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

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ABSTRACT

Background/Purpose: Checkpoint antagonist therapeutics have transformed the field of oncology while advancing the understanding of adequate checkpoint activity in preventing autoimmunity. Checkpoint agonism represents a promising and expanding class of therapies for the treatment of autoimmune diseases, including rheumatoid arthritis (RA), where unmet needs persist despite available therapies. The characteristics that confer agonistic properties to an antibody targeting a checkpoint receptor is an evolving science. Binding to membrane-proximal regions of suppressive 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 activating phosphatases from the immune synapse and promoting receptor clustering. Optimization of these characteristics results in improved agonism and depletion carrying the potential for restoration of immune balance and differentiated clinical efficacy. Rosnilimab has been engineered to leverage these important characteristics. It is a PD-1 agonist antibody with an IgG1 backbone designed to optimize inhibitory signaling through the PD-1 receptor and to deplete PD-1-high pathogenic T cells. It is currently in clinical development for RA and other inflammatory conditions. **Methods**: Mutations were targeted to surface exposed regions of the PD-1 extracellular domain where antibodies are likely to bind. Surface plasmon resonance of PD-1 structural mutants were used to locate the epitopes of PD-1 agonist molecules. Membrane-proximal and distal binding antibodies were studied in in vitro functional assays to assess T cell proliferation and antibody-dependent cellular cytotoxicity (ADCC). **Results**: Epitopes of agonistic antibodies were mapped to locations on the PD-1 receptor. The membrane-proximal epitope location of rosnilimab was confirmed and binding epitopes for other reference antibodies were identified. Rosnilimab and a membrane-distally binding antibody (Reference 1 Antibody) were selected for comparison in functional assays. Rosnilimab demonstrated better inhibition of T cell proliferation and depletion of PD-1+ T cells compared to Reference 1 Antibody, consistent with the hypothesis that membrane-proximal binding improves agonistic activity and target cell depletion. **Conclusion**: By therapeutically targeting and leveraging natural 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 optimized inhibition of T cell proliferation, inhibition of cytokine signaling, and PD-1-high T cell depletion. These mechanistic data, robust Phase 1 healthy volunteer safety, PK and translational PD data, and recognized persistent unmet needs in the treatment of RA provide rationale for an ongoing global Phase 2 dose-ranging study of rosnilimab in RA patients.

BACKGROUND/PURPOSE

- Programmed cell death protein 1 (PD-1), a T cell checkpoint receptor, functions to down regulate activated T cells by inducing a negative signaling pathway when engaged with its ligand PD-L1[1,2]
- PD-1 expressing T cells are elevated in the synovium and in the periphery, and PD-1 is a clinically validated target in the treatment of rheumatoid arthritis (RA)[3-4, 7]
- 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 in inflammatory cytokine secretion **(Figure 1)**
- Binding to a membrane-proximal region of suppressive receptors such as PD-1, together with Fc receptor engagement on an opposing cell, can contribute to tight immune synapse formation between an immune cell and an antigen presenting cell[5]
- To demonstrate 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

