XIX CONGRESSO NAZIONALE SIES 2026

IMMUNE CELLS AND EXTRACELLULAR VESICLE-BASED LIQUID BIOPSY REVEAL PROGNOSTIC AND IMMUNOMETABOLIC BIOMARKERS OF EARLY RESPONSE TO AZACITIDINE AND VENETOCLAX IN ACUTE MYELOID LEUKEMIA

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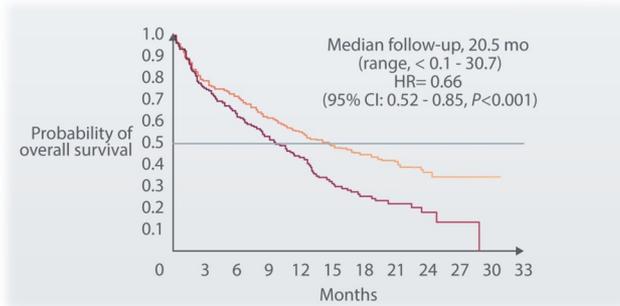Firenze | **4-6 marzo 2026**
Palazzo degli Affari

Disclosures of Federico Pasini

| Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

Nothing to declare

Azacitidine + Venetoclax in Acute Myeloid Leukemia: Innovative, though not without limitations

- Acute myeloid leukemia (AML) is an aggressive malignancy with high relapse rate

- Treatment with the oral BCL2 inhibitor venetoclax (VEN) represents the current standard-of-care for AML patients unfit for intensive chemotherapy, often associated to Azacitidine (AZA)
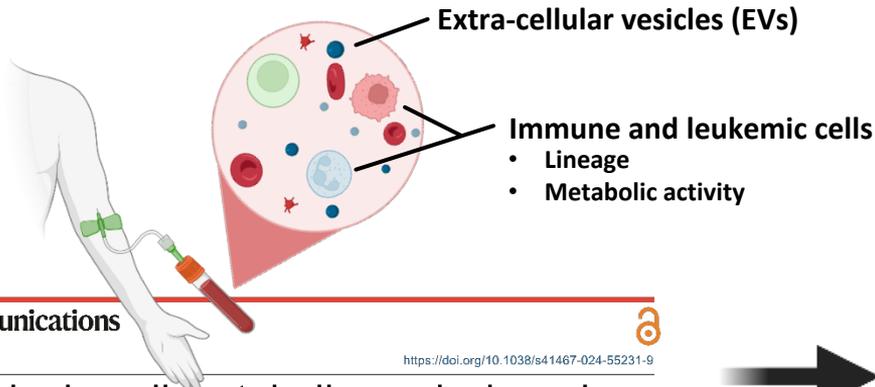

Forsberg et al. Haematologica 2024

- **Relapse prediction remains a clinical challenge**
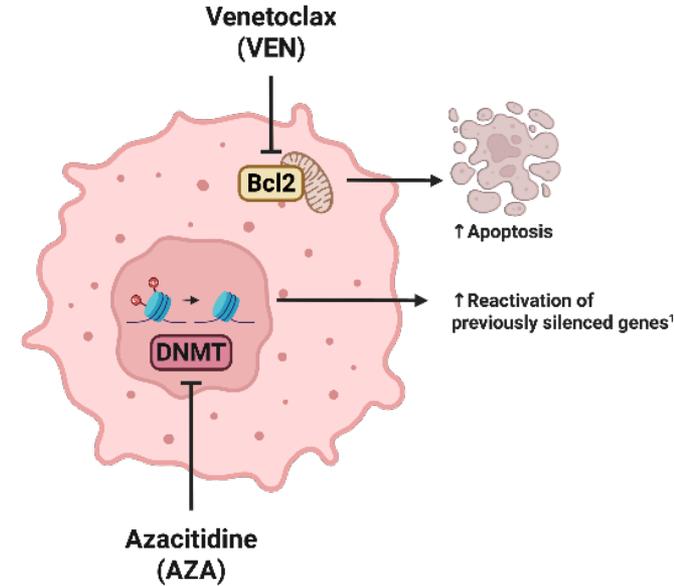
- The metabolism of leukemic stem cells (LSCs) plays a pivotal role in leukemogenesis, influencing relapse and shaping therapeutic response.

