UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

Hannah Edelen, JD
Director, Research & Fellowship Administration
Focused Ultrasound Surgery Foundation
213 Seventh St. NE
Charlottesville, VA 22902
Phone: (434)-220-4993
Fax: (434)-220-4978
E-mail: hedelen@fusfoundation.org

Peter R Eby, MD
Assistant Professor
University of Washington
Department of Radiology
Seattle Cancer Care Alliance
Phone: (206) 288-1257
Fax: (206) 288-6473
E-mail: preby@u.washington.edu

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RE: Progress report for FUS foundation grant #FUS61R1

CONFIDENTIAL RESEARCH FINDINGS

Dear Ms. Edelen,
As June begins, we are excited to provide a 6 month progress report for our project that is currently supported by the FUS foundation: "Induction of an immune response to breast cancer with magnetic resonance-guided focused ultrasound tumor ablation in a mouse model." Our funding start date was December 1st, 2009.

As you know, we have been developing an experimental protocol that has not previously been attempted anywhere to our knowledge. This protocol has required a multi-disciplinary team to achieve multiple goals towards an intersection of cutting edge science. Our endeavors have included establishing the animal tumor model, adapting the MR-guided HIFU system to the animal, and optimizing immunologic testing. Since the start of the funding period, 12/1/09, we have had challenges in each realm. However, we have made steady progress in all areas and look forward to accomplishing the project objectives by the end of the project period.

A summary of our progress accompanies this letter. Please let us know if you have any questions or need additional information. I will look to your direction on how to proceed in receiving the remainder of the project funding.

Sincerely,

Peter R Eby, MD

# Summary of Progress

**Project Title:** Induction of an immune response to breast cancer with magnetic resonance-guided focused ultrasound tumor ablation in a mouse model.

## 1. Animal Tumor Model

Progress to date:

a. Acquired experimental animals
b. Grown the tumor cells in culture
c. Implanted the tumor cells in animals -
d. Grown tumors in the expected location and size (Fig 1)

We began by acquiring a specific immuno-competent mouse model from a vendor. Then we obtained a very unique mouse breast cancer cell line from one of our UW colleagues. This required us to learn how to maintain the cell line at adequate levels and health to deliver 10,000,000 cells per animal. It also required us to learn how to implant the tumor for optimal growth.

At this point in the project, we feel very confident in our technique and ability to culture and implant tumor cells in our experimental animals. Over the course of refining our technique, we experimented with different locations of tumor implantations, but have settled on a location that produces optimal results with HIFU. We can now routinely grow tumors that are 1cm in the subcutaneous tissue that is easily accessible to HIFU (Fig 1).

Next Steps: We will continue to utilize our methods for culturing and implanting tumor cells in all future experiments. We do not anticipate any further challenges with tumor cell culturing and implantation.

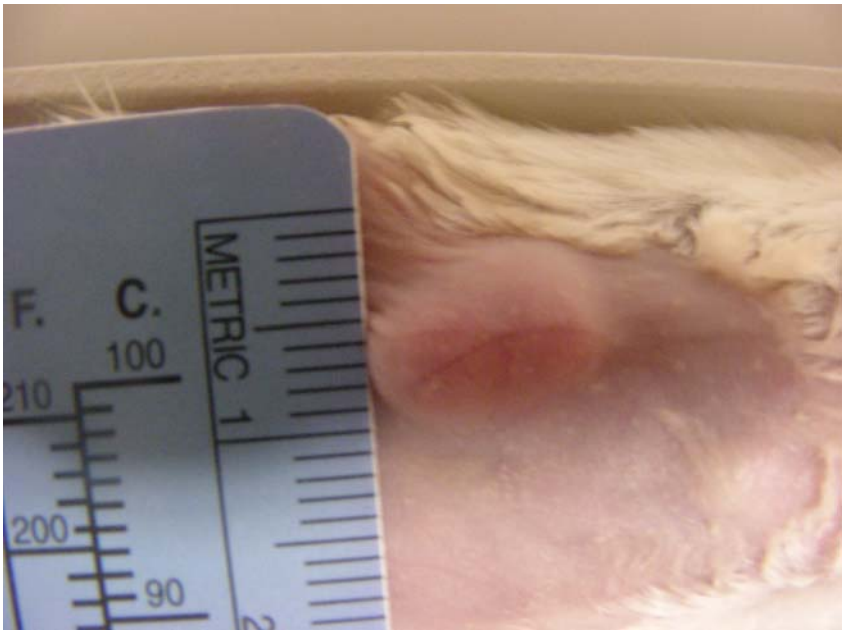


Figure 1. Experimental mouse demonstrating tumor that was grown from a controlled injection of 10,000,000 cells in the subcutaneous mammary fat pad.

