I received LRC funding for an application submitted in May of 2011 entitled "Optimizing digital quantification of tissue microarrays in order to evaluate early signaling consequences of ILK inhibition in an in vivo model of breast cancer". This project has been completed and is currently under peer review for publication. As a brief summary, this project was undertaken to; 1) establish the proof of principle that digital imaging is able to improve analysis of biological tissue samples in the context of tumour marker expression in preclinical and clinical specimens; and 2) validate the use of a drug candidate (a small molecule inhibitor targeting integrin linked kinase) in an animal model and as a potential drug in the treatment of breast cancer patients. My previous studies have shown that a chemical small molecule inhibitor (herein known as 0267), targeting the signaling adaptor protein Integrin Linked Kinase (ILK), is able to slow the growth of breast tumours in cell culture and animal models [1]. More recently it has been established that the optimal use of this drug candidate will likely be in a primary tumour setting before the cancer has metastasized [2]. The mechanism of action for this drug candidate in vivo has yet to be determined. As ILK is a signal transduction molecule with many effectors, determining the mechanism of action of inhibitors of this pathway would require a high throughput methodology of IHC such as TMA, and an automated quantification method such as the digital processing provided by the Aperio ImageScope software. In the studies described in my upcoming publication, I used an orthotopic mouse model of breast cancer to establish that 0267 is most effective at early time-points after administration. By 24 hours after treatment, growth of tumors is suppressed, and signal transduction is inhibited. Specific effectors of ILK that are silenced include AKT, TWIST and GSK. The mechanism of action of this growth suppression appears to be through induction of the apoptotic pathway. Illustration of early effects of this drug candidate and the demonstration of in vivo efficacy in ablating key signaling cascades provides support for the use of 0267 in clinical trials and furthermore future application in the treatment of breast cancer patients. I was also able to validate the methodology of digital imaging and analysis of immunohistochemical (IHC) samples providing a high-throughput method of analyzing molecular alterations in large numbers of samples that can be used in preclinical testing in the future.

My current research for which I received funding in January of 2012, is part of an ongoing study designed to explore how tumours growing in different areas of a body (a primary tumor versus metastases to the brain, lungs or bone) differ in their phenotypes, and if so, I am looking at determining how would use this information to properly model preclinical studies to reflect these differences. Until very recently it was assumed that the characteristics of breast cancer is stable during progression to invasive and then metastatic disease. Thus, 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 [3]. 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 herceptin. This group suggests that it is necessary to perform Her2/neu testing both on primary tumor and samples obtained from breast cancer metastases [4], 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. The tumor-type specific alterations of the angiogenic phenotype of cancers and metastases to the bone, is clinically significant especially when angiogenesis suppressive therapies are being used [5]. 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.

The experiments I am working on compare 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). For this research, multiple cell lines engineered to express a bioluminescent enzyme are required to create a robust experimental design. These cells will then be used to inoculate animals in order to visualize tumour growth in a non-invasive manner. At this time I have already characterized and used one breast cancer cell line (MDA MB 435 LCC6) to inoculate mice and create a metastatic model of human breast cancer. I have tried to establish a second model with another breast cancer cell line, MDA MD 231, however these cells were unable to establish disease in animals. Currently, I am using a third breast cancer cell line, JIMT-1, and these are proving to be more useful in vivo to model primary and metastatic breast cancer. Concurrently, I have designed and engineered two fluorescent nanoparticle delivery systems that can be visualized in a live animal. I am working on characterizing the pharmacokinetic and pharmacodynamics profiles of both molecules in animals. Once these tools (breast cancer cell model, and nanoparticles) have been established, the final experiment will begin. Animals with a primary or metastatic disease will be treated with the nanoparticle and delivery to primary tumors and metastases will be compared. Finally tumours will be collected and evaluated for blood vessel and microenvironment characteristics. The final experiment is scheduled to proceed in September 2012.

1. Kalra, J., et al., *QLT0267, a small molecule inhibitor targeting integrin-linked kinase (ILK), and docetaxel can combine to produce synergistic interactions linked to enhanced cytotoxicity, reductions in P-AKT levels, altered F-actin architecture and improved*

treatment outcomes in an orthotopic breast cancer model. Breast Cancer Res, 2009. 11(3): p. R25.

2. 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.
3. Shah, S.P., et al., *Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution.* Nature, 2009. 461(7265): p. 809-13.
4. Lorincz, T., et al., *Microvascular density of breast cancer in bone metastasis: influence of therapy.* Anticancer Res, 2005. 25(4): p. 3075-81.
5. 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.
6. Jubb, A.M., et al., *Vascular phenotypes in primary non-small cell lung carcinomas and matched brain metastases.* Br J Cancer, 2011. 104(12): p. 1877-81.