[1]: Issa et al. *Nat Rev Drug Discov*, 2005
[2]:DiNardo et al. N Engl J Med. 2020

Extra-cellular vesicles (EVs)

Immune and leukemic cells
- Lineage
- Metabolic activity

Parallel single-cell metabolic analysis and extracellular vesicle profiling reveal vulnerabilities with prognostic significance in acute myeloid leukemia

Dorian Forte [1], Roberto Maria Pellegrino [2], Paolo Falvo [3,4], Paulina Garcia-Gonzalez[5], Husam B.R. Alabed[2], Filippo Maltoni[1,11], Davide Lombardi[3,4], Samantha Bruno [1], Martina Barone [6], Federico Pasini[1], Francesco Fabbri[7], Ivan Vannini[7], Benedetta Donati[8], Gianluca Cristiano[1], Chiara Sartor[1,11], Simona Ronzoni[9], Alessia Ciarrocchi [8], Sandra Buratta[2], Lorena Urbanelli [2], Carla Emiliani[2,10], Simona Soverini[1], Lucia Catani[1,11], Francesco Bertolini [3,4], Rafael José Argüello [5], Michele Cavo[1,11] & Antonio Curti [6] ✉
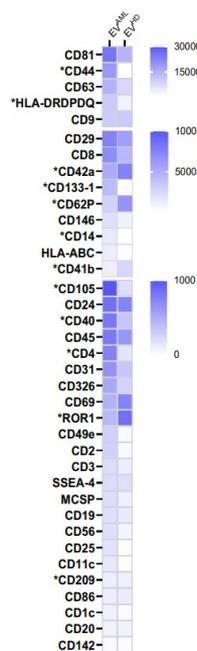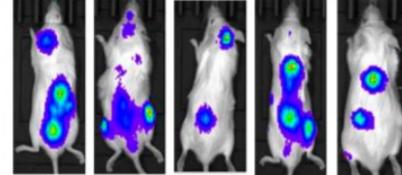
MOLM-13+ adv EV^AML
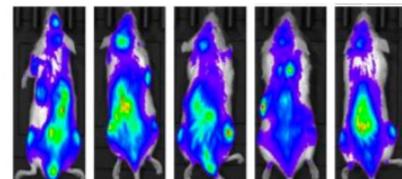
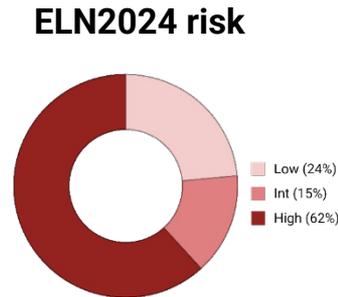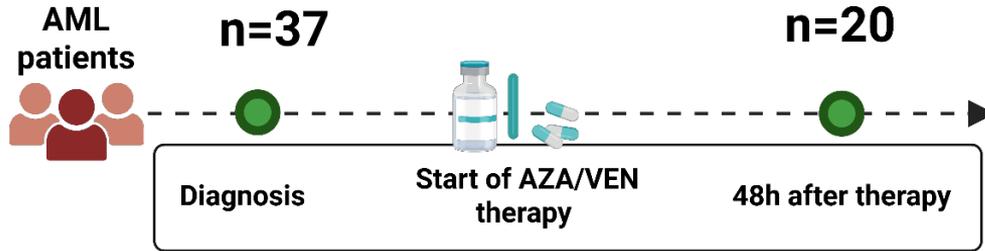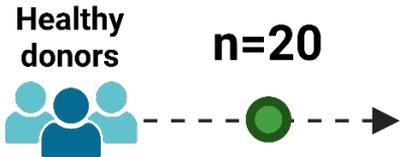MOLM-13+ inter EV^AML
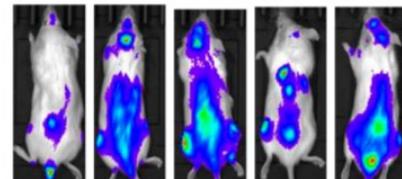
MOLM-13+ fav EV^AML

Can we use liquid biopsy at early timepoints to monitor patients' response to AZA/VEN in AML?

