RRx-001, a specific macrophage-targeted immunotherapeutic, orchestrates a Cd45+ anti-endometriosis impact

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Introduction/background

We previously demonstrated that RRx-001 (erythrophagoimmunotherapeutic with intrinsic hypoxic region targeting), reduces lesion growth, pain-like behaviour, and modifies peritoneal macrophages in a preclinical endometriosis mouse model. Here, we aimed to confirm specificity to disease-modified macrophages and determine the immune interactions in blood and peritoneal fluid that coordinate the anti-endometriosis impact.

Materials and Methods

We evaluated $Cd45^+$ cells isolated from a 'menses' mouse model of endometriosis using 10x scRNA-seq, to assess i) the specificity of RRx-001 by analysing immune populations in distal tissues (endometrium, liver, lungs and spleen) and ii) the impact of RRx-001 on immune populations and cell-cell interactions in peritoneal fluid and blood using communication inference. Experimental groups were: endometriosis mice treated with RRx-001 (i.v 2x weekly, 10mg/kg, n = 4) and vehicle (DMA-PEG400, n = 4).

Results

Single cell analyses using Seurat revealed 14,670 high-quality cells and all expected immune populations. RRx-001 treatment did not induce any pronounced off-target impacts on distal intra-organ immune composition. Specifically in the endometrium, RRx-001 did not modify NK, B cell or monocyte/macrophage proportions, and we observed no impact to endometrial macrophage gene expression, with <10 significant DEGs detected. Conversely, immune cell composition and macrophage populations were specifically modified within the peritoneal fluid (PF), with >3000 significant DEGs, and gene set enrichment analysis of gene ontology revealing enhanced establishment of lymphocyte polarity. We also detected corresponding changes to the peripheral blood immune composition including enhanced $Ly6c^+$ monocyte abundance and transcriptional modification (>200 significant DEGs). CellChat revealed RRx-001 modified $Cd45^+$ cell-cell communication only in the blood and PF.

Conclusion

Preclinically, RRx-001 exhibits specificity to disease-modified macrophages, orchestrates a macrophage-led anti-endometriosis immunological impact in PF and enhances circulating monocyte abundance. RRx-001 minimally impacts the endometrium, supporting our previous findings of unaffected fertility. Taken together, RRx-001 represents a promising new therapy for endometriosis and is soon to enter a clinical trial.

Key words

Therapeutic, transcriptomics, immunology