Settima edizione di

G.d.L. Biologia Cellulare Molecolare

AIEOP

ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

AIEOP...

...in Lab

Caratterizzazione multi-omica della malattia residua minima in pazienti pediatrici con B-ALL

Dr. Jolanda Sarno, PhD

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

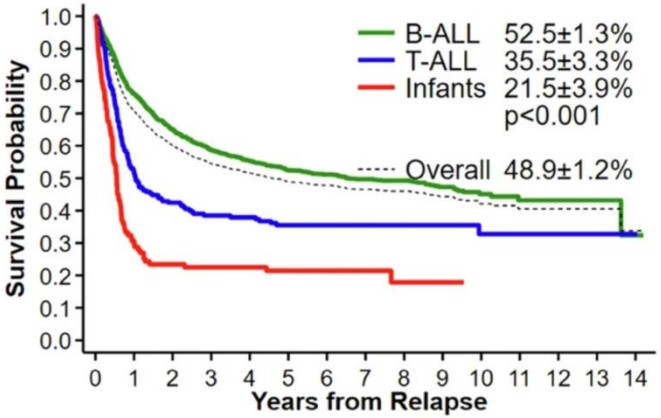
FONDAZIONE TETTAMANTI

Milano, 22 e 23 maggio 2026

Nothing to disclose

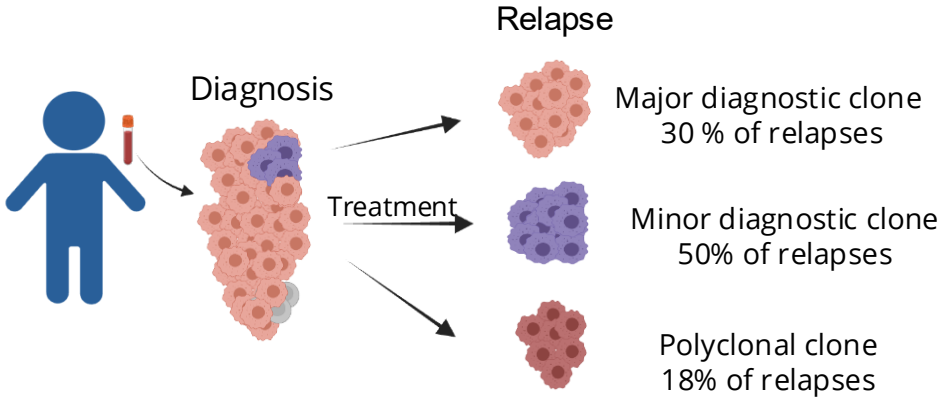
Relapsed ALL remains an unmet clinical need

Survival rates of Relapsed B-ALL patients from 1996 to 2014 in COG trials



Main causes of cancer-related death in children

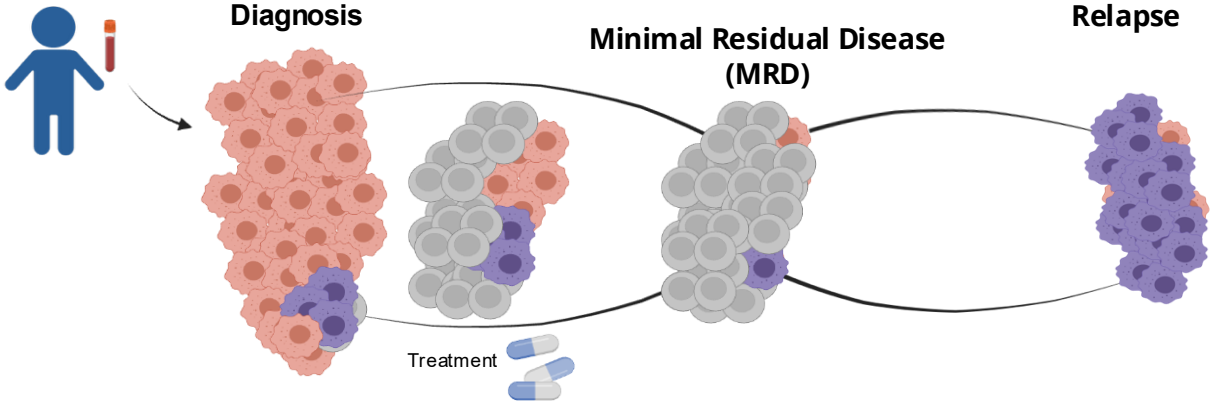
INTRA-Tumor Heterogeneity



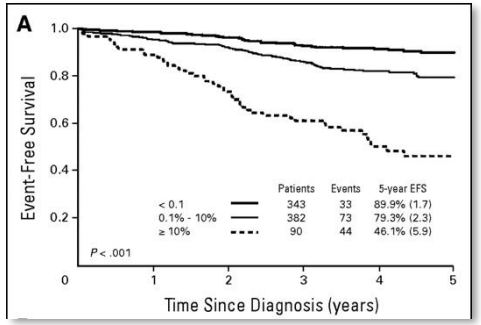
- Current clinical trials do not take into account Intra-tumor heterogeneity
- Minor diagnostic clones are present at relapse

Rheingold SR et al. Leukemia 2024. Waanders E et. Al. Blood Cancer Discov. 2020. Dobson SM et al. Cancer Discov. 2020

MRD: powerful readout of individual treatment response



EFS based on FCM at Day 15



30-40% relapses → Not-high risk MRD patients

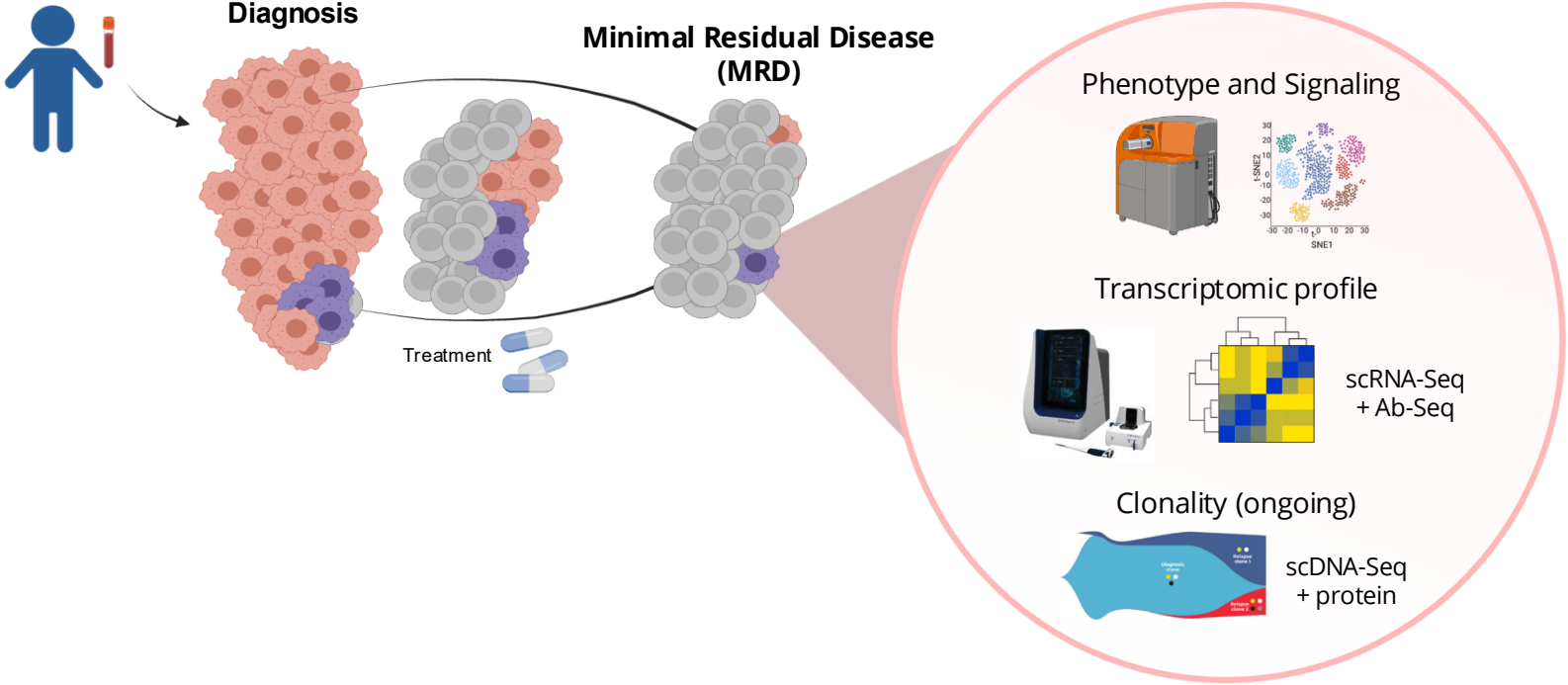
MRD assessment does not inform:

