Optimizing PD-1 Agonist Signaling With Membrane Proximal Binding of Rosnilimab, a Clinical Stage PD-1 Agonist IgG1 Antibody

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Disclosures: All authors are employees and stockholders of Anaptys

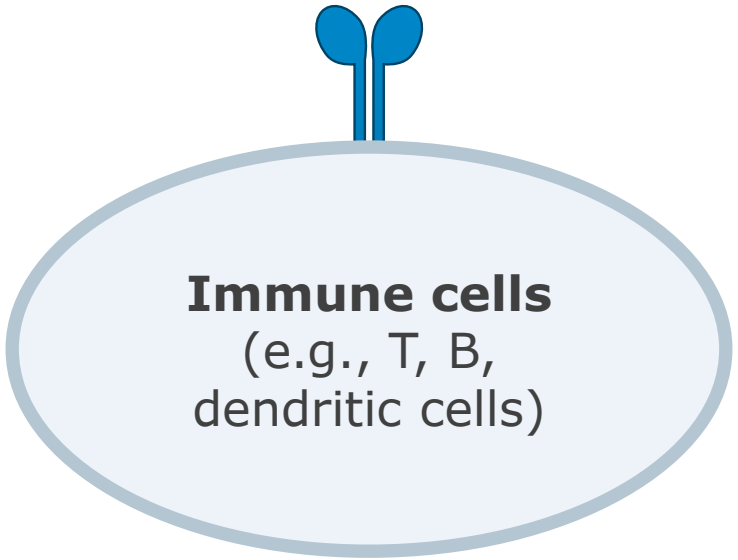
Checkpoint Receptors Modulate Immune Cells

Checkpoint antagonists:
"release the brakes"

Treat cancers:

Unleash immune response

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

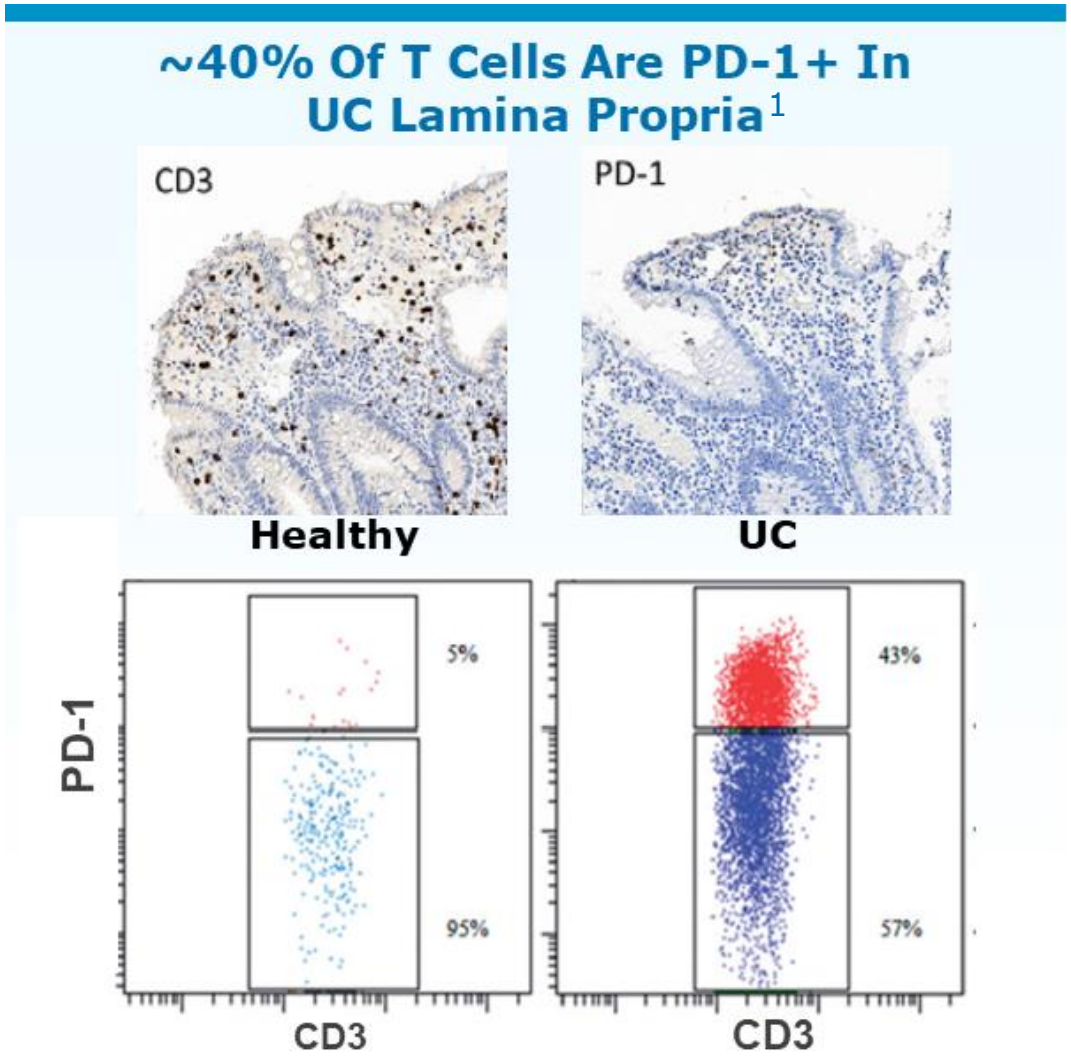


Checkpoint agonists:
"tap the brakes"

Treat inflammation:

