SINGLE-CELL GENOTYPING IDENTIFIES PERSISTENT ACUTE MYELOID LEUKEMIA POPULATIONS AFTER INDUCTION CHEMOTHERAPY

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Background: Relapse remains a major clinical challenge in the treatment of acute myeloid leukemia (AML). Although mechanisms of treatment resistance including immune escape have been described, the evolutionary trajectory of measurable residual disease (MRD) toward overt relapse remains poorly understood.

Purpose: Single-cell genomic approaches provide a framework for MRD detection and molecular profiling of residual leukemic cells, potentially enabling timely and informed therapeutic intervention. Here, we established proof-of-principle for single-cell MRD.

Methods: 5’ 10x Genomics single-cell RNA sequencing (scRNA-seq) and Oxford Nanopore long-read sequencing-based genotyping (nanoranger) of mutations in NPM1, SRSF2, or U2AF1 were performed to determine cell identity and mutation status. Integrated analyses enabled the definition of cellular states and characterization of clonal persistence across treatment response groups.

Results: 7 patients with paired diagnosis and post-induction AML samples were selected for longitudinal analysis. These included cases with varying treatment response to intensive chemotherapy. Overall, we obtained 119,077 scRNA-seq profiles, with mutation status successfully assigned to 75,447. At diagnosis, AML cells were identifiable within progenitor, differentiated myeloid, and erythroid-like compartments, with their distribution being patient- and mutation-specific. Within these compartments, we reached a theoretical limit of detection for MRD of 0.006-0.05%. At follow-up, refractory disease cases retained mutated cell fractions across all leukemic lineages. In one case, we observed elimination of an NPM1mut subclone despite persistence of SRSF2mut cells, consistent with differential sensitivity to chemotherapy. In contrast, in 2 cases of complete remission, small NPM1mut populations (1.1 – 2%) remained exclusively detectable within monocyte and pro-monocyte populations, consistent with persistence of post-mitotic cells. Of note, one case was MRD negative by conventional qPCR.

Conclusions: Single cell MRD tracking using scRNA-seq and targeted genotyping is feasible and defines residual leukemia phenotypes. Characterization of residual leukemia will improve our understanding of AML evolution and support informed therapeutic interventions at MRD.