a. Our specific aims were to:

1. Determine the exact protocol to maximize ultrasound enhancement of the accumulation of 60-100 nm particles in a tumor and surrounding tissue. In order to accomplish this goal, we will vary the instantaneous and time-averaged field within the region and measure the resulting accumulation and the full body biodistribution and pharmacokinetics (PK).

2. Characterize the changes in the tissue that increase the accumulation of 100 nm particles. We have preliminary indications that the effect of the sound waves is to dilate venules and veins and draining lymphatic channels. Our preliminary estimates indicate that these changes last for ~4 hours, during which accumulation is enhanced. We will complete this analysis in order to fully characterize tissue changes and characterize any off-target effects on surrounding tissues.

3. Conduct a preliminary therapeutic study with the optimized parameters using the commercially-available Doxil in order to assess the effect of ultrasound on efficacy.

b. Preliminary results

Specific aim 1. We have conducted a sequence of studies to find the optimal protocol for ultrasound-enhanced drug delivery. Our preliminary results indicate that over a range of temperature up to ~50°C, particle accumulation increases with the peak temperature of the insonified region. Within our studies, we achieved a local maximum of ~46% injected dose per gram of tissue at this higher temperature (as compared with 42°C), with the maximum occurring ~24 hours after the application of ultrasound and the injection of the particles. The mean tumor accumulation in this case was ~15%ID/g, which was ~3 fold greater than the contralateral tumor.

Based on work completed thus far, the optimal protocol involves rapid (2-3 minute) temperature escalation of the entire region, along with maintenance of the peak temperature for at least 2 minutes. Also, the optimal protocol involves injection of the particles before or very shortly after insonation (with accumulation decreasing if injection is further delayed). We achieved this increase by rapidly scanning the ultrasound beam within the region, using a peak pressure of 2.2 MPa, a center frequency of 1.5 MHz and a duty cycle of ~33%.

When the temperature in the region of interest is limited to 42°C (in order to avoid tissue damage due to hyperthermia), increasing the ultrasound pressure also can increase delivery and we continue to evaluate this strategy and will further explore the protocol over the coming months.

Specific aim 2. We have now acquired a substantial set of histology for the tumor and lymphatics following ultrasound thermal therapy in the Met-1 model. In this model, the sentinel lymph node is often very close to the tumor and significant changes in the structure of the node are observed, include dilated channels. We observe continued structural changes within the

lymph node several days after treatment and will analyze the immunohistochemistry of these samples over the coming months.

Specific aim 3. We have begun to evaluate the efficacy of therapy using Doxil in our model system. The IC50 for Doxil is ~1.5 μM in the Met-1 and Doxil alone delays tumor growth but does not eradicate this aggressive metastatic tumor. We will evaluate the efficacy of combined therapy in the remaining months. Our preliminary studies have indicated that complete tumor eradication can be achieved within this model system with a combined therapy.

We also find that in many cases small islands of viable tumor remain. Using positron emission tomography, we are attempting to find these small regions.

c. Changes to planned research

Our only change to the planned research has been to add parameters in which ultrasound resulted in a higher peak temperature to the plan, as we have discovered enhanced accumulation in local hot spots. We find that these higher temperatures further increase drug accumulation and likely efficacy.

d. Publications

No publications have yet resulted from this project but three are nearing submission and these are the following. All three should be submitted within a few weeks.

1. Chun-yen Lai et al, Ultrasonic hyperthermia and temperature measurement *in vitro* and *in vivo*, in preparation.
2. Azi Khierolomoom et al, Tumor regression via a combined therapy in a mouse model, in preparation.
3. Cecilie Rygh et al, Evolution of vascular permeability with the transition from *in situ* to invasive breast cancer, in preparation.