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Use of a single-cell multi-omics approach to determine MRD-markers of AML

Preliminary results from a pilot study

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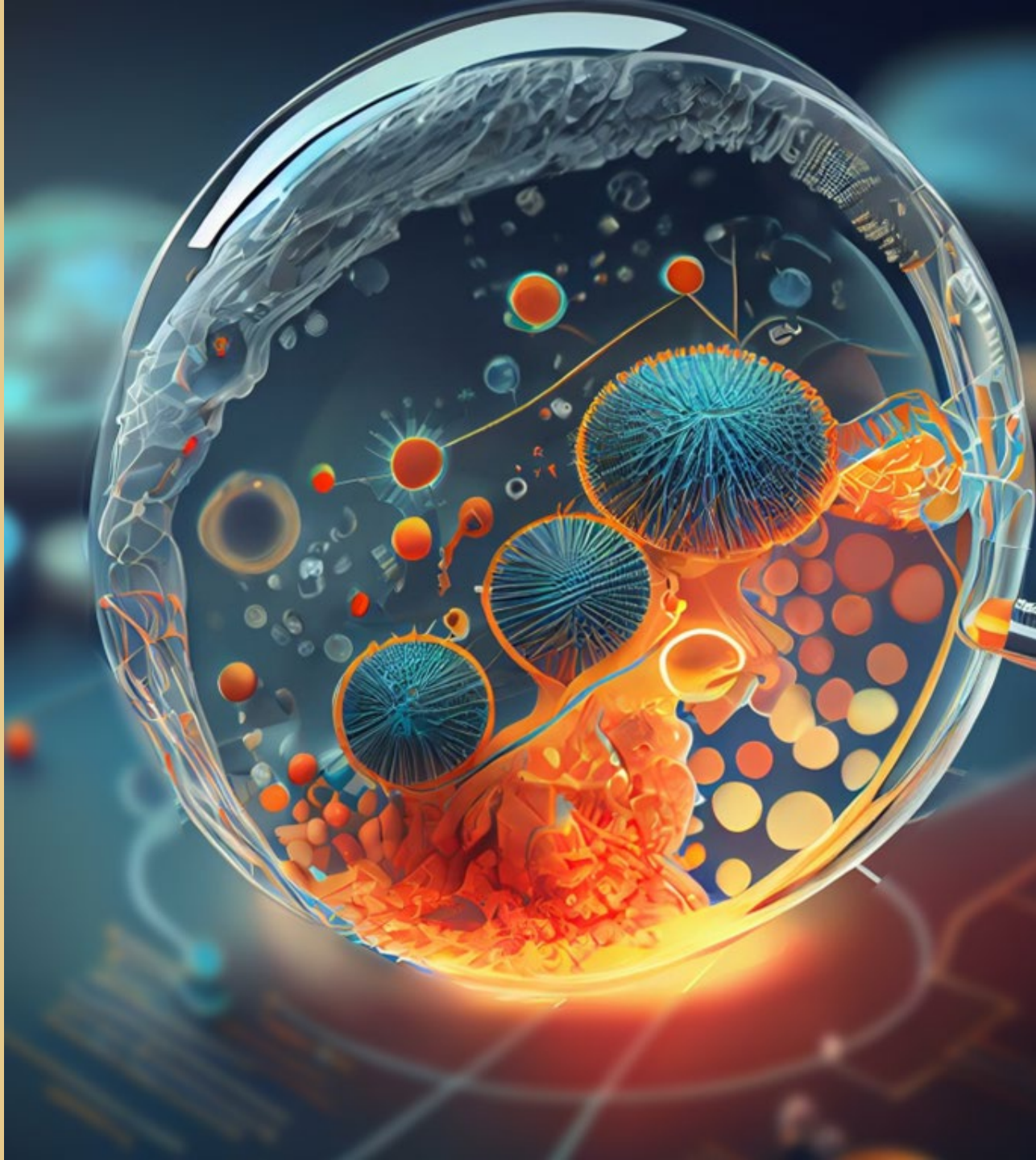

