

Repurposing eliglustat as an anti-cancer agent for triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) represents a heterogeneous subtype of breast cancer characterized by high pathological grade, strong invasiveness, local recurrence, high metastasis rate, and poor prognosis. Chemotherapy remains the main choice of systemic treatment. However, response to chemotherapy among TNBC patients is variable, lacks durability, and acquired resistance to chemotherapy is a frequent manifestation. There remains an unmet need to establish novel alternative therapies that can both exert targeted effects on cancer cells as well as stimulate an anti-cancer immune response that could prime tumors for combination with emerging immunotherapies.

We have uncovered an onco-metabolic biochemical feature in TNBC wherein cancer cells increase chemical conversion of sphingomyelin to ceramide with subsequent glycation to glycosphingolipid derivatives. Our mechanistic studies have established that this biochemical program is essential for survival mitophagy. The activation state of this program in cancer cells opens a metabolic vulnerability that is targetable through biochemical inhibition of glucosylceramide synthase (UGCG), a rate-initiating enzyme that mediates glycation of ceramides to glycosphingolipid derivatives **(Figure 1).** The primary objective of the current study was to evaluate the anti-cancer efficacy of eliglustat using orthotopic syngeneic mouse models of BRCA1co/co; MMTV-Cre; p53+/- TNBC and 4T1 as well as patient-derived and human cell-line derived xenograft models of TNBC. The combination of eliglustat with PARP inhibitors and anti-PD1 was also evaluated.

Methods: In vivo efficacy of eliglustat was tested using an orthotopic syngeneic mouse model of BRCA1co/co; MMTV-Cre; p53+/- TNBC as well as human cell line-derived and patient-derived xenograft models of TNBC. Electron microscopy and single-cell RNA sequencing was performed on tumors collected from the BRCA1co/co; MMTV-Cre; p53+/- mouse model of TNBC following intervention to evaluate changes in mitochondrial morphology and to assess for changes in the composition of immune cell subtypes. Tumors, spleens, and draining lymph nodes were collected from an independent cohort of BRCA1co/co; MMTV-Cre; p53+/- tumor-bearing mice for further characterization of the tumor immunophenotype and host immune response following eliglustat treatment. Efficacy of the combination of eliglustat plus Olaparib and anti-PD1 compared to monotherapy was evaluated in the BRCA1co/co; MMTV-Cre; p53+/- mouse model of TNBC.

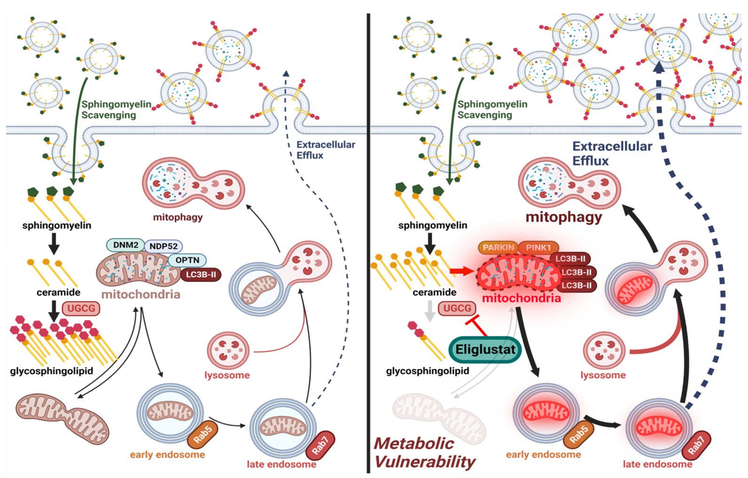


Figure 1. Proposed mechanism of sphingolipid oncometabolism. Essential components include (1) scavenging of sphingomyelin, (2) increased tumor catabolism of sphingomyelins to yield glycosphingolipids that support vesicle biogenesis that (3) intersects with 'survival' clearance of damaged mitochondria. *Metabolic Vulnerability:* Selective targeting of UGCG using small molecule inhibitors results in accumulation of mitotoxic ceramides in TNBC cells that promotes lethal mitophagy leading to direct anti-cancer effects as well as potentiation of the anti-tumor immune response.

