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Rosnilimab, a PD-1 Agonist Antibody that Binds to a Membrane Proximal Epitope Leading to Optimized PD-1 Agonistic Signaling

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Disclosures: All authors are employees and stockholders of Anaptys

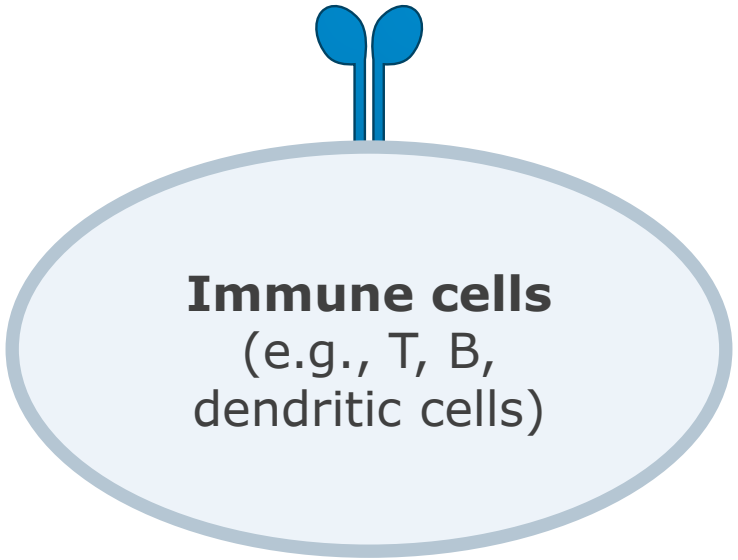
Checkpoint Receptors Modulate Immune Cells

Checkpoint antagonists:
"release the brakes"

Treat cancers:

Unleash immune response

Checkpoint receptors (e.g., PD-1, BTLA)



Checkpoint agonists:
"tap the brakes"

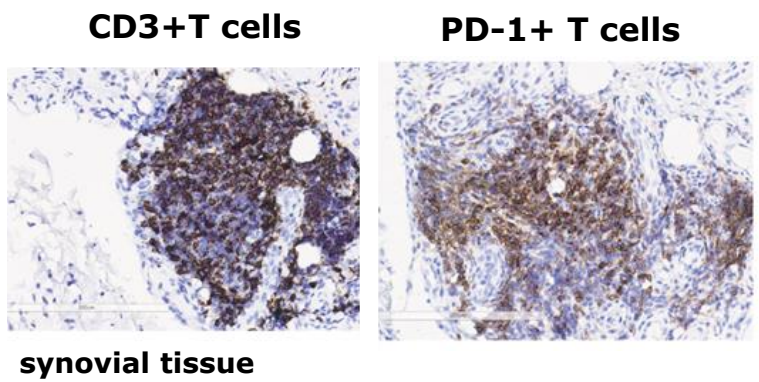
Treat inflammation:

Attenuate overactive/persistent immune response

Role of PD-1 in Rheumatoid Arthritis (RA)

Higher Prevalence of PD-1+ T Cells in RA[1]

Inflamed Tissue and Periphery	PD-1+ T Cell Population
RA	>80%



- PD-1+ T cells broadly impact multiple clinically validated drivers of RA pathogenesis[2]
- PD-1high Tph cells are elevated in RA synovium and secrete cytokines driving autoantibody production[3,4]
- PD-1 pathway gene expression is dysregulated in RA synovium[5]