Attenuate overactive/persistent immune response

PD-1 Pathway is Dysregulated in Ulcerative Colitis



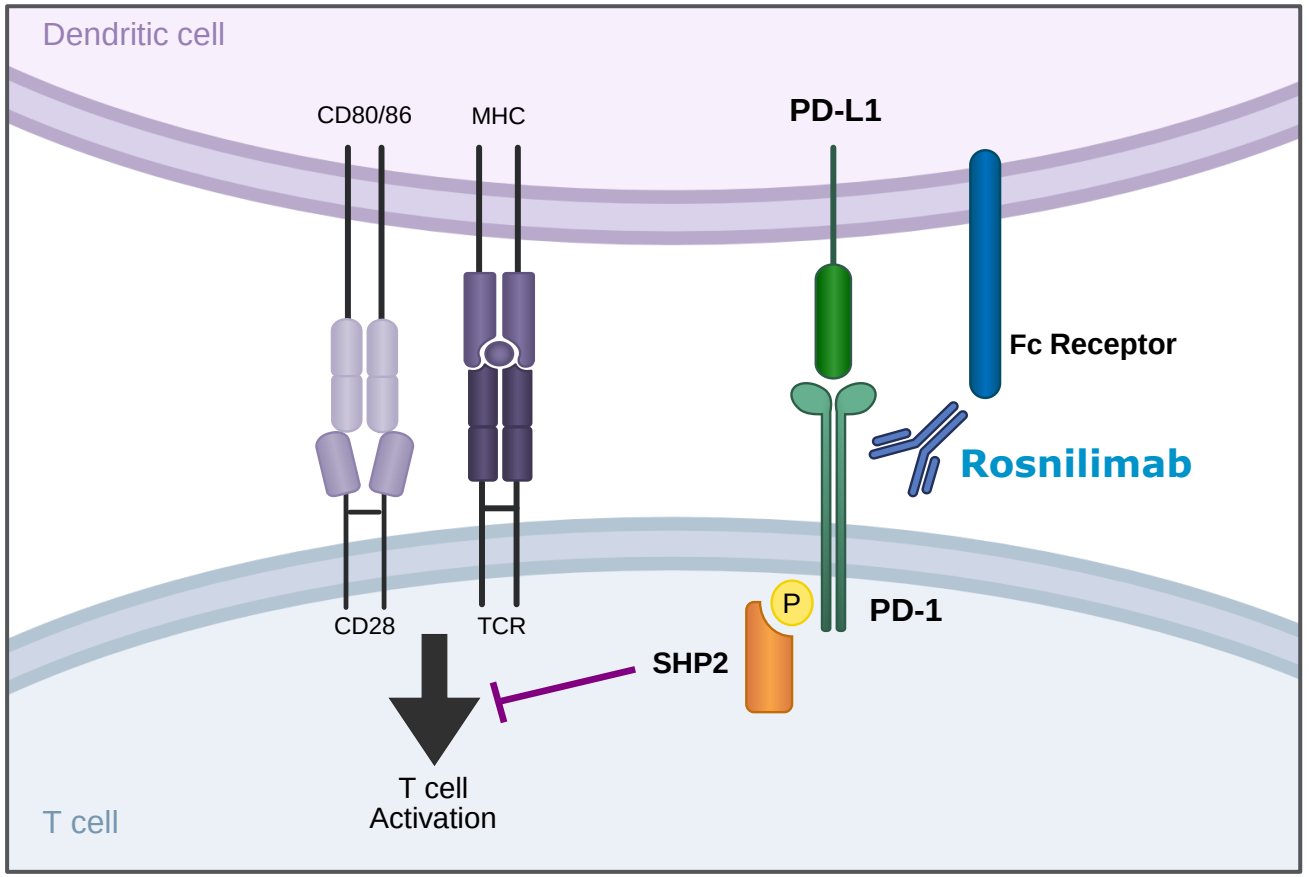
- Elevated PD-1 high Tfh and Tph cells in the peripheral circulation in UC positively correlate with Mayo clinical score, erythrocyte sedimentation rate, C-reactive protein [2,3,4]
- Diarrhea and colitis are frequently reported AEs with PD-1 antagonists[5]
- PD-1 pathway gene expression is dysregulated in UC tissues, similar to RA synovium[6]

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

- PoC for PD-1 agonism has been achieved in rheumatoid arthritis[7]
- Reduction of elevated PD-1 high Tph cells in both UC colon and periphery correlates with remission [3,4]

1. Roosenboom et al, Scand J of Gastro. 2021; 56(6):671 6793, 2. Uzzan et al, Nature Med. 2022:28(4): 766 779, 3. Long et al, Immunology Letters. 2021: 2 10; 4. Rao et al, Nature. 2017, 5. Martins et al, Nat Rev Clin Oncol. 2019:1 6(9):563 580 542(7639):110 114, 6. Massimino et al, Nat Comput Sci. 2021:511 515, 7.Tuttle et al, N Engl J Med 2023;388:1853-1862 ,

Rosnilimab (PD-1 agonist, IgG1)



Antibody Characteristics

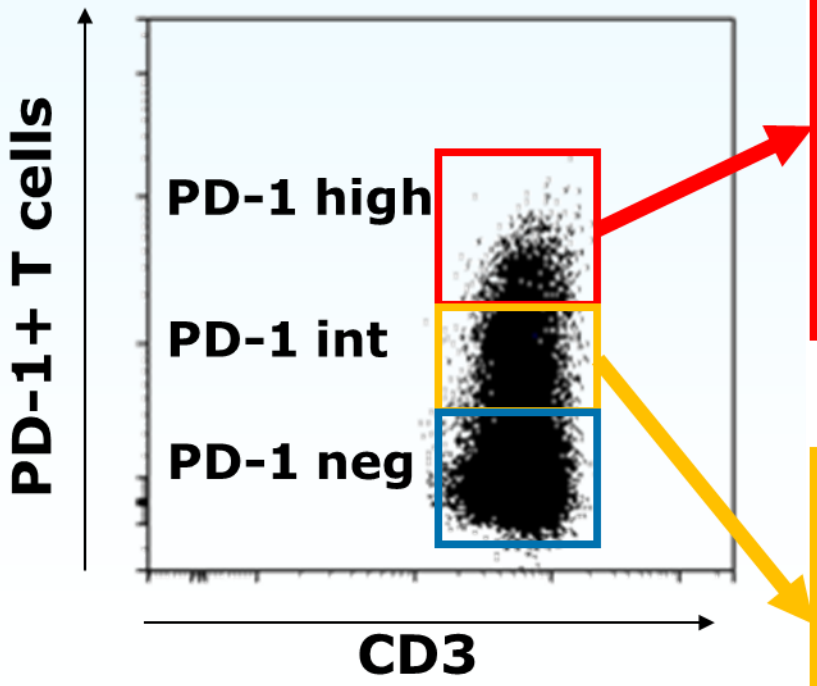
- Checkpoint ligands bind to receptors, forming tight synapses to enable clustering and exclusion of large phosphatases resulting in checkpoint agonism[1]
- Rosnilimab binds to PD-1 at a membrane proximal epitope[2]
- Fc Receptor engagement via the IgG1 Fc domain potentiates agonism and depletion activity[3]

