TH121

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

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ABSTRACT

Checkpoint agonism represents a promising class of therapies for the treatment of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA) and ulcerative colitis (UC), where unmet needs persist despite available therapies. Rosnilimab is an investigational PD-1 agonist IgG1 antibody designed to optimize inhibitory signaling through the PD-1 receptor. Rosnilimab depletes PD-1high pathogenic T cells and agonizes remaining PD-1int T cells. Binding to membrane proximal regions of checkpoint receptors and simultaneous Fc interactions with Fc receptors on opposing cells contributes to immune synapse formation between the immune cell and the antigen presenting cell, leading to clustering and agonistic activity. Similar binding properties also optimize the potential for depletion of high PD-1 expressing T cells. The binding epitope of rosnilimab was mapped to a membrane proximal region of the PD-1 receptor, while the epitope of a reference antibody (ref1) was mapped to a membrane distal region. Rosnilimab demonstrated greater reduction of T cell proliferation, inflammatory cytokines and genes related to T cell activation, and depletion of PD-1+ T cells compared to ref1 in vitro, consistent with the hypothesis that membrane proximal binding improves agonistic activity and target cell depletion. 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).

BACKGROUND & OBJECTIVE

- 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-L[1,2]
- There is a high prevalence of PD-1+ T cells in inflamed tissue and periphery in rheumatoid arthritis (RA)[3,4] and ulcerative colitis (UC)[5,6]
- In addition, PD-1 pathway gene expression is dysregulated in the synovium of patients with RA[7] and in inflamed tissue from the colon in patients with UC[8]
- 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 PD-1 clustering and agonism[9]
- Rosnilimab depletes PD-1high T cells and agonizes remaining PD-1+ T cells in tissue and in the periphery (**Figure 2**)

Objective: To evaluate the contribution of the membrane proximal binding properties of rosnilimab when compared to a reference PD-1 agonist antibody with a membrane distal binding epitope (Reference 1) via T cell proliferation and antibody-dependent cellular cytotoxicity (ADCC) in vitro assays

Rosnilimab Optimizes PD-1+ T Cell Inhibitory Signaling by Enabling Tight Immune Synapse Formation

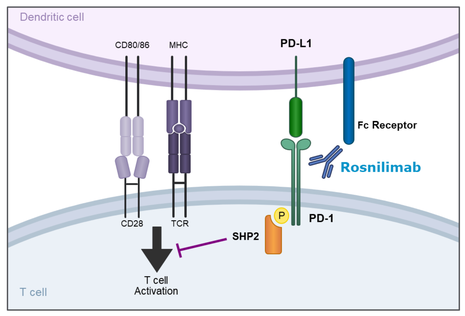


Figure 1. Proposed mechanism of action of rosnilimab

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

Rosnilimab has Dual Mechanisms of Depletion and PD-1 Agonism

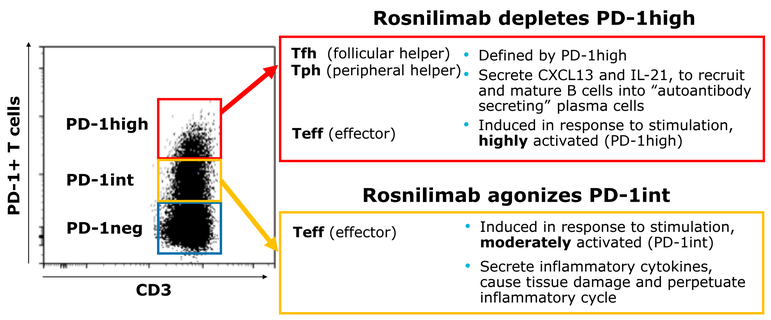


Figure 2. Summary of rosnilimab's effects on PD-1+ T cells

METHODS

Epitope Mapping
PD-1 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 3**)

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

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 PD-1 antibodies or an IgG1 isotype control antibody. Surviving cells were analyzed by FACS for the presence of PD-1+ T cells (**Figure 5**)

Gene Expression Analysis
Monocytes were purified from healthy donor peripheral blood mononuclear cells (PBMCs), polarized to DC, and co-cultured with isolated PanT cells in the presence of 30nM PD-1 antibodies or isotype control, and then cultured. Anti-CD3 antibodies were used to isolate CD4 and CD8 cell subsets for RNA extraction. Sequencing reads were aligned to the GRCh38 reference genome for quantification. Fold expression of select genes in antibody-treated samples was calculated versus isotype control (**Figure 6**)

