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Phenotyping of Human UC Colonic Tissue Reveals Inflammatory Pathway Gene Expression in PD-1+ Conventional and Regulatory T Cells Which Overlap with Those Regulated by Rosnilimab in a Mouse Model of Colitis

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BACKGROUND & OBJECTIVE

PD-1 and Ulcerative Colitis (UC)

- Activated T cells express PD-1 (e.g., PD-1+); those expressing high levels of PD-1 (e.g., PD-1^{high}) are highly inflammatory and implicated in the pathogenesis of UC[1]
- In UC lamina propria, multiple T cell subtypes express PD-1, including T peripheral helper cells (Tph) and T effector memory cells
  - The reduction of PD-1+ Tph cells has been shown to correlate with remission[2]
- In many inflammatory diseases, including IBD, regulatory T cells (Tregs) and specifically PD-1+ Tregs have been reported to be dysfunctional and demonstrate a proinflammatory phenotype[3,4]

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 PD-1+ T cells resulting in:
    - Reduced T cell migration, proliferation, and inflammatory cytokine secretion (e.g. IFNγ)
    - Reduced Tfh and Tph-derived cytokines (IL-21 and CXCL13)
- In a mouse model of colitis, mice treated with rosnilimab showed reduced body weight loss, reduced inflammation of the colon, and reduced infiltration of CD4 T cells[5]

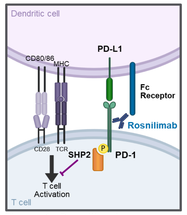


Figure 1. Rosnilimab proposed mechanism of action

Objective: Characterize PD-1+ T cell subsets (conventional T cells [Tcon] and Tregs) from active UC patient-derived colonic tissue and evaluate inflammatory pathways that may overlap with those regulated by rosnilimab in a mouse model of colitis

METHODS

Secondary analyses of active UC patient-derived colonic tissue for differential gene expression

- A published dataset of CD3-sorted single-cell proteomics and transcriptomics from UC patients (N=14)[6] was used to characterize PD-1+ CD4 Tcon and Tregs
- Tcon and Treg (PD-1+ and PD-1 neg) pathway activity was estimated from gene expression via footprint analysis with Progeny[7]
- Treg pathway enrichment was performed with Gene Ontology (GO) 'Biological Processes' (BP) gene sets **(Fig. 2, Fig. 3)**

Gene expression and pathway enrichment analyses of colonic tissue from mice in human PD-1 (hPD1) CD4 T cell transfer murine model of colitis[5]:

- Bulk RNA-sequencing of mouse colonic tissue and subsequent gene expression and pathway enrichment analyses were performed to assess overlap with Reactome pathways involved in human UC[8], including barrier function,[9] fibrosis,[10] and inflammatory myeloid genes **(Fig. 4, Fig. 5)**
  - Treatment groups: naïve, isotype control mIgG2a, rosnilimab mIgG2a, or control anti-mIL-12 p40

RESULTS

Proinflammatory Genes were Upregulated in PD-1+ Tcon and PD-1+ Tregs Compared to PD-1 neg

- PD-1 protein expression analyses of T cells from UC patient lamina propria showed that 51% and 30% of Tcon and Tregs were PD-1+, respectively; both had increased JAK-STAT, TNFα, and NF-κB activity (not shown)
- Genes upregulated in PD-1+ Tcon and PD-1+ Tregs are associated with T cell activation and inflammatory pathways **(Fig. 2)**

PD-1+ T cells (Tcon and Tregs) Have Increased Expression of Genes Associated with Proinflammatory Processes

PD-1+ Tcon

Process Categories	GO Biological Processes
Regulation of Cell-Cell Adhesion	Leukocyte Cell-Cell Adhesion Regulation of Leukocyte Cell-Cell Adhesion
T Cell Regulation and Activation	Regulation of T Cell Activation Positive Regulation of Leukocyte Activation Positive Regulation of Cell Activation Positive Regulation of Lymphocyte Activation
Mononuclear Cell Proliferation and Regulation	Leukocyte Proliferation Mononuclear Cell Proliferation Lymphocyte Proliferation\ Regulation of Leukocyte Proliferation Regulation of Mononuclear Cell Proliferation
Mononuclear Cell Differentiation	Lymphocyte Differentiation Mononuclear Cell Differentiation
B Cell Activation	B Cell Activation
Type II Interferon Regulation and Production	Regulation of Type II Interferon Production Type II Interferon Production Positive Regulation of Type II Interferon Production
Positive Regulation of Cytokine Production	Positive Regulation Of Cytokine Production
Regulation of Immune Effector Process	Regulation of Immune Effector Process
Immune Response-Activation Signaling Pathway	Immune Response-Activation Signaling Pathway

