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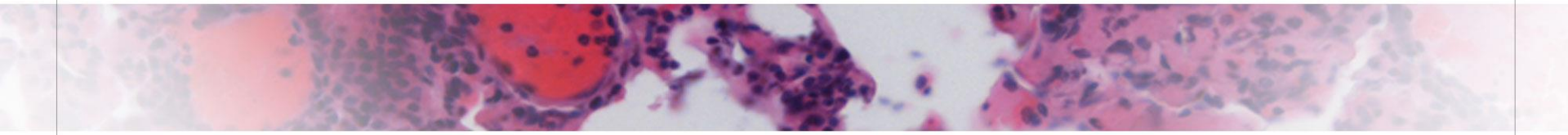

NEO-TRA1: A CD25-Targeted *De Novo* Non-Alpha Agonist of the IL-2 Receptor Selectively Expands Regulatory T Cells

Giacomo Tampella, Alex Chen, Alfredo Quijano-Rubio, Matt Walker, Irene Barber, Justin Hare, Justin Huard, Danielle Van Citters, Aaron Moss, Hector Rincon, and Bill Arthur

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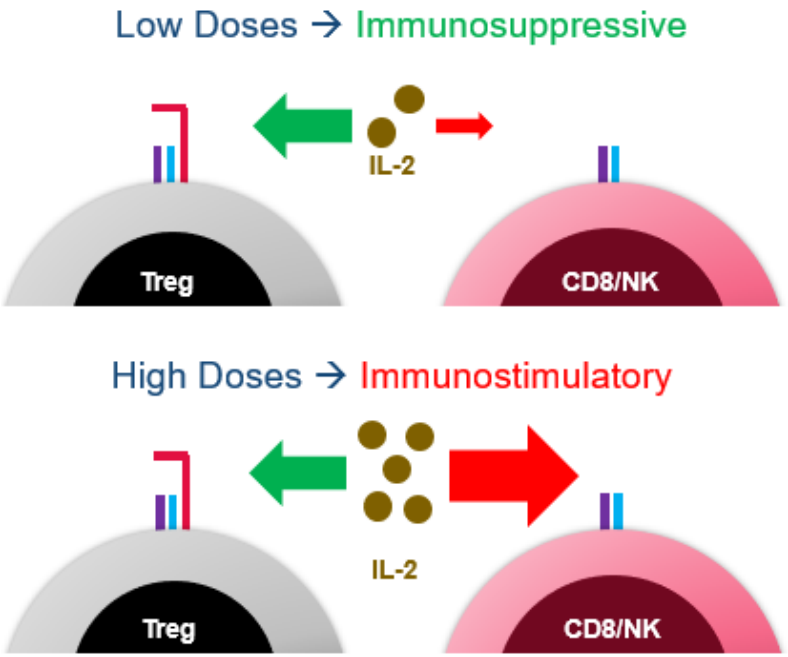
Monday, December 12, 2022

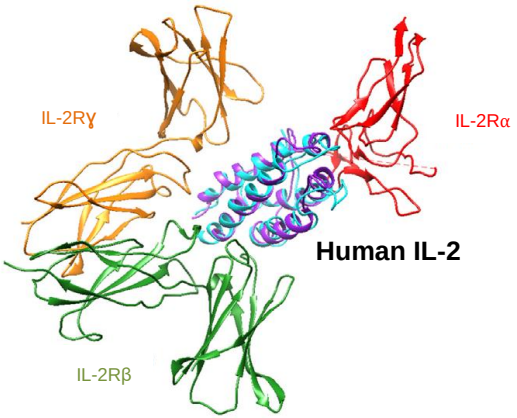
Background

- Regulatory T cells (Tregs) maintain immune tolerance; their dysfunction contributes to inflammatory and autoimmune conditions, such as SLE and graft versus host disease (GVHD)[1]
- Interleukin-2 (IL-2) is a pleiotropic cytokine that activates both immunosuppressive Tregs and pro-inflammatory cells (cytotoxic T & NK cells)[2,3]
- Low-dose IL-2 (LD-IL2) therapy has shown promise in multiple inflammatory diseases with high unmet need[4]
- LD-IL2 success has been limited by its short half-life, narrow therapeutic index, and stimulation of pro-inflammatory cells



