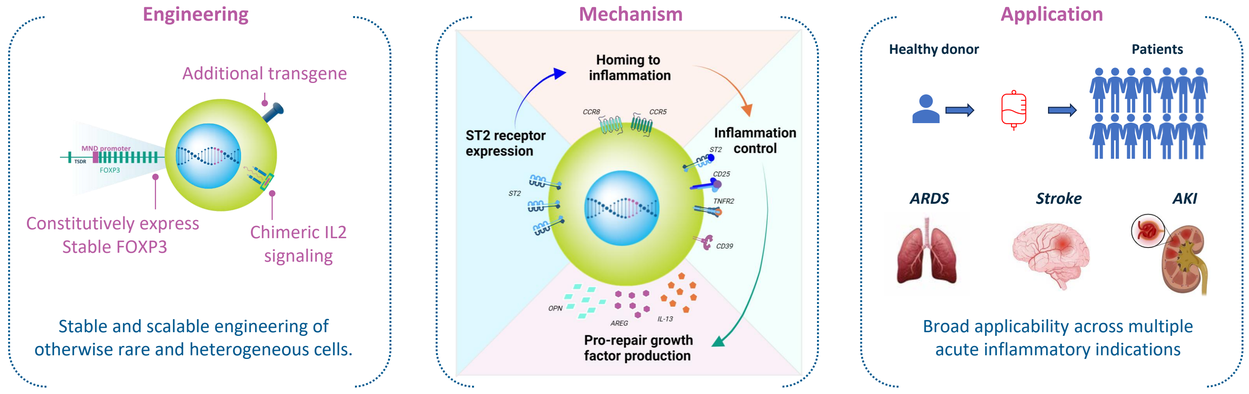
Allogeneic Tissue Engineered Tregs for the Treatment of Ischemic Stroke and Neurodegenerative Diseases

Maegan Hoover, Priya Saikumar, Yash Agarwal, Madison Milaszewski, Nicole Reed, Carlos E Frias, Abigail Doherty, Dalia Gaddis, Marko Repic, Xiaoming Hu, Jun Chen, Tiffany F Chen, Thomas Wickham, Payam Zarin