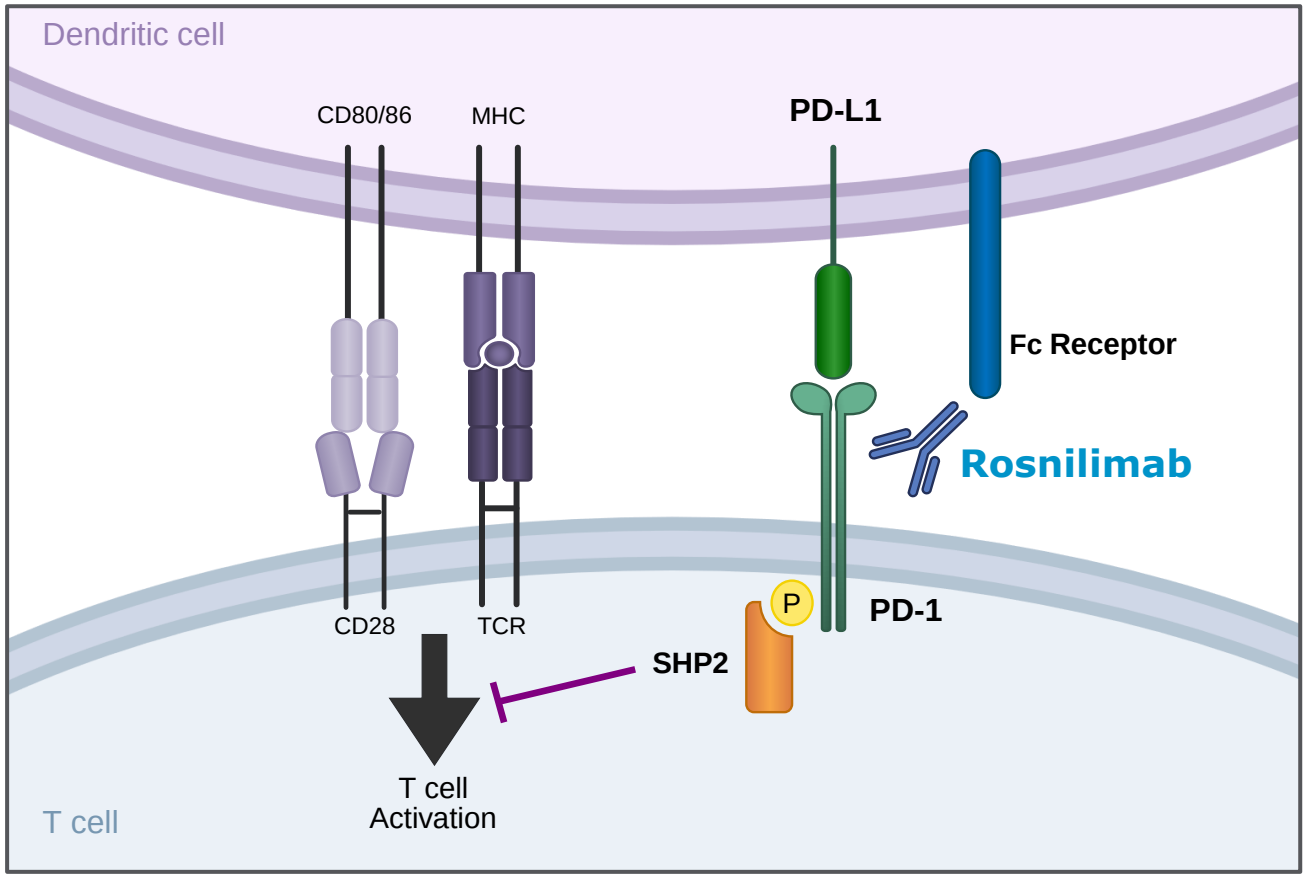
PoC for targeting PD-1 positive T cells has been achieved in RA[6]

Opportunity:
Leverage endogenous immune cell regulatory mechanisms to restore homeostasis via **PD-1 agonism**

PoC: Proof-of-concept

1. Roosenboom et al, Scand J of Gastro. 2021; 56(6):671 6793, 2. Aletaha and Smolen, JAMA, 2018, 3. Murray-Brown et al, RMC Open, 2022, 4. Rao et al, Nature, 2017, 5. Straube J, et al. Arthritis Res Therapy 2024;26:32, 6.Tuttle et al, N Engl J Med 2023;388:1853-1862

Rosnilimab (PD-1 agonist, IgG1)



Antibody Characteristics

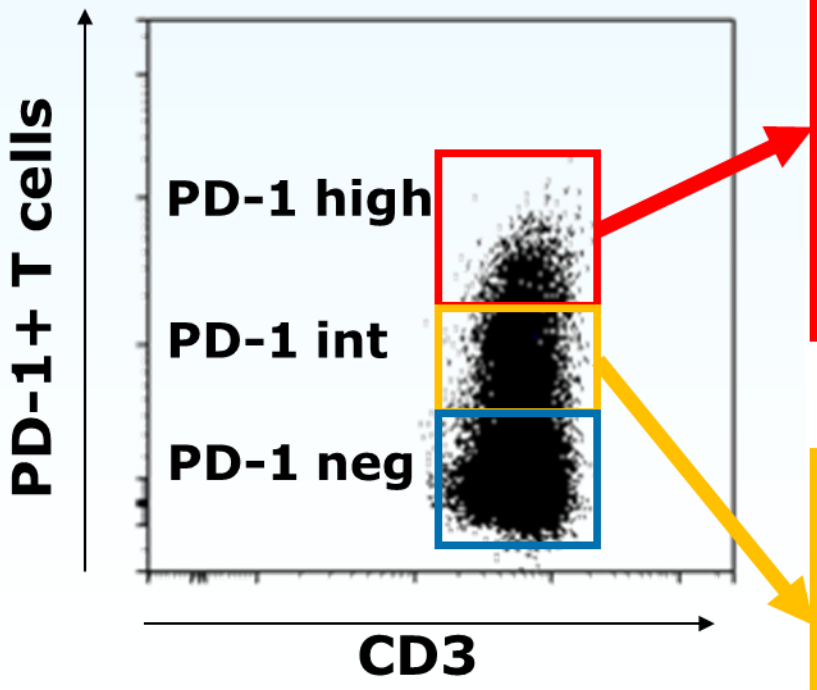
- Checkpoint ligands bind to receptors, forming tight synapses to enable clustering and exclusion of large phosphatases resulting in checkpoint agonism[1]
- Rosnilimab binds to PD-1 at a membrane proximal epitope[2]
- Fc Receptor engagement via the IgG1 Fc domain potentiates agonism and depletion activity[3]

Proposed Mechanism of Action

- Depletes PD-1 high T cells and agonizes remaining PD-1+ T cells, in tissue and in the periphery
- Broader T cell targeting agents, such as abatacept, have not demonstrated a safety risk for infection or cancer

1. Suzuki K, et al. Sci Immunol 2023;8(79):eadd4947. 2. Parmley S, et al. Arthritis Rheumatol 2023;75(suppl 9):Abstract 0086. 3. Cleary K, et al. J Immunol 2017;198(10):3999-4011.

