**\*\*Required for METAvivor’s board: Lay Description of Important Outcomes**

The majority of breast cancer deaths are due to metastatic disease. The high incidence and mortality associated with metastatic breast cancer (MBC), coupled with an overwhelming lack of effective therapies, warrant the exploration of novel treatments capable of impacting MBC patient survival. Immunotherapy, wherein the patient’s immune system is activated to eliminate cancer cells, has led to impressive clinical benefits in certain cancers. Strategies include: 1) immune checkpoint blockade (ICB), relying on inhibitors to checkpoints, such as programmed cell death protein 1 (PD-1), that regulate antitumor immunity; and 2) adoptive cell transfer (ACT), wherein the patient’s T cells (TCs) are activated and expanded *ex vivo* and re-introduced into the patient. Both approaches aim to increase the number of CD8+ TCs that can engage and eliminate cancer cells. Immunotherapy has been applied to MBC, but responses have proven modest. A major obstacle to immunotherapy is immunosuppression and TC exhaustion upon tumor entry. The tumor microenvironment leads to mitochondrial defects and a loss of mitochondrial content in TCs, contributing to metabolic dysfunction and energy imbalances. This ultimately limits the cancer cell killing potential of TCs. Our objective was to metabolically prime TCs to bolster their antitumor immunity in ACT. Nuclear respiratory factor-1 (NRF1) drives the creation of new mitochondria. Increased mitochondrial numbers ultimately improve mitochondrial respiration and ATP production. We hypothesized that treatment of isolated TCs with NRF1 messenger RNA (mRNA) would increase mitochondrial biogenesis and boost their bioenergetic fitness, protecting TCs against mitochondrial dysfunction and exhaustion, ultimately enhancing their antitumor immunity in ACT.

Our work so far has demonstrated that NRF1 overexpression increased mitochondrial mass in TCs undergoing exhaustion. NRF1 overexpression maintained mitochondrial quality control and homeostasis in TCs exposed to exhaustive factors and preserved mitochondrial health. NRF1 overexpression reduced TC exhaustion-associated glycolysis and increased ATP production. NRF1 overexpression in TCs led to increased resistance to exhaustion and exhaustion-associated apoptosis. Importantly, NRF1 induction in TCs undergoing exhaustion had increased production of effector cytokines and increased breast cancer cell killing potential.

This work is of significant clinical relevance. We have demonstrated that increasing mitochondria reinforces favorable TC bioenergetics needed for effective immunotherapy against MBC, potentially enhancing TC persistence and antitumor immunity, which may ultimately improve patient outcomes. TC exhaustion is a major limitation to immunotherapy in MBC and our findings show that NRF1 overexpression nullifies exhaustion-associated processes in TCs, enabling effective immunotherapy in MBC. We believe that metabolically potentiated TCs may synergize with immune checkpoint blockade strategies such as PD-1 inhibitors, increasing efficacious outcomes in MBC patients. Notably, our approach relies on clinical strategies, pharmacotherapies, antibodies, and cell therapies currently in clinical use, and thus, there is the potential for expeditious clinical translation. Continued successful completion of our work will highlight a strategy that can improve immunotherapy and efficacious outcomes in metastatic breast cancer patients.