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

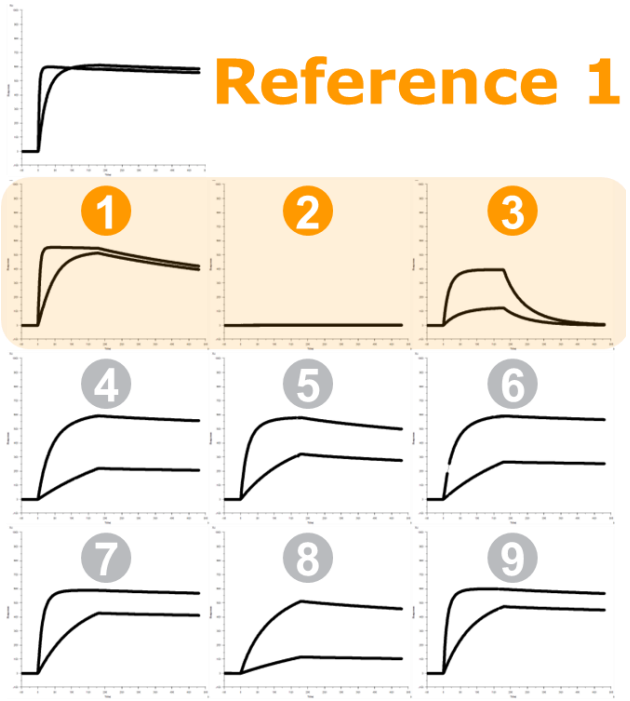
Teff (effector)

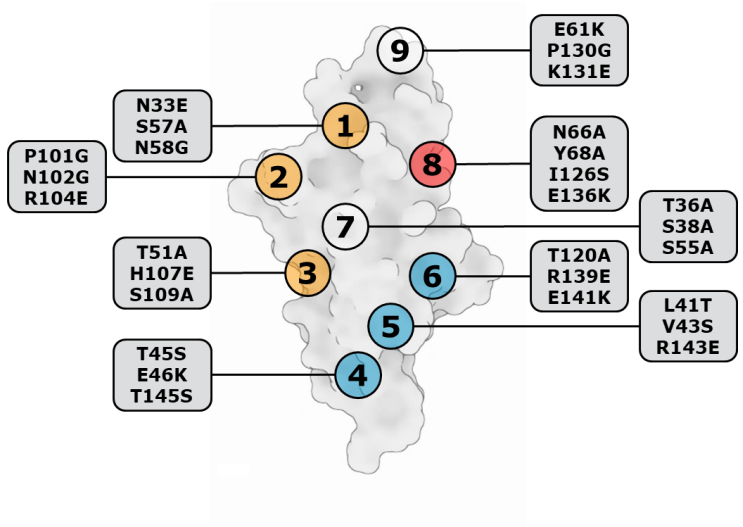
- Induced in response to stimulation, **moderately** activated (PD-1 int)
- Secrete inflammatory cytokines, cause tissue damage and perpetuate inflammatory cycle

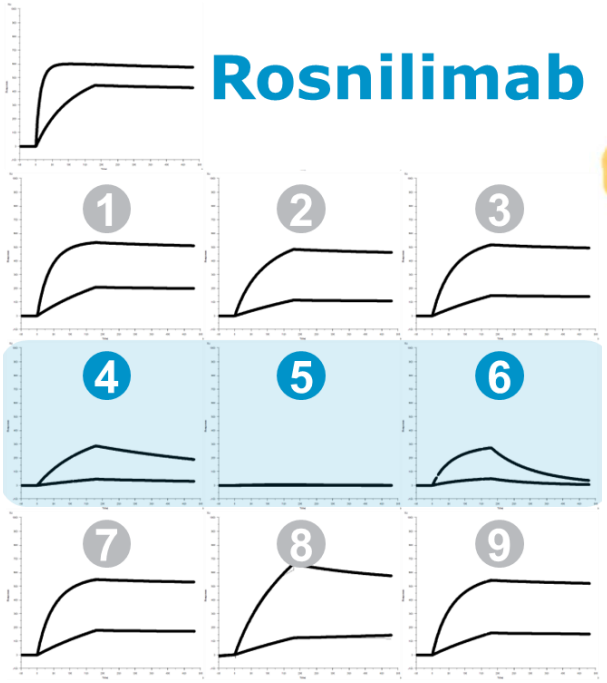
- Targeted approach to T cells already affected by approved, safe and efficacious MOAs such as anti-TNFα, anti-$\alpha 4\beta 7$, and S1P modulators

Rosnilimab Binds to a Membrane Proximal Epitope of PD-1, Distinct from Binding Epitope of PD-L1 and Membrane Distal Binding Epitope of Reference 1