- Why MRD cells are treatment resistant (features)
- Actionable targets for therapeutic intervention

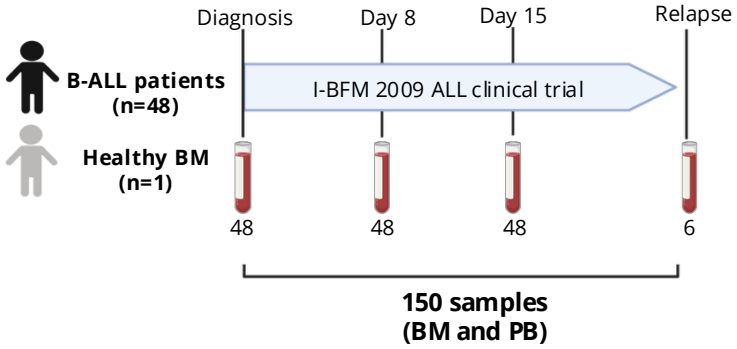
→ **Qualitative analysis of MRD cells** → Improve risk stratification; Inform therapeutically

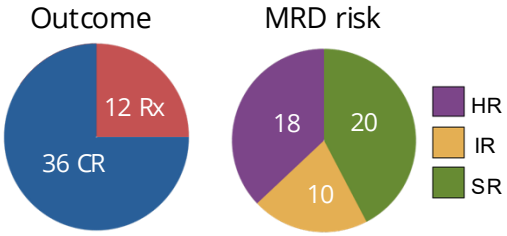
Basso G et al. JCO 2009

Multi-omic integrated approach to dissect resistance in leukemia

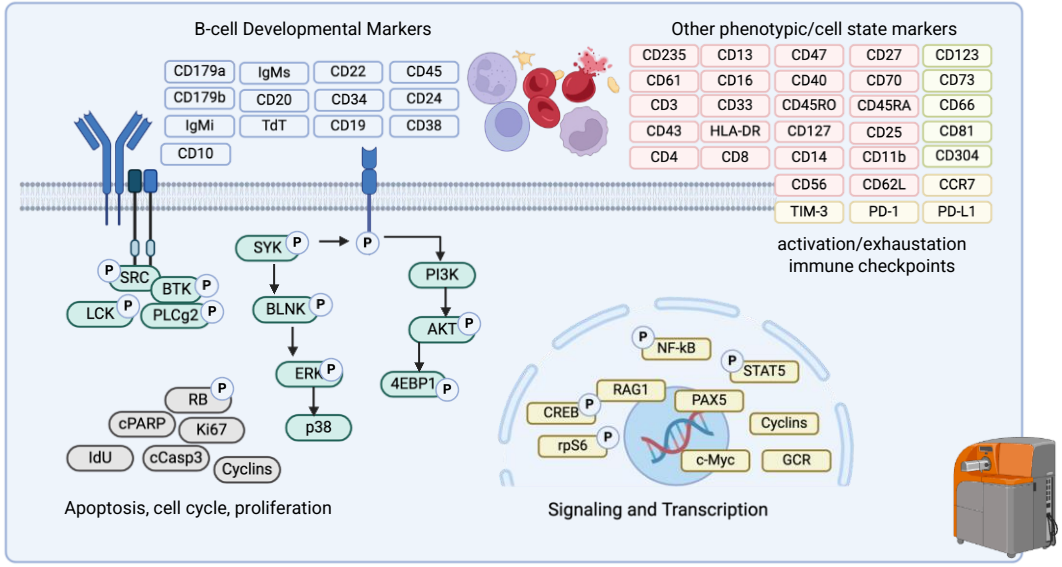


CyTOF analysis of longitudinal B-ALL samples





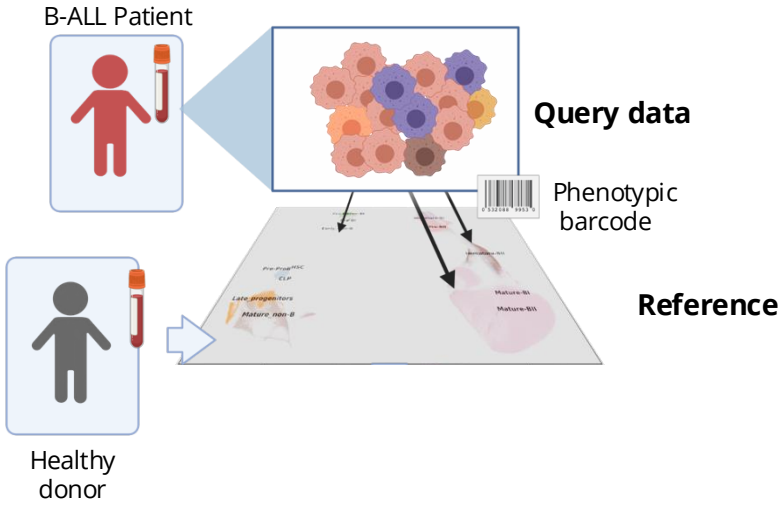
Single cell mass cytometry analysis of B-ALL samples



