Public Abstract

Rationale and goal: Among all ethnic groups, women of African American (AA) origin have highest breast cancer-related mortality rates. AA women have twice as high (38/100,000) incidence of Triple-negative breast cancer (TNBC) in comparison to White American (WA) women (19/100,000). Not only do AA women have higher TNBC incidence, but the survival rate for TNBC is also significantly lower in AA women in comparison to WA women (5-year relative survival of only 14% for AA in comparison to 36% for WA). This difference is observed even in places where the standard of care is the same. Irrespective of stage at diagnosis, AA-TNBC is more aggressive with higher risk of metastasis and inferior survival outcomes compared to WA-TNBC. Thus, an overarching challenge in the field is to develop effective therapy for metastatic AA-TNBC.

Currently, TNBC has limited therapeutic options as it lacks the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). We believe that AA-TNBCs have unique biology that results in an aggressive metastatic progression of these tumors. Our study is designed to understand the unique biology of AA-TNBC and we plan to exploit this knowledge to develop effective therapy against metastatic AA-TNBC tumors. In preliminary analyses, we uncovered that AA-TNBC cells are inherently aggressive, exhibiting elevated growth, migration, invasion and cancer stem-like phenotype compared to WA-TNBC cells. RNA-sequencing of multiple AA and WA TNBC cell lines showed an enrichment of GLI1 and Notch1 pathways in AA-TNBC cells. In line with this observation, analysis of The Cancer Genome Atlas (TCGA) dataset revealed a positive correlation between GLI1 and Notch1 in AA-TNBC and a negative correlation in WA-TNBC. Encouraged with our novel preliminary findings, we tested whether GANT61 (a preclinical GLI1-Hedgehog inhibitor) and DAPT (a preclinical γ-secretase inhibitor being used to target Notch1) inhibit AA-TNBC metastasis. Indeed, combined treatment of AA-TNBC-derived tumors with GANT61 and DAPT resulted in significant inhibition of tumors and distant metastasis. Also, tumor-dissociated cells showed mitigated migration, invasion, mammosphere-formation and CD44+/CD24- stem cell population. In fact, secondary tumors derived from GANT61+DAPT treated AA-TNBC tumors showed diminished stem-like phenotype. Overall, goal of our study is to understand the unique biology of AA-TNBC and exploit this knowledge to develop effective strategies against metastatic AA-TNBC.

Approach: Based on our novel findings, *we hypothesize that aberrant overexpression of GLI1 and Notch1 leads to 'oncoprotein addiction' and forms a relentless molecular axis that drives stemness and metastatic progression of AA-TNBC, and rendering metastatic AA-TNBC vulnerable to therapeutic strategies aimed at GLI1 and Notch1 inhibition.* We propose to test our hypothesis via two specific aims. In Aim 1, we will investigate *GLI1 and Notch1*-molecular targets involved in AA-TNBC metastasis, and examine the alterations in *GLI1 and Notch1 pathway proteins* in AA-TNBC and WA-TNBC and correlate it with clinical outcomes. We plan to combine genomic (GLI1/Notch1-binding profile), transcriptomic (RNA-Seq) and functional (CRISPER-mediated silencing) approaches to uncover the transcriptional program orchestrated by GLI1 and Notch1 in AA-TNBC metastatic progression. We will utilize our extensive clinically annotated tumor bank to examine the differences in Notch1 and GLI1 pathway genes' expression in AA-TNBC and WA-TNBC tumors and metastasis, and correlate it with clinical outcome. We will also evaluate if overexpression of GLI1-Notch1 axis serves as biomarkers to predict clinically aggressive tumor progression and a negative outcome in AA-TNBC compared to WA-TNBC. In Aim 2, we plan to examine the effectiveness of pharmacologic inhibition of GLI1 and Notch1 using Vismodegib and MK-0752 in AA-TNBC using patient-derived-xenografts (PDX) models. TNBC are currently being treated with combination of chemotherapy. We will also examine if combining Vismodegib and MK-0752 with chemotherapy presents a superior outcome. Testing clinically viable inhibitors of GLI1 and Notch1 (Vismodegib and MK-0752) will provide the necessary preclinical data for the development of a clinical trial.

Anticipated Clinical applications to benefit AA women with metastatic TNBC: The proposed studies will provide new molecular understanding regarding AA-TNBC and establish key molecular nodes that drive the aggressive metastatic progression of AA-TNBC. Defining the utility of Vismodegib and MK-0752 for AA-TNBC will move the field in a new direction to repurpose these drugs and potentially providing new therapeutic options for AA-TNBC based on their unique biology. Also, future translation of our translational findings can move faster (within 2-3 years) as both Vismodegib and MK-0752 are clinically available drugs, and a clinical trial is expected to start at the end of this project to treat metastatic AA-TNBC patients with high GLI1-Notch1 using Vismodegib and MK-0752 combination along with chemotherapy. Breast cancer clinical team at Johns Hopkins is very committed to bring novel therapeutic options to clinic.