## 2. MR-guided HIFU System

Progress to date:

a. Acquired the small animal adaptor for the 3 Tesla scanner –
b. Organized animal support to include (Fig 2)
   i. General anesthesia
   ii. Acoustic coupling
c. Optimized the MR imaging protocol
d. Successfully imaged tumors (Fig 3)
e. Applied HIFU to tumors to achieve partial ablation (Fig 4)
f. Confirmed ablation with tumor histology (Fig 5)

During the first six months of the project, we addressed the technical challenges associated with performing MR-guided HIFU treatments in mice. We acquired a small animal adaptor and arranged our general anesthesia system to safely immobilize the animals in combination with adequate acoustic coupling (Fig 2a and 2b).

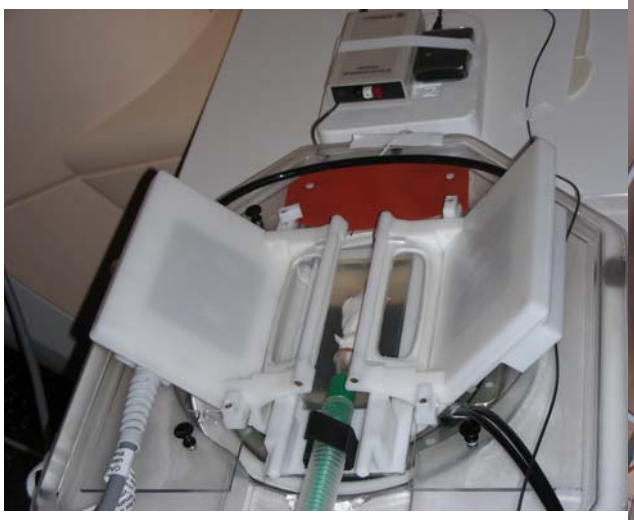

Figure 2a.

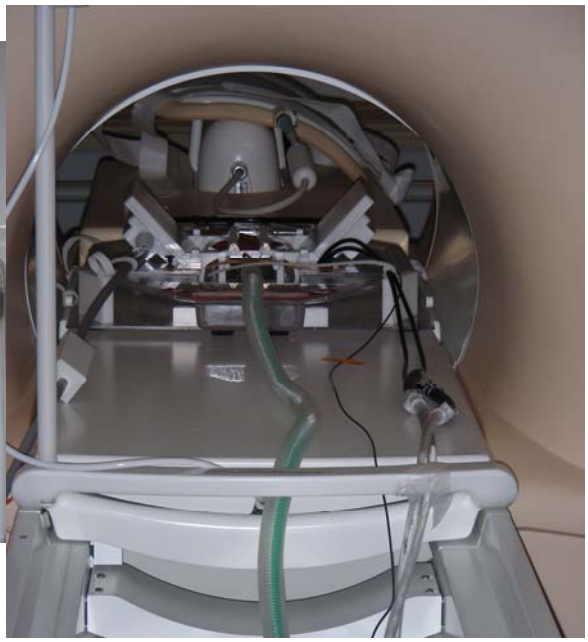

Figure 2b

Figure 2a and 2b. The experimental set-up for performing MR-guided HIFU on our mice.

Then we optimized the MR imaging protocol to reliably show us a 1cm tumor target. We are now able, as shown in Figures 3 and 4, to routinely image, target and perform HIFU treatments on mice.