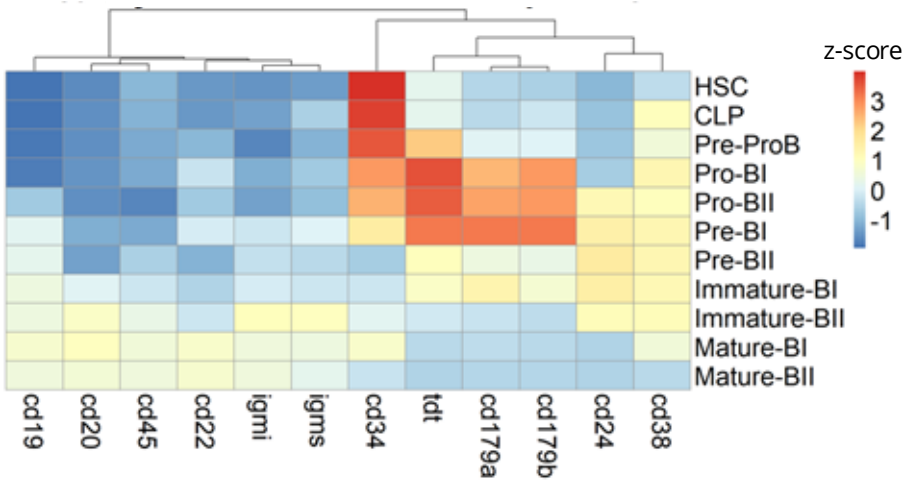
Leukemic cells were detectable at Dx, Day 8 and Day 15 in 98% of the samples (range 0.008% - 96.1%)

B-ALL classification of the gated blasts

Single cell proteomic B cell classifier

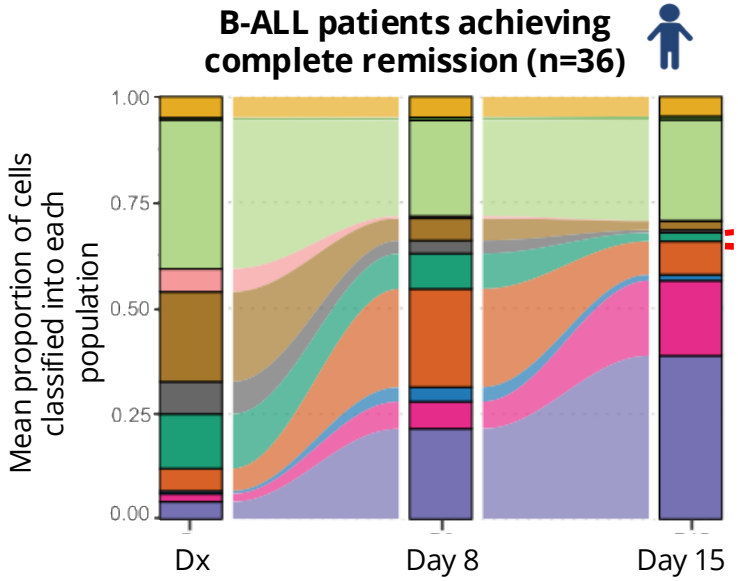


Phenotypic markers expression across gated populations in healthy BM



Good Z & Sarno J et al. Nat Med 2018; kara-davis-lab/DEVclassifier

Developmental classification identifies differentially abundant populations in relapsing patients

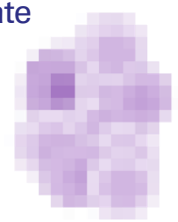


Gated blasts
(CD45^{low}/CD19^{+}/CD10^{+})

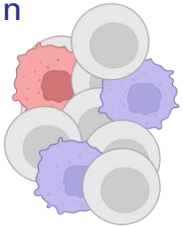
EdgeR analysis
- p<0.05
** p<0.01

Identify relapse-associated cells across patients using machine learning

Population-level aggregate



Single-cell resolution

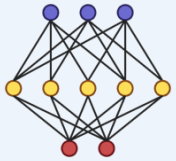


1. Model training (n=41 samples)

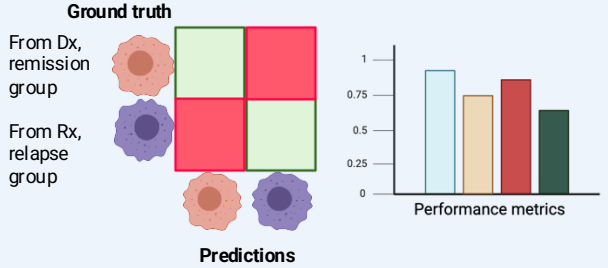
Treatment Sensitive
CR pts at Dx
label 0

Treatment Resistant
Relapsed pts at D8, D15 and Rx
label 1

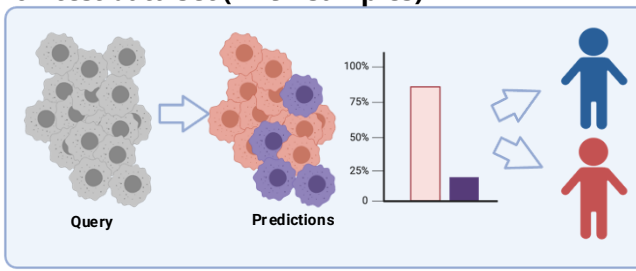
XGBoost model



2. Leave one-out cross validation on relapsed patients (n=12 samples)

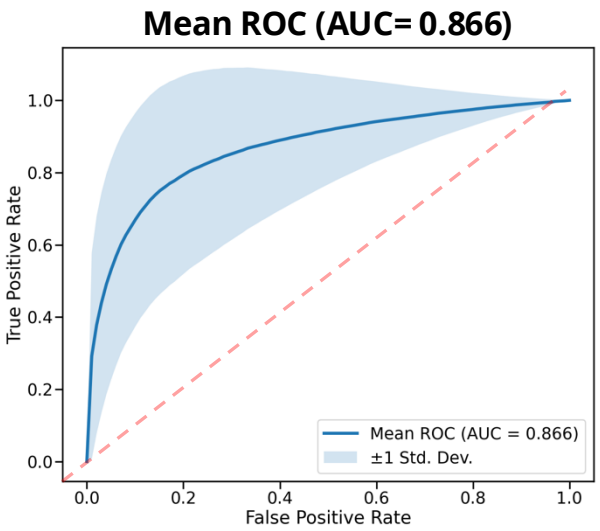


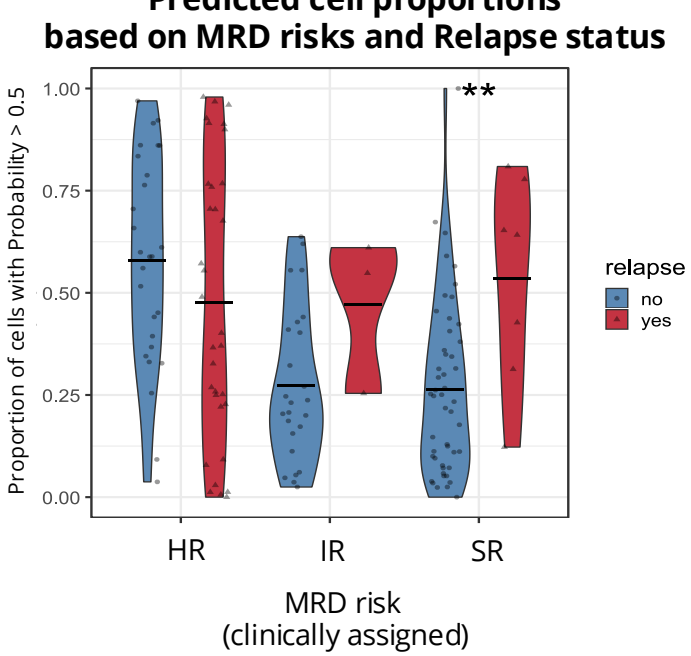
3. Prediction of diagnostic and MRD timepoints on test data set (n= 97 samples)



