PP0358

Rosnilimab, a Selective and Potent Depleter of Pathogenic T Cells, Downregulated an Inflammatory Myeloid Gene Signature in a Murine Model of Colitis that was Upregulated in Human UC Colon Tissue

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BACKGROUND & OBJECTIVE

Pathogenic T Cells and Ulcerative Colitis (UC)

- In UC lamina propria, multiple T cell subtypes are highly inflammatory and implicated in the pathogenesis of UC[1], including T peripheral helper cells (Tph) and T effector memory cells
  - Tfh/Tph cells drive the recruitment and activation/maturation of B cells to differentiate into autoantibody producing cells. Downstream B cell activity and autoantibody secretion further perpetuate and contribute to the inflammatory cascade
  - The reduction of Tph cells has been shown to correlate with remission in UC[2]

Rosnilimab

Mechanism of action and proposed impact on pathogenic T cells (Fig. 1):

- Selective and potent depletion of pathogenic Tfh/Tph and Teff cells resulting in:
  - Reduced cell numbers, proliferation, migration, and inflammatory cytokine secretion (e.g. IFNγ) of these target cells
  - Reduced Tfh and Tph-derived cytokines (IL-21 and CXCL13)
- In a murine model of colitis, mice treated with rosnilimab showed reduced body weight loss, reduced inflammation of the colon, and reduced infiltration of CD4+ T cells[3]

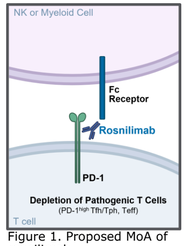


Figure 1. Proposed MoA of rosnilimab

Objective: To derive a myeloid gene signature in a murine model of colitis and evaluate the therapeutic impact of rosnilimab on genes associated with myeloid activation

METHODS

Previously reported study of rosnilimab in a human PD-1 (hPD1) CD4+ T Cell transfer murine model of Colitis (Fig. 2)

- Rosnilimab was 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
- Starting on Day 21, isotype control mIgG2a or rosnilimab mIgG2a, were dosed intraperitoneally twice weekly for 4 weeks

Derived human UC myeloid gene signature

- A UC myeloid gene signature was derived via secondary analyses of single-cell RNA sequencing data from a published atlas of human UC tissue[4] to identify 1,652 genes that were upregulated in inflamed myeloid cells compared to non-inflamed cells (Fig. 3A)

Derived myeloid gene signature in a murine model of colitis and evaluation of therapeutic impact of rosnilimab

- Bulk RNA-sequencing of mouse colonic tissue and subsequent gene expression and pathway enrichment analyses were performed to assess overlap with inflammatory myeloid genes involved in human UC (Fig. 3B)
- Overlapping genes and myeloid cell gene transcription from mouse colonic tissue were analyzed for regulation following rosnilimab treatment via spatial transcriptomics
- Spatial transcriptomics was performed using 2 mice (with 2 fragment) per condition (isotype control and rosnilimab treated). Macrophage cell types were identified in each Visium spot via annotation from Hong et. al[5]

RESULTS

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

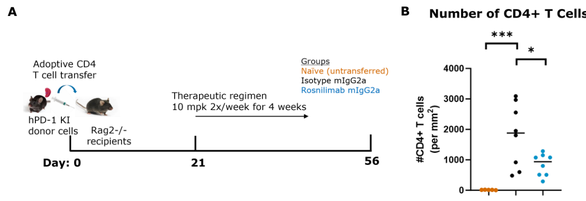
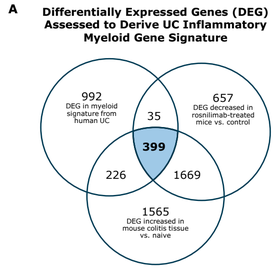


Figure 2. Schematic of colitis disease induction and treatment schedule (A) and quantification of CD4+ T cell numbers at Day 49 (B)

RESULTS

Inflammatory Myeloid Genes Upregulated in Human UC and in a Mouse Model of Colitis are Reduced When Treated with Rosnilimab mIgG2a



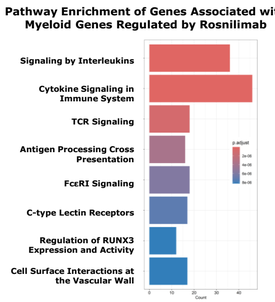
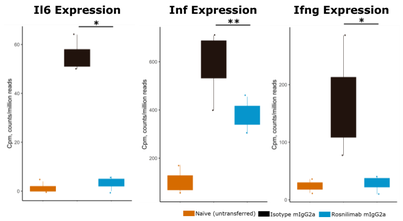


Figure 3. In a murine model of colitis, rosnilimab modulated 399 genes that overlap with a human UC inflammatory myeloid gene signature derived from human UC single cell analysis and mouse colitis colon genes (A). The corresponding genes were used to identify the top 8 most statistically significant Reactome pathways that were enriched for the rosnilimab myeloid signature. The color indicates the adjusted p-value and the count indicates the number of genes in the geneset (B)

- A myeloid gene signature of 399 genes was derived from the intersection of inflamed myeloid signature from human UC datasets[4], genes upregulated in mouse colon tissue, and genes downregulated in mice treated with rosnilimab (Fig. 3A)
- Of the 399 myeloid genes, genes involved in pathways including interleukin signaling, TCR signaling, and antigen cross presentation were prevalent (Fig. 3B)

