LAY ABSTRACT

The Goal: The goal of this project is to address the unmet need of effective non-toxic treatments for patients with triple-negative breast cancer (TNBC) who have developed brain metastases. Breast cancer brain metastases (BCBM) is a major clinical challenge and a devastating diagnosis for breast cancer patients. As many as 30-50% of HER2-positive and TNBC patients develop BCBM and endure poor prognosis, which is attributed to the fact that we still do not fully understand factors that drive and sustaining BCBM, and the lack of drugs that effectively penetrate the blood-brain barrier (BBB) and blood-tumor barrier (BTB). Although both HER2-enriched breast cancer and TNBC are more likely to develop BCBM (30-50%), TNBC brain metastases are associated with dismal prognosis of 4.9 months of survival, shorter than the brain metastases of other breast cancer subtypes. Thus, the goal of this Expansion Award project to identify and validate a new effective non-toxic BCBM therapy using preclinical studies, laying the foundation for a potential treatment for TNBC patients with brain metastases.

The Molecular Targets and Respective FDA-approved Orally Active Drugs:

TrkA receptor tyrosine kinase is encoded by the *NTRK1* gene that is prone to forming oncogenic *NTRK1* fusions; however, overexpression of wild-type TrkA is sufficient to enhance breast cancer progression. Consequently, TrkA is an important therapeutic target in several cancers. Trk selective inhibitor **Entrectinib** (ROZLYTREK) is an orally active, small molecule inhibitor that received FDA's tumor-agnostic approval for *NTRK*-altered solid tumors. Entrectinib inhibits both wild-type TrkA (detected in most breast cancer) and TrkA fusion, crosses the BBB/BTB, and has shown an impressive **intracranial objective response rate of 80% (20/25)** in Phase I/II trials with lung cancer patients with brain metastases (ALKA-372-001, STARTRK-1, and STARTRK-2 trials) . Our original **Breakthrough Award** suggested anti-BCBM of the Entrectinib and the need to combine it with another inhibitor to reach higher efficacy against TNBC BCBM.

RET is also a receptor tyrosine kinase that plays a major role in progression of many cancers including breast cancer. In search for a drug co-target with TrkA therapy, we found that is the top pathway co-activated with TrkA in breast cancer. Orally active RET selective inhibitor **Pralsetinib** (GAVRETO) received FDA approval for RET fusion-positive lung and thyroid cancers, and showed a promising **70% (7/10) intracranial objective response rate** in a Phase III trial with lung cancer patients with measurable brain metastasis at enrollment. Pralsetinib inhibits both wild-type RET (detected in most breast cancer) and RET fusion (ARROW trial).

Novel Combination of Entrectinib and Pralsetinib, two FDA-approved drugs with high clinical efficacy for lung cancer patients with brain metastases:

Whether Trk or RET kinase inhibitors are effective towards BCBM is not known. The combination of Trk and RET inhibitors has not been investigated for treating any tumor types, which will be tested for the first time in this project. The novel combination of Entrectinib and Pralsetinib is supported by the following **preliminary data**. Activated TrkA and activated RET are highly co-expressed in brain-metastatic breast cancer cells (100%) and in BCBM specimens (87%; 13/15) whereas adjacent normal brain tissues were negative for both receptors. Co-activation of both kinases is enriched in TNBC tumors and associated with a shorter time to develop brain metastases. Using brain-metastatic breast cancer cells, we observed a treatment synergy between Entrectinib and Pralsetinib in inhibiting cell growth and migration, and inducing cancer cell death.

Translational Impact: **(1)** Our proposal addresses a significant medical challenge in managing BCBM. BCBM treatment remains an immense medical challenge and the National Comprehensive Cancer Network (NCCN) does not have established guidelines for BCBM. TNBC brain metastases is associated with dismal prognosis of only 4.9 months of overall survival, worse than the brain metastases of other molecular breast cancer subtypes. **(2)** If the project is successful, we will establish preclinical evidence to support future clinical testing of the combination of orally active Entrectinib and Pralsetinib for patients with TNBC BCBM, and possibly HER2-positive and ER-positive BCBM. Drs. Anneliese Gonzalez (breast oncologist and collaborator) and Jay Zhu (neuro-oncologist and co-investigator) could lead this clinical trial. **(3)** The clinical trial with Entrectinib and Pralsetinib could be initiated within a short period of time since both drugs are already approved by the FDA with excellent safety profile. **(4)** Given the majority of BCBM tumors express the drug targets (87%), 87% of patients with existing BCBM could benefit from the Entrectinib+Pralsetinib treatment combination.