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





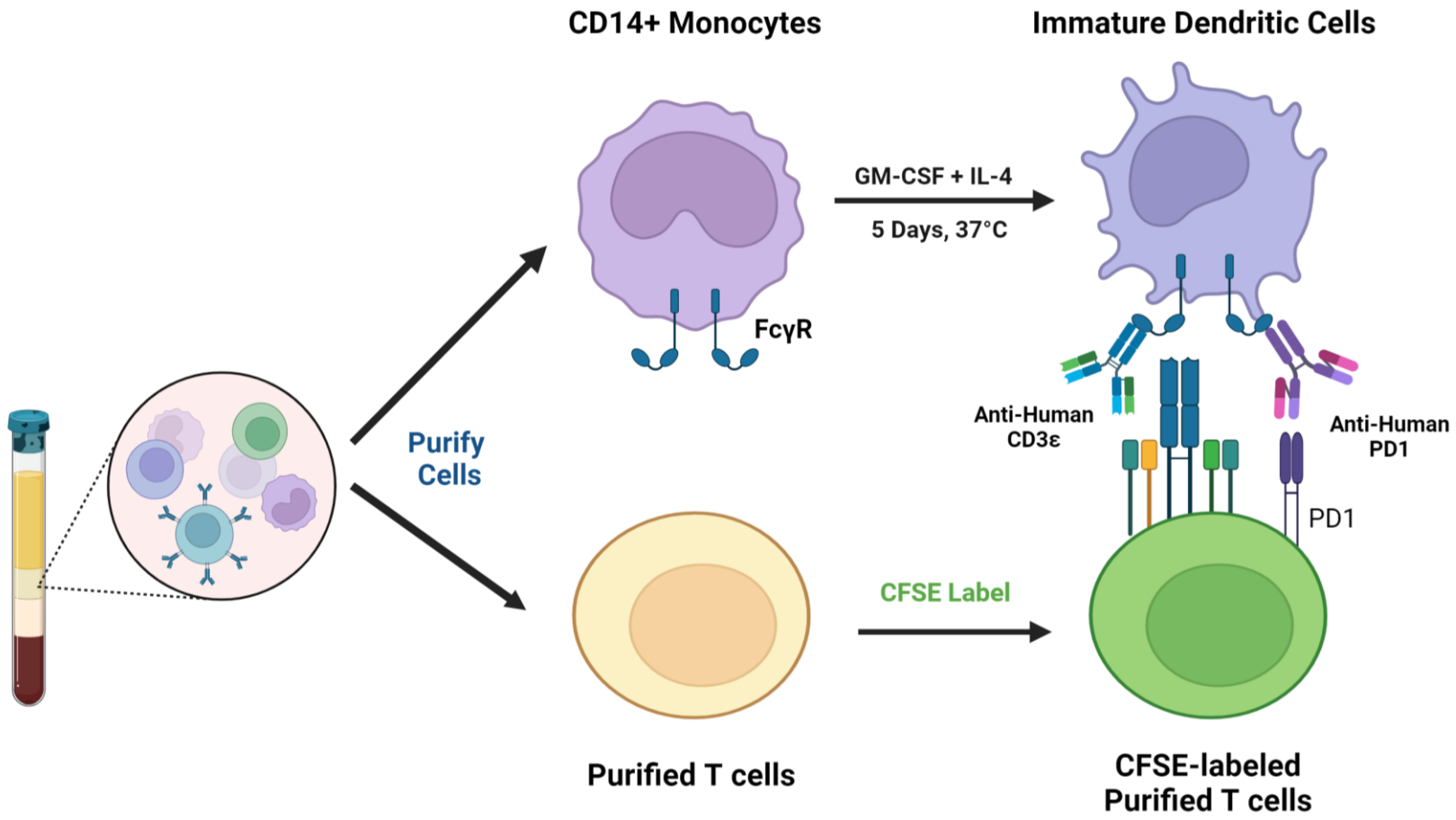


Cell membrane

Rosnilimab epitope
Reference 1 epitope
PD-L1 binding region

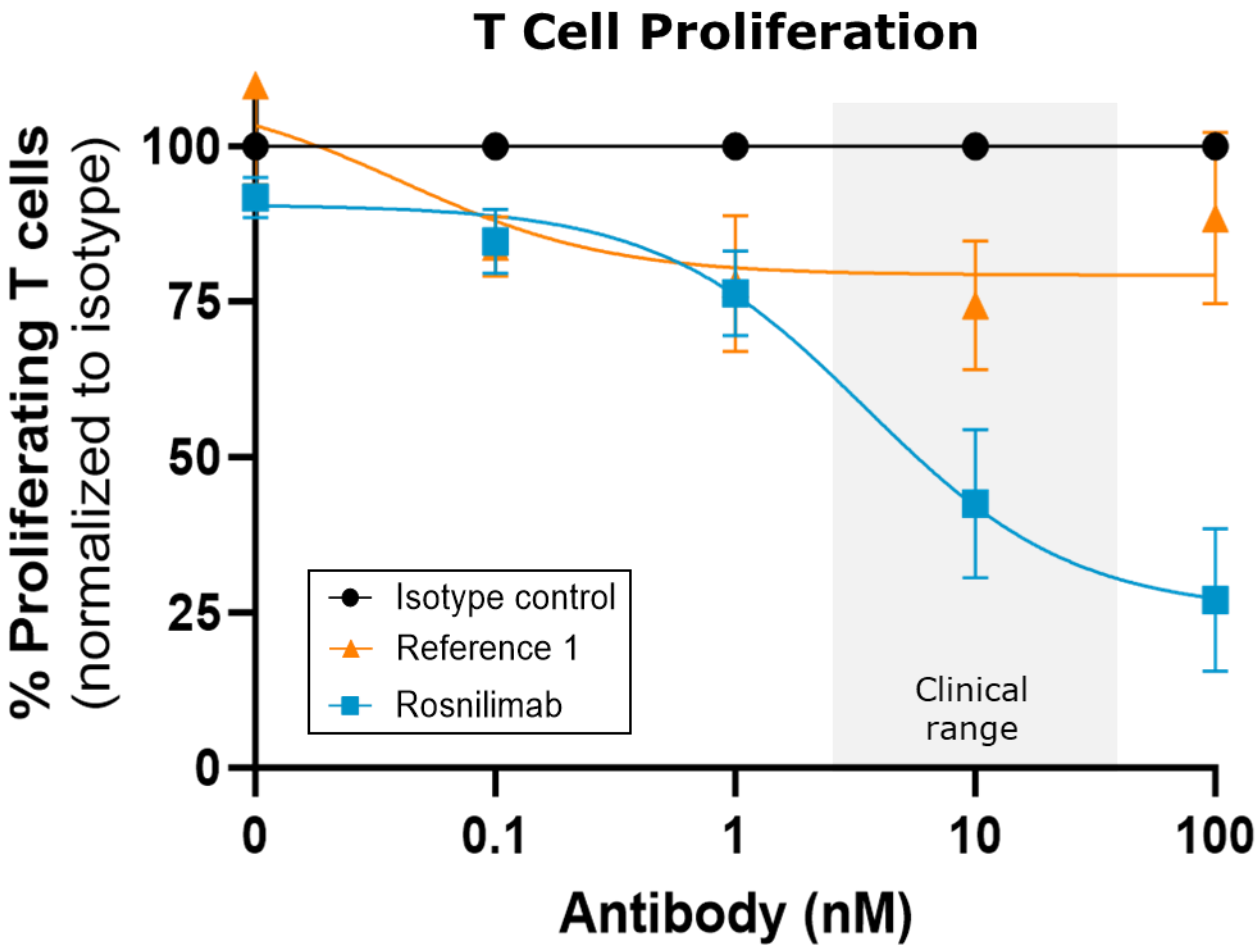
PD-1 agonist antibodies (rosnilimab or reference 1) were captured on independent flow cells using a Protein A biosensor and were subjected to wild-type or mutant human PD-1. Sensorgrams were generated after subtraction of both the reference flow cell as well as an injection of buffer over the active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

Membrane Proximal Binding Mediates Greater Depletion Mechanisms

Common mechanisms seen in antibodies, T cell engagers, and CART

Antibody distance from the cell membrane regulates antibody effector mechanisms

Kirstie L.S. Cleary, **H.T. Claude Chan**, **Sonja James**, **Martin J. Glennie**, and **Mark S. Cragg**
Antibody & Vaccine Group, Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, SO16 6YD, UK

