mission bio

THE FUTURE OF MRD IS SINGLE-CELL

Multi-omic scMRD assay offers novel molecular characterization of AML MRD

PRECISION MEDICINE: TAPESTRI® scMRD TECHNOLOGY

Precision medicine demands single-cell multi-omics

Acute myeloid leukemia (AML) measurable residual disease (MRD) is difficult to detect accurately, and current methods such as flow cytometry (FCM) and bulk next-generation sequencing (NGS) are commonly challenged by both false-positive and false-negative results. Even when these single-metric assays agree on an MRD result, too often they are still discordant with clinical outcome.[1]

COMPARISON BETWEEN FCM MRD & NGS MRD (POST-CONSOLIDATION) OVERALL SURVIVAL:

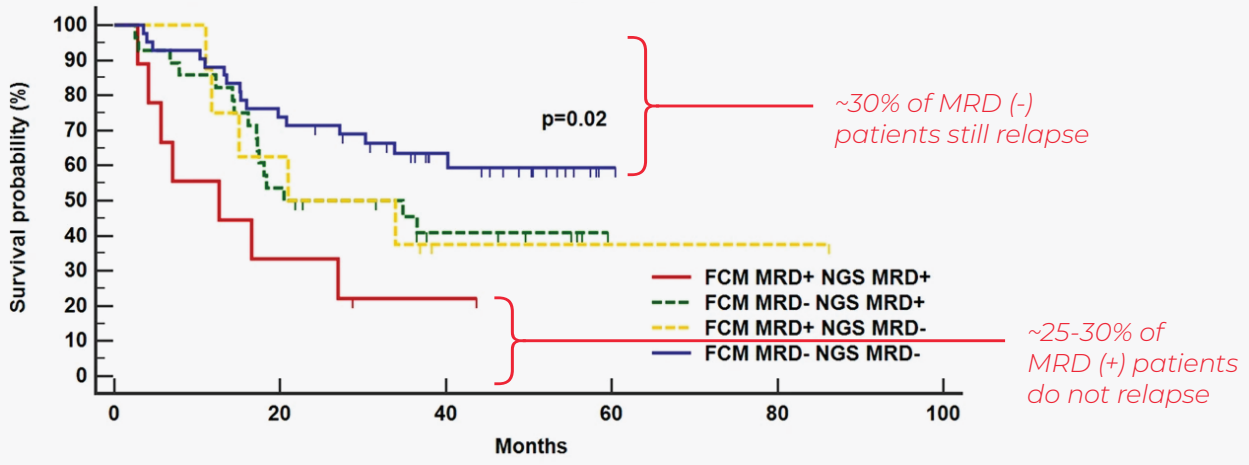

Figure 1: The clinical relevance detection of MRD during complete remission when measured by FCM or error-corrected NGS at post-consolidation. Patkar, N. et al., Leukemia (2021).

Utility of single-cell proteogenomics meets clinical research needs

High-resolution, integrated molecular profiles bring unprecedented clarity to complex disease biology:

- **Detect rare cells:** Confidently detect rare MRD cells typically obscured by the averaging effect of variant allele frequency (VAF) readout of bulk NGS
- **Resolve genetic heterogeneity with clonal architecture:** Understand the dynamics of treatment response, selective pressure, and therapy-resistant clones
- **Trace genotypic and phenotypic evolution:** Track changes in surface phenotype, the expression level of targeted antigens, identify clone-specific phenotypes
- **Monitor donor-host transplant chimerism**
- **Determine variant zygosity:** Spotlight aggressive clones wielding bi-allelic inactivation of tumor suppressor genes

INTEGRATING SURFACE IMMUNOPHENOTYPE & GENOTYPE YIELDS NOVEL INSIGHTS:

In the below example, bulk NGS detects only clonal hematopoiesis-associated (CHIP) variants within *DNMT3A*. During the same time, scMRD identifies distinct and exclusive clones each containing a single *DNMT3A* variant in addition to a rare *DNMT3A/NPM1* mutant clone and a *JAK2* mutant clone. These variants were later corroborated by bulk NGS in a clinical relapse sample, highlighting the potential of scMRD to yield valuable insights that may one day impact the clinical management of AML.[2]

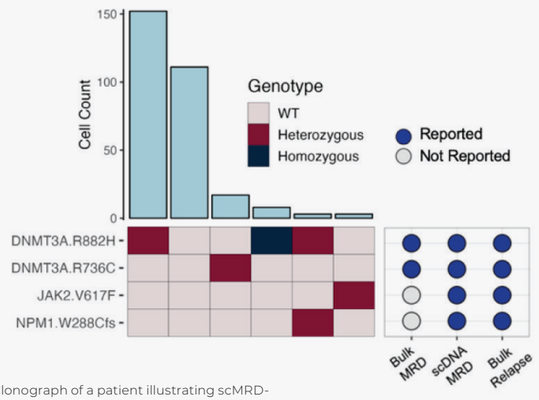

Figure 2: Clonograph of a patient illustrating scMRD-specific detection of *NPM1* and *JAK2* mutations that were present at late relapse. Robinson, T.M. et al., biorXiv (2022).