Riccardo Scribano
PhD student

XGBoost identifies resistant cells accurately in not-high risk patients

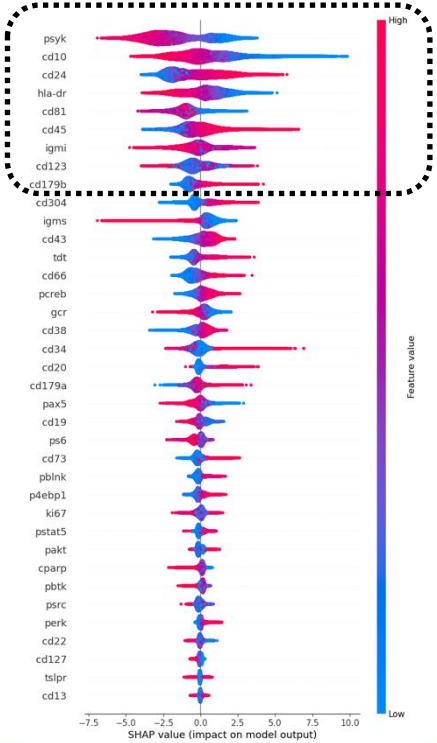




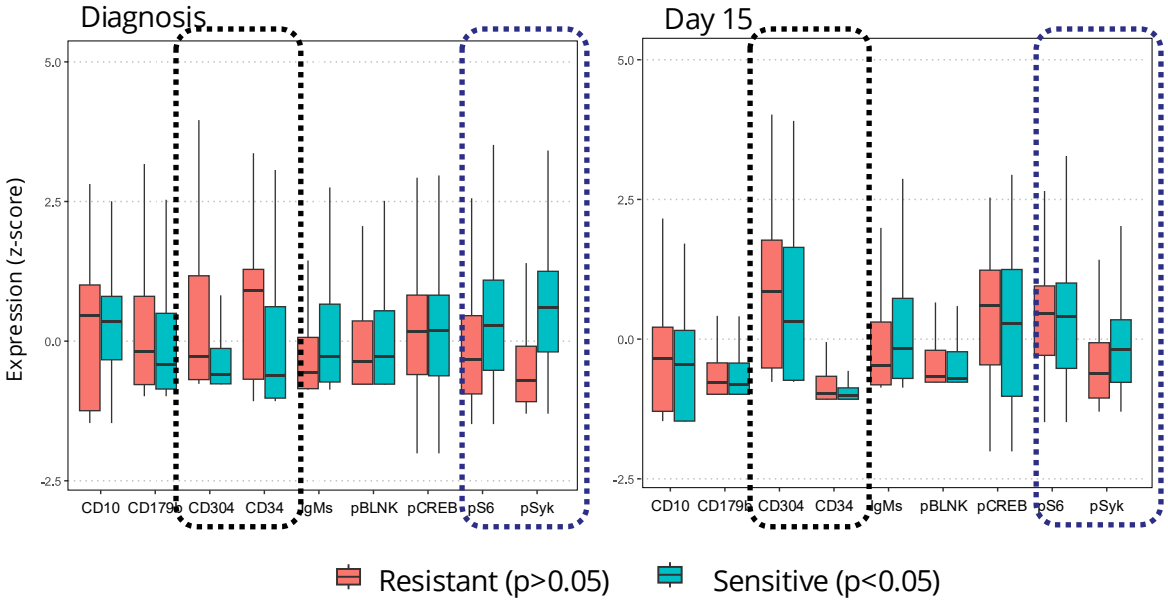
Please do not post

XGBoost features identify immature phenotypes with quiescent signaling state as resistant

Feature importance (SHAP)



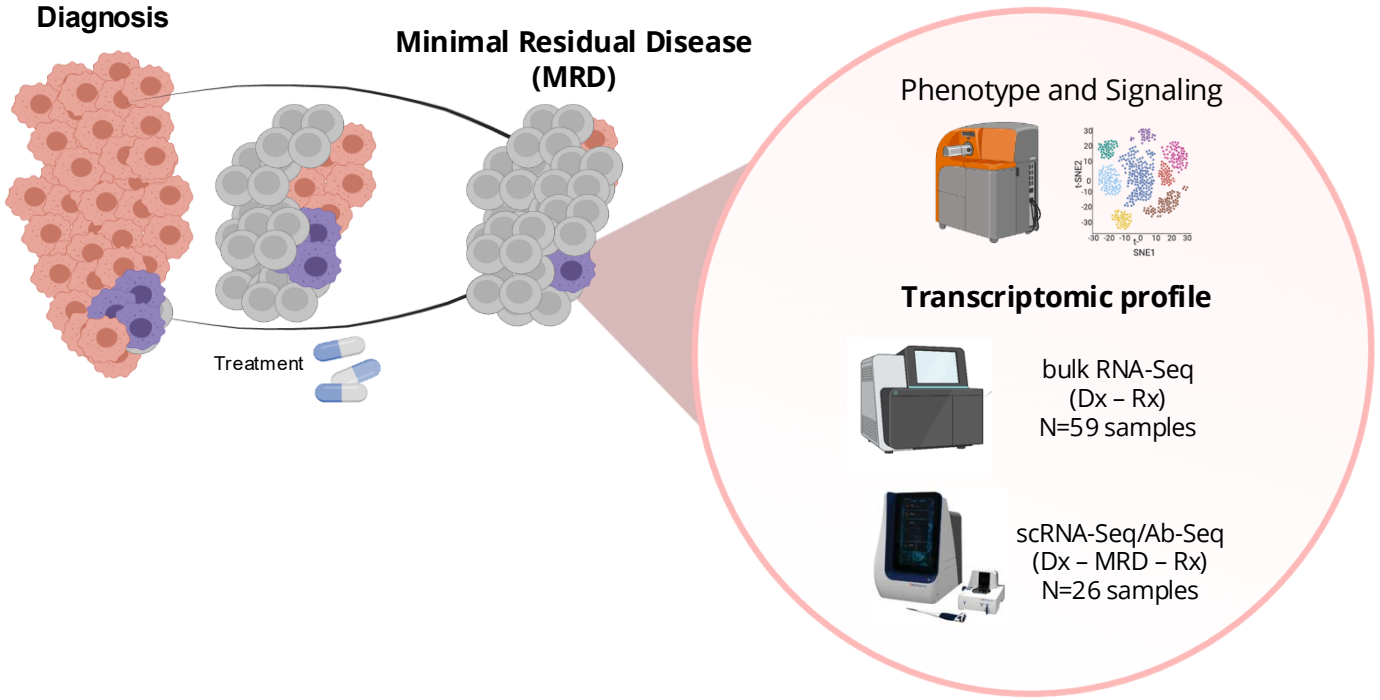
Marker expression in resistant vs sensitive XGBoost predicted cells



Decision threshold (0.5)

