The Overarching Goals: There is an urgent and unmet need to identify effective treatments for patients with triple-negative breast cancers (TNBC) and HER2-enriched breast cancers that develop breast cancer brain metastases (BCBM). BCBM occurs in over 50% of metastatic TNBC and HER2-enriched breast cancers and confers poor prognoses. Due to poor therapeutic response or lack of effective targeted therapies, BCBM has a dismal median survival of 2.3 months following initial diagnosis. Therefore, the overarching goal of our study is to identify novel treatment strategies to effectively treat BCBM without toxicity or side effects, and eliminate mortality of patients with metastatic breast cancers.

The Molecular Targets: Our pilot studies have identified two cellular pathways that are druggable and activated in BCBM: the RET (Rearranged during transfection) and tGLI1 (truncated glioma-associated oncogene homolog 1) signaling pathways. (1) RET is frequently overexpressed or hyperactive in metastatic breast cancers, especially in those that preferentially metastasize to the brain. Despite the clinical evidence that FDA-approved selective RET inhibitors (RETis; selpercatinib and pralsetinib) can reduce brain metastases in over 90% of lung cancer patients, we found that RET monotherapy is insufficient to treat BCBM in vivo, which suggests that established BCBMs gain therapeutic resistance to selective RETis through an unknown mechanism. (2) In our efforts to identify a druggable molecular target that is co-activated with RET in BCBM and contributes to BCBM resistance to RET monotherapies, we examined tGLI1, a tumor-specific and oncogenic variant of the GLI1 transcription factor. It was previously reported that tGLI1 is required for brain-tropism of breast cancer cells and is associated with BCBM incidence and progression. Datamining analysis of 1100 breast cancer patient datasets revealed that RET and tGLI1 are co-activated in TNBC and HER2-enriched breast cancers and that the RET-tGLI1 co-activation is associated with higher risk of developing BCBM.

The Therapeutic Agents: Our preliminary results showed exciting synergistic therapeutic effects from combining selective RETis, which are FDA-approved for other cancer types, with ketoconazole (KCZ), an FDA-approved anti-fungal that is being evaluated for repurposing as an anti-cancer drug. The RETis we will use are the orally active selpercatinib and pralsetinib, which are FDA-approved for treatment of RET-altered lung and thyroid cancers. KCZ is an anti-fungal that received FDA approval in 2000 for treatment of fungal infections, and was recently demonstrated to have selectivity against tGLI1-expressing breast cancer cells. KCZ is currently being evaluated in early Phase I clinical trials for patients with BCBM (NCT03471364).

Proposed Preclinical Studies: Our proposed study will be the first to determine the therapeutic utility of combining RETis and KCZ to inhibit BCBM of metastatic TNBC and HER2-enriched breast cancers. For this, we will use cell line-based in vitro models, including knockdown and overexpression models, to determine the role of tGLI1 expression in the therapeutic response of breast cancer cells to RET inhibition. We will validate our findings in vivo, using intracardiac inoculation model of metastasis, which measures the ability of circulating tumor cells to form distant metastases to various organs including the brain, lung, bone, and liver (Aim 1). We will test the efficacy of the RETi-KCZ combination therapy in two complementary mouse models of experimental brain metastasis: intracarotid injection, which will inject breast cancer cells into the common carotid artery (which supplies blood to the brain) and more specifically models brain metastasis without metastasis to other organs, and intracardiac inoculation (Aim 2). We will also perform transcriptomic analysis of breast cancer cells using RNA-Seq following treatment with the RETi-KCZ combination therapy, in order to identify novel downstream effectors of the RET-tGLI1 pathway crosstalk (Aim 3).

Translational Impact: (1) Our results will have high clinical relevance due to the use of experimental brain metastasis mouse models using authenticated human breast cancer cell lines and FDA-approved therapeutic compounds. (2) Our results could prove that co-inhibiting RET and tGLI1 pathways are more effective in treating developed BCBM of TNBC and HER2-enriched breast cancers compared to single agent treatment. (3) Our preliminary results indicate that over 90% of BCBM patients have co-activation of RET and tGLI1 pathways and could benefit from co-inhibition of RET and tGLI1. (4) The novel RETi-KCZ combination therapy could be further validated in early Phase I clinical trials within a relatively short period of time and conducted by an experienced breast oncologist. (5) Both RETis, selpercatinib and pralsetinib, and KCZ are non-toxic to tumor-bearing mice.