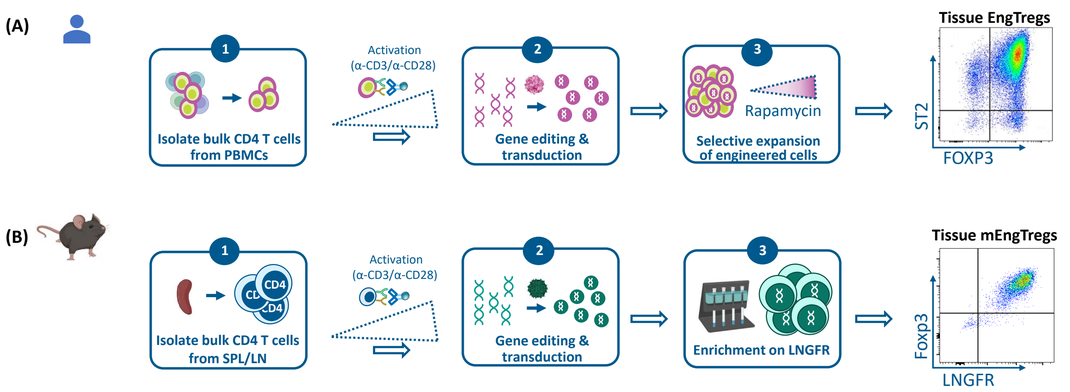
GentiBio, Inc., Cambridge, MA, USA

Premise



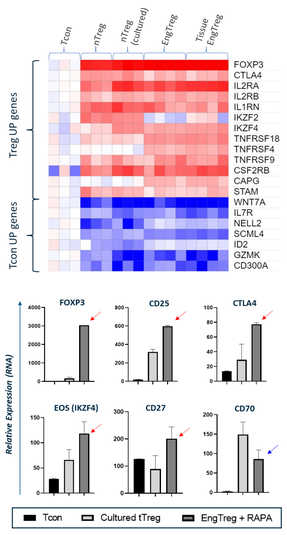
Human Engineered Tissue Tregs as a therapeutic approach for acute inflammatory and ischemic diseases. Panel 1: Gene edited CD4⁺ T cells express stable FOXP3 and a rapamycin-activated signaling complex (CISC) that provides tunable IL-2 signal, divorcing FOXP3 expression from existing regulatory elements known to promote Treg instability under inflammatory conditions (Honaker S, *Science Translational Medicine*, 2020, Cook P, *Molecular Therapy*, 2023). Panel 2: Expression of further transgenes, such as the IL-33 receptor ST2, enables effective tissue homing and enhanced expression of programs associated with activation, function, and repair in response to inflammatory signals. Panel 3: Scalable manufacturing of an allogeneic cell product from healthy donors would allow for an off-the-shelf treatment applicable across a broad range of acute inflammatory and ischemic diseases often associated with poor prognosis.

Engineered Treg Generation

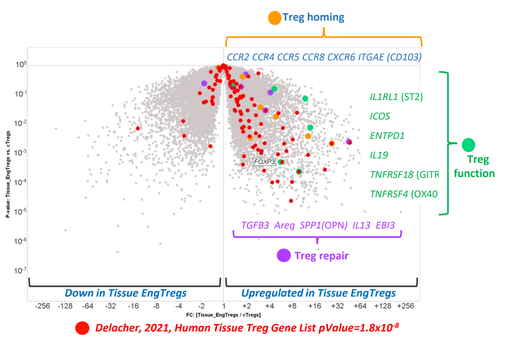


(A) Human or **(B)** murine CD4+ T cells are isolated and activated via α-CD3/α-CD28 microbeads (Panel 1). Cells are genetically modified using lentiviral transduction and/or CRISPR-Cas9 mediated knock-in of transgenes delivered by AAV vectors (Panel 2). For human EngTregs, CISC receptor expression enables selective expansion of edited cells, while mouse surrogate EngTregs rely on enrichment by the extracellular LNGFR tag (Panel 3).

1. Enrichment of Tissue Treg gene signature in Tissue EngTregs

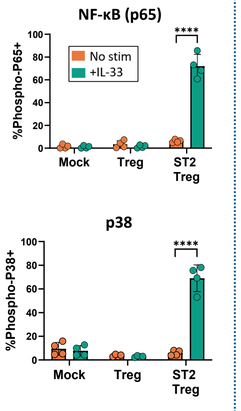


✓ **Tissue EngTregs express core Treg genes by transcriptome profiling (left) and resemble tissue Tregs by bulk RNAseq (volcano plot)**

