6-12 Month Progress Report:

A New Application for MR-guided Focused Ultrasound: Amplification and Localization of Blood Biomarkers

Aim 1: Optimize the MRg-FUS ExAblate 2000 system (InSightec) for use with living mice and the sonication of murine liver tumors.

As discussed in the half-year progress report we maintained the transplantation of the subcutaneous LS174T cell line's tumor piece into the liver to obtain the metastatic liver tumor model. The use of the in-table fibroid system in ExAblate 2000 was able to easily detect the tumors in the T2-weighted image. In treating the mice with the same system at a low intensity, we experienced an increase in the post Carcinoembryonic antigen (CEA) biomarker levels in the non-sonicated control group as well as the treated group. The intensity used was identical to our published non-focused ultrasound treatment of subcutaneous tumors in mice, which showed a significant increase in the treated group when compared to the untreated controls. The increase in the controls with the FUS system was indicative that the increase in the biomarker was due to the set up of the mice within the system and not only due to the sonication.

We then decided to try the prostate MRg-HIFU set up instead of the fibroid system. This system would allow less use of water surrounding the mouse, allowing the mouse to be kept relatively drier and warmer. The initial set up included the mouse on its side with its abdomen area in a small container of water to allow for the unobstructed penetration of the ultrasound from the prostate transducer placed on the top of the mouse. The transducer is surrounded by deionized water balloon, the water in which can be kept at 37ºC. The mouse was also kept warm with heated pads around it. This set up worked but we were still concerned with the mouse being kept at a comfortable temperature. We then tried another set up, which worked better for the mice in which the water bath was exchanged for a gel bed, which could absorb the transduced waves that pass through the mouse. (Fig.1). The mouse was kept warm with gloves filled with warm water kept around it. All interfaces between mediums were layered with warmed ultrasound coupling gel. The set up worked well for imaging the tumor and treatment, as well as following temperature changes at the treatment site.

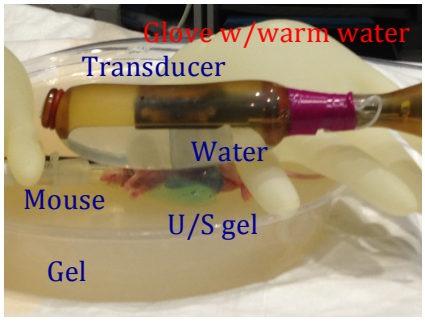

Fig.1: Setup of mouse using the prostate transducer

Aim 3: Study the effects of high intensity ablative FUS, similar to those currently used in clinical settings, on the release of blood biomarkers in an orthotopic liver tumor model.

Using the above liver model and set up, the mice were imaged and treated using the prostate HIFU system. Trial mice were first treated in the system and survived the 30-40 min of time within the system and recovered well. We then did a cohort of 10 mice and implanted the LS174T tumors within the mice livers. The

mice were sonicated till there was a 15ºC rise in temperature for 2 min. The temperature was measured using MR-thermometry. This rise in temperature mimics high intensity tissue ablation. The implanted tumors grew in 7 of the 10; the mice were randomized and 4 were sonicated and 3 were used as non-sonicated controls. The controls were set up and imaged the same way as the treated mice and then kept in the instrument for the same amount of time taken for sonication of the treated mice. Both the controls and the treated mice showed an increase in post CEA content in the blood compared to the pre-blood content (Fig.2).

