**Half-Year 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.

### Orthotopic liver tumor formation:

Initial methods in formation of the liver tumors involved ultrasound-guided injections of the colon cancer cell line LS174T into the liver of living mice. The tumors formed well but the seeding of the tumor as well as metastisis caused inconsistencies in the expected tumor growth. We have since decided to do a laparotomy and seed the cells directly into the liver lobe. The colon cancer cell line LS174T transfected with firefly luciferase 2 and enhanced green fluorescent protein (luc2-eGFP) reporter gene to be able to follow the tumor formation using bioluminescence in the living mice. Cells suspended in matrigel were either injected directly or a piece of subcutaneous tumor was transplanted into the liver of nude mice. The tumor growth was followed using bioluminescence imaging and tumor size was determined using ultrasound imaging.

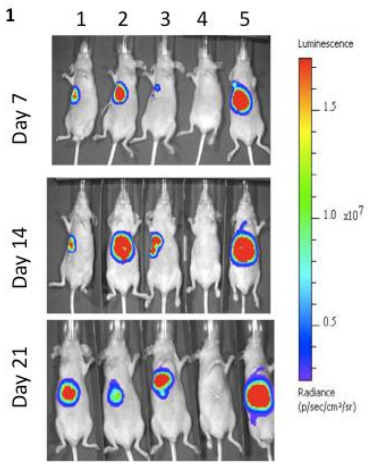


Fig 1: Bioluminescence of mice with LS174T tumor-tissue implanted into the liver followed over 21 days.

Both the methods used to form the liver tumors showed an 80% rate of tumor establishment within 1 week when observed using bioluminescence. The other 20% of the mice also had tumors but they were slower growing and not easily visualized by either bioluminescence or ultrasound imaging. The transplantation of a previously established subcutaneous tumor fragment showed a better control of the tumor size when compared to a fixed number of cells injected. The tumors were followed over 3 weeks and showed exponential growth (week-3 mean tumor volume = 0.815 $cm^3$).

### Pilot sonication of mice using the ExAblate 2000:

The pilot experiments of the mice in the ExAblate 2000 system using the in-table fibroid system showed that the tumors were well formed and were easily detected in the T2-weighted MR image. For the first few mice-trials we looked at treatment and survival of the mice on the instrument. For the initial experiment we used mice with subcutaneous tumor to optimize the set up and to see animal survival. The mice were placed on the FUS table inside a 3T scanner. A 3-inch surface coil was placed underneath the mice and the tumor was extended through the coil into a water bath for coupling to the transducer (Figure in proposal). The imaging and treatment of each mouse took a total of 30 – 45 min. FUS treatment was done at 256 J for 128 sec at 2 W using the 1.35 MHz transducer. At these conditions, which are similar to our unfocused preliminary studies, we found that there was no rise in temperature. In fact we could not distinguish a change in temperature as it was within the background noise levels. The mice survived well but rectal temperatures of the mouse done after removal from the instrument showed that they were cold respite using heating pads around the mice and keeping the tail between

two warm pads. The temperature of the water bath was around 72°F and was a factor to consider for the next set of experiments. Blood collected pre- and post-sonication was tested for the levels of the biomarker Carcinoembryonic antigen (CEA). Out of a total of 4 mice tested we were able to get blood from 3 and all 3 showed an increase in the post-CEA concentration when compared to pre-CEA levels.

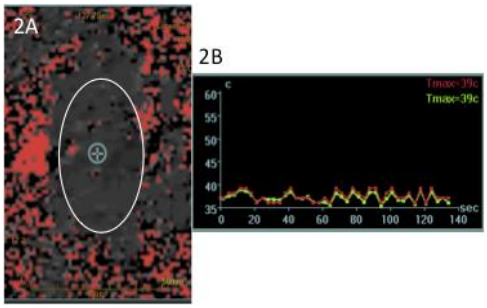


Fig 2A: MR temperature map of sonication of mouse liver tumor. The white oval outlines the body of the mouse while the cross hairs indicate the sonication location and the pixel from which the temperature is recorded.
Fig 2B: Temperature profile of focal spot during sonication. The red line indicates the temperature of the pixel at the intended location. The green line is the average value of the eight surrounding pixels.

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

The LS174T cells were injected into the liver of mice using ultrasound-image guidance for our initial trials. The water bath used for coupling was warmed in order to keep the mice warmer than the previous trial. A couple of heated pads were also kept at the sides of the mouse besides the tail to keep it warm. Out of the 10 mice that tumors grew within the liver, we were able to sonicate 5 and used 2 as non-sonicated controls.

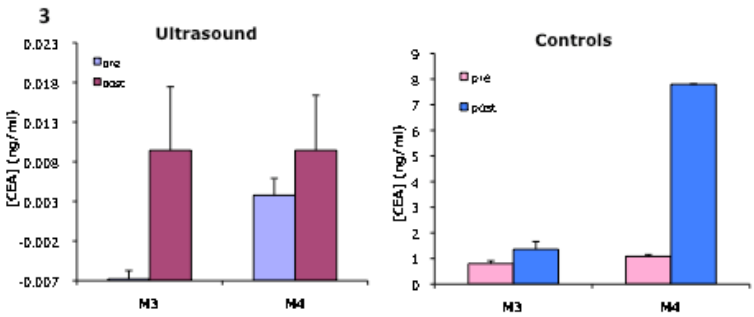


Fig 3: Representative values of pre and post CEA concentration. Both the ultrasound treated and the controls showed increases in the post levels.

The sonicated and the non-sonicated mice showed an increase in the post-sonication CEA levels when compared to the pre-sonication levels. We suspect the reason for the increase in the controls is due to the heating of the coupling water as this caused increased circulation and vasodilation in the animal. This vasodilation was also observed when obtaining the post-sonication sample from the mouse. The water temperature was not closely monitored and we would need a better-controlled alternative to keeping the water warm. Liver tumor treatments using an unfocused ultrasound did not show the same increase in the control mice and we therefore conclude that this is due to our set up for the MRg-FUS. In order to overcome this issue we are presently trying the prostate system as a set up. This system uses ultrasound-coupling gel for coupling the transducer and the mice and prevents the use of the large body of water. Our initial set up using the prostate system was able to show survival of the mice even after an hour on the instrument. If the need be we will look into other ways of keeping the animal warm and real time monitoring of the temperature of the mice while being treated.

**Presentation:** World Molecular Imaging Conference 2013 “Murine Liver-Tumor Characterization by Ultrasound-Induced Biomarker Release. Aloma L. D’Souza, Xinrui Yan, Huaijun Wang, Sanjiv S. Gambhir