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

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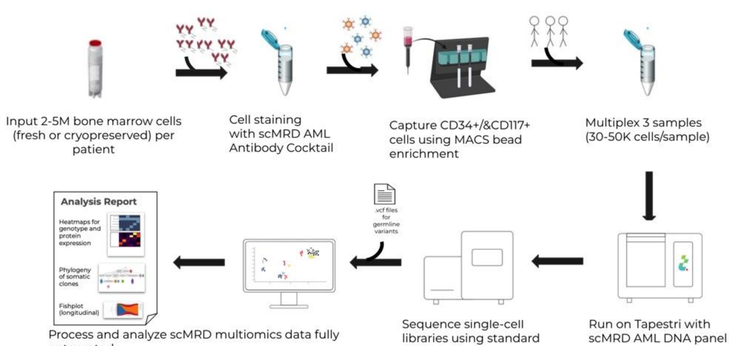
Aim

- 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.
- By combining high sensitivity with multiomics, this assay provides researchers with comprehensive and clinically actionable insights into AML MRD.

Methods

Fig 1: Tapestri® scMRD AML assay workflow:



The scMRD workflow leverages:

(i) MACs bead enrichment protocol to enrich for CD34+ and/or CD117+ cells.

(ii) a DNA and protein panel specifically designed for AML MRD diagnosis and treatment[1, 2, 3]

(iii) the ability to multiplex up to three patient samples in a single run.

(iv) a new, automated analysis pipeline to evaluate single-cell multiomics output. The pipeline uses each patient's known germline SNP genotypes for demultiplexing samples.

Results

Fig 2: **(A)** Genes covered by the scMRD DNA panel. **(B)** The protein AOCs covered by the scMRD protein panel.

(A)

ASXL1	FLT3	MYC	SF3B1
BCOR	GATA1	MYH11	SMC1A
BRAF	GATA2	NF1	SRSF2
CALR	IDH1	NPM1	STAG2
CBFB	IDH2	NRAS	TET2
CBL	IL6R*	PHF6	TP53
CHEK2	IP6K1*	PPM1D	TRPC4*
CSF1R	JAK2	PTPN11	U2AF1
CYP4F3*	KIT	RAD21	UBA1*
DNMT3A	KMT2A	RUNX1	WT1
ETV6	KRAS	SETBP1	ZEB2*
EZH2	MEIS2*	SF3A1*	ZRSR2

Germline SNPs for sample demultiplexing
Grey: The European LeukemiaNet (ELN), World Health Organization (WHO) & International Consensus Classification (ICC)
*Germline SNPs for sample de-multiplexing
Blue: scDNA-seq studies (Lenine, Smith, Takahashi) identified mutations that co-occur to provide a fitness advantage leading to disease progression, treatment resistance and relapse

(B)

Name	ID	Name	ID
CD2	0367 anti-human CD2	CD33	0052 anti-human CD33
CD3	0034 anti-human CD3	CD34	0054 anti-human CD34
CD7	0066 anti-human CD7	CD38	0389 anti-human CD38
CD10	0062 anti-human CD10	CD45RA	0063 anti-human CD45RA
CD11b	0161 anti-human CD11b	CD56	0047 anti-human CD56 (NCAM)
CD13	0364 anti-human CD13	CD123	0064 anti-human CD123
CD14	0081 anti-human CD14	HLA-DR	0159 anti-human HLA-DR
CD19	0050 anti-human CD19	CD117	anti-human CD117 (A3C6E2)
CD22	0393 anti-human CD22		

Table 1: Performance of the scMRD assay on samples containing CD34 + or CD117+ cell lines spiked in at 0.01 %. The enrichment ratio is the ratio of the spike in % post enrichment divided by the spike-in % before enrichment.

Sample type	Number of runs	Immuno-phenotype	Spike-in %	Number of spike-in cells (average)	Variant specificity (average)	False positive variants (average)	Fold Enrichment (average)
Cell line (KG1)	**15**	**CD34+**	**0.01%**	**11.3**	**99.9**	**0.36**	**27.8**
Cell line (HMC-1.2)	**13**	**CD117+**	**0.01%**	**10.4**	**99.9**	**0.39**	**25.2**

Fig 3: Enrichment protocol shows the expected change in cell type percentages in healthy bone marrow. Myeloid progenitors increase and T cells decrease. Each point is from a different Tapestri run. Sticky cells are those that express most proteins, and so are likely dead cells.

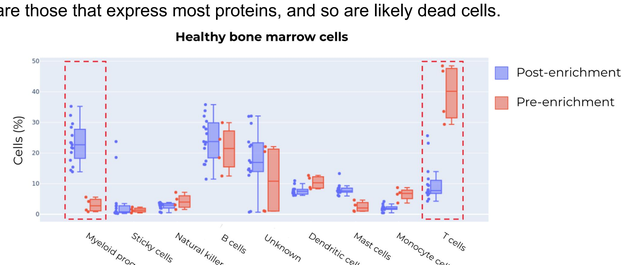
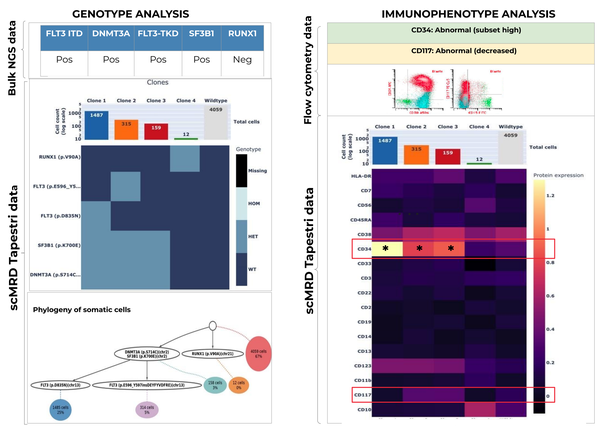


Fig 4: Example bioinformatics analysis of Tapestri® data from 1 clinical sample that contains 4 known somatic variants, where the corresponding somatic clones express high levels CD34+ and decreased levels of CD117+.



· Statistically significantly higher expressed compared to wild type

Conclusions

- **The scMRD assay resolved the clonal architecture identifying multiple leukemic clones with co-occurring mutations.**
- **The integration of genotype and immunophenotypic further enhanced MRD detection by identifying genotype-specific protein expression patterns.**
- **The assay demonstrates a limit of detection of 0.01%, specificity of ≥ 99% and a false positive rate of ≤ 3 variants per sample.**
- **Clinical sample genotype and phenotype align with known truth**
- **Samples run on the new V3 chemistry yielded high cell capture rate (26,252 cells).**
- ***By combining high sensitivity & specificity with multiomics, the scMRD AML assay offers a potential scalable solution for comprehensive MRD detection that guides therapeutic decision-making.***

References

1. Heuser, Michael, et al. "2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party." Blood, The Journal of the American Society of Hematology 138.26 (2021): 2753-2767.
2. Döhner, Hartmut, et al. "Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN." Blood, The Journal of the American Society of Hematology 140.12 (2022): 1345-1377.
3. Arber, Daniel A., et al. "International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data." Blood, The Journal of the American Society of Hematology 140.11 (2022): 1200-1228.