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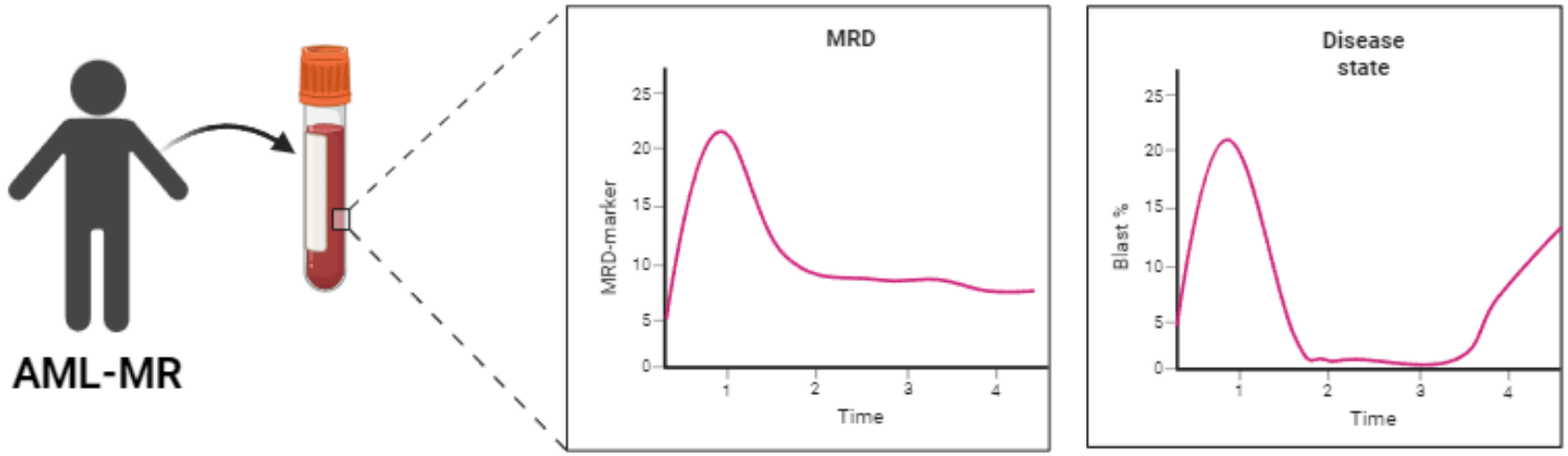
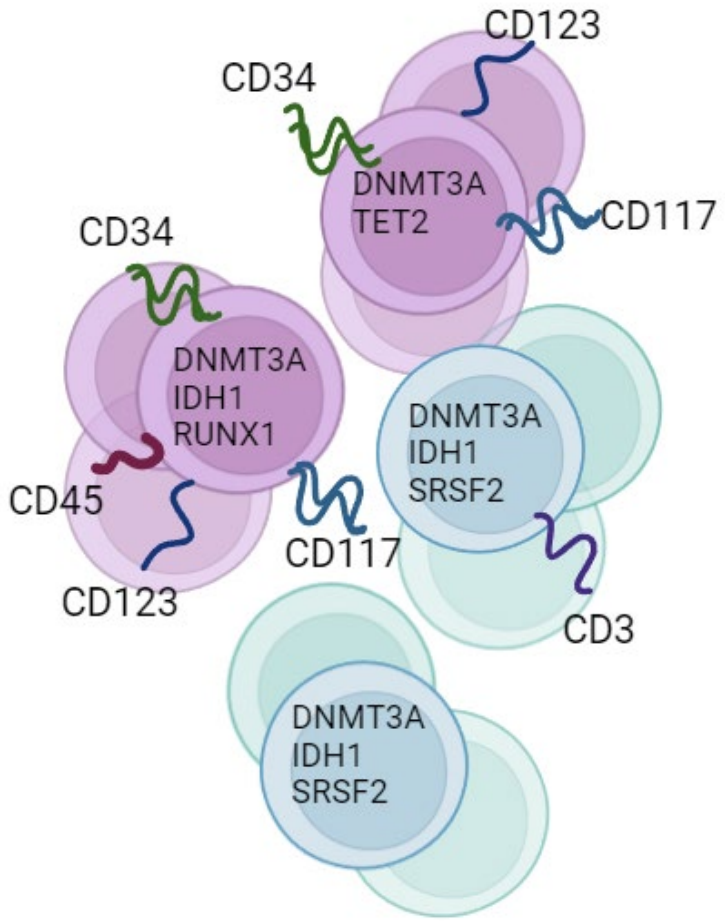
Identifying MRD-markers for MDS/AML and AML-MR patients