Rosnilimab has Dual Mechanisms of Depletion and PD-1 Agonism



Rosnilimab depletes PD-1 high

Tfh (follicular helper)
Tph (peripheral helper)

- Defined by PD-1 high
- Secrete CXCL13 and IL-21, to recruit and mature B cells into "autoantibody secreting" plasma cells

Teff (effector)

- Induced in response to stimulation, **highly** activated (PD-1 high)

Rosnilimab agonizes PD-1 int

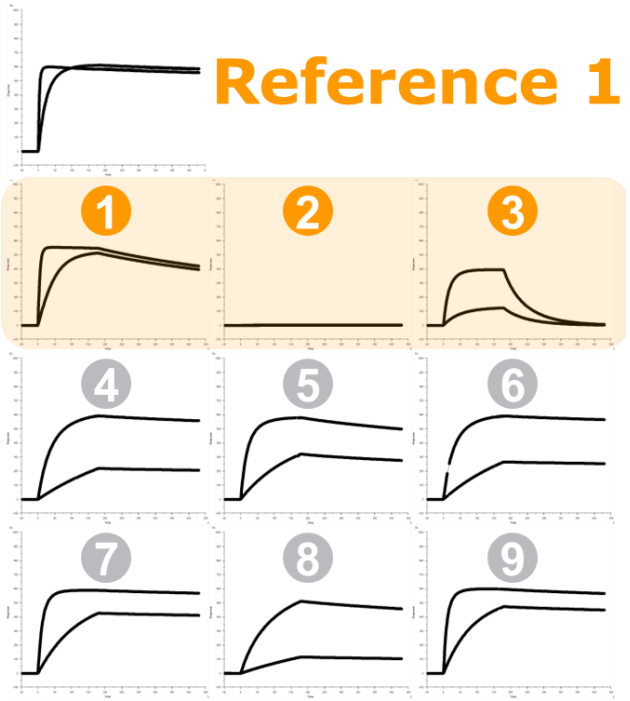
Teff (effector)

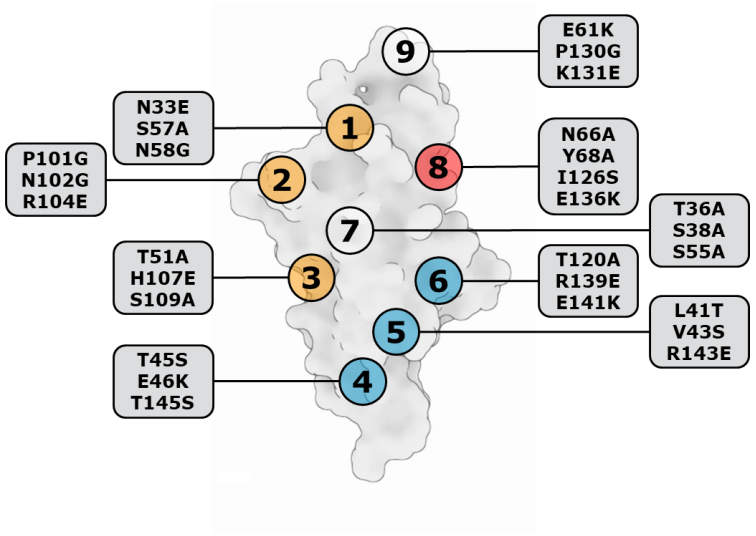
- Induced in response to stimulation, **moderately** activated (PD-1 int)
- Secrete inflammatory cytokines, cause tissue damage and perpetuate inflammatory cycle

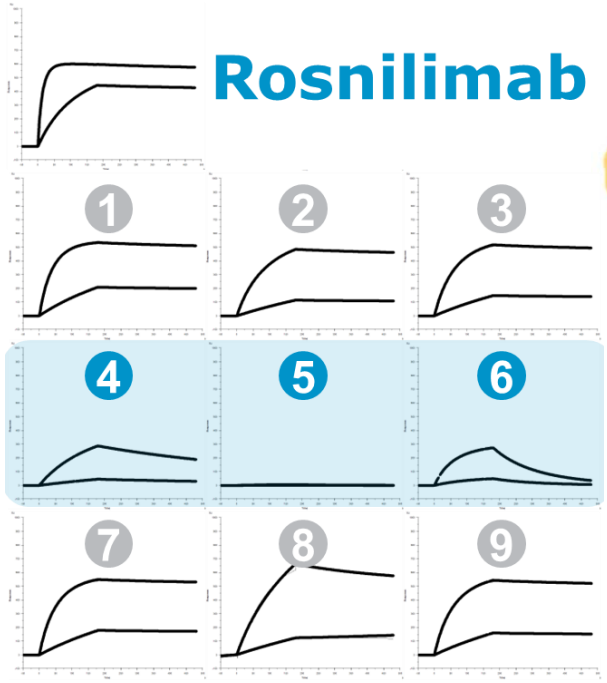
Rosnilimab Binds to a Membrane Proximal Epitope of PD-1 while Reference 1 Binds a Membrane Distal Epitope