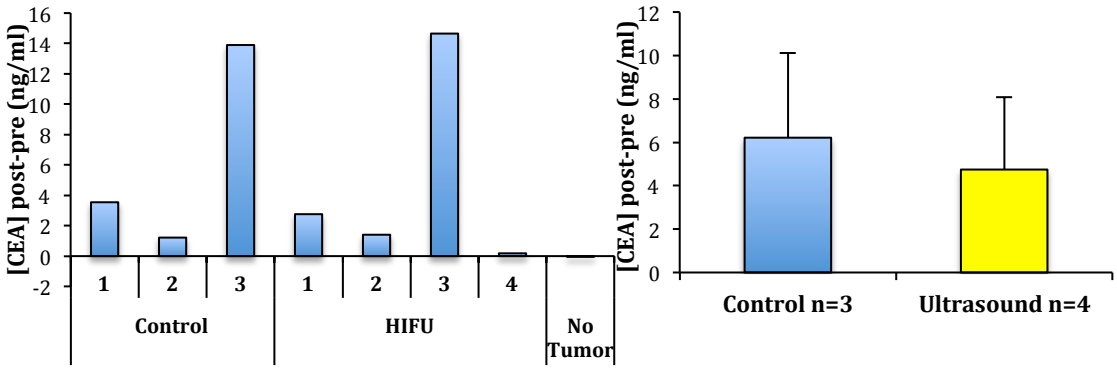

Fig.2: Post-Pre CEA blood levels in mice liver tumors treated with high intensity focused ultrasound (HIFU) or untreated (Control) mice.

Aim 2: Study the effects of low intensity non-ablative FUS on the release of biomarkers in an orthotopic liver tumor model.

Since the high intensity FUS treatment gave us results similar to the controls we went back to the low intensity non-ablative FUS. A couple of mice with liver tumors were treated with low intensity ultrasound similar in conditions to our non-focused ultrasound experiments. These control and treated mice showed similar increases in post CEA levels (Fig.3). A couple of mice with tumors were also kept warm with a glove filled with warm water as done in the MR setup to see if the warming of the mice without being in the MR setup caused the increase in the maker and these mice did not show a significant increase in post CEA. We also did a cohort of 10 mice with liver tumors and treated them with the non-focused ultrasound instrument that we had used for our subcutaneous tumor model.

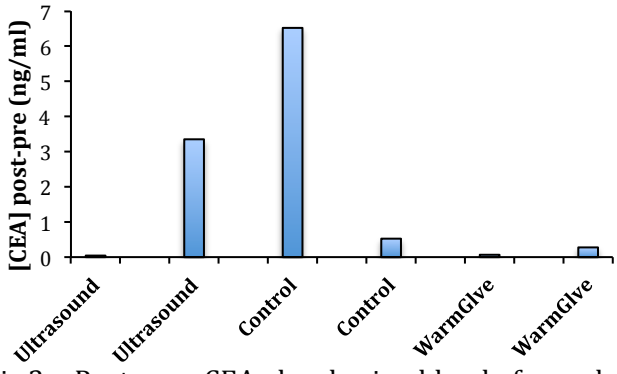

Fig.3: Post-pre CEA levels in blood from low intensity ultrasound treated (Ultrasound) or untreated (Control) liver tumors in mice. Mice with liver tumors kept warm with warm-water filled glove did not show much post CEA increases.

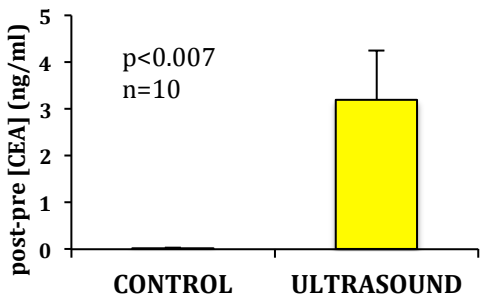

Fig.4: Post-pre CEA levels in blood of mice with liver tumors treated (Ultrasound) or not treated (Controls) with non-focused low intensity ultrasound.

This was done to confirm that the issue of the controls showing a spike in the post CEA levels was not due to the difference between the liver and subcutaneous tumors. The liver tumors with the non-focused ultrasound, showed a significant increase in the CEA levels in the treated group compared to the non-treated group ($p<0.007$).

Conclusions:

We conclude that the set up of the mice in the MR instrument was causing some distress in the mice enough to cause a spike in the post CEA levels in the blood of even the control mice that were not treated with ultrasound of any type. We did not move to larger rodents at this time as it was not possible within the scope of this grant but it is something we would like to pursue. We are also in the process of buying a dedicated small animal MR-HIFU instrument, which may help in solving some of our set up issues of the animals in the human instrument.

Presentation:

American Association for Cancer Research Annual Meeting 2014. Novel method of liver tumor detection and characterization using ultrasound-induced biomarker release. Aloma L. D'Souza, Xinrui Yan and Sanjiv S. Gambhir