- Identifying MRD-markers for MDS/AML and AML-MR patients can be problematic
  - Previously acquired somatic variants
  - Aberrant immunophenotypes of cells due to myelodysplasia



Single-cell multi-omics for MRD in AML

- What if we could combine immunophenotyping and sequencing on a single-cell level?
  - Distinguish between MDS- and AML-associated variants
  - Identification of MRD-marker



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Robinson, TM et al. *Science advances* vol. 9,38 (2023): eadg0488. doi:10.1126/sciadv.adg0488
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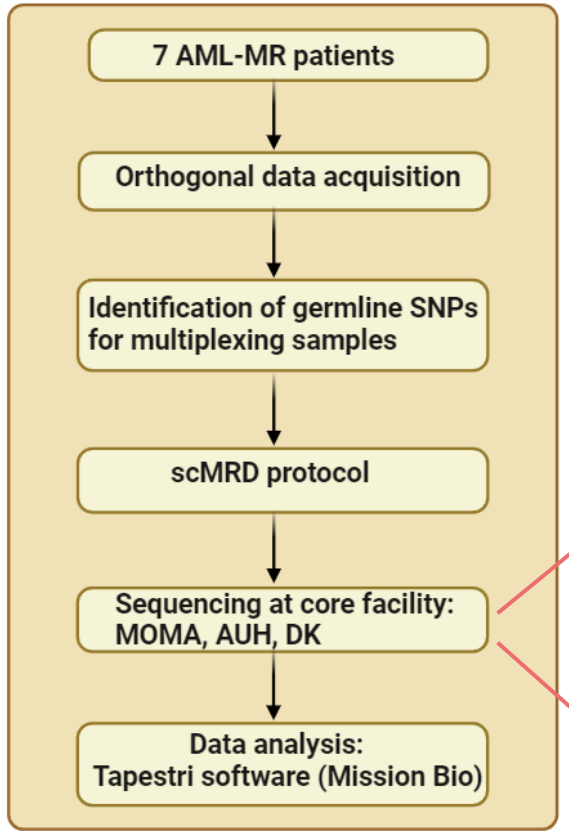
Patients & workflow

Inclusion criteria

- MDS/AML or AML-MR[1]
- Available bone marrow biopsy material from diagnosis (Dx) sample and first follow-up sample (MRD)
- Orthogonal data: NGS, MFC



DNA Panel, 40 genes

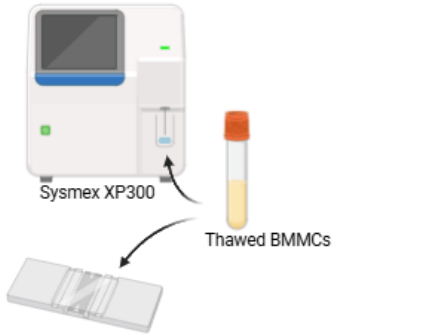
ASXL1	FLT3	MYC	SF3B1
BCOR	GATA1	MYH11	SMC1A
BRAF	GATA2	NF1	SRSF2
CALR	IDH1	NPM1	STAG2
CBFB	IDH2	NRAS	TET2
CBL	IL6R*	PHF6	TP53
CHEK2	IP6K1*	PPM1D	TRPC4*
CSF1R	JAK2	PTPN11	U2AF1
CYP4F3*	KIT	RAD21	UBA1*
DNMT3A	KMT2A	RUNX1	WT1
ETV6	KRAS	SETBP1	ZEB2*
EZH2	MEIS2*	SF3A1*	ZRSR2

Antibody-oligonucleotide conjugate (AOC) panel, 17 proteins

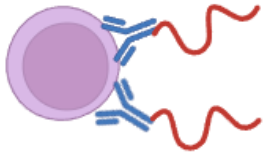
CD2	CD14	CD38	CD117
CD3	CD19	CD45RA	
CD7	CD22	CD56	
CD11b	CD33	CD123	
CD13	CD34	HLA-DR	

1 Döhner, Hartmut et al. “Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN.” *Blood* vol. 140,12 (2022): 1345-1377. doi:10.1182/blood.2022016867

