Early warning

Gene therapy

Status and potential in clinical medicine

SINTEF REPORT

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ABSTRACT

The report 7/2000 is an assessment of the potential of gene therapy in clinical medicine. A multidiciplinary group of experts have carried out the report.

KEYWORDS	ENGLISH	NORWEGIAN
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Preface

In the spring of 1998, the Reference Group of the Norwegian Centre for Health Technology Assessment (SMM) proposed that an assessment of the potential of gene therapy in clinical medicine be carried out. In the autumn of the same year, the SMM commissioned an 'early warning' report on this field from a multidisciplinary Group of Experts. The Ministry of Health and Social Affairs has been kept informed on the progress of this project on a continuous basis.

The interdisciplinary Group of Experts had these members:

Professor Erlend B. Smeland, the Norwegian Institute for Cancer Research, the Norwegian Radium Hospital, Chair
Professor Hans Prydz, the Biotechnology Centre of Oslo
Karen Helene Ørstavik, Chief Attending Physician, Ullevål Hospital
Professor Stig Frøland, National Hospital of Norway
Steinar Aamdal, Chief Attending Physician, the Norwegian Radium Hospital
Professor Ola Myklebost, Norwegian Board of Health
Professor Jaran Apold, Haukeland Hospital
Professor Anne-Lise Børresen-Dale, the Norwegian Institute for Cancer Research, the Norwegian Radium Hospital
Professor Erling Seeberg, National Hospital of Norway
Eivind Hovig, Research Scientist, the Norwegian Institute for Cancer Research, the Norwegian Radium Hospital (from April 1999)
Professor Hans Krokan, the Norwegian University of Science and Technology
Professor Kåre Berg, Ullevål Hospital
Håvard Attramadal, Research Director, National Hospital of Norway
Professor Jan Helge Solbakk, Centre for Medical Ethics, University of Oslo

The experts all have relevant experience, as they are all working on genetics-related issues and have a professional interest in the field .

Scientific adviser at the SMM, Anita Lyngstadaas, PhD PharmD has served as project co-ordinator.

Sveinung Løkke has translated the report.

All group members support the report.

The report was submitted to the SMM Steering Committee in December 1999.

The report has received the approval of the SMM Steering Committee.

Berit Mørland
Director

Anita Lyngstadaas
Scientific adviser

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A comment from the SMM Steering Committee

During the last few decades great advances have taken place in our knowledge and understanding of how the cell functions at the molecular level. It has taken some time before this has had an impact on clinical medicine; now, however, this latency period is definitely over. Today molecular biology makes inroads in all fields of clinical medicine. Practical implementation of molecular medicine includes procedures/strategies in therapy, diagnostics, and/or prevention. The present report was prepared by a Group of Experts for the Norwegian Centre for Health Technology Assessment (SMM), chaired by Professor Erlend B. Smeland and with Dr Anita Lyngstadaas as project co-ordinator. The report provides an account of potential therapeutic regimens based on new approaches in molecular biology, i.e. gene therapy**.** The report gives an account of the present status of somatic gene therapy, that is gene therapy on body cells. Moreover, on the basis of documentation derived by a systematic survey of protocols and findings, the group provides an assessment of the prospective uses of gene therapy in clinical medicine.

Gene therapy means that functional genetic material is transferred into a foreign cell in order to correct a genetic defect or to introduce a new function. In most cases, a prerequisite for using gene therapy is that the gene in question has been identified and studied in detail, and that the function and regulation of expression of the gene is known. Established in 1989, HUGO (the Human Genome Organisation) provides important contributions to the development of clinical gene therapy by sequencing the human genome. A first draft of the DNA sequence of the human genome is expected to be available in the course of the year 2000 (sequencing the first human chromosome was recently completed, in December 1999). The genome consists of an estimated 150,000 genes. The assumption is that the identification of all human genes will accelerate the speed of development of new pharmaceuticals. Pharmaceuticals based on gene therapy strategies include the transfer of therapeutic proteins by direct introduction of the genes in question in transport vehicles (vectors) and/or the introduction of small DNA/RNA molecules.

The concept of gene therapy has developed from only representing treatment of rare genetic diseases caused by changes in a single gene, to encompass all nucleic acid based treatment. Monogenic hereditary diseases, primary immunodeficiencies and cystic fibrosis are important groups, but gene therapy today also encompasses other disease groups such as cancer, infectious diseases (mainly HIV), and diseases of the cardiovascular system. Gene therapy studies of autoimmune diseases and certain neurological diseases have also been started. Most gene therapy studies have been carried out on cancer, but several disease groups and categories are expected to be candidates for future gene therapy. This report describes promising results observed in early clinical studies of cancer and of certain types of cardiovascular disease. However, the Steering Committee would like to emphasise that the effects of the method and its place in the health service can only be properly assessed in phase III studies designed as randomised controlled trials.

In the early 1980s, gene therapy was introduced in the health service with the promise of a 'new age' in medicine with potential therapies for all serious diseases. Quite clearly, this has not come about. So far, no patient has been cured of his disease through gene therapy. With one small exception, gene therapy is not an established therapy for any disease today. This report shows that extensive preclinical work has been carried out. This work, and a considerable number of early phase clinical trials have provided a solid scientific basis. Preliminary trials indicate that gene therapy is a safe method with surprisingly few, mostly local side effects reported. However, one death was recently reported which may be associated with the gene therapy itself. This death has led to a new revision of the safety aspects of gene therapy.

The most important barriers to achieving clinical effect have been identified. The main problem in today's gene therapy is the problem of gene transfer. The main focuses in gene therapy research are more efficient and targeted gene transfer, and controlled gene expression in the target cell. Seen in this light, the question is rather one of 'when' gene therapy becomes a therapeutic reality than a question of 'if'. There is optimism among scientists in the field, although no one dares to predict when the breakthrough will come.

The conclusion of the Group of Experts and the Steering Committee is that gene therapy is still in its infancy. The field is characterised by preclinical and clinical research focusing on preclinical issues. Early phase trials have shown limited clinical effects, but no results from phase III trials have been published. Therefore it is hard to predict what place gene therapy will have in Norwegian medicine in the future. A very small number of Norwegian patients have so far been treated with gene therapy in clinical research protocols. At the moment, few Norwegian professionals are active contributors in clinical gene therapy research. Gene therapy related research in molecular biology in Norway is mainly preclinical. The Steering Committee would like to point out that the recommendations given are the Group of Experts' own contribution; however, the Steering Committee recommends that the Group of Experts' recommendations are taken under advisement, and recommends its conclusions.

1. Summary

It has been approximately 10 years since the first patient was treated using gene therapy. Since then between three and four thousand patients have been treated with different gene therapy strategies in more than 400 clinical studies. In Norway the three first patients to be treated with gene therapy were recruited at The Norwegian Radium Hospital, as part of two approved protocols for cancer treatment. Both studies are large international multicentre studies.

Gene therapy is today dominated by preclinical and clinical research. Development of gene therapy must, like other new therapeutic methods, go through a series of different steps before it can be introduced for the regular treatment of patients. Preclinical research involves the use of both cell cultures and research animals. The next step is clinical trials in humans. Clinical trials can be subdivided into different phases (phase I, II and III). The effect of the methodology under assessment and its place within the health care system is primarily determined from phase III randomised controlled trials. Several thousand articles have been published regarding gene therapy. Of these only approximately 80 articles describe results from clinical trials. This indicates that most gene therapy-related research is focused on preclinical issues. The majority of gene therapy studies have been performed in the USA. Recent years have, however, witnessed a considerable increase in gene therapy-related research and development in Europe.

In this health technology assessment report a total of 401 gene therapy protocols were identified. Most of these studies are phase I or II with few patients in each study, and only three of the protocols represent phase III studies. It should be pointed out that none of the existing international databases of gene therapy protocols are complete, and that this report excludes gene marking in its definition of gene therapy.

Apart from the use of soluble antisense oligonucleotides against cytomegalovirus (CMV), which has been approved in USA for use in eye infections among AIDS patients, gene therapy is not an established treatment modality for any disease today. Phase III studies are on-going and results have not yet been published. It will probably take more than three years before definitive answers from independent phase III studies within the same disease subgroup have been generated. In Norway, gene therapy will probably remain at the clinical research stage for the next three to five years. Thus no patient in Norway today should be offered treatment with gene therapy unless they are included in clinical protocols (except for CMV antisense therapy).

1.1 Disease types

Gene therapy has been tested for different types of disease, in particular in different genetic diseases. This includes both inherited diseases, resulting from mutations in a single gene, and diseases where mutations in several genes can contribute to disease development, such as cancer. In addition, gene therapy can also be implemented in other diseases in order to add a drug or an active component. While most gene therapy studies

have been performed in the treatment of cancer, some have targeted viral infections (HIV in particular, but also others) and monogenic diseases where the gene is characterised and cloned. During the past few years several studies within cardiovascular diseases have been initiated and recently trials have also been commenced in autoimmune diseases and certain neurological diseases such as amyotrophic lateral sclerosis (ALS). What the current studies have in common is that all relate to patient groups having serious diseases that lack adequate alternative treatment. Promising results have already been observed in early cancer clinical trials and also recently in certain forms of cardiovascular diseases[1].

1.2 Summary of the most important conclusions and recommendations from the expert group

Three Norwegian patients have been treated with gene therapy against cancer. Gene therapy is today, with a minor exception (treatment of a special viral eye infection), not an established treatment modality in Norway. Norwegian patients should only be offered gene therapy as part of clinical studies

Gene therapy has developed to a point where it is important to build up national competence in the field. The Norwegian Ministry of Health and Social Affairs has recommended that government funds be directed towards competence building at two centres in Norway, Haukeland Hospital and The Norwegian Radium Hospital.

The expert group thoroughly discussed a national gene therapy effort in a five-year perspective. It was agreed that two different strategies should be used:

– To build up infrastructure at certain selected milieus.
– To start a national program for gene therapy research, which should include both preclinical and clinical research.

When gene therapy eventually becomes an established treatment modality, it will be necessary to establish facilities at the different regional hospitals and at The Norwegian Radium Hospital. It is suggested that the Ministry establish a scientific steering committee for the evaluation of projects that will be generated from these different centres.

Ethical considerations

– The expert group considers somatic gene therapy to be ethically sound and finds that this does not represent a new ethical principle. The expert group confirms that gene therapy, for the time being, must be reserved for treatment of serious disease.
– It is important to point out that both children and adults should be evaluated for gene therapy research. Gene therapy research should not be avoided among in children just because the subjects are children.
– The expert group finds that gene therapy *in utero* should not be performed until more knowledge is available.

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[1] After completion of the SMM report French scientists reported a breakthrough in April 2000. Two infants suffering from SCID (severe combined immuno-deficiency) were successfully treated for at least up to 10 months.

– Germline gene therapy or gene manipulation of embryos or germ cells should be forbidden in accordance with international consensus and Norwegian law.
– Genetic enhancement should not be allowed under any circumstances.
– It is important that the ethical discussions concerning gene therapy be on-going, in particular with respect to new gene therapy protocols.
– Research on the ethical issues related to gene therapy should be supported.
– The expert group maintains the necessity for a continued and informed public debate regarding these issues, in addition to the formal regulations and routines for approval which today regulate human gene therapy.

The expert group states that it is important that the safety and side effects of gene therapy continue to be thoroughly investigated and supervised.

The expert group considers it important to obtain more convenient procedures regarding the approval of gene therapy studies while at the same time ensuring that the process still covers the needs of society with respect to control and ethical considerations. It is suggested that three applications should be sufficient, one for laboratory approval, one for the ethical committee and yet another for approval of drug testing. It is recommended that the Norwegian Medicines Control Authority be augmented with a specific advisory committee for gene therapy, similar to the current situation in England.

The different procedures on gene therapy should be regulated with different safety restrictions. Thus, treatment with ribozymes and antisense oligonucleotides in soluble form, which is reminiscent of conventional drug treatment, should have more simplified safety demands than other gene therapy methods. It is recommended that smart viruses and gene marking studies, which are not included in the definition of the gene therapy in this report, should be regulated like gene therapy.

Gene therapy is still in an early phase of clinical research and it is difficult to foresee the influence of gene therapy in the Norwegian health care system in the future. As the results of several phase III clinical studies will be available in the next couple of years, it is recommended that a new evaluation of this field be performed within three to five years. This includes updating of the gene therapy databases.

A national strategy for the development of the gene therapy field is important to achieve. This will make it possible to systematically prioritise different gene therapy methods, to build up national competence in the field and to establish gene therapy as regular treatment whenever this will be indicated. A strengthening of the transfer between basic and clinical research (translational research) within gene therapy will prevent unnecessary pressure for establishment of such new and exciting methods before their clinical effect has been demonstrated, and thus also prevent the inappropriate and uncoordinated establishment of new methods at the national level.

2. Introduction

Gene therapy represents a new medical methodology and a rapidly expanding field. There are great hopes attached to clinical applications of gene therapy, but also considerable uncertainties. At the present stage there is no agreement on what role gene therapy might have in the treatment of disease. In this 'early warning' report, a multidisciplinary Group of Experts conducts a methodological assessment of gene therapy. The report charts and describes the present status of clinical gene therapy protocols and identifies important results from preclinical research. On this basis, the Group of Experts assesses the future potential for clinical application of gene therapy and the disease groups most likely to benefit from this type of intervention. Gene therapy is also discussed in relation to its impact on the Norwegian health services. The report is organised in introductory chapters on mandate, concepts and approaches. Then follow two general chapters on gene transfer and DNA vaccination, followed by chapters on various diseases with brief sketches of the causes of disease, existing therapeutic alternatives, strategies for gene therapy intervention, and experience so far with concluded or ongoing clinical studies, with a focus on published results and protocols as well as trends in the various fields. Two charters on legislation and ethics are included, and a chapter on status, potential and limitations and a chapter on the impact on Norwegian health services conclude the report. The report starts with a brief summary and the unanimous conclusions and recommendation of the Group of Experts.

The Group wanted the report to be as accessible to the public at large as the field allows. Each chapter on diseases is introduced by a short summary outlining the most important trends. An appendix provides simple explanations of the most important gene therapy terms.

2.1 Concepts

Health Technology Assessment

Health technology assessment (HTA) is an assessment of effects, side effects, costs and other impacts of procedures which are used or proposed for use in the health services in order to prevent, diagnose and treat disease. The essential aspect of the assessment is a systematic review (SR) of the literature, subjecting the available scientific evidence to a *critical* and *systematic* assessment. In order to minimise all types of bias, established procedures and quality criteria have been developed for use in the evaluation of the scientific 'evidence' on a clinical issue. Such assessments of existing research data and documentation are called secondary research and are conducted in collaboration between experts in the field and experts in methodology.

A systematic review focuses on clinical effects and is important for helping the scientific community systematising and compiling international documentation. However, decisions in the health services are not only based on medical/scientific knowledge; they are also affected by financial, ethical, organisational and legal concerns. Health technology assessment takes such aspects into account and balances the medical and

scientific conclusions against other framework conditions. The purpose of a health technology assessment of a given issue is to provide the best possible professional basis for decision-makers in the health service (clinicians, administrators, politicians). The themes may include areas where there is uncertainty or disagreement in the medical/scientific communities or among administrative or political policy-makers. The aim is to provide the best possible standardised treatment of the individual patient under the given framework conditions.

The Norwegian Centre for Health Technology Assessment (SMM) has national responsibilities for health technology assessment in Norway. The SMM is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international network linking non-profit publicly funded and sponsored centres.

Early identification and assessment

Identification and assessment of new methods *before* they are implemented in the health services is an important area for health technology assessment. This process is often termed 'early warning' or 'horizon scanning' and complements systematic reviews of procedures that are already introduced/established in the health services. Such 'early' methodological assessment of potential future procedures will often include interventions for which there is as yet no scientific evidence in terms of published, conclusive randomised controlled trials, or for which the clinical documentary basis is predominantly early phase studies.

There is a steadily increasing number of new medical methods. This makes it difficult for health-service decision-makers to keep abreast of the development of new knowledge. When new methods are rapidly implemented, decision-makers may have a weak scientific basis for assessing investments and resource allocation. Thus there is a growing interest in *early* and *independent* assessments of medical methods before they are introduced in the health services. Early assessments of a future medical method are primarily designed to facilitate communication between experts and policy-makers (politicians, administrators, clinicians) on issues related to the method. The assessments may, however, also be of interest to other stakeholders, be they doctors, industry, media, patients, or the public at large.

Relevant areas for early warning-type health technology assessment are those that are expected to be of great importance and imply major changes in the health services, including methods that

- have considerable financial consequences;
- are of concern to a large patient group or address a widespread health problem;
- have great impact on the structure and organisation of the health services;
- have ethical implications;
- are based on a scientific explanation which instigates debate;
- imply a medical breakthrough.

At a given stage, early warning may also include methods that are expected to spread more rapidly than desirable on the basis of present knowledge, or more slowly than desirable judges by its advantages.

Gene therapy

The Group of Experts defines gene therapy as: *Transfer of DNA/RNA to target cells for treatment purposes (nucleic acid based treatment)*. The definition is limited to apply to *somatic gene therapy as part of the treatment of a serious disease.*

Gene therapy on somatic cells implies a genetic change which is not passed on to future generations, hence *somatic gene therapy* is limited to *the individual undergoing treatment.* On the other hand, *germline based gene therapy*, i.e. gene transfer to germ cells or fertilised oocytes, will cause a genetic change that will be inherited. There is broad national and international consensus to the effect that germline based gene therapy is not ethically defensible today; hence Norway unambiguously prohibits germline based gene therapy. This report is thus only about somatic gene therapy, and the gene therapy concept is used synonymously with somatic gene therapy. Furthermore, there is agreement to the effect that gene therapy at the present stage should only be tried out for serious disease. The definition includes both DNA and RNA as genetic material. Moreover, the Group of Experts emphasises that only experimental *treatment* is allowed. The definition of gene therapy has changed over time; as of today there is no commonly accepted definition of the concept. The definition used by the Group of Experts conforms with the Norwegian Board of Health's definition of gene therapy and the definition used in most international forums, except that many countries include gene marking studies in the concept of gene therapy.

The Group of Experts subdivides gene therapy into four levels:

1. Gene transfer with integration (the gene is incorporated into the DNA)
2. Gene transfer without integration (the gene is not incorporated into the DNA)
3. The use of small synthetic oligonucleotides, so-called ribozyme/antisense molecules without regulatory elements which modify gene expression
4. Therapeutic DNA vaccines

Ribozyme/antisense molecules, incorporated into vectors (transport vehicles) belong at levels 1 or 2, while ribozyme/antisense molecules without replication sites of their own (i.e., potential for breeding) or regulatory elements belong at level 3. It is a debatable point whether the use of small synthetic ribozyme or antisense molecules should be included in the definition of gene therapy. The Group of Experts has, however, found it expedient to include studies using ribozyme or antisense molecules without regulatory elements. Such studies are, in fact, included in the big international databases on gene therapy studies.

Marking studies, 'smart viruses', and prophylactic (preventive) vaccines are not included under the definition. Marking studies have no therapeutic effect, but are used to mark certain cells. Smart virus studies have a principle which borders closely on gene therapy, but they do not come under the usual definition. As the use of these viruses probably should be regulated in a similar manner as for gene therapy, it seems warranted to include such viruses in this report. We therefore include a review of such viruses and the status of clinical trials. However, the protocols are not included in the evidence table, neither are prophylactic DNA vaccines, as they have a preventive, not a therapeutic effect. As the principles behind prophylactic and therapeutic DNA vaccines are the same, we have, however, included a brief account of DNA vaccines in the report.

Transfer of cells and tissue to patients for therapeutic purposes (cell therapy, transplantation) does not fall under the concept of gene therapy if the cells/tissue are not genetically modified before transfer to the patient. Other types of intervention which do not fall under the gene therapy concept, but still offer a potential for correcting genetic defects, are methods based on transplantation of the cell nucleus. In case of defects in the mitochondrial genome, the cell nucleus (which is free of gene defects) may be transferred to another cell which does not have a nucleus – a fertilised ovum with normal mitochondrial genome from which the original nucleus has been removed.

2.2 Clinical trials of new therapies

Present-day clinical trials of new drugs follow an established pattern. Substances which show promising effects *in vitro* (i.e., in a test tube) are tested on laboratory animals. These tests on animals are meant to provide information on acute or subacute/chronic toxicity and the pharmacokinetics and efficacy of the substance.

Those substances that seem most promising after testing in animal models, go on to clinical trials. These are usually step-by-step trials in which one tries to find answers to these questions:

I. What is the human tolerance of the substance?
II. Which disease group is most susceptible to treatment?
III. Does the substance constitute an improvement compared to today's treatment?

The answers to these questions may be found in so-called phase I to phase III trials.

Trial protocols

All scientific trials of new drugs require elaborate trial protocols which describe in detail which patients should be included, treatment regimen, criteria for response etc. For the patients, a detailed information brochure is developed which includes information about the objective of the trial, what benefit the patients may derive from the treatment, description of side effects, and an unambiguous statement to the fact that participation in the trial is voluntary, and that the patient may at any time withdraw from the trial. It is a requirement that the patient (or, if the patient is a child, next of kin) signs an informed consent statement.

Effect measurements

In phase II and phase III trials of new substances for cancer therapy, the focus is usually on a tumour that may be measured. Treatment is ranked on a response scale as sketched below.

CR: Complete remission, i.e. complete absence of tumour after the treatment.
PR: Partial remission, i.e. reduction of tumour size in relation to predetermined criteria.
NC: No change in tumour size.
PD: Progressive disease, i.e. increase in tumour size in relation to predetermined criteria.

For other diseases, similar effect measurements have to be defined.

Phase I trials

The primary objectives of phase I trials are to identify side effects, to estimate the maximum permissible dose in humans, and to search for signs of response.

Participation in phase I trials is offered to patients when all established therapies have failed or no such therapy exists. The first patients receive a low dose, expected to have minimal side effects on the basis of experience from animal models. Usually three patients are treated on each dose level. The dose level is not increased in the individual patient, but escalated in new groups of three patients each until unacceptable side effects occur. More patients (up to six) are then treated on this dose level or just below it until a reasonable estimate of the 'maximum permissible dose' is reached. Very comprehensive records of subjective and objective side effects are kept.

Gene therapy gives few objective side effects, hence the 'maximum permissible dose' concept is often not suitable for such treatment.

Phase II trials

Even if a new treatment has not shown unambiguous signs of therapeutic effect during the phase I trial, it has to go into further trials on groups of patients who have not received so much previous treatment.