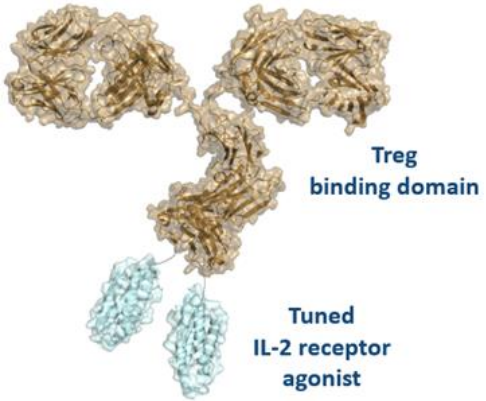
1. Nishimori H, et al. *Acta Med Okayama*. 2013;67(1):1-8. **2.** Antony PA, et al. *J Immunother*. 2005;28(2):120-128. **3.** Malek TR. *Annu Rev Immunol*. 2008;26:453-479. **4.** Graβhoff H, et al. *Front Immunol*. 2021;12:648408.

Neoleukin's Next-Generation Treg Agonist: NEO-TRA



Human IL-2

Decouple & Optimize



Treg Agonist NEO-TRA

Small size; rapid clearance

Difficult to independently vary the strength of IL-2Rα (CD25) binding and IL-2Rβγ agonism

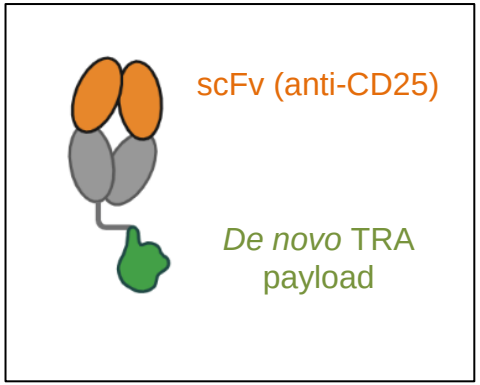
Fixed orientation of CD25 binding

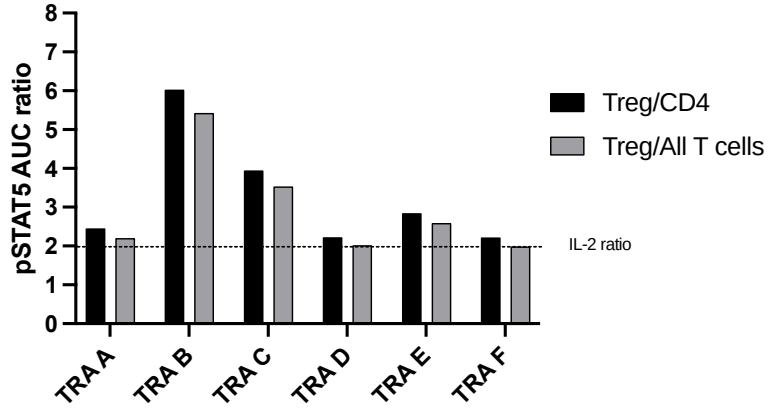
Detuned mimetic with reduced binding affinity to IL-2Rβ (CD122) and IL-2Rγ (CD132)

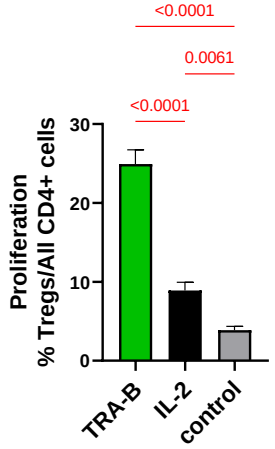
Potential modular system to pair with different targeting domains (e.g., CD25)

