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ORAL ABSTRACTS

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

Multimodal Atlas of Paired Diagnosis and Relapse AML Samples Enables Novel Therapeutic Targeting of Surface Antigens

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AML is an aggressive clonal malignancy characterized by combinations of chromosomal abnormalities, gene mutations, and cell surface antigen (Ag) expression profiles. This heterogeneity contributes to refractory or relapsed disease that presents a major challenge when treating AML. Previous single cell sequencing studies of primary AML samples have provided remarkable insight into the clonal architecture of AML cell populations and how their mutational, transcriptomic, and surface Ag profiles vary between patients. However, information addressing clonal shifts during progression from diagnosis to relapse in large cohorts is lacking. Here, we adapted single cell RNA sequencing with surface Ag feature barcoding to analyze more than 450,000 cells from 28 paired AML patient bone marrow mononuclear cell samples collected at diagnosis and relapse (56 samples). To our knowledge, it is the largest and most comprehensive single cell AML atlas to date. This atlas contains rich clinical metadata including cytogenetics, mutation status of canonical AML genes, treatment history, and survival information. We leveraged these data to identify potential correlations with clonal heterogeneity during progression to relapse and to propose novel strategies for targeting the surface of AML cells.

The feature barcoding panel consists of a comprehensive list of 81 surface Ags reported in clinicaltrials.gov or mined from literature, including large proteomics mass spectrometry datasets of AML patient bone marrow samples (deBoer et. al. Cancer Cell 2018; Jayavelu et al, Cancer Cell 2022). Feature barcoding produces antibody derived tag (ADT) counts which are interpreted as relative values but alone does not indicate absolute number of Ags per cell. We addressed this limitation by measuring absolute Ag density of AML Ags, CD33, CLL1, CD123, and EMR2 in patient samples using the flow cytometric QuantiBRITE assay. Focusing on these four Ags, we modeled the relationship between ADT expression and antibodies per cell (ABC) Ag density from QuantiBRITE. The model was applied to impute absolute Ag density for the remaining 77 Ags in all samples.

UMAP visualization after sample transcriptome integration revealed distinct clustering of CD45-dim myeloblasts, T cells, B cells, and erythroid cells. Longitudinal analysis identified 28 surface Ags that were differentially expressed (absolute $\log_2 FC > 1$, $p < 0.01$) between relapse and diagnosis myeloblasts across multiple patients (Fig. 1A). An additional 19 Ags were differentially expressed in no more than one patient while the other 19 had no significant change in any patient, highlighting the inter-and intra- heterogeneity of AML at diagnosis and relapse in these patients. Targeting multiple antigens simultaneously may help address the issue of antigen heterogeneity of tumor cells and help avoid potential antigen escape. Because multi-targeting therapies are a potential solution to this, weleveraged the single-cell resolution of our atlas to systematically identify Ags that in combination provide maximum coverage and are significantly co-expressed in myeloblasts. From this analysis, we found 48 and 52 co-expressed Ag pairs in diagnosis and relapse blasts, respectively ($r > 0.4$, $p < 0.01$; Fig. 1B). Of note, CD44 and EMR2 were co-expressed at both diagnosis ($r = 0.49$ $p < 0.01$) and relapse ($r = 0.3$, $p < 0.01$) with >90% of their blasts expressing CD44, EMR2, or both at targetable antigen levels of >1000 Ags/cell. Furthermore, 10 samples had >40% of their blasts co-expressing both Ags simultaneously at >1000 Ags/cell at diagnosis or relapse. 6 of these patients had a >10% increase in co-expression at relapse, 3 had >10% decrease at relapse, and one patient had no co-expression change. Our comprehensive profiling of AML has provided high resolution insight on cell surface at diagnosis and during disease progression and produced novel hypotheses about targetable combinations of surface Ags based on their expression changes between timepoints, co-expression, and surface densities. Furthermore, our reference atlas can be further dissected to characterize the transcriptome and mutational profiles of myeloblasts at diagnosis and relapse thereby prodding an important

resource to create surface antigen protein - transcript correlations within AML blasts and to trace molecular signatures during disease progression.