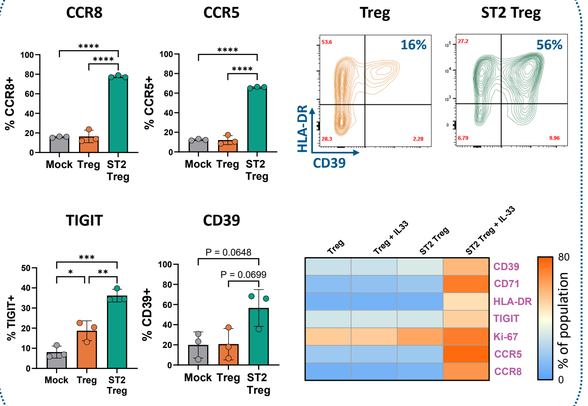


2. Tissue EngTregs signal through ST2 and express higher levels of homing, activation, and tolerogenic markers

✓ **ST2 Signaling**

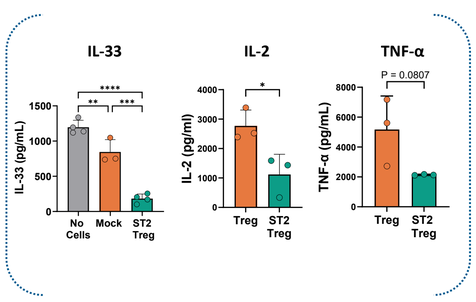


✓ **Tissue Treg Phenotype**

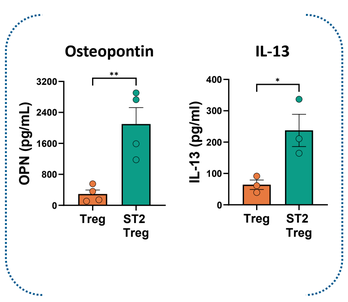


3. Tissue EngTregs show enhanced response to inflammatory cytokines and express repair mediating factors

✓ **Clearance of inflammatory cytokines**

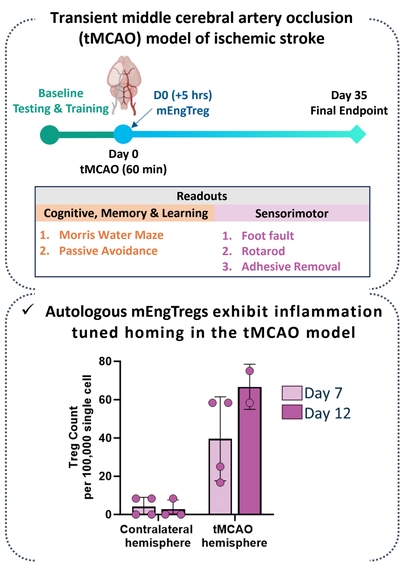


✓ **Production of pro-repair factors**

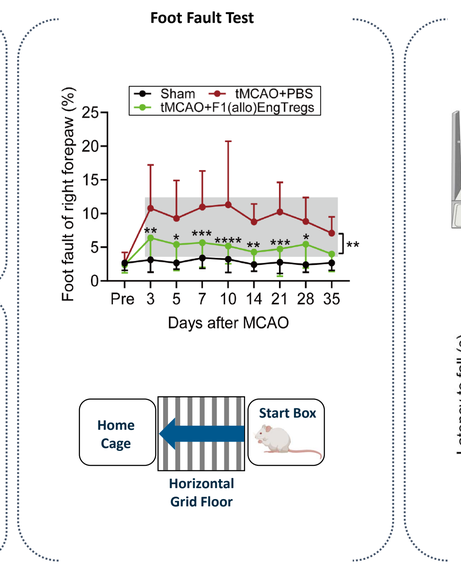


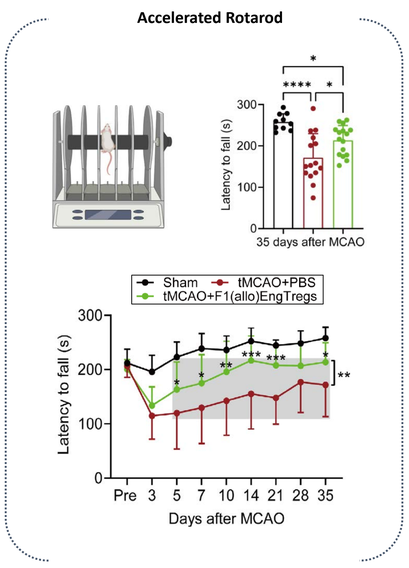
4. Allogeneic Tissue mEngTregs significantly improve sensorimotor recovery and cognitive readouts in the tMCAO model of ischemic stroke

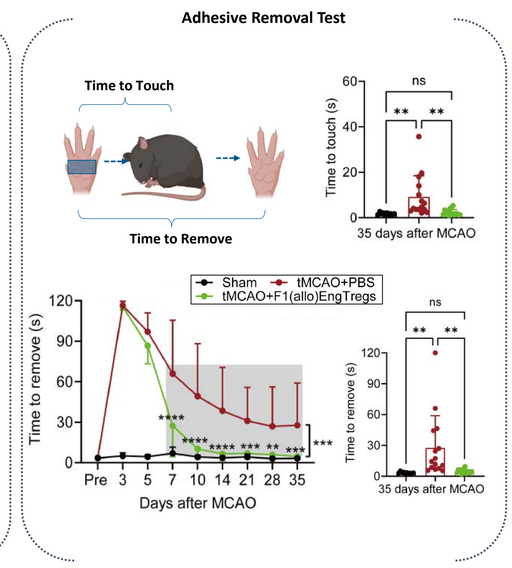
✓ **Improved sensorimotor readouts in allogeneic mEngTreg treated tMCAO animals**



