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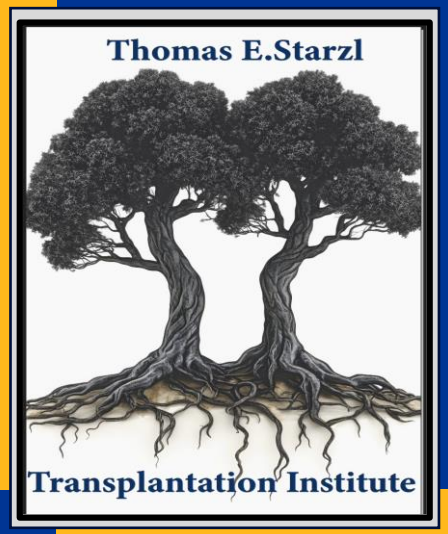
Off-the-Shelf Reparative Regulatory T Cell Therapy Has Clinical Potential for Resolution of Damage following Acute Lung Injury



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Disclosures

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Learning Objectives:

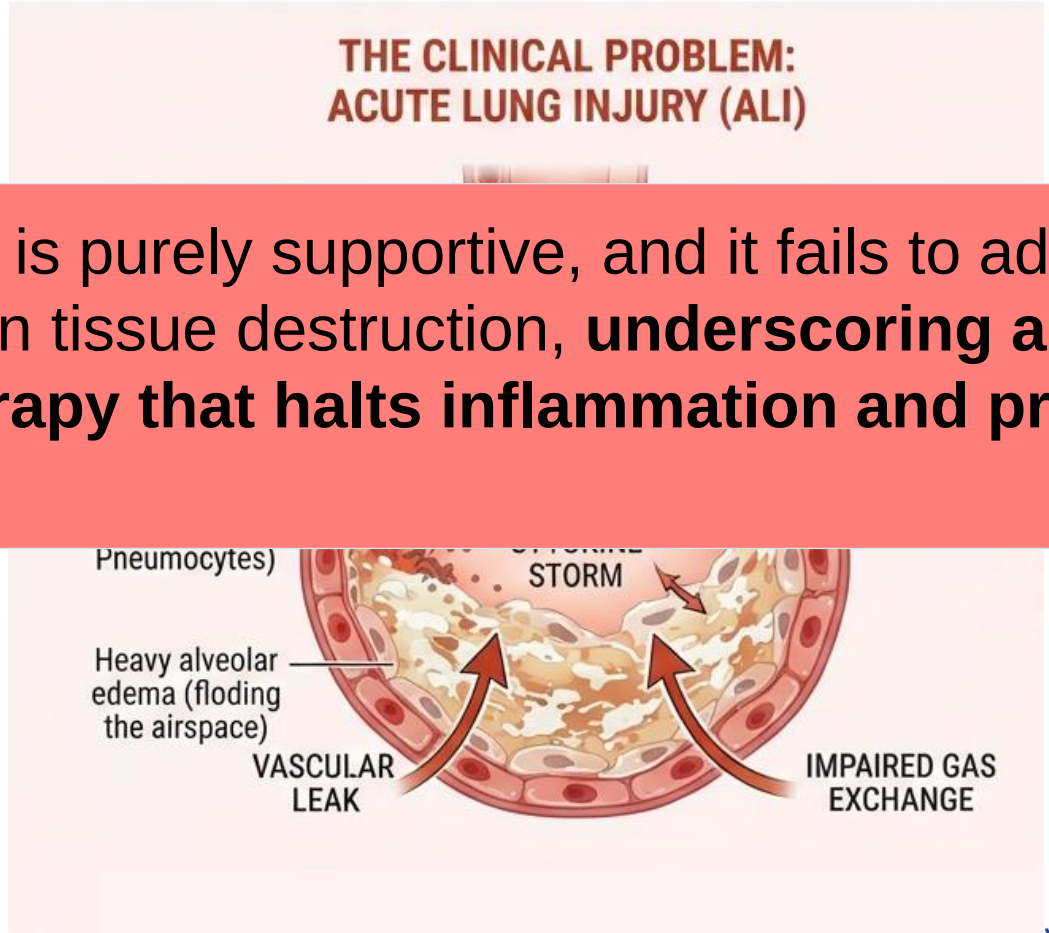
- **Describe** the reparative mechanisms of IL-33-stimulated Treg, including ST2-driven amphiregulin and IL-13 secretion, and how these differ from conventional IL-2-expanded Treg.
- **Analyze** preclinical efficacy data comparing cryopreserved allogeneic and syngeneic IL-33-expanded Treg in ALI, including engraftment, alveolar macrophage preservation, and morbidity outcomes.
- **Discuss** the translational pathway for IL-33-expanded "off-the-shelf" Reparative Treg Immunotherapy as a treatment for military-relevant acute lung injury, including cell engineering strategies targeting ST2 expression.

Clinical Problem: Lack of effective therapies targeting underlying immunopathology in Acute Lung Injury (ALI)

Lung Injury:

- I
- T
- P
- P

Current standard-of-care is purely supportive, and it fails to address the underlying immune-driven tissue destruction, **underscoring an urgent need for a targeted therapy that halts inflammation and promotes repair.**



ction

Death

High **mortality** 30-40%

Long term functional impairments
40-60% of ARDS survivors have impaired lung function

Understand endogenous regulatory mechanisms controlling early inflammation and the shift towards tissue repair

If effective therapies can:

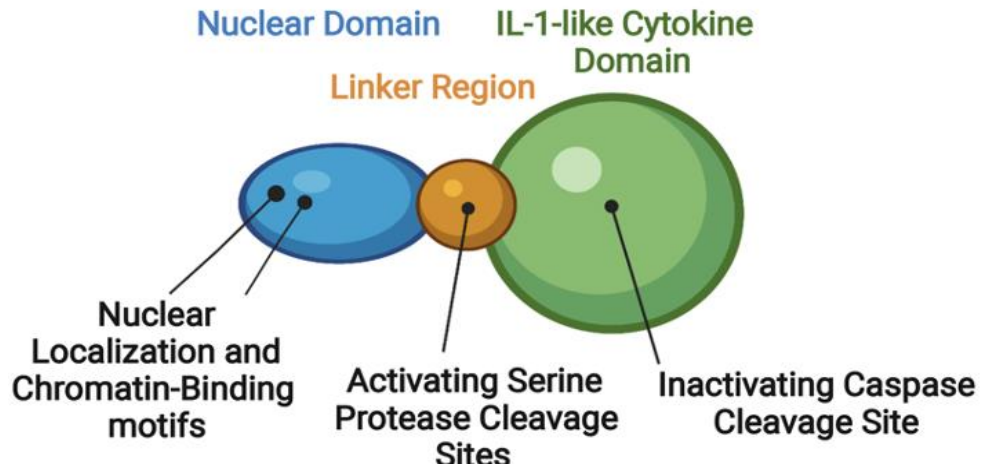
- Limit inflammation
- Rapidly resolve damage

Then outcomes will include:

- Limit morbidity
- Improve Tissue Function
- Reduction in systemic immunosuppression

Figure created with the assistance of generative AI technology.

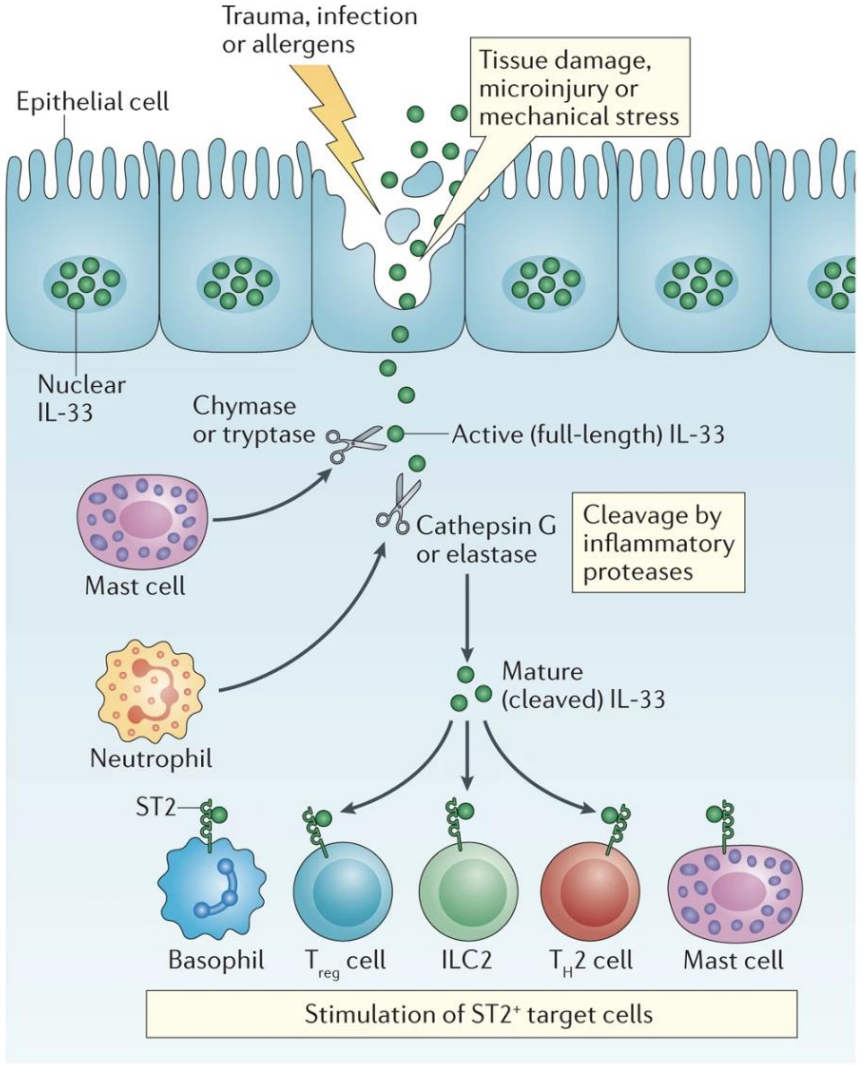
Alarmin IL-33 is an important injury signal



