**Specific Aims**

More than one hundred years ago, Dr. Stephen Paget reported that there exists a measurable inclination for secondary breast cancer metastases to occur in specific organs: the liver, lungs, bone, ovaries, and spleen [[1](#_ENREF_1)]. This non-random metastatic spread is commonly referred to as *tissue tropism*. More recently, a thorough study by Kennecke et al. has demonstrated that metastatic lesion formation is correlated with clinical subtype [[2](#_ENREF_2)]. Neither Paget’s nor Kennecke’s findings can be explained by simple physical trapping of circulating tumor cells in areas of dense vascularization, and to this day, there is no biophysical explanation for metastatic site preference. This proposal aims to test the **hypothesis** that the ability of a breast cancer cell to form a successful metastatic lesion depends on the physical and chemical properties of these diverse metastatic tissue sites. It is proposed here that cell-extracellular matrix (ECM) interactions at the secondary tissue site regulate the ability of extravasated cancer cells to invade and proliferate within these tissues, resulting in an observed tissue tropism in metastatic lesion formation. To test this hypothesis, the following specific objectives are proposed:

*Aim 1:* **Quantify subtype-specific tissue tropism in breast cancer in Engineered Metastatic Microenvironments.**

Hypothesis: The physical and chemical properties of secondary metastatic lesion sites promote the invasion and proliferation of extravasated breast cancer cells in a subtype-specific manner

Method: Objective 1 will design highly controllable, reproducible biomaterial systems, which mimic the *in vivo* physicochemical properties of metastatic tissue. These biomaterials will be poly(ethylene glycol) (PEG)-based, and called Engineered Metastatic Microenvironments, or EMMs. Using twelve human breast cancer cell lines that represent the clinically relevant disease sub-types and quantitative 3D microscopy, this proposal will systematically quantify the migration and proliferation response to these controlled physicochemical cues.

*Aim 2:* **Quantify integrin-mediated signaling pathways implicated in force transduction across breast cancer subtypes.**

Hypothesis: Subtype-specific migration and proliferation of cancer cells is dependent on the ability of cells to activate force transduction pathways, and generate intracellular tension.

Method: High-throughput biochemical signaling analysis techniques will be used to measure activation of key intracellular signaling pathways implicated in force transduction and cytoskeletal assembly.

*Aim 3:* **Build statistical models to discover signals that contribute to metastasis across combinations of cell type and ECM composition.**

Hypothesis: Correlations between cell signaling and measured markers of metastasis exist and are detectable using a multivariable statistical framework.

Method: Multivariable models based on robust statistical methods will be used to relate cell type, ECM composition, and signaling nodes contributing to cell migration, proliferation, and, therefore, poor patient prognosis. Measurements associated with cell phenotype will be used as the dependent variables in a partial least squared regression (PLSR) analysis, resulting in an identification of signals that are most highly identified with successful metastatic lesion formation. Statistical models will be validated with a small subset of rationally designed experiments of single cell knockdowns in 3D EMMs.

The long-term goals of this proposal are to 1) generate new biomaterial and mathematical tools for widespread use in studies of metastasis in cancer, and 2) to identify novel signaling nodes to block tissue tropism for patient-specific therapeutics. Completion of these studies will generate never-before-realized experimental information on how the physical properties of the matrix feedback to regulate cell biology, which will be strengthened by a predictive statistical model to correlate physical characteristics of a material to cell state. Importantly, the predictive model and controllable biomaterial systems generated from this proposal may extended to cancer metastasis paradigms across many other carcinoma subtypes not characterized here.