Therapeutic Dosing of Rosnilimab mIgG2a Reduced Inflammatory Cytokine Gene Expression Associated with T Cell Activation

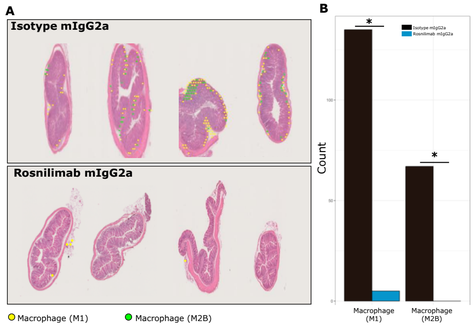


In a murine model of colitis, mice treated with rosnilimab compared to isotype control (Fig. 4):

- Significantly reduced Il6 and Tnf expression, which are predominantly produced by macrophages and antigen presenting cells
- Significantly reduced Ifng, a T cell cytokine involved in macrophage activation

Figure 4. The expression levels (Cpm, counts per million reads) of Il6, Tnf, and Ifng are shown in either naïve (non-disease control), isotype, or rosnilimab treated colitis tissue. N=3 for each group.; *adjusted p-value < 0.1, **adjusted p-value <0.05 for limma-voom analyzed data

Rosnilimab mIgG2a Reduced the Number of M1 and M2B Infiltrating Macrophages in Murine Colon Tissue During Colitis



- Rosnilimab treatment in a murine model of colitis significantly reduced the number of infiltrating M1 and M2B macrophage cells in colon tissue (Fig. 5)

Figure 5. 10X Visium spatial gene expression was performed on 2 replicates of 2 tissue fragments from isotype (control) or rosnilimab treated mice at day 56 post-transfer. Each spot is 55µm in diameter and contains 5-10 cells, indicating macrophage M1 (yellow) or M2B (green) are the predominant cell type (A) and was quantified (B). *adj. p value <1e-10

Rosnilimab mIgG2a Treatment Reduced Inflammatory Myeloid Gene Signature in a Murine Model of Colitis

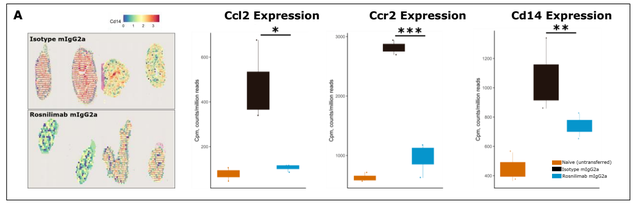
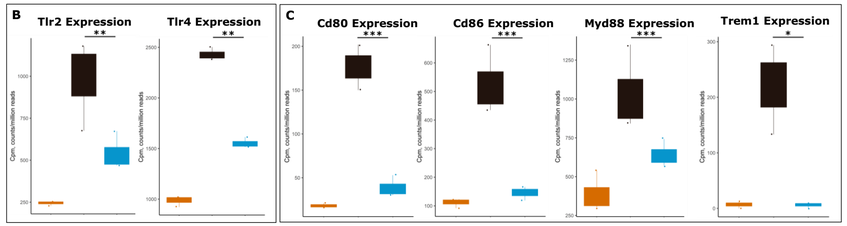


Figure 6. Spatial analyses of relative Cd14 expression from colon tissue of mice treated with isotype control or rosnilimab, where red and blue indicate high and low expression, respectively (A). Bulk RNA-seq analyses of genes involved in macrophage and inflammatory monocyte recruitment (A), pathogen detection (B), and immune amplification (C) from colon tissue of naïve, isotype, or rosnilimab treated mice



In a murine model of colitis, treatment with rosnilimab decreased inflammatory myeloid genes that are known to be elevated in disease state, including:

- Macrophage recruiting chemokine (Ccl2) and its receptor, Ccr2 (Fig 6A), resulting in reduction marker of inflammatory monocytes (Cd14) that are recruited to active inflammatory tissue in human UC
- Pathogen detection genes Tlr2 and Tlr4 (Fig. 6B)
- Immune activation and amplification genes Cd80, Cd86, Myd88, and Trem1 (Fig 6C)

CONCLUSIONS

- In a murine model of colitis, treatment with rosnilimab:
  - reduced pathogenic T cell numbers in the colon
  - reduced the number of proinflammatory macrophages and the inflammatory myeloid gene signature in the colon
- These data support previous preclinical findings demonstrating the potential therapeutic benefits of selectively depleting pathogenic T cells to reduce inflammation in a mouse model of colitis
- These results, together with existing rosnilimab safety data in humans, support the scientific rationale for evaluating rosnilimab in an ongoing Phase 2 study in UC (NCT06127043)

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REFERENCES

1. Roosenboom B, et al. *Scand J Gastroenterol* 2021;56:671-79.
2. Long Y, et al. *Immunol Letters* 2021;233:2-10
3. Parmley S, et al. *United European Gastroenterol J* 2024;12:482 (Abstract MP448)
4. Thomas, T, et al. *Nat Immunol* 2024; 25:2152–2165
5. Hong, D, K, et al. *Commun Biol* 2024; 7:731

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