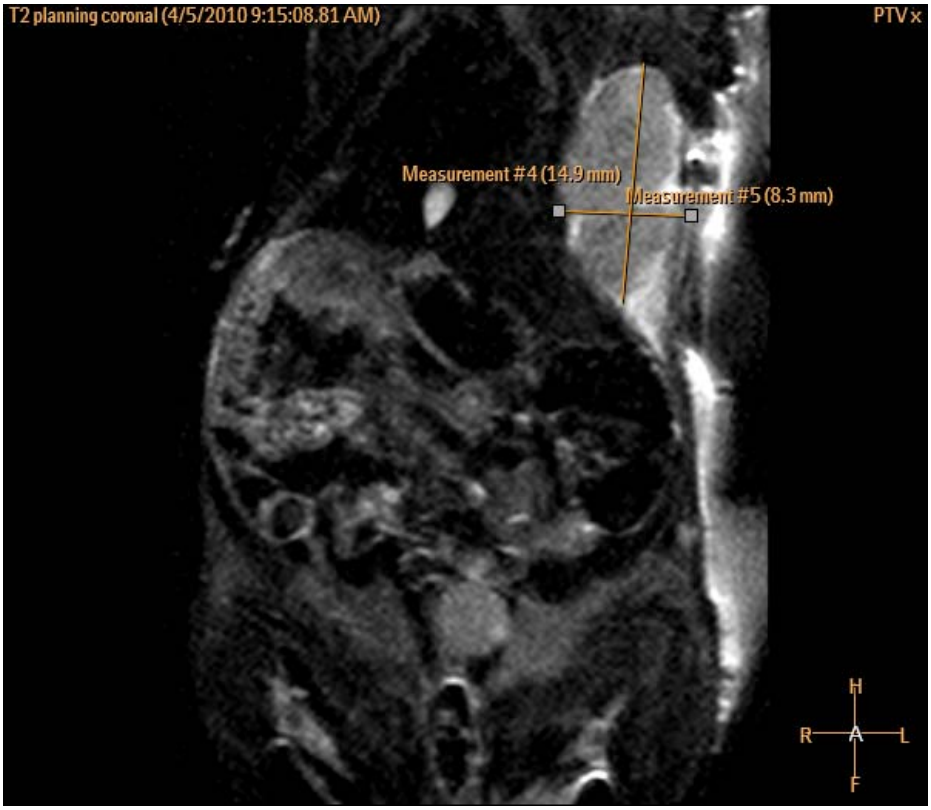


Figure 3. Coronal T2 weighted non-contrast image of the animal and tumor at the time of targeting and treatment.

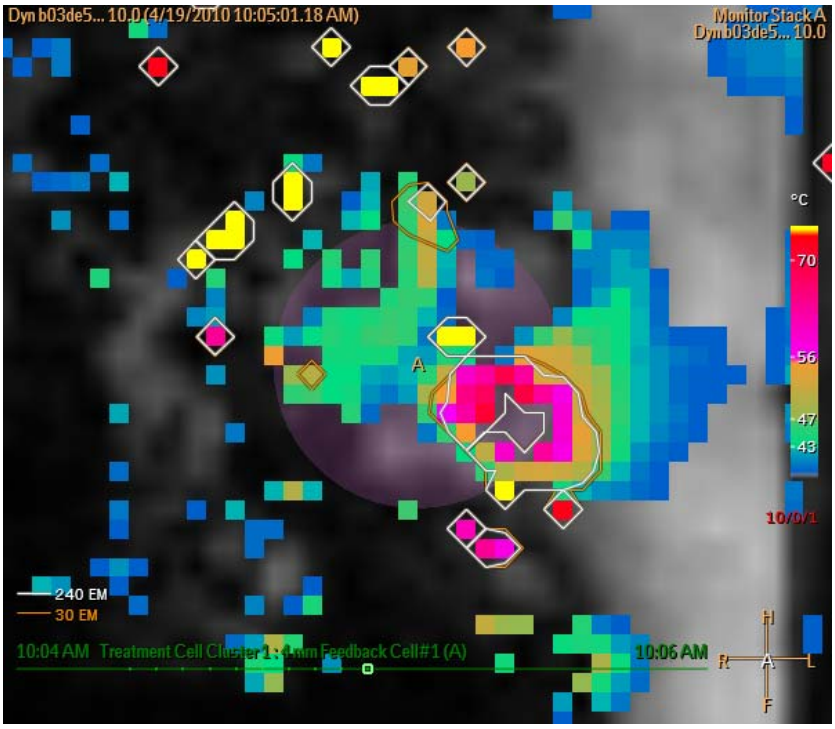


Figure 4. Temperature map of tumor during HIFU therapy.

One week after HIFU treatments we harvested tumors from experimental and control animals. We used specialized histology and scientific imaging labs at the Fred Hutchinson Cancer Research Center to determine if the HIFU treatments produced the expected necrosis. Fig 5 shows a treated tumor (purple) with central tissue necrosis (pink) secondary to HIFU ablation.

