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 varied the instantaneous and time-averaged field within the region and measured 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. Results

Specific aim 1. We have conducted a sequence of studies to find the optimal protocol for ultrasound-enhanced drug delivery. Our 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 (ID/g) 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.

The optimal protocol depends on the specifics of the tumor. In a subset, a rapid (2-3 minute) temperature escalation of the entire region, along with maintenance of the peak temperature for at least 2 minutes enhances accumulation. In a second subset, the duration or temperature must be increased. We are finalizing a paper to be submitted within the next few weeks summarizing this.

When the temperature in the region of interest is limited to 42°C (in order to avoid tissue damage due to hyperthermia), accumulation increases with increasing treatment time.

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. This has led us to define sub-populations and to analyze the effect of ultrasound in these subpopulations.

Specific aim 3. We have evaluated the efficacy of therapy using liposomal doxorubicin and ultrasound in our model system and have shown substantial regression with an optimized combination of parameters.

c. Changes to planned research

We have now completed our work and will finish paper submissions. We have performed the proposed studies. Further, we have used these data to submit an R01 application to the NIH.

d. Publications

Two papers are now published and a third is nearing submission.

(1) Kheirolomoom, A., Mahakian, L. M., Lai, C.-Y., Lindfors, H. A., Seo, J. W., Paoli, E. E., Watson, K. D., Haynam, E. M., Ingham, E. S., Xing, L., Cheng, R. H., Borowsky, A. D., Cardiff, R. D., and Ferrara, K. W. (2010) Copper−Doxorubicin as a Nanoparticle Cargo Retains Efficacy with Minimal Toxicity. *Molecular Pharmaceutics 7*, 1948-1958.

(2) Lai, C., Kruse, D. E., Caskey, C. F., Stephens, D. N., Sutcliffe, P. L., and Ferrara, K.W. (2010) Noninvasive thermometry assisted by a dual-function ultrasound transducer for mild hyperthermia. *Ultrasonics, Ferroelectrics and Frequency Control, IEEE Transactions on 5*7, 2671-2684.

(3) Kate Watson et al, Efficacy of ultrasound-enhanced drug delivery, in preparation.