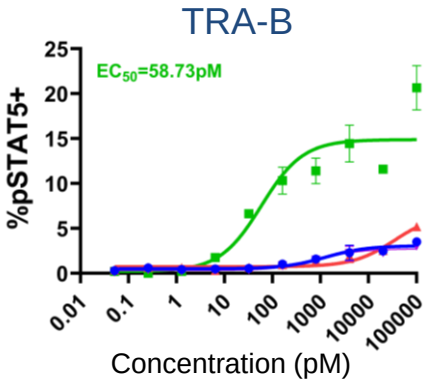
Designed for improved selectivity, wider therapeutic window, and longer half-life compared to LD-IL2

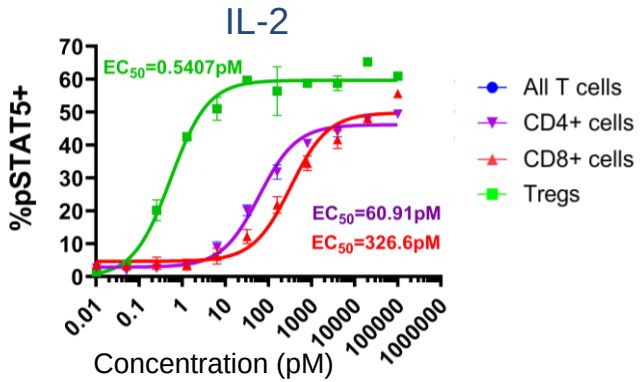
TRA Screening Through scFv Fusion Targeting Domain Identified Highly Selective Treg Agonists





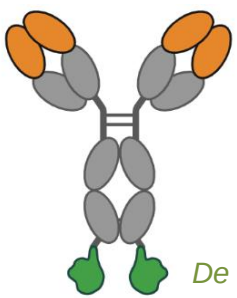






Treg Agonist Fused to a Full-Length Antibody (NEO-TRA1) Displays Signaling Specificity

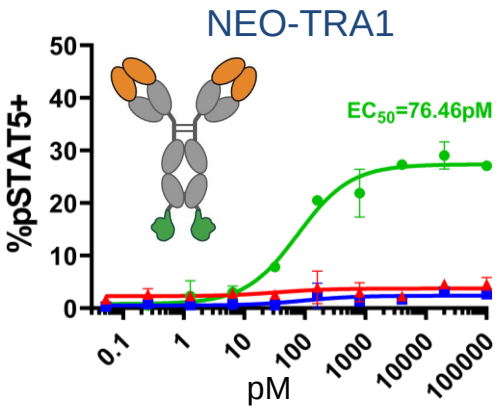
NEO-TRA1

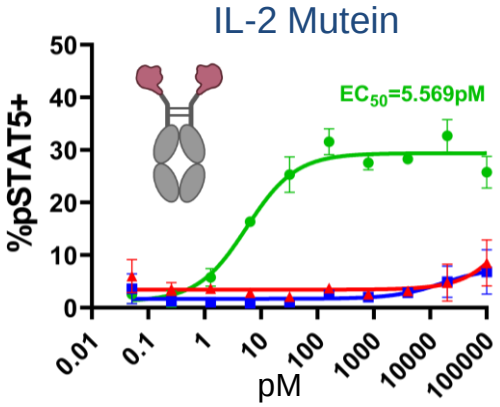


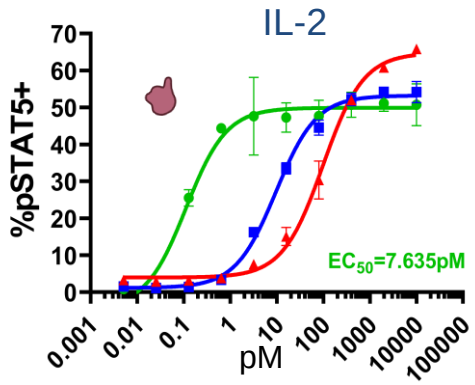
Targeting domain
(e.g., anti-CD25)