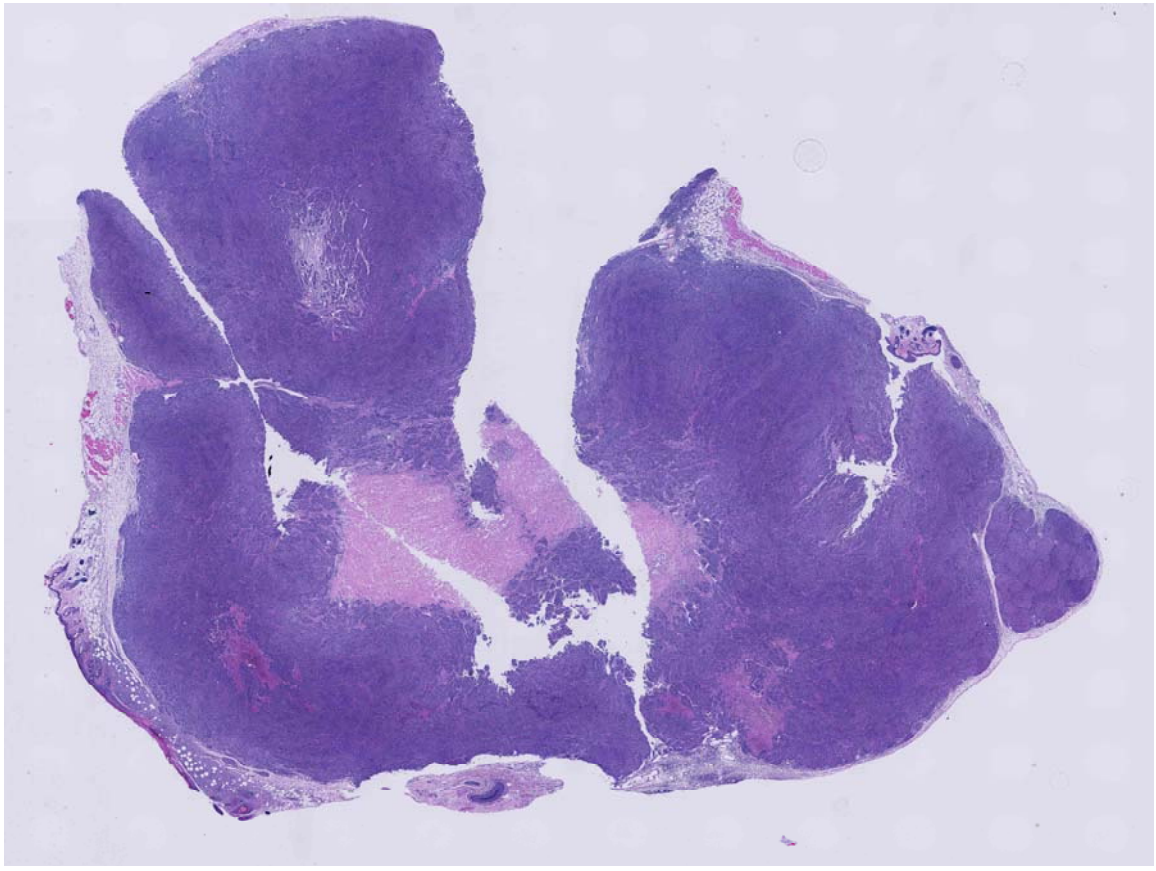

Figure 5. H & E staining of the excised tumor 7 days after therapy demonstrates uniform sheets of purple malignant cells surrounding a pink area of coagulative necrosis from HIFU treatment.

Next Steps:

For the remainder of the project, we will continue to perform MR-guided HIFU ablations of tumors and assess accuracy of ablative energy delivery.

**3. Immunologic Experiments**

Progress to date:

a. Optimized the assay for IL-2 and IFNg (Fig 6)
b. Awaiting comparison of control animals to experimental animals

Now that we have reached a point in our research where we can reliably produce and treat tumors, we are starting to apply immunologic testing methods. We have been evaluating the lymphocyte cell count from the spleens of our animals. Further, we have been testing the relative numbers of cells that produce

interferon-gamma (IFNg) and interleukin-2 (IL-2) in control and treated animals (Fig 6). We are optimizing the assay with each experiment. It is too early to tell if we have found significant differences.

Next Steps:

We will be running paired animals for overall analysis of immune response to HIFU treatment. We will also be adding an assay that quantifies the level of lymphocyte reactivity to specific breast cancer proteins.