Member of the IL-1 super family

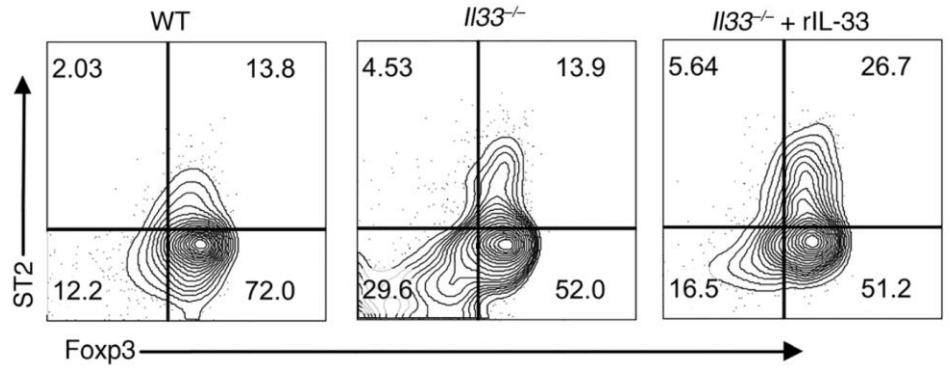
Classified as a **DAMP** (Damage-Associated Molecular Pattern)

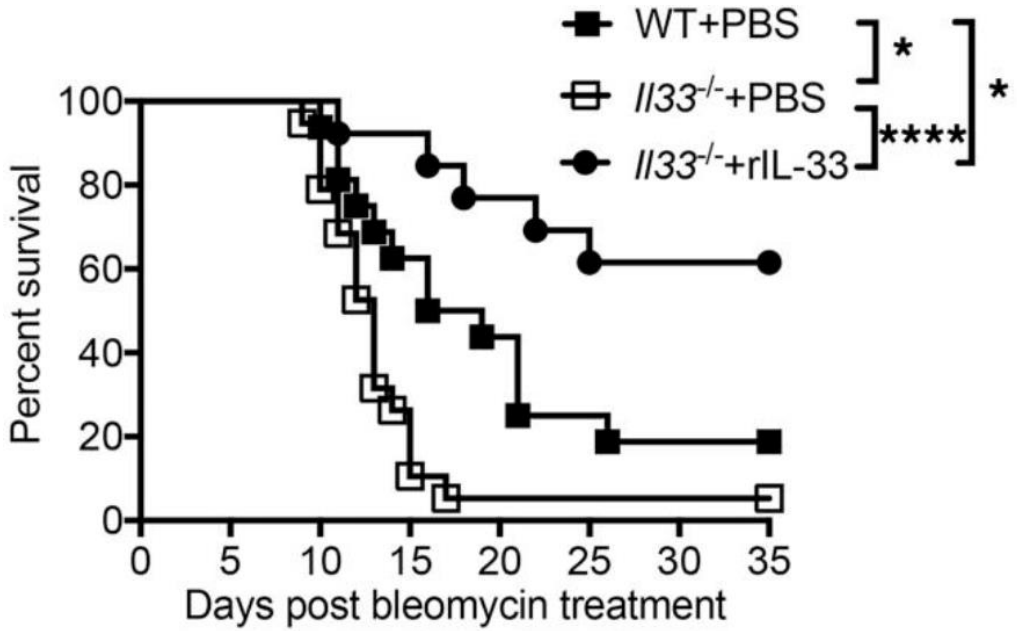
Sequestered in the nucleus of stromal cells

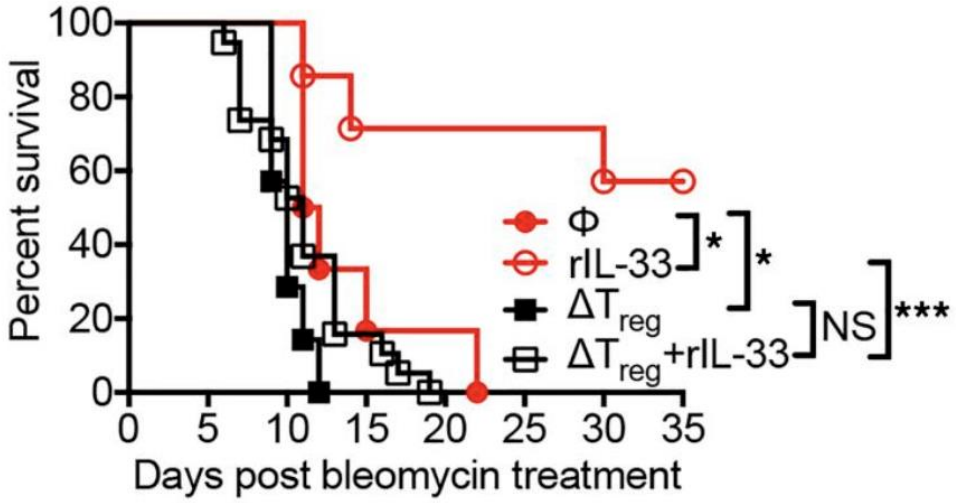


Dwyer *et al., Annual Rev. Immunol.* 2022
Liew, F., Girard, JP. & Turnquist, *Nat Rev Immunol*. 2016

IL-33 and Tregs are required to prevent mortality after chemical lung injury







Lui et al. *JCI Insight.* 2019

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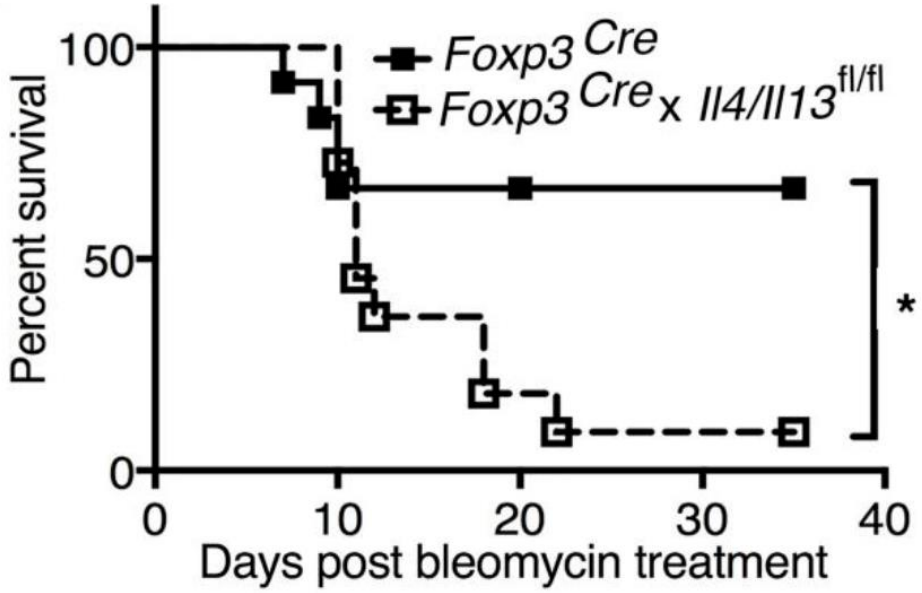
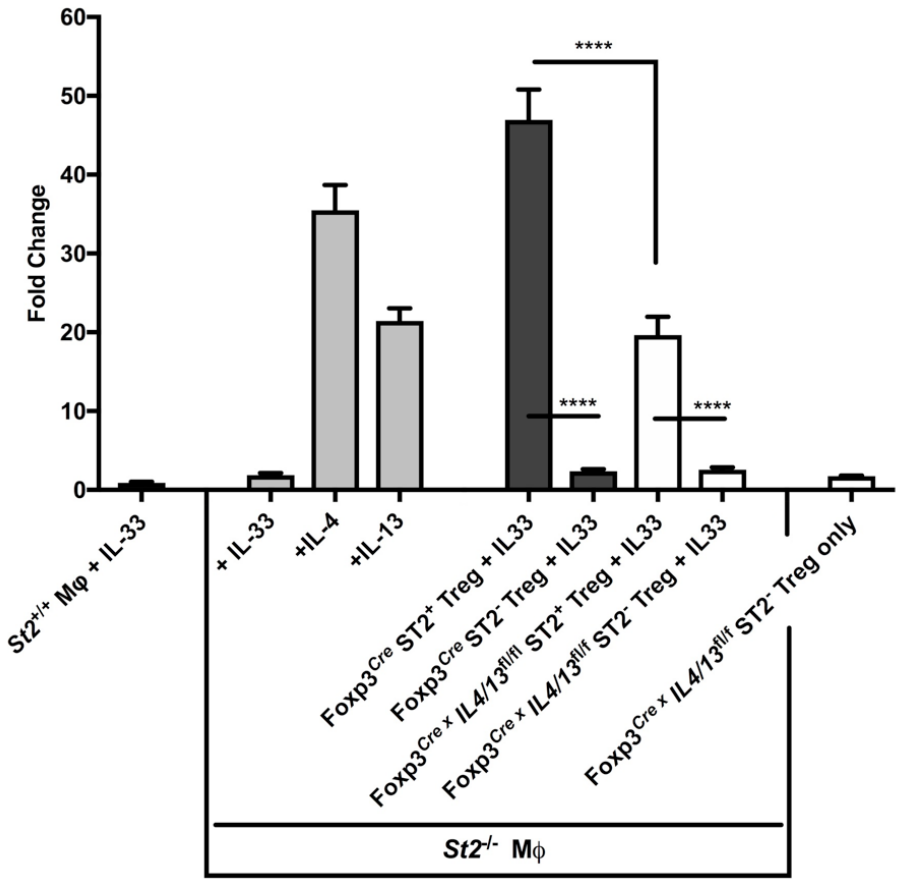
Treg derived IL-13 prevents mortality in chemical lung injury