Epitope mapping using wild-type or mutant human PD-1

Rosnilimab binds to a PD-1 epitope distinct from the PD-L1 binding pocket





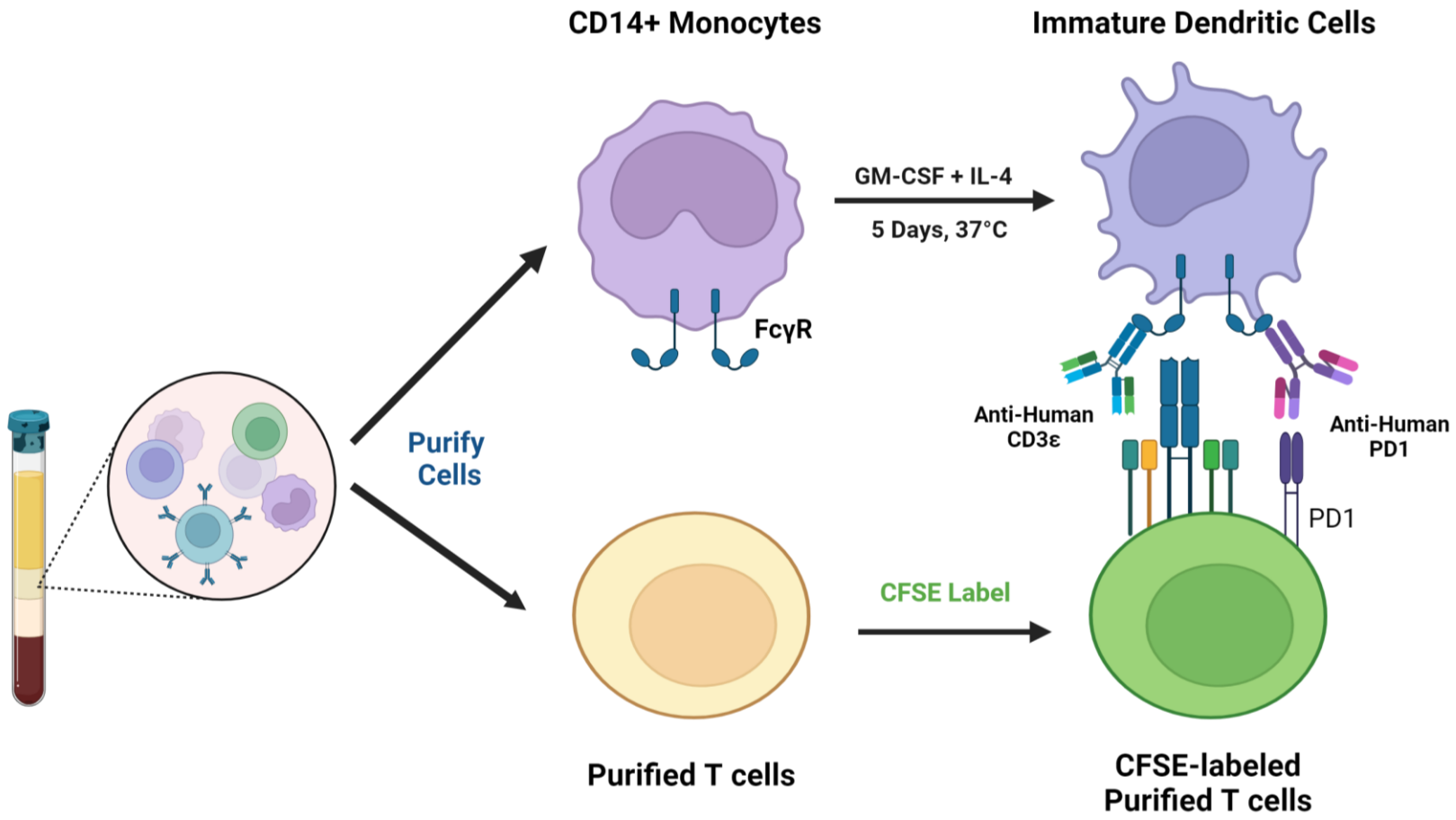


Cell membrane

Rosnilimab epitope
Reference 1 epitope
PD-L1 binding region

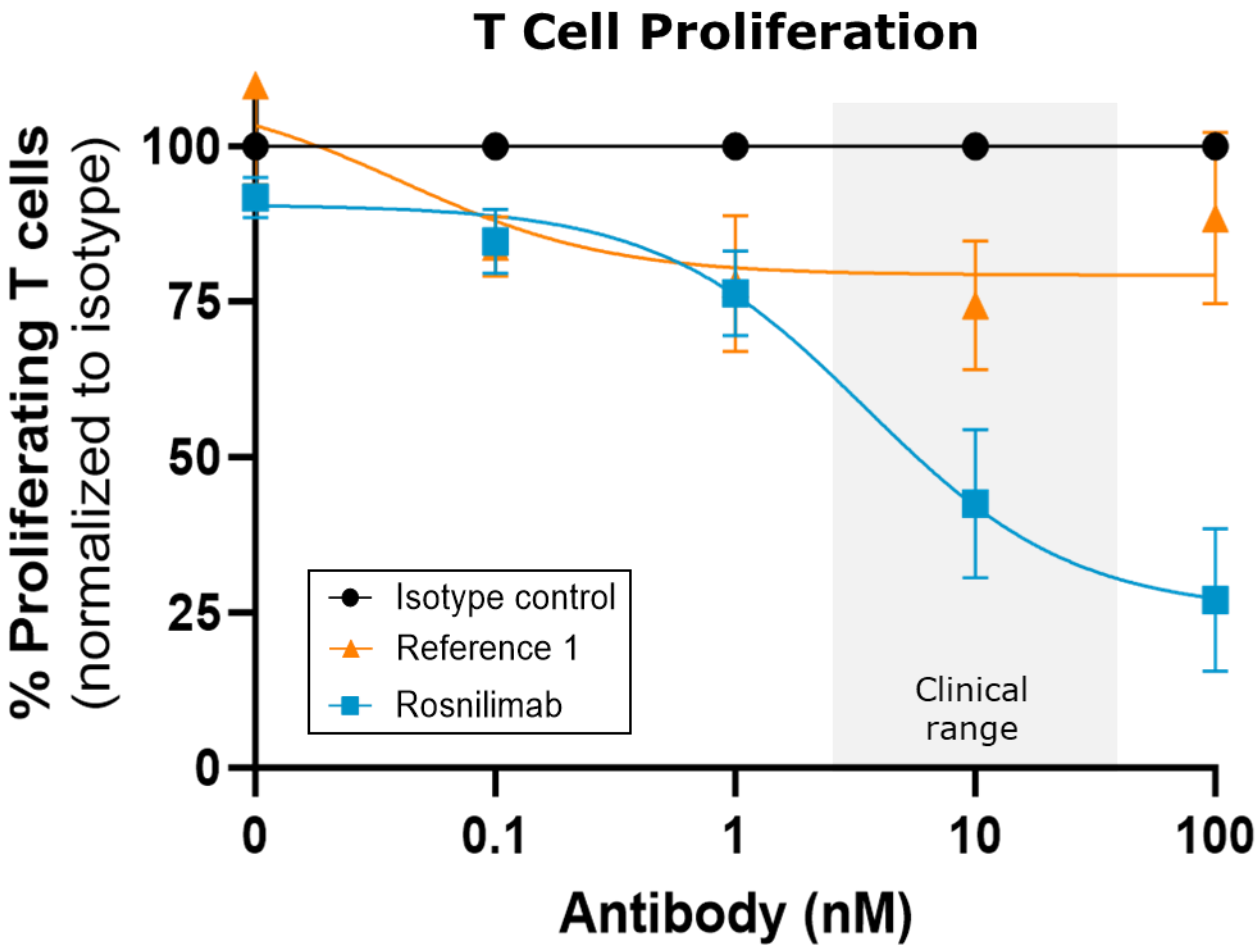
PD-1 agonist antibodies (rosnilimab or reference 1) were captured on independent flow cells using a Protein A biosensor and were subjected to wild-type or mutant human PD-1. Sensorgrams were generated after subtraction of both the reference flow cell as well as an injection of buffer over the active surface

Evaluating PD-1 Agonism Using Primary Immune Cells



- Objective: Evaluate the contribution of PD-1 membrane proximal binding and FcR engagement to PD-1 agonism, when there are no cells capable of mediating depletion in this assay

Greater Potency of Agonism (Reduced T Cell Proliferation) by Membrane Proximal Binding Rosnilimab

