MP448

Rosnilimab, a PD-1 Agonist Antibody in Clinical Development for Ulcerative Colitis, Reduces Pathogenic PD-1+ T Cells and Inflammatory Cytokine Secretion in Patient Blood and in a Mouse Model of Colitis

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ABSTRACT

Introduction: Checkpoint agonism represents a promising class of therapies for the treatment of autoimmune and inflammatory diseases, including ulcerative colitis (UC), where unmet needs persist. Engagement of checkpoint receptor PD-1 by its natural ligand PD-L1 is critical for regulation of T cells. Rosnilimab is an investigational PD-1 agonist IgG1 antibody designed to optimize inhibitory signaling through PD-1, does not block PD-1 binding to PD-L1, and depletes PD-1high pathogenic T cells while agonizing remaining PD-1+ T cells. The percentages of PD-1+ T cells in the peripheral blood of moderate and severe UC patients are higher than in mild UC patients (1-4). Reduction of elevated PD-1high Tph cells in both UC colon and peripheral blood correlates with remission (2). Therapeutic reduction of PD-1high Tph cells and inflammatory cytokines from PD-1+ T cells, by depletion and agonism signaling together, has the potential to reduce overactive immune responses and induce remission. **Aims & Methods:** The potency of the agonism and depletion mechanisms of rosnilimab was evaluated in vitro using peripheral blood mononuclear cells (PBMCs) from UC patients. Rosnilimab is a human IgG1 antibody with Fc effector function. A version of rosnilimab that lacks Fc effector function was generated by mutating the IgG1 Fc domain at L234A and L235A (LALA). PBMCs from UC patients were activated with anti-CD3 and anti-CD28, cultured for several days with rosnilimab IgG1, rosnilimab IgG1-LALA, or isotype control and analyzed for the number of PD-1+ T cells and levels of inflammatory cytokines. In addition, the therapeutic potential of depletion and agonism of pathogenic PD-1+ T cells was evaluated in a mouse model of UC using a surrogate of rosnilimab. The surrogate consists of the fab domains of rosnilimab formatted with a mouse IgG2a Fc domain which enables immune cell depletion in mice. On Day 0, hPD1 CD4+CD45Rb+ donor T cells were adoptively transferred into Rag2-/- recipients to elicit UC-like disease. Starting on Day 21, 10 mpk of isotype control mIgG2a, rosnilimab-mIgG2a, or control anti-mIL-12 p40 were dosed intraperitoneally twice weekly for 4 weeks. This model system allowed assessment of the therapeutic potential of depletion and agonism mechanisms of rosnilimab on hPD1+ T cells, with a depletion-enabled mouse IgG2a isotype, in the context of T cell-driven gut inflammation and pathology. **Results:** In PBMCs from UC patients, at therapeutically relevant concentrations, rosnilimab IgG1 reduced PD-1high T cells by 90%, while rosnilimab IgG1-LALA reduced PD-1high T cells by 14%. Isotype control did not reduce T cells. Furthermore, rosnilimab IgG1 reduced secretion of inflammatory cytokines interferon gamma (IFN-γ) and CXCL13 compared to rosnilimab IgG1-LALA or isotype control. In the hPD-1 mouse model of UC, rosnilimab mIgG2a reduced body weight loss, colon pathology, IFN-γ and CXCL13 as assessed by spatial transcriptomics of the colon, and infiltration of hPD-1 CD4+ T cells into the colon as assessed by immunofluorescence, compared to isotype control. In addition, rosnilimab mIgG2a demonstrated efficacy comparable to anti-IL-12 p40 antibody treatment. **Conclusions:** Rosnilimab, a PD-1 checkpoint agonist antibody, functions to reduce pathogenic PD-1+ T cells by both depletion and agonism. These in vivo and in vitro results are consistent with the mechanisms of depletion and agonism and support the strong rationale for evaluating the therapeutic potential of rosnilimab in an ongoing phase 2 study in UC (NCT06127043).