The aim of phase II trials is to investigate whether the drug has therapeutic effect. In trials of substances for cancer therapy, the requirement usually is that at least 20 per cent of patients with a certain tumour type shall have shown objective response (CR or PR) before a new therapy is recommended for further trials.

Phase II trials usually use a slightly lower dose than the 'maximum permissible doses' in phase I trials. Painstaking recording of side effects is also maintained during phase II trials.

The standard indication that a therapy works is decreasing tumour size, complete remission or partial remission, while no change in tumour size (NC) is deemed insufficient.

In trials of what is referred to as biological therapies, such as gene therapy for cancer, the usual effect measurement criteria are not always suitable. No change in tumour size, but prolonged life, could be a sign that a treatment is effective.

Phase III trials

Phase III trials are a more elaborate identification of the place of a new therapy among medical therapeutic modalities. The treatment has to be tested against the established modalities, usually in randomised clinical trials. This means that patients are randomly assigned to either established treatments or to the new treatment, and that results are compared.

Future clinical gene therapy trials will probably follow a pattern that is different form

the classical phase I–III trials. Changes in the standard clinical trials procedure were recently proposed in order to better address the special concerns relating to clinical trials of gene therapy (James W. Wilson, 1999 American Society of Gene Therapy Presidential Address, http://www.med.upenn.edu/ihgt/info/asgt99.html). Reasons for this includes that phase I trials, which are focused on biological safety, often do not provide interesting answers to gene therapy approaches. This is because optimal dose does not necessarily correspond to maximum permissible dose, and because the intervention, given enhanced understanding of the biology, should be studied in the context of disease pathogenesis. Furthermore, given the increasing molecular specialisation, there will not be sufficiently large disease groups available for a strict phase I, II and III regimen. Among the proposals put forward by Dr Wilson is a change in the classical phase I and phase I/II trials in a way that relates the first clinical trials to a broader biological understanding of the disease. Dr Wilson uses the concept of 'BETA trials' ('Biological Efficacy and Toxicity Assessment'), thus proposing that biological efficacy should be studied in the first clinical phase. This places higher requirements on biological relevance in early clinical trials. This might lead to new methodological approaches with more rapid progress as a potential result.

3. Mandate and approach

3.1 Mandate

The Group of Experts was given this mandate by the SMM Steering Group:

- The Group of Experts shall identify and give an account of the status of completed and ongoing clinical protocols in gene therapy.
- On the basis of this identification, the Group of Experts shall assess the prospect for clinical application of gene therapy and the groups of disease for which it holds most promise.
- The Group of Experts shall discuss gene therapy in relation to its consequences for the Norwegian health service.

In order to provide better forecasts of the future use of gene therapy in clinical medicine, the Group of Experts shall identify important trends by also including preclinical research data in the assessment.

3.2 Approach

The approach chosen for responding to the various elements in the mandate was two-fold, a systematic and a non-systematic approach:

- systematic approach: identification of protocols/results
- non-systematic approach: general searches in the literature

Identification of protocols/results

The assessment is based on a systematically identified body of documentation consisting of clinical gene therapy protocols, ongoing or completed with published findings, if any. The Group of Experts' definition of gene therapy formed the basis for including or excluding protocols. The process of identifying the protocols that should be included had four stages:

- identification of descriptive protocols;
- identification of *publications* (protocols and/or findings);
- inclusion in a database;
- presentation of data in evidence tables.

Identification of descriptive protocols

Clinical gene therapy trials, concluded as well as ongoing, and results from such trials, were identified in the Human Gene Therapy Protocols of the National Institutes of Health (NIH) (http://nih.gov/od/orda) and the *Journal of Gene Medicine* website (http://www.wiley.co.uk/genmed). The Human Gene Therapy Protocols of the NIH are a list of gene therapy protocols approved by US authorities or for which an application for approval has been submitted. The NIH includes marker studies in its definition of gene therapy, and the list (which as of 18 May 1999 counted 313 protocols) gives a detailed description of each protocol. The *Journal of Gene Medicine* web page includes a database (Wiley's database) which aims at providing a comprehensive survey of all gene therapy protocols in the world. The database also includes protocols for marker studies;

as of 1 September 1999 it held a total of 396 protocols, though the information relating to the individual protocols is of varying quality. The NIH list and the *Journal of Gene Therapy* are updated at regular intervals; however, identification of identical protocols in these two sources is complicated as 'Wiley's database'

- does not refer to the protocol number of the NIH;
- in some cases has incomplete information about protocols;
- does not include all US protocols;
- is not complete with regard to European protocols;
- includes several partial protocols under a main protocol or multicentre trial;
- is updated at other intervals than the NIH list.

Hence the first stage towards identifying gene therapy protocols was to

- compile an inventory of protocols complying with the Group of Experts' definition of gene therapy:
- identify identical protocols in order to eliminate duplicates.

Identification of publications

Publications describing protocols and/or findings from protocols were identified by literature searches in Medline (http://www.nlm.nih.gov) and Embase (DIALOG). The searches in Medline (Grateful Med and PubMed) were conducted using the search words *gene therapy, gene transfer, DNA vaccines, antisense, ribozymes, cancer vaccines* – all combined with *clinical trials* as type of publication. Additional searches were carried out in Embase. The content pages of mainstream journals in the field, such as *Human Gene Therapy*, *Science*, *Nature*, *Nature Medicine* and *Journal of Gene Medicine* were checked thoroughly for additional protocols/findings. In addition, members of the group contributed by identifying publications within their fields of expertise. Relevant publications identified through such additional searches are often indexed in Medline as other types of publications, e.g. *journal article* or *review.*

Protocols identified in the form of publications were combined with protocols retrieved from the NIH and 'Wiley's database', and new findings registered. The literature search revealed considerable lacunas in the NIH list and in 'Wiley's database', particularly in relation to protocols under *DNA/cancer vaccines, antisense* or *ribozymes*.

Generation of a database

As no international database for gene therapy protocols is complete, an English-version database was set up containing gene therapy protocols, which fall under the Group of Experts' definition of gene therapy. Generation and classification of protocols was carried out in a FileMaker database. Identical protocols were identified and duplicates deleted. A total of 401 protocols were identified. The information available on each protocol was systematised according to:

- publication of protocol and/or findings;
- identification number in the NIH list and/or 'Wiley's database', and title/objective of the study;
- disease group and disease category;
- principle;
- details of the protocol: phase, gene transferred, vector, *in vivo/in vitro*, co-

intervention, mode of administration, target cell, number of patients, date;
- findings; side effects, effect on clinical outcome.

Presentation of data in evidence tables
Information on all identified gene therapy protocols is presented in an evidence table by disease group, clinical trial phase, and disease category or principle. A comprehensive evidence table may be obtained from The Norwegian Centre for Health Technology Assessment (SMM). In a methodology assessment context it is important to present the documentary basis in the field; thus the report only presents an evidence table that includes protocols with *published* results (a total of 81 protocols; Annex 1).

Data on clinical gene therapy identified through systematic mapping of protocols in evidence tables form the documentary basis for the Group of Experts' reply to the mandate items relating to i) the present state of clinical protocols and ii) an assessment of the prospect of clinical application and the disease groups for which it has most relevance.

Other searches in the literature

For the purpose of replying to the items in the mandate, more general non-systematic Medline searches has been carried out in addition to the systematic mapping of clinical gene therapy protocols.
- In its description of the status of clinical protocols in the various disease groups/disease categories and its assessment of the potential in clinical medicine, the Group of Experts also used relevant literature obtained through non-systematic searches, e.g. review articles.
- The identification of important trends in gene therapy is completely based on an assessment of preclinical literature retrieved through such general searches.
- Non-systematic searches also form the basis for the chapters devoted to gene transfer, DNA vaccines, legislation and ethics.

4. Gene transfer – modes of administration, advantages, potential drawbacks and dangers

For DNA in genes or gene fragments to be of use in the treatment of disease, one needs good methods of transfer to target cells in humans where the new DNA must be expressed in the form of RNA and/or protein. Right now, developing safe and efficient transfer systems is the biggest obstacle to exploiting the large potential for treatment which gene therapy represents. A detailed introduction to transfer systems used in gene therapy lies beyond the scope of this report, which only present a brief overview of major principles. Are more detailed introduction may be found in several recent review articles (1–3).

In gene therapy the gene or the gene fragment that is of interest is usually inserted in a vector (often modified virus DNA/RNA or plasmid DNA) which is a crucial part of the transfer system (transport vehicles). Therapeutic DNA is inserted in the vector at a place where it often replaces DNA that is not essential for the transfer or multiplication of virus. Work is in progress on a range of different transfer systems. So far research and development work has concentrated on various virus-based systems, in particular various retrovirus, adenovirus, adeno-associated virus, herpesvirus, pox virus and polyoma virus, or DNA bound up with chemically defined substances, notably liposomes (lipid membranes). Experiments have also been made, to some extent with promising results, with transfer of 'naked' DNA, either through injection, gene cannons, or other methods. The most prevalent transfer systems are listed in Table 4-1.

Table 4-1. Vectors used in gene therapy*

Virus-based vectors:
• Retrovirus (RNA virus), including lentivirus
• Adenovirus (DNA virus)
• Adeno-associated virus (DNA virus)
• Herpes simplex virus (DNA virus)
Vaccinia virus (DNA virus)
Non-viral vectors:
• Plasmid DNA
• Liposome-bound DNA
• Protein DNA conjugates
• Artificial chromosomes

* Modified from Desnick et al., 1998

For some frequently used virus-based transfer systems it has proved difficult to make sufficient quantities of genetically modified virus particles. The prime examples in this respect are retroviral derivatives. These systems have, however, been extremely important in terms of demonstrating gene therapy as a potential therapeutic principle. Therapeutic effect, safety and potential problems can only be sufficiently studied when efficient transfer systems have been developed.

The administration of synthetic, relatively small DNA molecules (synthetic oligonucleotides) for targeted action on the gene expression represents another therapeutic modality, which most professionals now count as gene therapy. In this therapeutic modality, inserted DNA cannot be multiplied in the cells or be incorporated in the DNA. Relatively large amounts of oligonucleotides must be injected. The oligonucleotides will then gradually be eliminated from the cells through various mechanisms. In point of principle, this type of gene therapy has much in common with conventional drug treatment, but it can have much higher selectivity and increases dramatically the number of potential targets for therapy.

Somatic gene therapy may be divided into three categories: *Ex vivo:* Removing cells from the body, treating them with a vector carrying the therapeutic DNA, and returning the genetically modified cells to the organism. So far this has mostly been an option with blood cells and skin cells. *In situ:* Placing the vector carrying the therapeutic DNA directly into the tissue in which the gene will function. This has been tried out for airways/lungs in cystic fibrosis and some forms of cancer; and *in vivo;* here the therapeutic DNA-carrying vector might for instance be injected into the bloodstream to be taken up by target cells. So far there are few examples of this latter type of gene therapy. However, this is assumed to become a major type of gene therapy when suitable transfer systems have been developed. Synthetic oligonucleotides will probably only be relevant for *in vivo* treatment.

The present view is that the safety problems are mainly related to the potential disease-inducing properties of virus-based systems, and to gene inactivation caused by insertion of DNA in places where the new DNA may inactivate genes in the chromosome. One also has to assume that the expression of a new gene in large quantities, or in cells where it normally is not expressed, in some cases may lead to unintended side effects. It has also been shown that therapeutic DNA taken up in cells in some cases functions only for a period of time and then disappears. This is a usual problem when new DNA is not integrated in chromosomal DNA. With some systems it is a problem that the vector is only taken up by and/or expressed in some of the target cells, i.e. only in cells that divide.

4.1 A short introduction to transfer systems

Viral vectors

Both RNA viruses and DNA viruses have been used as transfer systems in gene therapy; the most widely used viruses are listed in Table 4.1. Ideally, a viral vector will give a regulated expression of the therapeutic DNA in the target cells only and not in other cells; it should give a stable and continuous expression and not be harmful to the host. It should also be practically feasible to produce sufficient quantities of the transfer system in a cost-efficient manner and with minimal variation between the units of production.

As of today, no system meets these requirements. In order to minimise the risk of the virus causing disease, the virus used is modified so that genes causing disease are not present, or viruses which do not cause any known disease in humans. The virus should be replication-deficient, so that the potential risk is reduced as much as possible. The gene of interest is inserted into the viral genome in a region in which it replaces genetic material which is not necessary for the reproduction of the virus within the cell, for integration in host cell DNA (for viruses able to integrate), or for the formation of the proteins that form the capsid (the viral shell), The proteins in the viral shell are needed for uptake in the target cell. In order to achieve selective uptake of the virus in the target cell, viruses may be used that are naturally only taken up by the target cells. For example, many adenoviruses will normally only be taken up by cells in the airways. The vector may also be equipped with new genetic material which forms modified shell proteins which result in recognition and selective uptake in certain target cells. It is also possible to achieve selective effect in certain target cells by expressing the therapeutic gene under the control of a promoter only active in the target cells.

RNA viruses

Retrovirus: Retroviral vectors have been used in about 60 per cent of all clinical gene therapy protocols (1). Several different genetically modified retroviruses have been tried; genetically modified murine leukaemia virus (MuLV) is the most widely used. Retrovirus particles contain a polymerase (reverse transcriptase, RT) which may generate a DNA copy of the RNA genetic material through 'reverse transcription', hence the term retrovirus. The virus particle also contains an integrase needed for integration. In principle, the ability to integrate make retroviral vectors well suited for gene therapy. A double-stranded DNA copy of retrovirus RNA is integrated into the chromosomes of the host cell as a 'provirus'. The integration implies that the new DNA is copied and inherited by both daughter cells through division, thus the DNA is inherited in a stable manner, contrary to DNA from viral vectors which does not integrate. In modified retroviral vectors one may add up to 8 kilobases (kb) of new DNA. Retroviruses have mostly been used in *ex vivo* contexts, e.g. for gene therapy on blood cells removed from the patient. The limited uptake in relevant cells via the naturally occurring MuLV receptor is one problem with such vectors. Other problems are related to low uptake in non-dividing cells. One must expect problems related to inactivation of genes in sites where retroviral DNA is integrated.

The problem with low uptake of vectors in non-dividing cells may possibly be solved by using new retroviral vectors based on lentivirus, the type of retrovirus to which HIV-1 (4) and many other retroviruses belong. Lentivirus-based vectors are taken up by dividing and non-dividing cells. As with the other retroviruses, these vectors integrated into the genome in the target cells. Lentivirus having a limited scope in terms of target cell (to CD4+ cells), hybrid vectors have been made in which the 'content' is taken from, e.g., HIV-1, while the 'packaging', which determines which cells the virus may invade, have been taken from Vesicular stomatitis virus or from MuLV. By taking away all unnecessary (in the context of vectors) genetic material from lentivirus vector RNA, one has made the vector as harmless as possible without reducing the transfer efficiency to non-dividing cells.

Cost-efficient production of large quantities of modified retrovirus has proved to be difficult. It is difficult to see short-term solutions to this problem for 'standard'

retrovirus (e.g. MuMLV), hence such retrovirus will probably mainly be used for *ex vivo* gene therapy which requires transfer of the vector to a much lower number of cells than *in vivo* therapy. The vector can only be produced in living cells. Because it is a replication defect in itself, it has to be produced in special 'package cells' that compensates for the defect in the vector. A potential hazard is that replication-competent viruses may develop in recombination processes during production. Mammalian cells often contain endogenous retrovirus, or at least remnants of retroviral sequences in the chromosome, hence pathogenic retrovirus could possibly develop through recombination processes in the cells. In a production context and in view of the hazard of unwanted recombination, the new 'stripped' lentivirus-based vectors may prove easier to handle.

DNA virus

Adenovirus: Adenovirus (AV) is a double-stranded DNA virus which infects several cell types, cells in the airways in particular. Known adenoviruses give mild airway symptoms only or do not cause disease. They are the most widely used viruses (serotype 2 and 5 in particular) for *in situ* transfer of therapeutic DNA, e.g. in trials of therapies for cystic fibrosis in which airway problems are a major concern. AV vectors also have the advantage of absorbing larger quantities of therapeutic DNA (up to 35 kb) than retroviral vectors.

AV is not integrated into chromosomal DNA, but replicates in the cell's nucleus. One advantage with AV is that, unlike retrovirus, it infects both dividing and non-dividing cells. Experiments with AV vectors have shown that considerable expression of marker genes may be achieved, e.g. b-galactosidase, or therapeutic genes, e.g. cystic fibrosis transmembrane protein (CFTR); but the response is short-term. This implies that repeated transfers are needed. This may complicated by immunological and inflammatory responses as described below.

First generation vectors with defects in 'early region' (E1), making them replication-deficient, and defects in E3 (to make room for inserted therapeutic DNA) have been shown to cause considerable inflammatory responses and immune responses. This will limit their potential for repeated *in situ* treatment. Efforts have been made to genetically modify AV vectors in order to eliminate inflammatory and immune responses by removing as many AV genes as possible, but the problem is not yet unambiguously solved. Probably the first death in the context of a gene therapy trial took place after intravenous injection of an adenovirus-based vector with high titre (10^{13} particles). However, AV vectors are promising in spite of these problems, and they are the objects of intense research and development work.

Adeno-associated virus (AAV): AAV contains single-stranded DNA and are non-pathogenic parvovirus that requires coinfection with AV auxiliary viruses or some types of herpesvirus in order to replicate. They are common in humans; about 80 per cent of the human population are infected. They replicate as double-stranded DNA. In the absence of auxiliary viruses, AAV-DNA integrates in a special region on the short arm of chromosome 19. The almost site-specific integration is among the factors, which make AAV attractive candidates as vectors for gene therapy. AAV are small viruses (double-stranded replication intermediate is only 4.7–4.8 kb), but more than 95 per cent of viral sequences may be removed and give room for about 4.7 kb of therapeutic DNA.

However, this is still too little for a large number of genes; space is a problem. Another problem is that integrated AAV may be reactivated by later infection with AV that may function as an auxiliary virus. AAV will then be cut out from the chromosome. Furthermore, many deleted AAVs lack the ability for specific integration. Also, so far one does not have suitable package cells for efficient vector production (review by Desnick et al., 1998). In spite of these limitation, intense research and development work is carried out on AAVs. In animal models (mice and dogs), outstanding results have been achieved with long-term partial correction (more than eight months for dogs and more than 17 months for mice) of haemophilia B after infusion of recombinant AAV vectors in the portal vein or the caudal vein.

Other transfer mechanisms

Several other transfer mechanisms for DNA have been tried; a number of them are under continuous improvement. This applies to insertion of plasmids ('naked' DNA), through injection or direct unmediated uptake, cationic liposomes or liposomes with antibodies against the target cell's surface component, electric discharges, or directly by firing on the target cells with small, DNA-coated particles from gas-propelled pistols. Binding plasmids to the surface of target cells through receptor binding or antibody binding might greatly increase specificity.

Plasmids are only rarely incorporated in the genome. Having entered the target cell, they are able to express the gene they have been equipped with; however, they slowly degrade because of the cell's own enzymes. Plasmids may easily be equipped with tissue-specific control elements (promoters, positive or negative regulating elements) which may limit the expression of the inserted gene to one cell type.

The number of such tissue-specific promoter systems is steadily increasing. In an increasing number of systems, the expression of the inserted gene may also be turned on and off. Given the advances in knowledge about gene regulation, we may expect that over time, plasmids will be developed to a high degree of precision in terms of where they are taken up, and when, where and how much the genes that they are carrying are expressed. Genes that have been used or could be used, may be related to a range of possible strategies ('prodrug', increased stimulation of the patient's immune system, reduced chemotherapy resistance in cancer cells/increased chemotherapy resistance in for instance bone marrow cells).

The so-called 'Human Artificial Chromosomes' (HACs) are new and fundamentally different tools for insertion of genes in a cell. These are model chromosomes, made on the basis of knowledge about which elements in a chromosome are necessary for its function (telomeres, centromeres, arms in which more or less complex gene sequences are inserted). In animal models with the more or less similar MACs (Mouse Artificial Chromosomes), such chromosomes have been made to replicate (each chromosome is copied) and to segregate (the artificial chromosomes are divided equally on the two daughter cells and are inherited). This indicates that artificial human chromosomes may be made in which genes may be inserted in a well-controlled and regulated manner. Well into the nucleus, the genes in the artificial chromosomes may be expressed and regulated as realistically as possible. By using artificial chromosomes one avoids the problems of introducing mutations in the genome (the therapeutic gene is integrated in the site of another gene and may destroy it).

4.2 Antisense oligonucleotides and ribozymes

Short sequences of the DNA nucleotides, A, C, G, T, may be selected so that they correspond (are complementary and antiparallel) to the sequence of nucleotides in a certain segment of a certain gene whose expression we wish to influence (the target gene). If these sequences are built into a chain of suitable length (at least 18–20 nucleotides), this chain may bind with a high degree of specificity to the corresponding segment on the target gene or to the copy of the target gene in the form of messenger RNA (mRNA). This causes destruction of the mRNA and a delay or stop in the regeneration of this RNA.

The expression of a gene may be even more efficiently suppressed through the use of ribozymes, in which the antisense effect is combined with an enzymatic cleaving of mRNA (5). Ribozymes are short (30–50) chains of modified DNA or RNA building blocks with the property of binding to certain segments of the RNA strand and cut it so that it can no longer serve as a messenger with instructions from the genetic material in the nucleus to the cell cytoplasm. Such ribozymes may seem to be effective during 12 to 24 hours; one might imagine suppressing the expression of a gene with daily supplies of ribozyme. It is a debatable point whether strategies based on antisense and ribozyme technology should be included in the concept of 'gene therapy'. In Norwegian terminology they so far are; in other countries there are several ongoing clinical trials based on these principles.

4.3 Other strategies

As knowledge of the human genome increases, we have a growing number of examples of genes which may step in for each other with respect to function, wholly or in part, but which are not expressed simultaneously in the patient's life or at the same place in the patient's body. In monogenic diseases caused by one gene falling out, e.g. b-thalassaemia, the corresponding gene which is expressed during fetal life, may step in if it is allowed expression. Here, no insertion of a new gene is required, but an influence on or change of the regulatory mechanism of the gene.

Methods based on nuclear transplantation are a different type of intervention, usually not included under the gene therapy concept, but still having the potential for correcting genetic defects. In cases of defects in the mitochondrial genome, the cell nucleus, which is free of genetic defects, may be transferred to another cell without a nucleus – a fertilized ovum with normal mitochondrial genome from which the original nucleus has been removed.

