Targeted therapy guided by single cell transcriptomic analysis

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) is a rare but severe reaction to a drug, often manifesting as skin rash. In a patient with refractory DiHS/DRESS, single cell sequencing was used to identify promising therapeutic targets, use of which led to resolution of DiHS/DRESS symptoms. D Kim et al., *Nat. Med.* (2020).

Snapshot	10x Genomics product
Research area: Allergies & inflammation **Organism:** Human **Sample type:** Patient skin biopsies and PBMCs isolated from blood **Research questions:** How can single cell sequencing help guide treatment decisions? What signaling pathways initiate T-cell proliferation in DiHS/DRESS?	**Chromium Single Cell Immune Profiling Solution** • Chromium Single Cell 5′ Library and Gel Bead Kit • Chromium Single Cell V(D)J Enrichment Kit, Human T Cell • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines **Chromium Single Cell Gene Expression Solution** • Chromium Single Cell 3′ Library and Gel Bead Kit v2 • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines

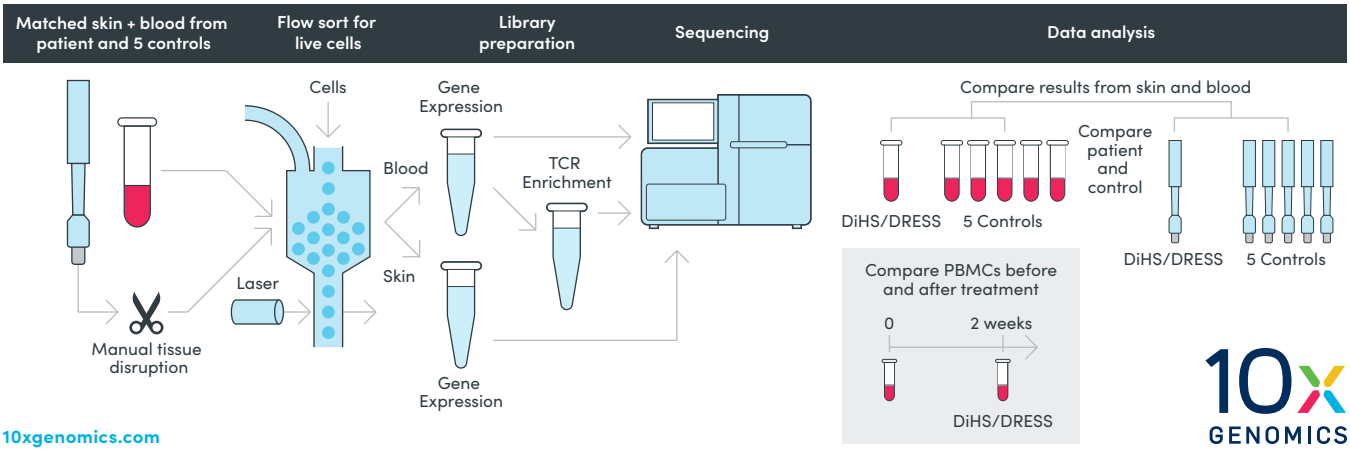
Experiment overview

Preparation of skin and blood samples

- Collection of skin biopsy from flank of patient with DiHS/DRESS and five healthy volunteers
- Dissociation of skin sample using manual mincing followed by enzymatic digestion
- PBMCs isolated from blood with density gradient centrifugation
- All samples enriched for live cells by FACS

Single cell gene expression and immune profiling analyses

- Single cell gene expression of skin and blood samples and full-length, paired T-cell receptor sequencing from blood using Chromium Single Cell Gene Expression and Immune Profiling Solutions
- Confirmation of single cell gene expression results in cell-culture model of disease onset
- Sequenced on Illumina HiSeq 3000 instrument



Why single cell?

In cases where patients present with disease that is unresponsive to traditional treatments, single cell analysis may help ascertain the specific molecular pathways driving an individual's disorder. Here, single cell gene expression data from affected skin and blood were compared to that of healthy individuals to discover cell-specific misregulated pathways that provided potential drug targets. Additional single cell information from paired, full-length T-cell receptor sequences helped exclude possible disorders, further refining diagnosis and treatment. Single cell data were complementary to clinical history and helped refine treatment options, resulting in successful therapy.

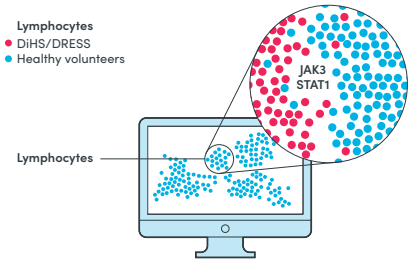


Figure 1. Comparison of gene expression in skin from DiHS/DRESS and healthy volunteers. Cells are pooled across samples, clustered based on gene expression, and annotated by cell type. Isolation and subclustering of lymphocytes show clear separation based on donor and increase in JAK/STAT signaling in DiHS/DRESS sample.

Results

Unique lymphocyte gene signature in DiHS/DRESS

Single cell whole transcriptome profiling was performed on DiHS/DRESS patient skin biopsy and compared to healthy volunteer controls. Relative to controls, patient skin had expanded populations of keratinocytes and immune cells, as well as sweeping transcriptomic changes in lymphocytes. Subclustering of lymphocytes showed clear segregation between patient and control samples and upregulated JAK/STAT signaling (Figure 1). Comparison of gene expression for PBMCs showed clear differences in several signaling pathways, but notably JAK/STAT signaling was not altered, indicating the importance of sampling the right tissue for gene expression analysis.

Single cell-guided treatment resolves symptoms

DiHS/DRESS is typically treated with steroids, but these medications can be ineffective and come with their own side effects. In this case study, scRNA-seq provided an important tool for clinicians to successfully resolve refractory DiHS/DRESS by pinpointing its molecular drivers, leading to treatment with JAK/STAT inhibitors.

Computational analysis

To compare changes in cell populations before and after treatment, gene count matrices were generated in Cell Ranger. Then, cluster analysis and annotation was performed using Seurat on aggregated PBMC samples taken before and two weeks after treatment. Aggregated cells were color-coded based on collection time, and the proportion of each cell-type population was directly compared. A drastic reduction in inflammatory-associated cell types was seen in post-treatment PBMCs (Figure 2).

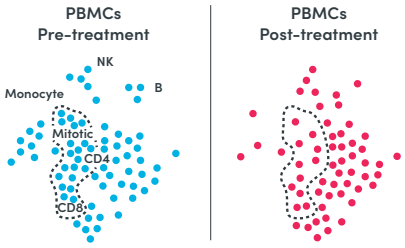


Figure 2. Shift in PBMC populations after treatment. Clustering was performed on pooled samples, with clusters annotated by cell type. After annotation, cells were distinguished as pre- or post-treatment. T cells that were depleted after treatment are highlighted by the dotted line and include clusters that expressed higher levels of DiHS/DRESS-associated genes, including *CCR4* and *CCR10*.

References

1. D Kim et al., Targeted therapy guided by single-cell transcriptomic analysis in drug-induced hypersensitivity syndrome. *Nat. Med.* 26, 236–243 (2020).

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