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

Stephen Parmley, Benjamin Szlyk, Richard T. Frank, Matthew Hsu, Polina Brodsky, Cailin Sibley, Paul Lizzul, and Martin Dahl
AnaptysBio, Inc., San Diego, CA, USA

## ABSTRACT

**Background**: Checkpoint agonism represents a promising class of therapies for the treatment of autoimmune and inflammatory diseases, including ulcerative colitis (UC), where unmet needs persist despite available therapies. Binding to membrane proximal regions of checkpoint receptors, together with Fc interactions with receptors on opposing cells, can contribute to tight immune synapse formation between the immune cell and antigen presenting cell. This has been proposed to improve potency of agonistic signaling by excluding phosphatases such as CD45 from the immune synapse and promoting receptor clustering. Optimization of these characteristics results in improved agonism and depletion, with the potential for restoration of immune balance and broader clinical outcomes. Rosnilimab was engineered to leverage these important characteristics. It is a PD-1 agonist IgG1 antibody designed to optimize inhibitory signaling through the PD-1 receptor and to deplete PD-1$^{high}$ pathogenic T cells. Rosnilimab is in Phase 2 clinical development for UC and rheumatoid arthritis. **Methods**: Mutations to surface exposed regions of the PD-1 extracellular domain were made and surface plasmon resonance was used to infer the epitopes of PD-1 agonist molecules from resulting changes to binding. Membrane proximal and distal binding antibodies were studied in in vitro functional assays to assess T cell proliferation and antibody-dependent cellular toxicity. **Results**: Epitopes of agonistic antibodies were mapped to locations on the PD-1 receptor. The membrane proximal binding epitope of rosnilimab was confirmed and binding epitopes for other reference antibodies (ref) were identified. Rosnilimab and a membrane distally binding antibody (ref 1) were selected for comparison in functional assays. Rosnilimab demonstrated greater reduction of T cell proliferation and depletion of PD-1+ T cells compared to ref 1, consistent with the hypothesis that membrane proximal binding improves agonistic activity and target cell depletion. **Conclusion**: By targeting and leveraging inhibitory immune regulatory mechanisms to modulate the pathogenic T cells driving disease, there is an opportunity to dampen the inflammatory cycle and restore immune balance via agonism. Rosnilimab binds to a membrane proximal region of the PD-1 receptor, resulting in potent reduction of T cell proliferation and depletion of PD-1$^{high}$ T cells. These mechanistic data, translational in vivo and in vitro data, robust Phase 1 healthy volunteer data, and unmet needs in UC provide rationale for an ongoing global Phase 2 study of rosnilimab in UC (NCT06127043).

## BACKGROUND

- Programmed cell death protein 1 (PD-1), a T cell checkpoint receptor, functions to downregulate activated T cells by inducing negative signaling when engaged with its ligand PD-L1[1,2]
- PD-1 expressing T cells are elevated in the lamina propria of the inflamed colon and in the periphery in patients with ulcerative colitis (UC) vs. healthy controls, and thought to contribute to disease pathogenesis[3,4]
- Rosnilimab, a PD-1 agonist, IgG1 isotype monoclonal antibody, mimics the function of PD-L1 by inducing negative signaling on activated T cells resulting in reduction of T cell proliferation and reduction of inflammatory cytokine secretion (**Figure 1**)
- Binding to membrane proximal regions of checkpoint receptors such as PD-1, together with Fc interactions with receptors on opposing cells, can contribute to tight immune synapse formation between the immune cell and antigen presenting cell, inducing agonism and PD-1 clustering[5]

**Objective:** To demonstrate the contribution of the membrane proximal binding properties of rosnilimab, T cell proliferation and antibody-dependent cellular cytotoxicity (ADCC) in vitro assays were used to compare rosnilimab to a reference PD-1 agonist antibody (reference 1) with a more membrane distal binding epitope

