Preclinical Development of a T Cell Receptor-Engineered Regulatory T Cell (Treg) Therapy for Progressive Pathology in MS

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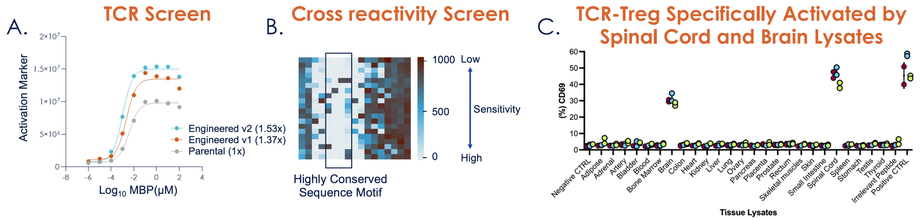
Abstract

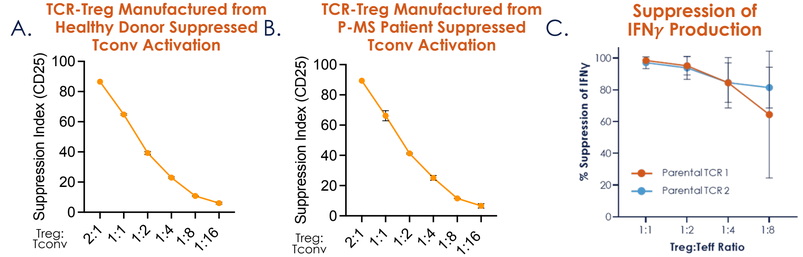
- **Background:** Disease-modifying therapies have revolutionized care for relapsing-remitting Multiple Sclerosis (MS) largely by preventing the influx of peripheral immune cells into the central nervous system (CNS). However, progressive pathology in MS is driven by chronic CNS-compartmentalized processes for which no effective therapies exist. Meningeal lymphoid aggregates play an essential role in this pathology by supporting chronic-active lesions in the CNS parenchyma. CD4$^+$ Regulatory T cells (Tregs), given their intrinsic ability to suppress immune-mediated inflammation through diverse mechanisms and their phenotypic stability, are well-suited to address this aspect of MS pathophysiology. We hypothesize that CNS-targeted Tregs will disrupt meningeal lymphoid aggregates.
- **Objective:** To develop a tissue-targeted Treg cell therapy for the treatment of progressive MS (P-MS).
- **Methods:** A TCR-engineered CD4$^+$ Treg (TCR-Treg) product was designed to target the CNS tissue-specific antigen Myelin Basic Protein (MBP) in the context of HLA-DRB1*15:01. Candidate T cell receptors (TCRs) were screened through a pipeline of assays for their MBP-reactivity and ability to induce suppressive activity in human Tregs. The best-performing TCR was introduced into Tregs isolated from healthy donor and MS patient leukopaks. The suppressive activity, phenotypic stability, and manufacturability of TCR-engineered Tregs from healthy donor and MS patient materials were characterized.

MBP Reactive TCRs Screened and Selected



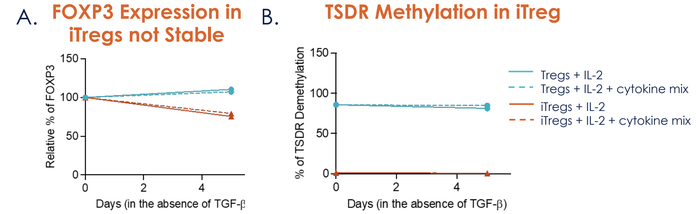
MBP-reactive TCRs were validated and selected (A). Selected TCR is highly specific to MBP antigen (B). TCR-Treg is only activated by human brain and spinal cord tissue lysates (C).

TCR-Tregs Suppress Conventional T Cell Activation and Inflammatory Cytokine Production



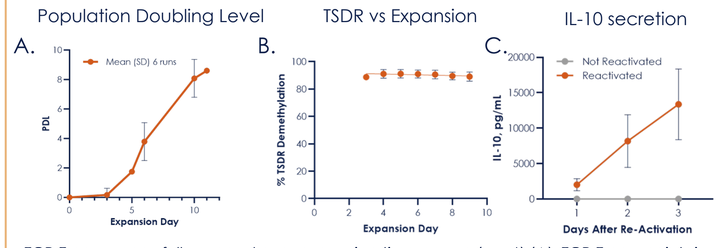
In response to antigen stimulation, TCR-Tregs functionally suppress conventional T cell activation and cytokine production.