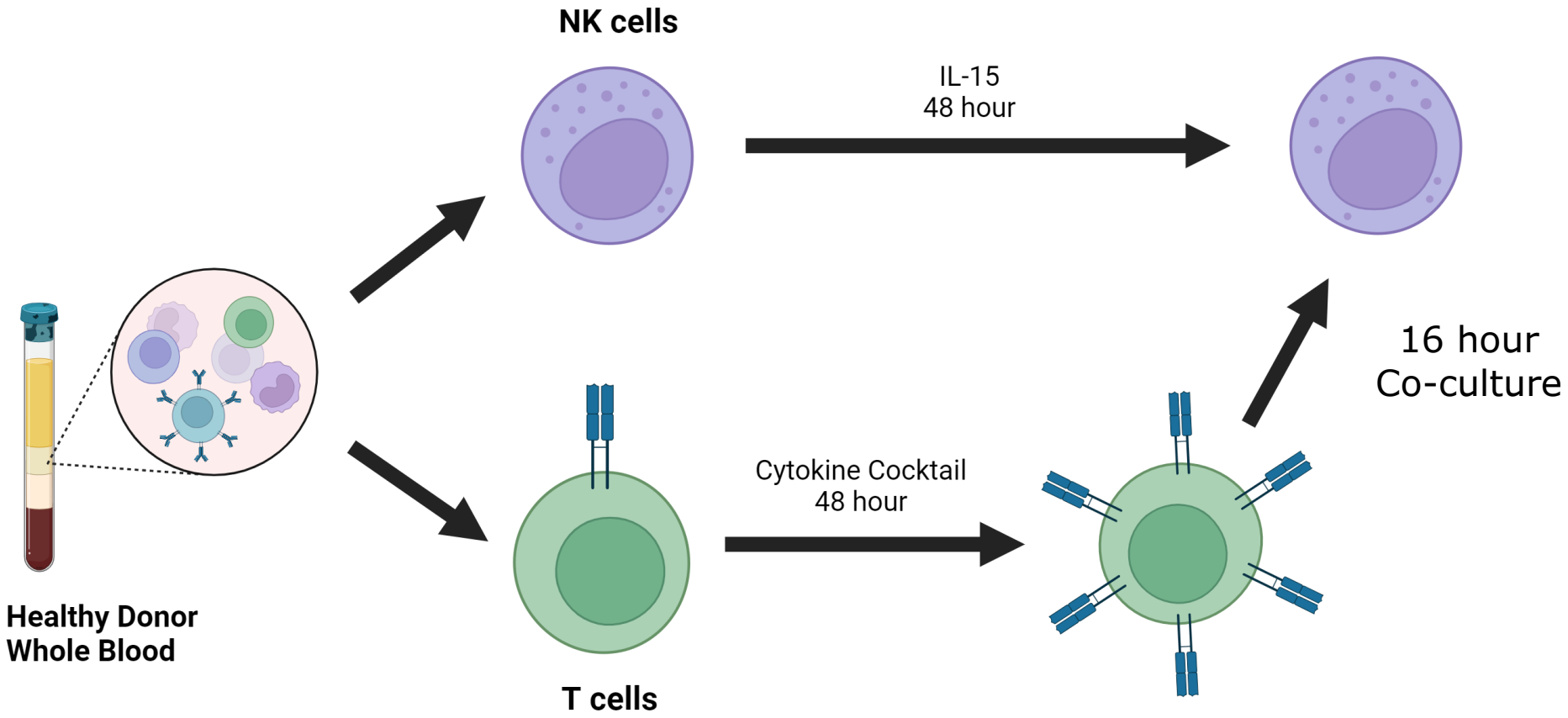


Test Article	PD-1 Membrane Binding	T Cell Proliferation Reduction*
Reference 1	Distal	~20%
Rosnilimab	Proximal	**~75%**

*Compared to isotype control

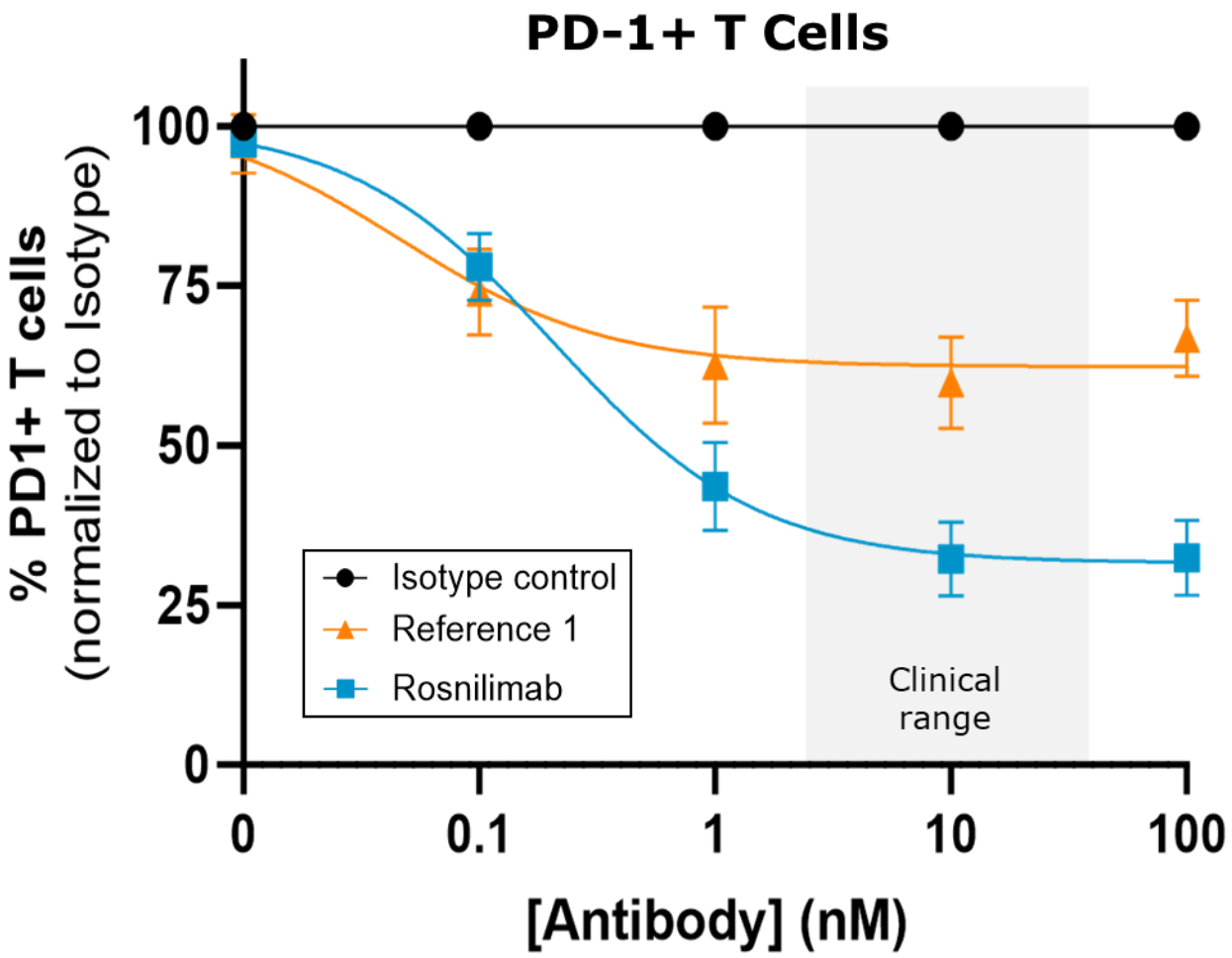
Parmley, et al. Presented at the ECCO meeting, Stockholm, Feb 21-24 2024

