

**ABSTRACT****Single-cell RNA-sequencing reveals Transcriptional Changes and Clonal Architecture associated with Post-Transplant Relapse in Acute Myeloid Leukemia**Ziheng Xu<sup>1</sup>, Christopher A. Miller<sup>2,3</sup>, Sridhar N. Srivatsan<sup>2</sup>, Catrina C. Fronick<sup>3</sup>, Robert S. Fulton<sup>3</sup>, Timothy J. Ley<sup>2,3,4</sup>, and Allegra A. Petti<sup>2,3</sup><sup>1</sup>Washington University School of Medicine, St. Louis, MO<sup>2</sup>Division of Oncology, Washington University School of Medicine, St. Louis, MO<sup>3</sup>McDonnell Genome Institute, Washington University School of Medicine, St. Louis, MO<sup>4</sup>Department of Genetics, Washington University School of Medicine, St. Louis, MO

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Acute myeloid leukemia (AML) is a malignancy characterized by overproduction of myeloid precursors at the expense of more differentiated, functional hematopoietic cells, resulting in anemia, thrombocytopenia, and neutropenia. Despite initial sensitivity to chemotherapy, a majority of patients with AML ultimately relapse. Among the challenges associated with relapse, post-allogeneic stem cell transplant relapse is particularly intractable because of our relative lack of understanding -- and thus lack of effective treatment options -- of the underlying mechanisms.

**METHODS**

Single-cell RNA-sequencing (scRNA-seq) enables us to examine the heterogeneity of disease processes on a single-cell level. Here we report a preliminary analysis of transcriptomic and clonal changes associated with post-transplant AML relapse, using whole genome sequencing data and high-throughput scRNA-seq data (Chromium Single Cell 5' Gene Expression workflow, 10x Genomics) obtained from paired

presentation and relapse bone marrow samples from one AML patient.

**RESULTS**

We first compared the whole-sample scRNA-seq data of the presentation to the relapse bone marrow sample. Using PCA followed by UMAP for dimensionality reduction and data visualization, we have shown that AML presentation and relapse are two transcriptionally distinct events. A further test on the differentially expressed genes with enrichment analysis suggested that the principal down-regulated biological pathways associated with relapse include antigen presentation and lysosomal degradation, whereas the up-regulation is comparatively modest and is centered around pathways involved in nucleic acids catabolism. These are potential pathways that the relapsed AML utilizes to achieve immune-escape.

To leverage the single-cell resolution of scRNA-seq, we sought to elucidate the trajectory of transcriptional changes during evolution from presentation to relapse using the pseudotemporal ordering algorithm Monocle3. The analyses yield one cluster of

AML cells in the presentation sample that appears to give rise to the relapse.

We then focused on this putative relapse-driving cluster and examined its associated expression changes. We used differential gene expression analysis (Seurat) and functional enrichment analysis (ToppGene) to identify genes with significant changes specific to this cluster and group them into functional modules. These include a significant down-regulation of *LYZ*, the lysozyme encoding gene, and *S100A8* and *S100A9*, key regulators of inflammation and immune response. Combined with further orthogonal validation, this list could further provide guidance in the development of potentially cluster-oriented prophylactic treatment of AML relapse at disease presentation.

## CONCLUSION

In summary, our study has identified transcriptional changes and key clonal architecture associated with post-transplant relapse in AML. Pseudotemporal ordering and differential gene expression analysis further identified the key dysregulated subpopulations and expression signatures leading to relapse. Studies are currently underway to extend this analysis to a larger number of samples. We hope that this study can contribute to our knowledge of AML relapse at a new level and ultimately, the development of novel therapeutics of relapsed AML.