J Immunol. 2017 May 15; 198(10): 3999–4011. doi:10.4049/jimmunol.1601473

Targeting a membrane-proximal epitope on mesothelin increases the tumoricidal activity of a bispecific antibody blocking CD47 on mesothelin-positive tumors

Eric Hatterer, Xavier Chauchet, Françoise Richard, Leticia Barba, Valéry Moine, Laurence Chatel, Lucile Broyer, Guillemette Pontini, Tereza Bautzova, Flora Juan, Sebastien Calloud, Nicolas Bosson, Maud Charreton, Krzysztof Masternak, Vanessa Buatois & Limin Shang

MABS 2020, VOL. 12, NO. 1, e1739408 (13 pages)

Membrane-Proximal Epitope Facilitates Efficient T Cell Synapse Formation by Anti-FcRH5/CD3 and Is a Requirement for Myeloma Cell Killing

Ji Li,[1] Nicola J. Stagg,[1] Jennifer Johnston,[1] Michael J. Harris,[2] Sam A. Menzies,[2] Danielle DiCara,[1] Vanessa Clark,[1] Maria Hristopoulos,[1] Ryan Cook,[1] Dionysos Slaga,[1] Rin Nakamura,[1] Luke McCarty,[1] Siddharth Sukumaran,[1] Elizabeth Luis,[1] Zhengmao Ye,[1] Thomas D. Wu,[1] Teiko Sumiyoshi,[1] Dimitry Danilenko,[1] Genee Y. Lee,[1] Klara Totpal,[1] Diego Ellerman,[1] Isidro Hötzel,[1] John R. James,[2] and Teemu T. Junttila[1,3,*]

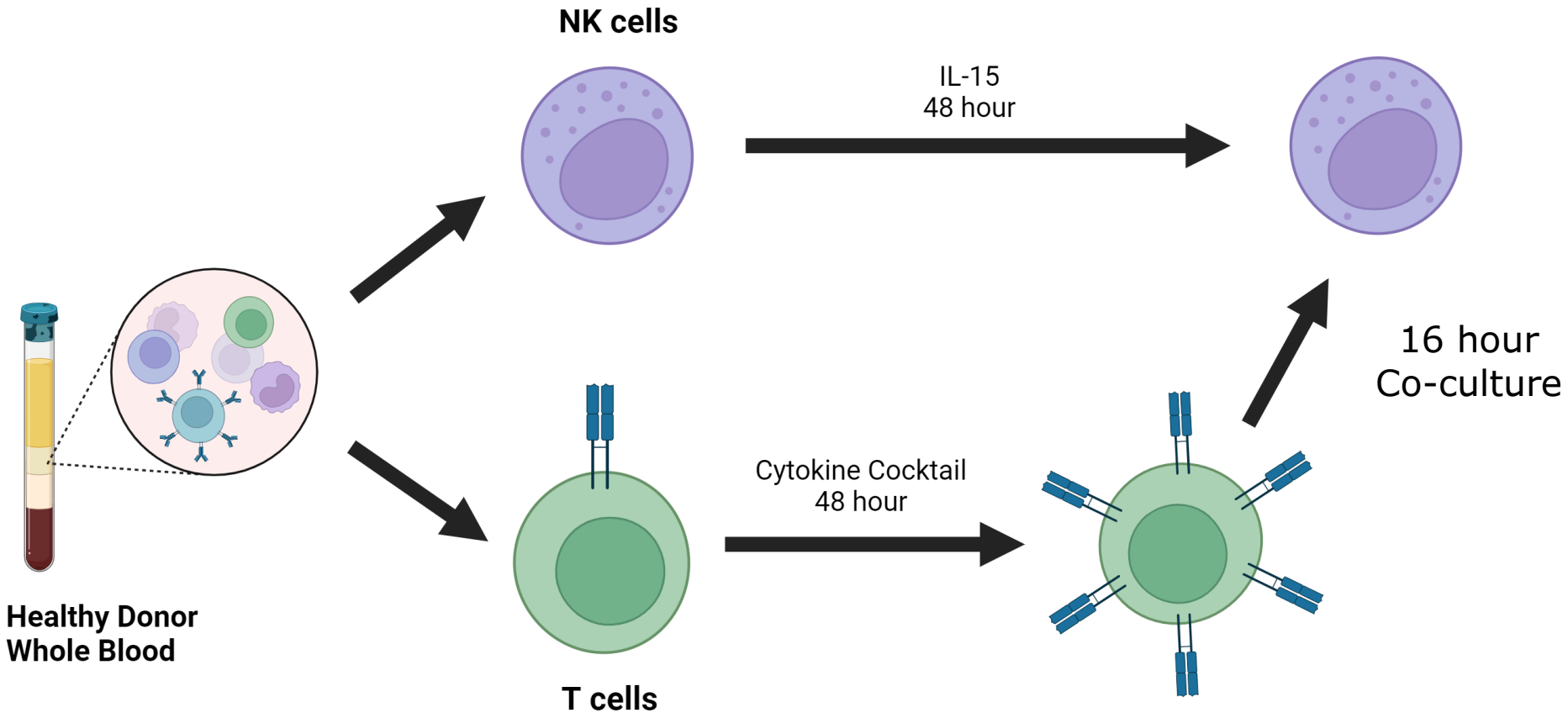
Cancer Cell 31, 383–395, March 13, 2017

Anti–PD-1 antibodies recognizing the membrane-proximal region are PD-1 agonists that can down-regulate inflammatory diseases

Kensuke Suzuki[1,2†], Masaki Tajima[1,3†], Yosuke Tokumaru[1,2], Yuya Oshiro[1,2], Satoshi Nagata[4], Haruhiko Kamada[4], Miho Kihara[5], Kohei Nakano[5], Tasuku Honjo[6], Akio Ohta[1*]