IL-33 → ST2⁺ Treg → IL-13





Lui et al. *JCI Insight.* 2019

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Treg derived Amphiregulin (Areg) limits tissue damage

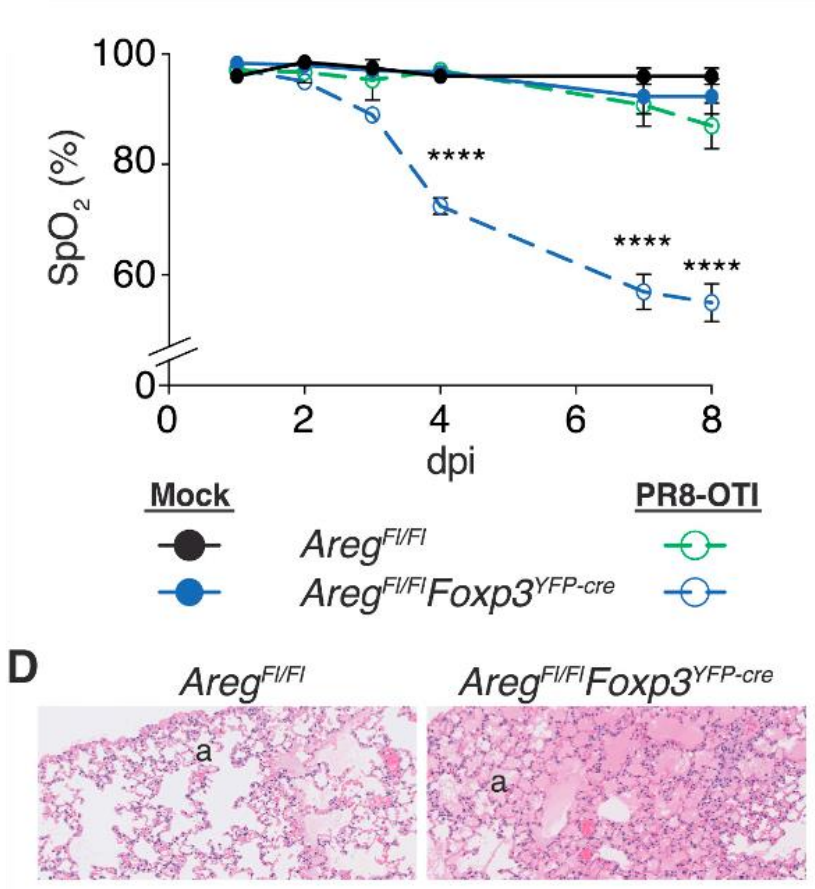
Areg

↓

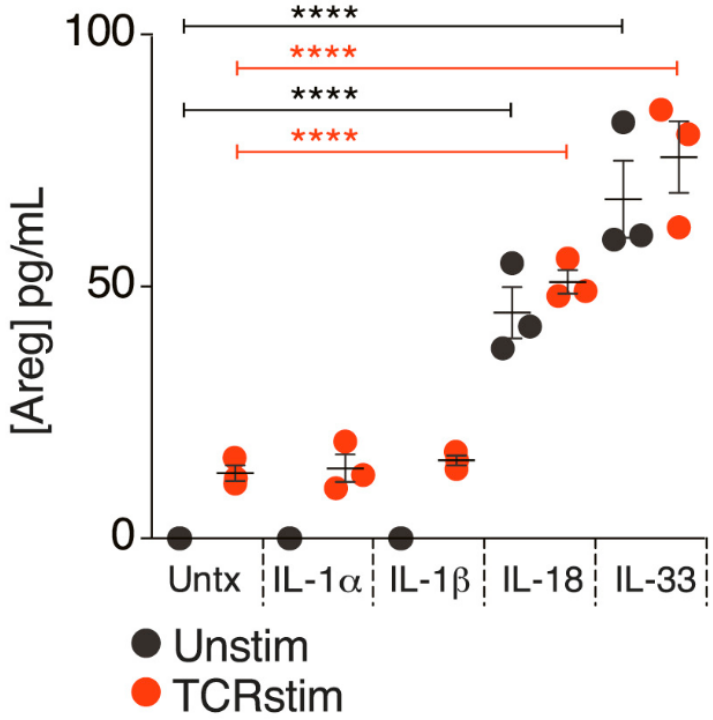
EGFR

↓

Proliferation of Stem cells, and fibroblasts; **Epithelial Tissue repair**



Treg cell production of amphiregulin is IL-33 dependent



Arpaia et al. *Cell.* 2015

Reparative Tregs and Tissue Injury: The roles of IL-33, IL-13, and Amphiregulin (Areg)

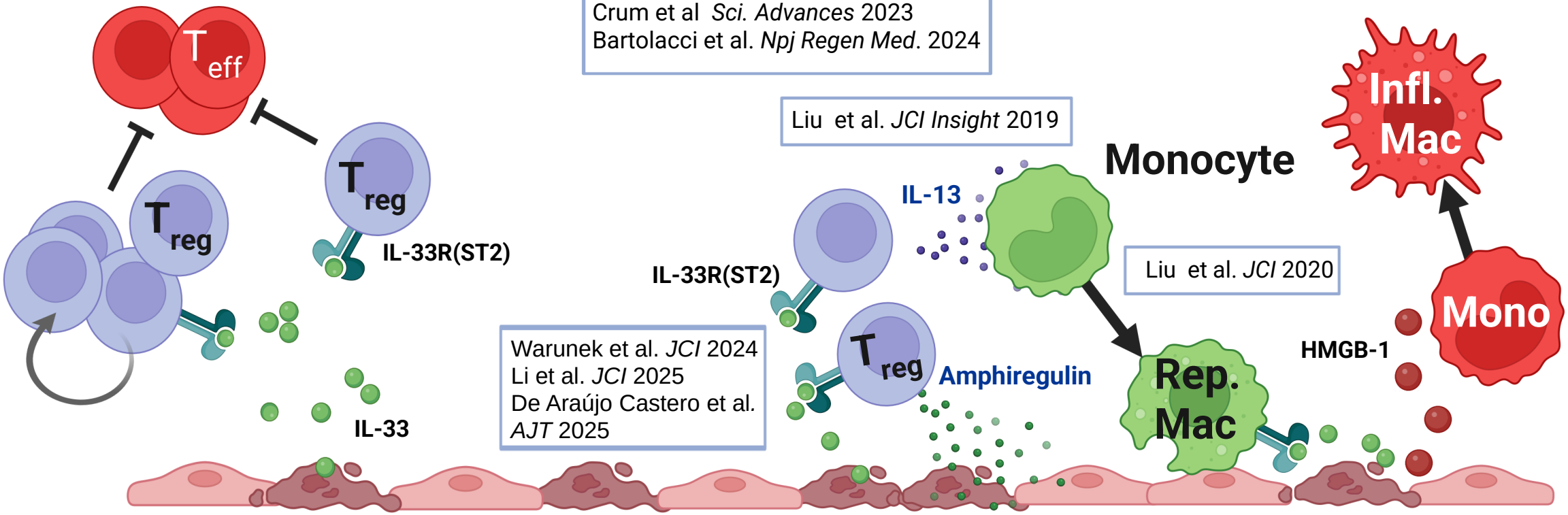
Turnquist et al. *JI* 2011
Reichenbach et al. *Blood* 2015
Matta et al. *Blood* 2016
Dwyer et al. *JCI* 2022

Hussey et al. *J. Imm. Reg. Med.* 2019
Crum et al *Sci. Advances* 2023
Bartolacci et al. *Npj Regen Med*. 2024

Liu et al. *JCI Insight* 2019

Liu et al. *JCI* 2020

Warunek et al. *JCI* 2024
Li et al. *JCI* 2025
De Araújo Castero et al. *AJT* 2025