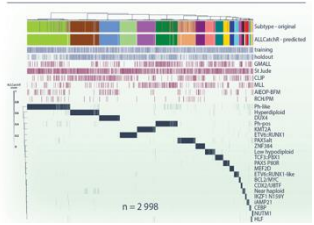
Please do not post

Transcriptomic profile to identify additional resistance features



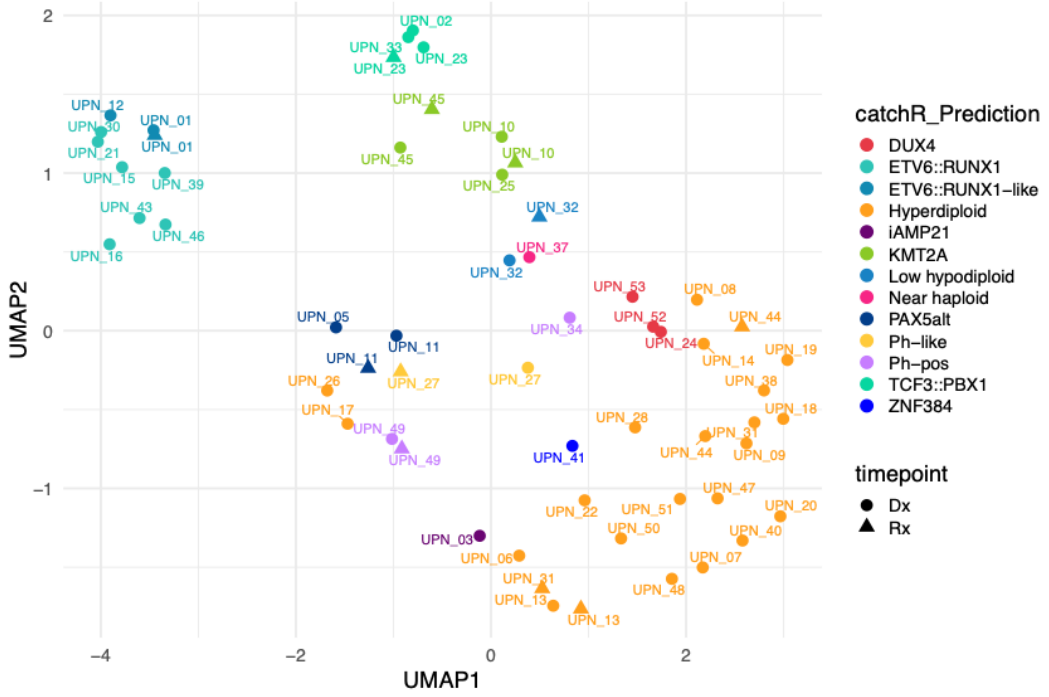
Bulk RNA-Seq: subtype driven transcriptomic profile

B-ALL subtype classification
ALLCatchR

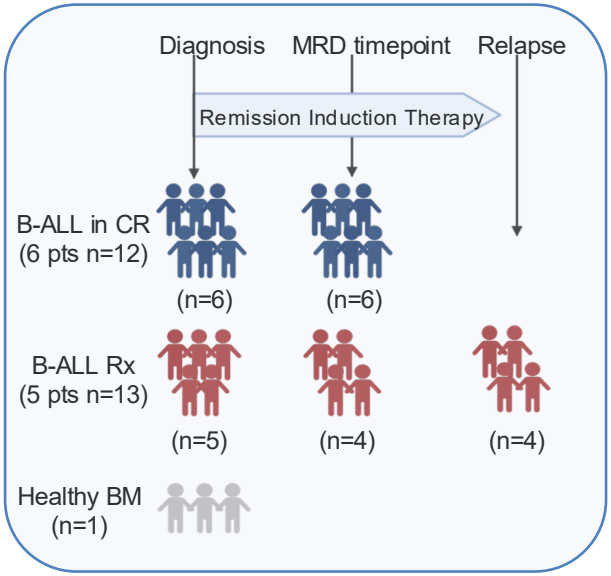


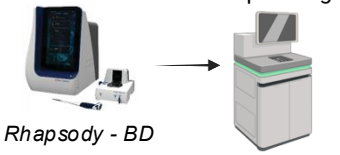
Beder T et al. HemaSphere 2023

UMAP of the top 1000 variable genes in diagnostic and relapse samples



scRNA-Seq/Ab-Seq to study transcriptomic profiles of early resistant cells



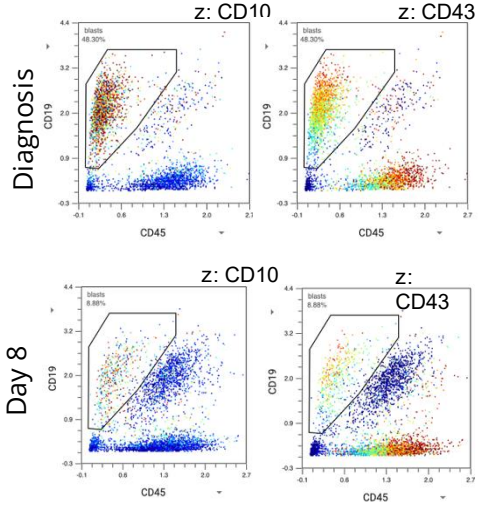


- WTA
- Abs
- BCR
- Sample Tags

Gating blasts using Ab-Seq expression

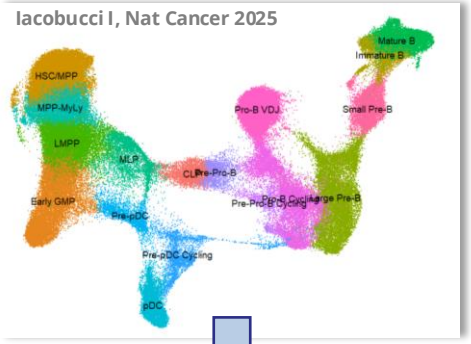
CD10
CD19
CD22
CD20
CD24
CD38
CD127
CD43
CD45
CD73
CD66
CD304
CD123
CD81
IgM

oligo-conjugated antibody staining

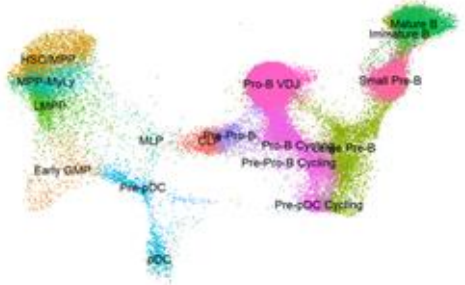


Unfiltered: 221030 (76.6%)
Blasts: 67406 (23.4%)

Healthy BM B-cell enriched reference

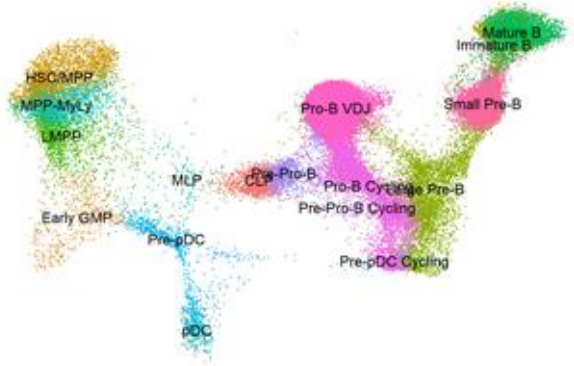


B-ALL blasts projected data

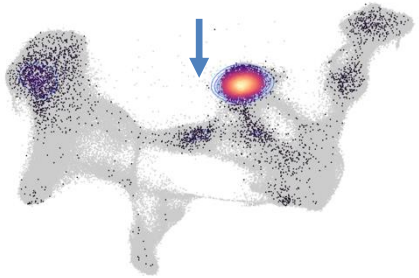


Early MRD cells from relapsing patients are more enriched in early Pro-B populations

