TGWSM who were initiating FHT. Similar primary and secondary outcomes are to be elucidated in both cohorts. We anticipate the RM environment of TGWSM using FHT in both cohorts compared to the RM environment of cisgender MSM in the cross-sectional cohort will be associated with greater percentages of activation/co-receptor expression of CD4+ T cells that express biomarkers of interest. In the longitudinal cohort, we similarly anticipate increased percentages and activation/co-receptor expression of CD4+ T cells expressing biomarkers of interest in TGWSM after in comparison to before initiating FHT. DISCUSSION/SIGNIFICANCE OF IMPACT: This is the largest study of its kind to compare HIV target cells in RM of TGSWM, which challenges prevailing perspectives suggesting to group cisgender MSM with TGWSM. Anticipated results will inform HIV prevention strategies and future vaccine studies in this high-risk population.

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Elucidating the altered metabolism of NAD+ in the selective targeting of glioblastoma with the NQO1-activated drug β-lapachone*†

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OBJECTIVES/GOALS: NAD+ synthesis is enhanced in glioblastoma (GBM) allowing GBM to resist chemotherapy. NQO1 is upregulated in GBM and may be targeted by β-lapachone (β-lap) to induce NAD-depletion and cell death. This project investigates NQO1 as a selective target for GBM and the contributions of glucose and uridine to NAD+ synthesis. METHODS/STUDY POPULATION: Survival Studies and NQO1 expression. RNA-seq and survival data from TCGA of glioma patients (n = 667) was obtained using the UCSC Xena platform. Western blots were utilized to determine expression levels of NQO1 and NAMPT in normal human astrocytes, U87 cells, and patient-derived GBM cell lines. Immunocytochemistry: γ-H2AX staining was used to evaluate β-lap induced DNA damage. NQO1-dependence was evaluated with the NQO1-inhibitor dicoumarol. Cytotoxicity measurements. Cells were exposed to β-lap and other inhibitors, and cell survival was determined by trypan-blue exclusion assay. Co-culturing experiments were performed with fluorescently labeled U87 cells and unlabeled astrocytes. NAD+ quantification. Intracellular NAD+ was acid extracted and quantified by an enzyme-cycling reaction. RESULTS/ANTICIPATED RESULTS: NQO1 overexpression is linked to decreased survival in glioma patients. In glioma patients, high NQO1 expression was associated with a decreased overall survival and high-grade tumors. β-lap induces selective NAD+ depletion and cell death in NQO1-expressing GBM cells. Western blots demonstrate NQO1 expression to be elevated in GBM cell lines compared to normal human astrocytes. β-lap induces NQO1-dependent NAD+ depletion and cell death in GBM compared to astrocytes in mono- and co-culture experiments. Glucose and uridine facilitate NAD+ regeneration in GBM. We demonstrate extracellular glucose and uridine facilitate NAD+ regeneration and cell survival in β-lap exposed GBM cells. Utilizing inhibitors, we determined that glucose and uridine facilitate NAD+ regeneration through the NAD+ salvage pathway. DISCUSSION/SIGNIFICANCE OF IMPACT: GBM is the most common primary adult CNS tumor with a median survival of 14 months. Despite significant research in therapeutic strategies, treatment has not improved in 2 decades. There is a significant need to discover new targets that may improve GBM treatment. We demonstrate here that targeting NQO1 with β-lap induces selective GBM toxicity.

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Calcium channel blockers for treatment of triple-negative breast cancer*

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OBJECTIVES/GOALS: Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer (BC) with limited treatment options. Mortality rate is especially high in African American (AA) women of reproductive age. High levels of intracellular calcium (Ca2+) have been shown in TNBC cells. This study is to investigate Ca2+ channel blockers (CCBs) as therapy for TNBC. METHODS/STUDY POPULATION: Two human TNBC cell lines obtained from ATCC – HCC1806, and MDA-MB-453 are treated with CCBs, Cilnidipine (Cil), and Mibefradil (Mib), in a concentration- and time-dependent manner. Cell proliferation assays are performed by the MTS cell viability assay. Intracellular Ca2+ levels are measured using the fluorescent dye: Fluro 4-AM. Apoptosis is determined by flow cytometry using Annexin V staining and mitochondrial permeability will be assessed by the Mito JC-1 assay. Expression of Ca2+ signaling genes will be quantitated by real-time polymerase chain reaction (RT-PCR). Potential pathways of CCB efficacy will be identified by ingenuity pathway analysis (IPA). RESULTS/ANTICIPATED RESULTS: Our findings show both CCBs decrease cell proliferation in a concentration- and time-dependent manner to a maximum of 80% vs. control in both TNBC cells. Flow cytometry findings on both TNBC cells treated with both drugs at 20 μM for 24 hours depicts late apoptosis. Interestingly, Mib did not change the intracellular Ca2+ level in HCC1806 cells yet decreased in MDA-MB-453 cells by fivefold, while Cil increased the intracellular Ca2+ level in both cells almost twofold. It is anticipated that Mito JC-1 assay depict decreased mitochondrial potential in both cells. For reverse transcription polymerase chain reaction, it is anticipated that CCB treatment will increase transient receptor potential Ca2+ channels and decrease voltage-gated Ca2+ channels in both cells. IPA analysis is expected to show apoptotic pathways are involved in TNBC via CCB treatment. DISCUSSION/SIGNIFICANCE OF IMPACT: TNBC lacks the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. Treatment options for TNBC remain severely limited. Our findings that both Cil and Mib can inhibit proliferation of human breast cancer cell lines indicate repurposing CCBs as treatment for TNBC warrants further investigation.

https://doi.org/10.1017/cts.2024.728 Published online by Cambridge University Press