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618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

A Multiomic, Single-Cell Measurable Residual Disease (scMRD) Assay for Phasing DNA Mutations and Surface Immunophenotypes

Charlie Murphy[1], *Kate Thompson*[1], *Lubna Nousheen*[1], *Divya Rao, PhD*[1], *Todd E. Druley, MD*[2]

[1] Mission Bio, SOUTH SAN FRANCISCO, CA
[2] Mission Bio, South San Francisco

The small population of cancerous cells that remain following treatment, known as measurable residual disease (MRD), is the major cause of relapse in acute myeloid leukemia (AML). Usually, these refractory cells have gained additional resistance mutations or changed their surface immunophenotypes in ways that preclude detection and phasing by current gold standard flow cytometry or bulk next-generation sequencing assays. For this reason, a multiomic single-cell MRD (scMRD) assay could offer a more comprehensive indicator of relapse and the potential for faster response. Here, we present a new scMRD assay with a 0.01% limit of detection that provides single-cell clonal architecture and immunophenotyping to not only identify residual leukemia cells, but also identify putative DNA or protein targets for salvage therapy. The assay enables rare-cell detection on a standard Mission Bio Tapestri run by adding (i) an upfront bead-based protocol to enrich for blast cells, (ii) a DNA and protein panel specifically designed for AML MRD diagnosis and treatment, and (iii) a new, automated analysis pipeline to evaluate single-cell multiomics output. By utilizing Mission Bio's single-molecule DNA sensitivity for single cells, this pipeline can identify and correlate co-occurring *de novo* variants, thereby reducing false positive rates over bulk assays that do not correlate variants. It furthermore can create phylogenetic trees of the detected MRD cells and present their surface protein signature and arm-level copy number. In addition, the multiplexing of up to three patient samples combined in one run via germline identification further reduces per sample costs and increases throughput. To demonstrate these features on 0.01% MRD, samples were constructed by titrating diseased cells into healthy bone marrow cells before processing them with the scMRD assay. We detected 0.01% spike-in (CD34+) and 0.1% spike-in (CD117+) in 6 of 6 samples, with an average enrichment of 41x and 13x, respectively. Further testing detected CD34+ 0.1% spike-ins in 10 of 10 samples (32x average enrichment). We applied the scMRD assay to banked bone marrow aspirate samples from 3 AML patients. The scMRD assay resolved the clonal architecture identifying multiple leukemic clones with co-occurring mutations. The assay readily distinguished pre-leukemic from leukemic clones thereby increasing the specificity of MRD results. The integration of genotype and immunophenotypic further enhanced MRD detection by identifying genotype-specific protein expression patterns. By combining high sensitivity with multiomics, this assay offers a potential scalable solution for comprehensive MRD detection that guides therapeutic decision-making.

Disclosures No relevant conflicts of interest to declare.

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