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

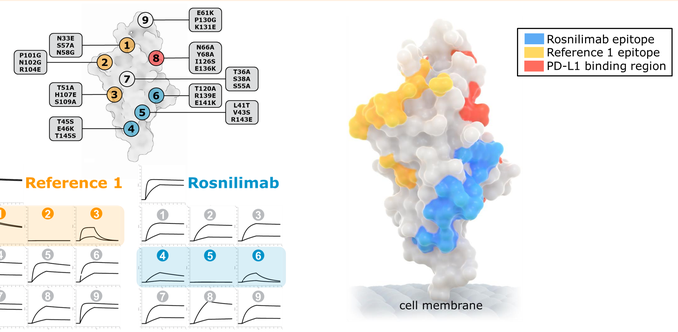
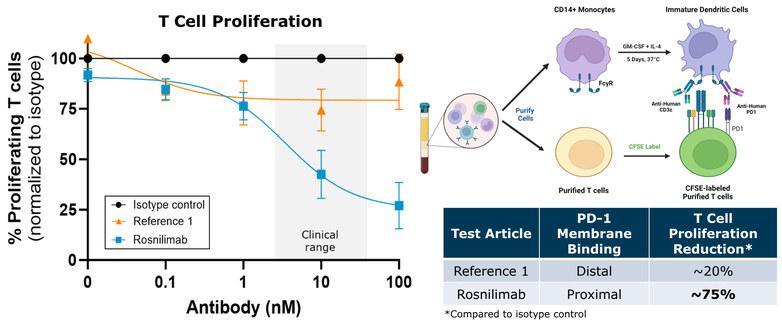


Figure 3. Epitope mapping of rosnilimab and Reference 1. Changes in dissociation were attributed to weakened interaction and relevance to epitope was inferred.

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

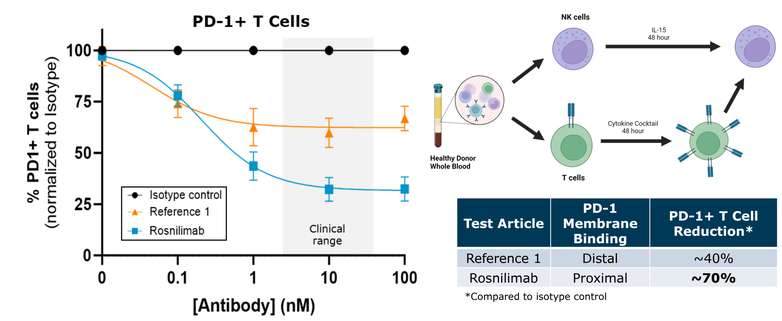


Test Article	PD-1 Membrane Binding	T Cell Proliferation Reduction*
Reference 1	Distal	~20%
Rosnilimab	Proximal	**~75%**

*Compared to isotype control

Figure 4. Rosnilimab reduced T cell proliferation by ~75% and Reference 1 reduced T cell proliferation by ~20% when compared to isotype control in an assay with no cells capable of mediating depletion

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

Figure 5. Rosnilimab reduced PD-1+ T cells by ~70% while Reference 1 reduced PD-1+ T cells by ~40%. Isotype control did not mediate any depletion

RESULTS

Membrane Proximal Binding Rosnilimab Decreased Expression of Genes Associated with T Cell Activation in DC-T cell Co-cultures

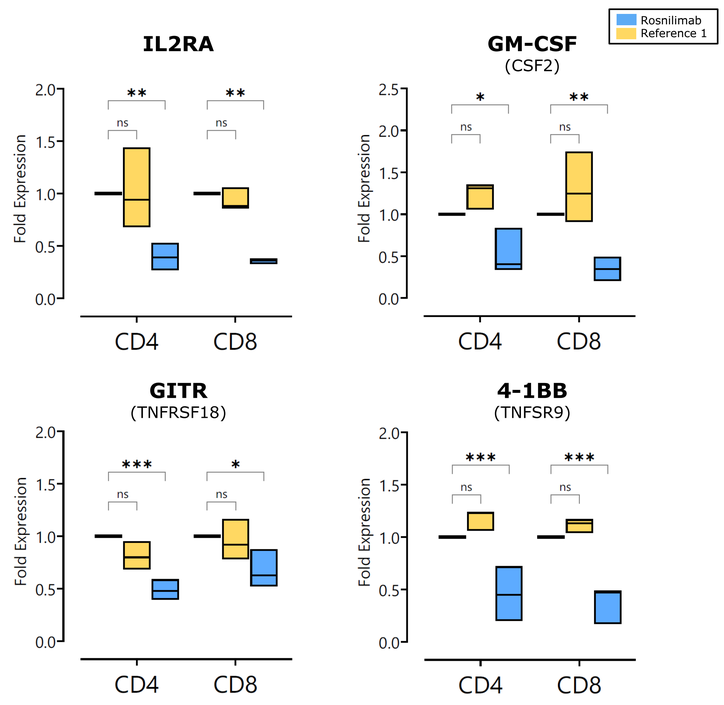


Figure 6. Expression of selected genes associated with T cell activation on CD4+ and CD8+ T Cells from DC-T cell co-cultures treated with PD-1 antibodies and quantified by RNAseq. Statistical analysis performed using ordinary two-way ANOVA followed by Dunnett's multiple comparisons test with four comparisons per gene and thresholds for significance relied on multiplicity adjusted P values.

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 demonstrated membrane proximal binding of PD-1 antibodies improved PD-1 agonistic activity[9] and enhanced target cell depletion[10]
- Proof of concept for PD-1 agonists have been demonstrated in RA
- These mechanistic data, translational in vivo and in vitro data, robust Phase 1 healthy volunteer data, and unmet needs in RA and UC provide rationale for an ongoing global Phase 2 study of rosnilimab in patients with RA (NCT06041269) and UC (NCT06127043)

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