Unlike TCR-Tregs, Induced Tregs (iTregs) Do Not Retain Treg Phenotype



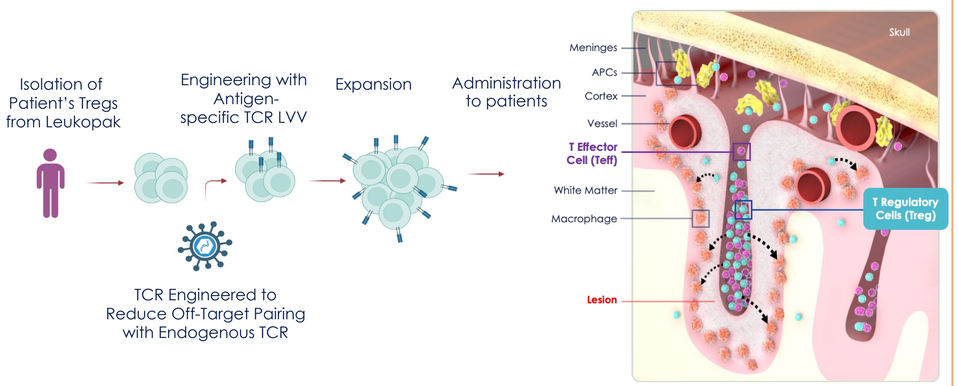
Induced Tregs (iTreg) converted to conventional T cell phenotype after 5 days with/without cytokine stimulation, with TSDR highly methylated.

Success in Manufacturing Natural, Stable Tregs



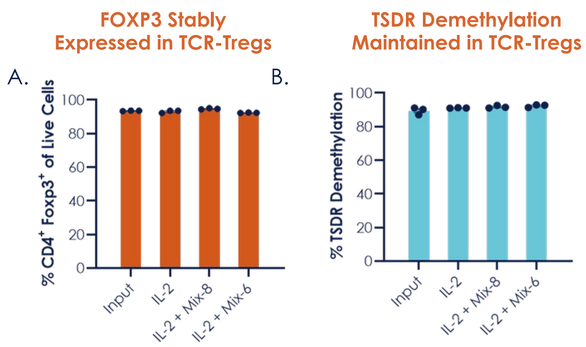
TCR-Tregs successfully expand over expansion time course (n = 6) (A). TCR-Tregs maintain high purity and stability over expansion (n = 6) (B). Post-thawed TCR-Tregs functionally produce IL-10 post activation (n = 6) (C).

Autologous TCR-Engineered Treg to Treat Progressive MS



TCR-Tregs designed to infiltrate CNS targeted inflammation by antigen specific activation

TCR-Tregs Remain Stable Under Inflammatory Conditions



TCR-Tregs maintain high levels of FOXP3 expression (> 93.1 ± 2.6 %) (A) and high levels of TSDR demethylation (> 91.0 ± 8.0 %) after five days in *in vitro* cell culture with IL-2 alone, or IL-2 plus inflammatory cytokine mixes.

Summary

A TCR-engineered Treg therapy is designed for the treatment of progressive MS (P-MS).

- TCRs specifically reactive to MBP antigen were screened and selected for engineering TCR-Tregs.
- TCR-Tregs displayed suppressive activity against conventional T cell activation and cytokine expression, upon exposure to MBP peptide/MHC.
- TCR-Tregs remained stable, unlike iTregs, by maintaining Foxp3 expression and high TSDR demethylation upon exposure to inflammatory cytokine mixes.
- TCR-Tregs can be manufactured at high purity, stability, and functionality for both healthy donors and MS patients.

Disclosure: This work was supported by Abata Therapeutics, a for-profit company dedicated to translating the biology of Regulatory T cells (Tregs) into transformational medicines for patients with autoimmune diseases. Juliana Barrios is an employee and shareholder in Abata Therapeutics.