BACKGROUND & OBJECTIVE

PD-1 Pathway is Dysregulated in Ulcerative Colitis (UC)

- Programmed cell death protein 1 (PD-1) is a coinhibitory receptor that reduces the activation status of T cells when engaged with its ligand PD-L1[1-3]
- High expressing PD-1+ T cells (PD-1^{high}) represent dysregulated pathogenic cells that are elevated in the gut lamina propria and in the periphery in UC[4-6]
- Reduction of elevated PD-1^{high} Tph cells correlates with remission in UC[7]

Rosnilimab (PD-1 agonist, IgG1)

- Mechanism of action and proposed impact on PD-1+ T cells (**Fig. 1**):
  - Depletion of PD-1^{high} Teff, Tfh, and Tph cells and agonism of remaining moderately activated PD-1+ T cells resulting in:
    - Reduced pathogenic T cell migration, proliferation, and inflammatory cytokine secretion (e.g. IFNγ)
    - Reduced Tfh and Tph-derived cytokines (IL-21 and CXCL13) preventing subsequent plasmablast & plasma cell generation and autoantibody levels
- Regulation through PD-1 may restore immune homeostasis in numerous autoimmune and inflammatory indications, including UC

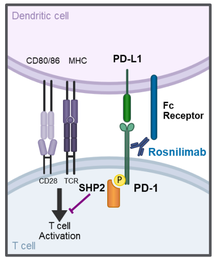


Figure 1. Proposed MoA of rosnilimab

Objective: Assess the therapeutic potential of PD-1 depletion and agonism mechanisms of rosnilimab in vitro using PBMCs from UC patients and in a mouse model of colitis

METHODS

In Vitro Evaluation of Rosnilimab's Depletion and Agonism Mechanisms in UC Patient-Derived Peripheral Blood Mononuclear Cells (PBMCs)

- A version of rosnilimab was generated to lack Fc effector function by mutating the IgG1 Fc domain at L234A and L235A (LALA)
- UC patient-derived PBMCs were activated with anti-CD3 and anti-CD28 and cultured with rosnilimab IgG1, rosnilimab IgG1-LALA, or isotype control
- PD-1+ T cells, proliferation and inflammatory cytokine levels (CXCL13, IL-21, IFNγ) were evaluated

Assessment of Rosnilimab Efficacy in a human PD-1 (hPD1) CD4 T Cell Transfer Murine Model of Colitis

- The fab domains of rosnilimab were formatted with a mouse IgG2a Fc domain (mIgG2a) to generate a surrogate which enabled immune cell depletion in mice
- On Day 0, hPD1 CD4+CD45Rb+ donor T cells (from hPD-1 transgenic mice) were adoptively transferred into Rag2-/- recipients. Body weight (BW) and clinical observations were recorded biweekly. BW loss was the determinant of colitis induction
- Starting on Day 21, 10 mpk of isotype control mIgG2a, rosnilimab mIgG2a, or control anti-mIL-12 p40 were dosed intraperitoneally twice weekly for 4 weeks
- Efficacy was evaluated by maintenance of body weight, distal colon histology scoring, immunofluorescence (IF) staining for hPD-1 CD4+ T cells, and gene expression analysis for inflammatory cytokines (Ifng and Cxcl13) via spatial transcriptomics and bulk RNAseq at Day 49

RESULTS

Rosnilimab IgG1 Reduced PD-1^{high} T Cells and Inflammatory Cytokine Secretion in UC Patient-Derived PBMCs

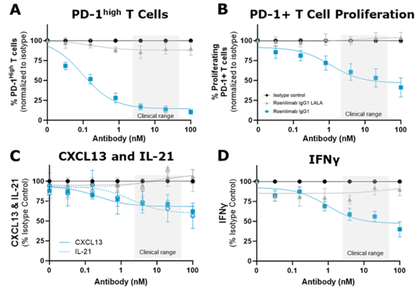
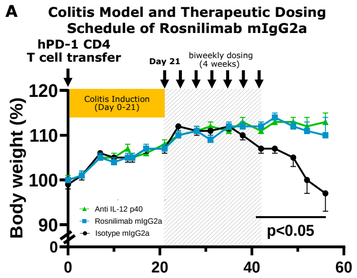


Figure 2. In vitro effect of rosnilimab on PD-1^{high} T cells (A), PD-1+ T cell proliferation, and secretion of inflammatory cytokines (C,D) from UC patient-derived PBMCs

In UC patient-derived PBMCs:

- Rosnilimab IgG1 reduced PD-1^{high} T cells by 90% and rosnilimab IgG1-LALA reduced PD-1^{high} T cells by 14% when compared to isotype control (**Fig. 2A**) Rosnilimab IgG1 also reduced proliferating PD-1+ T cells while rosnilimab IgG1-LALA had no effect on proliferation, confirming the important role of FcR engagement via the IgG1 domain of rosnilimab (**Fig. 2B**)
- Rosnilimab IgG1 reduced secretion of inflammatory cytokines CXCL13, IL-21 (**Fig. 2C**) and IFNγ (**Fig. 2D**) when compared to rosnilimab IgG1-LALA or isotype control

Therapeutic Dosing of Rosnilimab mIgG2a Demonstrated Efficacy in a Murine Model of Colitis



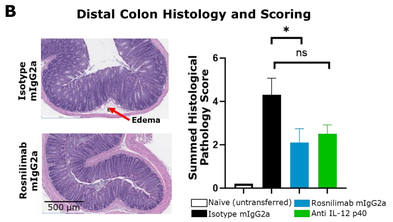


Figure 3. Schematic of colitis induction and treatment schedule (A) and evaluation of colon morphological pathology via H&E staining and scoring of the distal colon at Day 49 (composite measurement of inflammation, hyperplasia, gland loss, and erosion)

- Rosnilimab mIgG2a maintained body weight when dosed in a therapeutic regimen, compared to isotype control treated mice (**Fig. 3A**)
- Rosnilimab mIgG2a significantly reduced colonic inflammation measured by histological pathology score, compared to isotype control treated mice (**Fig. 3B**)

RESULTS

Rosnilimab mIgG2a Significantly Reduced CD4+ T Cell Infiltration into the Mouse Colon

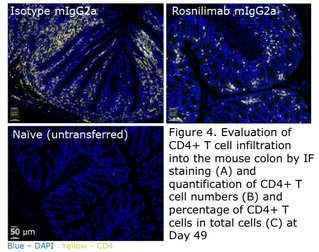
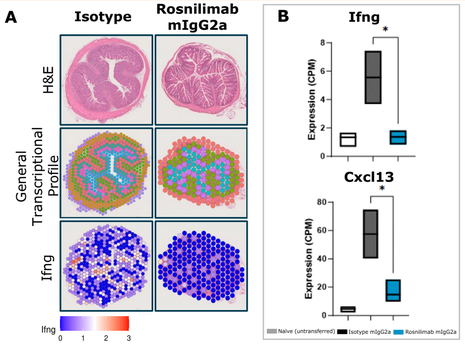


Figure 4. Evaluation of CD4+ T cell infiltration into the mouse colon by IF staining (A) and quantification of CD4+ T cell numbers (B) and percentage of CD4+ T cells in total cells (C) at Day 49

- Rosnilimab mIgG2a significantly reduced the number and percentage of CD4+ T cells in the colon in a mouse model of colitis (**Fig. 4**)

Rosnilimab mIgG2a Significantly Reduced Gene Expression of Ifng and Cxcl13 in the Mouse Colon



- Analyses of colonic gene expression using spatial transcriptomics demonstrated reduced Ifng with rosnilimab mIgG2a treatment compared to isotype control treatment (**Fig. 5A**)
- Inflammatory cytokine gene expression (Ifng and Cxcl13) was significantly reduced with rosnilimab mIgG2a treatment compared to isotype control treatment (**Fig. 5B**)

Figure 5. Spatial information of colons across treatments were mapped for differential gene expression (A) and evaluated for Ifng and Cxcl13 gene expression via bulk RNA-seq (B) at Day 49

CONCLUSION

- Rosnilimab reduced PD-1^{high} T cells and inflammatory cytokine secretion in UC patient-derived PBMCs in vitro
- In a murine model of colitis, at Day 49, rosnilimab mIgG2a:
  - Demonstrated efficacy with a therapeutic dosing regimen
  - Significantly reduced colonic inflammation measured by histology
  - Reduced CD4+ T cell infiltration, and Ifng and Cxcl13 expression
- These data, combined with results from a Phase 1 healthy volunteer study, support the rationale for evaluating rosnilimab in moderate-to-severe UC in an ongoing Phase 2 study (NCT06127043)

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