Title: **Targeting Occult Cancer Cell Subpopulations Driving Metastases Growth**
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Public Abstract

Metastatic triple negative breast cancer (TNBC) has a median overall survival of less than 2 years due to the aggressive behavior of this disease and resistance to standard of care (SOC) chemotherapy. Recent clinical trials using a combination of chemotherapy with targeted- or immunotherapy has shown promising data in TNBC. For example, the addition of an anti-PD1 immune checkpoint inhibitory antibody, pembrolizumab, to chemotherapy has improved event-free survival for metastatic and early stage TNBC and is now a new standard of care in this setting. However, only 20% of patients are good candidates for this combination therapy due to the absence of PDL-1 protein, the target of pembrolizumab, in most TNBC tumors. That said, therapy combinations are promising strategies that target multiple vulnerabilities to improve survival rates. **In this proposal, we aim to test inhibitors of a novel target in metastatic TNBC in combination with standard of care (SOC) chemotherapy and study its immune implications.**

The challenge with effective combination therapy is figuring out which reagents will best synergize against the drivers of metastatic disease. To determine the best combination, we will use **Synergy-Seq**, a platform that integrates data from multiple cancers on FDA approved drugs and their molecular targets to predict the best combinations that will synergize against driver signals. The platform has been successfully used to predict drugs' synergism in glioblastoma. This approach will expedite integrating our novel inhibitors into ongoing combination trials for metastatic TNBC.

In our recent studies, we identified TEAD, a gene expression regulator, in **a small subpopulation of cancer cells that drives and maintains metastatic growth without being selected as the dominant population.** These cells remain below detection and are thus unaccounted for in analyses that inform therapy decisions. We obtained novel TEAD inhibitors (iTEAD) that are currently in phase I clinical trial for solid cancers. Interestingly, in our studies mice bearing mammary tumors that were treated with iTEAD did not develop lung metastases compared to control group. Most strikingly, iTEAD treatment halted outgrowth from cancer cells that had disseminated to the lungs indicating a promising therapy for patients with metastatic disease. Importantly, animal studies show that TEAD inhibitors are well tolerated in mouse models. TEAD activity is mostly observed during early embryonic development and in diseased tissue, which would imply **low toxicity** levels of TEAD inhibitors in adult normal tissue. Additionally, iTEAD treated lungs (without metastases) showed a drop in an inflammatory pathway called TLR4 pathway. This may indicate a cooperation between TEAD and TLR4. The good news is that TLR4 is cell receptor that has several promising inhibitors currently tested in the clinic for inflammatory diseases which can be repurposed as an anti-metastases targeted therapy.

First, we will test iTEAD in our metastatic TNBC models alone and in combination with SOC chemotherapy reagents to assess their efficacy against established lung metastases. **Second**, we will examine the effect of TLR4 inhibitors in combination with therapies identified by Synergy-Seq on immune cell populations in metastatic lesions to determine their efficacy at boosting responses to current therapy.

Our proposed studies seek to amend current approved therapy for metastatic TNBC with a targeted therapy that is aimed at **clinically relevant cancer cell subpopulations** to improve outcome and survival of patients with lung metastases.