Evaluating Depletion (ADCC) of PD-1+ T Cells Using Primary Immune Cells



- Objective: Evaluate the contribution of PD-1 membrane proximal binding and FcR engagement to depletion of PD-1+ T cells

Greater Potency in Depletion of PD-1+ T Cells by Membrane Proximal Binding Rosnilimab

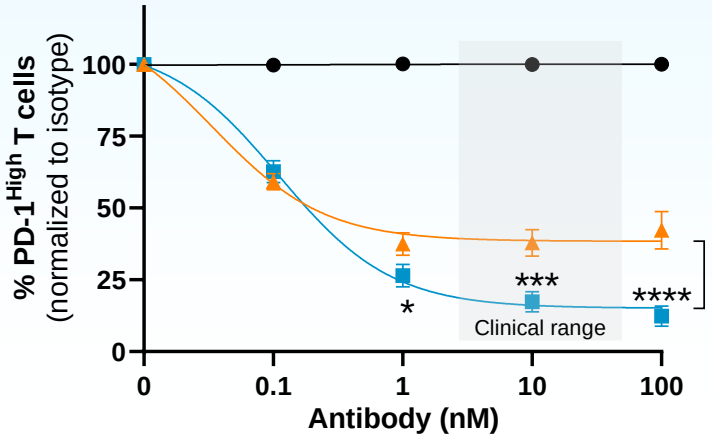


Test Article	PD-1 Membrane Binding	PD-1+ T Cell Reduction*
Reference 1	Distal	~40%
Rosnilimab	Proximal	**~70%**

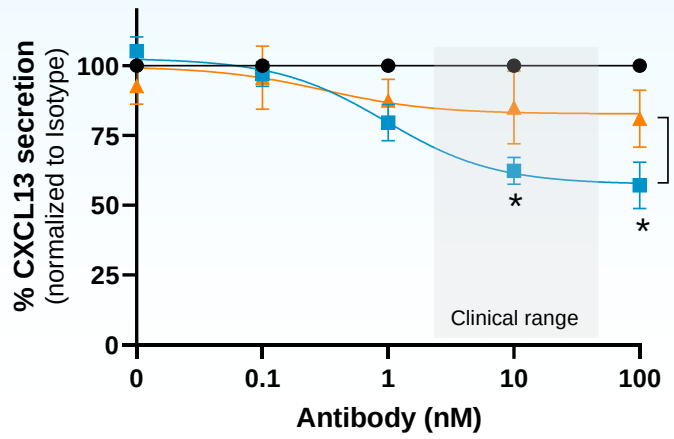
*Compared to isotype control

Rosnilimab's Potent Depletion and Agonism Reduced T Cell Proliferation and Inflammatory Cytokines from RA Patient PBMCs

Depletion of PD-1^{high} T cells

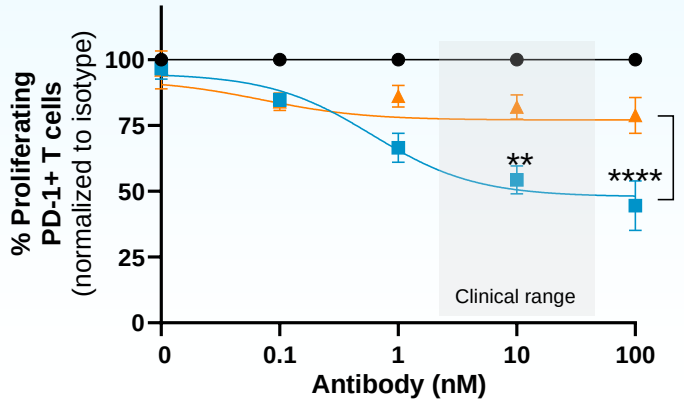


Inhibition of Tfh/Tph chemokine

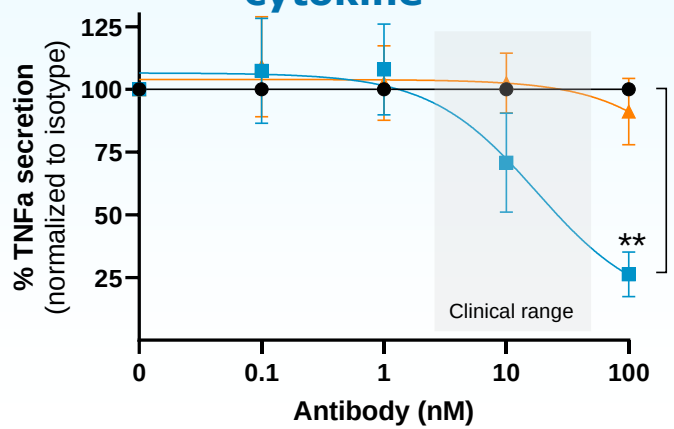


Isotype control
Reference 1
Rosnilimab

Inhibition of T cell proliferation



Inhibition of inflammatory cytokine[1]