**AML patients**

n=37

n=20

Diagnosis

Start of AZA/VEN therapy

48h after therapy

Healthy donors

n=20

ELN2024 risk

Low (24%)
Int (15%)
High (62%)

Mutational profile

RUNX1 18.92%
TET2 16.22%
TP53 16.22%
FLT3 13.51%
DNMT3A 24.32%
ASXL1 21.62%
IDH1 5.41%
CEBPA 10.81%
NPM1 16.22%
SRSF2 18.92%
IDH2 13.51%

Extracellular Vesicles (EVs) Represent a Promising Tool for Liquid Biopsy in AML

Extracellular vesicles in blood
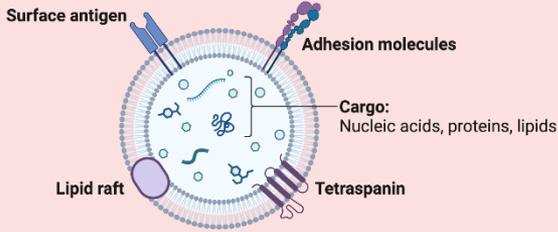
- **Actively released by viable cells**
- **"Cell Biopsy"** [1]
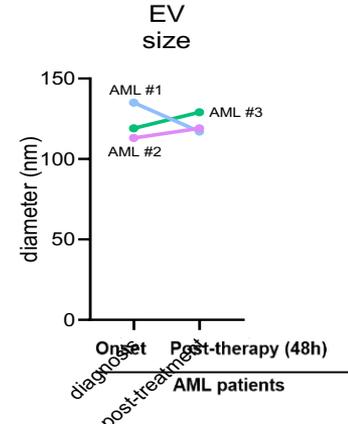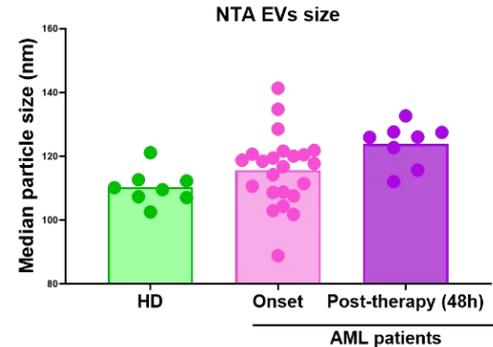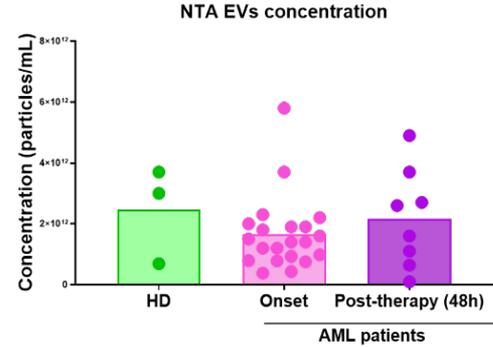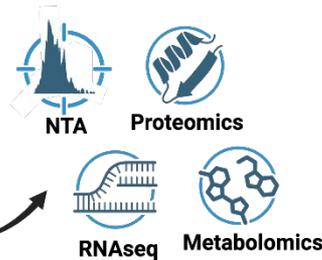- **Abundant in blood (10^4 from 1 cell)**
- **Stable in blood**

EVs characterization

Size-exclusion chromatography — Isolated EVs
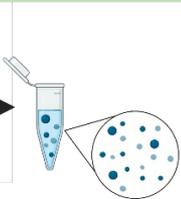
NTA — Proteomics
RNAseq — Metabolomics