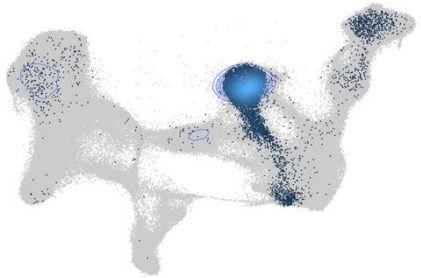
B-ALL blasts projected data

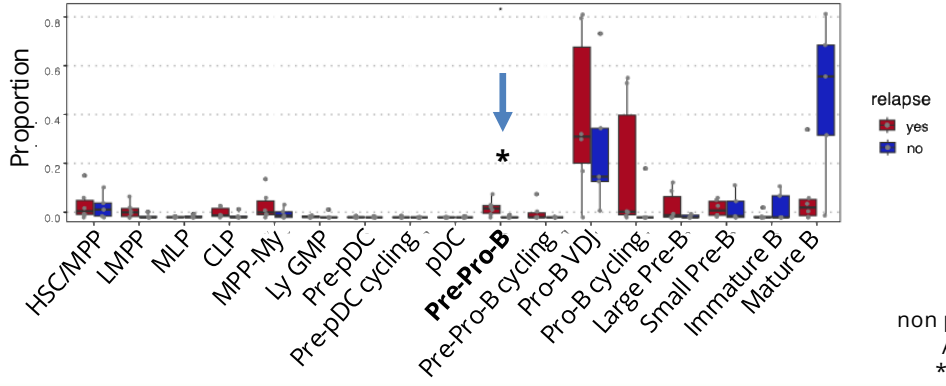


Early MRD cells of Relapsing patients (n=5)



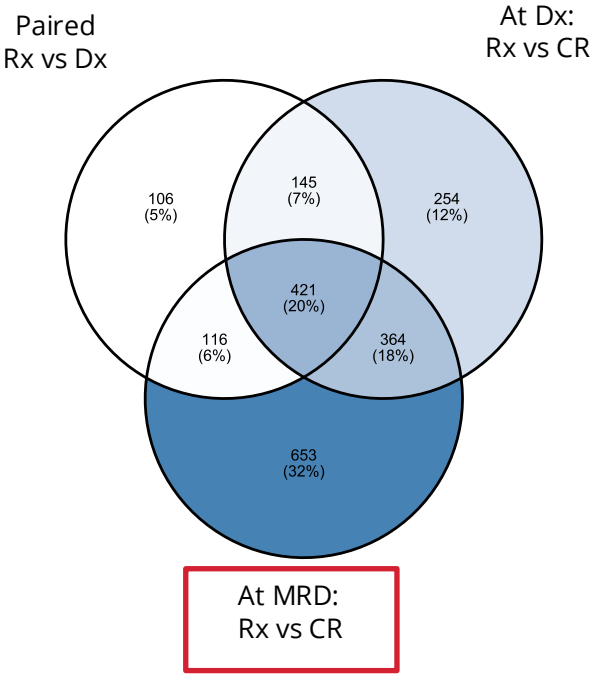
Early MRD cells of CR patients (n=6)