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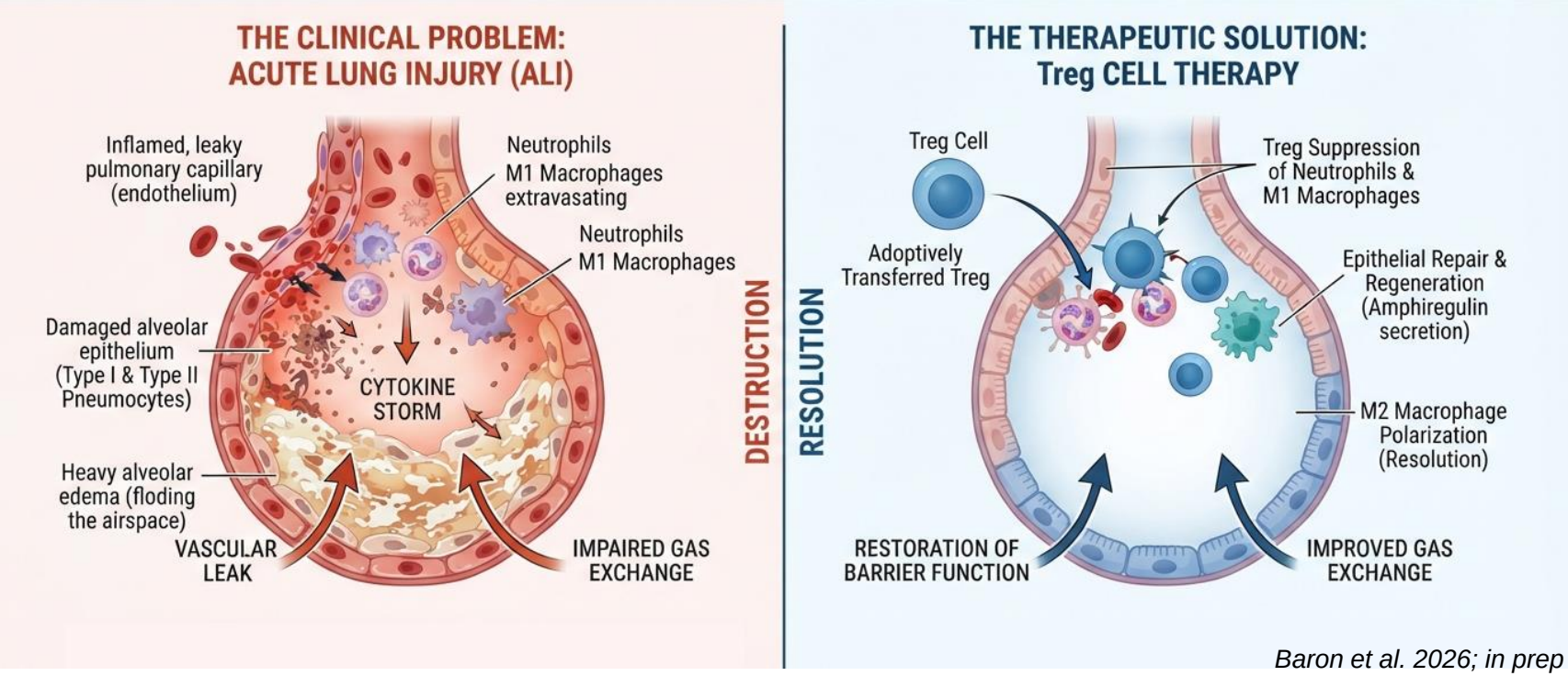
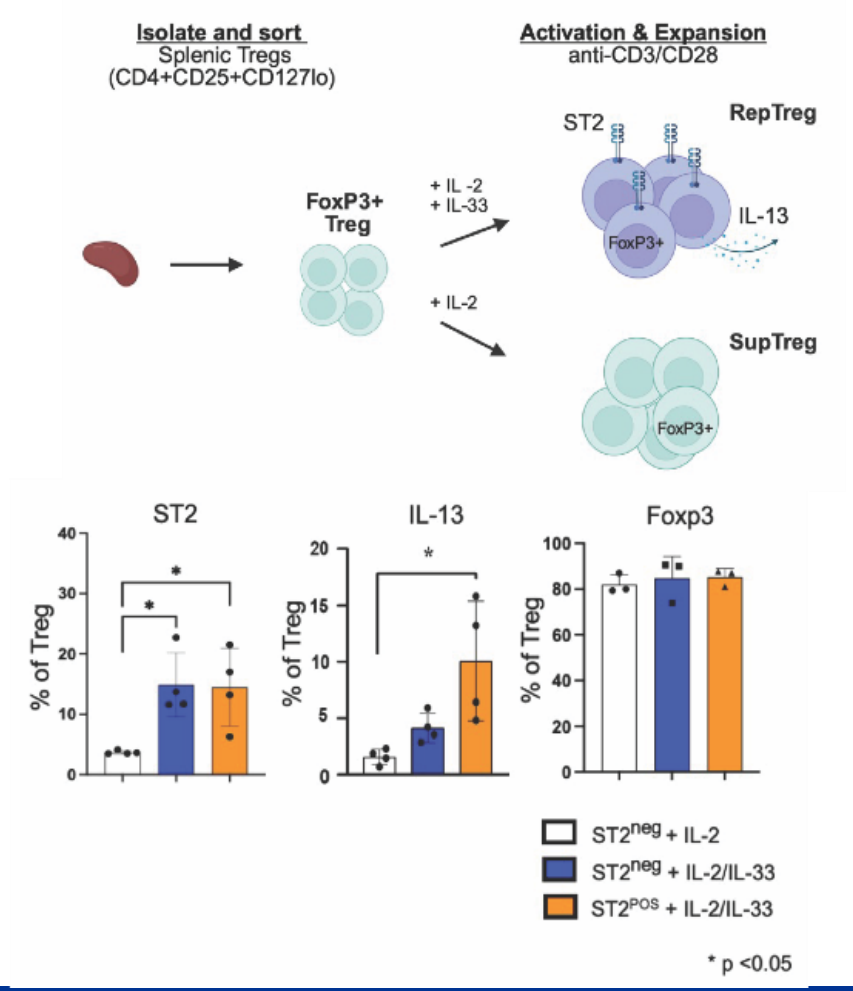
Hypothesis:

IL-33 can be used during ex vivo expansion to program Tregs to be reparative, in addition to suppressive, and effectively utilized as an "off-the-shelf" Reparative Treg Immunotherapy (RTI) delivered after ALI.

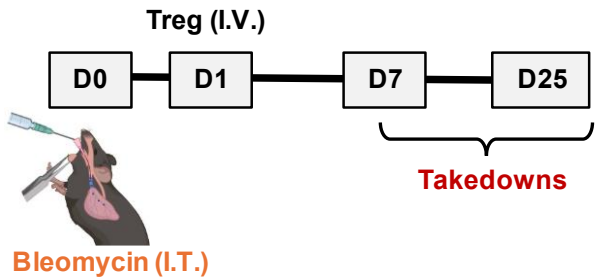


Baron et al. 2026; in prep

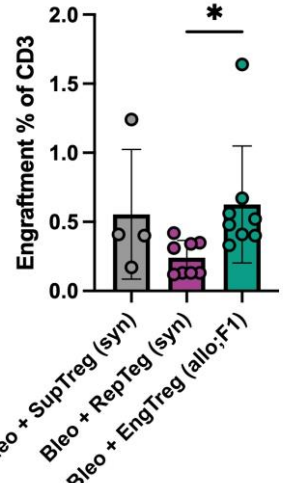
Generating Reparative Treg with IL-33: Increased ST2 and IL-13 Expression After Ex Vivo Expansion



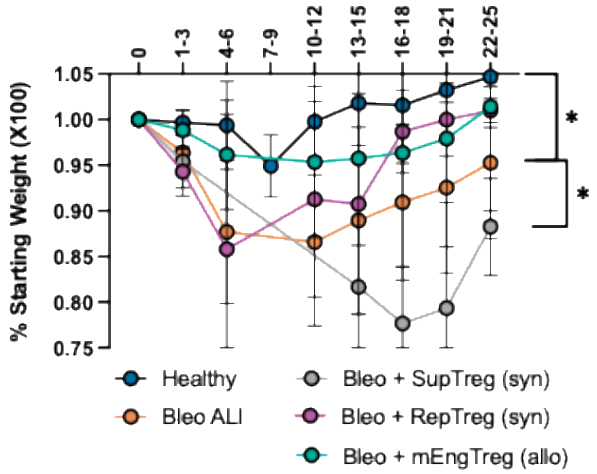
Reparative Treg Cell Therapy Protects Against Bleomycin-Induced ALI Morbidity



