The problem addressed by this proposal is drug resistance in HER2-positive (HER2+) breast cancer brain metastases (BCBMs). BCBMs are a major cause of death and disability in patients with metastatic breast cancer. Up to 50% of patients with HER2+ breast cancer are affected by brain metastases. Current treatments such as surgery, radiation and drug therapies are unable to cure HER2+ BCBMs because almost all tumors become drug-resistant over time. Thus, new approaches to tackle HER2+ BCBM drug resistance are needed.

We have found that, even when drugs penetrate the brain, BCBMs use genetic and non-genetic mechanisms to become resistant to different therapies. Thus, the most effective way to prevent the formation of a drug resistant tumor is to identify and inhibit the mechanisms that generate drug-resistant cells. Many tumors harbor reservoirs of non-dividing, or quiescent, cancer cells to form the seeds from which a drug-resistant tumor can arise, after most actively-dividing cells are killed. These 'persister', quiescent cancer cells eventually rearrange their genetic programs and form actively dividing drug-resistant cancer cells that create a fully drug-resistant tumor. We previously showed that a population of quiescent cancer cells marked by being AKT^{low} are found in breast cancer brain metastases. AKT^{low} quiescent cancer cells (QCCs) can promote whole-tumor drug resistance because they 1) are naturally resistant to many targeted therapies and chemotherapies; 2) form proliferative, drug-resistant cells that can repopulate a tumor; and 3) are able to evade the immune system, which plays a crucial role in killing cancer cells. We have found that formation of AKT^{low} QCCs can be inhibited using a mTOR1/2 inhibitor. Because QCCs promote tumor drug-resistance, we propose that inhibiting QCC formation with a mTOR1/2 inhibitor will improve drug sensitivity in HER2-positive BCBMs, leading to more effective therapy for patients with HER2-positive BCBMs.

Our goal is to evaluate whether targeting QCCs overcomes HER2-inhibitor drug-resistance in HER2-positive BCBMs. To achieve this goal we will: 1) evaluate whether QCCs are enriched in patient BCBMs treated with a HER2-inhibitor and are associated with immune cell (CD8+ T cells) exclusion, and 2) test if reducing quiescent cancer cells in a HER2+ BCBM mouse model overcomes eventual resistance to tucatinib, a promising brain-penetrant HER2-inhibitor that is undergoing testing in patients.

For Aim 1, we will quantify QCCs and surrounding immune cells in patient HER2+ BCBM tissues, using a novel single cell imaging technique (tissue cyclic immunofluorescence). We will compare QCCs and associated CD8+ T cells between HER2+ BCBM tissues from patients who previously received any HER2-targeting drug vs those that were untreated. We expect to find that QCCs, and associated CD8+ T cell exclusion from the tumor cells, are more frequent after treatment. Importantly, we will also be able to describe whether populations of quiescent cancer cells other than the AKT^{low} subset (e.g. $NR2F1^{high}$, $CD44^{high}/CD24^{low}$ etc.) are altered by treatment.

For Aim 2, we will test whether a brain-penetrant mTOR1/2 inhibitor, sapanisertib, can reduce QCCs and, in turn, overcome eventual tucatinib resistance in an immune-competent mouse model of HER2+ BCBM ($NIC/Pten^{null}$). The $NIC/Pten^{null}$ model that we developed recapitulates the most common genetic alteration found in BCBMs (loss of the tumor suppressor PTEN). When the $NIC/Pten^{null}$ mice are treated with the brain-penetrant HER2-inhibitor tucatinib, tumors initially respond but then grow again, suggesting that they have become resistant. We will use this mouse model of HER2+ BCBM resistance to test whether adding sapanisertib to tucatinib results in reduced QCCs and prolonged response to tucatinib.

Significance to patients with metastatic breast cancer: Successful results from this study will demonstrate that targeting QCCs is a feasible strategy to overcome drug resistance in HER2+ BCBMs. Given that tucatinib and sapanisertib both enter the brain and have been used in clinical trials (but have not yet been tested together to treat BCBMs), the approach in this study could be rapidly translated into human clinical trials. Early evidence also suggests that targeting QCCs may help awaken an anti-tumor immune response. Finally, the optimal strategy to target quiescent cancer cells is still an active area of research. Data from human BCBMs and our mouse experiment might suggest that more than one quiescent cell population may be present and contribute to BCBM drug resistance. Our work in identifying quiescent cancer cell diversity in patient tissues will be crucial to maximizing the efficacy of targeting quiescence to overcome drug resistance and improve outcomes for patients living with metastatic breast cancer.