EV-TRACK ID: *EV230004*

NTA EVs concentration

Concentration (particles/mL)

HD — Onset — Post-therapy (48h)
AML patients

NTA EVs size

Median particle size (nm)

HD — Onset — Post-therapy (48h)
AML patients

EV size

diameter (nm)

AML #1
AML #3
AML #2

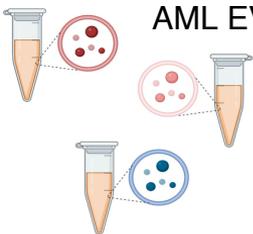Onset (diagnosis) — Post-therapy (48h) (post-treatment)
AML patients

[1]: Trino et al. *Leukemia*, 2021

Multiplex Proteomic Profiling of AML-Derived EVs at Onset Reveals Prognostic Markers

Multiplex Proteomic Profiling of EVs show a specific profile in AML compared to HD
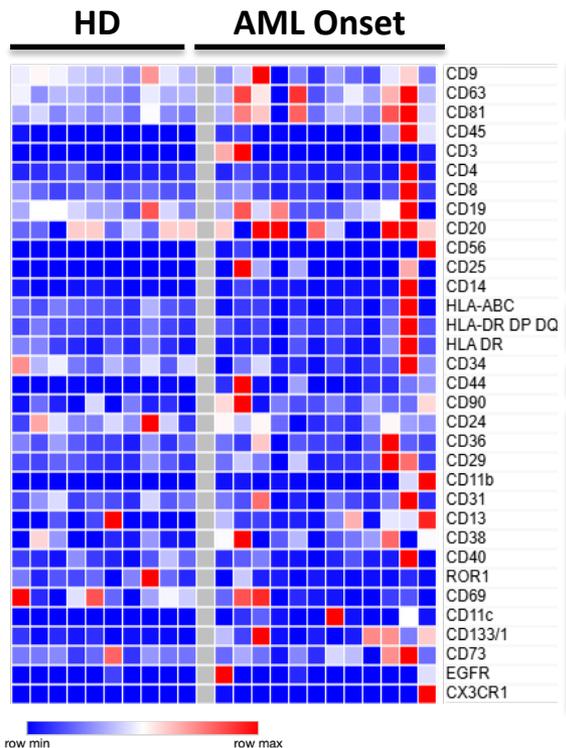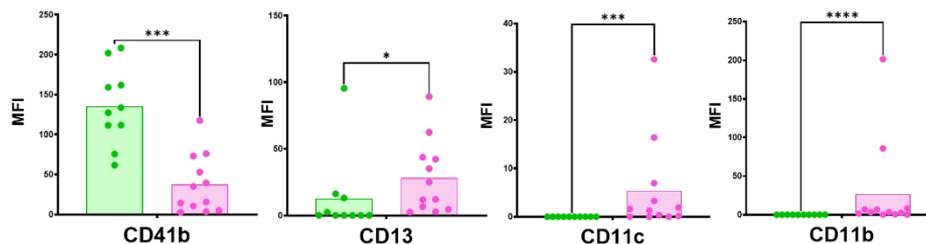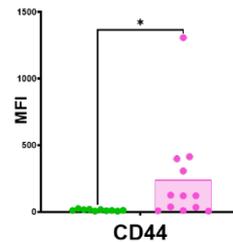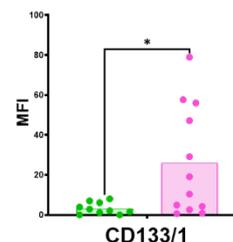
EV RNA sequencing highlights a T cell and metabolic involvement

Gene expression

Over-Representation Analysis (ORA)
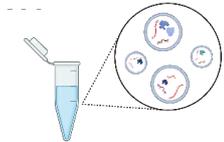
EVs RNA extraction
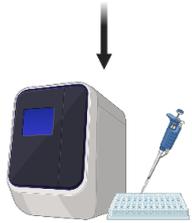
cDNA library preparation