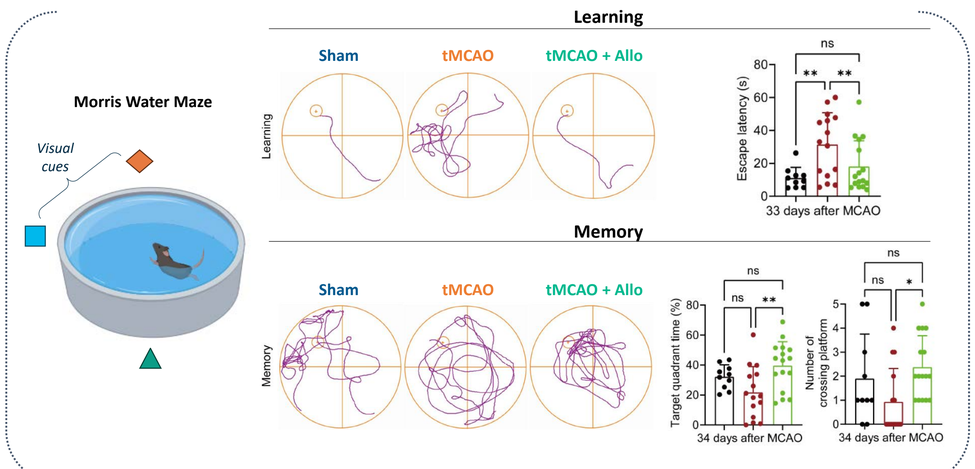
✓ **Autologous mEngTregs exhibit inflammation tuned homing in the tMCAO model**

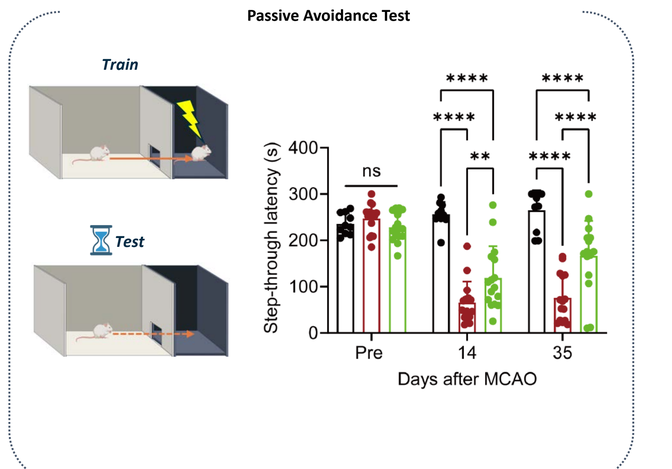






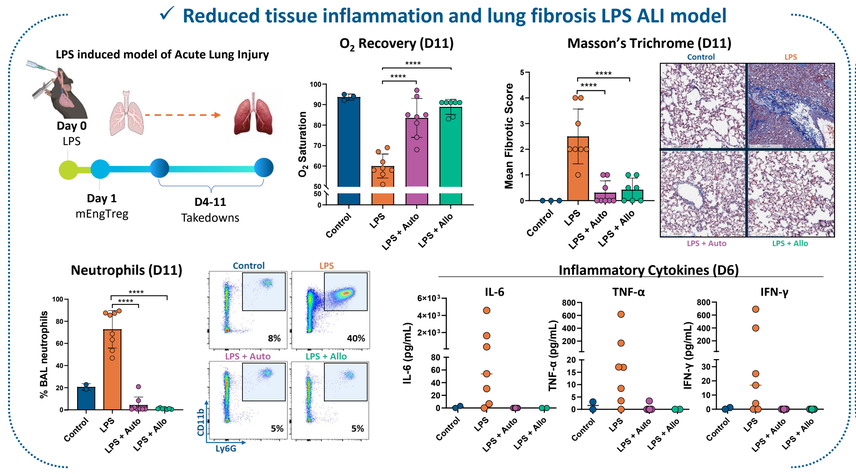
✓ **Improved cognitive and memory readouts in allogeneic mEngTreg treated tMCAO animals**