Methods



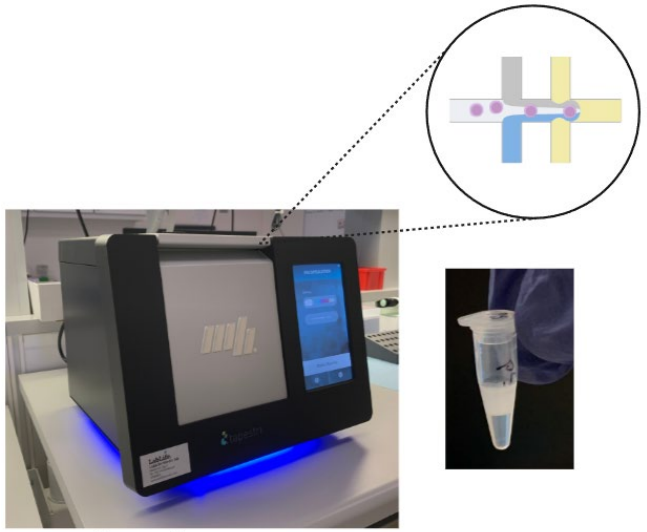
Thaw sample, quantify cells and asses viability



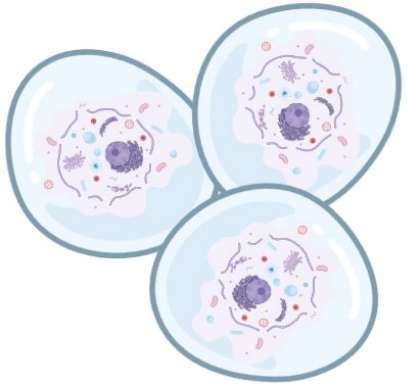
Staining cells with 17-plex AOC panel

Enrich CD34+ and CD117+ cells with MACS™

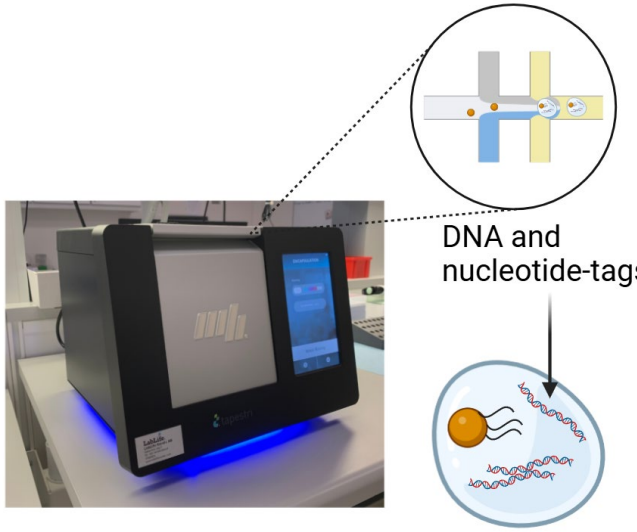
Multiplex cells from 3 samples



Encapsulate cells using the Tapestri instrument

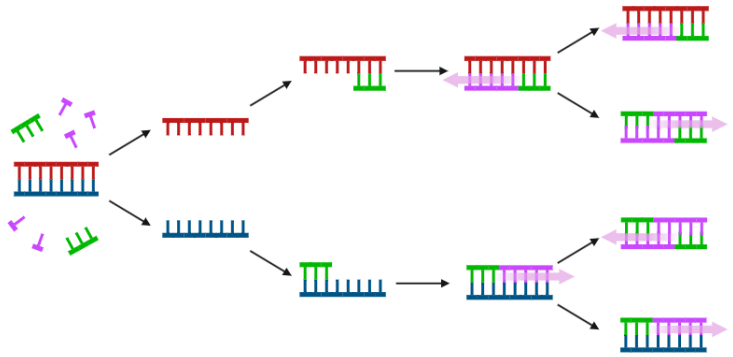


Cell lysis and protein digestion



Barcode cells using the Tapestri instrument

DNA and nucleotide-tags



Targeted PCR amplification,1st cleanup, library PCR, and 2nd cleanup

QC followed by sequencing on NovaSeq XPlus

Conclusion based on preliminary results

- **Remaining samples are to be analyzed!**
- Preliminary results indicate:
  - The scMRD-method (MissionBio) can distinguish between subclones in AML samples
  - It is possible to construct phylogenetic trees based on the clonality of samples
  - scMRD of diagnostic samples aids in identification of the dominant clone incl. genotype and immunophenotype
  - A scMRD method can possibly aid in identification of suitable MRD-markers for AML-MR patients?

Acknowledgements, affiliations & disclosures

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