Sequencing
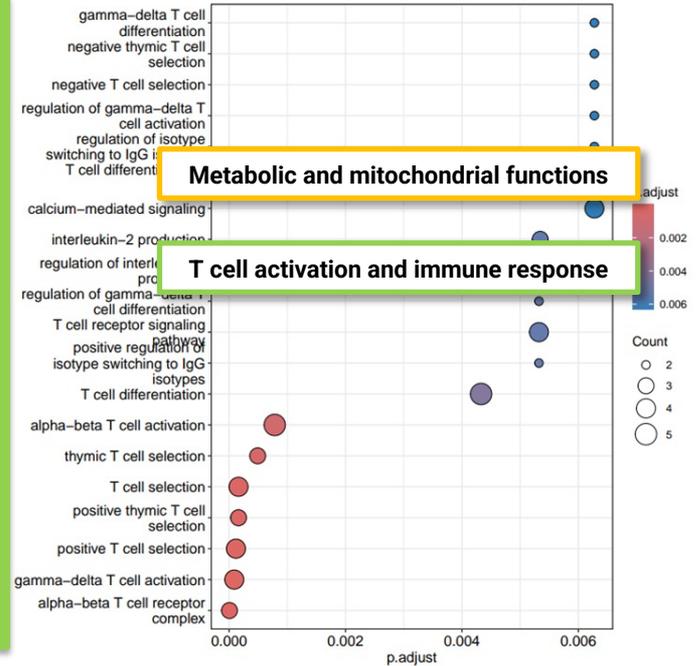
Metabolic and mitochondrial functions

T cell activation and immune response

T cell activation and immune response

N = 12 HD vs 25 AML ONSET

RNA analysis performed by F. Maltoni

Multi-parametric spectral flow cytometry allows to detect rare cell subsets

SONY ID7000 spectral flow cytometry

36 colors in a single tube (spectral unmixing)

SCENITH[1]

(Single Cell Energetic metabolism by profiling Translation inhibition)

Immunophenotype

- CD34+ CD117-
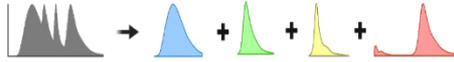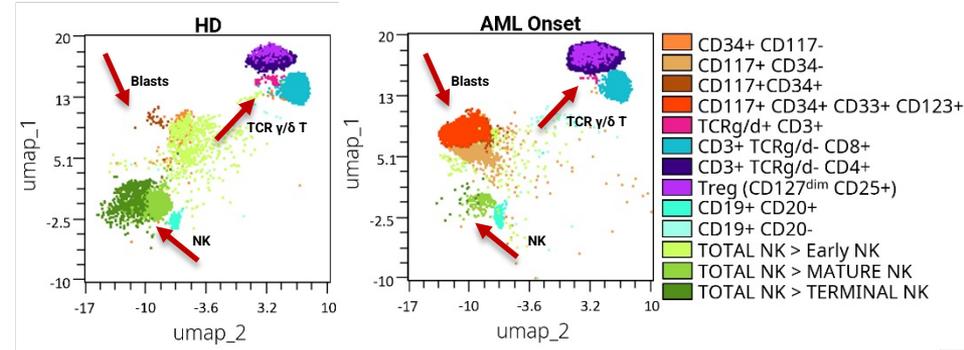- CD117+ CD34-
- CD117+CD34+
- CD117+ CD34+ CD33+ CD123+
- TCRg/d+ CD3+
- CD3+ TCRg/d- CD8+
- CD3+ TCRg/d- CD4+
- Treg (CD127dim CD25+)
- CD19+ CD20+
- CD19+ CD20-
- TOTAL NK > Early NK
- TOTAL NK > MATURE NK
- TOTAL NK > TERMINAL NK
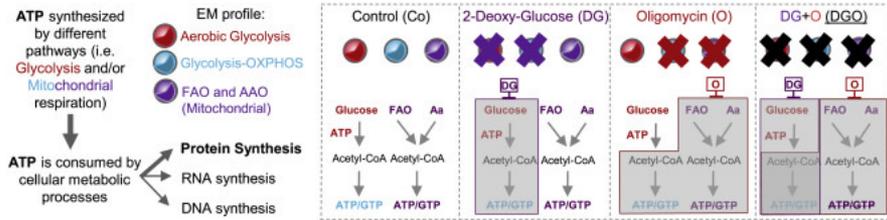
SCENITH metabolic analysis by cell type or risk