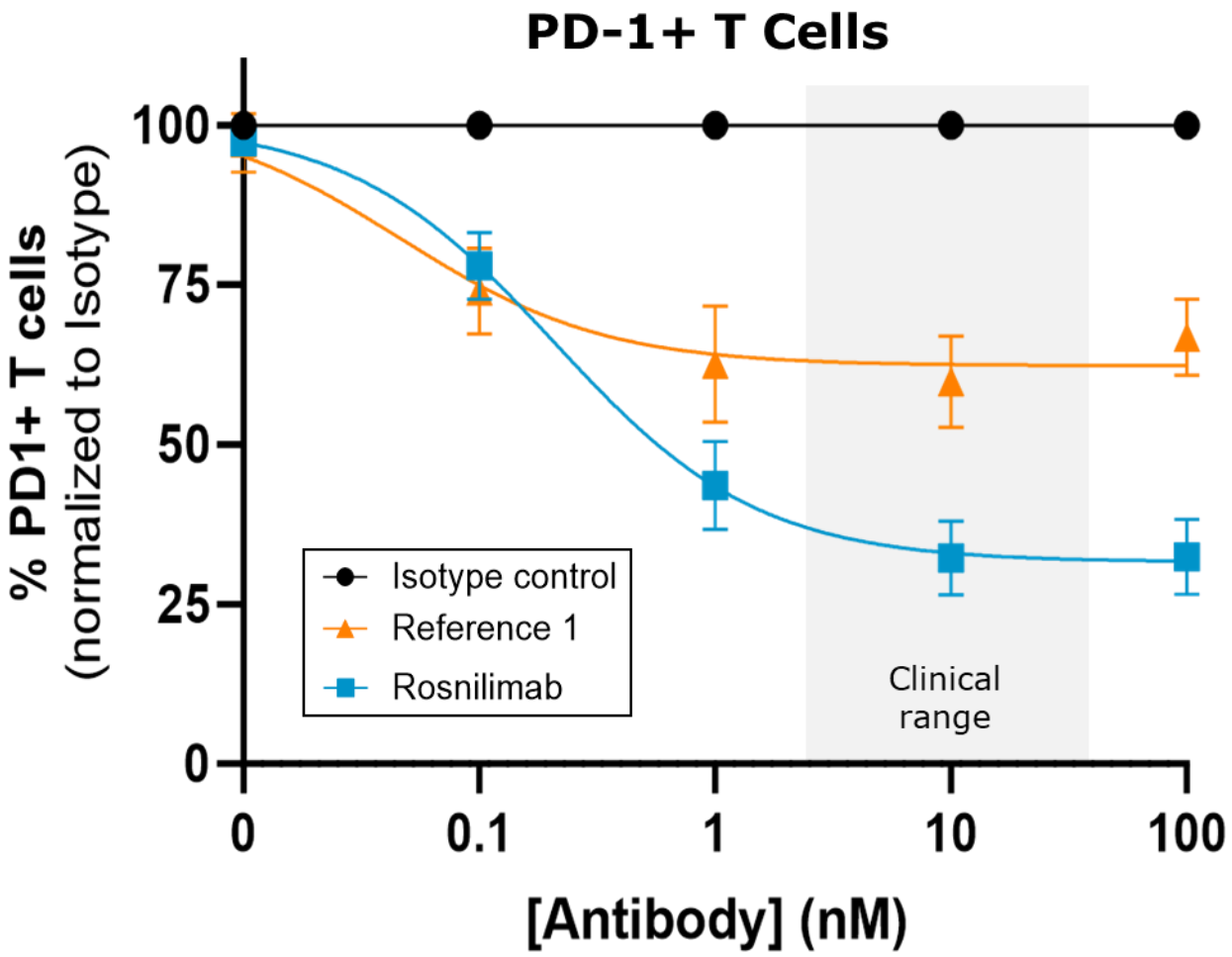
Suzuki et al., Sci. Immunol. 8, eadd4947 (2023) 13 January 2023

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



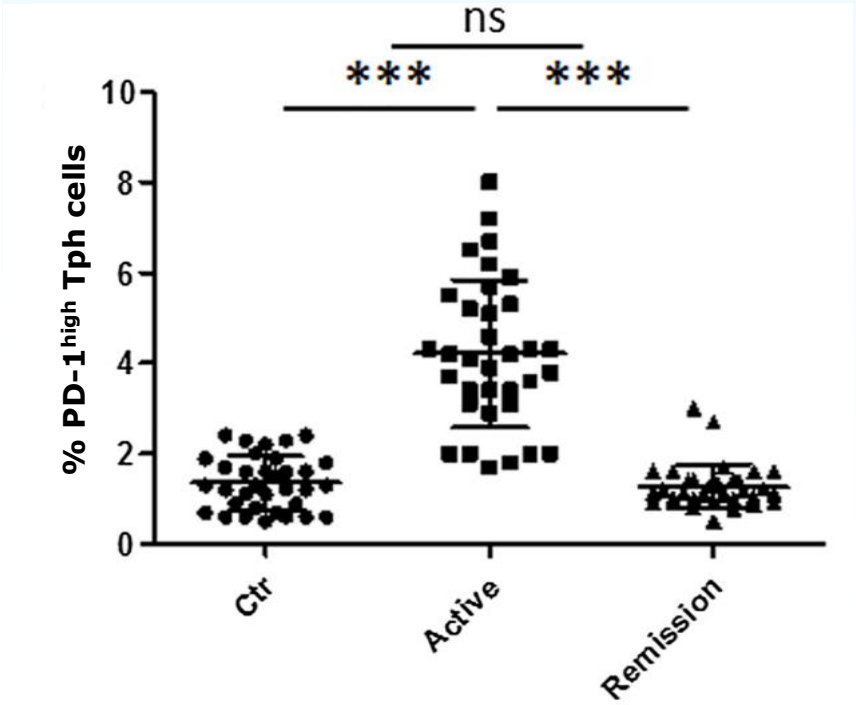
Test Article	PD-1 Membrane Binding	PD-1+ T Cell Reduction*
Reference 1	Distal	~40%
Rosnilimab	Proximal	**~70%**