PD-1+ Treg

Process Categories	GO Biological Processes
Regulation of Cell-Cell Adhesion	Regulation of Cell-Cell Adhesion Leukocyte Cell-Cell Adhesion Regulation of Leukocyte Cell-Cell Adhesion
T Cell Regulation and Activation	Regulation of T Cell Activation Positive Regulation of Leukocyte Activation Positive Regulation of Cell Activation Positive Regulation of Lymphocyte Activation
αβ T Cell Activation	CD4+, αβ T Cell Activation αβ T Cell Activation αβ T Cell Differentiation
αβ T Cell Regulation	αβ T Cell Regulation
Mononuclear Cell Differentiation	Lymphocyte Differentiation Mononuclear Cell Differentiation T Cell Differentiation
Type II Interferon Regulation and Production	Regulation of Type II Interferon Production Type II Interferon Production Positive Regulation of Type II Interferon Production
Positive Regulation of Cytokine Production	Positive Regulation Of Cytokine Production
Positive Regulation of Adaptive Immune Response	Positive Regulation of Adaptive Immune Response Regulation of Adaptive Immune Response

Figure 2. GO 'BP' pathway enrichment analyses of PD-1+ Tcon and Tregs. Top 20 pathways shown determined by statistical significance (fold change >1.5, adjusted p-value < .05)

RESULTS

PD-1+ Tregs in UC Colonic Tissue Expressed Higher Levels of Proinflammatory Genes

Selected Genes Differentially Regulated (PD-1+ vs PD-1 neg Tregs)

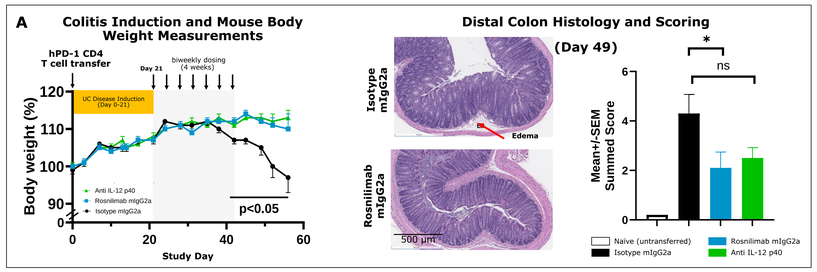
Gene	Function	Expression Level (PD-1+ Tregs)	log2FC	P value	Adjusted P value
IL17A	Inflammatory cytokine	↑	2.52	1.12E-08	1.65E-04
IL12RB2	Inflammatory cytokine receptor	↑	2.16	2.80E-20	4.10E-16
TNF	Inflammatory cytokine	↑	1.65	1.27E-28	1.86E-24
IL23R	Inflammatory cytokine receptor	↑	1.56	1.63E-08	2.38E-04
TBX21	Inflammatory transcription factor	↑	1.50	4.80E-06	7.03E-02
TGFB1	Pro-fibrotic/anti-inflammatory cytokine	↑	0.94	2.01E-10	2.94E-06
IL1R1	Inflammatory cytokine receptor	↑	0.70	1.48E-07	2.17E-03
SELL	Regulation of trafficking	↓	-1.11	3.95E-13	5.79E-09
IL2RA	Treg suppressive function	↓	-1.18	1.22E-14	1.79E-10
CCR7	Regulation of trafficking	↓	-1.86	1.10E-24	1.61E-20

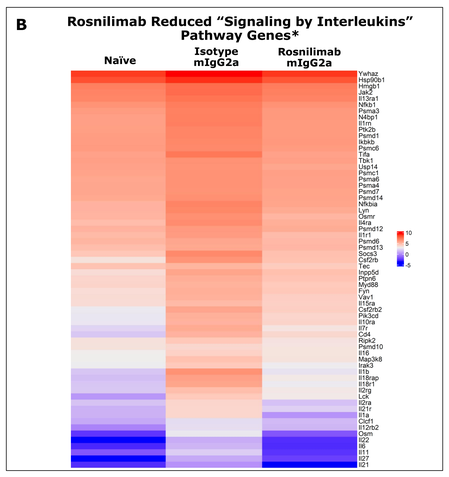
Figure 3. Panel of differential gene expression for key genes reported to be associated with dysregulated inflammatory Tregs or important for Treg suppressive function are shown in order of decreasing log2 FC (log2 fold change between PD-1+ and PD-1 neg Tregs)[4,11-13]

PD-1+ Tregs showed **(Fig. 3)**:

- Higher gene expression of several proinflammatory cytokines, cytokine receptors, and a transcription factor *Tbx21* (*Tbet*)
- Lower gene expression of surface molecules regulating trafficking and *IL2RA* (*CD25*), which contributes to Treg suppressive function

Genes in Pathways Involved in Human UC were Reduced to Naïve (non-T cell transferred) Levels with Rosnilimab Treatment in Murine Model of Colitis