N = 30 AML ONSET

[1]: Argüello et al., Cell Metabolism, 2020
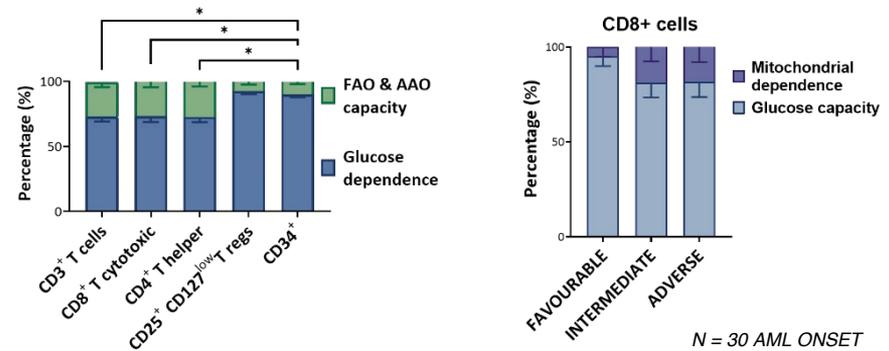
Immuno-metabolic shift after 48h of AZA/VEN therapy

**SCENITH analysis performed on whole peripheral blood
-> Analysis in real-time**
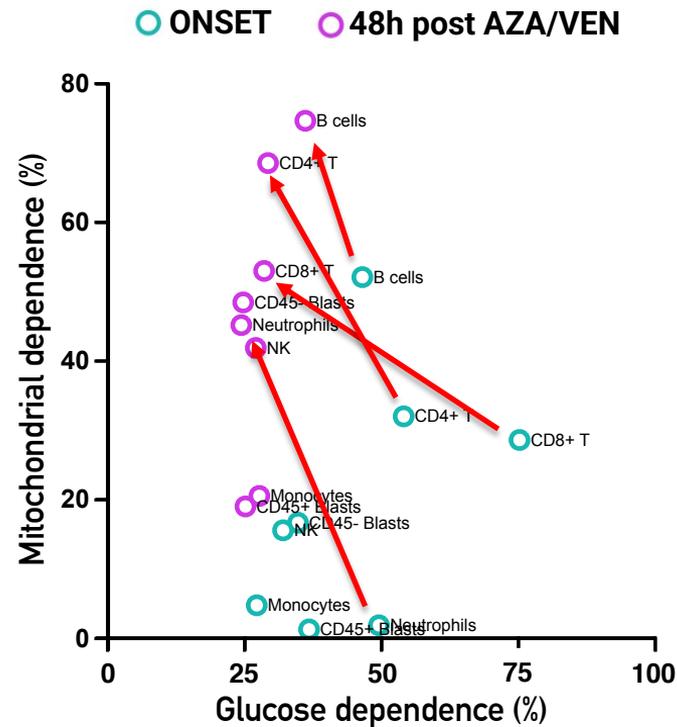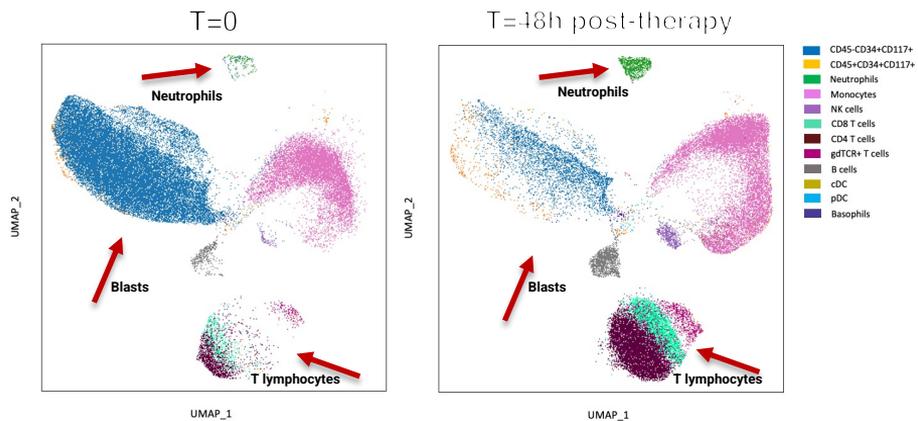
AML EVs 48h post AZA/VEN show a decreased level of immune-associated markers
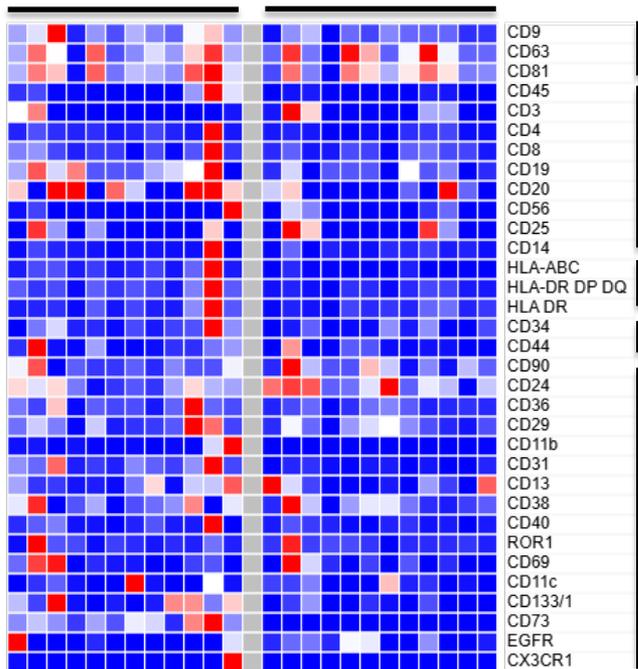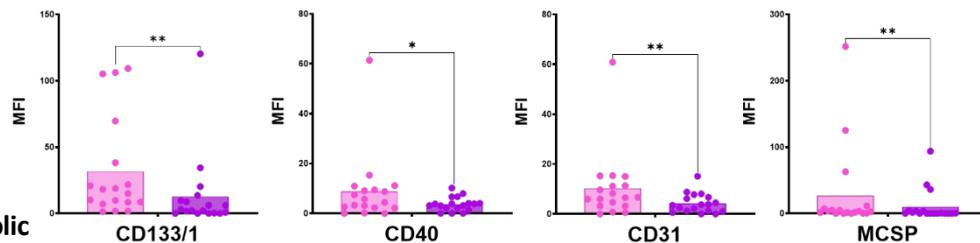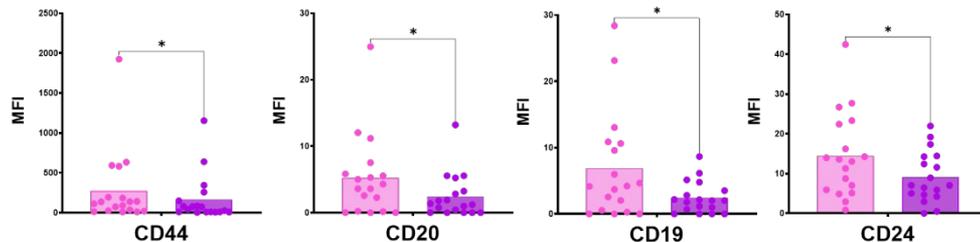