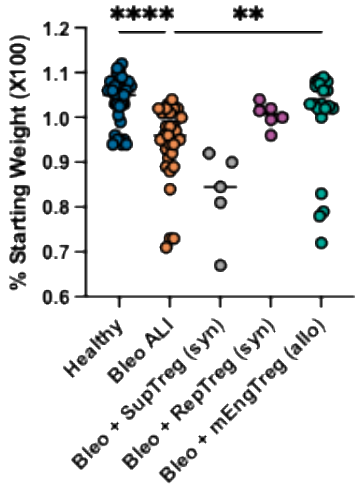
Lung Interstitium

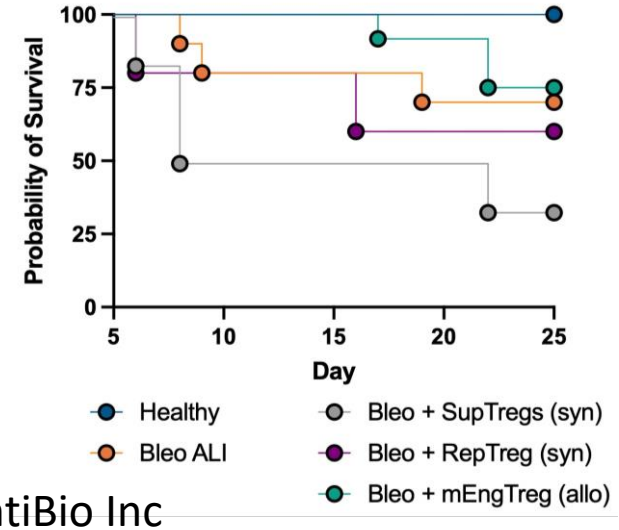


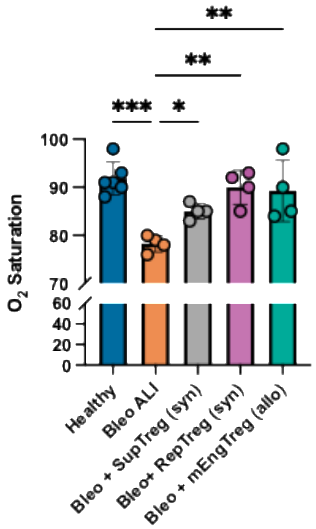
Day Post ALI



Day 22-25 Endpoint







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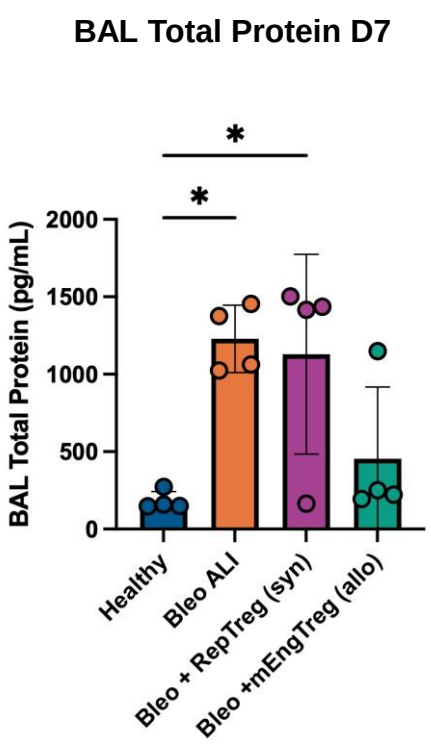
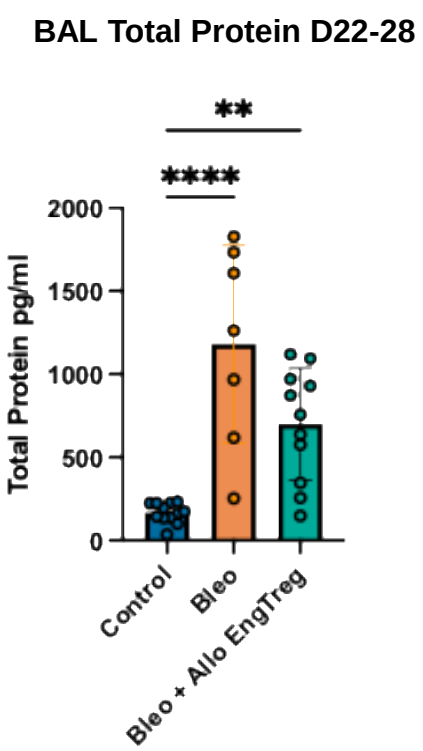
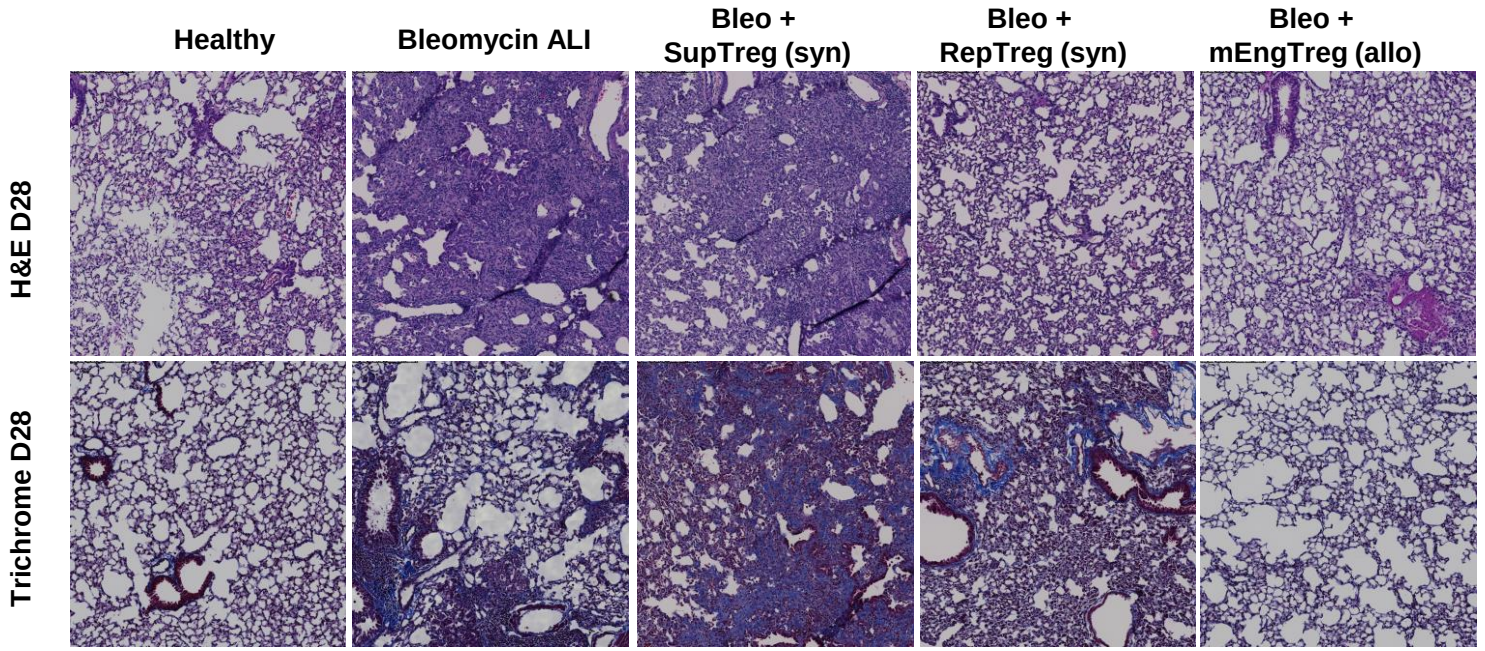
Reparative Treg Therapy Attenuates Alveolar Damage and Reduces Fibrotic Injury Following ALI







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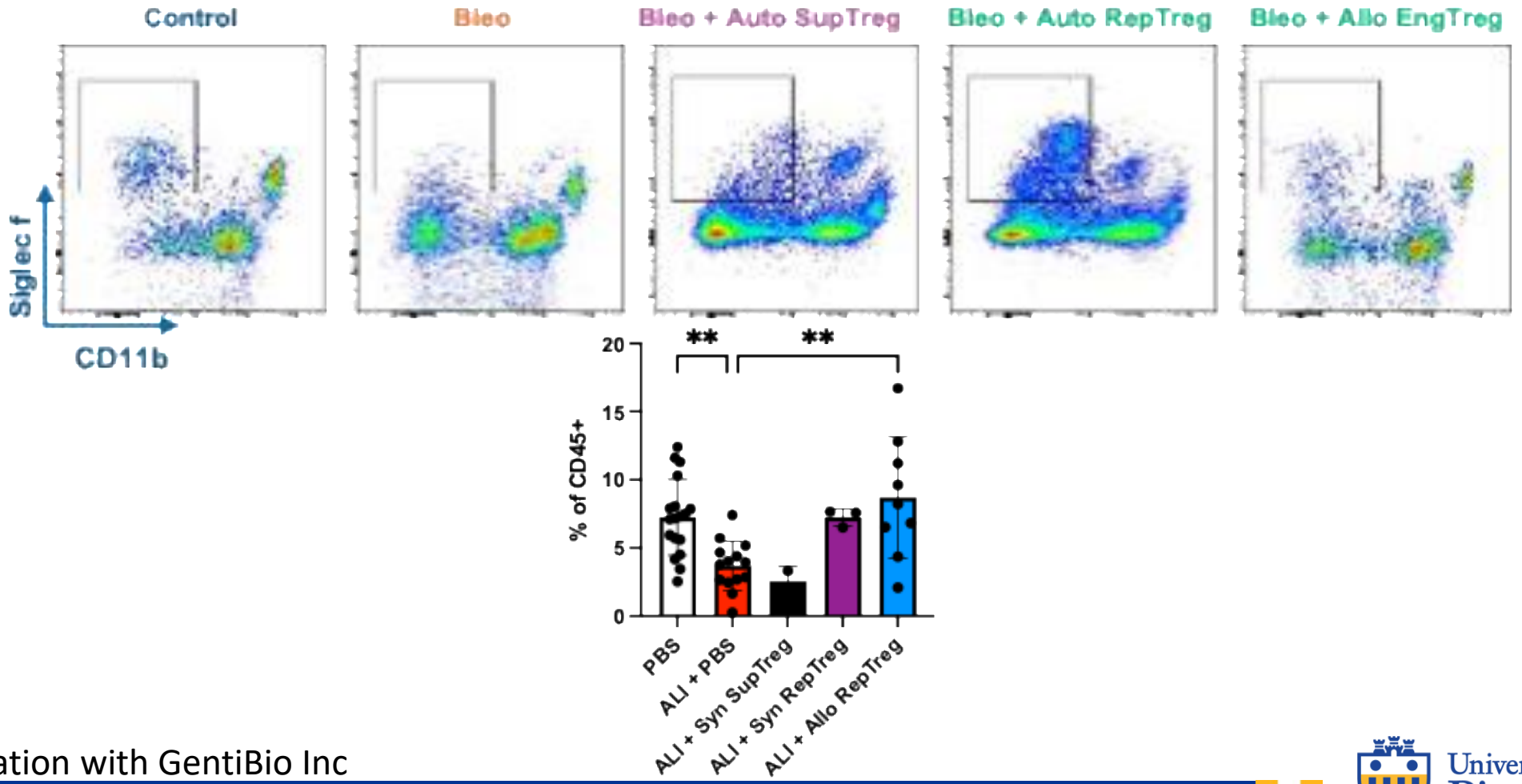
Adoptive Transfer of Syn or Allo RepTreg Protect Against Bleomycin-Induced ALI Through Modulation of Local Macrophages



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Reparative Treg Cell Therapy Protects Against Bleomycin-Induced ALI Morbidity

