**Overall Laboratory Objective:** Breast cancer, when confined to the breast at the time of detection, is largely curable, with greater than 90% of patients surviving at least 5 years. However, once breast cancer has spread to other organs, such as metastasis to the brain or lung, it is usually lethal. Our laboratories are committed to ending the suffering and death due to metastatic breast cancer by understanding and exploiting cancer-specific vulnerability points. We utilize cutting-edge molecular characterization technology, modeling of human breast cancer in animals with patient-derived xenografts and genetically engineered mouse models that faithfully recapitulate metastatic disease, and innovative methods for testing the functional consequences of depleting/inhibiting parts of pathways thought to be involved in cancer. Ultimately, by identifying what is different about cancer compared to normal cells and exploiting these differences, we hope to generate novel targeted therapies that have fewer unwanted side effects while still being effective at reducing tumor growth.

**Rationale for Proposed Experiments:** Our laboratory is focused on triple negative breast cancer (TNBC), an aggressive, often metastatic subtype of breast cancer. It is defined by low or no expression of estrogen receptor and progesterone receptor (two hormone receptors), and by an absence of amplification of HER2, a receptor tyrosine kinase. Because TNBC is historically and clinically defined by what it lacks, rather than what drives it, options for TNBC have been primarily limited to chemotherapy, radiation, and surgery. However, recent studies have started to characterize and functionally tease apart the molecular drivers of TNBC. Indeed, work from our laboratory has identified that the protein tyrosine phosphatase, PTPN12, is lost in ~60% of TNBC. We demonstrate that when PTPN12 is lost, multiple receptor tyrosine kinases (TK) including MET and PDGF work together to drive TNBC. Importantly, restoring PTPN12 expression experimentally blocks the activity of these TKs and inhibits TNBC growth. While PTPN12 restoration is not a tractable option in patients, we have identified a novel multi-TK inhibitor therapy that is highly effective across a large panel of patient-derived xenografts and mouse models of primary TNBC. Furthermore, this targeted multi-TK inhibitor (TKi) approach is now in Phase II clinical trials for breast cancer patients at our institution. Given the dearth of viable treatment options for metastatic TNBC patients, we tested the efficacy of this therapeutic combination on established models of lung metastases, the main site of metastasis of TNBC. Our preliminary results demonstrate that some TNBC lung metastatic models are responsive suggesting this therapeutic strategy may be a viable option for some patients with metastatic TNBC. However, other TNBC lung metastatic models do not respond, raising the important questions of: (1) what are the pathways that dictate sensitivity or resistance of TNBC metastasis to this therapeutic approach, (2) how broadly applicable is this therapy for patients with metastatic TNBC, and (3) what characteristics might serve as markers for predicting response to aid in selecting patients for this therapy who are likely to respond.

**Goal of the Research Proposal:** Resistance to therapy is common and presents a real clinical concern. Extensive studies with primary tumors have identified that resistance to therapy can stem from both intracellular events and cues from outside the tumor cell, called the “tumor microenvironment”. Less is known about the metastatic microenvironment and how external signaling factors present within the metastatic site govern response to therapy. Ultimately, a next-generation solution will need to include a comprehensive understanding of the mechanisms underlying resistance to therapy and our proposal seeks to identify this solution using the following objectives:

**AIM 1:** To determine the mechanism(s) of resistance to multi-TKI therapy driven by the metastatic niche. A variety of cell culture and metastatic mouse models will be used to directly test the effects of modulating cell intrinsic and microenvironment cues on drug potency.

**AIM 2:** To identify intrinsic and extrinsic mediators of mTNBC sensitivity to Sitratavinib. We propose to use our preclinical models to identify biomarkers and facilitate patient selection for future clinical work. We will elucidate molecular markers that correlate with TKi response of mTNBC and test whether pharmacologic inhibition of cell intrinsic or extrinsic modifiers reverses resistance of mTNBC to TKi therapy.

**Impact:** Through this proposal, we will identify how broadly applicable this therapeutic approach is to metastatic TNBC, determine key mechanisms of drug resistance in the context of lung metastasis, and identify potential biomarkers for patient selection. As Sitratavinib is currently undergoing clinical trials, we postulate that if the pre-clinical work herein is successful, there is a high likelihood of rapid translation of this approach to the clinic. Additionally, this work will preemptively identify mechanisms of resistance. If successful, this work could provide a viable option to help reduce the morbidity and mortality associated with metastatic TNBC.