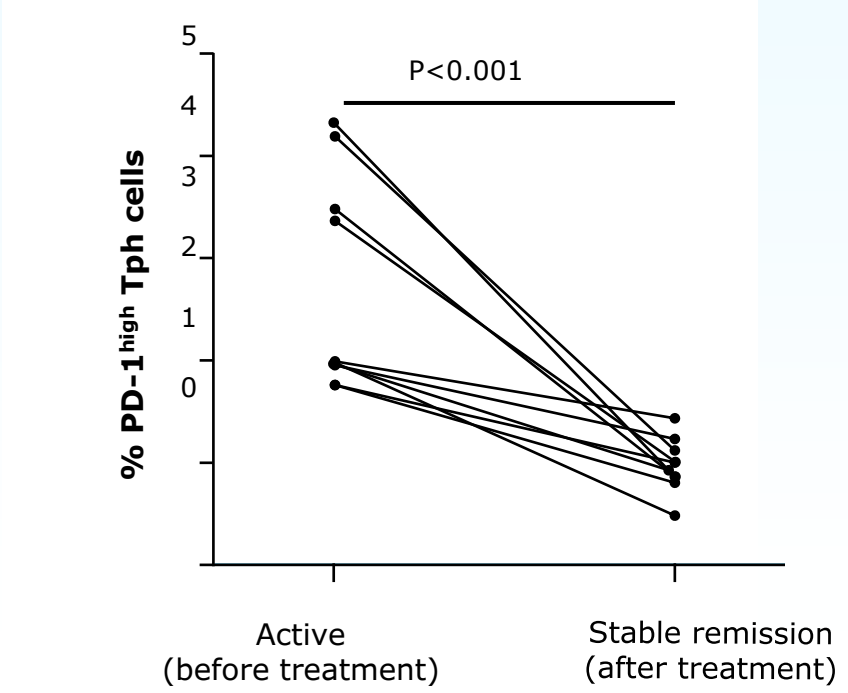
*Compared to isotype control

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

Reduction of Elevated PD-1 high Tph Cells Correlates with Remission

PD-1 high Tph cells are reduced with remission





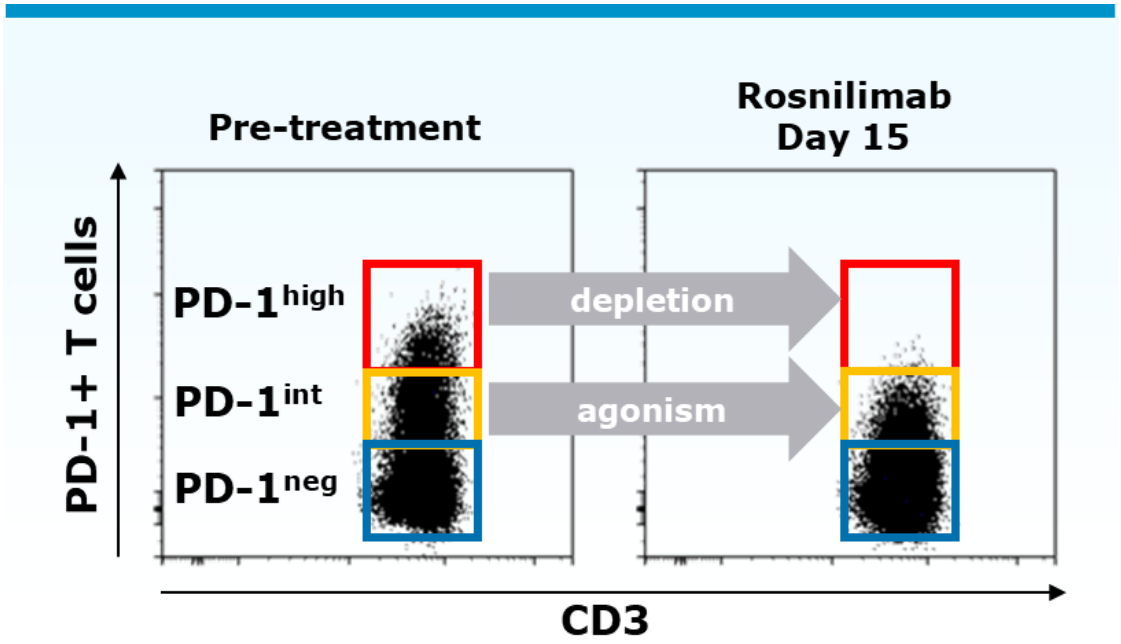
Reduction of plasma cell generation & autoantibody levels, including anti-microbial IgG antibodies contributing to colonic inflammation and barrier disruption

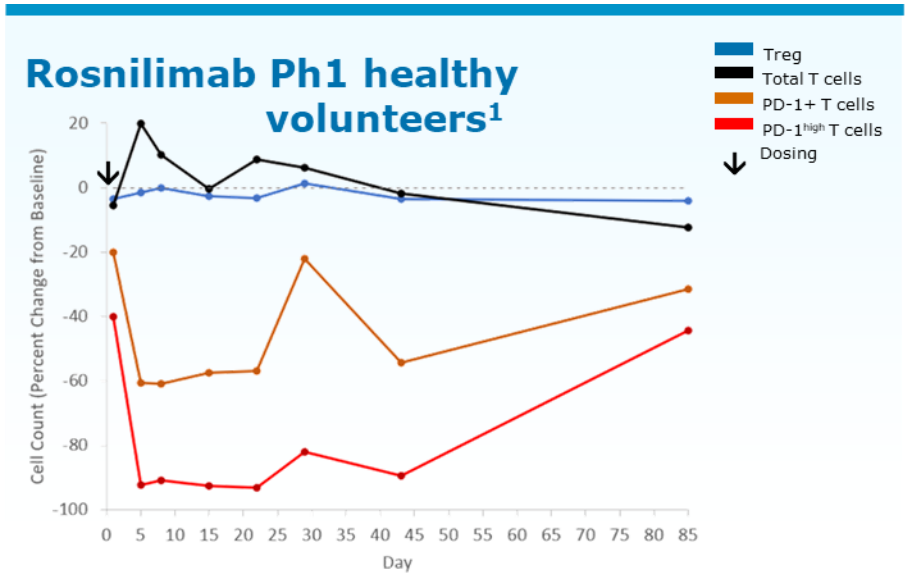
PD-1^{high} Tph cells defined by CD3+CD4+CD45RA-PD-1+TIGIT+ICOS+CXCR5-). Long et al, Immunology Letters 233 (2021) 2-10, *** p<0.001.