Results

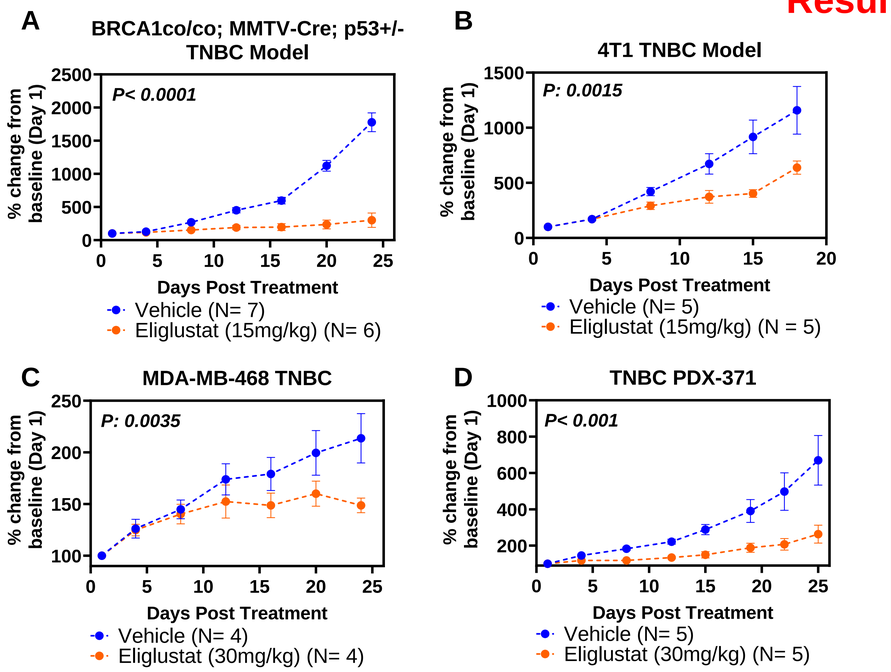


Figure 2. A-D) Tumor growth trajectories (% change from baseline (Day 1)) for BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ tumor-bearing mice (N=6-7 mice per group) (A), 4T1-tumor bearing mice (N= 5 mice per group) (B), human MDA-MB-468 tumor-bearing mice (N = 4 mice per group) (C), or in a patient-derived xenograft model of TNBC (N= 5 mice per group) following treatment with eliglustat (administered via daily oral gavage) or saline control. Statistical significance was determined by repeated measured two-way ANOVA.

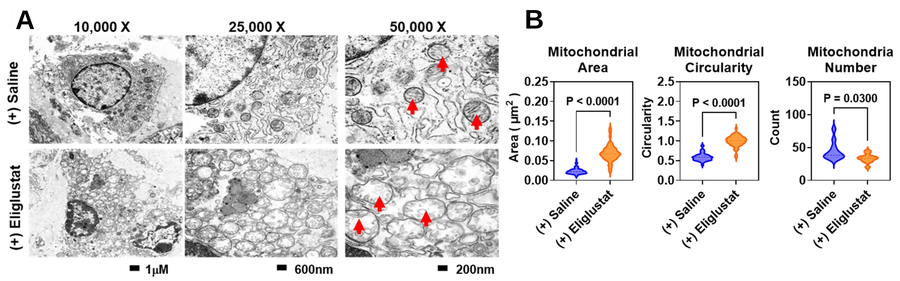


Figure 3. A) Electron microscopy images of tumors (N= 1 mice per group) from saline or eliglustat (7.5mg/kg) treated mice. Magnification (10,000X, 25,000X and 50,000X) are provided. Red arrows are provided for mitochondria. **B)** Mitochondrial morphology analysis of mitochondrial area, circularity and number from electron microscopy pictures. Statistical significance was determined by 2-sided Student T test.

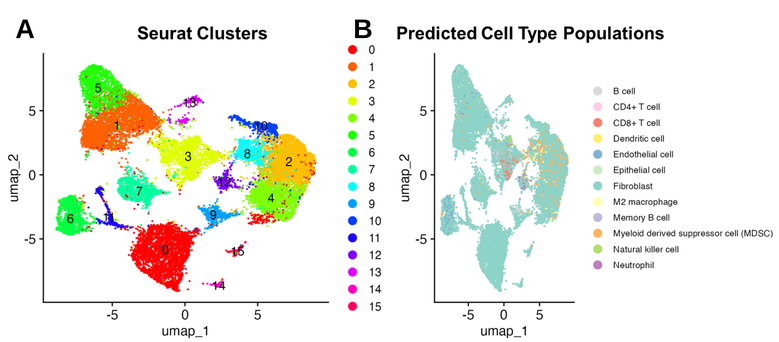


Figure 4. A-B) Umaps depicting 15 clusters (**A**) and cell-types (**B**) inferred from single-cell RNAseq data of tumors from BRCA1co/co; MMTV-Cre; p53+/- tumor-bearing.