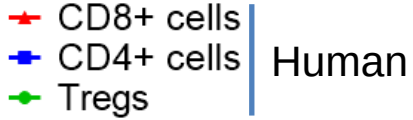
Effector-less
IgG1 backbone

De novo TRA-B payload





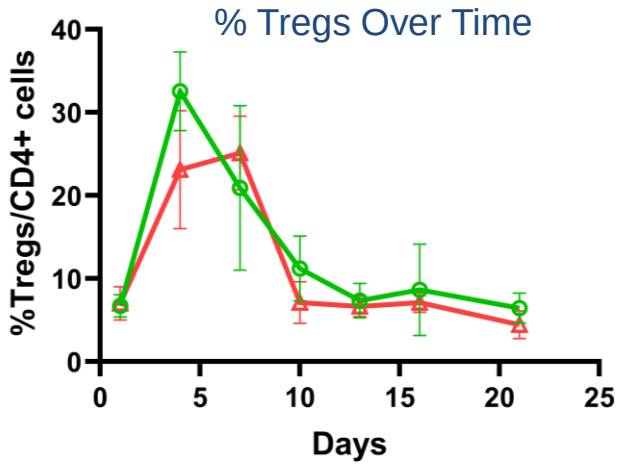


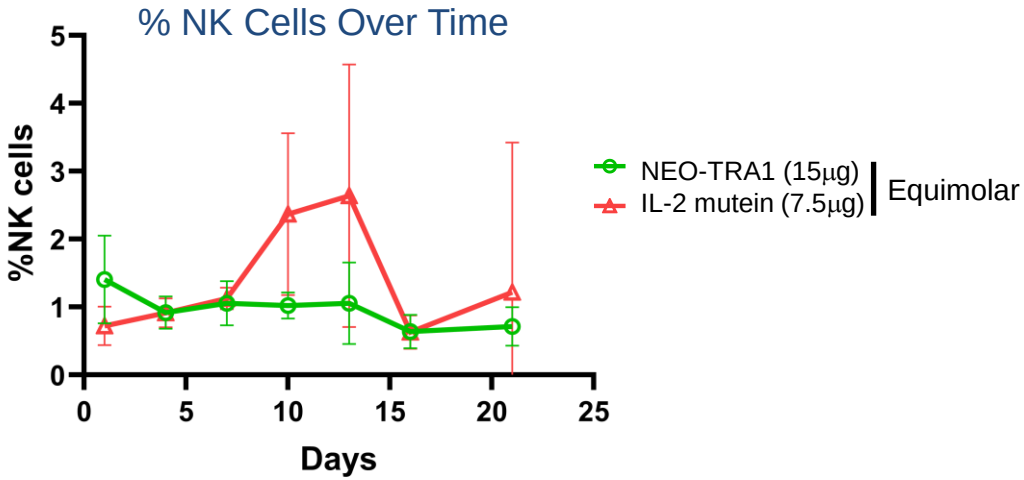


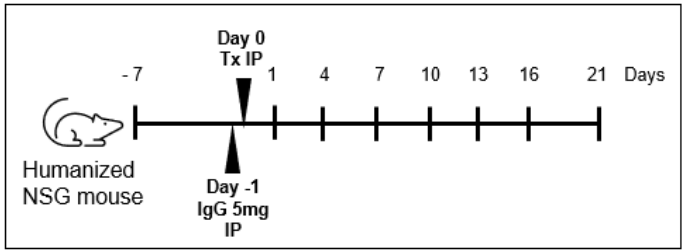
- Minor activation with high doses in resting CD25+ non-Treg cells
- Antibody targets CD25 with blocking functions and has human and monkey cross-reactivity