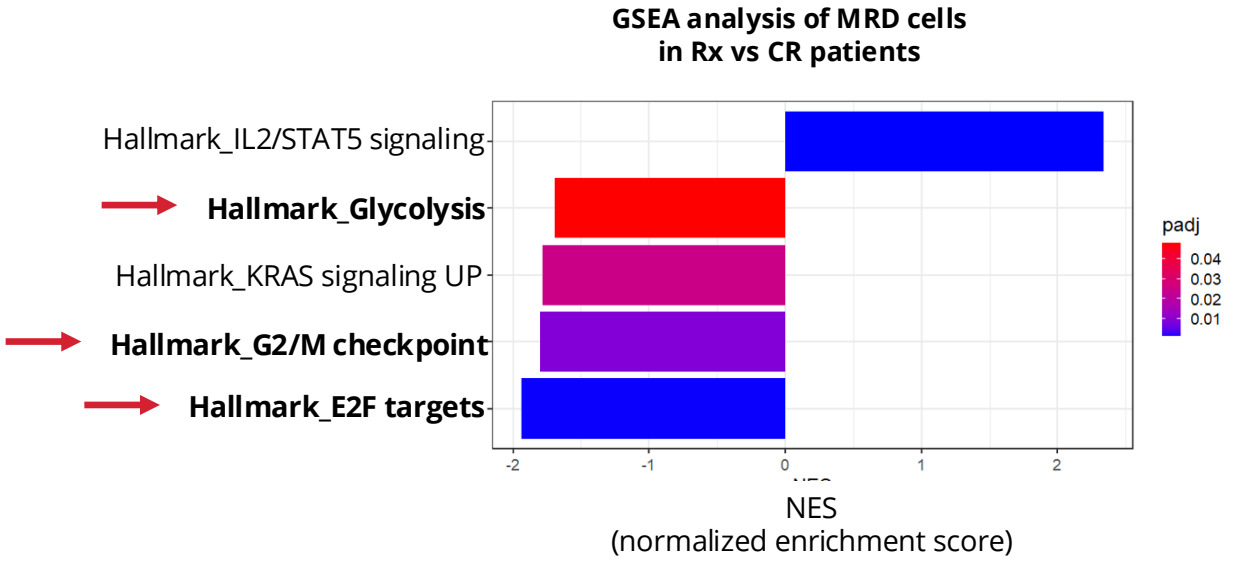




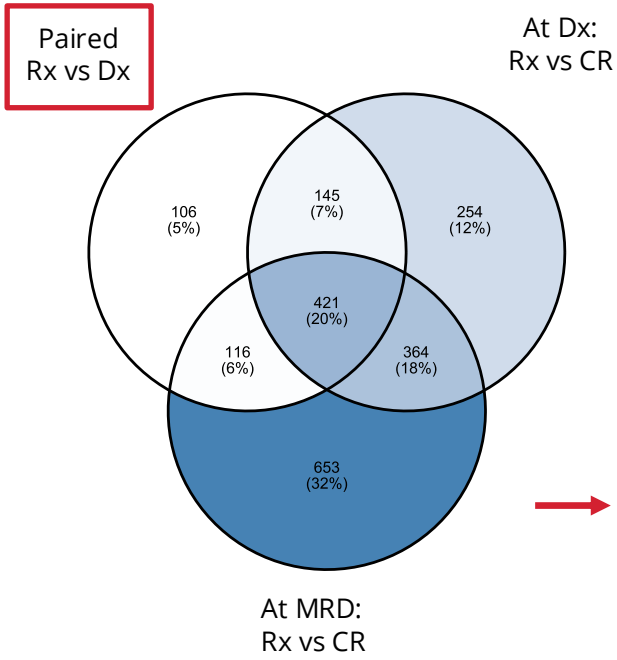
non parametric ANOVA
* $p<0.05$

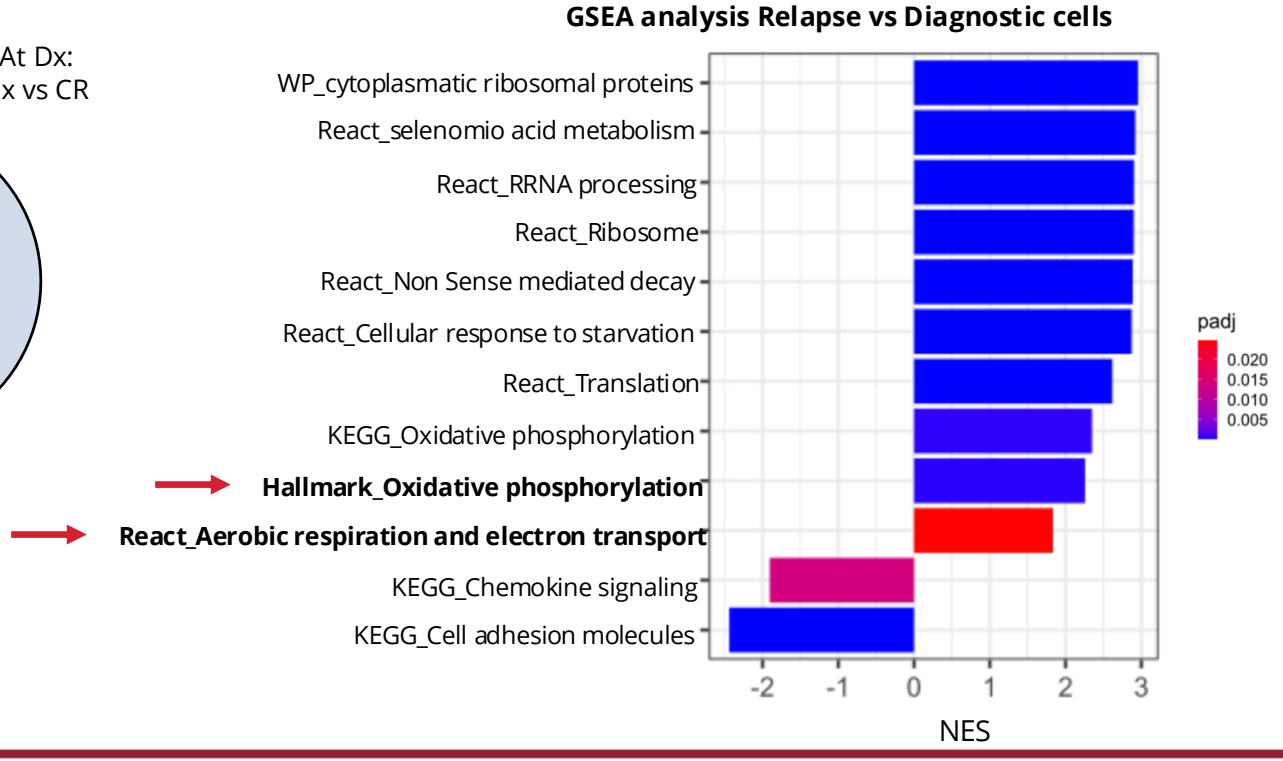
MRD cells display a quiescent state with metabolic reprogramming





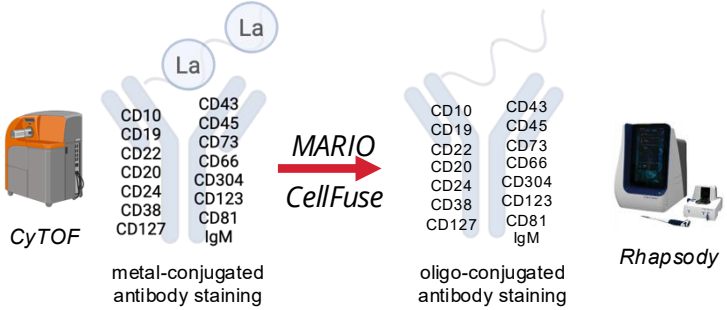
MRD cells display a quiescent state with metabolic reprogramming





Integrated data revealed leukemic cell plasticity of high predicted cells

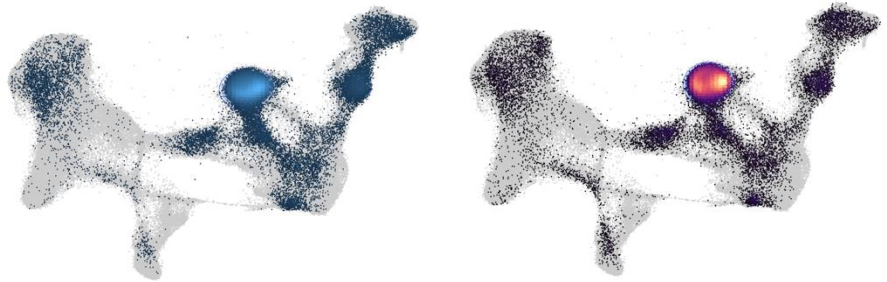
Proteomic/Transcriptomic Integration

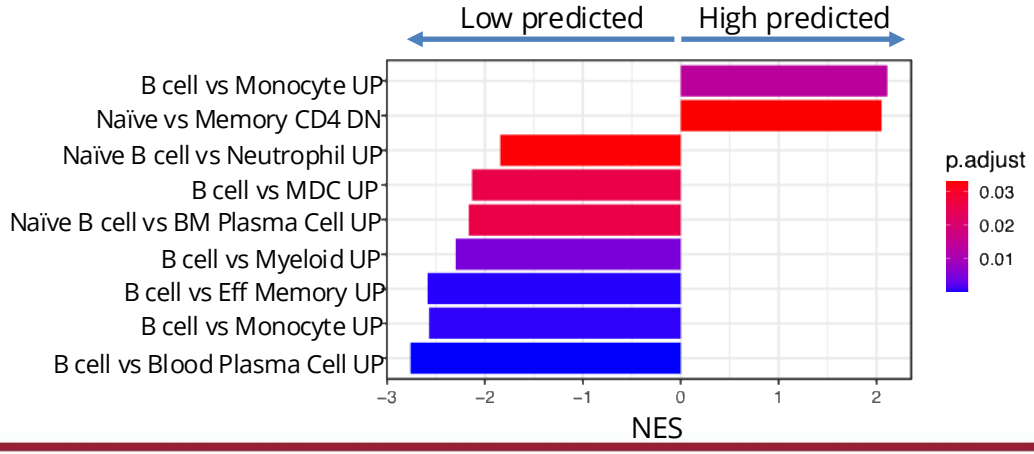


Zhu B. et al Nature Methods 2023;
Koladiya A. et al. BioRxiv 2025

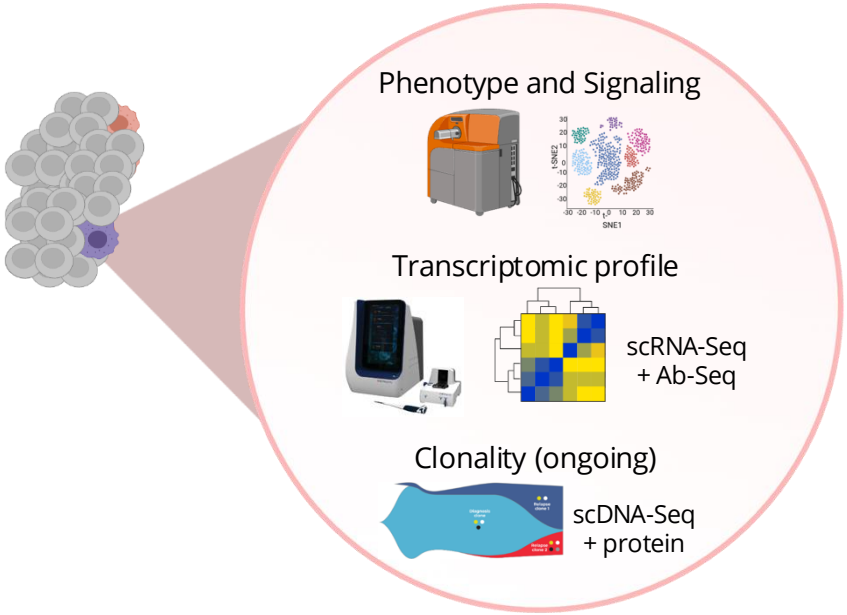
XGBoost prediction
High predictive (p>0.05)
Low predictive (p<0.05)

