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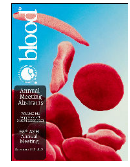

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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Single Cell Transcriptome Comparisons of Diagnosis-Relapse Pairs Uncover Programs Associated with Chemoresistance in Pediatric AML

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Background: Relapse rates for pediatric AML remain unacceptably high at nearly 50% after frontline therapy. Once relapse occurs, the disease is often refractory to further chemotherapy. Prior studies have shown that acquired mutations conferring chemoresistance are uncommon, and most relapses are thought to arise from the selection of clones already present at diagnosis. Therefore, characterizing chemoresistant disease at the gene expression level could lead to new insights and novel therapy targets. Profiling cases by bulk RNA sequencing has revealed important disease features such as novel gene fusions. However, identifying resistant subpopulations by bulk sequencing is challenging, particularly if they constitute small fractions of the leukemia cells at diagnosis. We hypothesize that by applying single-cell transcriptome profiling (scRNA-seq) to diagnosis-relapse pairs we can identify and characterize the subclones selected by treatment. Additionally, we will discover pathways and gene-expression programs that are associated with chemoresistance.
Methods: We profiled thirteen diagnosis-relapse pairs from pediatric AML patients who were treated on or similarly to the Children's Oncology Group (COG) study AAML1031 and consented to banking of cells for future research. Six pairs were banked at Texas Children's Cancer Center and 7 were obtained from the COG biobank. The COG cases were flow-sorted to enrich for blasts before undergoing scRNA-seq with 10X-Chromium and Illumina NovaSeq systems. In addition, two local normal bone marrow cases were profiled, one with prior sorting and one with and without prior sorting. We included scRNA-seq profiles of 8 pediatric AML patients and 4 non-cancer bone marrow samples from Bailur et al. (JCI insight, 2020) as additional controls. Sequencing data were analyzed using CellRanger and Seurat. Four of the COG cases were profiled at the protein level by mass cytometry (CyTOF) and analyzed in Cytobank.
Results: In total, for the 13 diagnosis-relapse pairs, 17,233 to 34,246 cells were profiled per patient, and 22,800 transcripts were detected. Unsupervised clustering of our samples and controls yielded 43 clusters (Figure 1) that could be confidently identified as either malignant or non-malignant (normal). We further classified all clusters into inferred hematopoietic developmental stages based on comparisons to publicly available expression reference data (Figure 2). We distinguished between patients with cell types at relapse that were also identified at diagnosis (Group 1), albeit often at a lower proportion, and patients whose cell types at relapse were not detected at diagnosis (Group 2). We also characterized 4 of the diagnosis-relapse pairs by CyTOF with an antibody panel that quantified surface lineage markers and intracellular signaling proteins. The protein expression data validated the RNA data in both the degree of differentiation of AML cells and the degree of similarity between diagnosis and relapse.
We identified a Relapse-Up signature composed of 139 differentially expressed genes that were upregulated in AML clusters that expanded between diagnosis and relapse, compared to clusters that diminished between diagnosis and relapse. The Relapse-Up gene signature is enriched in genes that are co-expressed with *FLT3*, *CDK6*, and *ILK* kinase genes. With publicly available TARGET gene expression data, we confirmed that this gene signature is upregulated in relapsed AML samples when compared to diagnosis samples. We further used the profiles of the cell types that expanded between diagnosis and relapse to construct a risk-predictive function that predicted patient outcomes based on bulk RNA-Seq profiles of their diagnostic samples with significant accuracy.
Conclusion: Single-cell RNA-seq of diagnosis-relapse pairs from pediatric patients with AML revealed distinct patterns of relapse that were also apparent at the protein level. The Relapse-Up signature was enriched in pediatric AML relapse samples in a large bulk RNA-seq data set, confirming its broader applicability. The kinase co-expression programs present in the

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signature are likely to be functionally associated with chemotherapy resistance and represent targets for future preclinical therapeutic studies.