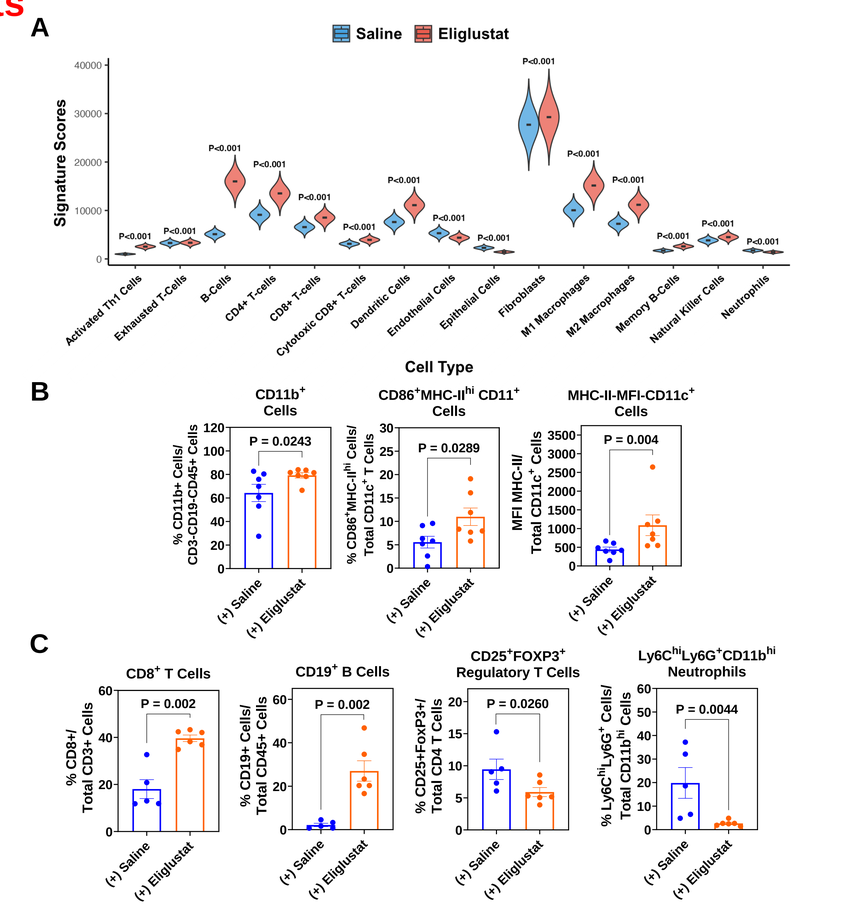


Figure 5. A) Representation of immune cells inferred from scRNA data of tumors from BRCA1co/co; MMTV-Cre; p53+/- tumor-bearing after 14 days of treatment with saline or 7.5mg/kg eliglustat. **B)** Representation of CD11b^{+}, CD86^{+}MHC-II^{hi}CD11^{+}, MHC-II-MFI-CD11c^{+}, and CD103^{+}CD4^{+} immune cells in tumors from BRCA1co/co; MMTV-Cre; p53+/- tumor-bearing mice after 3 days of treatment with saline or 7.5mg/kg eliglustat. **C)** Flow cytometry-based analysis of immune cells in tumors collected from BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ tumor-bearing mice after 14 days of treatment with saline or 7.5mg/kg eliglustat.

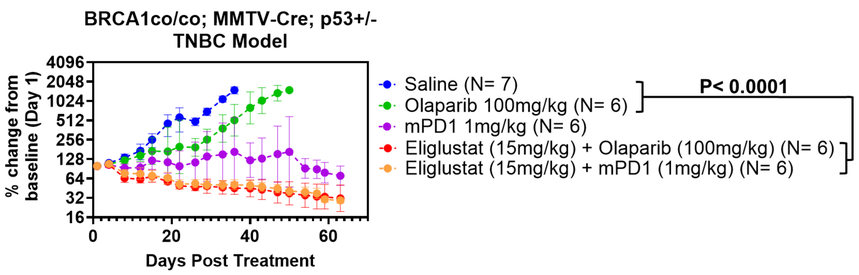


Figure 6. A) Tumor growth trajectories (% change from baseline (Day 1)) for BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ tumor-bearing mice (N= 6-7 mice per group) following treatment with saline control, 100mg/kg Olaparib, 1mg/kg anti-PD1, eliglustat (15mg/kg) + Olaparib (100mg/kg), or eliglustat (15mg/kg) + anti-PD1 (1mg/kg). Statistical significance was determined by repeated measures two-way ANOVA.

Conclusions: Targeting of the cancer cell sphingolipid onco-metabolic pathway via repurposing of eliglustat, a selective small molecule inhibitor of glucosylceramide synthase (UGCG), is a novel therapeutic strategy for TNBC.

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