4.4 References

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5. DNA vaccines

There is no doubt that vaccines represent one of the most important developments in modern medicine, completely or almost completely eradicating several extremely serious infectious diseases, e.g. smallpox and poliomyelitis. Traditional vaccines represent injections of whole inactivated/non-virulent pathogenic microbes or fragments of them, triggering an immune response stopping or preventing later attacks by the same virulent mechanism. It has, however, been shown that this method is not always applicable or successful; in some cases it has indeed caused serious disease. DNA vaccines are a relatively new concept in which DNA fragments/genes are introduced intramuscularly, triggering an immune response directed towards the gene product made by the foreign DNA fragment (1–3). When a microbe with the gene product in question later enters the body, its further growth will be prevented by the antibodies produced (humoral response) or activated T-cells (cellular response) directed specifically towards the foreign protein. As this method involves injections of genes and DNA, it may be seen as a special type of gene therapy, particularly if the patient is already infected or the vaccine is geared to killing cancer cells. As the technology will be the same in preventive contexts (i.e., inoculations against future disease), we have chosen to comment on DNA vaccines in a generic sense.

5.1 A short introduction to the technology

DNA inoculation as a principle was used in laboratory animals as early as 30 to 40 years back, as injection of DNA isolated from pathogenic microbes was found to trigger an immune reaction against the organism itself. The conclusion drawn from this finding was that proteins had to be made from isolated DNA which in turn triggered immune response. When recombinant DNA techniques were introduced it became feasible to ‘bring out’ defined DNA fragments/genes from pathogenic organism and link them to carrier molecules (plasmids) which later could be bred in large numbers in quite harmless bacteria. This way the vaccine could be isolated inexpensively and efficiently, and the plasmids have a long storage life, which makes them simple to produce and handle. Experiments on laboratory animals, mice in particular, have shown that when injected intramuscularly, the plasmids trigger an immune response against the gene products formed by DNA on plasmids. This means that plasmid DNA enters the cell nucleus; then the genetic material is decoded and the relevant gene products generated. These products are then secreted, or degraded and presented on the surface of T-cells and trigger a humoral response (formation of free antibodies) and/or a cellular response. The cellular response is probably domineering and usually also the most important in the immediate immune response against invading microbes. This concept has truly unique potential, given the possibility of engineering DNA on plasmids in quite specific ways and isolating genes for the most important surface proteins of pathogenic microbes. Over time attempt will be made at mounting on one plasmid several different antigen proteins from the same organism. It has also been shown that the combination of the gene for the antigen, if any, and genes for certain types of cytokines and chemokines (signal molecules) greatly stimulates immune response and thus improves the vaccine.

5.2 Preventing infectious diseases

Most clinical trials are in phase I or II (see Table 5-1). Generally speaking, a better immune response has been achieved in laboratory animals than in humans. There are still many steps in the technology, which have to be improved, for instance by achieving efficient implantation of DNA in order to trigger the best possible immune response. Work is also in progress on using RNA directly in order to circumvent the need for the gene reaching the cell nucleus. The DNA vaccine developed against malaria is so far probably the most promising (4). Malaria is caused by a parasite, *Plasmodium*, which is a considerably more complex organism than viruses and bacteria. The 'target gene' used in the DNA vaccine codes for a known surface protein (circumsporozoite protein, CS) in *Plasmodium*. It is known that this protein is a potent antigen and a serologic test for antibodies against CS is now used as an indicator of malaria infection.

Table 5-1. Examples of clinical trials (phase I/II) of DNA vaccines for the prevention of infectious diseases (adapted from Weiner and Kennedy, ref. 3)

Infectious agent	DNA/gene	Immune response
Hepatitis B	Hepatitis B surface antigen	Humoral and cellular response
Herpes simplex	Herpes glycoprotein	Ongoing immune analysis
HIV	Capsid and regulatory proteins, nucleic proteins and enzymes involved in DNA replication	Cellular response to nuclear proteins
Influenza	Haemagglutinin	Ongoing immune analysis
Malaria	Circumsporozoite protein	Cellular response

5.3 Treatment of infectious diseases

DNA vaccines also have considerable potential in the treatment of patients, especially in combination with other treatment modalities (Table 5-2). This might be particularly efficient in relation to HIV infection and other diseases causing immunodeficiency.

Table 5-2. Examples of clinical trials (phase I/II) of DNA vaccines for the treatment of viral infectious diseases (adapted from Weiner and Kennedy, ref. 3)

Infectious agent	DNA/gene	Immune response
HIV	Capsid, regulatory and nucleic proteins and enzymes involved in DNA replication	Combination with other drug treatment. Ongoing immune analysis
HIV	Capsid and regulatory proteins, nucleic proteins, or tat, nef and regulatory proteins	Humoral response in the first experimental design, cellular response in the second

5.4 Cancer therapy

DNA vaccines might also be used to stimulate the body's immune system against certain forms of cancer, which express specific surface proteins. Phase I/II trials are under development (Table 5-3).

Table 5-3. Cancer therapy with DNA vaccines. Clinical trials (phase I/II)

Type of cancer	Vaccine gene	Immune response
Adenocarcinoma	Carcinoembryonic antigen (CEA)	Cellular response
B-cell lymphoma	T-cell receptor	Humoral response
Cutaneous T-cell lymphoma	T-cell receptor	Ongoing immune analysis
Prostate cancer	Prostate-specific surface antigen	Ongoing immune analysis

5.5 Conclusion: future aspects

DNA vaccines are seen as very promising, with applications in preventive medicine as well as in therapy. Such vaccines will have potentials against infectious diseases and cancers in which the immune defence is of great importance. However, in spite of promising results so far considerable challenges remain in technology development. Among them are:

- Prediction, selection and identification of good antigens and combinations of these
- Increased plasmid stability and continuous antigen response
- Dosage and the most efficient mode of administration
- Cell-specific targeting of the vaccine and co-stimulation of immune response
- Combination therapies with other modalities for high efficiency and broad-spectrum immune response

It is expected that DNA vaccines will go on to phase III trials in the near future, and that there will soon be clinical data available on the clinical importance of DNA vaccines.

5.6 References

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6. Gene therapy – publications and protocols

Gene therapy is a rapidly expanding field of medical research. A literature search in Medline on the search word ‘gene therapy’ may serve to illustrate the interest in gene therapy among scientist world-wide. Figure 6-1 shows the number of hits (articles per year) from a search for ‘gene therapy’, in title, abstract and/or as an indexing concept.

Figure 6-1. Number of references in Medline; search word: gene therapy

Gene therapy emerged as a field in the 1980s; this is reflected in the low number of articles published during that decade. Activity in the field increased considerably in the 1990s and reached a temporary climax in 1998 with approximately 1800 published articles including gene therapy.

As other new medical methods, research on gene therapy must go through various stages before the method can be introduced in the health services. The earliest stage, preclinical research, comprises use of cell cultures as well as laboratory animals. The next stage is clinical trials on humans. Such experimental treatment is subdivided into different phases (phases I, II and III; see the introductory chapter). The method’s efficiency and application in the health service is first assessed in phase III trials through randomised controlled trials. Gene therapy articles identified in the Medline searches mentioned above reflect all the stages sketched out, but do not show the distribution on preclinical/clinical research or early/late phase trials.

Medical articles are indexed in Medline according to type of publication; the most widely used types are: i) review article, ii) editorial, iii) clinical trial (CT), iv)

randomised controlled trial (RCT), and v) letter. Not all articles are indexed this way, but are included in a wider group of scientific publications (journal articles). By including type of publication in the search, we get a picture of the share of the medical literature that may be of clinical relevance. Figure 6-2 shows the distribution by type of publication of articles in which 'gene therapy' is used as a search word.

Figure 6-2. Number of references in Medline by type of publication

Figure 6-2 shows a clear trend in terms of type of publication. Few articles report clinical gene therapy trials and of these, extremely few are indexed as randomised. For example, in 1998 only three out of 34 clinical studies were indexed as randomised-phase trials (results are not reported during this phase). This indicates that the clinical documentation is dominated by early phase studies, and that most of the research concentrates on preclinical issues. The large proportion of review articles illustrates the great interest in gene therapy issues. The 'All' group includes all types of scientific articles, journal articles as well as the types of publication mentioned.

In this methodology assessment exercise, a total of 401 completed and ongoing gene therapy protocols were identified. Figure 6-3 shows the distribution of the protocols by clinical phase.

Figure 6-3. Distribution of protocols by phase (I, I/II, II and III)

Extremely few protocols represent phase III trials. Figure 6-3 clearly shows that clinical documentation is dominated by studies in an early phase (phases I and II).

The 401 gene therapy protocols are conducted on five main groups of diseases: i) cancer, ii) monogenic diseases, iii) infectious diseases, iv) cardiovascular disease, and v) other diseases. Figure 6-4 shows the distribution by group of disease.

No. of protocols Cancer Monogenic diseases Infectious diseases Cardiovascular disease Other diseases

Figure 6-4. Number of protocols by disease group

Gene therapy protocols on cancer dominate; there are, however, also several protocols on monogenic and infectious diseases. The ‘Other diseases’ group includes two protocols on rheumatoid arthritis, one protocol on amyotrophic lateral sclerosis and one protocol on cubital tunnel syndrome.

7. Gene therapy against cancer

7.1 Summary

Internationally, about 70 per cent of gene therapy studies started are targeted at cancer; the two first gene therapy studies approved in Norway are also targeted at this disease category. So far, three patients in Norway have undergone gene therapy. Cancer is a highly prevalent disease, and both incidence and the numbers of people dying from cancer will increase in the years ahead. There has been a vast increase in our understanding of cancer and the development of cancer over the last few years, and new treatment modalities based on this knowledge are being tried out. Cancer is caused by changes in genes (the hereditary material); hence, correcting genetic defects in cancer cells through gene therapy is a fascinating thought. Gene therapy may also conceivably be used to strengthen the immune apparatus in order to destroy cancer cells and in order to make them more amenable to chemotherapy and radiotherapy.

However, before we get that far, several problems have to be solved. Most studies up until now are early clinical trials primarily designed to study safety, applicability and toxicity. Several of these phase I and II studies have, however, shown partial remission of cancer tumours and, in rare cases, complete remission, although complete restoration of health has not yet been shown. Several different principles are used, including immunotherapy with cytokines and foreign histocompatibility antigens, replacement of a defective tumour suppressor gene, 'suicide gene' therapy. In some trials, including p53 gene therapy trials, effects on tumour size have been observed in more than half of the patients. Up until now, only two phase III and one phase II/III studies have been started up, and results from these studies will not be available for some years. Several new trials based on improvements in earlier protocols and/or combination with other treatment modalities have also been started. The results from these trials are not yet published. It should be pointed out that most cancer patients included in gene therapy studies so far are patients in a late stage of the disease; probably they are not the patients most likely to profit from such treatment. As of today, the results should be seen as promising for some of the principles which are being tried out (1); their clinical importance must, however, be documented in larger controlled clinical trials.

7.2 Introduction

Cancer is a serious health problem and the second most common cause of death in Norway. The number of new cancer cases is expected to increase considerably in Norway over the next several years (Norwegian Cancer Plan – Care and Knowledge, Norwegian Official Report 1997:20). There has been a gradual improvement in the prognosis for cancer patients. While 30 years ago five-year survival without remission was about 30 per cent, it is now about 52 per cent. Mortality will still increase in the years to come because of a higher incidence of cancer. There are few reasons to expect considerable improvement in survival for cancer patients on the basis of modifications to traditional treatment modalities; hence there is a great deal of interest in developing new treatment strategies based on the explosion in biological knowledge relating to cancer and cancer development which has taken place over the last decades.

7.3 Strategies for cancer therapy

Cancer cells have an abnormal growth pattern in which regulation of cell growth and maturation is out of control. The molecular mechanisms leading to development of cancer have to a large extent been mapped during the last years. Cancer growth is a multistage process involving mutations in genes that code for molecules which govern cell growth, cell death and DNA repairs. In particular, there are three sets of genes that are mutated in cancer cells. **Oncogenes** (cancer genes) are genes that normally stimulate cell growth; in cancer cells they are over-activated. **Tumour suppressor genes** ('brake genes') inhibit normal cell growth and are inactivated in cancer cells to that cell division gets out of control. Additionally, genes that normally contribute to *repairing damaged DNA* may be mutated in cancer cells. Usually, changes must occur in several genes (4–10 different genes) for a cancer tumour to develop. The set of genes involved may vary from patient to patient. Some gene changes frequently occur in cancer tumours, such as mutations in the tumour suppressor gene p53, which are seen in about 50 per cent of all forms of cancer.

A characteristic feature of cancers is their ability to metastasize. In most forms of cancer, metastasis is what kills the patient. The risk of metastasis is at its lowest early in the course of the disease, when the tumours are small. Therefore, in point of principle a cure for metastasizing cancer should be systemic, i.e. with equal amounts administered to the entire body. This implies strong requirements for efficient targeting and gene transfer.

Several gene therapy trials aim at correcting genetic defects in cancer cells, either by introducing genes into the cancer cells that are inactivated in the tumour (e.g. p53), or by suppressing the function of over-activated genes. Although these are theoretically attractive strategies, it has turned out that efficient gene transfer to all cancer cells in a patient is difficult to achieve with the methodologies of today, not least because cancer cells are heterogeneous.

Another strategy for attacking cancer cells with gene therapy consists in transferring a gene to the tumour which splits an inactive substance and activates it into an active cell poison ('prodrug'), with a resulting high concentration of the cell poison in the tumour area. This principle is tried out in the treatment of brain tumours and in other areas.

Cancer growth will be affected by various factors in the body. In point of principle, the immune system is able to recognise cancer cells as foreign to the body, but when a cancer tumour develops, it escapes the immune system through various mechanisms. However, in recent years it has been shown that the body's immune response to cancer cells could be improved. A series of gene therapy experiments aim at stimulating immune response to cancer cells. A great advantage with immunotherapy is that it uses the specificity of the immune system to kill cancer cells, and that gene transfer takes place directly to the immune cells and not directly to the cancer cells. When the immune system is activated, the effects may be amplified through cascade effects and potentially be active in the entire body; they do not depend on optimal gene transfer to all cancer cells. It is not necessary that the gene transfer is one hundred per cent efficient, or that the gene remains expressed for a long time.

Some gene therapy protocols aims at protecting the cells in the body that have the highest sensitivity to cell poison, i.e. the blood cells. Here, genes are transferred which

counteract the effect of cell poisons on the bone marrow stem cells from which all blood cells emerge, from the toxic effects of chemotherapy. As bone marrow toxicity is dose limiting, protection of bone marrow stem cells allows the use of higher doses of drugs without destroying bone marrow function.

7.4 Clinical trials

Strategies for gene therapy protocols in cancer may be subdivided in the following three main groups:

1. Therapies targeted directly at cancer cells
2. Immunogen therapy
3. Protection of normal cells

Therapies targeted directly at cancer cells

'Prodrug'

The implantation of so-called 'suicide genes' is the approach best studied *in vitro* as well as *in vivo*, involving by far the largest patient groups up until now. 'Suicide genes' are a class of genes that produce proteins with negative selection properties, e.g. in the sense that they may convert a prodrug to an active mortal substance. The most widely used genes code for the tymidin kinase enzyme from Herpes simplex virus (HSV-TK). This enzyme, normally not present in human cells, very efficiently converts the drug gangciclovir (GCV) into a substance that is toxic to dividing cells.

In order to implant sufficient quantities of tymidin kinase into tumours, cells (mostly, mouse cells) must be infected *ex vivo* with a retroviral vector containing the gene for TK and then be implanted in the target organ. There the cells continue to produce virus, which is integrated into proliferating cells. The gene transfer efficiency is still low; however, because of a 'bystander effect' non-infected tumour cells might also perish. Possibly, this bystander effect is caused by channels between tumour cells and causes the toxic product of GCV to be transferred to not genetically modified tumour cells. Other mechanisms behind this bystander effect have also been suggested, and improved knowledge of this phenomenon may lead to a better effect of this type of gene therapy. The foreign, virus-producing cells are quickly broken down *in vivo*.

It has been assumed that this type of strategy, which attacks all dividing cells in the proximity of the producer cells, is best applied in the brain, in which almost only cancer cells divide. Thus, the first clinical trials employing this strategy were targeted at brain tumours, starting in December 1992. Since then, about 50 phase I/II trials have been started, including a total of between 250 and 300 patients. As the transfer turns out to be very local, the strategy has also been taken up for several other types of cancer. Around ten studies have also been started on patients with ovarian cancer; the vector is injected into the abdominal cavity. In one of these protocols, injections of inactivated, virus-producing human ovarian cancer cells are used. Three of the protocols use adenovirus. Indeed, an increasing number of studies seems to be using adenovirus, which has a far higher transfer efficiency, but so far with more pronounced side effects.

So far the results of retrovirus treatment of about 30 brain tumour patients have been published (2, 3). The procedure has only effect in patients with small tumour mass, and

the transfer efficiency is very low. The most important reported side effect was brain oedema. The results are not published, but preliminary reports are not particularly uplifting reading. On the basis of medical objections, the Norwegian Board of Health declined inclusion of Norwegian patients in this trial (see chapter on legislation).

Another gene/drug system used is the gene for cytosine deaminase (CD) which converts the drug 5-fluorocytosine (5FC) into the toxic 5-fluorouracil (5FU). Only one protocol on patients with liver metastasis from intestinal cancer has been published; no results are as yet available.

Gene transfer targeted at oncogenes; tumour suppressor genes
In order to restore important processes that have been lost, one may either implant defective genes (tumour suppressor genes) into cancer cells, or introduce genes that regulate and/or inhibit activated oncogenes or oncoproteins.

Tumour suppressor genes
The function of the p53 protein is particularly important in the control of the cell cycle, initiation of DNA repair after damage, the maintenance of genomic stability and the induction of programmed cell death (apoptosis). Injection of retrovirus or adenovirus with *TP53* in animal models results in remission of several different types of tumour, such as non-small-cell lung cancer, leukaemia, brain tumours, breast cancer, ovarian cancer, cancer of the colon and of the kidneys (4, 5). Several preclinical trials and early phase I trials have shown efficient gene transfer and few side effects. Adenovirus vectors in which the *TP53* gene is under the control of a cytomegaloviral promoter seem to be the more efficient. Growth inhibition has been shown to be selective for cells with endogenous p53 production. Implantation of p53 in cell lines from ovarian cancers has also shown increased sensitivity to chemotherapy and radiation therapy, which leads to increased apoptosis. A 'bystander effect' similar to the one which is demonstrated in HSV-TK 'suicide gene' therapy has been observed in several studies; however, the mechanism behind this effect is not clear. It also seems as if the implantation of endogenous p53 increases the chemosensitivity and radiosensitivity even in tumours with no p53 mutation or where only a fraction of the cells are mutated.

Approximately 20 clinical phase I/II trials are in progress, mainly on non-small-cell lung cancer and ovarian cancer. Repeated intratumoral injections are well tolerated, and remission or stabilisation of the tumour mass is observed in more than half of the patients. A larger multicentre phase II/III trial planned to include 360 ovarian cancer patients has been started. In this study, patients are randomised for treatment either with chemotherapy alone or chemotherapy plus intraperitoneal injection of an adenoviral vector with p53 into the abdominal cavity. Some Norwegian patients are also included in this trial. Results are first expected in the course of the year 2000.

BRCA1 is another tumour suppressor gene which is often mutated or inactivated in hereditary breast and ovarian cancer, but also somatic mutations occur in sporadic cases of these forms of cancer. Preclinical studies have shown that over-expression of *BRCA1* may function as a growth inhibitor. Intraperitoneal injection of a retroviral vector which expresses *BRCA1* inhibits growth of ovarian cancer. One phase I trial including 12 ovarian cancer patients has been initiated (6). Preliminary results show stabilisation of tumour growth in eight out of twelve patients and remission of tumours in three. There

are also ongoing phase 1 trials with *BRCA1* on other forms of cancer such as prostate cancer and breast cancer. Among other tumour suppressor genes, only the retinoblastoma gene has reached phase I trials.

Oncogenes
One of the oncogenes most frequently expressed in several different types of cancer, such as ovarian cancer, breast cancer, lung cancer and cancer of the colon, is *Her-2/Neu/c-erbB2*. The gene codes for a growth factor receptor and over-expression is mainly caused by gene amplification (from 20 to 100 times). Preclinical trials have shown that intracellular expression of antibodies aimed at the extracellular part of *c-erbB2* inhibits tumour growth. In an animal model of ovarian cancer in which the gene for the antibody against *c-erbB2* has been transferred, tumour reduction as well as longer survival have been shown. Clinical phase I trials on breast and ovarian cancer using this strategy have been initiated; however, no results are yet available. During the last few years, antibodies targeted against the extracellular part of *c-erbB2* have been developed, and such antibodies are now being tried out in patient treatment (herseptine). This strategy seems highly promising. However, this is immunotherapy, not gene therapy.

Another angle of attack in counteracting the effect of *c-erbB2* over-expression is the use of the viral protein E1A. Intraperitoneal injection of the E1A gene in plasmid form is packaged in a cation-liposome complex. One phase I trial (7) recently concluded used intraperitoneal injection of the E1A gene in plasmid form packaged in a cation-liposome complex; the results are not yet published. One multicentre phase II trial has recently been started; preliminary results show low toxicity and stabilisation of the disease. There are also indications to the effect that combined treatment with taxol and E1A may be an efficient treatment modality. Other forms of cancer than those with *c-erbB2* over-expression will also be included, as it has recently been shown that the E1A protein has a series of different cellular points of attack (8).

Downregulation of the expression of other oncogenes has also been tried, but through other strategies than gene transfer only (see antisense and ribozyme treatment).

Oncolytic 'smart viruses'
Differences between normal cells and cancer cells may be used in therapy. It is feasible to construct viruses, which infect and kill cancer cells only, though this strategy is perhaps not gene therapy in the usual sense. In contrast to the typical procedure in most strategies, one uses replication-competent viruses, which may replicate in the cancer cells; when the cell is destroyed, new viruses are released and may infect neighbouring cells. In principle, one single injection centrally in the tumour may start a domino effect, which destroys all cancer cells without affecting normal cells (9). Such viruses are often somewhat misleadingly termed 'smart viruses'. Oncolytic virus is a more appropriate term. As with other viral-based strategies, a problem is that the particles cannot diffuse very far in solid tissue: islands of cancer cells situated only slightly apart may be impossible to reach. Immune response will be a bigger problem here than with other techniques, as the virus needs time to spread.

One of the prototypes of such strategies is the so-called dl1520 adenovirus (11, 12). This virus is already in clinical trials in combination with chemotherapy. Adenovirus must

block the activity of p53 and pRB in order to replicate in the cells. As the dl1520 adenovirus has received the inactivated E1B-55k protein, it cannot block p53 and replicate in normal cells. Cancer cells, on the other hand, often have inactivated p53 and may be infected and lysed by the modified virus. In real life this is more complex, as intact p53 may be inactivated indirectly, and the life cycle of the virus may be affected by other factors in the cancer cells (12).