NEO-TRA1 Induces Treg Proliferation *In Vivo* While Avoiding NK Cell Expansion

Humanized NSG mice, single dose



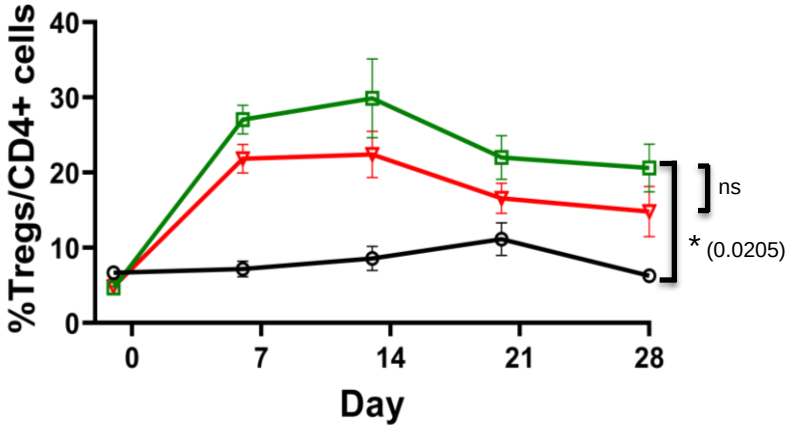




	Exposure comparison	
Time (hr)	**TRA-1 (15ug)**	**IL-2 mutein (7.5ug)**
~AUC (ng*h/mL)	5,753 (34 nmol)	2,494 (29.6 nmol)
MW (Kda)	169	84

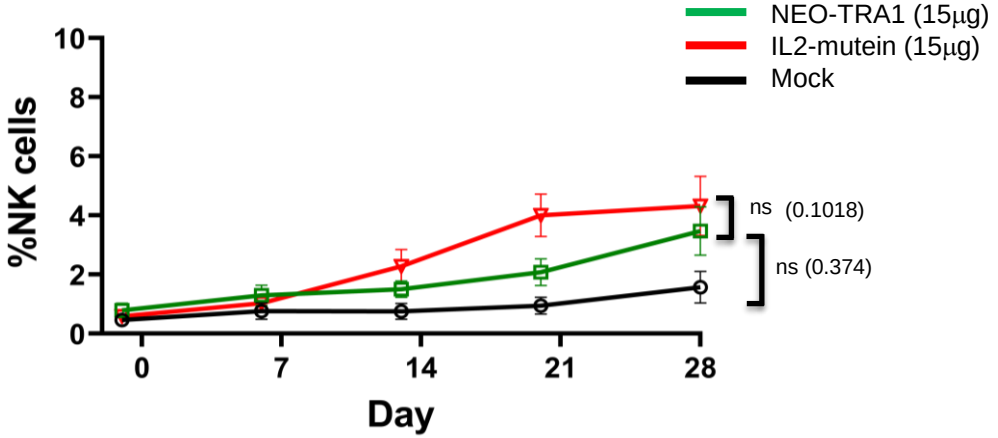
NEO-TRA1 Maintains Elevated Tregs Through Multiple Doses Over Time

% Tregs Over Time

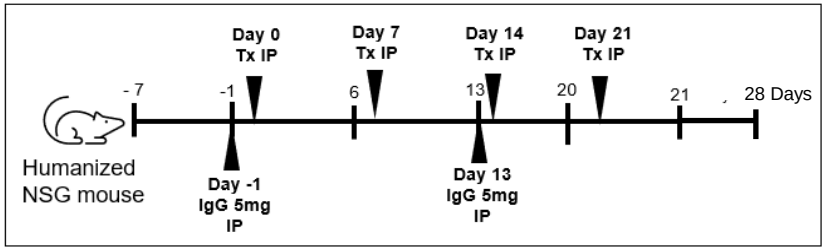


Similar or better Treg expansion was observed despite a 2:1 (IL-2 mutein: NEO-TRA1) molar ratio

