Targeting immune metabolism may represent a novel approach for therapeutic intervention in autoimmunity. The mitochondrial F$_{1}$F$_{0}$-ATPase is the final complex in the electron transport chain. Small molecule, allosteric modulators of this complex cause increase in superoxide formation and apoptosis of susceptible cells. Because of their distinctive metabolic phenotype, chronically activated lymphocytes are preferentially sensitive to ATPase Modulator-induced apoptosis vs resting or acutely activated lymphocytes. In inflammatory bowel disease (IBD), mucosal lymphocytes are chronically activated, overexpress anti-apoptotic proteins and show resistance to activation-induced cell death (AICD). Thus, restoring lymphocyte AICD may be a potential therapeutic strategy. Here a set of F$_{1}$F$_{0}$-ATPase Modulators was evaluated in murine IBD models and in gut-tropic T cells from IBD patients.

### METHODS

#### Acute Murine TNBS-Induced Acute Colitis
- TNBS treatment hastens tissue proteins making them immunogenic via a Th1-mediated inflammatory response.
- C57BL/6 male mice (n = 6 per group) were sensitized with topical TNBS then challenged with TNBS intracolonically 7 days later.
- The ATPase Modulator LYC-51194 was dosed once daily beginning 1 after TNBS challenge.

#### Peripheral Blood Mononuclear Cell (PBMC) Analysis
- PBMCs treated for 16 hrs with ATPase Modulators.
- Quantify cell death (TAD/Annexin V) in gut tropic (CD3+CCR9+) and non gut tropic (CD3+CCR9-) T cells by FACS.

### RESULTS

1. **LYC-51194 and LYC-51661 are Modulators of Mitochondrial F$_{1}$F$_{0}$-ATPase**
   - Modulation of the F$_{1}$F$_{0}$-ATPase leads to mitochondrial membrane potential hyperpolarization and an increase in superoxide production by complex III.
   - Apoptosis is triggered by an indirect effect on the mitochondrial respiratory chain.
   - Bioenergetic and redox abnormalities sensitize chronically-activated lymphocytes to F$_{1}$F$_{0}$-ATPase modulation resulting in preferential apoptosis.

2. **LYC-51194 Dosed Therapeutically Reduces the Severity of Murine TNBS-Induced Acute Colitis**
   - In PBMC from healthy subjects, LYC-51194 induces cell death selectively in gut tropic T cell

3. **LYC-51194 Induces Cell Death in Human Gut-Tropic Activated T Cells**
   - LYC-51194 induces cell death in gut tropic T cell
   - Consistent with the mechanism of action of other Lycera ATPase Modulators, LYC-51194 induces cell death via increased intracellular ROS. Addition of vitamin E (anti-oxidant) inhibits the ability of LYC-51194 to induce of cell death

   - Therapeutic dosing of ATPase Modulators reduces the severity of acute TNBS-induced colitis in mice.

5. **In PBMCs from IBD Subjects ATPase Modulators Induce Cell Death in Gut-Tropic T Lymphocytes**
   - ATPase Modulators induce cell death preferentially in the gut tropic T cells (CCR9+), found in peripheral blood of healthy subjects and IBD patients. These cells are considered a surrogate cell type for studying potential effects on lamina propria T cells.
   - Similar to what has been observed in chronically activated lymphocytes, ATPase Modulator-induced cell death in gut tropic T cells is dependent on the production of reactive oxygen species.
   - The preferential action of ATPase Modulators on chronically activated lymphocytes may provide an advantage over current immunosuppressive treatments and suggests that ATPase Modulators may be a promising approach for treating IBD.