A modification to this strategy is to make the activity of the E1A gene dependent on regulatory parts from the gene for prostate-specific antigen (PSA) (12). This virus is in clinical trials against prostate cancer; this type of cancer is slow-growing and hence has low sensitivity to traditional therapy.

Although reovirus is not known to cause disease in humans and does not kill normal cells in culture, it turns out that they may lyse cancer cells with overactive Ras oncogene (12). Very promising results have been achieved in preclinical studies (14); work is in progress on getting approval for clinical trials.

Herpesvirus and parvovirus, which may be pathogenic to humans, have also been put to use (9). Crucial in this respect is that when proliferation-competent virus is used, safety is an even more paramount concern than in other types of gene therapy. Oncolytic viruses can also be used to transfer immune-stimulating or prodrug-activating genes which might have considerable 'bystander effect' on those parts of the tumour which are not lysed.

Antisense and ribozyme treatment

Antisense and ribozyme treatment is rarely employed in clinical cancer trials. So far, about 10 phase I studies have been started with antisense targeted at various oncogenes, and then usually in combination with other types of conventional or gene therapy related treatment. Up until now, no data are published from these studies. As far as we know, no clinical studies have been started of ribozymes used on cancer patients. One angles of attack fast approaching the clinical trials stage is probably a ribozyme for downregulation of the angiogenic factor VEGF, a gene implicated in the metastasis process.

Most early antisense studies with antisense oligonucleotides were based on uptake in the cells of antisense molecules implanted in plasmids (lipids/retroviral vectors). Among ongoing plasmid-based studies, mention should be made of antisense treatment against insulin-like growth factor I in glioblastoma, antisense c-myc in prostate cancer and breast cancer, and intrapleureal and intraperitoneal antisense c-fos in breast cancer. Trials using antisense against epidermal growth factor receptor and against TGF-β2 have also been started. There is also an ongoing combination therapy introducing a new gene in combination with downregulation of another gene. Chronic myelogenous leukaemia is caused by a translocation between the bcr and abl genes in haematopoietic germ cells. Curing the patient implies removing all cells containing this translocation. This is extremely hard to achieve with conventional treatment. In this ongoing phase I study, a retrovirus containing the gene for the chemotherapy resistance factor dihydropholate-reductase is linked to an antisense sequence against the translocation gene. The objective here is to combine chemotherapy resistance to the normal cells and at the same time downregulate the expression of the transforming translocation gene in the remaining tumour cells (15).

A phase II trial has recently been started using a phosphorotioate antisense molecule targeted against protein kinase C α for treatment of patients with malign melanoma and non-small-cell lung cancer. The antisense molecule is administered intravenously. Results from phase I trials show effects on tumour growth in a couple of patients. The Norwegian Radium Hospital participates in this trial.

Immuno-gene therapy

Several ongoing gene therapy trials use various principles in order to stimulate immune response. The objective is to make cancer cells more immunogenic so that the specific immune apparatus is stimulated , i.e. the T- and B-lymphocytes. The unspecific immune apparatus could also be stimulated to take part in the fight against the cancer cells. A survey of the principles behind the ongoing studies on immunogen therapy for cancer is presented in Table 1.

Table 1. Principles for immunogene therapy studies

Type of gene product	Gene transferred
Cytokines	IL-2, IL-4, IL-6/sIL6-R, IL-7, IL-12, TNF, IFN gamma, IFN-b, GM-CSF, Lymphotactin
Alloantigens/xenoantigens (histocompatiblity antigens)	HLA-B7, HLA-A2, HLA-B13, H2-K(k)
Viral antigens	Human papillomavirus (HPV E6 and E7)
Tumour antigens	MUC-1, MART-1, Gp100, CEA, PSA, HPV, Tyrosinase, Idiotype
Cell interaction molecules	CD80 (B7.1), CD140 (CD40L)
Modified antibody molecules and T-cell receptors	sFv, TCR Ab, CC49-Zeta TCR

Cytokines

Cytokines are naturally occurring proteins which act as communication molecules between cells. Cytokines play an important role in regulating immune defence activity and has to some extent been of considerable effect against cancer in animal models. Cytokine therapy is complicated by the fact that many of them have serious side effects, high cost and limited effects in humans when applied in this way. By using cells that are transfected with one or more cytokine genes *ex vivo* before they are injected into the tumour, a local production of the cytokine may be achieved. Several different cell types have been used, including autologuous (the body's own) and allogeneic (from other individuals) tumour cells, fibroblasts, autologuous lymphocytes and dendritic cells (antigen-presenting cells). Autologous cancer cells are difficult to transfect in sufficient numbers, and the trend is towards increasing use of dendritic cells (D1 cells). The cytokines may also affect the cancer cells in various ways, directly or indirectly. Several phase II trials using cytokine genes have been started; in several studies an increased specific anti-tumour immunity has been observed and in some cases local tumour regression in some patients (IL-2, IL-12, GM-CSF, IFN gamma) (16–25). Several recent protocols use combinations of several cytokines.

Alloantigens

Foreign histocompatibility locus antigens (alloantigens) are very potent in stimulating an immune repose. By transferring genes for alloantigens to the tumours, either by direct injection of DNA in complexes with liposomes or via cells that have been transfected with such genes, one may achieve an anti-alloantigen response and also a response against other tumour antigens. A series of trials have used liposome-mediated transfection of the HLA-B7 gene to patients lacking HLA-B7 (93 per cent of Caucasians lack HLA-B7). Also under way are several phase II trials and one phase III trial combining HLA-B7 gene therapy with chemotherapy. Successful gene transfer and expression has been achieved in the treatment of malignant melanoma and various carcinomas (cancer emanating from the epithelium). Several patients had reduced tumour size (26–30). A few patients achieved regression also of non-injected tumours.

Tumour antigens

During the last few years, several molecules which are expressed by tumour cells and may induce specific immune response have been identified. Several clinical trials have been started in which one uses adenoviral or vaccinia-virus based vectors for transferring genes that code for established tumour antigens (CEA, MART-1, gp100; MUC-1 etc.) *In vivo* administration is mainly used. Most of these protocols are early-phase. Specific anti-tumour T-cell response has, *inter alia*, been shown by intradermal injection of the CEA gene in a vaccinia-virus vector, though in a small number of patients (31). Recent protocols partly use transfection of dendritic cells with genes that code for tumour antigen, partly also immunisation with naked DNA (DNA vaccination).

Modified antibodies/TCR genes

Some trials have used gene constructs of immunoglobulin (antibody) or T-cell receptor genes. An interesting principle consists in using sFv (single chain Fragment variable) of tumour idiotype in B-cell neoplasia. In a B-cell tumour, all cancer cells express an identical immunoglobulin molecule, hence this will represent a unique tumour antigen (idiotype). A certain amount of clinical success has been achieved with anti-idiotype based strategies in lymphoma of the B-cell type, and gene therapy trials based on idiotypic sFv gene constructs have been started. The use of sFv gene fragments alone gives modest immune response, but preclinical data from mice, in which sFv has been linked to a part of the tetanus toxin gene, show very promising results (32). A clinical trial based on this principle has recently been started. Preclinical experiments linking sFv til chemokines have also yielded interesting results.

Protection of normal cells

High-dose chemotherapy followed by autologuous bone marrow/stem cell transplantation is used for selected patients in whom other modalities have only a small probability of success. The best effect of high dose chemotherapy is achieved by high repeated doses, but with the risk of pancytopenia (low level of blood cells), associated with infections that may be fatal. In order to counteract this, several strategies have been tried out for transferring genes to bone marrow/stem cells to build resistance against the chemotherapy regimens used. The genes used are primarily the so-called ‘multidrug resistance gene’ (MDR1 gene) and the gene for a repair enzyme, O^{6}-methylguanine-DNA-methyltransferase (MGMT). *In vitro* studies have shown efficient gene transfer to stem cells *ex vivo*. However, most phase I trials show little or no gene transfer of the

transplanted cells *in vivo*. There are around ten ongoing phase I and phase I/II trials, including around 50 patients with ovarian cancer, brain tumour and lymphoma. Preliminary conclusions are that there are big problems related to achieving efficient *in vivo* transfer of stem cells, and if transfer is achieved, it only lasts for a very short time (33). Other genes are in preclinical testing, but it is doubtful whether this strategy will be sensible in the future. The most recent data on high-dose chemotherapy for the big cancers, including breast cancer and ovarian cancer, show that high-dose chemotherapy has less effect than previously assumed.

7.5 Conclusions/trends

For gene therapy targeted at tumour suppressor gene or oncogenes, the most promising strategies are those that are based on supplying a normal *TP53* gene to tumours in which this gene is inactivated. The results of the larger ongoing phase I/II trials will be important in the choice of direction in this type of therapy. It will be important to evaluate the effect of combination with other, conventional therapies, like chemotherapy and/or radiotherapy, as the therapy may seem to depend on a normally functioning *TP53* gene in order to function efficiently. Over time, identification of new tumour suppressor genes that turn out to be inactivated in a larger number of tumours will provide far more candidates for this type of treatment.

Most immunotherapy trials are early trials, and for most of them published results are not available. *Ex vivo* studies have dominated the picture; lately the share of *in vivo* trials has been increasing. Several have shown acceptable gene transfer, and specific immune response is reported in several trials. Partial tumour responses have been achieved in some trials, and also stabilisation of the disease, though the effect cannot be said to be dramatic. By and large, local effects on tumours have been observed (partial reduction in size), but in singular cases declines in distant metastases have also been observed. Transfection of HLA-B7/b2 microglobulin is used in 36 different protocols. Partial tumour responses are observed, also in melanomas and intestinal cancer. Results from phase III trials are not yet published. Transfection of cytokine genes has also given local and general immune responses; here, too, some partial responses have been observed. Most trials have shown modest side effects. Diffusion of replication-competent virus to the environment has not been observed. Most patients included in these trials have suffered from advanced-stage metastasizing cancer and have also been thoroughly treated with chemotherapy. Large tumour burden and deteriorating general condition are both unfavourable factors in terms of optimal immunotherapy, but so far no data are available from early phase patients with small tumour volume. Another problem is how to measure specific immune response, although progress has lately been made, also in relation to finding evidence of antigen-specific T-cells. Other trends include the use of therapeutic cancer vaccines based on DNA vaccination and gene transfer to dendritic cells, which are highly potent antigen-presenting cells. Furthermore, combination therapies are increasingly tried out.

Promising preclinical data have been reported from animal models of a combination of pro-drug gene therapy and radiotherapy. Clearly, however, the strategy of introducing suicide genes requires considerable preclinical efforts before it might be characterised as promising. The transfer efficiency has to be increased, either by increased communication between the tumour cells, or through the use of other vector system,

such as adenovirus or modified lentivirus. Side effects of adenovirus have to be surmounted, for instance by using new generations of virus.

In external delivery of antisense molecules, the localisation, distribution and degradation of the molecules, intracellularly and in the body, will depend on the chemical structure of the molecules. Artificial modifications tried out so far have been phosphorotioate-modified antisense molecules. Antisense molecules modified in several other chemical fashions will probably soon reach clinical trials. Generally speaking, phosphorotioate molecules are not very toxic; normally only 'immunostimulatory' effects are observed.

Hopes are also placed in the use of angiogenesis inhibitors in cancer therapy (34). Experiments indicate that primary tumour growth, as well as metastasizing of cancer cells require regeneration of blood vessels. Vessel regeneration can experimentally be prevented by the use of inhibitors, and modulation of angiogenesis has been shown to cause tumour regression in animal models. A possible strategy would be to add angiogenesis inhibitors by gene therapy.

7.6 References

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8. Gene therapy in monogenic diseases apart from primary immunodeficiency

8.1 Summary

Monogenic inherited disorders are caused by mutations in one single gene. Each disorder is rare, but as a group they represent a considerable health problem. Most of them are severe disorders with no curative treatment, hence gene therapy will often be the only possible treatment. Gene therapy for a monogenic hereditary disease requires that the gene implicated in the disease in question is identified. So far, only a limited number of genes is known for the more than 5,000 diseases caused by monogenic inheritance. In spite of many years of ongoing clinical trials on some diseases, e.g. cystic fibrosis, no patient with a monogenic hereditary disease has so far been cured by gene therapy.

8.2 Introduction

Each of the monogenic inherited disorders is rare; however, as there are more than 5000 different diseases, as much as 1 per cent of the population suffer from them. These diseases have in common that they are usually serious and there are limited treatment options for them, hence they constitute a considerable health problem. Symptomatic treatment may prolong life, but gene therapy will often be the only possibility of a curative therapy.

A precondition for gene therapy of monogenic inherited disorders is that the gene is known. For many of these disorders the gene is not yet known, thus experiments with gene therapy are not possible.

In this chapter we will concentrate on gene therapy for cystic fibrosis, the monogenic hereditary disease in which we have the greatest experience with clinical gene therapy trials. Monogenic inherited disorders caused by a primary deficiency in the immune system will be discussed in chapter 9.

8.3 Cystic fibrosis (CF)

CF is the most frequent of the serious autosomal recessive inherited disorders; with an incidence of 1/2000 to 1/4500 in children of Caucasoid origin. The disease is caused by a defect in a gene, which codes for a protein regulating the chloride transport through the cell membrane. This leads to dehydration of extracellular fluid and generation of thick mucus, resulting in malabsorption due to impeded function of the pancreas, repeated bacterial airway infections, and sterility in men. Most patients die of respiratory failure in early adulthood. The gene was identified as early as in 1989 (‘cystic fibrosis transmembrane conductance regulator gene’, CFTR); more than 800 mutations are known. The most frequent mutation, DF508, is found in 60 to 70 per cent of patients depending on ethnic background. Most of the other mutations are very rare. The

mutations may be divided into five different classes depending on the changes found in the CFTR protein. It is, however, not completely established what role this protein has in the development of the lungs.

Symptomatic treatment consists of antibiotics, physiotherapy and drugs that make the mucus less sticky. This symptomatic treatment has lead to an expected average lifespan of about 40 years for CF patients, a doubling over the last 20 years. However, CF is still a serious childhood and juvenile disease which usually leads to death in early adulthood. There is a great need for a curative treatment.

In vitro experiments have shown that the chloride transport defect may be corrected in epithelial cells from CF patients by insertion of a normal gene. Therapeutic effect may also be expected with limited expression of the gene as the epithelium may be normalised even when gene expression is only found in 6 to 10 per cent of the cells. As CF primarily affects the airways, i.e. tissue accessible to *in situ* treatment, CF is a disease that should be a good candidate for gene therapy.

Strategies

Most widely used vectors have been employed for transfer of the CF gene: adenovirus, adeno-associated virus, or liposomes. The target cells for gene transfer have been epithelial cells in the nasal mucosa, sinuses and bronchi. Most trials have been done on the nasal epithelium, which is the most easily accessible. Epithelium from sinuses is available for gene therapy in patients who has undergone antrotomy. There is however, an ongoing discussion of whether other target cells might not be better suited, e.g. submucosal serous cells, believed to be the lung cells that have the highest expression of CFTR.

How best to measure the effect of the gene transfer is also a matter under discussion. Several different measurements have been used, of mRNA or CFTR protein, the CFTR function, or of the binding of the Pseudomonas aeruginosa bacterium to the respiratory epithelium.

Clinical trials

Several clinical trials using adenovirus, adeno-associated virus, or liposomes have been published (1). These are phase I/II protocols; most of them are old. It has been shown that gene transfer takes place, but the efficiency is too low. Adenovirus gives a more efficient gene transfer, but leads to immunological complications; this is a major problem, as the therapy has to be repeated with some weeks' intervals. The biggest problem related to gene therapy for CF is, however, that the therapy is not efficient enough.

All clinical trials have been carried out on adult persons. Ideally speaking, gene therapy should probably start before lung changes occur, and they occur as early as four weeks after birth. Hence there is a debate on whether one should start clinical trials with children, and on the inherent ethical problems.

8.4 Other monogenic inherited disorders

Metabolic diseases are caused by a defect in metabolism, usually lack of an enzyme, which leads to progressing disease and often early death. In a few cases dietary

treatment can prevent the disease from developing. Other possible therapeutic modalities may be bone marrow transplantation and administration of the enzyme. In most cases, however, symptomatic treatment is the only option. For most metabolic diseases, gene therapy will be the only possibility for curative treatment. One problem in the treatment of metabolic diseases is that permanent damage has usually occurred by the time the disease is diagnosed. There is a special chance of early diagnosis of metabolic diseases when they affect siblings; then the diagnosis can often be established at birth on the basis of earlier affected siblings.

Strategies

This group of monogenic inherited disorders is highly heterogeneous, and the rationale for treatment will vary according to the nature of the disease.

Clinical trials

There are clinical protocols for a few metabolic diseases; only the most important will be mentioned here. The most prevalent of the lysosomal diseases is Gaucher's disease (1/55,000), an autosomal recessive hereditary disease caused by a lack of the lysosomal enzyme glucocerebrosidase. This enzyme deficiency leads to accumulation of glucosylceramide in reticuloendothelial cells, with spleen enlargement, liver and skeletal affection, and pancytopenia. Gaucher's disease has been considered well suited for gene therapy with gene transfer to stem cells. In several trials recombinant retrovirus has successfully been transferred to primitive haematopoietic cells *ex vivo*. However, the number of corrected cells has turned out to be too low for clinical effect to be achieved.

Hunter's disease (mucopolysaccaridosis II) is a rare (1/130,000 boys), X-linked recessive hereditary disorder caused by lack of the iduronate-2-sulfatase enzyme. This leads to accumulation of glucosaminoglycans and causes skeletal deformities, cardiopulmonary complications, mental retardation, and death before the age of 15. Recombinant retrovirus has successfully been transferred to peripheral lymphocytes *ex vivo*, but no results from clinical trials have been published.

Familial hypercholesterolaemia shows autosomal dominant inheritance; in its heterozygous form it is one of the most prevalent monogenic diseases. As many as one in 300 may be afflicted. The disease is caused by a mutation in the low density lipoprotein gene (LDL). Its far rarer homozygous form is very difficult to treat. Recombinant retrovirus has successfully been transferred to liver cells *ex vivo* which, when returned to the body, gave a reduced cholesterol concentration in homozygous patients; this suggests that gene therapy might become an option for this disease (2).

8.5 Preclinical data

Phenylketonuria (PKU, Følling's disease; 1/15,000) is caused by lack of the liver enzyme phenylalaninehydroxylase leading to accumulation in the blood of phenylalanine and mental retardation. Today this disease is treated with a diet low in phenylalanine. This treatment has greatly improved prognosis, but it still has limited effect, and there is a need for a curative treatment. Recombinant adenovirus and retrovirus have successfully been transferred to liver cells in mice, but the treatment has turned out to have the same limitations as with CF: immunological complications from adenovirus and low efficiency from retrovirus (3).

Haemophilia B is a rare bleeding disorder (1/25,000 boys) due to lack of coagulation factor IX. The disease may be treated by administering factor IX concentrate from blood with the risk of complications implied. A plasma concentration of 10 per cent of the normal value being sufficient to cure the disease, this disease should be a suitable candidate for gene therapy. Recent experiments in a canine model with haemophilia B have given promising results for gene therapy using a recombinant adeno-associated virus injected into the portal vein or intramuscularly (4).

Most monogenic diseases start early in life, and it is important to start treatment before permanent damage has set in. However, some monogenic diseases have their onset later in life; here a treatment which postpones development of damage would be essential for the patient. One example is retinitis pigmentosa, which strikes young adults in particular and is the most frequent hereditary cause of blindness. If gene therapy can postpone the loss of vision some years, this will be a great boon to the patient. Several promising experiments with gene therapy in animal models suggest that gene therapy for retinitis pigmentosa may be possible (5).

8.6 Conclusion

Gene therapy has great potential in the treatment of the monogenic inherited disorders for which no other curative treatment exists. Although gene transfer to several different target cells has been successfully performed, clinical experience is so far limited. Gene therapy in cystic fibrosis reflects the development of gene therapy in general. Faulty vector technology and the ability of epithelial cells to defend themselves against foreign matter are important reasons why gene therapy for this serious disease does not seem to be available in the near future. In spite of clinical trials going on for more than five years and over 100 persons having received the CFTR gene, it remains to be demonstrated that the therapy has clinical effect (6), nor has therapeutic effect been shown for any other monogenic disease.

Many monogenic inherited disorders are very rare. Establishing gene therapy for these diseases will only benefit a limited number of patients. However, experience from experiments with gene therapy for these rare diseases has been of importance to the development of gene therapy for other, far more prevalent diseases that may be candidates for gene therapy.

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9. Gene therapy for primary immunodeficiency diseases

9.1 Summary

Primary immunodeficiency diseases constitute a group of rare, usually congenital diseases which in most cases are caused by mutations in genes essential for the normal functioning of the immune system and therefore lead to deficient defence against microbes. During the last decade, the genetic defects in several primary immunodeficiency diseases have been studied in depth, and the functions of the relevant genes in the immune system have been identified. These diseases are often extremely serious, and curative therapy is not available unless bone marrow transplantation is feasible. Hence, primary immunodeficiency diseases are seen as an attractive target for gene therapy, and it was for one of these diseases (ADA deficiency) that gene therapy was first tried clinically (1990).

There are ongoing gene therapy trials in several primary immunodeficiency diseases, and preliminary results are available for several of them. So far, no primary immunodeficiency disease has been cured by gene therapy, but clinical experience has given valuable insights that will probably lead to improved therapeutic strategies.The difficulties that must be overcome are partly related to general problems in human gene therapy, partly related to specific difficulties associated with the complexity of the human immune system.

9.2 Introduction

Most cases of primary immunodeficiency are caused by mutations in genes that are essential for the development and/or functioning of important cells in the immune system (1). Over the last ten years, a genetic defect has been demonstrated for several primary immunodeficiency diseases; some of the genes in question have been thoroughly studied and their function identified.

These diseases are usually extremely serious, and no curative treatments exist except in those cases where bone marrow transplants are possible. During the last few years one has tried to develop gene therapy strategies for the treatment of several primary immunodeficiency diseases. Results are already available for some of these diseases.

