I received LRC funding for an application submitted in May of 2012 entitled **"Animal Models in Oncology Research: The influence of inoculation site on Proliferation, Inflammation, Necrosis, Vascularity, and Molecule Delivery"**

This project is near completion. As a brief summary, this project was undertaken to compare tumours growing in different areas of a body (a primary tumor versus metastases to the brain, lungs or bone). A better understanding of phenotypic variations that arise with tumor location may shed light on why preclinical models fail to predict clinical success and therefore can be used as a guide for preclinical study design in drug discovery.

Until very recently it was assumed that the characteristics of a breast cancer are stable during progression to invasive and metastatic disease. Thus, the treatment of women with breast cancer has traditionally been based on the physical and genetic characteristic of their primary tumours. However, recent data suggest that the gene expression profile of the metastatic lesion can be very different compared to that of the primary breast cancer [1]. A study conducted by Lorcinz et al showed that in breast cancer patients with bone metastases, the bone metastases had a loss of the primary marker used to determine treatment of the patient, a marker called Her2/neu. Her2/neu positive patients are treated successfully with a drug known as Herceptin. With a loss of Her2/neu expression in bone metastases, the patients systemic disease would no longer be responsive to treatment with this widely used agent. Lorcinz's group suggests that it is necessary to perform Her2/neu testing both on primary tumor and samples obtained from breast cancer metastases [2], a practice that is not currently performed. Another group evaluated micro-vessel density of bone metastases of various cancer types (all adenocarcinomas) compared to their primary tumors. This data demonstrated that the vascularization of bone metastases is frequently altered compared to the primary tumors, and patterns of vascularization are different in the case of various cancer types [3]. The tumor specific alterations of the angiogenic phenotype of cancers and metastases to the bone, is clinically significant to drug delivery, as drugs use new blood vessels as a means of entering the tumor. Additionally, angiogenesis suppressive therapies would only be effective under angiogenic conditions. For example, Jubb et all show that brain metastases have a significantly higher proportion of mature vasculature then the primary lung cancer, suggesting that the patient with advanced metastatic disease will be refractory to anti-angiogenic therapy.

In a previous study, I illustrated that animals with tumors growing in different sites respond differently to the same treatment of the drug Docetaxel [4]. The key message of this research paper was that metastatic disease responds poorly as compared to orthotopic disease. The pharmacokinetic profiles of tumors growing at different sites suggested that the difference in efficacy is the result of variable drug delivery and clearance. It was my intent to examine this phenomenon more carefully. The experiments I conducted from May 2012 to this time, compared the characteristics of tumor growth, nanoparticle delivery and microenvironment of tumour cells implanted in the mammary fat pad (which represents a primary human breast cancer) with tumours that arise in various tissues after injection in

to the heart (which represents metastatic disease). In the first experiment for this project, I show for the first time that the human breast cancer cell line JIMT-1 can be inoculated within the mammary fat-pad to form a localized breast tumor, or injected into the left ventricle of the heart to form metastatic disease. When comparing the tumors collected from the mammary fat pad to those at distant sites such as the adrenal gland and ovaries, it was noted that tumor phenotypes vary with location. These phenotypes include blood vessel density, vessel perfusion and tumor marker expression such as Her2/neu. To probe this further, multiple cell lines engineered to express a bioluminescent enzyme were required to create a robust experimental design. These cells were used to inoculate animals in order to visualize tumour growth in a non-invasive manner. To this end three breast cancer cell lines were manufactured and characterized; MDA MB 435 LCC6, MDA MD 231, and JIMT-1. The cell lines were inoculated into the mammary fat-pad or left ventricle of immune-compromised mice. Subsequently disease progression was compared using bioluminescent imaging. Immediately the differences in growth could be appreciated which suggested that not only would the site of tumour inoculation impact a preclinical drug study, the chosen cell line could also influence outcome. For the second stage of this study, I designed and engineered two fluorescent nanoparticle delivery systems that can be used to visualize, and compare delivery to tumour sites in a live animal with a disease burden. Using these labelled nanoparticles, I would be able to establish how metastatic or primary tumours might influence drug delivery. First, the nanoparticles were characterized in terms of the pharmacokinetic and pharmacodynamics profiles in normal animals. Once my tools (three breast cancer cell models, and two florescent labelled nanoparticles) were established, animals with a primary or metastatic disease were monitored for disease progression for up to 45 days, and then treated with the nanoparticle. Tumour progression was monitored using bioluminescent imaging in vivo, while nanoparticle delivery to primary tumours and metastases was monitored over 24 hours using fluorescent imaging in vivo. The results of the imaging showed that physiologically, orthotopic tumours were able to collect the nanoparticle while metastases were not. At the end of this study, animals were sacrificed and tumours were collected. The tumours were evaluated for blood vessel density and microenvironment characteristics. At the tissue level, major differences in blood vessel density, vascular perfusion and drug delivery were observed when comparing different cell lines as well as location of tumours in vivo. The conclusion of this study suggests that when designing a preclinical study for drug efficacy research, investigators must consider cell lines as well as cell inoculation routes in order to recapitulate human disease and successfully predict drug behaviour in clinical trials.

The completion of this paper requires two more animal experiments, which will begin October of 2013 and be completed by December of 2013. The manuscript describing these experiments will be submitted for publication before March of 2014.

1. Shah, S.P., et al., *Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution.* Nature, 2009. **461**(7265): p. 809-13.
2. Lorincz, T., et al., *Microvascular density of breast cancer in bone metastasis: influence of therapy.* Anticancer Res, 2005. **25**(4): p. 3075-81.
3. Lorincz, T., J. Timar, and M. Szendroi, *Alterations of microvascular density in bone metastases of adenocarcinomas.* Pathol Oncol Res, 2004. **10**(3): p. 149-53.
4. Kalra, J., et al., *Validating the use of a luciferase labeled breast cancer cell line, MDA435LCC6, as a means to monitor tumor progression and to assess the therapeutic activity of an established anticancer drug, docetaxel (Dt) alone or in combination with the ILK inhibitor, QLT0267.* Cancer Biol Ther, 2011. **11**(9): p. 826-38.