### Rosnilimab optimizes PD-1+ T cell inhibitory signaling by enabling tight immune synapse formation

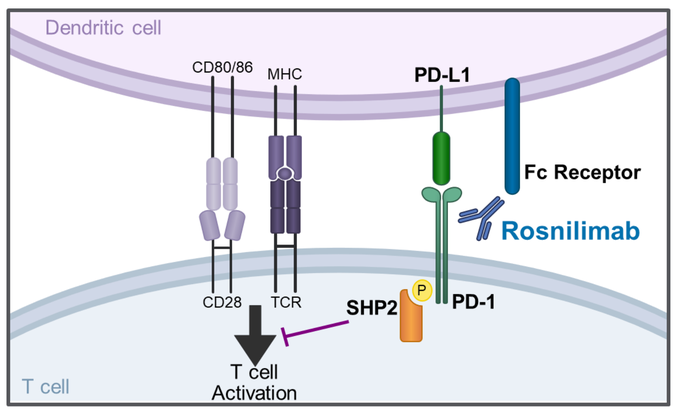


**Figure 1.** Proposed rosnilimab mechanism of action.

**Rosnilimab:**

- Depletes and agonizes PD-1+ T cells in inflamed tissue and in the periphery
- Does not block PD-L1 engagement
- Effector function is enabled via IgG1 isotype
- Potential to restore immune balance in numerous autoimmune and inflammatory indications

## METHODS

### Epitope Mapping

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 (**Figure 2**)

### T Cell Proliferation Analysis

Whole blood from healthy donors were sorted into T cells and monocytes. Monocytes were cultured with GM-CSF and IL-4 to polarize them to immature dendritic cells. Immature DC and autologous CFSE-labelled T cells were co-cultured in the presence of anti-CD3 antibody and anti-PD-1 antibodies or an IgG1 isotype control antibody. Proliferating cells were analyzed by FACS based on CFSE dilution (**Figure 3**)

### PD-1+ T Cell Analysis

Whole blood from healthy donors were sorted into T cells and NK cells and cultured with a cocktail of cytokines or IL-15, respectively. NK cells and T cells were co-cultured in the presence of anti-PD-1 antibodies or an IgG1 isotype control antibody. Surviving cells were analyzed by FACS for the presence of PD-1+ T cells (**Figure 4)**

## RESULTS

### Rosnilimab binds to a membrane proximal epitope of PD-1, distinct from the binding epitope of PD-L1 and the membrane distal binding epitope of reference 1

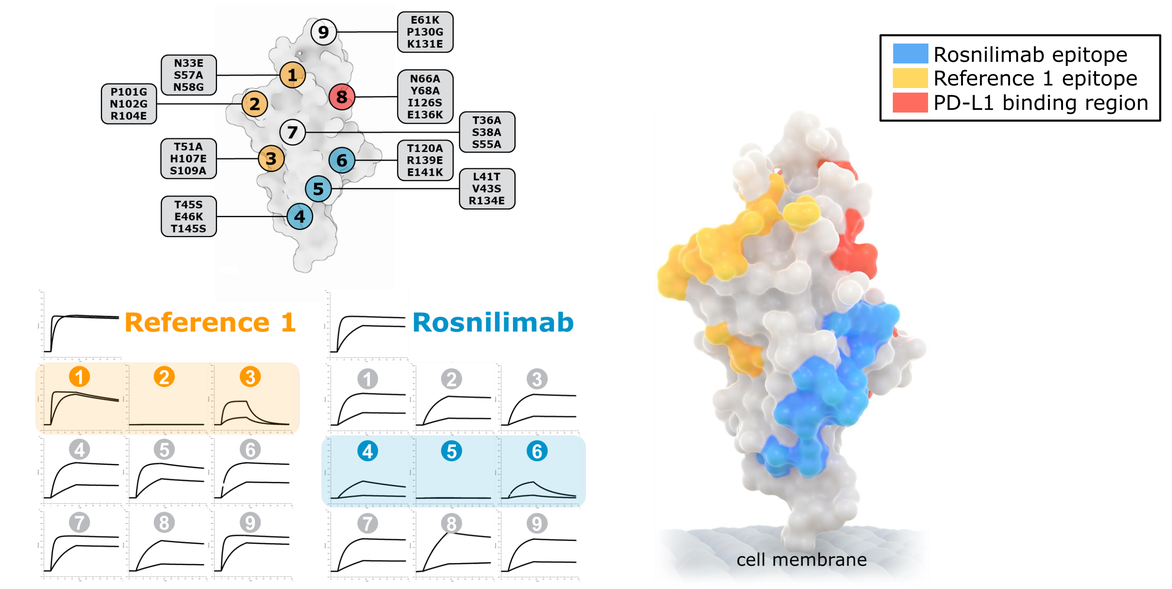


**Figure 2.** Epitope mapping of rosnilimab and reference 1. Changes in dissociation were attributed to weakened interaction and relevance to epitope was inferred.