Disclosures No relevant conflicts of interest to declare.

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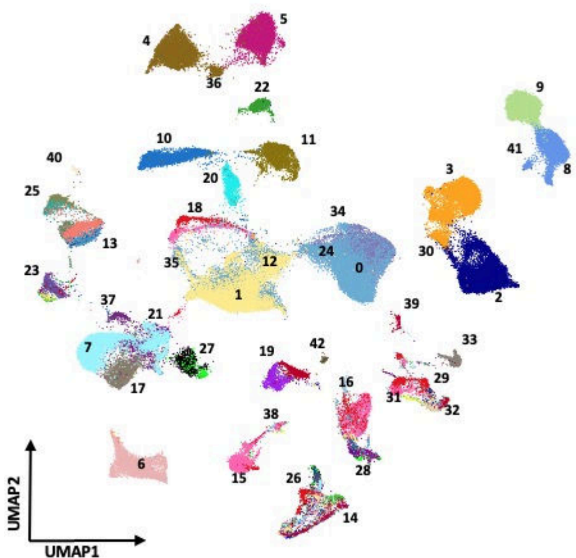

Figure 1. UMAP representation of 43 clusters. Numbers indicate clusters. Colors correspond to the 42 samples listed in Figure 2, including 13 pediatric AML diagnosis-relapse pairs, 8 pediatric AML diagnosis samples and 7 normal bone marrow samples.

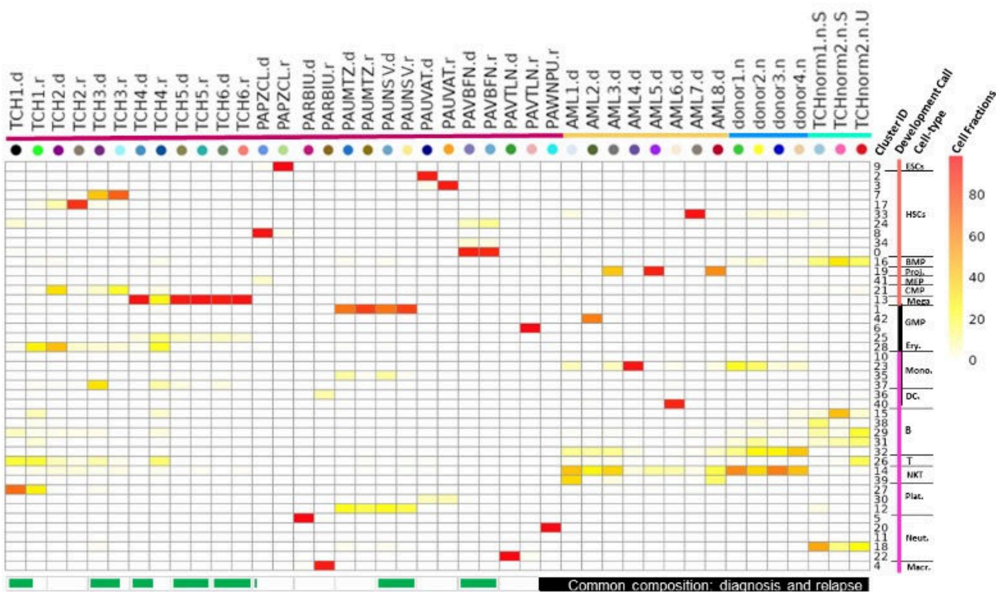

Figure 2. Proportions table demonstrating the fraction of cells in each cluster that are derived from each sample. The public AML and normal bone marrow data correspond to samples AML1.d-AML8.d and donor1.n-donor4.n, respectively. The cell type assigned to each cluster is indicated along the right margin. The color of the dot below each sample name corresponds to the colors in Figure 1. The green bars at the bottom indicate the degree of cluster overlap between diagnosis and remission samples for each pair.

Figure 1

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