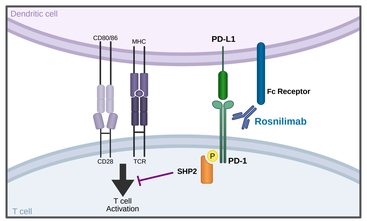

Figure 1. PD-1 pathway and rosnilimab proposed mechanism of action

Rosnilimab

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

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

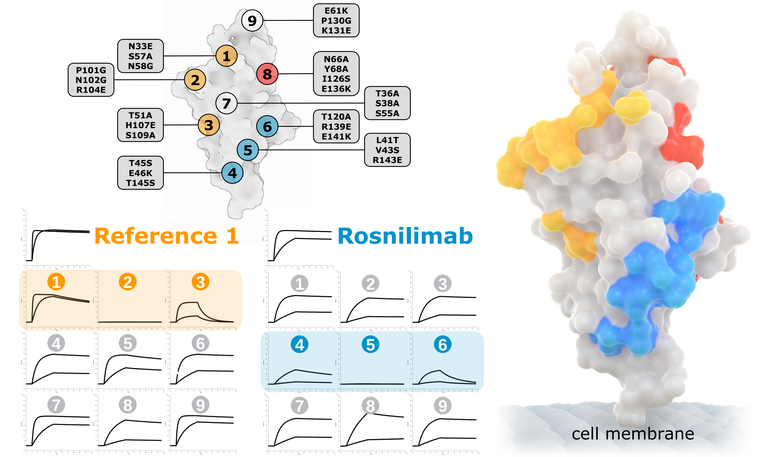

Figure 2. Anti-PD-1 agonist antibodies (rosnilimab or reference 1) were captured on independent flow cells using a Protein A biosensor (Cytiva) and were subjected to 50 and 500 nM wild-type or mutant human PD-1. Sensorgrams show the response after subtraction of both the reference flow cell as well as an injection of buffer over the active surfaces. Changes in dissociation were attributed to weakened interaction and relevance to epitope was inferred. PD-L1 binding region is indicated in red. Rosnilimab and reference 1 epitopes are indicated in blue and orange, respectively.

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

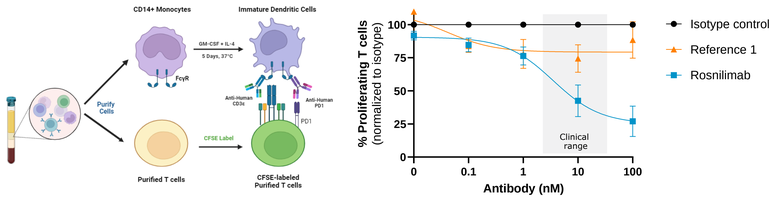

Figure 3. Whole blood from healthy donors was sorted into T cells and monocytes. Monocytes were polarized to immature dendritic cells by culturing for 5 days with GM-CSF and IL-4. Immature DC and autologous CFSE-labelled T cells were co-cultured in the presence of anti-CD3 antibody and anti-PD-1 antibodies or a IgG1 isotype control antibody. After 3 days, proliferating cells were analyzed by FACS based on CFSE dilution. Compared to isotype control, reference antibody 1 reduced T cell proliferation by 20.8%, while rosnilimab reduced T cell proliferation by 74.0%.

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

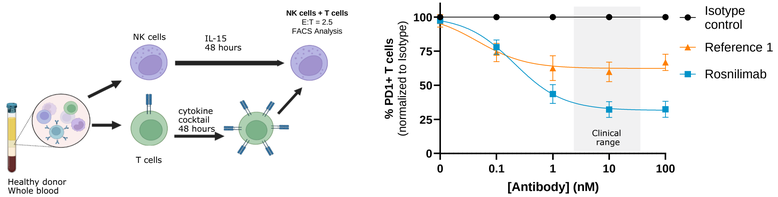

Figure 4. Whole blood from five healthy donors was sorted into T cells and NK cells and cultured for 48 hours with a cocktail of cytokines or IL-15, respectively. NK cells and T cells were co-cultured in the presence of anti-PD-1 antibodies or a IgG1 isotype control antibody at an effector:target ratio (E:T) of 2.5. Surviving cells were analyzed by FACS for the presence of PD-1+ T cells. Reference antibody 1 reduced PD-1+ T cells by 37.7%, while rosnilimab reduced PD-1+ T cells by 68.5%. 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
- These results are consistent with published studies that demonstrate membrane-proximal binding of PD-1 antibodies improves PD-1 agonistic activity[5] and enhances target cell depletion[6]
- These mechanistic data, robust Phase 1 data (see Luu, et al. ACR2023 poster 0455), and clinical validation of PD-1 agonism in RA[7] provide rationale for an ongoing global Phase 2 study of rosnilimab in RA patients (NCT06041269)

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