AML EVs 48h post AZA/VEN show a decreased level of immune-associated markers
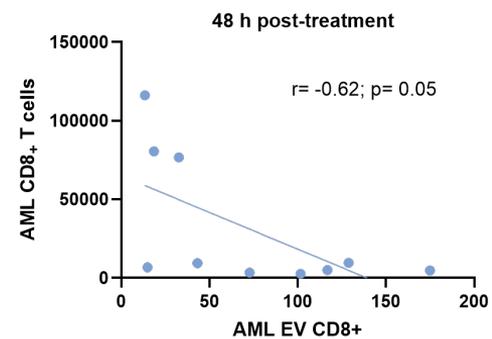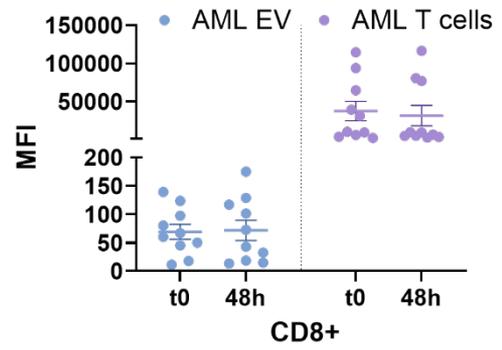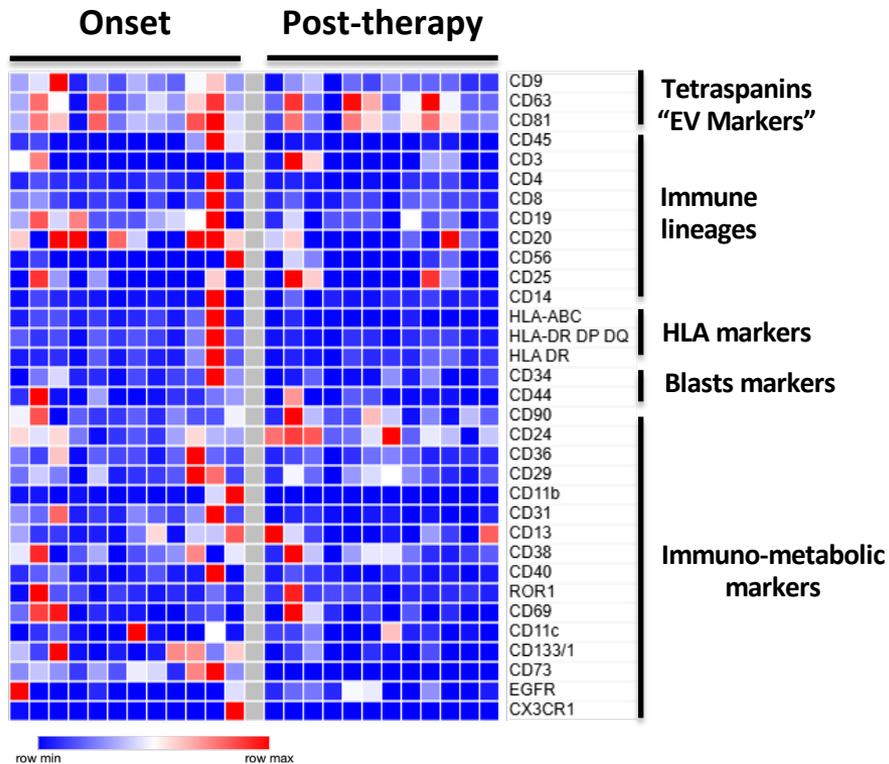
Conclusions

- Liquid biopsy provides useful insights in the monitoring of therapy response at early timepoints

- Immune cells metabolism at diagnosis shows prognostic value and after therapy shows a different metabolic profile

- AML EV surface markers correlate with ELN risk classification and show a different profile after therapy at early timepoints, suggesting an immune involvement

Clinical potential: Integrating EV signatures with immune-metabolic crosstalk may reveal novel prognostic biomarkers and allow to monitor therapy response

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Read our paper on EVS from AML at Onset

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