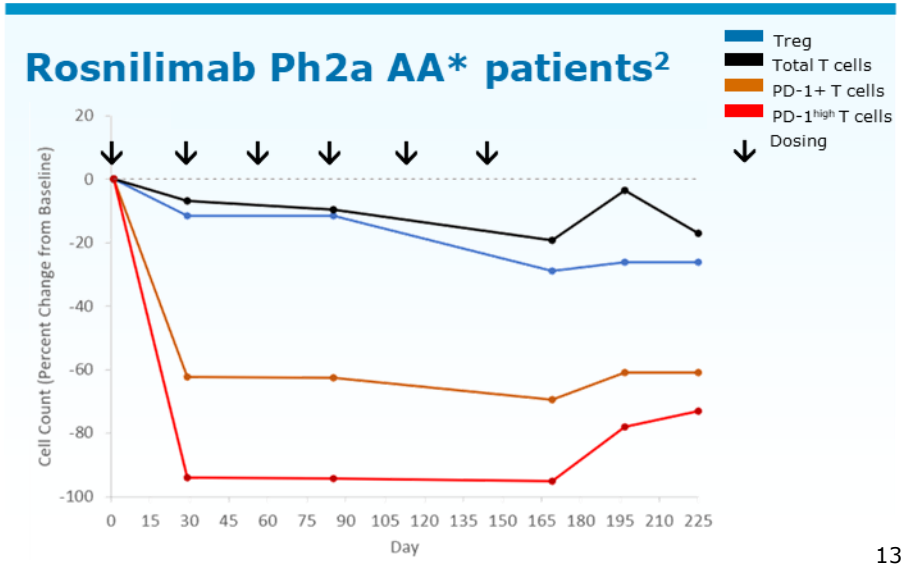
Rosnilimab Demonstrated Reduction of Peripheral PD-1 high and PD-1+ T Cells in Humans

Safety, Tolerability, and PK

- Rosnilimab was well tolerated; no dose-limiting toxicities
- No carcinogenic events observed; no infection risk signal
- Favorable PK with 2 wk half-life in IV and SC injections
- Receptor occupancy increased in a dose-dependent manner and consistent with PK

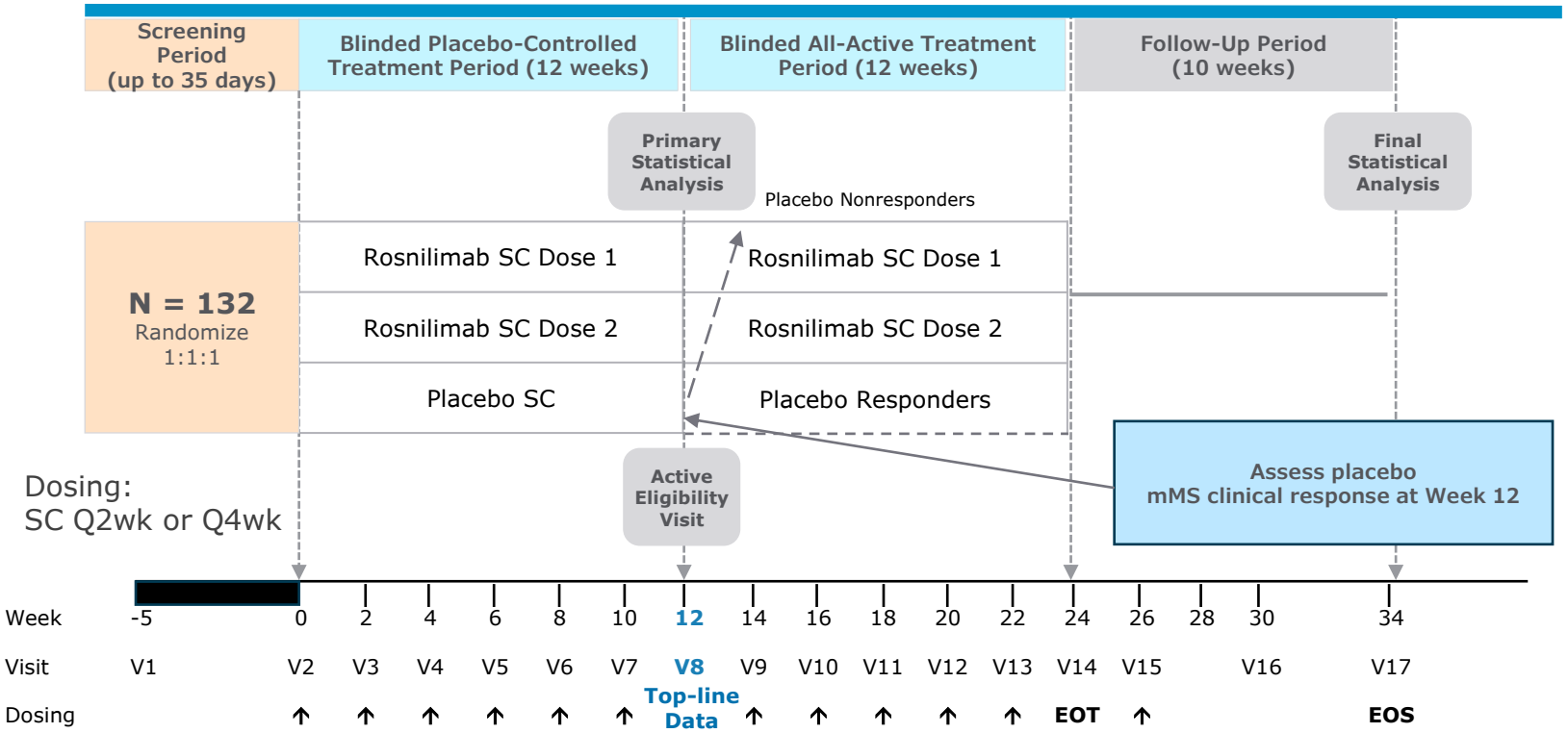






1. Luu, et al. Presented at the ECCO meeting, Stockholm, Feb 21-24 2024; 2. Data on file ; *AA=Alopecia Areata

ROSETTA:
Rosnilimab Phase 2 in Moderate-to-Severe UC



Patient population		• Adults with moderate-to-severe ulcerative colitis • Inadequate response to, loss of response to, or intolerance to as least 1 conventional or advanced UC therapy (~50% advanced UC therapy experienced)
Endpoints	Primary	• Mean change from Baseline in modified Mayo Score (mMS) at Week 12
	Secondary	• Clinical remission on mMS • Clinical response on mMS • Endoscopic remission • Mucosal healing
Exploratory endpoints		• Mean change from Baseline in colonic tissue and peripheral biomarkers

ClinicalTrials.gov Identifier: NCT06127043

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
- The class of PD-1 agonists, including rosnilimab, have not demonstrated increased safety risks in terms of infection or cancer, in numerous phase 1 and phase 2 studies
- These mechanistic data, translational in vivo and in vitro data, robust Phase 1 healthy volunteer data (see Su1784), and unmet needs in UC provide rationale for an ongoing global Phase 2 study of rosnilimab in participants with UC (NCT06127043)