Glioblastoma multiforme (GBM) is an aggressive malignant brain tumor with poor prognosis. Like other difficult-to-treat cancers, GBM utilizes glucose (glycolysis) at a high rate (the Warburg effect), but can adapt to stresses or starvation by using alternatives, including fatty acids or amino acids, as mitochondrial substrates. The unique features of GBM-specific metabolism may serve as novel therapeutic targets. Temozolomide (TMZ), along with surgery and radiation, are current standards for treating GBM. TMZ induces DNA damage, resulting in cell cycle arrest, and apoptosis. However, GBM can overcome TMZ by activating DNA repair enzymes. Our hypothesis is that combining TMZ with metabolic disrupting agents will potentiate TMZ cytotoxicity.

To test this hypothesis, C6 (rat) and 263 (human) GBM cell lines were treated with TMZ, with or without inhibitors of fatty acid oxidation or autophagy. Treated cells were stained and analyzed by flow cytometry for mitochondrial membrane potential (ΔΨm); uncoupling protein 2 (UCP2); Fas (CD95) and FasL (CD178); LC3A/B, detecting autophagy; and for DNA damage quantification. Explanted human GBM was stained for UCP2. We found that C6, 263, and explanted GBM tissue express significant levels of UCP2. In C6, etomoxir increased ΔΨm and decreased UCP2 expression. As predicted, etomoxir enhanced TMZ-induced DNA damage and the combination treatment elicited a greater response. Each treatment altered Fas and FasL expression, but the combination of etomoxir plus TMZ were necessary to achieve significant differences. Interestingly, we observed one high and one relatively low population of cells with regard to LC3A/B. However, treatment with etomoxir caused the largest increase in accumulation of LC3A/B, an indicator of autophagy.

Finally, we determined the optimum combinations of combined drugs to achieve the greatest level of toxicity. We demonstrate that combination treatment using TMZ with metabolic inhibitors potentiates the effectiveness of TMZ on GBM.