9.3 Preconditions for development of gene therapies

A range of preconditions has to be met before one can make an effort at developing a gene therapy for a given primary immunodeficiency disease (2, 3). First, the gene has to be studied in detail so that the DNA sequence and the organisation of the genome is mapped. For several important immunodeficiency diseases, e.g. common variable immunodeficiency, i.e. the most common form of primary hypogammaglobulinaemia, these conditions are not yet filled. Furthermore, the gene's function must be known, since otherwise manipulation of the gene might have un-foreseen and potentially serious consequences. There are also serious problems related to the special regulatory mechanisms that are operative for many essential genes in the immune system. Some

genes for which gene therapy might be relevant, are only expressed in certain types of cells, e.g. the so-called BTK gene in B-lymphocytes, or only in a special functional phase of one cell type, e.g. the CD40L gene in activated T-lymphocytes. Present gene therapy technology does not allow such selective regulation of the gene's expression *in vivo*; hence gene therapy might have serious consequences if the therapeutic gene and gene product is expressed in the 'wrong' cells at the 'wrong' time during an immune response. It has for instance been shown in animal models that increased expression of CD40L may cause lymphoproliferative disease. In certain genes there are also narrow quantitative physiological regulation of gene expression; in such cases a therapeutic manipulation causing substantially higher expression of the gene and its gene products than what is normal might also cause serious side effects.

Extensive cell culture experiments have been performed with many of the genes identified in primary immunodeficiency diseases. If it is at all possible, such experiments have to be supplemented by studies in appropriate animal models which now are available for some important primary immunodeficiency diseases.

9.4 Strategies for protocols

In clinical protocols established for gene therapy in primary immunodeficiency diseases, two cell types have been defined as target cells for gene transfer: haematopoietic stem cells and lymphocytes. The former cell is the progenitor cell from which all other cells in the immune system originate. It is self-renewing, thus manipulating the genes in this cell type is theoretically attractive. However, gene transfer to these cells is not very efficient with existing vector systems, and this has hampered development in the field.

Because lymphocytes are essential effector cells in immunological reactions and the defect in primary immunodeficiency diseases is often in the lymphocytes, this cell type is also a natural target cell for gene therapy. However, one problem here is that the number of available lymphocytes is often greatly reduced in the most serious forms of primary immunodeficiency diseases; this limits the prospects for using lymphocytes as target cells. Certain forms of primary immunodeficiency disease are caused by defective monocytes, also potential target cells for gene therapy. One problem is the limited lifespan of such cells since they do not renew themselves by cell division, thus passing on the therapeutic gene. All existing clinical experience is derived from gene therapy targeted at lymphocytes or CD34 positive haematopoietic stem cells.

Below a short account is given of the type of primary immunodeficiency disease for which clinical trials of gene therapy have already been tried or are ongoing.

9.5 Severe combined immunodeficiency (SCID)

SCID is a group of serious primary immunodeficiency diseases which attack both the B- and the T-cell systems in the immune defence, causing a breakdown in the total specific immunological defence against infection. Several different genetic defects have now been described as causes of SCID. They have differing inheritance patterns and different immunological and clinical consequences. Common to all these conditions is that they are fatal if the patient does not receive treatment. In principle, all forms of SCID are treated with bone marrow transplantation. For ADA deficiency there is also an

alternative to bone marrow transplantation: substitution therapy with the enzyme, a highly expensive therapy which is not curative.

ADA deficiency

As early as in the mid-1980s, ADA deficiency was identified as a prime candidate for gene therapy, and it was the first disease in humans for which such therapy was tried out clinically (4). Its advantage in terms of gene therapy is that the function and regulation of the ADA gene is thoroughly studied and well known. Moreover, normal functioning of the T-lymphocytes is seen over a broad range of enzyme concentrations (from 5 per cent of normal values to about 50 times normal average values); therefore side effects from too high gene expression should not be a problem. After successful preclinical studies, clinical trials in patients were started in 1990 and are now carried out according to five different protocols (2, 3). Supportive therapy with ADA is given to the patients. In two protocols, including the first, T-lymphocytes from peripheral blood have been used as target cells. One of the others uses haematopoietic stem cells from bone marrow; in another CD34 positive stem cells isolated from umbilical cord blood are used; in a third, haematopoietic stem cells as well as T-lymphocytes.

The preliminary conclusion from therapy in about ten patients is that no serious side effects have been associated with the gene therapy procedures. None of the patients seems to have been cured, neither has it been possible to terminate substitution therapy with ADA. This complicates the assessment of the effect of the therapy. The trials have, however, shown that genetically modified T-lymphocytes may be detectable in the organism several years after gene therapy. Observations also suggest that the cells to which the normal ADA gene has been transferred, have a competitive survival advantage compared to similar cells with a defective ADA gene; in the long run it may be hoped that this normal cell type will increase in numbers. Only more observations of attempts at terminating supplementary ADA therapy may tell whether gene therapy according to existing protocols is of substantial clinical value in this type of SCID.

X-linked recessive SCID

In X-linked recessive SCID there is a mutation in the gamma chain constituting part of the lymphocyte receptor for the important cytokine interleukin 2. This molecule is also a component of the lymphocyte receptors for several other important cytokines, including IL-4, IL-7, IL-9 and IL-15. Recent studies indicate that this common gamma chain (gamma c) is essential for intracellular signal pathways after stimulation of the respective cytokine receptor on the membrane of the cell. Therefore mutations in the gamma chain may lead to serious disturbances in the lymphocyte responses to a number of important cytokines which are also essential for the normal growth and differentiation of T- and B-lymphocytes. Such patients usually have a profouind reduction of the number of T-cells, but normal or even increased numbers of B-lymphocytes, which, however, are functionally deficient.The only curative treatment available is bone marrow transplantation which may yield very good results, particularly with a histocompatible donor. Not infrequently, however, the result of the bone marrow transplantation is only incomplete immunological reconstitution, and the development of a gene therapy is greatly to be desired. Preclinical experiments have shown that transfer of the normal gene to haematopoietic stem cells *in vitro* is possible, with good functional results (2). Very recently favourable results were reported in two infants with gene therapy utilizing a retroviral vector associated with the normal gene for the gamma chain (7).

JAK 3 defect SCID

Another variant, JAK 3 defect SCID, is caused by a mutation in the gene for the JAK 3 enzyme which plays a crucial role in the biochemical activation mechanism of the T-lymphocytes. Also in this rare condition, bone marrow transplantation is the only lifesaving treatment, but not always possible. Experiments in cell cultures have shown that it is possible to transfer the normal gene to stem cells from 'knock-out' mice with JAK 3 defect, and that the reinfusion of such stem cells causes a considerable degree of immunologic normalisation (2). For this condition, too, a clinical protocol has been approved and therapy trials are under way. No results are available.

9.6 Purine nucleoside phosphorylase (PNP) deficiency

Another rare cause of life-threatening primary immunodeficiency disease is a genetic defect in the enzyme called purine nucleoside phosphorylase (PNP), which also causes a broad defect in T-cells as well as B-cells (1). No really efficient treatment is available for this immunodeficiency disease. Even bone marrow transplantation from histocompatible donors usually gives fairly poor results. Experiments in cell cultures have shown that transfer of genes to stem cells is possible. A clinical protocol has been developed for gene transfer and inclusion of patients has started, but no results are available.

9.7 Primary immunodeficiency diseases with deficient phagocytic function

Several primary immunodeficiency diseases are caused by gene defects which lead to reduced functioning of phagocytic cells, i.e. monocytes, macrophages and neutrophilic granulocytes. These conditions often lead to serious problems with infections.

Chronic granulomatous disease

In chronic granulomatous disease there are defects in an important enzyme system, the NAPDH oxydase system. This leads to serious problems with infections. Four different gene defects have been described as causes of this condition. Three different clinical protocols are now operative, each of them aiming at gene therapy correction of one of the known genetic defects in chronic granulomatous disease. From one of these trials preliminary results are available which show that gene therapy has lead to a certain, although very limited, production of phagocytes, with expression of the normal gene several month after treatment (8).

Leukocyte adhesion defect

In leukocyte adhesion defect, a rare primary immunodeficiency disease, there is a defect in a gene for an important adhesion molecule (CD18) which causes deficient granulocyte functioning and leads to serious infection. The disease can be cured by bone marrow transplantation, but this is not always possible. Clinical gene therapy trials are in progress (2).

9.8 Other primary immunodeficiency diseases

For some primary immunodeficiency diseases, gene therapy is not possible in spite of the fact that the gene defect is known, as the gene's function is not yet properly mapped. This group includes X-linked recessive hypogammaglobulinaemia (Bruton hypogammaglobulinaemia) and Wiskott-Aldrich syndrome.

9.9 Conclusion

For several of these pathological conditions in the immune defence, many of them extremely serious, gene therapy will probably be the only possible curative treatment. For some primary immunodeficiency diseases alternative treatment modalities are available; however, only in those cases when bone marrow transplantation is possible, can a curative effect be achieved. If such treatment is not possible or does not have the desired effect, gene therapy could be a valuable alternative. In some primary immunodeficiency diseases the patient can survive more or less disease-free with other treatment modalities, e.g. immunoglobulin therapy in forms of hypogammaglobulinaemia, gamma interferon therapy in chronic granulomatous disease, frequent use of antibiotics etc. Also in these cases, gene therapy may become a therapeutic alternative, partly because of enhanced quality of life for the patient, partly as a consequence of the economics of the health services, as many existing therapies are very expensive. Thus there is reason to believe that gene therapy will find its place in the treatment of several primary immunodeficiency diseases in the years ahead.

9.10 References

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10. Gene therapy for HIV infection and other viral infections

10.1 Summary

HIV is an important cause of human disease, as are several other viruses. Although the HIV epidemic in Norway has taken a less drastic course than in many parts of the world, this infection is an important challenge for us, medically as well as in economic terms. Large problems remain to be solved, even though significant progress has been made over the last three or four years with drugs for treating HIV infection. However, by no means all HIV patients respond to existing drugs, the treatment does not eradicate the virus in patients, relapses are frequent, and the side effects can be considerable. It should also be noted that the present therapeutic drug regimens are very expensive. Thus there is a great deal of interest in developing new therapeutic modalities.

The characteristics and disease-inducing capacity of the HIV virus have been studied in depth over several years, and there is clearly a theoretical basis for attacking the infection with gene therapy. Several different strategies are relevant. A series of gene therapy protocols are now in phase I trials, in some cases also in phase II trials. So far, no unambiguous clinical therapeutic results have come out of these trials, but useful information has been gathered which will be of value in the ongoing work on gene therapy for HIV infection.

Also with regard to some other viral infections, the focus is now on the prospect of using gene therapy as a supplement or alternative to antiviral drugs which often have limited or no effect. Gene therapy with synthetic oligonucleotides is already available in clinical medicine for eye infection with cytomegalovirus, though so far experience is limited.

10.2 Introduction

The HIV epidemic has struck in most countries of the world and is out of control in Africa, Asia and Latin America. In the Scandinavian countries the epidemic is contained, but HIV infection represents a considerable problem here, too, medically as well as financially. Great advances have been made in the treatment of HIV infection in western countries since 1996, as new and more potent drugs against the virus have become available, as well as new methods from molecular biology for quantifying the viral load in the blood. These advances have brought a decline in mortality of AIDS (the collective term for cases of HIV infection with pronounced immunodeficiency and resulting serious immunodeficiency complications) as well as significantly reduced morbidity.

Present-day HIV therapy has, however, a number of inherent problems. By no means all HIV patients respond satisfactorily to drug therapy, and relapses are often seen after successful initial treatment. Furthermore, the drugs are expensive, and several of them

have side effects which may be considerable. The mutation frequency of the virus implies a great risk of resistance development against the drugs now used. A final point is that with today's therapeutic regimens it does not seem possible to eradicate the virus in the patients, who will probably have to take the drugs for the rest of their lives. One reason for this is that the HIV virus can survive in latently infected cells and in so-called 'privileged' sites in the body where drugs and the immune system cannot reach them. There is a need for new therapeutic modalities in addition to conventional drug therapy; hence the great interest in developing new gene therapy strategies against HIV infection.

For several other viral infections as well, work is in progress on developing gene therapy as there are no efficient drug modalities, and the infectious diseases in question lead to considerable morbidity and mortality.

10.3 Strategies for protocols for HIV infection

The design of gene therapy strategies is based on the great advances made over the last few years in the understanding of the genetics of the HIV virus, its molecular biology, and the pathogenesis of HIV infection (1). The virus has an RNA genome. The most important target cells which are attacked by HIV and over time destroyed in the organism, are the CD4 positive T-lymphocytes, which play a crucial part in the immune defence. When the virus has penetrated the cell, its genetic material in RNA form is 'translated' into DNA (by reverse transcriptase, a virus enzyme), which is then built into the host cell's DNA genome.

This integrated viral genome then directs the synthesis of new viral particles in the cell by coding for three sets of viral proteins: structural proteins (Gag, Pol and Env), regulatory proteins (Tat, Rev, Nef), and so-called viral maturation proteins (Vif, Vpu, Vpr).

Theoretically, the replication life cycle of the virus may be stopped by blocking or inhibiting the function of one or more of the genes and their corresponding proteins. There is no good animal model for HIV infection; a sizeable number of preclinical gene therapy studies have been based on *in vitro* experiments with cell cultures. Primarily based on such experiments, some of these strategies have already been tried in phase I and in a few cases also in phase II trials on HIV-infected individuals (Table 10-1). The emphasis of the presentation below will be on gene therapy studies that already are or have been in clinical trials.

Two main strategies for gene therapy against HIV infection are being tried out:

1. Introduction of new genes in order to make target cells for HIV infection resistant against HIV replication.
2. Genetic modification of immune cells in order to strengthen the individual's immune defence against HIV.

For each of these main principles there are several different types of strategies, as shown in Table 10-1 (2, 3).

Table 10-1. Gene therapy against HIV infection – main principles

Intracellular inhibition of HIV
- Based on viral nucleic acids
  - Antisense, ribozymes, 'decoys'
  - Synthetic DNA oligonucleotides
- Based on intracellular proteins with anti-HIV effect
  - Dominant negative proteins
  - Cellular proteins
  - Intrabodies
  - Suicide genes

Immunotherapy
- HIV-specific
  - Cytotoxic T-lymphocytes
  - DNA vaccines
- Non-specific

10.4 Intracellular inhibition of HIV

Gene therapy targeted at intracellular inhibition of HIV is based either on viral nucleic acids or on viral or cellular proteins (2, 3).

Therapy based on viral nucleic acids

These forms of therapy fall into two main groups:
1. RNA-based strategies based on antisense, ribozymes, or 'decoys'
2. Exogenously introduced synthetic DNA oligonucleotides with antisense properties

Antisense

In antisense therapy, a gene is introduced into the target cells which triggers expression of RNA molecules that are complementary to the viral RNA one wants to attack (4). Inside the cell, the therapeutic RNA will bind specifically to the viral target molecule and block the function of and/or break down the molecule. This type of strategy has been shown to have a certain effect on various forms of viral RNA in cell cultures. Phase I trials in HIV patients are in progress with antisense therapy targeted at these important viral target molecules: the so-called TAR sequence, messenger RNA for the Rev gene, Pol gene, and viral genomic RNA (5). The TAR sequence is the binding site for Tat, a crucial regulatory viral protein that is of great importance to the speed of viral replication in the cell. The Rev protein binds to the so-called RRE element, which is broadly represented on viral mRNA and has a crucial role in the formation of new virus particles in the cell (1). So far, no published clinical results from these trials are available.

There are a number of problems with antisense strategies. First, antiviral effect requires relatively high intracellular concentrations of the expressed antisense RNA. This problem could possibly be solved through improved technology in terms of the efficiency of the gene expression. Another important problem, well known in conventional anti-HIV drug therapy, too, is the great risk of resistance development in the genetic material of the virus which will reduce or neutralize the effect of the antisense RNA used. In all probability, antisense strategies will be used in combination with other forms of therapy. One reason for this is to prevent resistance development.

Ribozymes
Ribozymes are antisense molecules with enzymatic effect. After binding to the target (the viral RNA molecules), they cause their destruction. Ribozymes are so small that several different ribozymes with different angles of attack may be built into the same vector system; at the same time it is possible to design multitargeted ribozyme molecules which simultaneously attack in different places in the viral RNA.

The ribozymes represent a potentially potent anti-HIV therapy, for the reasons mentioned above and because ribozymes can attack viral RNA both before and after the genetic material of the virus has been built into the host cell's chromosomal DNA (2, 3). There are, moreover, promising preliminary results from attempts at manipulating the ribozymes so that they can be guided directly to the areas in the HIV-infected cell where the viral RNA is.

Several phase I studies of ribozyme therapy are now in progress, but results have so far not been published (7, 8). Here, too, a crucial problem is that the virus might develop mutants resistant to the ribozyme used. In all probability, this form of therapy will also have to be used in combination with other modalities.

Decoys
As mentioned above, the TAR and RRE sites on viral RNA are important binding sites for Tat and Rev, crucial regulatory viral proteins,. This binding can be blocked by gene therapy through a decoy strategy based on the introduction of a gene in the host cells expressing short RNA molecules, structurally identical with viral TAR and RRE (1, 2, 3). The decoy molecules will bind the regulatory viral proteins Tat and Rev and hence 'tempt' them away from TAR and RRE. This principle has considerable antiviral effect in cell cultures. There is, however, a certain amount of concern that this form of gene therapy may have potentially serious side effects, as the 'natural' binding between Tat and Rev on the one hand and their corresponding RNA on the other also involves the binding of host cell proteins which may be diverted from normal cell functions (6). Work is in progress on designing decoy molecules that no longer bind to such cell proteins. The first phase I trials are now under way with the decoy strategy outlined; clinical results are not available.

DNA oligonucleotides
An antisense principles is involved here, too, as the oligonucleotides used are designed so that they specifically bind to the viral mRNA which are the target molecules (6). This blocks the mRNA so that the corresponding viral protein is not synthesized in the cell. These forms of therapy have great potential. They are efficient in cell culture experiments and have also been shown to amplify the effect of conventional anti-HIV drugs *in vitro*. Introductory phase I experiments with one such oligonucleotide (GEM 91) targeted at mRNA for the gag gene in the viral genome have already been published (9), and an improved version of this molecule (GEM 92) will probably soon enter clinical trials.

Therapies based on intracellular proteins with anti-HIV effect

Different strategic options exist aiming to inhibit the function of viral or cellular proteins in the infected cell (2, 3).

Dominant negative proteins
The approach here is to introduce genes or gene fragments which trigger synthesis of proteins closely related to important viral proteins, but without certain essential properties of 'natural' viral proteins (5, 6, 10). Such modified proteins will have antiviral effect, partly by competing with 'natural' viral proteins for binding sites on mRNA, partly by capturing cellular factors involved in the 'natural' binding between viral protein and viral RNA, and finally by building complexes with the naturally occurring viral protein and thus neutralizing it. Several viral proteins have been attacked with this strategy in cell culture experiments. In the clinically most advanced trial, a gene is introduced that codes for a modified Rev protein (Rev M10). There are at least two ongoing clinical phase I trials (10, 11); clinical results with this strategy have not yet been published. Interestingly, one has managed to manufacture a protein that represents a combination of modified Tat and Rev proteins. Implantation of the genetic sequence for this protein in cells in culture has a significant anti-HIV effect, and the gene clearly has a potential for gene therapy (3, 6).

A potential problem in gene therapy based on dominant negative proteins is that these proteins, which are foreign to the host, might conceivably trigger an immune response when they are expressed in modified host cells and thus be destroyed, so that the therapeutic effect disappears.

Cellular proteins
Implantation of genes that code for cellular proteins, i.e. proteins of host origin with anti-HIV effect, might conceivably be used in gene therapy for HIV infection (6). The CD4 molecule is expressed on the surface of CD4-positive lymphocytes and is able to bind HIV. In cell cultures, cells in which the gene is introduced for a modified CD4 molecule retained in the cell's cytoplasm, have shown a certain anti-HIV effect, but this has not yet been tried *in vivo*. Introduction of the gene for human a-2 interferon, which clearly has an anti-HIV effect in cell cultures, has not been taken further to clinical trials, partly because there is a risk of side effects with this strategy. Moreover, interesting results have been reported with the introduction of the gene for b interferon in cell cultures from HIV patients as well as healthy individuals. This way a sustained low production of b interferon is achieved in the cells. This clearly has an anti-HIV effect, and it also improves the immunological function of the cells. Clinical trials using this strategy have not yet been published.

Intrabodies
It is feasible to introduce a gene in host cells, including CD4 lymphocytes, which gives a synthesis of so-called 'intrabodies', i.e. antigen-binding single-chain fragments derived from ordinary antibody molecules. These intrabodies can be designed so that they react selectively with essential HIV proteins and may inhibit viral replication in cell cultures (3, 6). The first phase I trials in HIV patients are already in progress with gene therapy based on intrabodies against the Rev protein and against HIV-1 gp 120 (Env). No clinical results are as yet known.

Suicide genes
Introduction in cell cultures has been achieved of several different suicide genes which directly or indirectly cause the death of the cell if it is infected with HIV. This may be done by for instance introducing the gene for diphtheria toxin and by introducing the thymidin kinase gene from Herpes simplex virus, which causes cell death if the cells are

exposed to the antiviral drug ganciclovir (3, 6). Clinical trials with this strategy have not yet been performed.

10.5 Immunotherapy

Apart from the many possibilities for inhibiting the intracellular life cycle and replication of the virus, gene therapy could also be used to boost the patient's own immune defence against HIV. This could be done through strategies that either boost immune reactions specifically against HIV, or generally strengthen cell-mediated immune defence.

HIV-specific immunotherapy

Cytotoxic T-lymphocytes

It is not yet finally established what elements in the immune system are decisive in combating HIV, but several observations indicate that cytotoxic CD8 lymphocytes are important in this respect. Relatively early on it was tried to obtain CD8 lymphocytes from patients, increase the number of cells by cultivating them *ex vivo*, and then returning the cells to the patients (3). No clinical effect could be demonstrated. A new approach to therapeutic use of CD8 lymphocytes is based on gene therapy modification of collected CD8 lymphocytes, by inserting a gene that leads to expression of a surface receptor which binds selectively to HIV-infected CD4 lymphocytes (3, 6). After the binding, the infected cells are killed. A problem with this modality is that CD8 lymphocytes have a limited lifespan in the organism. At least three different phase I studies based on this principle are in progress; results are not known.

DNA vaccines

Several therapy protocols are operative with therapeutic DNA vaccines in HIV infection. This principle is discussed in chapter 5.

Non-specific immunotherapy

Several HIV patients respond satisfactorily to modern HIV drugs according to the usual criteria, but without full reconstitution of immunological function This may impair the patients' ability to combat HIV as well as other microbes which may cause problems. Designing gene therapy strategies targeted at boosting the overall immune defence in HIV patients may be an interesting approach, but such therapeutic modalities are still in the preclinical phase.

