

PUBLIC ABSTRACT

The Goals: There is an unmet urgent need to identify effective treatments for patients with breast cancer brain metastasis (BCBM), a major clinical challenge; BCBM constitutes approximately 10-30% of metastatic breast cancer cases and associated with poor prognosis. The poor prognosis is attributed to the fact that we still do not fully understand the factors driving breast cancer cells to the brain, the biology of brain metastases, and the lack of drugs that effectively penetrate the blood-brain barrier (BBB) and blood-tumor barrier (BTB). The goal of our study is therefore set to **identify novel treatment strategies that can be used to effectively treat women with established BCBM without unwanted toxicity and side effects.** To achieve our study goal, we plan to **generate preclinical evidence to support future clinical utility of ketoconazole (KCZ),** an antifungal drug already approved by the FDA for other diseases and/or being evaluated in multiple types of cancers including metastatic breast cancer, and novel KCZ derivative WF-229A. Based on clinicaltrials.gov, there are 61 active clinical trials using KCZ for treating cancer patients, including a phase II trial (NCT00212095; KCZ with docetaxel) for metastatic breast cancer and our **Phase 0 clinical trial entitled “Ketoconazole before surgery in treating patients with recurrent glioma or breast cancer brain metastases”** (NCT03796273; Lo, Co-Investigator). If proven effective in our preclinical studies, KCZ in combination with surgery or radiation therapy can be moved to Phase I/II trials relatively quickly at the Wake Forest University Comprehensive Cancer Center. There are currently 107 active oncology clinical trials at various stages being conducted at our Cancer Center in which 19 of them are for breast cancer patients.

The Molecular Target: The Principal Investigator’s laboratory made the discovery of a novel oncogenic transcription factor **truncated glioma oncogene homolog 1 (tGLI1)** as a **tumor-specific, gain-of-function, master regulator of tumor growth, vascularity, and breast cancer brain metastasis (BCBM).** Our published and pilot studies using >1,000 breast tumor and metastases samples and mouse models of breast cancer metastasis have established the following rationale to pharmacologically target tGLI1 for brain-metastatic HER2-enriched and triple-negative breast cancers. **(a) tGLI1 strongly promotes breast cancer metastasis to the brain and shortens patients’ time to develop brain metastases. (b) tGLI1 is highly expressed in metastatic HER2 and triple-negative breast cancers (84-91%) and brain metastases (86%). (c) tGLI1 expression is tumor-specific. (d)** Datamining of over 1,200 patients showed tGLI1 is activated in the majority of triple-negative and HER2-enriched breast carcinoma samples, the two subtypes with high risks of brain metastasis.

Drug Screen and Preclinical/Clinical Data: There is no available tGLI1 inhibitor. To identify inhibitors that selectively target tGLI1 that is only expressed in cancerous tissues but not normal tissues and strongly promotes BCBM, we conducted synthetic lethality screens of 1,520 compounds and subsequently identified an FDA-approved orally active, antifungal **KCZ** that selectively and strongly inhibits tGLI1(+) HER2-enriched and tGLI1(+) triple-negative breast cancer cells, particularly cancer stem cells that are known to be more metastatic and more resistant to therapies compared to the non-stem cell populations. tGLI1 is required for KCZ effects. Using preclinical mouse models of BCBM, we showed that **KCZ crossed the BBB and BTB, and selectively suppressed tGLI1-high BCBM without liver or cardiac toxicities.** Clinical results of our Phase 0 clinical trial with BCBM patients showed that KCZ readily penetrated the BBB/BTB to accumulate in the human brains. To improve KCZ’s ability to accumulate in the brain and to mitigate potential liver toxicity, we collaborated with Dr. Terrence Smalley, an experienced medical chemist, to create novel KCZ derivatives and found two promising derivatives WF-229A, with potentially higher efficacy than KCZ and diminished liver toxicity.

Proposed Preclinical Studies: Our study will be the first to determine the utility of KCZ and its novel promising derivative WF-229A as a novel adjuvant treatment with surgery for treating BCBM. For this, various animal models will be used.

Translational Impact: **(1)** Our results will have high clinical relevance largely because of the uses of PDX, authenticated cell lines, cell line-derived xenograft mouse models, and clinical samples. **(2)** Our results could prove that KCZ monotherapy and KCZ-based adjuvant therapy are effective in preventing brain metastasis of triple-negative breast cancer and HER2-positive tumors, and treating developed existing BCBM. **(3) More than 80% of the women with metastatic triple-negative and HER2-positive breast cancers plus >85% of BCBM patients** could benefit from the treatments. **(4)** The treatment could be further validated in Phase I/I trials within a relatively short period of time and conducted by an experienced breast oncologist. **(5)** The promising derivative and WF-229A could be further tested in human patients after approval for Investigational New Drug (IND). **(6)** Both KCZ and WF-229A are non-toxic to tumor-bearing mice.