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A novel single-cell measurable residual disease (scMRD) assay for simultaneous DNA mutation and surface immunophenotype profiling

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Introduction

The Mission Bio AML single-cell MRD assay quantitatively characterizes SNVs and surface protein expression simultaneously across thousands of individual cells. In contrast, bulk NGS requires averaging across the entire population, preventing co-localization of joint genetic lesions or changes in cellular immunophenotype, and leukemia-associated immunophenotyping by flow cytometry can miss residual AML cells that have the same genotype, but different immunophenotype from the diagnostic population. These methods are commonly discordant, leading to clinical questions for individual surveillance and treatment. Combining these assays with single-cell resolution overcomes these limitations and reveals subclonal populations that may harbor resistance mutations or unique therapeutic targets.

Aim

We sought matched clinical and remission specimens that yielded discrepant MRD results by flow cytometry. Either MRD+ by flow without relapse; or MRD- by flow with relapse.

Our aims were as follows:

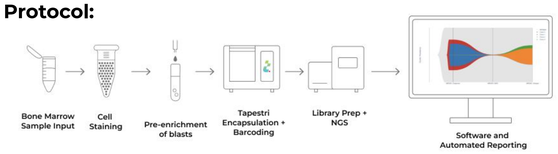
1. Compare Tapestri MRD results to flow cytometry
2. Compare clonal profiling from diagnostic specimens with the relapse clone(s) at remission
3. Benchmark Tapestri MRD sensitivity and specificity against duplex sequencing data from the same specimens (PMID: 37534515)

Methods

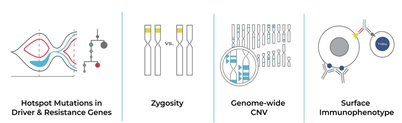
Samples: 20 pairs of matched diagnostic and remission samples from patients treated on SWOG S0106 where patients were treated with standard 7+3 with or without gemtuzumab.

Panels: 40-gene MRD AML panel
17-plex antibody heme markers.

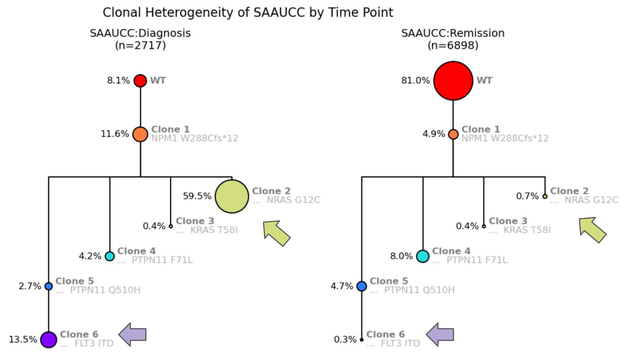
Protocol:



Analytes:



Example: Competing MRD Clones



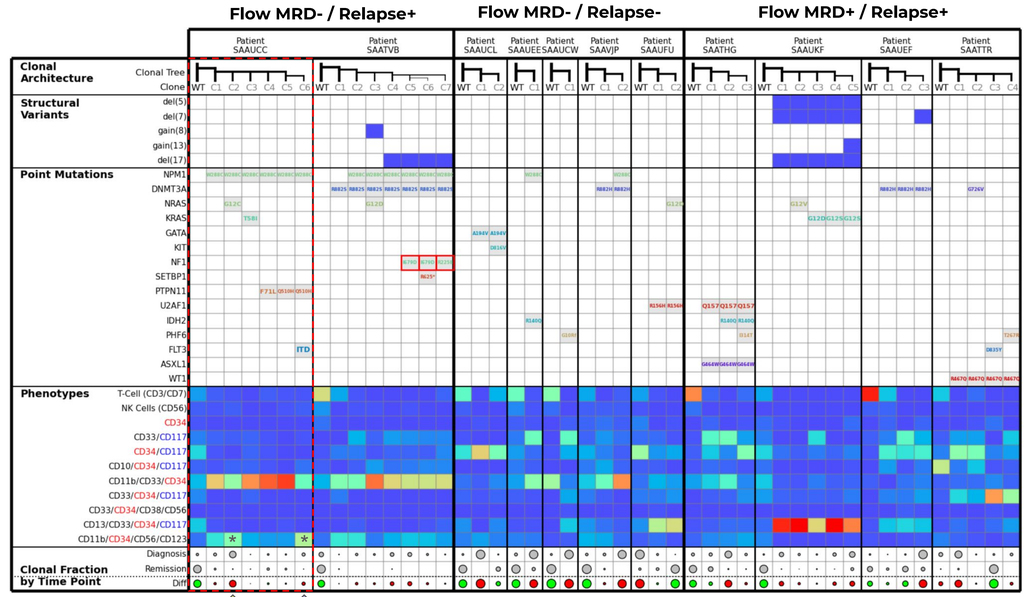
Shown above are clonal trees constructed from thousands of single cells from the diagnosis (left) and remission (right) time points of patient SAAUCC. The patient was found to be MRD- by flow cytometry at remission but nonetheless relapsed.

Observations:

- The trees indicate a diverse set of branching daughter clones originating from an NPM1 W288C founding mutation.
- The dominant clones at diagnosis are clone 2 (NRAS G12C, yellow) and clone 6 (FLT3 ITD/PTPN11 Q510H, purple).
- Post treatment, the wild type clone (WT) expands and clones 2 & 6 diminish to still detectable levels: the FLT3 ITD was **0.0025%** VAF by duplex sequencing (0.03% here with enrichment).
- Other clones were relatively stable through treatment.

Clonal diversity and non-overlapping FLT3 ITD/NRAS mutations might suggest poor response to FLT3 inhibitors alone.

Cohort Analysis



Cohort heterogeneity is tabulated above. Each column is a sample clone, with columns grouped by patient and more broadly by flow cytometry MRD status/relapse outcome. Each row is an analyte: structural variants, point mutations, phenotypes fraction, and clonal fraction by time point.

Observations:

- WT clones often have significant T-cell populations (CD3+/CD7+, first phenotype) that show despite blast enrichment.
- Patient SAAUCC from before (outlined red) has responding clones 2 and 6 with aberrant phenotype (*) while others clones do not.
- For relapse-negative patients, the clonal architecture is relatively simple, suggesting diversity might be a prognostic biomarker.

Conclusions

Single cell characterization of AML via the Mission Bio AML MRD assay enables the simultaneous assessment of SNVs and indels in genetic regions recommended by ELN guidelines along with surface immunophenotyping. Whereas bulk NGS and flow are frequently discrepant, this assay resolves genotype:phenotype ambiguity with greater resolution and without the need for averaging across the entire sample, revealing low frequency subclones or changing in the diagnostic immunophenotype that are often the cause of false negative results and recurrent leukemia. In this cohort, we observed a correlation between relapse and greater clonal diversity, presumably due to greater genetic variability. In summary, Mission Bio has developed a novel, flexible, high-resolution single cell assay for the characterization of clonal diversity and identification of putative therapeutic targets.

References

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2. L. Miles, et al., “Single-cell mutation analysis of clonal evolution in myeloid malignancies.” *Nature* **587**, 477 (2020).
3. T. Robinson, et al., “Single-cell genotypic and phenotypic analysis of measurable residual disease in acute myeloid leukemia.” *Science Advances* **9**, eadg0488 (2023).

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Contact

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