10.6 Problems with gene therapy for HIV infection

In addition to the points of attack focused on in protocols up until now, gene therapy might also be targeted at other important processes in the life cycle and immunopathogenesis of the HIV infection. However, several problems have to be solved before gene therapy is an available therapeutic option for HIV infection (3, 6). Many of these problems are generic to today's gene therapy, others are related to the complexity of the HIV infection itself. CD4 T and CD8 T-lymphocytes are the target cells that so far have been studied for cell culture studies and clinical trials, the first because they represent the most important point of attack of the virus, the latter because they are seen as important in the immune defence against HIV. These are hardly ideal target cells,

however, as both CD4 and CD8 T-lymphocytes have a limited lifespan in the organism, and as genetic modification of these cells will probably not trigger a substantial increase in numbers through cell division. Moreover, the gene transfer systems used so far are not very efficient in relation to these cells, partly because they are not proliferating during the procedure Finally, by far the largest number of CD4 T-lymphocytes are not found in the peripheral blood stream, but in lymphoid tissue in the organism which also holds large amounts of virus, and only a small proportion of the patient's CD4 T-lymphocytes can be genetically modified this way.

In HIV infection, it would be far more attractive to use haematopoietic stem cells as target cells, instead of T-lymphocytes Genetically modified stem cells might in principle generate a vast number of daughter cells with the same gene, so that a considerable part of the patient's immune system would be repopulated with modified cells. Experiments have already shown that it is feasible to extract a significant number of CD34-positive stem cells from peripheral blood and umbilical cord blood in HIV patients (12). The transfer systems used so far in gene therapy are not very efficient in relation to CD34-positive stem cells, but new results indicate that lentiviral vectors may yield satisfactory results in this respect. However, the safety aspects of using lentivirus for gene transfer are not fully elucidated.

The heterogeneity of the HIV virus causes quite crucial problems. A large number of variants of the virus may be present in a single HIV patient; theoretically, they may react differently to one gene therapy strategy. Still more problematic is the fact that this virus has a great propensity for mutations which may cause resistance against the gene therapy strategy used in the individual case. The existence of so-called 'privileged' locations of the virus in the organism, e.g. in the central nervous system where the therapy does not reach in, is a significant problem in gene therapy, as it is in conventional drug therapy. All these concerns indicate that future gene therapy will probably be combined with other therapeutic modalities, including drug therapy.

The problems and obstacles encountered in gene therapy against HIV should in no way be underestimated. It is, however, probable that such treatment modalities will become available in the years ahead.

10.7 Gene therapy for other viral infections

Work is in progress on the development of gene therapy strategies for several other important viral infections. The principles involved are by and large the same as those sketched out for HIV infection.

CMV infection

Cytamegalovirus (CMV) is a very frequently occurring virus which usually only gives mild or subclinical infection in individuals with a normal immune defence, but often causes serious, possibly fatal infection in patients with immunodeficiency. Patients who have undergone transplantation or patients with uncontrolled HIV infection are important groups in this respect. Over the last few years, several drugs have become available for treatment of CMV infection. However, the drugs have side effects and may lead to resistance, and they do not always have therapeutic effect, thus there is clearly a need for new treatment modalities for CMV infection.

Development work on gene therapy strategies against CMV infection has been going on for several years and has recently produced results. Today, CMV infection is the first and so far the only infection in humans for which there is an efficient gene therapy available: synthetic DNA oligonucleotides with antisense properties targeted at the IE2 region in human CMV mRNA (3). *In vitro*, the effect of this therapy is about 30 times more potent than the effect of ganciclovir, the most frequently used CMV drug. An antisense oligonucleotide of this type (fomivirsen) is commercially available, designed for injection in the eye in cases of CMV infection in the retina, a serious complication in AIDS (13). *In vitro* experiments have also shown that other forms of oligonucleotide therapy may be possible (6). Clinical data are not available.

EBV infection

This herpesvirus is an important pathogen in humans, causing acute mononucleosis and various forms of malignant disease in immunodeficiency patients and in patients with apparently normal immune systems. Although some recent drugs have a certain effect on EBV infection, there is a need for new therapeutic modalities, for instance against infections in patients with immunodeficiency. It has been shown *in vitro* that DNA oligonucleotides with antisense properties in important regions in EBV mRNA are very potent in inhibiting viral replication and hence possible candidates for clinical trials (6). Reinfusion of genetically modified CD8 lymphocytes with EBV specificity is also a possible strategy (6).

HBV infection

The hepatitis B virus is a frequent cause of acute hepatitis. Most cases are cured without permanent injuries, but in some, the infection turns chronic. This is a possible cause of serious chronic hepatitis, which may develop into cirrhosis and into liver failure. Chronic HBV infection can also lead to cancer of the liver and to chronic immune-mediated inflammatory diseases in other organs. Some drugs may have a certain effect in HBV infection, but there is a definite need for new and more efficient therapeutic modalities. *In vitro* experiments have shown that certain synthetic DNA oligonucleotides with antisense effect in relation to the gene that codes for the pre-S antigen of the virus, very efficiently inhibit viral replication, and thus may be candidates for further development (6). Years of development work on anti-HBV DNA vaccines have resulted in clinical trials, but results are not yet available. An efficient DNA vaccine will probably have prophylactic as well as therapeutic applications in HBV infection.

HCV infection

Like HBV, hepatitis C virus (HCV) causes acute as well as chronic hepatitis and a predisposition for cancer of the liver. The infection can also cause immune-mediated disease in other organs. The relevant drugs, a-interferon and ribavirin, often have limited or no effect; not infrequently they have disturbing or serious side effects. There is a great need for a new and better therapy. Studies *in vitro* with synthetic oligonucleotides have so far not yielded relevant results (6). Work on a DNA vaccine based on the so-called HCV core region is, however, well under way, although clinical results are not available. An efficient DNA vaccine against HCV will probably have prophylactic as well as therapeutic applications.

Human papilloma virus (HPV)

This virus causes skin and mucosa infections and is associated with malignant diseases, including carcinomas in the oral cavity and the cervix of the uteri. Preclinical experiments indicate that various types of antisense strategies might be pursued (6). Clinical trials are now in progress with a synthetic DNA oligonucleotide (aforvirsen) which is injected directly into the affected skin.

Herpes simplex virus

This virus is associated with a series of infections ranging from trivial ailments to serious or fatal conditions, particularly in immunodeficiency patients. Efficient drugs are available, but resistance development is an increasing problem, hence gene therapy is a relevant option. Good effect from DNA oligonucleotides with antisense properties has been shown *in vitro* and in animal models. *In vitro* experiments with transdominant negative viral proteins have also yielded promising results (6). A DNA-based vaccine against the Herpes simplex virus is now in a clinical trial. Such a vaccine might also be relevant as a therapy in serious infections.

10.8 Conclusion

A large number of gene therapy protocols on HIV infection are now operative. The experience gained from clinical trials will be useful in the development of gene therapy for HIV infection and other viral infections. There is reason to hope that gene therapy in the years ahead will become a valuable alternative or supplement to antiviral drug therapy.

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11. Gene therapy in diseases in the cardiovascular system

11.1 Summary

Cardiovascular disease will probably remain the leading cause of death in the industrialised world. Gene therapy is a new therapeutic principle in this patient group, and there are only a few on-going clinical trials of gene therapy for diseases in the cardiovascular system. However, encouraging preliminary results have been reported from early phase gene therapy studies (phase I and a few phase II studies) on patients with cardiovascular disease. There is also considerable research activity on cardiovascular disease. Great advances have been made during the last few years, and it is not improbable that gene therapy in relation to cardiovascular disease to a significant extent may yield clinically important results in the near future. It should, however, be pointed out that phase III trials have not yet been started in this disease group.

11.2 Introduction

Cardiovascular disease is the most frequent cause of death in the western world. Most cases are caused by ischaemia due to arteriosclerosis, which leads to stenosis or occlusion of the vessels. There are no accurate data on the incidence and changes in incidence of ischaemic cardiovascular disease. New drugs such as b-blockers, ACE inhibitors and cholesterol-controlling drugs (statins) introduced over the last 10 to 15 years have contributed to reducing cardiovascular disease mortality; however, it is expected that this group of diseases will continue to be the leading cause of death in the western world. The main reason is demographic: The elderly will constitute an increasing proportion of the population in the years ahead. Thus, cardiovascular disease has significant socio-economic implications in a population. Cardiovascular research is a driving force in the development of conceptually new drugs aimed at reducing the morbidity and mortality from these diseases. Gene therapy in the cardiovascular system is a particularly enticing idea, as the endothelial cells are in direct contact with the bloodstream; and gene therapy is a new potential treatment principle.

11.3 Background: pathogenesis

Coronary artery disease has a special place among the cardiovascular diseases. It is caused by arteriosclerosis with occlusion of the coronary arteries which impedes the blood supply to the heart, with ensuing angina pectoris or myocardial infarction. In myocardial infarction, a part of the heart muscle is lost, and if the infarction is sufficiently large, the patient may develop heart failure so that the heart is no longer able to cope with its pumping function and supply a sufficient amount of oxygen to the body. After myocardial infarction and loss of a part of the heart muscle, changes will often occur in the remaining healthy part of the heart muscle. These changes include enlargement of the left ventricle and pathologic hypertrophy with myocardial fibrosis. This remodelling of the myocardium is in itself detrimental to the myocardial function, and modern post-infarction treatment aims at limiting myocardial remodelling and thus postponing the development of heart failure.

The arteriosclerotic pathogenesis usually takes place over several years and is influenced by lifestyle and several polygenic factors. A number of risk factors for arteriosclerotic disease are known, including increased total cholesterol level and level of low density lipoprotein (LDL) cholesterol, low high-density lipoprotein (HDL), low level of apolipoprotein A, too high level of homocysteine, and hypertension. Cigarette smoking and low physical activity are also considered risk factors. In some cases in which monogenic factors have a decisive role, arteriosclerosis can develop far more rapidly and cause manifest disease in early adulthood. This applies to, e.g., familial hypercholesterolaemia, Tangier disease (familial high density lipoprotein deficiency), and greatly increased levels of Lp (a) lipoprotein.

Arteriosclerosis with accumulation of lipids in the arterial wall also involves inflammatory mechanisms with growth of smooth muscle cells and thickening of the intima layer of the arterial wall. This may cause complications, for instance that plaques rupture and causes arterial thrombosis. Hence, ischaemic cardiovascular disease has a highly complex pathogenesis which involves atherogenesis, thrombogenesis and inflammatory mechanisms. Efficient treatment will probably imply interventions modulating all the three mechanisms mentioned. In theory, gene therapy could be an efficient mode of attack.

PTCA (percutaneous transluminal coronary angioplasty) is a minimally invasive method increasingly used to open up stenotic coronary vessels in order to revascularize the myocardium in cases of unstable angina and imminent or acute myocardinal infarction. However, with this therapy reocclusion of coronary vessels occurs within six months in 20–35 per cent of the patients. Early reocclusion seems to involve inflammatory mechanisms, including intima hyperplasia. When such a complication occurs, aortocoronary bypass (ACB) surgery may be necessary. In ACB procedures, veins from the lower extremities or arteria mammaria interna are usually use to bypass the stenotic area. Even these procedures are often followed by reocclusion in a not insignificant percentage of cases over a five to ten-year period after the procedure (1). The research on reocclusion after PTCA or ACB surgery aims at finding interventions, which may prevent reocclusion. Right now gene therapy is tried out with a view to modulating the mechanisms that lead to reocclusion after a surgical intervention. A recently published prospective, randomised trial used decoy oligonucleotides which bind up transcription factor E2F and thus block its normal effect. E2F normally causes up-regulation of several cell cycle genes, and reduced activity of E2F will inhibit cell division in the arterial wall. Significantly fewer reocclusions were observed after 12 months in the group treated with decoy E2F oligonucleotide than in the control group (2).

11.4 Gene therapy for cardiovascular disease

Most published studies on gene therapy for cardiovascular disease are experiments in animal models. Several gene therapy protocols on human subjects are now under review, and some phase I and phase II trials have been reported. The problems with gene therapy in the cardiovascular system are by and large the same as those known from other attempts at gene therapy, not least the difficulties with incorporating the gene and getting gene expression in a reasonable number of cells in the receiving organism. *In vivo* as well as *ex vivo* gene transfer have been tried, and several different vectors for gene transfer have been tested. There are also problems with achieving stable gene

expression. For some applications, this limitation may serve a purpose; one example is the treatment of restenosis after PTCA for which transient gene expression is probably sufficient, perhaps in fact desirable.

The following pathological processes in the cardiovascular system are currently under assessment as candidates for gene therapy:

1. Gene therapy targeted at monogenic diseases affecting lipid metabolism, e.g. familial hypercholesterolaemia (LDL receptor deficiency) and Tangier disease (ABC-1 deficiency). Gene therapy for familial hypercholesterolaemia is discussed in more detail in the chapter on monogenic diseases.
2. Gene therapy targeted at preventing reocclusion of coronary arteries after PTCA.
3. Revascularization of hypoxic or ischaemic tissue through gene therapy.
4. Gene therapy for preventing the development of heart failure because of dilated cardiomyopathy.

Gene therapy could also be relevant for modulating the inflammatory and thrombogenic mechanisms in ischaemic coronary disease. Moreover, gene therapy may conceivably be used to modulate several disturbances in lipid metabolism in addition to the monogenic diseases mentioned. Below we describe in more detail some of the strategies for using gene therapy.

11.5 Gene therapy targeted at preventing reocclusion of coronary arteries after PTCA

Most attempts at gene therapy in relation to cardiovascular disease have focused on preventing the frequent reocclusion during the first six months after PTCA. Pharmacotherapeutic interventions with anticoagulants and acetylsalicylic acid have minimal effect. However, new platelet inhibitors that competitively inhibit the gp2b/3a receptor have been reported to reduce the restenosis problem to a certain extent. On the basis of preclinical experiments we may conceive of several different targets for gene therapy. In human subjects, attempts have been made at transferring the gene for VEGF, vascular endothelial growth factor, in order to stimulate re-endothelialization of damaged arterial walls after PTCA. These trials use naked (3) plasmid DNA that codes for transfer of a 165 amino acid isoform of VEGF. In this setting, transient gene expression seems to be an advantage rather than a disadvantage. Early-phase trials with transfer of VEGF are promising, but it is too early to express any definite opinion of whether this may be a topical treatment for preventing restenosis. Larger trials are in progress, and we have to await the results from these.

11.6 Attempts at revascularization of hypoxic/ischaemic damaged tissue

Ischaemic cardiovascular disease can cause dysfunction in tissue because of oxygen deficiency (hypoxia). Strategies for increasing the formation of new blood supply to hypoxic tissue through collateral development will be interesting candidates for gene therapy. In this context, several growth factors have been relevant, including FGF (fibroblast growth factor) and VEGF. Apart from being tried out as an instrument of preventing restenosis of coronary arteries after PTCA, transfer of the gene for VEGF has been used in order to stimulate collateral formation from endothelial cells. There are

recent reports of promising results with increased collateral formation after direct intramyocardial injection of naked plasmid DNA or recombinant adenovirus coding for VEGF in human subjects (4, 5). These injections have been used as an add-on treatment to coronary bypass surgery or as the only treatment in minimally invasive procedures. It should be noted that these reports represent clinical phase I trials, trials in which the primary concerns are tolerability and safety. In both reports, improved functioning was achieved for most patients. Moreover, improved myocardial function was shown in the area in which the gene vector had been injected. No serious side effects of the treatment were reported. The result of these treatments are promising and provides a basis for going on with clinical phase II trials aimed at investigating whether beneficial effects that are superior to existing modalities can be achieved through this gene therapy intervention.

Multiple intramuscular injections of naked DNA that codes for VEGF in the lower extremities in patients with occluding intermittent claudication and ischaemic calf ulcers have also given encouraging results (6, 7). Isner et al. have shown that VEGF stimulated increased collateral formation and healing of leg ulcers in most patients receiving this treatment. Phase I trials have given no evidence of significant problems in terms of tolerance of the genetic material administered. In the near future we will probably have results form larger trials and from more centres, results that might indicate whether such interventions should become established treatment modalities.

11.7 Gene therapy for the prevention of heart failure

There is a great deal of interest in a new treatment principle which may prevent the development of dilated cardiomyopathy after, e.g., myocardial infarction. As of today, the most promising preclinical results are related to the myocardium-specific transfer of the carboxy-terminal fragment of G-protein-linked protein kinase-2 (bARK-CT). The carboxy-terminal region of bARK-CT contains a plextrin homology domain (binding domain for Gbg). Overexpression of this domain competitively inhibits the activity of G-protein-linked receptor kinases and hence desensitization of G-protein-linked receptors, including b-adrenergic receptors. Heart failure has been associated with reduced response through b-adrenergic receptors. It is not clear whether the interaction with receptor desensitization is in fact the cause of the beneficial effect of overexpression of bARK-CT in heart failure. However, administering recombinant adenovirus coding for bARK-CT directly into the coronary artery circulation has been shown to have a highly beneficial effect on the development of dilated cardiomyopathy in several experimental models of heart failure. This interaction could probably be tried out in patients with terminal heart failure.

11.8 Other potential gene therapies for cardiovascular disease

Intervention related to modulation of the inflammatory component in ischaemic coronary disease will probably be tried out through the use of gene therapy targeted at adhesion molecules. For example, phase I trials using antisense oligonucleotides to inhibit expression of ICAM-1 have already been carried out (8).

Cardiovascular disease usually has a complex chain of causation. With more detailed knowledge of the molecular mechanisms in the pathogenesis of coronary disease, we

could probably use gene therapy to modulate the expression of genes which will greatly influence development of coronary artery disease and the remodelling of the myocardium after myocardial infarction.

There is not much doubt that a great deal is expected from gene therapy and modulation of gene expression in relation to ischaemic cardiovascular disease, and that clinical success is expected from these efforts. Relatively large patient groups might benefit, and a moderate effect in terms of mortality may imply great gains in the number of lives saved. Preliminary results from gene therapy on patients with cardiovascular disease are promising, and significant advances have been made during the last few years, thus it is not improbable that gene therapy for cardiovascular disease might generate a considerable number of clinically important results far earlier than gene therapy targeted at other diseases.

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12. Gene therapy in other disease groups

12.1 Summary

Gene therapy is also topical for other disease groups, including some autoimmune diseases and certain diseases in the central nervous system. Significant preclinical research results are available from both fields. As yet, trials in humans have only been started for some autoimmune diseases, including amyotrophic lateral sclerosis and cubital tunnel syndrome.

12.2 Gene therapy in neurodegenerative diseases

Gene therapy has great potential as efficient treatment for Parkinson's disease and Alzheimer's disease. These are serious and relatively prevalent diseases without a big genetic component. Their basic causes are still largely unknown, though we know about some substances that are not present in these patients, and that are key substances in the pathogenesis. An obvious objective for gene therapy will be to insert genes coding for these substances into the relevant structures of the brain. Gene delivery is effected by stereotactic injection either of genes that have been genetically modified *in vitro*, or by direct injection of vector/gene *in vivo*.

In **Parkinson's disease**, there is a lack of l-dopa/dopamine in the striatum of the brain. Gene therapy has by and large been aimed at administering the tyrosine hydroxylase enzyme to the striatum, as this enzyme slows down the production of l-dopa/dopamine. Experiments in animal models have successfully increased the levels of these substances, but long-lasting and high expression has not been achieved. Nerve growth factor (NGF) may also have the effect of reducing nerve degeneration in animal models of Parkinson's disease. The same may apply to an anti-apoptotic protein, Bcl-2, which normally has an important role in regulating apoptosis.

All the main types of vectors have been used. On the background of the immune-protected state of the brain, it has been suggested that adenovirus does not cause the immunological problems known from its use in other tissues, though recent results with suicide-gene therapy on brain tumours may indicate development of a more chronic inflammatory condition. In these trials, adenoviral vectors have been used; it is not established whether these are responsible for the inflammatory reaction (1). Herpes simplex virus is perhaps particularly attractive, as it naturally infects nerve cells. The virus can carry large genes into the cell, establish a latent infection and cause protracted gene expression in spite of the fact that the virus is not integrated into chromosomal DNA. Adeno-associated virus is also relevant, as it also infects cell in the dormant phase and is integrated in the chromosome.

In **Alzheimer's disease**, a crucial aspect of the pathogenesis is the degeneration of cholinergic cells which need a steady supply of nerve growth factor. It has been shown in animal models that introduction of the gene for this growth factor can delay the degeneration of cholinergic neurones. Experiments also suggest that introduction of the antiapoptotic peptide Bcl-2 delays cell degeneration.

A new therapeutic principle has recently been introduced for both diseases: replacing dead nerve cells with stem cells from nerve tissue (or other types of stem cells). These stem cells may be isolated, grown in cultures, genetically modified if necessary, and then returned to the body, either in a local injection or in the bloodstream.

Amyotrophic lateral sclerosis (ALS) is a neurological disease with increased death of motor nerve cells. A beneficial effect of neurotrophic factors, including ciliary neurotrophic factor (CNTF), has been observed in preclinical trials. A phase I gene therapy trial has been started on this disease (2). The CNTF gene is transferred to suitable cells *ex vivo*, and the transfected cells are then transferred to patients in polymer capsules. However, no clinical effect was observed on the progression of the disease in the first patients (2).

12.3 Autoimmune diseases

There are several strategies for moderating the effect of an overactive immune system in autoimmune diseases. Several indications suggest that changes in the cytokine balance may have an important role in the pathogenesis of several of these diseases, hence it is rational to inhibit pro-inflammatory mediators like tumour necrosis factor (TNF) and interleukin-1 (IL-1) or to stimulate/increase the amount of anti-inflammatory mediators like transforming growth factor b (TGF-b), IL-4, IL-13, Fas-ligand and IL-10. TNF antagonists, i.e. anti-TNF antibodies or a recombinant TNF receptor fusion protein, has recently been introduced in the treatment of rheumatoid arthritis and in Crohn's disease (3). A fair number of gene therapy studies of autoimmune diseases have been carried out in animal models (4–5). Along with modification of the cytokine balance, other angles of attack are also tried, e.g. blocking the expression of important interaction molecules on the cells, like ICAM-1, or inhibiting the proliferation of synovial cells (6). Treatment could be systemic or local. Clinical gene therapy trials on human subjects have been started on rheumatoid arthritis, Crohn's disease and ulcerative colitis; no results are as yet available.