H1N1 induced model of Acute Lung Injury

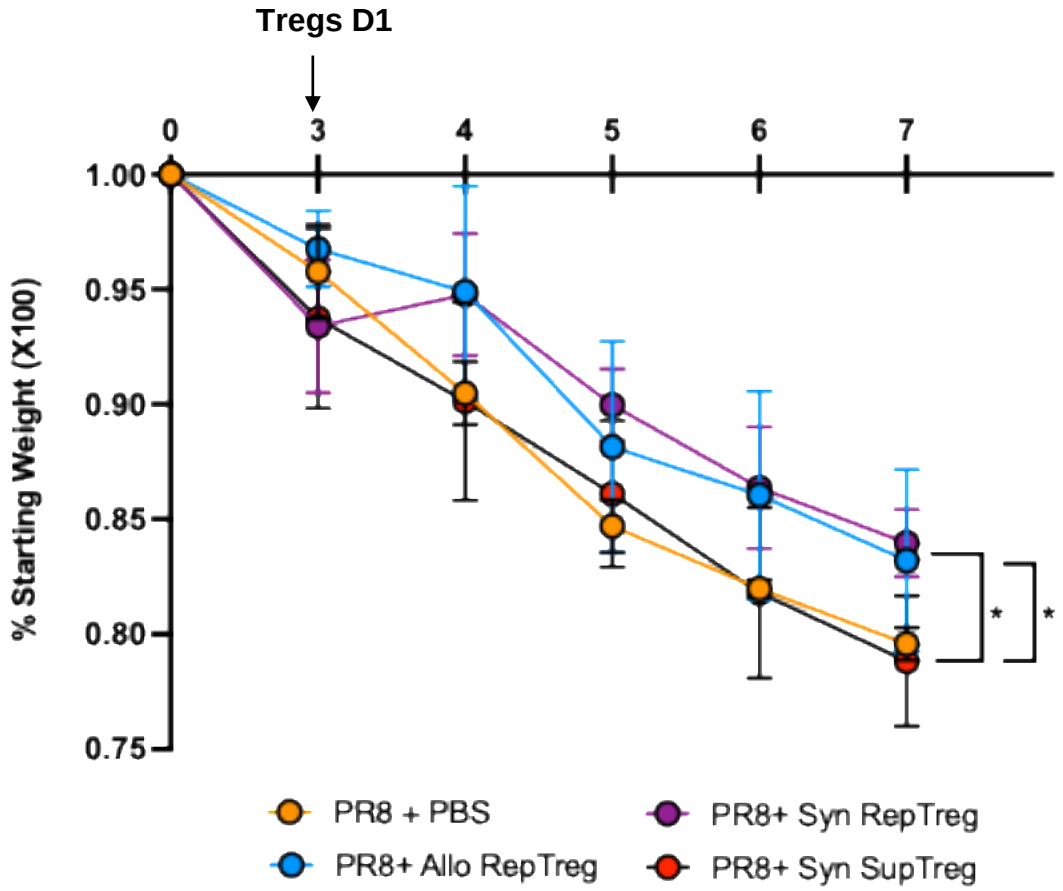
Treg (I.V.)

D0 D1 D7

P8 (I.T.)

Readouts:
Flow Cytometry, H&E, Masson's Trichrome

Days post H1N1 (PR8) Treatment



representative result from two independent experiments

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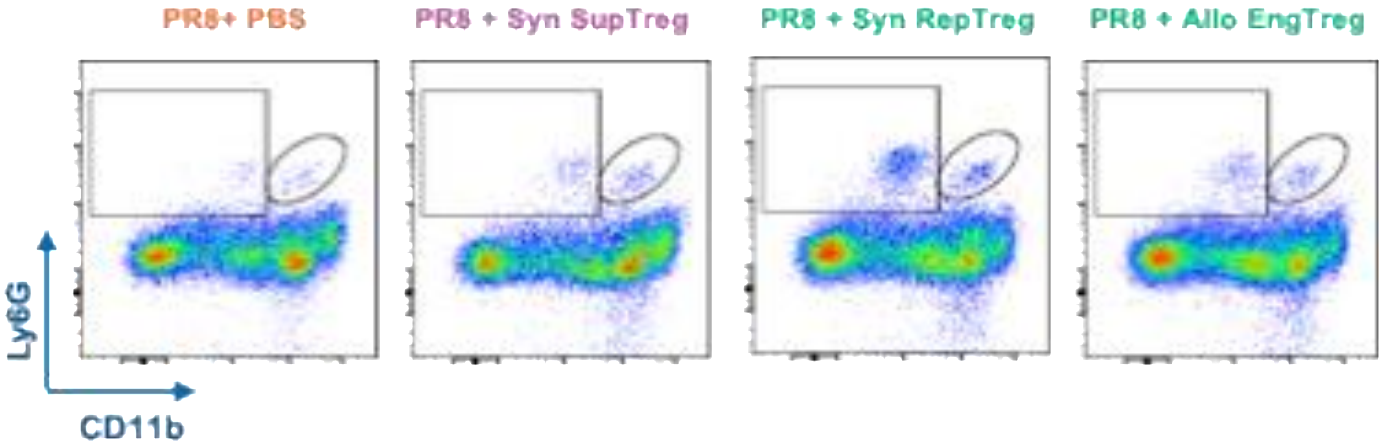
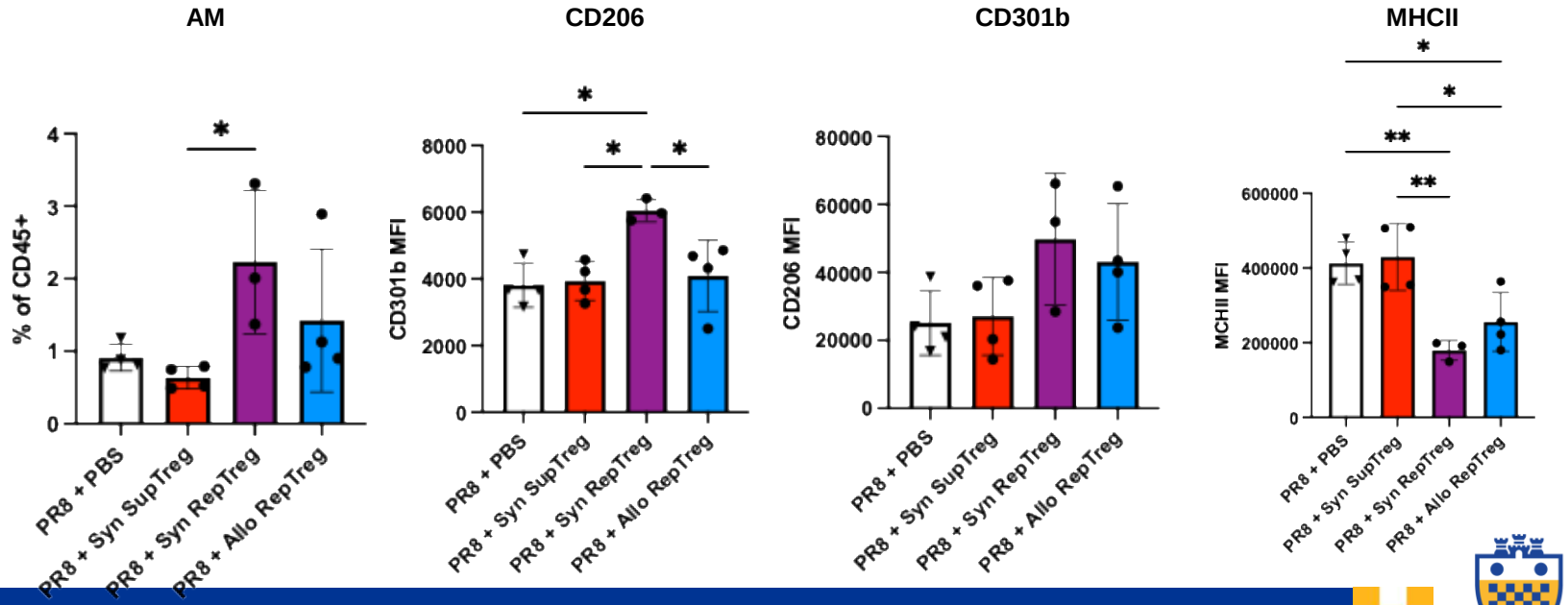
Reparative Treg Protect Against Viral ALI and Augment Local Reparative Macrophages





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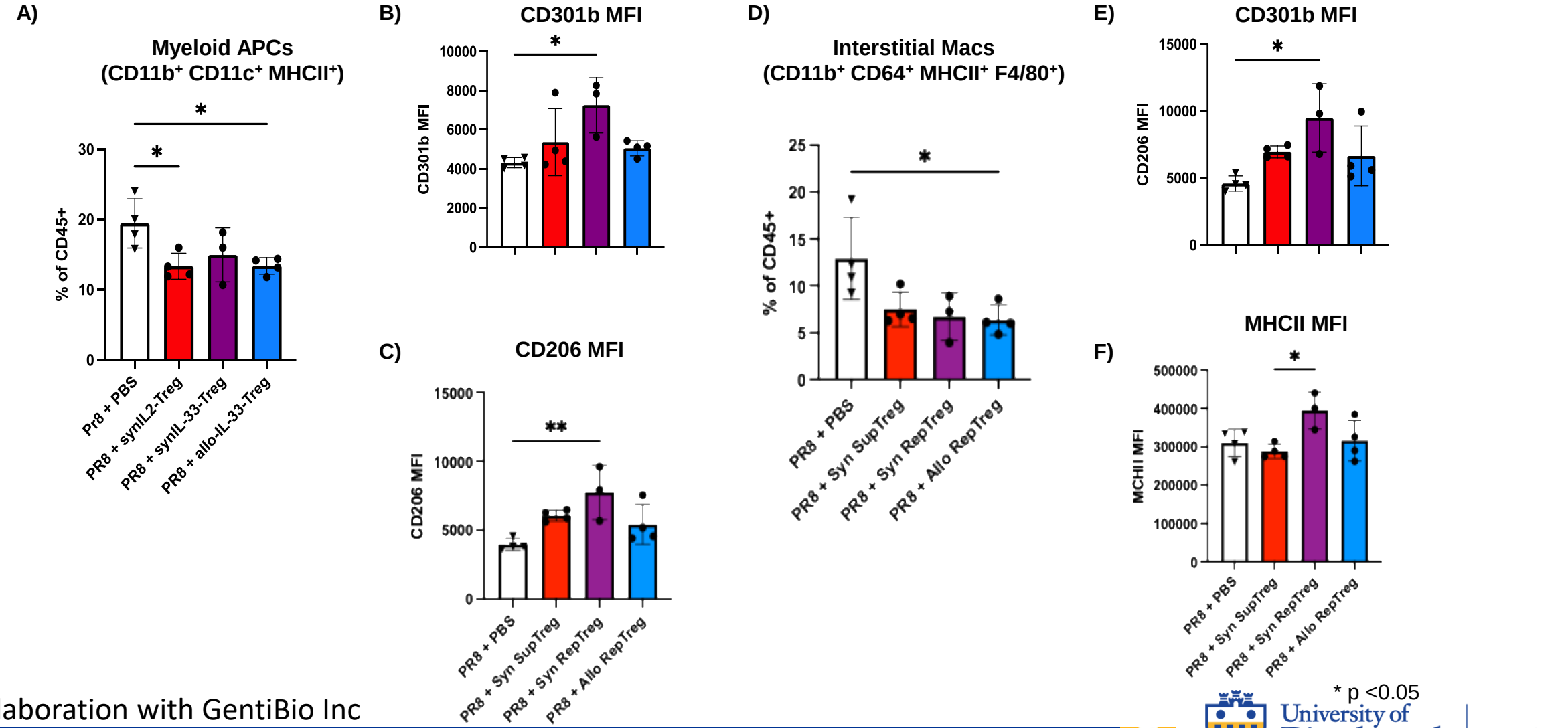
Reparative Treg Promote Reparative Macrophages Following Viral ALI