Low predicted **High predicted**





Conclusion and future perspectives



- Early MRD cells, detected by CyTOF and scRNA-Seq, bear phenotypic and intracellular features predictive of future relapse
- Transcriptomic data reveal quiescence, aerobic respiration, and lineage infidelity as main pathways of resistance

- Test other predictive models (MIL)
- Test identified signature of resistance on bulk RNA-Seq data using deconvolution methods
- BCR repertoire (ongoing in collaboration with Dr. Stomatopoulous)
- Clonality studies with Tapestri (ongoing)

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University of Michigan MI, USA

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Giuseppe Gaipa
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Cristina Bugarin

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Stefano Rebellato

Angela Maria Savino
Sarah Tettamanti

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AIRC

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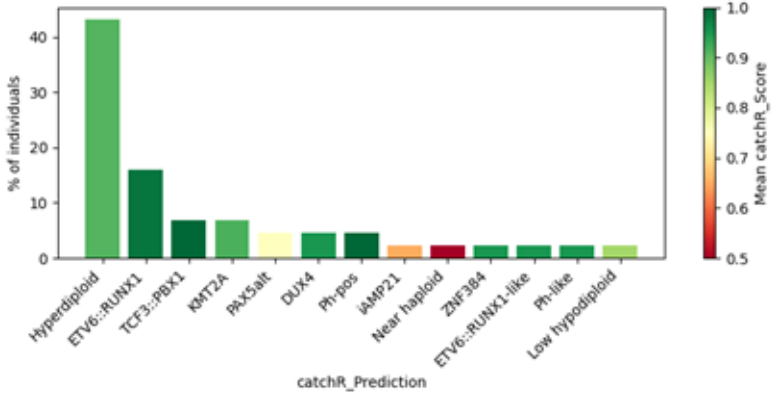
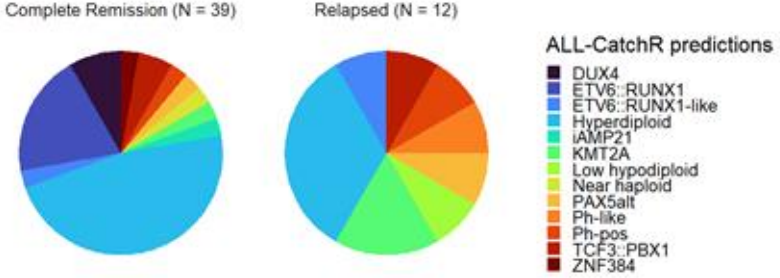
HUMAN TECHNOPOLE

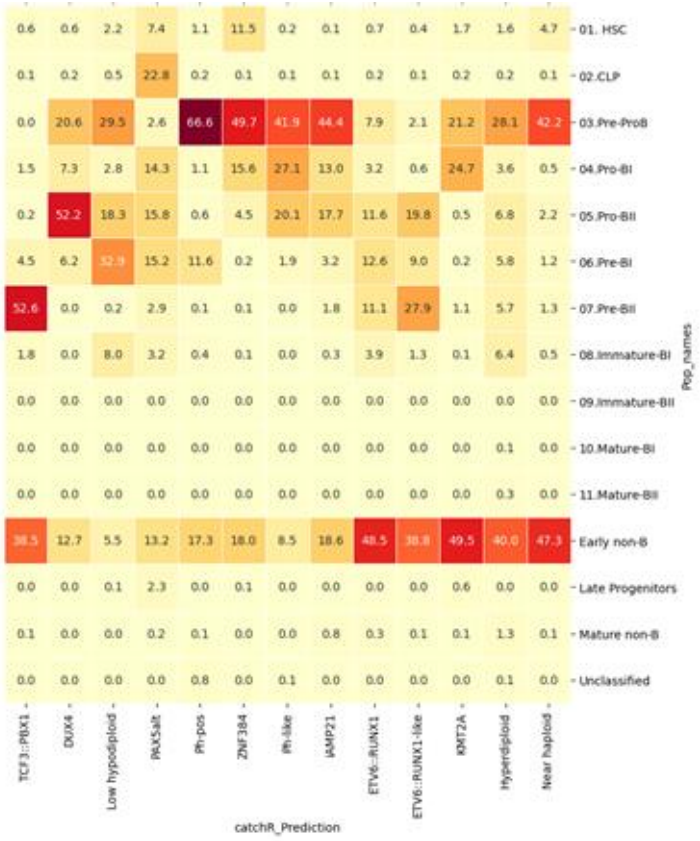
TRANSLATIONAL RESEARCH TRAINING IN HEMATOLOGY

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

CatchR prediction vs B-cell classifier





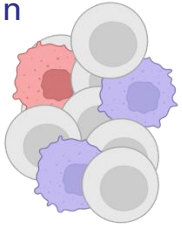


Identify relapse-associatated cells across patients using machine learning

THE APPROACH > ML projects high-dimensional single-cell profiles from multiple patients into a common embedding to reveal FEATURES that separate relapse-associated states.

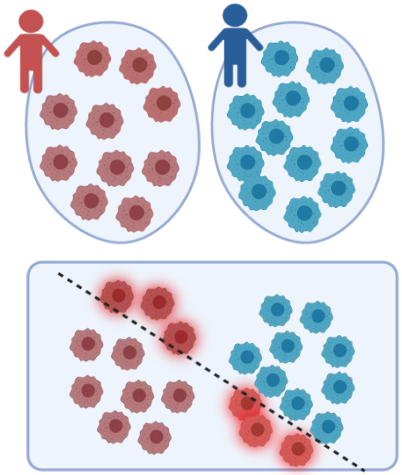
Population-level aggregate

Single-cell resolution



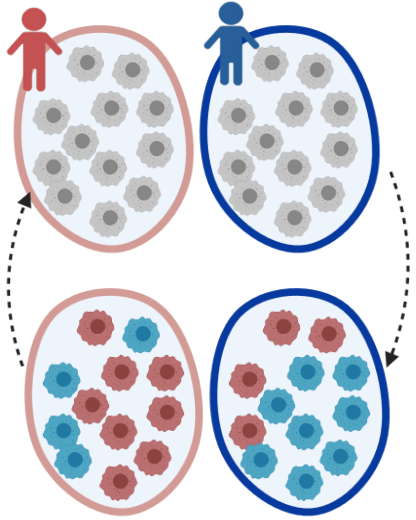
SEMI-SUPERVISED

Ground truth: assumed labels based on the training



MULTI-INSTANCE LEARNING (MIL)

Ground truth: clinical outcome



Only patient labels are known.
The model iteratively learns single-cell labels

OUTPUT > single cell predictions that we compare to the clinical outcome