### Agonism by membrane proximal binding rosnilimab more potently reduces T cell proliferation than the membrane distal binding reference 1

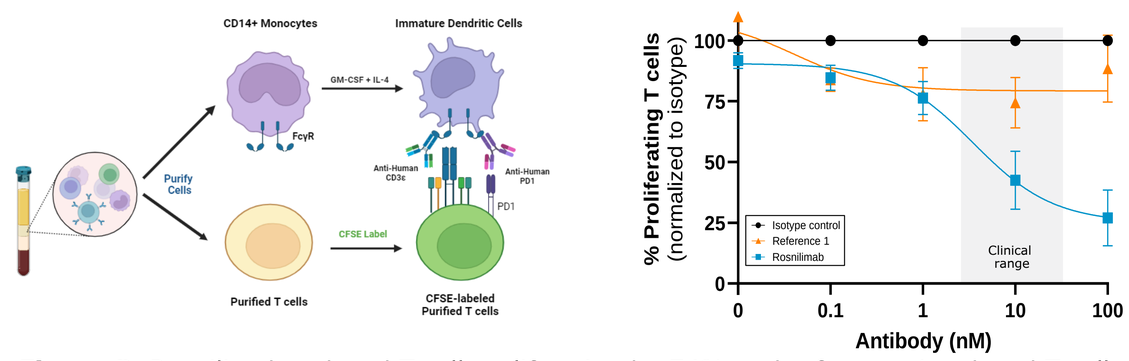


**Figure 3.** Rosnilimab reduced T cell proliferation by 74% and reference 1 reduced T cell proliferation by 20.8% when compared to isotype control.

### Depletion of PD-1$^{+}$ T cells by membrane proximal binding rosnilimab is more potent than the membrane distal binding reference 1

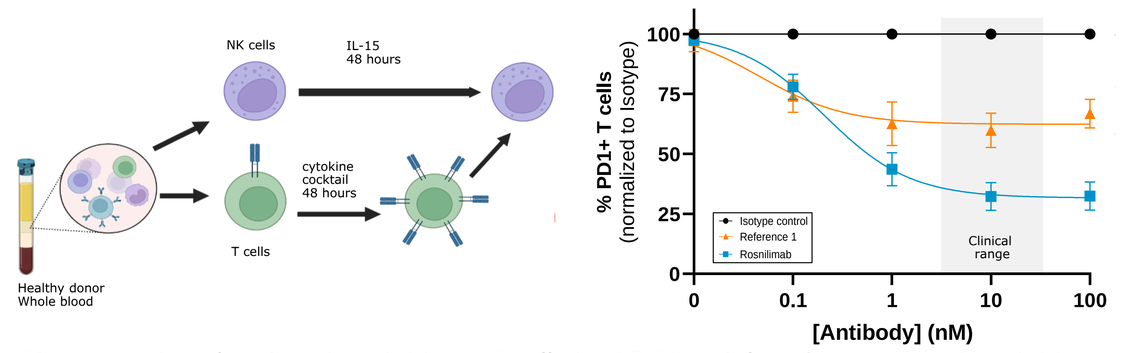


**Figure 4**. Rosnilimab reduced PD-1+ T cells by 68.5% while reference 1 reduced PD-1+ T cells by 37.7%. Isotype control did not mediate any depletion.

## 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[5] and enhance target cell depletion[6]
- These mechanistic data, translational *in vivo* and *in vitro* data, robust Phase 1 healthy volunteer data (*see DOP81*), and unmet needs in UC provide rationale for an ongoing global Phase 2 study of rosnilimab in participants with UC (NCT06127043)

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