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* p <0.05

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ALI Conclusions

Treatment with Syn or Allo Reparative Treg protected against ALI morbidity compared to conventional suppressive Treg which was detrimental

Transferred syn and allo Reparative Treg are identifiable in the lungs of mice in both ALI injury models where they:

- Sustained or restored alveolar macrophages
- Promoted local reparative myeloid cells in the injured lung

These data demonstrate that Reparative Treg has the potential to be a clinically relevant therapeutic intervention for promoting tissue repair after injury

Vascular Composite Allograft Transplantation (VCA)

Transplantation of **skin, muscle, vessel, nerve, tendon, skin, and/or bone**

1998 First Human hand transplantation (*Dubernard JM et al., 1999*)

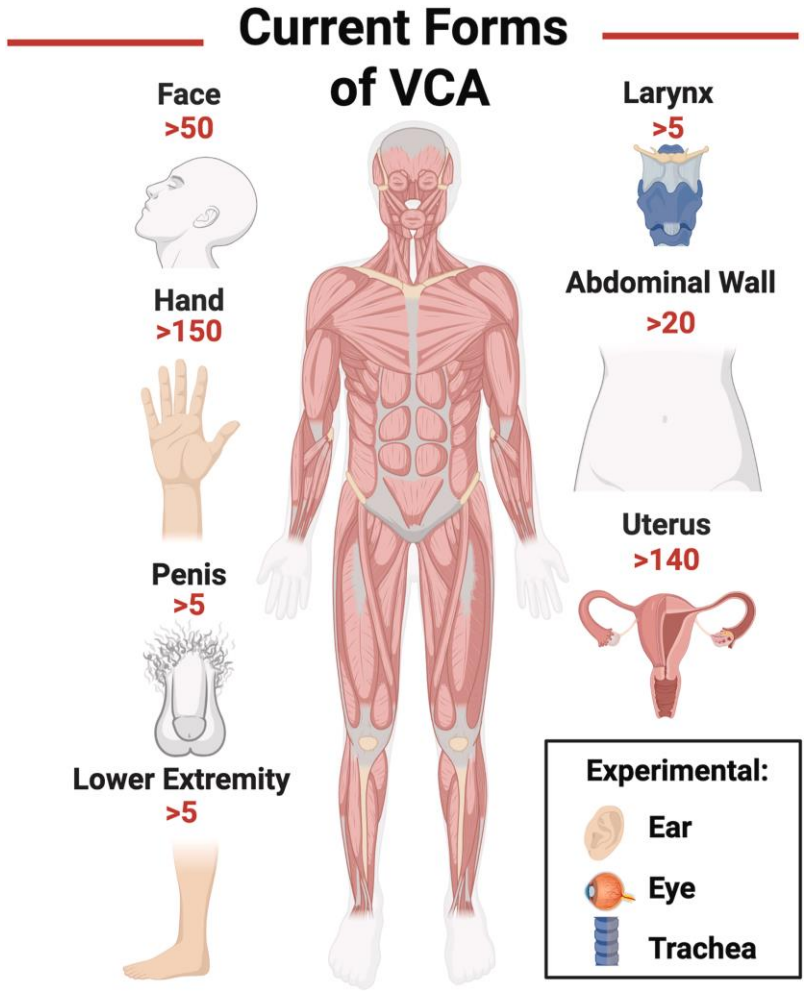
2005 First Human face transplantation (*Dubernard JM et al., 2007*)

Potential to restore appearance, tissue/limb function, quality of life

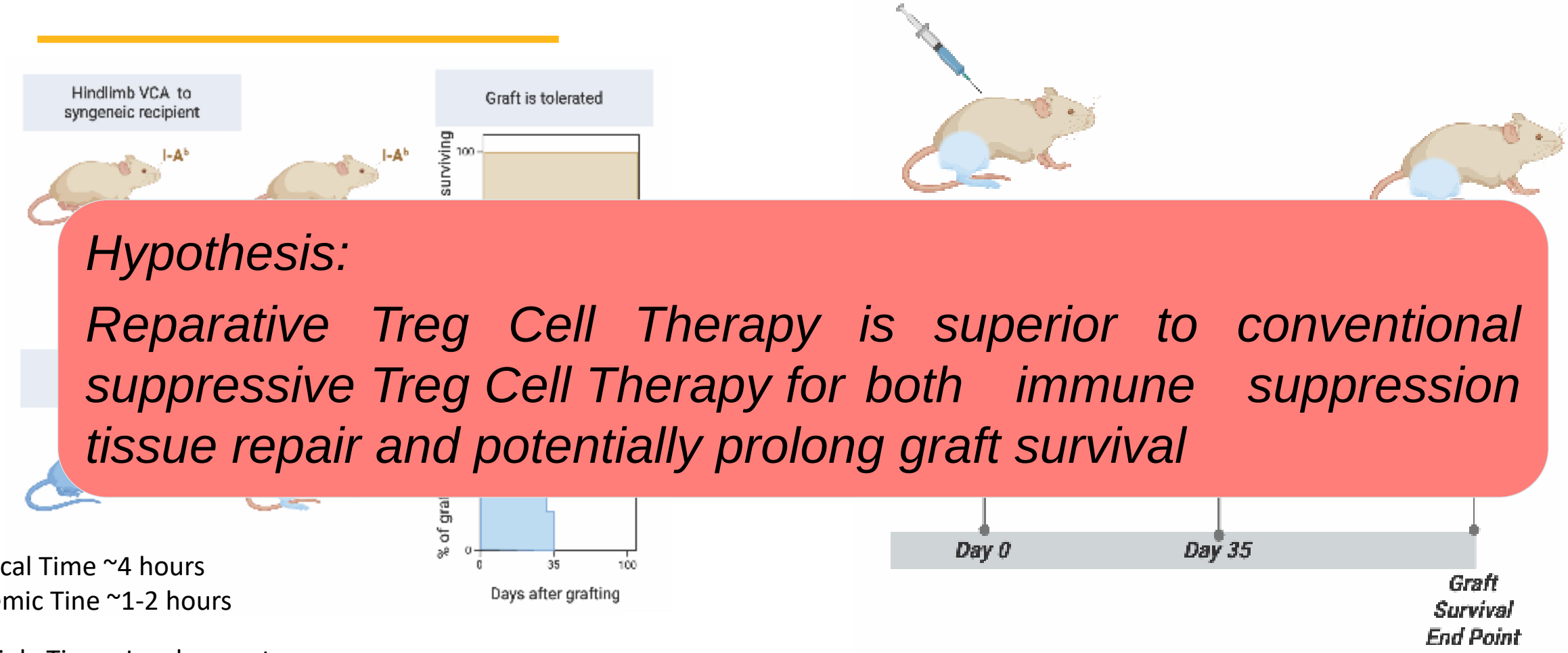
Requires lifelong multidrug immunosuppression that have severe toxic side effects

85-90% of current hand transplant recipients still experience at least 1 episode of acute rejection during the first year

Vasculopathy and muscle atrophy



Rodent hindlimb VCA model



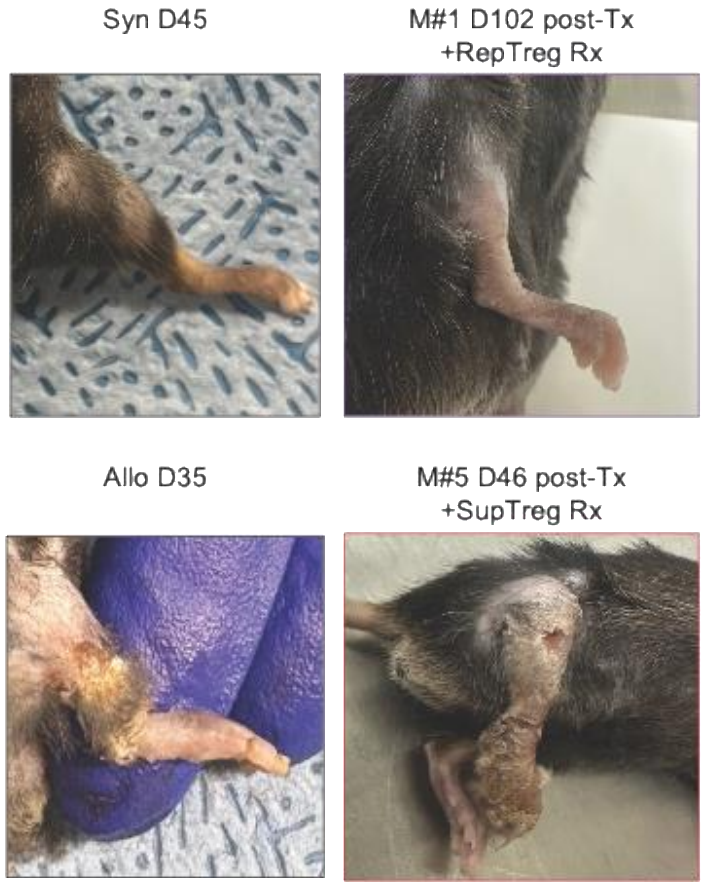
Hypothesis:

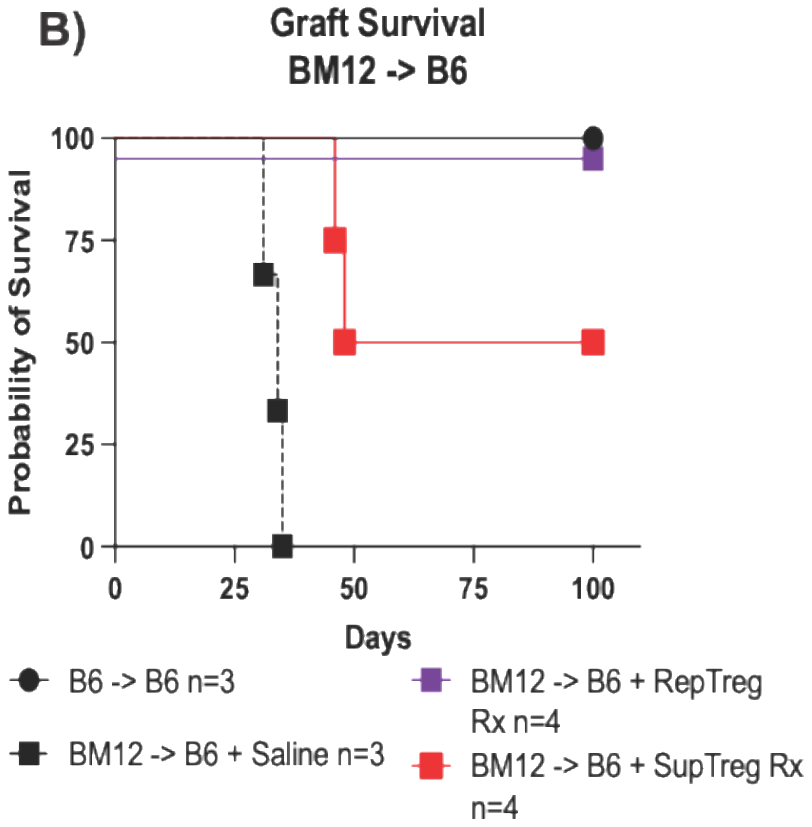
Reparative Treg Cell Therapy is superior to conventional suppressive Treg Cell Therapy for both immune suppression tissue repair and potentially prolong graft survival

Surgical Time ~4 hours
Ischemic Tine ~1-2 hours

Multiple Tissue Involvement:
- Skin
- Vascular
- Muscle

Reparative Treg Therapy Prolongs VCA graft survival

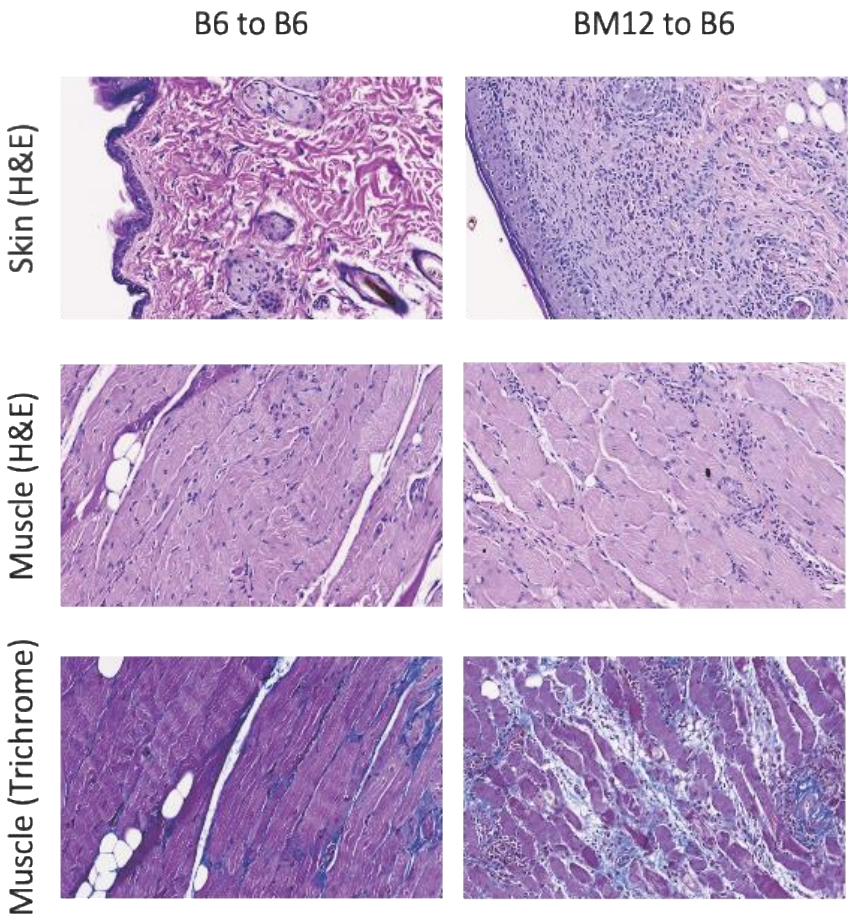


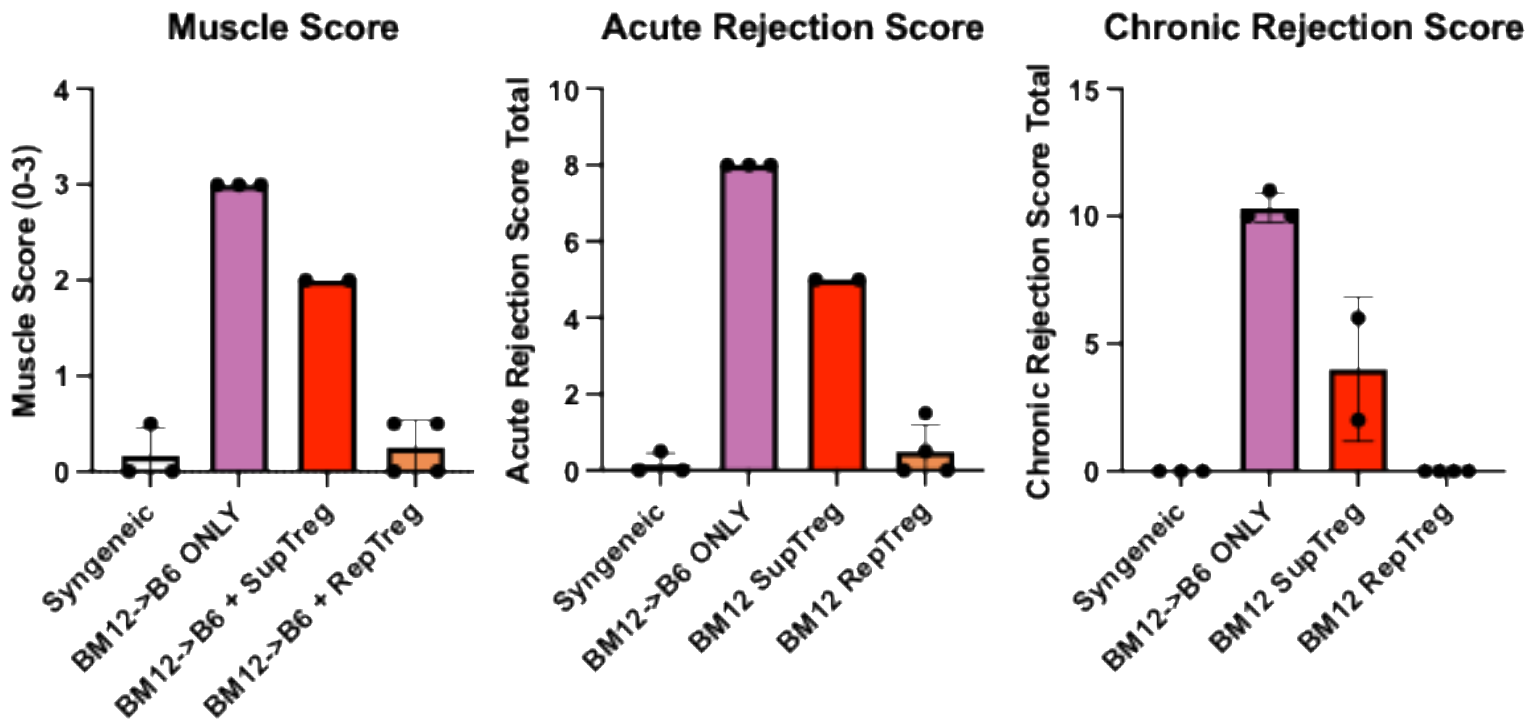


Adoptive transfer of Reparative Tregs (RepTreg) dramatically prolonged allogeneic VCA graft survival, a striking contrast to conventional Suppressive Treg (SupTreg).

RepTreg potentially promote local effector suppression while supporting tissue repair in VCA transplant, supporting their development as a clinically implementable immunotherapy for promoting tissue repair after Tx.

Reparative Treg Therapy Prolongs VCA graft survival





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Questions?

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