infants and young children (<4 years) with malignant brain tumours are further indications.

Very controversial indications include high-grade glioma. Currently, there is little or no indication for HDT/HCT in ependymoma, brain stem glioma or pineoblastoma. More investigations are required to define the optimal HDT for each tumour type. Most groups use similar HDT regimens, i.e. BU/TT (SFOP, Spain), VP/TT/CBDCA (US/CCG, Germany, Spain) or a tandem approach Vp16/CBDCA—TTP/L-PAM (Italy).

#### 94.2.9 Extra and Intracranial Germ Cell Tumours

CNS germ cell tumours (GCTs) can be divided into major groups including germinomas (having a superior prognosis) and non-germinomatous GCTs (NGGCTs), with teratomas often considered a separate category- and represent approximately 3% of primary paediatric brain tumours.

##### 94.2.9.1 Recommended Indications in in Germ Cell Tumours

As paediatric patients with extracranial GCTs may expect an excellent outcome with conventional chemotherapy approaches, there is no stan-

standard indication for HDT/AHCT. Based on previously published reports on adults, or the few reports for children, treatment with HDT combined with aHCT has a beneficial effect on R/R GCTs. However, the strategy of HDCT used as a frontline treatment has no demonstrated benefit for children in the poor-risk group and requires further research.

High-risk patients with extracranial GCTs are initial non-responders or poor responders (no local control achieved) and patients after relapse failing to achieve second CR.

In high-risk CNS GCT patients <18 years, the following criteria for HDT may be adopted: recurrent CNS GCT when biological remission is achieved prior to HDC and insufficient response to primary chemotherapy.

Since carboplatin and etoposide have been included in the front-line chemotherapy of paediatric GCTs, other drug combinations should be considered for conditioning regimens, such as melphalan, thiopeta, or paclitaxel. The treatment of paediatric poor-risk GCTs is still a challenge for paediatric oncologists. HDCT combined with AHCT in this group of patients requires further study.

#### Key Points for Solid Tumours in Children and Adolescents

- In neuroblastoma and Ewing sarcoma, there is clear evidence for the advantage of HDT/auto-HCT with an increasing interest in tandem transplants.
- In other paediatric solid tumour, indication still lacks randomized trials, and indications are based on observational studies, case reports and EBMT database only.

## 94.3 Solid Tumours in Adults

Paolo Pedrazzoli, Giovanni Rosti.

### 94.3.1 Auto-HCT in Adults

Supported by a strong rationale from laboratory studies and apparently ‘convincing’ results of early phase II studies, in the 1990s auto-HCT was uncritically adopted as a potentially curative option for solid tumours. For this reason, randomized trials comparing high-dose therapy with conventional control arms were difficult to conduct. As a result, the number and size of clinical studies initiated (and often unfortunately abandoned before completion) to prove or disprove its value were largely insufficient. Nowadays, after 30 years of clinical research and thousands of patients receiving auto-HCT, the benefit of auto-HCT in solid tumours, except for germ cell tumours (GCTs) and possibly in selected patients with breast cancer (BC) is still unsettled (Fig. 94.1), (Sureda et al. 2015; Snowden et al. 2022).

#### 94.3.1.1 Breast Cancer (BC)

The role of intensified chemotherapy with auto-HCT for primary BC at high risk of recurrence (at least four involved axillary lymph nodes) has been assessed by several randomized trials, evaluated by a meta-analysis of individual patient data (Berry et al. 2011a; Pedrazzoli et al. 2015). Overall, it was shown that auto-HCT prolonged DFS when used as adjuvant therapy and showed a benefit on BC-specific survival and OS in selected cohorts of patients (Nitz et al. 2005; Pedrazzoli et al. 2015). Whether auto-HCT has benefits in the context of contemporary targeted therapies is largely unknown.

In the metastatic setting, seven phase III studies have been published in peer-reviewed journals. Most of these trials showed improved PFS in the auto-HCT arm, but only one OS advantage. Six randomized trials, including 866 metastatic breast cancer (MBC) patients, have been analyzed in the parallel meta-analysis of individual patient data (Berry et al. 2011b) showing a significant improvement in PFS, but no improvement in OS.