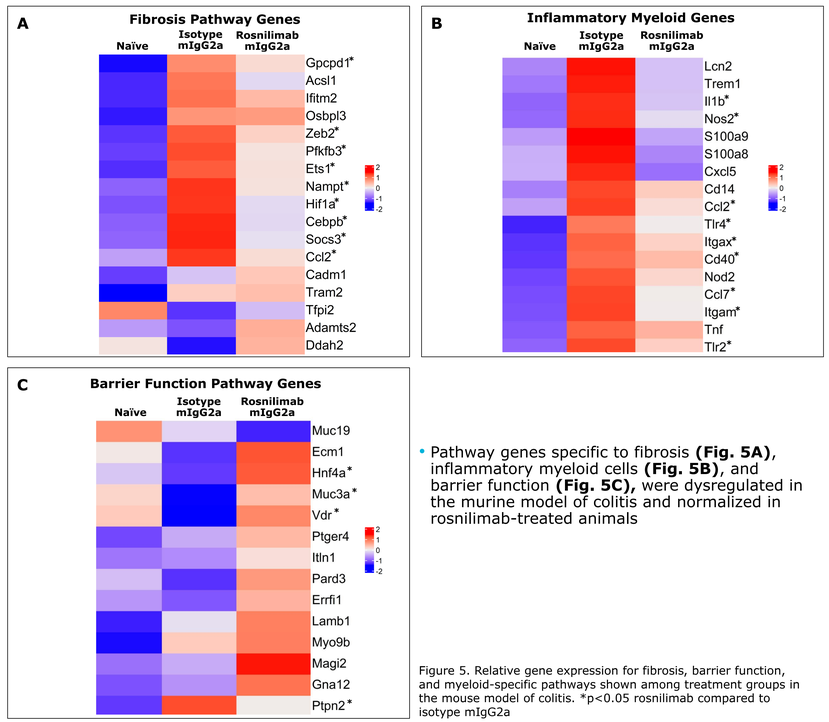


In a murine model of colitis:

- Rosnilimab mIgG2a maintained body weight and significantly reduced colonic inflammation when dosed in a therapeutic regimen, compared to isotype control treated mice **(Fig. 4A)**
- Genes in pathways involved in human UC ("Signaling by interleukins" (e.g., *Il6*) and "Chemokine receptors bind chemokines" (e.g., *Ccl20), not shown*) in mice treated with rosnilimab were comparable to naïve animals **(Fig. 4B)**

Figure 4. Schematic of colitis disease induction and treatment schedule 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) (A). Relative gene expression for "signaling by interleukins," shown among treatment groups in mouse model of colitis (B). *p<0.05 rosnilimab compared to isotype mIgG2a

Pathway Genes Specific to Fibrosis, Barrier Function, and Inflammatory Myeloid Cells were Normalized by Rosnilimab



- Pathway genes specific to fibrosis **(Fig. 5A)**, inflammatory myeloid cells **(Fig. 5B)**, and barrier function **(Fig. 5C),** were dysregulated in the murine model of colitis and normalized in rosnilimab-treated animals

Figure 5. Relative gene expression for fibrosis, barrier function, and myeloid-specific pathways shown among treatment groups in the mouse model of colitis. *p<0.05 rosnilimab compared to isotype mIgG2a

CONCLUSIONS

- In UC colon tissue, PD-1+ Tcon and Tregs had increased proinflammatory gene expression compared to respective PD-1 neg cells
- In a murine model of colitis, rosnilimab treatment normalized dysregulated pathways associated with human UC to naïve levels, consistent with previous preclinical findings
- Together, these data support the concept that PD-1+ Tcon and Tregs are proinflammatory and the ability of rosnilimab to reduce these cells, while also normalizing dysregulated fibrosis, barrier function, and inflammatory myeloid cell pathway gene signatures, may contribute to the potential therapeutic efficacy of rosnilimab in UC
- Combined with results from a Phase 1 healthy volunteer study, these data support the rationale for evaluating rosnilimab in moderate-to-severe UC in an ongoing Phase 2 study (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. Lord James D; *World J Gastroenterol* 2015;21:11236-45.
4. Yu Qi T., et al. *Inflamm Bowel Dis* 2007;13:191-9.
5. Parmley S, et al. *United European Gastroenterol J* 2024;12:482 (Abstract MP448).
6. Gupta et al. *Cancer Cell* 2024;42:797-814.
7. Schubert M, et al. *Nat Commun* 2018;9:20.
8. Linggi B, et al. *Sci Rep* 2021;11:18243.
9. McCole DF. *Inflamm Bowel Dis* 2014;20:1829-49.
10. Dovrolis N, et al. *Front Immunol* 2022;13:1058237.
11. Viglietta V, et al. *J Exp Med* 2004; 199:971-979.
12. Lowther DE, et al. *JCI Insight* 2016;1(5):e85935.
13. Kitz A, et al. *Cold Spring Harb Perspect Med* 2018;8:a029041.