12.4 References

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13. Legislation relating to the development of gene therapy

In Norway, development and implementation of gene therapy is the object of several regulations and approval systems. The types of genetically modified organisms (GMO) that can be used in preclinical research and development are regulated by the Genetic Engineering Act; laboratories need to be approved. Production and testing in humans of pharmaceutical drugs in general requires the approval of the Norwegian Medicine Control Authority. In clinical trials involving the use of GMO, only approved laboratories may be used for preparing injections and analysis of samples taken from patients. In addition to these rules, and the legislation governing pharmaceutical drugs and drug development, the *Act relating to the medical use of biotechnology* specifically regulates the use of gene therapy on human subjects. Under this Act, approval by the Norwegian Board of Health following a preliminary assessment from the Advisory Board on the Application of Biotechnology in Medicine is required. Gene therapy testing must also be approved by the Regional Committee for Research Ethics in Medicine, and the Ministry of Health and Social Affairs must be notified in writing.

13.1 The Biotechnology Act

As its name implies, the Act relating to the medical use of biotechnology (the Biotechnology Act[2]) regulates the 'medical use' of biotechnology, but this concept is not clearly defined. The interpretation used by the Norwegian Board of Health is that the Act and the legislative history (the *'travaux préparatoires'*) regulates clinical use of biotechnology, i.e. biotechnology methods with concrete therapeutic or diagnostic consequences for the individual patient. Hence, preclinical research is beyond the scope of the Act. The Legislation Department in the Ministry of Justice has, however, concluded that the Act covers all medical research in biotechnology in the areas within its scope. This would, *inter alia*, imply that research on gene therapy, including preclinical research, could only be carried out in approved institutions, and that approval is needed for all treatment modalities under investigation. As a consequence of the Opinion given by the Legislation Department, the Ministry of Health and Social Affairs has prepared a bill[3] which clarifies the scope of the Act in relation to research. In this bill, the definition of medical use is in line with the Board of Health's interpretation. If this is enacted by the Storting, Norway's parliament (in the spring session of 2001), only use of gene therapy on human subjects will be included. An assessment of the Biotechnology Act is in progress; some of the Board of Health's experience with the Act is sketched out below.[4]

The Act includes a special chapter on gene therapy, which states that (section 7-1) «The genetic material of human beings can only be changed through gene therapy on somatic cells for the treatment of serious disease or to prevent that such disease breaks out.

2 http://www.helsetilsynet.no/regelver/lover/biotekno.htm
3 http://www.odin.dep.no/repub/98-99/otprp/93/innhold.htm
4 http://www.helsetilsynet.no/htil/avd2/fagradeval.htm#kap7

Treatment for the purpose of changing the genetic material in a fertilized ovum is prohibited.»

What is meant by gene therapy is not clearly defined in the Act. It is included among the comments relating to section 7-1 in the *travaux préparatoires* (Proposition to the Odelsting no. 37, 1993–94) that «Gene therapy on somatic cells should only be used for the treatment of serious disease, under the prerequisite condition that the new gene has the potential *of correcting the genetic defect*, and that it will be expressed in the cells in a safe manner» (our italics). This might give the impression that the primary concern has been gene therapy in the context of correcting heritable disease. However, chapter 6.4.1 of the *Proposition to the Odelsting* also enumerates other types of disease for which gene therapy may be relevant; one would hardly be justified in saying that these forms of gene therapy should not be allowed or regulated.

On the basis of this, one must assume that there is no doubt that therapy which leaves long-term or permanent changes in the genetic material in somatic cells is regulated under the Act. As with treatment that (only) kills cancer cells, it is, however, not obvious that e.g. DNA vaccination and antisense treatment or implantation of genetically modified cells is covered by this provision. Further s doubts could also be raised as to whether DNA vaccination is covered by the Act, on the grounds that it is not «treatment of disease», but prophylaxis. The Act only allows gene therapy for the treatment of *serious disease*, and one must assume that DNA vaccination will primarily be used for prevention of ordinary diseases. In the opinion of the Board of Health, these matters might indicate that such treatment should be beyond the scope of the Act.

There are no special provisions for gene therapy on fetuses (*in utero*). Legally speaking it is not clear whether gene therapy on fetuses would have to be regulated in the same way as gene therapy on those already born, as jurists usually are of the opinion that «the genetic material of *human beings*» only refers to the genetic material of those already born.

13.2 Administrative review of applications for approval

At present, applications for gene therapy are subject to a highly comprehensive review procedure. The Norwegian Medicine Control Authority reviews and approves projects relating to clinical drug trials, and the Regional Committee for Research Ethics will also normally review research projects in this field. The various bodies approve or recommend the project on the basis of differing guidelines/regulations and authority in law; the division of labour between them is not defined in any detail in the Act or the *travaux préparatoires*. Gene therapy applications must also be submitted to the Advisory Board on the Application of Biotechnology in Medicine for an opinion before the Board of Health makes a final review of the application. The Board of Health has recommended that it should be considered whether it might be sufficient that applications for gene therapy are reviewed by the Norwegian Medicine Control Authority and the regional research ethics committees only.

The highly comprehensive nature of the review process makes it unnecessarily time-consuming. However, now that the first applications have been approved, one may expect a more expedient procedure for new applications, but it could hardly be expected that the review will take less than 60 to 90 days, the usual review period in other European countries as well.

13.3 On what basis is approval granted?

Beyond what follows from the requirement in section 7-1, the Act does not specify the concerns that should be emphasised in the review of applications for gene therapy. According to the *Proposition to the Odelsting*, page 57, «[e]ach individual treatment modality shall be the object of approval. In this type of therapy, there will be a need for a comprehensive safety assessment. A decision on whether or not approval will be granted should be based on ethical, societal and safety assessments.»

The Board of Health has established an Advisory Board on the Application of Biotechnology in Medicine[5] to assist the Board of Health in its work on the Biotechnology Act, and the Advisory Board has in its turn set up a Working Group on Gene Therapy. The Advisory Board and the Working Group have recommended criteria which should be emphasised in the review of applications for clinical gene therapy projects, and have also assisted in the assessment of applications in the field. The recommendations attach great emphasis to the medical/scientific merits of a given project, although the Act does not specifically authorise this emphasis. The Board of Health is of the opinion that there is statutory authority for rejecting applications even if they satisfy the Act's explicit minimal requirements, but that there may be some doubt concerning the extent to which the expertise of the scientists involved, generally and in relation to the specific project, should be assessed and accorded weight in relation to the decision.

«Safety assessments» could refer to spread of any genetically modified organisms used for therapy to the environment or to other humans, matters regulated by the Genetic Engineering Act. The term could, however, also refer to the patient's safety, with a view to undesirable integration of genes into areas of the genome where they should not be. A matter of principle not yet settled is whether treatment of human subjects with genetically modified organisms constitutes 'dispersal' in the sense of the Genetic Engineering Act, which otherwise does not regulate gene therapy on human subjects.

A more complicated matter is safety assessment in relation to the ban on gene therapy on germ cells. What should be required in terms of documentation to the effect that the gene therapy agent does not reach germ cells? Pollution of the gonads may be a special concern in fetal therapy, as the risk of effects on immature germ cells moving about in the body may be greater, and small size may increase the risk of contamination.

These aspects were assessed in the application review procedure:

- the extent to which the documentation submitted gave an adequate description of the objective, procedure and expected benefit of the study;
- whether the gene therapy study was concerned with the treatment of serious disease or with the prevention of such disease;
- side effect, if any;
- scientific use of the data;
- collaboration, if any, with other health care institutions, research institution or groups, or pharmaceutical companies;
- ethical issues;
- whether the gene therapy study was to be carried out in an institution with adequate and relevant expertise in relation to the study;

5 http://www.helsetilsynet.no/htil/avd2/fagrad.htm

- information in writing designed for participants in the study;
- the opinion of the Advisory Board on the Application of Biotechnology in Medicine.

The review process in practice

The three applications so far reviewed by the Board of Health are all related to the treatment of cancer. The first application was for a gene therapy project designed to include 10 to 15 patients with *glioblastoma multiforme*, a brain tumour. In short, the treatment given under this project consisted of surgical removal of most of the tumour and insertion of mice cells producing a modified virus in the cavity left by the tumour. The virus would transfer the thymidine kinase gene to the cancer cells. After some time, the patient would be treated with ganciclovir, which would kill thymidine kinase holding cells, thus there would be no genetically modified cells left in the patient after successful treatment.

The Board of Health had no objections in point of principle to the proposed gene therapy modality; however, the Board found that several aspects should be clarified and improved through preclinical experiments before approval for a trial could be given, and that the proposed project could only provide a limited amount of new knowledge. On this basis, extensive brain surgery on patients was considered unacceptable. The Board of Health did, however, express the view that in point of principle, providing a treatment is not necessarily unethical even if one does not expect an effect from the gene therapy, as long as the patients are well informed and personally wish to participate, and the study will provide valuable new insights.

Since then, the Board of Health as reviewed two gene therapy applications, the first of them for injection of an antisense oligonucleotide against the PKC-a enzyme directly into the tumour in patients with advanced malignant melanoma (an extremely aggressive type of cancer of the skin), or non-small-cell lung cancer. This enzyme is a prerequisite for growth and division in cancer cells. The other application was for the use of an adenovirus containing the cloned human gene for p53. This is a ‘brake protein’ for preventing cancer development. It is of particular importance that p53 may induce programmed cell death during chemotherapy. The gene therapy would be combined with cytostatic drugs to see if would lead to increased survival rates in patients with ovarian and peritoneal cancers. This trial also involved injections directly into the tumour.

The Board of Health received no objections to these applications, both of which were assumed to have few gene therapy-related side effects, and both were approved.

13.4 Legislation in other European countries

A network for regulatory issues in gene therapy in Europe has been set up under the EU’s BIOMED 2 programme. This network aims at providing an overview of gene therapy regulation in the European countries, a newsletter is published, and the network has a web site with an overview of regulatory efforts in various European countries.[6] Meetings and contact sessions are organised for professionals involved in national

[6] http://193.48.40.240/www/eurogenethy/eurogenethy.html

approval procedures for gene therapy applications. One long-term objective is to achieve a harmonisation of regulations across Europe.

Several countries, including Sweden, Denmark and Germany, let their drug control authorities be in charge of the approval of gene therapy; protocols need the recommendation of committees for research ethics. Some countries have also set up advisory bodies reviewing gene therapy protocols, for instance the Gene Therapy Advisory Council (GTAC) in the UK, and the Kommission für somatische Gentherapie in Germany, which issue recommendations in the field.

14. Ethical issues in gene therapy

In the literature on gene therapy a distinction is usually drawn between somatic gene therapy trials and germ-line therapy. The ethical justification for this distinction has been that somatic gene therapy trials do not represent anything new in terms of ethical principle; they should therefore be regulated according to already existing principles for therapeutic research, such as informed consent and adequate risk/benefit assessments.

A specific type of risk given special attention in somatic gene therapy trials is the risk of *unintentional* manipulation of germ-line cells, especially in relation to the use of viral transfection vectors. Though interventions on germ-line level go beyond the definition of gene therapy in the present report, this potential side effect requires a brief definition of what is implied by the term germ-line therapy. The first thing to note is that the term 'germ-line therapy' is misleading. For one cannot speak of therapy in the proper sense of the word, if the trials do not involve individual patients with identifiable diseases, but rather reproductive cells like spermatozoa, their precursors and eggs, or eventually (in the case of preimplantation diagnostics) totipotent embryonic cells at the 8 to 16 cells stage. «Genetic manipulation in germ-line cells can never be specifically therapeutic, because an individual who is affected by a disease does not exist. Genetic interventions into germ-line cells are preventive strategies for potential diseases» (1). It has been suggested on these grounds that the term 'germ-line therapy' should be replaced by the term *'germ-line gene manipulation'* for this type of intervention (1). Moreover, germ-line based interventions differ from somatic gene therapy in the sense that a permanent change in the DNA or RNA is established, a change that may be inherited by offspring. When germ-line cells are the target of intervention, an adequate assessment of risks and results is harder to undertake. A number of ethical arguments for genetic intervention at germ-line level have been advanced – such as cost-efficiency and the potential of eradicating a genetic disease from whole families (preventing vertical spread), or of giving people with genetic disease the chance of having healthy children; the *safety aspect* however and the lack of potential for adequate risk assessment has lead to an international consensus to the effect that genetic experiments at germ-line level remain banned.

These issues raise the question whether the ban might also have consequences for somatic gene therapy trials, as the risk of *unintended* damage at germ-line level in such trials cannot be ruled out. The view has been put forward in the literature that the probability of such damages is negligible (2), effectively of the same order of magnitude as the risk of iatrogenic mutation at germ-line level in high-dose chemotherapy (3); we however still lack sufficient experience to be able to estimate the risk of such damages occurring or their extent. However, this kind of uncertainty has not lead to demands for a universal ban on somatic gene therapy trials, though some scientists have proposed that *prenatal gene therapy in utero* should be banned, for this very reason (4). The main argument for a differentiated ban of this type is that the potential for damage increases and risk assessment is even harder when the 'patients' are bound to the mother's life. These issues show that it is necessary to differentiate between types of gene therapy trials, not only in relation to the severity of the disease, or the risks involved in each

trial, but also in relation to *when* and on *whom* the trials are conducted. At the very least, patients participating in such trials – or the parents, in the case of trials on children– should be informed about possible risks and discomforts, including that of accidental damage at germ-line level.

It should be emphasised in this context that although the distinction between children and adults is ethically relevant, children and adults should in point of principle be seen as methodologically equal. This implies that one should not refrain from including children in gene therapy trials *only because they are children*. This equality principle seems particularly relevant in cases of monogenic diseases that manifest themselves in early childhood, in which case trials on adults cannot be carried out. According to the Helsinki Declaration, medical trials can only be conducted when the objective of the trial «stands in a reasonable relationship to the risk for the person included in the trial» (5). Even in the case of gene therapy trials involving children should the severity of the disease and the relationship between expected benefit and estimated risk, in the final analysis, be decisive for whether or not the trial should be allowed. This is also in line with the International Council of Medical Sciences' (CIOM) international guidelines for biomedical trials on human beings: «Risks are to be justified in relation to anticipated benefits to the child» (6).

A third distinction often introduced in the literature is between gene therapy interventions targeted at an identifiable disease and interventions targeted at 'improving' natural features, usually referred to as 'genetic enhancement' or quality improvement. The above distinction, together with that between somatic and germ-line level interventions, has resulted in the introduction of four categories or forms of genetic intervention:

1. somatic gene therapy,
2. preventive intervention at germ-line level,
3. enhancement in relation to somatic cells, and
4. enhancement at germ-line level.

In the present report, we have chosen to limit the definition of gene therapy to *somatic* gene therapy for *serious* diseases; hence categories 2, 3 and 4 fall beyond our scope.

A fourth and for the time being last distinction introduced in the literature is between somatic *gene therapy at germ-line level* and *mitochondrial gene therapy* (7, 8).It is still not clear whether this distinction will require ethical assessments different in kind from those already established in relation to the three distinctions mentioned above. Some researchers maintain that gene therapy in relation to mitochondrial diseases deserves special attention, as the inherent methodological potential (transfer of nucleus combined with *in vitro* fertilization) might serve as a 'door opener' for ethically acceptable forms of intervention at germ-line level: «… the therapeutic approach entails enucleation of the patient's oocyte; enucleation of a donor's oocyte to provide normal mitochondria in a cytoplast; transfer of the patient's nucleus to the donor's cytoplast; *in vitro* fertilization (IVF) of the nuclear transplanted oocyte; and implantation into the patient's uterus» (9).

The main argument for this type of intervention at germ-line level is that some risks associated at present with somatic gene therapy trials are thus avoided, e.g. the risk involved in the use of viral transfection vectors or the risk of penetration of the nuclear

membrane. If correct, this of course weakens the argument for maintaining an absolute ban on genetic interventions at germ-line level.

A preliminary conclusion which may seem to follow from these reflections is that attempts at genetic intervention should be assessed on the basis of four different dimensions: *cell type* (somatic cells vs. germ-line cells), *objective* (therapy vs. enhancement), *type of DNA* (mitochondrial vs. nuclear DNA), and *when* an intervention is carried out (prenatal vs. postnatal, children vs. adults). In a next step, these dimensions allow us to differentiate between six categories of genetic intervention, characterised by increasing risk and hence diminishing ethical acceptability as we move down the list:

1. postnatal gene therapy on children or adults on nuclear DNA or mitochondrial DNA in somatic cells;
2. prenatal attempts at gene therapy *in utero* on nuclear DNA or mitochondrial DNA in somatic cells;
3. mitochondrial germ-line manipulation (transplantation of the nucleus + IVF);
4. germ-line intervention on nuclear DNA;
5. enhancement in relation to nuclear DNA in somatic cells; and
6. enhancement in relation to nuclear DNA in germ-line cells.

The Group of Experts sees reasons to emphasise that these dimensions, categories and the ethical ranking of gene therapy will probably require adjustments as the field develops. This calls for an ongoing ethical discussion of new gene therapy protocols in the scientific community. The Group also wishes to point out that it considers the last two categories of genetic manipulation mentioned above, i.e. the so-called genetic enhancement or quality improvement, as ethically reprehensible under any circumstances. Last, but not least, the Group acknowledges the importance of funding research on the *ethical aspects* of the field. Not only is such research of professional relevance; it could also contribute to an open and informed public debate on this important field.

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15. Clinical applications of gene therapy – status, potential and limitations

15.1 Status

The first patient was treated with gene therapy 10 years ago. Since then, three to four thousand patients have been treated with various gene therapy methods in more than 400 different clinical trials. As of today, gene therapy is not an established treatment modality for any disease, with the exception of soluble antisense oligonucleotides against cytomegalovirus (CMV), approved for use in the USA. The gene therapy field today is characterised by clinical research. Most gene therapy trials have been carried out in the USA; however, during the last few years the field has gathered momentum in Europe.

The great majority of clinical trials are early-phase trials with a few patients in each. Three phase III trials have been started, but results are not yet available. It will probably take three to five years before conclusive answers are obtained from independent phase III trials in the same category of disease. This means that gene therapy in Norway will continue to be clinical research in the three to five years to come, and that it should not be offered to patients in Norway unless they are included in defined clinical trials (CMV antisense therapy is an exception).

15.2 Disease groups

Gene therapy has been tested out for diseases in which there is a fault in the genetic material, such as monogenic diseases and cancer, and for non-heritable disease in which gene therapy can be used to administer a drug or therapeutic agent. Most gene therapy studies have been done on cancer. There are also a large number of gene therapy protocols on viral infections (HIV in particular, but other viral infections as well) and on heritable monogenic diseases. Trials in cardiovascular diseases have arrived, and gene therapy trials have been started on the treatment of autoimmune diseases and certain neurologic diseases, for instance amyotrophic lateral sclerosis (ALS). In other neurologic diseases, i.e. Parkinson's disease and Alzheimer's disease, clinical trials are forthcoming. Common to existing gene therapy trials is that all apply to patients with serious disease without satisfactory alternative treatment modalities. Promising results have been observed in early-phase clinical trials in cancer, and recently also in certain forms of cardiovascular disease.

In cancer, tumour responses have been observed in several patients in phase I/II studies, especially in various immunotherapy protocols and with administration of tumour suppressor genes, while the effect of prodrug gene therapy is more uncertain. In several different gene therapy trials, remission of tumours has been observed, mainly locally in the injection site (partial remission), and in rare cases also complete remission (the patient no longer has demonstrable tumours). However, it has not yet been shown that gene therapy has cured cancer. In cardiovascular disease, there are recent reports of

interesting preclinical findings and promising results in early clinical trials of treatment of restenosis. It should be noted that these trials have not come very far, and results have not been published. In heritable monogenic diseases, e.g. adenosine deaminase (ADA deficiency), it has been shown that the transferred gene can function *in vivo* over a protracted period of time and produce ADA, but cannot yet replace administered conventional enzyme (Peg-ADA)[7]. In other immunodeficiency conditions, cystic fibrosis, haemophilia and Gaucher's disease, no certain clinical effects of gene therapy have so far been shown. There are, however, interesting preclinical data from experimental models in big animals on haemophilia and other conditions. In HIV, a phase II trial has been carried out without conclusive results, one reason being that the patients received other types treatment in parallel. Conclusive clinical effects are not reported for gene therapy on patients with HIV or other viral diseases (except CMV), but several interesting principles are tested in trials.

15.3 Limitations and potential

The impediments to successful gene therapy today can at large be ascribed to problems with gene transfer, hence optimisation of gene transfer is the main focus in gene therapy research. Technology and knowledge advances at a fast pace, and the potential of gene therapy is great.

Optimisation of gene transfer

More efficient and better targeted gene transfer

As unaided gene fragments are poor at passing through the cell membrane and into the nucleus in which they are intended to function, they are often linked to vectors which facilitate uptake. The vectors may be looked upon as vehicles transporting the cell fragments into the cells. Viruses have a specific design for going into and transferring genetic material to cells; hence modified, inactivated viral particles are often used as vectors. More efficient gene transfer and protracted gene expression in the cells are necessary. Therefore, a main focus in gene therapy research is to improve gene transfer to human cells, through better modified viral vectors and through enhanced non-viral based delivery systems. Higher target cell selectivity is also called for. Work is in progress on linking gene constructs to molecules that bind specifically to the requisite target cells in order to promote better and more selective uptake in the target cells. During the last few years, we have gained much better insight into how molecules are transported between cell compartments. This insight has been used to link small peptides with localising signals to the cell nucleus. This makes for more efficient transport of the transferred genes into the cell nucleus and thus increases the gene transfer efficiency. A similar principle could be used to guide the expressed protein to other parts of the cell by linking it up with gene fragments coding for specific signal sequences. Such inner guiding to the target has been used to express antibodies against growth factor receptors in the endoplasmatic reticulum in order to prevent the receptor from rising to the surface of the cell and thus prevent biological activation of the receptor.

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[7] In a phase I trial french scientists recently reported that gene therapy has been successfyully used to treat babies suffering from x-linked SCID. The treatment has reconstituted the infants' immune system for up to one year, and the babies are no longer in isolation and live at home without any treatment. (The success is being seeing as a milestone for genetherapy).

Protracted gene expression *in vivo* in humans has so far been difficult to achieve, though effect over several years has been observed in adenosine deaminase deficiency. It should, however, be pointed out that not all therapeutic strategies require protracted gene expression in the target cells. One area in which this applies is immunogen therapy; it is assumed that activation of the specific immune defence only requires short-term expression of the therapeutic gene to trigger immune responses. Similarly, a short-term gene expression could be preferable for restenosis, as too vigorous stimulation may lead to inappropriate proliferation of vessels something, which has been shown in animal models.