Disclosures Halfond: *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Ung:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Etchin:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Keschner:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **DiFazio:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Pyclik:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Mundelboim:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Ge:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Lydeard:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company. **Chakraborty:** *Vor Biopharma:* Current Employment, Current equity holder in publicly-traded company.

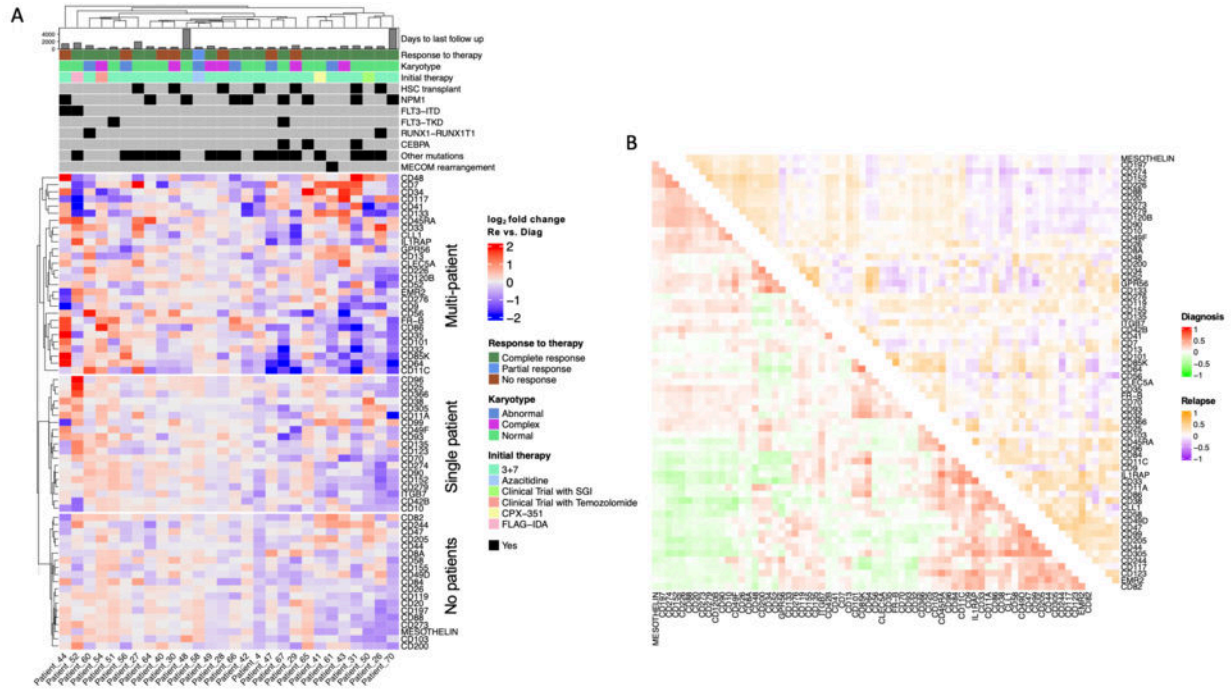

Figure 1. Surface ag expression in AML patient myeloblasts across clinical timepoints. A. Heatmap showing surface ag log2-fold changes between relapse and diagnosis blasts for all AML bone marrow samples. Positive values indicate marker is upregulated in relapse bone marrow. Clinical annotation for each patient is displayed above heatmap. Ags are categorized into three groups based on whether they are differentially expressed between timepoints in multiple, one, or no patients. B. Heatmap showing pairwise correlation of ag marker expression in AML blast population. Bottom and top triangles show Pearson correlation coefficients for diagnosis and relapse samples, respectively.

Figure 1

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