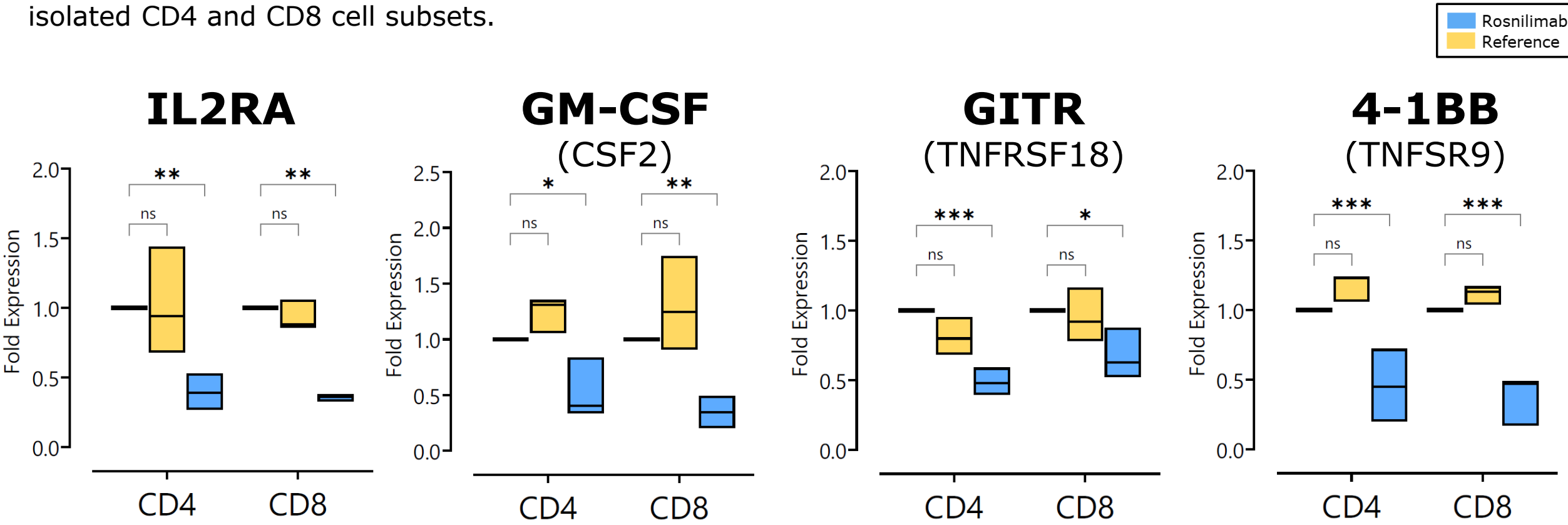


Anti-CD3+ anti-CD28 stimulation of RA patient PBMCs for assessment of depletion and agonism MOA, representative data from N=8 donors. Two-way ANOVA, Tukey's multiple comparison test. ****P<0.0001, ***p<0.001, **p<0.01, *p<0.05.
1. TNFa secretion measured in anti-CD3+ anti-CD28 stimulation of purified DC+T cells from N=4 healthy donors.

PD-1 Agonism By Membrane Proximal Binding Rosnilimab Reduced T Cell Activation Associated Genes

Gene Expression Analysis

Monocytes were purified from healthy donor PBMCs, polarized to dendritic cells, and co-cultured with isolated PanT cells in the presence of 30nM PD-1 antibodies or isotype control, and then cultured. RNA was extracted from isolated CD4 and CD8 cell subsets.



Statistical analysis performed using ordinary two-way ANOVA followed by Dunnett's multiple comparisons test with four comparisons per gene and thresholds for significance relied on multiplicity adjusted P values.
PBMCs: peripheral blood mononuclear cells

Conclusions

- Rosnilimab binds to a membrane proximal region of PD-1 while reference 1 binds to a more membrane distal region
- Optimization of rosnilimab's binding characteristics results in more potent agonism and deeper depletion of PD-1 expressing T cells compared to reference 1
- Results were consistent with published studies that demonstrate membrane proximal binding of PD-1 antibodies improve PD-1 agonistic activity and enhance target cell depletion
- PoC for PD-1 agonism has been demonstrated in RA
- These mechanistic data, translational in vivo and in vitro data, robust Phase 1 healthy volunteer data, and unmet needs provide rationale for ongoing global Phase 2 studies of rosnilimab in RA (NCT06041269) and UC (NCT06127043)