Molecular profiling of peripheral immune cell subsets in patients with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that affects 1% of the world’s population. Several key biological functions are dysregulated in RA, manifesting clinically as pain, fatigue, and synovitis, with articular destruction, organ-based comorbidities, and functional decline. Defining immune dysregulation in the peripheral blood of patients (pts) with RA will help inform future work to assess the extent to which immune homeostasis can be therapeutically achieved for these pts.

Objectives: To identify baseline molecular characteristics of the peripheral immune system, at the level of individual immune cell subsets, in pts with RA recruited to clinical trials of the oral, selective Janus kinase 1 (JAK1) inhibitor, filgotinib.

Methods: Peripheral blood mononuclear cells (PBMC) were collected from 324 pts with moderate to severely active RA, who had an inadequate response to methotrexate ([MTX], FINCH-1; NCT02889796; n=109) or who were MTX naïve (FINCH-3; NCT02886728; n=215). PBMC were also collected from 50 demographically matched healthy volunteers (HV). The Immune Profiler platform was used to sort PBMC into 24 immune cell subsets, then quantify their gene expression and chromatin accessibility using RNA-seq and the assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq), respectively. Differentially expressed genes (DEGs) and differentially accessible regions (DARs) were identified among immune cell subsets from pts with RA versus HV. Gene set signature scores of Molecular Signatures Database hallmark pathways were calculated using single sample gene set enrichment analysis (ssGSEA) to examine differences in pathway activity between groups.

Results: A total of 14,500 sequencing datasets were generated from the pt and HV immune cell subsets. Among these, over 62,000 DEGs and 410,000 DARs were identified in RA versus HV (false discovery rate <0.05) across the 24 immune cell subsets. DEGs were identified in all immune cell subsets tested and were most pronounced in natural killer (NK) subsets; most DARs were detected in myeloid and NK subsets. ssGSEA revealed differential pathway signaling in RA versus HV across multiple functions at the immune cell subset level. Myeloid subsets from pts with RA often showed elevated pathway activities versus HV whereas B, T and NK subsets showed a general decrease. In particular, monocyte populations from pts with RA versus HV had elevated pathway activities involved in inflammatory response and interleukin-6/Janus kinase/signal transducer and activator of transcription 3 signaling. The B, T and NK subsets showed a general decrease in tumor necrosis factor-α signaling; conversely, monocyte subsets showed an increase. Prior MTX exposure did not have a notable impact on the detected molecular profile.

Conclusions: Differences in gene expression, hallmark pathway activity, and chromatin accessibility were identified in RA versus HV at the immune cell subset level. Significant contributions to differences in chromatin accessibility identified in the myeloid and NK cell populations suggest that there are more active regulatory sequences in these cell types that are associated with RA. Further investigations based on these findings may increase understanding of the immune regulatory paradigm in the context of RA.

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