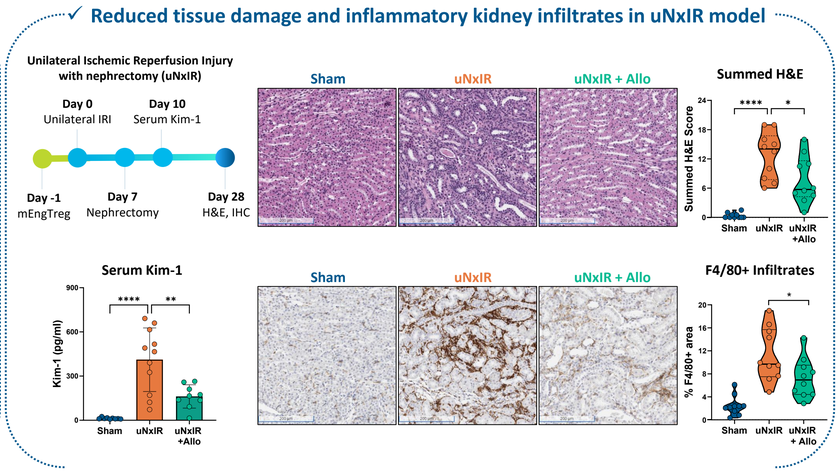


5. Tissue mEngTregs reduce inflammatory infiltrates and cytokines and improve histopathology in additional models of acute inflammation

✓ **Reduced tissue inflammation and lung fibrosis LPS ALI model**



✓ **Reduced tissue damage and inflammatory kidney infiltrates in uNxIR model**



CONCLUSIONS

- GentiBio's Engineered Treg platform overcomes scaling and stability limitations of Treg therapeutics by starting with more abundant T cell sources and enriching FOXP3+ edited cells with an engineered IL-2 signaling receptor.
- Additional ST2 engineering bestows Tissue EngTregs with a pro-repair phenotype, expressing greater levels of Treg activation markers, tissue homing receptors, and tolerogenic factors.
- In preclinical studies of ischemic stroke, allogeneic murine Tissue EngTregs demonstrate potent efficacy as measured by sensorimotor and cognitive readouts.
- In additional mouse models of acute inflammation, Tissue mEngTregs drive efficacy by reducing local inflammation, limiting cellular infiltration, and improving overall tissue integrity.
- Improved lung fibrotic scores in the later stage of disease in Tissue mEngTreg treated ALI mice suggests potential mEngTreg driven protection against downstream fibrosis following acute tissue injury.
- These data support the use of allogeneic CD4 derived Engineered Tregs as a powerful off-the-shelf therapeutic approach for acute onset inflammatory and ischemic diseases including ARDS, AKI, and ischemic stroke.

ACKNOWLEDGEMENTS: The laboratory of Dr. David Rawlings at Seattle Children's Hospital pioneered the gene editing approach to produce engineered Tregs. The research groups of Dr. Jason Mock and Dr. Heth Turnquist contributed scientific discussions regarding models of acute lung injury. Histology and surgical AKI methodology performed at Inotiv. The research group of Dr. Gregory Bix contributed scientific discussions regarding stroke models. tMCAO modeling performed in the laboratory of Dr. Jun Chen.

*, **, ***, **** = p-value < 0.01, 0.005, 0.001 and 0.0001 respectively.

We make Tregs. *Better.*