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POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

A Rapid Test to Evaluate the Tumor Differentiation State of Acute Myeloid Leukemias

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Introduction: The combination of the hypomethylating agent Azacitidine with Venetoclax, a BCL2 inhibitor (Aza+Ven), is the frontline therapy for patients with acute myeloid leukemia (AML) who are elderly or unfit for intensive chemotherapy. However, identifying responsiveness to Aza+Ven *a priori* remains a clinical challenge.Aza+Ven effectively targets the primitive leukemia stem cell population (Pollyea, 2018), whereas resistance is observed in differentiated tumors (Pei, 2020). Further insights into the hierarchical organization of hematopoiesis have led to a definition of malignant AML cell types that follow the progression from hematopoietic stem cell to myeloid differentiated cells based on gene expression signatures, and these have been used to classify AML tumors (van Galen, 2019). Classification schemes using gene expression to capture variations in leukemic composition represent key biomarkers of response to targeted agents (Zeng, 2022; Bottomly, 2022). However, the prompt availability of nuanced cell state signatures to guide clinical decision making is limited by the laborious nature of performing and analyzing RNA-sequencing studies. Rapid AML stratification based on these classifications permits the possibility of identifying patients most likely to benefit from a drug or combination.
Methods: To classify cell states based on transcriptomic signatures, we developed two assay formats, involving custom Nanostring and TaqMan Array card panels, for evaluation of tumor cell states for AML patients. Both formats contained gene sets representative of the six hematopoietic lineage states (van Galen, 2019) and both provide outputs that are compatible with the timeline for clinical decision-making. Assays were run according to the manufacturers' specifications, and enrichment of each of the six gene signatures was assessed using the Singscore stable method (Foroutan, 2018). Banked AML patient samples with matched RNA-seq and ex vivo Aza+Ven drug sensitivity data were used to validate the assay formats. Data obtained from the rapid assays were validated against cell states determined by deconvolution of bulk RNA sequence and compared with respect to clinical immunophenotype data where available.
Results **:** Both the Nanostring and quantitative PCR-based assay formats detected gene expression representative of the appropriate cell state as previously determined by bulk RNA sequencing (Fig. 1A). Correlation of Aza+Ven ex vivo sensitivity with tumor cell state signatures revealed patterns where primitive but not differentiated cell states associated with sensitivity. In contrast, RNA isolated from healthy donor samples showed expression of all six cell state signatures. Across a cohort of 50 primary AML patient specimens, enrichment scores for each gene set correlated well with those derived for the same patients using deconvolution of bulk RNA-seq (Pearson r values: 0.785 to 0.944). Cell state characterization by rapid gene expression assays also aligned well with clinical immunophenotype, a routinely available diagnostic characterization of tumor blasts. Surface expression of primitive (CD117) and mature (CD11b) markers associated with Aza+Ven ex vivo sensitivity and resistance overall, respectively (p=0.03 & 0.04); however, ex vivo sensitivity could be significantly further stratified among samples expressing these markers when considering the gene expression-based enrichment scores for progenitor- and monocyte-like gene signatures (low vs high p-values: 0.02-0.05) (Fig. 1B). Analysis of matched samples from individual patients before and after treatment also showed cell state signature changes that reflected their temporal disease progression and correlated with differences in ex vivo drug sensitivity and clinical immunophenotype.
*Conclusion:*Incorporation of cell state into prospective evaluations of AML patients provides an early indication of responsiveness to Aza+Ven. With rapid turnaround, assays such as these may augment treatment stratification and improve patient outcomes. These assays offer additional versatility to detect changes in AML cells after forced differentiation or acquired drug resistance.

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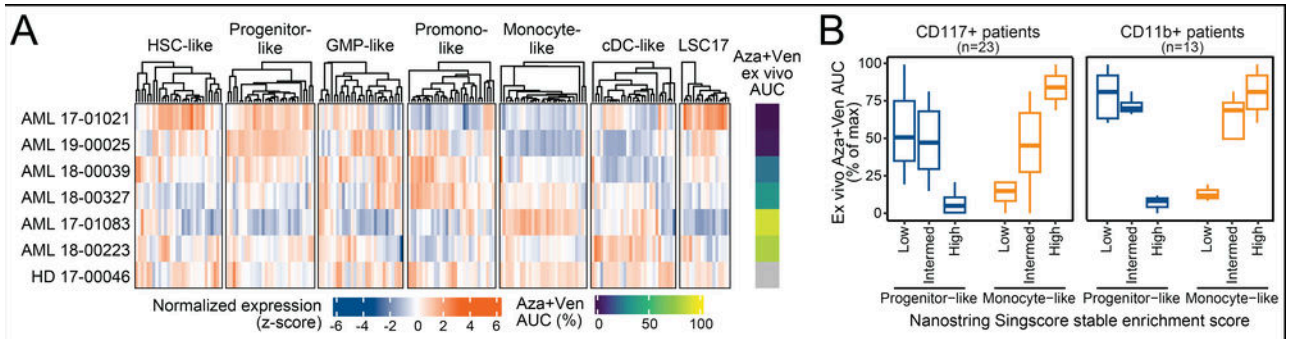

Figure 1. (A) Heatmap depicting hematologic cell states identified by the Nanostring cell state gene panel on AML patient samples and correlated with ex vivo sensitivity to Aza+Ven. HD denotes healthy donor sample. LSC17 denotes a previously described 17-gene signature for leukemic stem cells (Ng et al, Nature 2016), shown here for comparison. **(B)** Breakdown of Aza+Ven ex vivo sensitivity in patients positive for either CD117 or CD11b (by clinical flow) according to relative enrichment scores for the progenitor- (blue) and monocyte-like (orange) gene sets by Nanostring assay. Low, intermediate, and high indicate samples with Singscore stable scores in the lowest 25%, middle 50%, and top 25% of all values for that gene signature across the surveyed cohort (n=50).

Figure 1

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