Overall, based on the randomized studies so far, meta-analyses and retrospective studies, auto-HCT may still represent a therapeutic option for younger patients harbouring HER2-negative tumours and having gross involvement of axillary nodes (adjuvant setting) or highly chemo sensitive disease (advanced setting) (Martino et al. 2016, 2022).

A recent update (20 years) of the Dutch trial (Steenbruggen 2020) on 885 patients below 56 years of age did provide improved statistically significant overall survival in very high-risk patients (i.e. with  $\geq 10$  involved axillary lymph nodes) compared to standard adjuvant therapy. High-dose chemotherapy did not affect the long-term risk of a second malignant neoplasm or major cardiovascular events.

#### 94.3.1.2 Germ Cell Tumours (GCTs)

Auto-HCT is not recommended as first-line therapy in GCT.

In relapsed GCT, high-dose chemotherapy (HDCT) is considered a therapeutic option, especially when poor prognostic factors are present (Lorch et al. 2011; Necchi et al. 2015; De Giorgi et al. 2017a, b). In this setting, a randomized trial (Tiger study) comparing conventional-dose therapy with high-dose therapy has recently (2023) completed its accrual of over 400 patients worldwide, but results are not available yet. Auto-HCT is a standard of care for patients that are (primarily) refractory to platinum-based chemotherapy or for those with a second or further relapse (Necchi et al. 2015). Multiple intensified cycles with carboplatin and etoposide are recommended as the standard conditioning regimen for GCT also due to concerns that using a three-drug regimen would require dose reductions of the two most active drugs in this disease (Einhorn et al. 2007; Feldman et al. 2010).

Furthermore, auto-HCT can be safely administered in high-risk patients older than 45 years. However, since the prognosis is poorer for older patients with non-seminoma histology, a comprehensive risk–benefit evaluation should include co-morbidities and the patient’s risk category.

The assessment of a large series of EBMT centers, including 46 cases with pure seminoma,

seems to support the notion that auto-HCT may represent a valuable therapeutic option after failure of standard-dose chemotherapy in this patient category (Necchi et al. 2017).

Both HDCT with peripheral blood stem cell transplant and conventional-dose chemotherapy (CDCT) are recommended treatment options for relapsed GCTs. Consistently reported cure rates from phase II and large retrospective studies support the use of HDCT in the hands of an experienced team of oncologists (Adra et al. 2017; Chovanec 2023).

The role of auto-HCT in the mediastinal non-seminoma (MnS) GCT disease category is under evaluation due to the rarity of the disease. Data from the EBMT confirmed that the MnS was characterized by the poorest outcome with 5-year OS ranging from 40 to 45% (Rosti et al. 2019). The use of auto-HCT as both early intensification and at disease recurrence proved to be effective, given upfront and may produce a 15–20% absolute improvement in survival compared with standard-dose CT (Bokemeyer et al. 2002, 2003; De Giorgi et al. 2005). The EBMT is currently conducting a registry-based retrospective international study on primary mediastinal GCTs.

#### 94.3.1.3 Soft Tissue Sarcoma (STS)

STS accounts for about 1% of adult cancers. Based on the observation of a dose–response correlation for some drugs used in STS, e.g., DOX and IFO, HDT with auto-HCT has been investigated in some, mostly non-randomized phase II trials. Most of these trials found few patients to possibly benefit from auto-HCT but, owing to the small patient numbers of each of the included histologic subgroups, they could not establish robust markers for identifying these patients.

A recent large retrospective analysis from the EBMT Registry (Heilig et al. 2020) found no evidence for a clear benefit of auto-HCT in STS but, again, did not sufficiently report on outcomes in the different histologic subgroups. However, considering that the current WHO classification differentiates more than 50 histological subtypes of STS, it might be hypothesized that clinical response to auto-HCT may vary significantly depending on the histological differentiation.