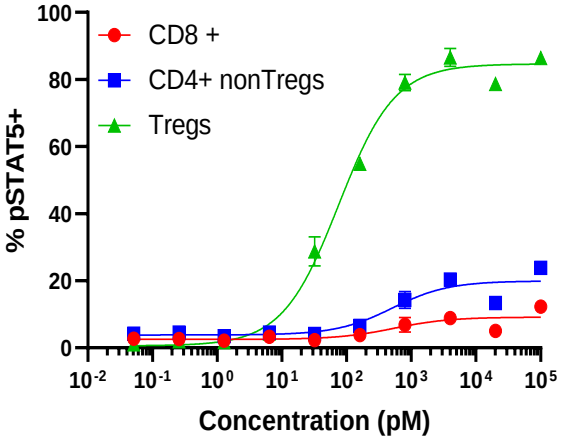
% NK Cells Over Time

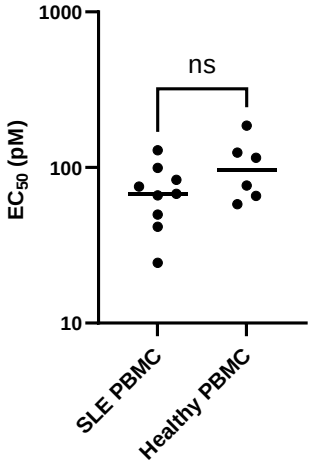


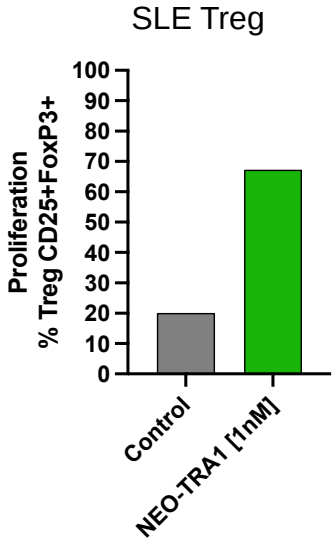
No significant expansion of NK cells observed

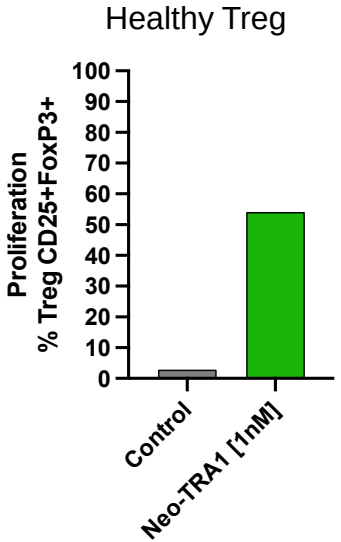


TRA Increases Treg Expansion and Activity in Systemic Lupus Erythematosus (SLE) PBMCs







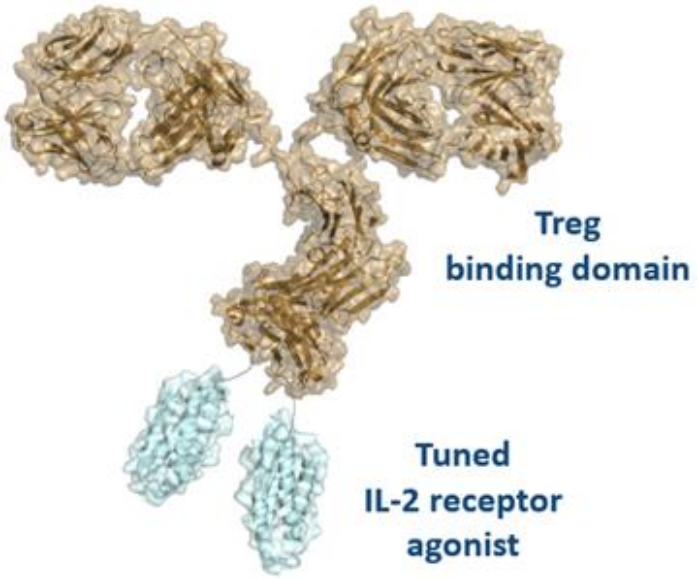


NEO-TRA1 activates Tregs purified from PBMC of SLE patients (with similar potency as healthy donors)

NEO-TRA1 stimulates the proliferation of Tregs isolated from PBMCs of SLE patients

NEO-TRA1 Summary

- Neoleukin's first Treg agonist based on a computationally designed *de novo* protein.
- Highly specific agonist of IL-2 signal transduction pathway in Tregs and not other lymphocyte cell types.
- Selectively expands Treg populations from both healthy donors and SLE patients *in vitro* and in humanized mouse models.
- A promising therapeutic candidate for the treatment of autoimmune and inflammatory conditions, including GVHD, SLE/lupus, and other diseases.



Acknowledgments

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