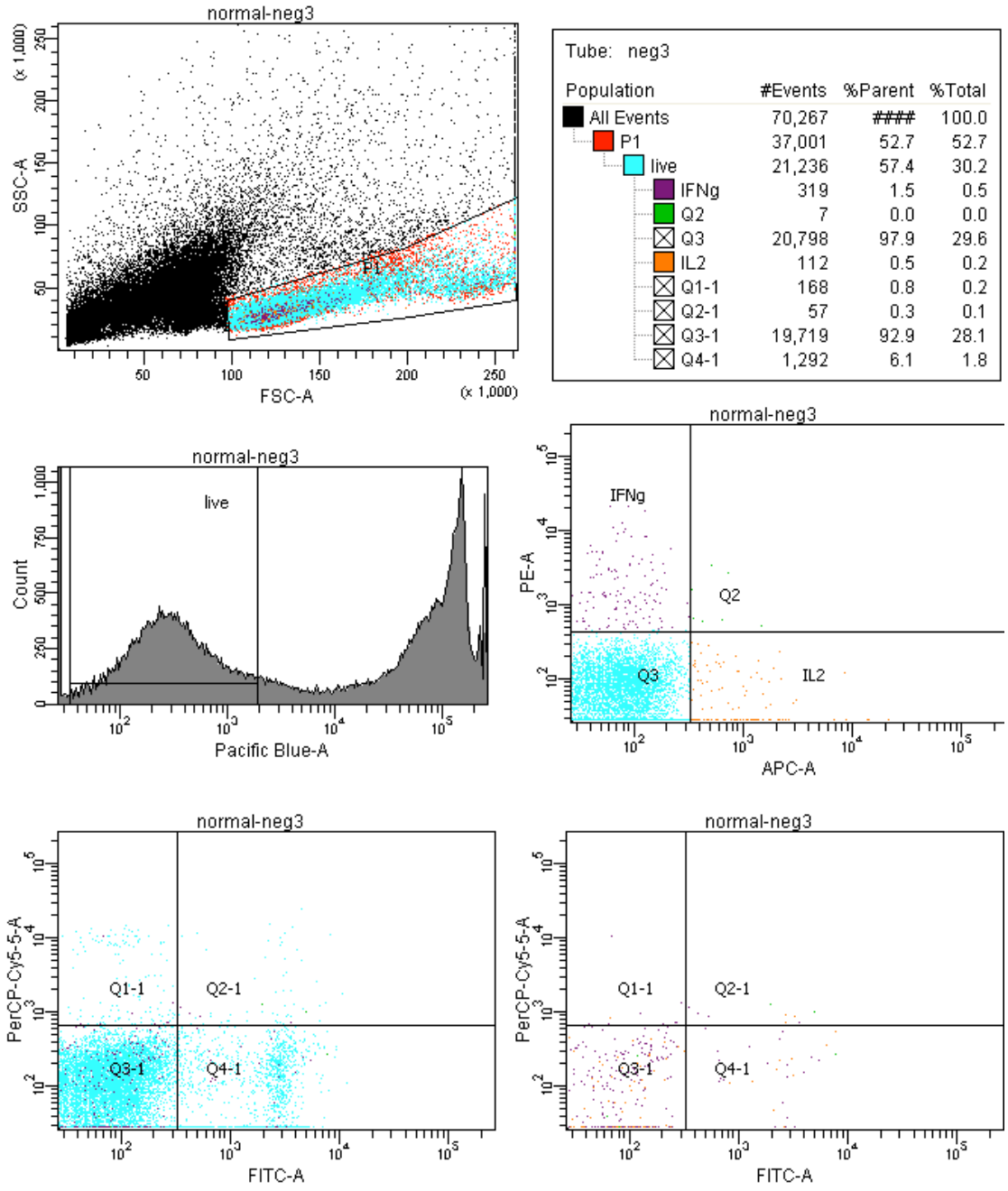


Figure 6. Sample results for a normal animal indicating the relative percentage of lymphocytes producing Interleukin-2 (IL2) and Interferon-gamma (IFNg).

Overall, we have made significant progress in creating a reliable animal model as well as adapting the HIFU system to treat very small animals and targets. We will be turning our attention now to the immunologic response associated with HIFU treatment in hopes of answering the seminal question of our project. We are thankful for the support of the FUS Foundation and look forward to completing our research.