Another new and conceptually different tool for insertion of genes in a cell is the use of artificial chromosomes. These are model chromosomes able to carry relatively large gene fragments. It has recently been shown in animal models that artificial mice chromosomes inserted into the cell nucleus of a fertilized ovum are inherited as ordinary chromosomes. However, artificial chromosomes could also be important tools in conventional gene therapy. If such artificial chromosomes could be efficiently transferred into the target cells, the genes inserted into these chromosomes could be expressed and regulated as realistically as possible.

Use of stem cells and dendritic cells

For long, a considerable problem has been how to achieve efficient uptake in dormant cells for instance in human haematopoietic stem cells which are topical target cells for several forms of gene therapy. Right now there is great enthusiasm surrounding the use of modified lentivirus (the HIV virus family) as it has been shown that even if one removes by far the greater part of the toxic genes from e.g. the HIV virus, it still efficiently infects dormant cells. Experiments are now carried out on modified lentiviral vectors for gene transfer to haematopoietic stem cells, and results from animal models and preclinical experiments are promising. There is an important unsettled safety issue involved in the use of modified lentivirus: whether or not they can recombine back to potentially virulent wild-type virus.

The new understanding of stem cells, and the stem cell concept emerging over the last few years, provides new angels of attack. It has been shown in animal models that mesenchymal stem cells from bone marrow can settle in different organs; these cells might thus be attractive target cells for gene therapy on heritable bone diseases. It has also been shown that various stem cells can be developed into other types of cells; bone marrow stem cells can be developed into muscle and nerve cells, and nerve stem cells in the brain can be developed into blood cells *in vivo*. Finally, cloning of human, embryonic stem cells provides, in theory, a potential for using genetically modified embryonic stem cells for various therapeutic objectives. Such cells might conceivably be genetically modified and differentiated to the point when they function as effector cells. Modification of donor cells in the context of organ transplantation in order to reduce reactions is a case in point, especially in relation to xenotransplantation. Dendritic cells are increasingly used in immunotherapy; these are cells that have a crucial role *in vivo* for triggering immune responses. Transfer of genes that give immune response to dendritic cells *in vivo* or *in vitro* might enhance the efficiency of immunogen therapy protocols.

Reduced immune response to the vector

Work is also in progress on developing vectors that reduce the immune responses to the vector itself. Immune responses against vectors may cause side effects and limit the

effect of the therapy, as the genetically modified cells are destroyed. Immune responses are mostly caused by the vector, and a great deal of work is going on internationally aimed at developing better vectors which give rise to less immune response. More modern modified adenoviruses are under development; some of the genes that provoke immune response have been taken out without compromising cell uptake ('gutted' virus). Preclinical attempts have also been made at adding to known vector genes that turn down an immune response.

Regulated expression

A significant problem in present gene therapy is the lack of regulated expression of the gene in the target cells; hence genes have to be used that are not toxic when overexpressed, e.g. adenosine deaminase (ADA deficiency). If gene therapy were to be used against diabetes, it would be desirable to insert the insulin gene in cells with intact regulatory sequences, so that insulin production would show normal regulation relative to blood hormone and sugar levels. Conceivably, insulin-producing genetically modified cells might be biologically encapsulated in the body in order to prevent rejection. Moreover, several experiments are now in progress on using regulatory sequences to achieve expression of the gene in certain cells types whose regulatory sequences normally are active, for instance the use of tyrosinase promoter in the treatment of malignant melanoma.

DNA vaccination – a new principle for vaccines

It has been shown for malaria and some viral infections that DNA vaccines used prophylactically can give immune responses. There is, however, a significant degree of uncertainty about the safety of DNA vaccines, especially for prophylactic use in the population at large, but for serious disease the threshold for trials of DNA vaccines as therapy is lower, and several introductory clinical trials with therapeutic DNA vaccines have been started. In order to obtain even stronger immune responses trials are being carried out in which a therapeutic gene has been linked to other immunostimulating molecules, for instance genes for cytokines.

Treatment with soluble antisense molecules and ribozymes

Soluble antisense molecules are the most advanced concept in gene therapy, in the sense that antisense oligonucleotides against CMV are available as a pharmaceutical drug. Interesting trials are also going on of soluble antisense molecules against other viral infections and certain forms of cancer. Using antisense molecules and ribozymes against factors affecting the formation of vessels may be important in relation to cardiovascular disease as well as in cancer therapy. Recently, trials have been started which use antisense molecules against Nf-kB in autoimmune gastric diseases (Crohn's disease and ulcerous colitis). There is little experience with ribozymes in clinical trials, but antisense molecules and ribozymes are expected to give higher efficiencies through improved uptake and reduced breakdown, on the basis of modifications in the chemical composition.

New gene therapy strategies – preclinical studies in animal models

There is a considerable amount of ongoing research on *oral gene therapy*, i.e. delivery of therapeutic genes through the gastrointestinal tract. Experiments in animal models have been carried out in relation to peanut allergy; the gene that codes for the most important allergen is administered orally to the animals. This has lead to weakened allergic immune response in the animals when they are exposed to the peanut allergen

afterwards. It has also been shown that oral administration of the b-galactosidase gene in an adeno-associated viral vector gave long-term clinical improvement in an animal model for lactose intolerance. Thus, oral gene therapy with use of ‘genetic pills’ clearly has a potential in the treatment of various diseases. Trials like these have so far not been started on human subjects.

Normally, a radical change in the circulation takes place after birth. This is caused by the closure of a channel, ductus arteriosus, which is only open in the fetus and is normally closed right after birth. In certain types of congenital heart deformity, it might be preferable that the ductus arteriosus is not closed at once. Preclinical experiments on lamb fetuses have shown that it is possible to prevent closure of the ductus arteriosus after birth through *in utero* gene therapy. A gene construct inhibiting the formation of fibronectin was injected into the ductus arteriosus of lamb fetuses well over halfway into the gestational period. This way, closure of the ductus arteriosus in the lambs was prevented. These experiments show with some elegance the potential of gene therapy.

Present corrective gene therapy consists of inserting a new gene in addition to the defective gene. Methods have been developed for replacing certain defective genes with a normal gene (homologous recombination). Today, this technology is only used in animal models. For the time being, it is far from efficient enough to be applied in human studies, but it represents an interesting future goal. Conceivable, one might also correct minor genetic defects through gene therapy on mutated DNA. In certain settings, artificial chromosomes have clear advantages, as they may contain large bits of genetic material and thus transfer big genes as well as complex control areas. These will replicate and segregate in a stable manner in cell division; moreover, they have the advantage that the rest of the genetic material is not damaged by integration. Delivery is, however, a problem; the molecules are so large that they have to be injected directly into the cell. This limits application to the transformation of stem cells.

It is important to point out that better a biological understanding of disease and pathophysiology will contribute to a better and more logical approach to gene therapy methods. It is expected that the mapping of the human genome, completed in a few years time, and knowledge about the biologic function of the various genes will contribute to a significantly enhanced understanding of disease and disease pathogenesis.

16. Consequences for the Norwegian health services and medical research

In Norway, the first patients were treated with gene therapy at the Norwegian Radium Hospital under two approved protocols for cancer treatment. Both protocols are part of large, multinational multicentre trials. Gene therapy is not an established treatment against any disease today (with the exception of antisense against cytomegalovirus on special indications). Gene therapy will most probably still be clinical research during the next five years. However, the development of gene therapy is now so far advanced that it is important that Norway participates in systematic testing of some gene therapy principles, and at the same time develops expertise in order to implement and develop future gene therapy as a therapeutic principle. On this basis, the Group of Experts is of the opinion that a national effort at building expertise in gene therapy is important, in line with the recommendation in the National Cancer Plan and the preliminary political decisions on state funding for two centres in Norway (Haukeland Hospital and the Norwegian Radium Hospital).

In its review of Proposition to the Storting no. 61 (1997–98) on the National Cancer Plan and a plan for investments in equipment in Norwegian hospitals, the Storting, Norway's parliament, accepted the Government's proposal for spending NOK 95 million during the period covered by the plan, 1999–2003, in order to strengthen and enhance gene therapy expertise at regional hospital level, with regard to basic research as well as clinical research on gene therapy for cancer and other diseases.

The 1999 fiscal budget included an appropriation of NOK 5 million which was split between Haukeland Hospital and the Norwegian Radium Hospital, to be used for developing expertise in the use of gene therapy against cancer. The year 2000 budget includes funds for further development of expertise in the proposed NOK 226 million for follow-up of the National Cancer Plan.

16.1 Gene therapy related activity in Norway

So far, gene therapy development has mostly taken place in the USA, but other countries, European countries in particular, have lately made efforts at developing gene therapy. During the last few years, Sweden has increasingly focused on gene therapy, particularly in three centres, Stockholm, Lund and Uppsala, and invested considerable sums on facilities. Building costs for the new centre for genome and gene therapy research in Uppsala amounted to SEK 50 million, and the two other centres have also been greatly expanded.

In Norway, research in molecular biology, including preclinical gene therapy related research, is carried out in the regional hospitals and in the Norwegian Radium Hospital. These hospital reported their activities and plans to the Ministry of Health and Social Service in the context of the allocation of state funding for 1999. The Group of Experts

has received copious reports from the National Hospital of Norway, Tromsø Regional Hospital and the Norwegian Radium Hospital. A report from Ullevål Hospital was submitted at the Group's last meeting. In spite of reminders, no reports or plans have been received from Haukeland Hospital or Trondheim Regional Hospital. In addition to the earmarked funding from the Ministry, some of the hospital contribute their own funds to the financing of gene therapy related research and skills enhancement.

16.2 Development of the field of gene therapy in Norway

The Group of Experts has discussed in depth approaches to a national effort at developing gene therapy, agreeing on recommending two different approaches:

1. Development of infrastructure in some milieus
2. A national programme for building expertise in gene therapy

Development of infrastructure

Gene therapy is a large and complex high technology field requiring international collaboration. Considerable multidisciplinary expertise is needed as well as special facilities for gene therapy trials and preclinical experiments. As a consequence, international centres have been developed with a critical mass in terms of expertise in the field. The view of the Group of Experts is that the first thing to do is to build infrastructure in Norway for gene therapy trials. A milieu for gene therapy has to be interdisciplinary and draw on expertise in a number of fields, including basic molecular biology, and have its own full-time staff. Also needed are facilities for work with vectors at various safety levels and special facilities for work with patients. Gene therapy requires an exceptional amount of resources, and it is recommended that the infrastructure initially is concentrated in a few institutions. It follows that the Group of Experts is of the opinion that priority should be given to building expertise rather than to specific groups of disease, and that resources should go to the best groups. This is in line with the Norwegian Board of Health's recommendation of developing gene therapy centres in three locations at the most. The funding should be stable, confere the importance of dedicated positions. There should be a follow-up in terms of assessment of the results achieved with infrastructure funding, for instance in a review process conducted in collaboration with the groups after about two years.

In the present situation, building expertise in the field of cancer is of particular importance. Likewise, building groups for cardiovascular disease should be considered, possibly also for monogenic diseases and infectious diseases. A national gene therapy network will be important. If and when gene therapy becomes an established treatment, a regional solution will be necessary in which gene therapy could be performed in all regional hospitals and in the Norwegian Radium Hospital.

The Swedish view is that it is of critical importance to build two laboratories for construction of viral vectors and one laboratory designed for Good Manufacturing Practice. The National Hospital of Norway has plans for a P3 laboratory and the Norwegian Radium Hospital is building an advanced P2 laboratory for gene therapy; a new wing will include a P3 laboratory. Both institutions will be able to work with most vectors relevant for preclinical studies and in clinical trials. Scientists in Bergen now have access to P3 laboratories for preclinical experiments, but no facilities for the

treatment of patients. In those few cases when GMP manufactured vectors and gene constructs are not supplied by industrial partners for clinical trials, it is recommended that the services are acquired on a commercial basis; the Group is of the opinion that a national GMP laboratory in Norway is not necessary over the next few years; establishing this type of laboratory should, however, be considered in a five-year perspective.

A national programme for building expertise in gene therapy

Gene therapy is an expensive modality which should be developed in collaboration with international groups. In Norway, several research groups have ideas for potential gene therapy protocols on the basis of their own research, but as far as we know, none of these ideas are close to clinical trials. It is important that Norway has contributions of its own in the field of gene therapy, in addition to introducing expertise from abroad into Norwegian groups (training research). The Group of Experts sees it as important that the Ministry, beyond the infrastructure investments, also funds clinical as well as basic research in the field. In Sweden, a proposal has come for a national programme with collaborative funding from the Cancer Society, the Medical Research Council, the Foundation for Strategic Research, and the Wallenberg Foundation. Grants totalling SEK 60 million over a five-year period are awarded to gene therapy projects on the basis of project proposals assessed by an international peer review committee. A similar solution might be of interest in Norway. One of the focal points of the Swedish programme is research relating to vectors and gene transfer. Additional funding is provided for PhD students (ten per year) and postdocs (five per year) and scientists of particular eminence or promise (one per year for setting up a research group). It would seem appropriate that some of the Ministry's funding of gene therapy were set aside for a programme like this. The Group emphasises the importance of quality assurance of activities and grants being awarded on the basis of applications.

It is recommended that the Ministry sets up a steering group which may advise of the use of the funding. Sweden also has an international committee which reviews applications under the programme, an arrangement which should also be considered in Norway. Building networks through national and international collaboration is important.

16.3 Safety and ethics

There has been considerable interest in and concern over the safety aspects of gene therapy. In most early studies, safety and side effects are important parameters. So far, there have been some reports on fatal outcomes associated with gene therapy trials. One of them was a young man treated in the USA for a monogenic heritable disease who died some days after receiving gene therapy; however, onset of symptoms was some hours after the injection. There is an overwhelming probability that his death was related to the treatment, but so far the causation is not finally established. Another patient died under a gene therapy trial in cardiovascular disease; in this case it is not clear whether the fatality could be directly associated with the treatment[8]. In treatment of brain

[8] It was later concluded that the death of this patient, a 18 year old man known as Jesse Gelsinger, was a direct consequence of the gene treatment. However, investigators simultaneously revealed that there were multiple protocol violations during the conduct of this clinical trial.

tumours with prodrug gene therapy, inflammatory responses have been observed around the injection site, and animal models have shown that this inflammation can be considerable and long-lasting.

Apart from these, surprisingly few side effects have been reported. The side effects are largely local and related to the injection, or mild fever and influenza symptoms. Development of symptoms or signs of autoimmunity have not been observed. In trials from which reports are available, no spread of replication-competent virus from patients to their surroundings has been demonstrated. One important safety aspect is the potential of passage to germ-line cells. In systemic therapy especially, there will be a possibility of the therapeutic gene passing to germ cells and so potentially being heritable. However, preclinical experiments in animal models indicate that the risk of passage to germ cells is minimal in the forms of gene therapy that are of topical interest today. It should be added that there are as yet no reports of patients who have had children after undergoing gene therapy. Close monitoring of the safety and side effect aspect is, however, most important[9].

As of today, the only topical gene therapy is somatic gene therapy for serious disease. The US National Institutes of Health have recently approved a protocol with a quality of life objective (an attempt at saving eyesight in patients with bilateral retinoblastoma). What should be the therapeutic objective of gene therapy is indeed an important discussion; the boundaries are not set once and for all. While gene therapy on germ cells and the fertilized ovum is prohibited, there is now an intense debate, especially in the USA, on gene therapy on the fetus. The argument for is that gene transfer as well as therapeutic efficiency in some conditions might be higher during fetal life. The primary drawbacks are the risk of spread to immature stem cells and germ cells, as they are more immature in a fetus and because of the small anatomy. So far, applications for two preliminary protocols have been received by the US regulatory agencies, which have concluded that there are still too many uncertainties for fetal gene therapy to be acceptable. The Group agrees that fetal gene therapy should not be allowed before more knowledge is available. It should, however, be noted that fetal gene therapy could be an alternative to abortion if it can be performed in an acceptable manner.

A considerable source of fear relating to gene therapy is the potential for genetic enhancement, not only treatment of disease. In animals, introduction of hormone genes or muscle genes could result in increased bodily size or muscular mass. There is an express fear that gene therapy might be used on human beings for such ends, for instance in athletics. There is reason to point out that today this is only theoretically possible in humans through germ-line cell therapy, which is illegal in all countries. Other qualities, like intelligence, speed, strength and staying power, will depend on the interplay of many different genes, in addition to the environmental factors. The present consensus is that genetic enhancement is ethically unacceptable. Still, definite indication boundaries for allowing gene therapy will at all times be important. The Group of

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9 The death of the 18-year-old triggered a thorough investigation of data on all patient adverse events involving gene transfer to patients. Unexpectedly, it was discovered massive under-reporting of adverse events. The revelation has become a major concern to the federal regulatory agencies, and as a consequence clear directives about adverse events reporting are now being established.

Experts maintains that gene therapy must be reserved for the treatment of serious disease, and that genetic enhancement is not ethically defensible. An ongoing ethical discussion of new gene therapy protocols will be important.

As of today there is an enormous technological potential for genetic manipulation of animals, including cloning and the development of germline-changed, genetically modified animals. Cloning of human embryonic stem cells is a fantastic advance; however, in theory it could also be used to make genetically modified human beings. The Group has not discussed in depth germ-line based gene therapy that is unethical and illegal. One should, however, be aware of the increasing potential for 'improving' the embryo, or curing its heritable diseases. Newly established techniques for microinjection of artificial chromosomes into mammalian cells might, for instance, lower the threshold for such 'therapy', the risk of damage to the other chromosomes being minimal. Apart from the strong ethical objections to 'enhancing' human beings, there is the scientific risk that such foreign chromosomes might get into the species' genome. This calls for caution and a thorough debate on the ethical aspects of the field. The Group of Experts is of the opinion that research on these ethical aspects should be financially supported. Moreover, the Group will point to the necessity of a continuous and informed public debate on these issues, in addition to the formal legislation and approval systems that regulate the activity.

16.4 Legislation

Many European countries are regulating gene therapy under their general legislation on pharmaceuticals; one might ask whether it is necessary to have a special act of parliament for it Norway. There is indeed a need for simpler and better co-ordinated rules of administrative procedure in this area. Scientist have taken an initiative to a Europe-wide co-ordinated legislation on gene therapy protocols; the EU is supporting this project. Although the Norwegian rejection of the Sandoz trial of thymidine kinase treatment for glioblastoma was justified, it is reported that Norway has acquired a reputation as a country with a strict attitude to gene therapy. Cumbersome administrative procedures impeding Norwegian participation in international multicentre trials will contribute to cementing this reputation and could reduce the interest in including Norway in such trials. From a medical/scientific viewpoint it is important that Norwegian clinical centres can join such trials in order to build up the multidisciplinary centres that are so essential for developing expertise. The Group of Experts emphasises the need for simpler, more expedient administrative procedures for approval of gene therapy trials, while at the same time ensuring that the process still covers the needs of society with respect to control and ethical considerations. A system in which three applications have to be submitted seems would be completely adequate: one for laboratory approval, one to the Committee on Research Ethics, and yet another for approval of drug testing. It is recommended that the Norwegian Drug Control Authority be augmented by a special advisory committee for gene therapy, on the lines of the British system.

The different procedures on gene therapy should be regulated with different safety restrictions. Thus, treatment with ribozymes and antisense oligonucleotides in soluble form, which is reminiscent of conventional drug treatment, should have more simplified safety demands than other gene therapy methods. It is recommended that smart viruses

and gene marking studies, which are not included in the definition of the gene therapy in this report, should be regulated like gene therapy, not least because of the risk of spread of replication-competent virus. Likewise, gene marking studies should be covered by the same body of regulations.

16.5 Some issues relating to health economy

To a significant extent, central government is involved in the development of gene therapy, in the USA and in European countries. The pharmaceutical industry takes a great deal of interest in some fields of gene therapy, but the industry interest is rather more lukewarm when it comes to small disease groups. Several smaller biotechnology companies that have been formed also take an interest in the field. The development of gene therapy will probably have to be funded on an international basis in a collaboration between research agencies, the health services and the pharmaceutical industry. Parts of the industry maintain that public funding should cover a larger proportion of the development costs for gene therapy than for other treatment modalities, not least because of the drug development costs incurred by strict testing requirements and the safety concerns in this field. This may be particularly applicable for rare, heritable diseases in which development costs are considerable and for which, in theory, one round of medication might be sufficient.

The costs of developing gene therapy procedures are high indeed. Estimates indicate that the present cost of vector manufacturing for only ten patients is between NOK 1 and 1.5 million. However, this is about the same as the cost of six courses of treatment with taxans, a new, important cancer drug. Existing HIV treatment is also indeed costly. Moreover, it is reasonable to assume that the cost will fall as the empirical basis widens, and especially if therapeutic breakthroughs are achieved. Though gene therapy may still be expensive, it could be cost-efficient, for instance in heritable diseases. For about half of these, there are at present few or no therapeutic options; gene therapy will often be the only possibility of curative treatment. Beside, the inefficient treatment modalities we now have are often very expensive. Gaucher's disease is a case in point, a heritable disease with changes in lipid metabolism which involves lifelong (i.e. 30–50 years) expenses for conventional treatment for all patients. Seen in this light, successful gene therapy intervention might definitely save costs. For other diseases the picture is more complex, depending on the cost-efficiency of present treatment and what effect gene therapy will have. It should also be noted that some gene therapy products will be less expensive to manufacture once the safety aspects are better known. This applies to, e.g., soluble antisense molecules and ribozymes as well as the use of DNA vaccines.

Small patient groups represent a problem, as there is less industry interest in developing gene therapy for these diseases. This suggest that society should accept more of the responsibility for these diseases, and it calls for international collaboration. One national centre of expertise for some of these diseases might be a sensible approach; one might also consider letting some of these patients receive treatment abroad, provided that the therapeutic effect is documented. Nordic collaboration for such patient groups could also be an option.

Even though gene therapy today is high cost medicine, the expense is still comparable to other expensive treatment options, hence the cost-efficiency of gene therapy should be

assessed and priority assigned in relation to other medical needs according to established practice.

A national strategy for the development of the gene therapy field is important to achieve. This will make it possible to systematically prioritise different gene therapy methods, to build up national competence in the field and to establish gene therapy as regular treatment whenever this will be indicated. A strengthening of the transfer between basic and clinical research (translational research) within gene therapy will prevent unnecessary pressure for establishment of such new and exciting methods before their clinical effect has been demonstrated, and thus also prevent the inappropriate and uncoordinated establishment of new methods at the national level.

16.6 New assessment

Gene therapy is still an early stage of clinical research, and it is difficult to tell what place it will have in Norwegian medicine in the future. Results from a number of late-stage clinical trials, including phase III trials, will be available in the course of the next few years. A new assessment of the field and updating of the databases after three to five years may be appropriate.

Appendix

Cancer
Monogenic diseases
Infectious diseases
Cardiovascular diseases
Other diseases