Building on a prior work[3] showing the capability of single-cell DNA sequencing to measure chimerism post allogeneic stem cell transplantation, the scMRD assay demonstrates the ability to correlate donor and host immunophenotype, differentiate donor-derived CHIP from host-derived CHIP, and resolve the complex clonal architectures with single-cell, multi-omic resolution.[2]

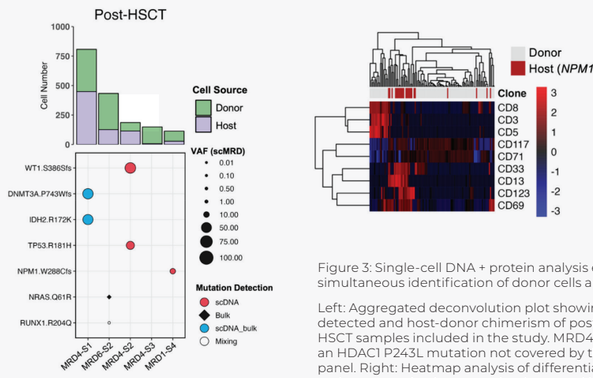

Figure 3: Single-cell DNA + protein analysis enables simultaneous identification of donor cells and MRD.

Left: Aggregated deconvolution plot showing mutations detected and host-donor chimerism of post-allogeneic HSCT samples included in the study. MRD4-S3 had an HDAC1 P243L mutation not covered by the scMRD panel. Right: Heatmap analysis of differential surface maker expression between the donor and host cells in MRD1-S4. Robinson, T.M. et al., biorXiv (2022).

Challenges of single analyte resolution for MRD detection

EXAMPLE OF A FALSE POSITIVE SCENARIO FROM USING BULK NGS ALONE:

Ancestral variants in premalignant cells often present with high VAF in bulk NGS data, which may lead to false positive MRD results without the context of the clonal architecture to clearly define disease-associated variant co-occurrence. Similarly, rare subclonal variants are commonly lost under the averaging effect of bulk NGS variant allele frequencies, ultimately obscuring MRD detection and potentially leading to false negative results.

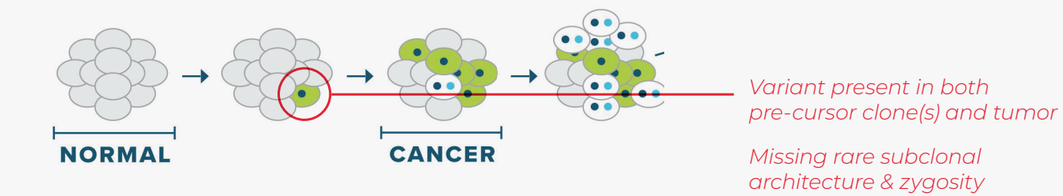

Figure 4: False positive results can arise when using bulk NGS alone.

EXAMPLE OF FALSE NEGATIVE SCENARIO FROM USING FLOW CYTOMETRY ALONE:

Phenotypic heterogeneity proves challenging for flow cytometry to maintain high MRD-specificity and may result in false negative results. While a phenotypically normal tumor cell may evade single-analyte detection by flow cytometry, Tapestri's integrated proteogenomic read-out readily identifies pathognomonic genotypes lurking behind normal surface immunophenotype profiles.[4]