#### 94.3.1.4 Other Solid Tumours

Data from randomized phase III studies comparing HDCT vs. CDC for first-line treatment of advanced ovarian cancer and limited or extensive small-cell lung cancer have shown no statistically significant difference in PFS or OS (Pedrazzoli et al. 2006). Limitations due to study design, difficulty in recruitment and toxicity may have accounted for the lack of favourable results that were expected based on previous phase II and retrospective analyses of such highly chemosensitive diseases.

In other chemo-sensitive histologies, including Ewing/PNET and certain CNS tumours, data regarding auto-HCT in adult patients are limited, again based on clinical trials without randomization and retrospective analyses. For this reason, auto-HCT cannot be recommended as a standard of care. High-dose therapy can be regarded as a potential clinical option in selected adult and AYA (adult young adolescents) patients harbouring paediatric tumours including Ewing's sarcoma and medulloblastoma (Haveman et al. 2021; Spreafico et al. 2005).

#### 94.3.2 Allo-HCT in Adults

Immune therapy for cancer is being pursued with extraordinary interest by researchers all over the world, given the recent scientific acquisitions on immune mechanisms that control cancer and the introduction in the marketplace of checkpoint inhibitor molecules, such as nivolumab/pembrolizumab (PD-1/PDL-1 inhibitors) and ipilimumab (anti-CTLA4). The paradigm for immune therapy of cancer is allo-HCT, whose therapeutic effect is carried out by immunocompetent T cells of the donor, an effect known graft-versus-tumour effect (GvT). Several studies of allo-HCT in selected solid tumours, namely, renal cell cancer (RCC), ovarian cancer, BC, colorectal cancer and others, with some evidence of GvT and occurrence of transplant-related toxicities, mostly GvHD have been reported. In RCC, a long-term survival effect in a fraction (20%) of patients was documented. Since 2004, when molecularly targeted drugs were introduced into the clinic for

renal cell cancer, patient referral for transplants dropped precipitously, and transplant rate evaluation for solid tumours from 2009 was limited to a few patients in Europe.

A survey provided a picture of the status of allo-HCT for solid tumours in EBMT centres (Bregni et al. 2016). In contrast to our expectations, allo-HCT for solid tumour indications has been nearly abandoned in adults and can be proposed only within clinical trials.

#### 94.3.3 Cell Therapy in Solid Tumours in Adults

Despite the encouraging success of industry-manufactured chimeric antigen receptor (CAR)-T cell therapy in haematology, clinical trials with advanced therapy medicinal products (ATMP) face unique challenges in solid tumours because of the immunosuppressive tumour microenvironment, the hurdle of T-cell trafficking and infiltration into scarcely accessible tumour sites and difficulties in identifying targetable antigens with optimal expression and a good toxicity profile (Comoli et al. 2019; Maher and Davies 2023). In addition, due to the variety of programs and infrastructures involved in ATMP manufacturing and delivery, the availability of information on ongoing studies in ST is limited (Comoli et al. 2023).

Currently, while waiting for breakthrough cellular products to treat ST, cellular therapy programs in solid tumours in adults can be offered only within clinical trials.

##### Key Points

- The benefit of auto-HCT in solid tumours of the adults, with the possible exception of selected high risk (more than 10 positive axillary nodes) and metastatic breast cancer patients and germ cell tumours in second or subsequent lines, is still unsettled.
- Despite the great potential, cell therapy programs, including allogeneic trans-



plant for cancer control still have a marginal, if any, role in the management of patients with solid tumours. This issue should be regarded as a priority for medical oncology and cell therapy/transplantation societies also in view of the recent development of immune checkpoint inhibitors that represent a major breakthrough in cancer treatment and may well be incorporated into cell therapy programs.

- The story of HCT in solid tumours demonstrates the importance of adopting an internationally coordinated approach to the investigation of this treatment modality. There needs to be an increased emphasis on prospective trials that are statistically robust and have well-defined criteria for patient selection. Only these will be able to demonstrate whether HCT, alone or incorporated into programs with novel therapeutic modalities, is worthwhile in patients for whom conventional treatments have often limited impact on survival.

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