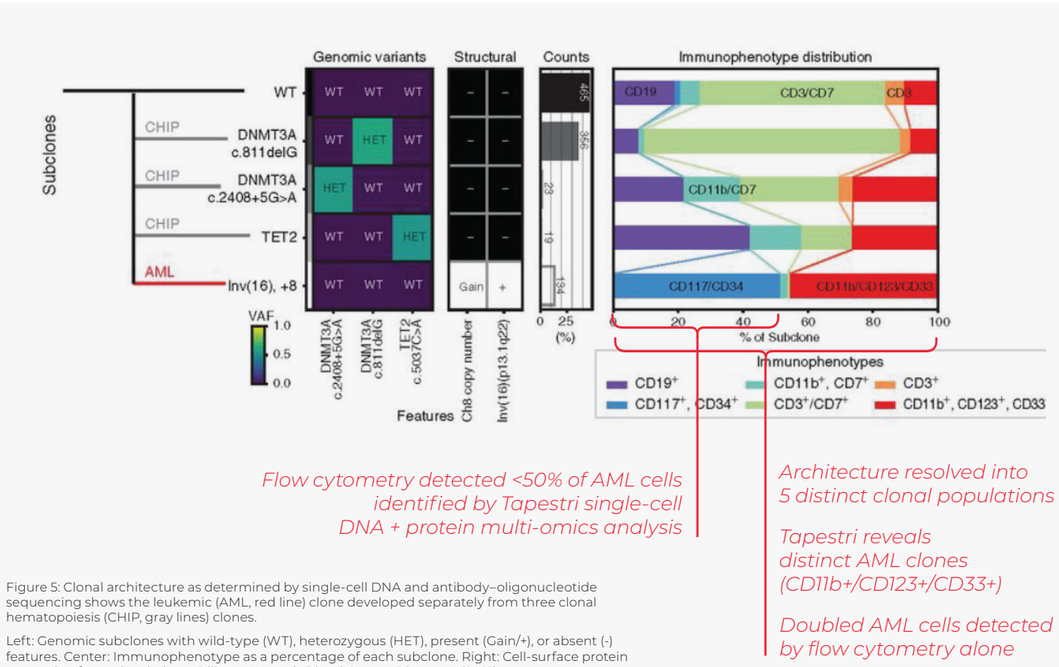

Figure 5: Clonal architecture as determined by single-cell DNA and antibody–oligonucleotide sequencing shows the leukemic (AML, red line) clone developed separately from three clonal hematopoiesis (CHIP, gray lines) clones.

Left: Genomic subclones with wild-type (WT), heterozygous (HET), present (Gain/+), or absent (-) features. Center: Immunophenotype as a percentage of each subclone. Right: Cell-surface protein expression for each subclone. Dillon, L.W. et al., Blood Cancer Discovery (2021).

mission bio

LET TAPESTRI TAKE YOUR CLINICAL RESEARCH TO THE NEXT LEVEL.

CONTACT US FOR MORE INFORMATION ON OUR EARLY ACCESS PROGRAM.

DIFFERENTIAL SURFACE MARKER EXPRESSION BETWEEN CHIP/PRELEUKEMIC AND LEUKEMIC CLONES

Genotype and immunophenotype integration enables the identification of differential immunophenotypic states in genetically distinct clones, allowing more precise discrimination between pre-leukemic and leukemic populations.[2]

Considering the increased use of cell surface targeting therapeutics in AML, the identification of subclone-specific expression of key antigens (differential CD34 and CD33 expression shown as an example below) could be exploited to track the expression of druggable targets in the different clonal populations; thus guiding further therapeutic options.[2]

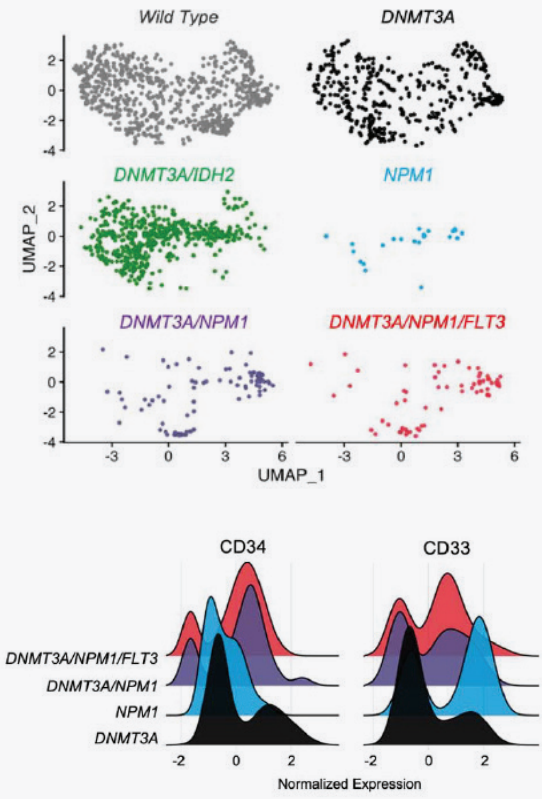

Figure 6. Clone- and mutation-specific immunophenotype.

Top: UMAP analysis of immunophenotypes of CHIP/preleukemic vs leukemic clones. Bottom: Differential surface marker expression between CHIP/preleukemic vs leukemic clones. Robinson, T.M. et al., biorXiv (2022).

REFERENCES

[1]Patkar, N., Kakirde, C., Shaikh, A.F. et al. Clinical impact of panel-based error-corrected next generation sequencing versus flow cytometry to detect measurable residual disease (MRD) in acute myeloid leukemia (AML). *Leukemia 35*, 1392–1404 (2021). https://doi.org/10.1038/s41375-021-01131-6

[2]Robinson, T.M., Bowman, R.L., Persaud, S. et al. Single cell genotypic and phenotypic analysis of measurable residual disease in acute myeloid leukemia. *bioRxiv* 2022.09.20.508786 (2022). https://doi.org/10.1101/2022.09.20.508786

[3]Xu, L., Durruthy-Durruthy, R., Eastburn, D.J. et al. Clonal Evolution and Changes in Two AML Patients Detected with A Novel Single-Cell DNA Sequencing Platform. *Sci Rep* 9, 11119 (2019). https://doi.org/10.1038/s41598-019-47297-z

[4]L.W. Dillon, L.W., Ghannam, J., Nosiri, C. et al. Personalized Single-Cell Proteogenomics to Distinguish Acute Myeloid Leukemia from Nonmalignant Clonal Hematopoiesis. *Blood Cancer Discovery* (2021) 2 (4): 319–325. https://doi.org/10.1158/2643-3230.BCD-21-0046

CONTACT US TO LEARN MORE

info@missionbio.com
415 854 0058

MISSIONBIO.COM