Multimodal atlas of paired diagnosis and relapse AML samples enables novel therapeutic targeting of surface antigens

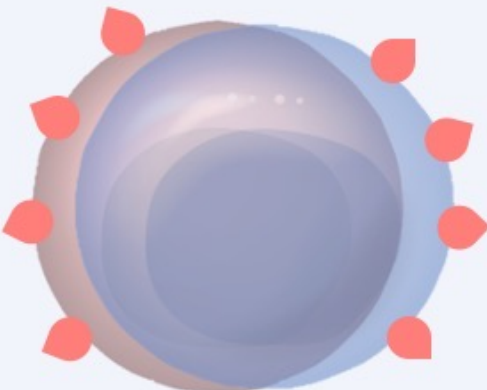**Matthew H. Ung, PhD**
Associate Director, Quantitative Biology

Vor Bio, Cambridge MA, USA

1

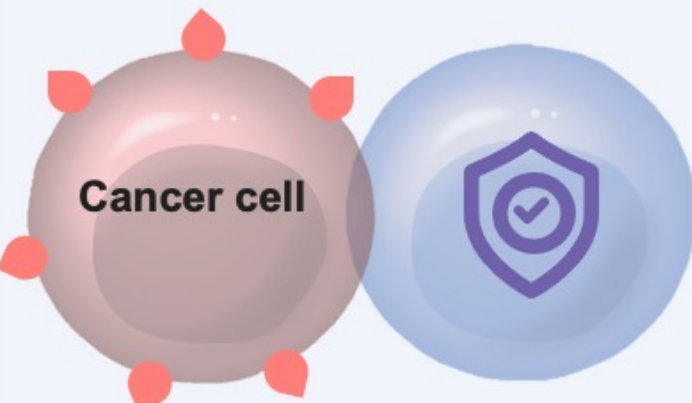# We use genome engineering to make healthy cells invisible to drugs

Problem

Few unique cancer antigens, so drugs kill both cancer and healthy cells through **on-target toxicity**

Genome engineering →

Vor Paradigm: Engineered HSCs (eHSCs)

Cancer cell

Remove target expression on healthy cells so that killing is **cancer-specific**

Patient to patient heterogeneity presents obstacles to treatment
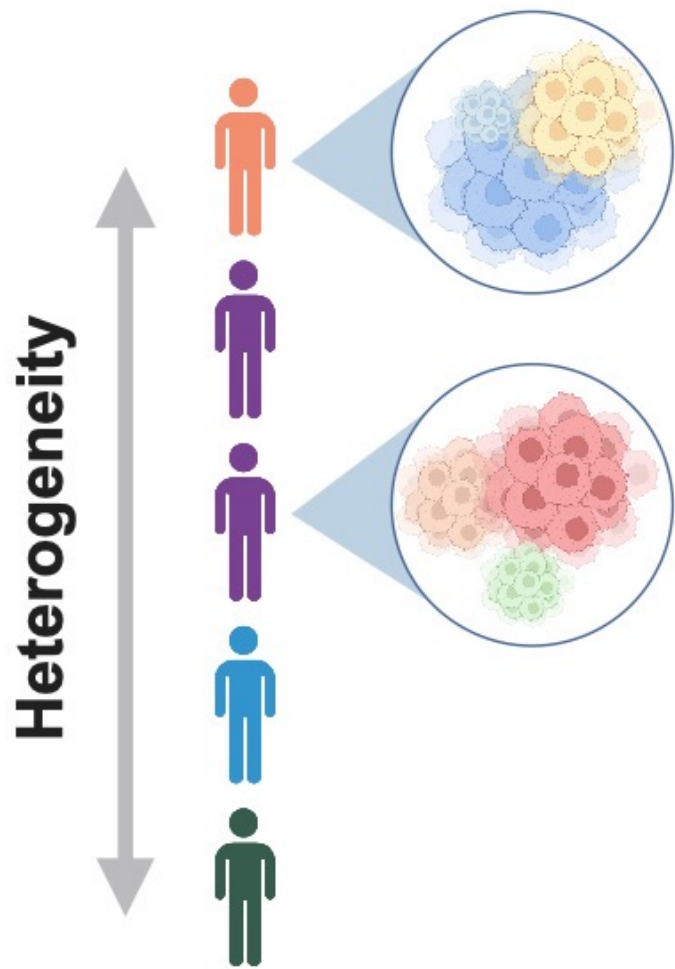
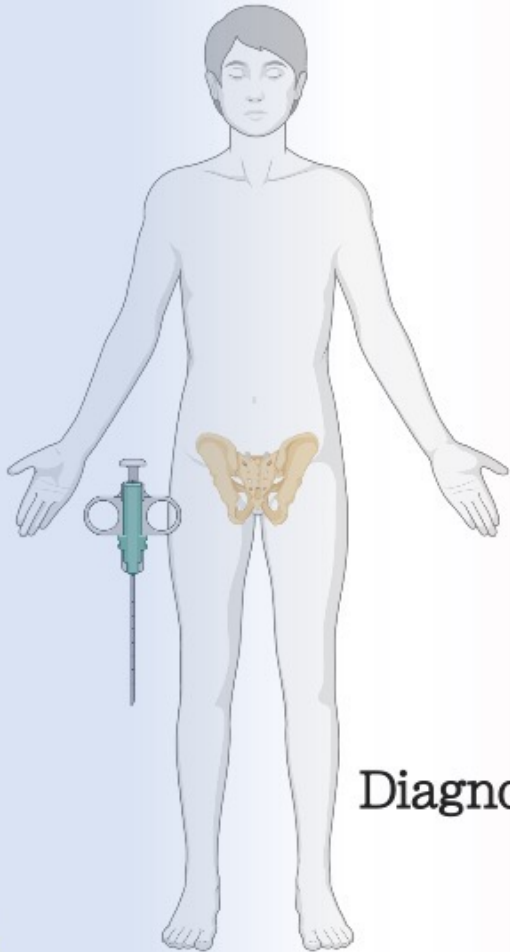# Evolution of leukemic blasts presents obstacles to treatment
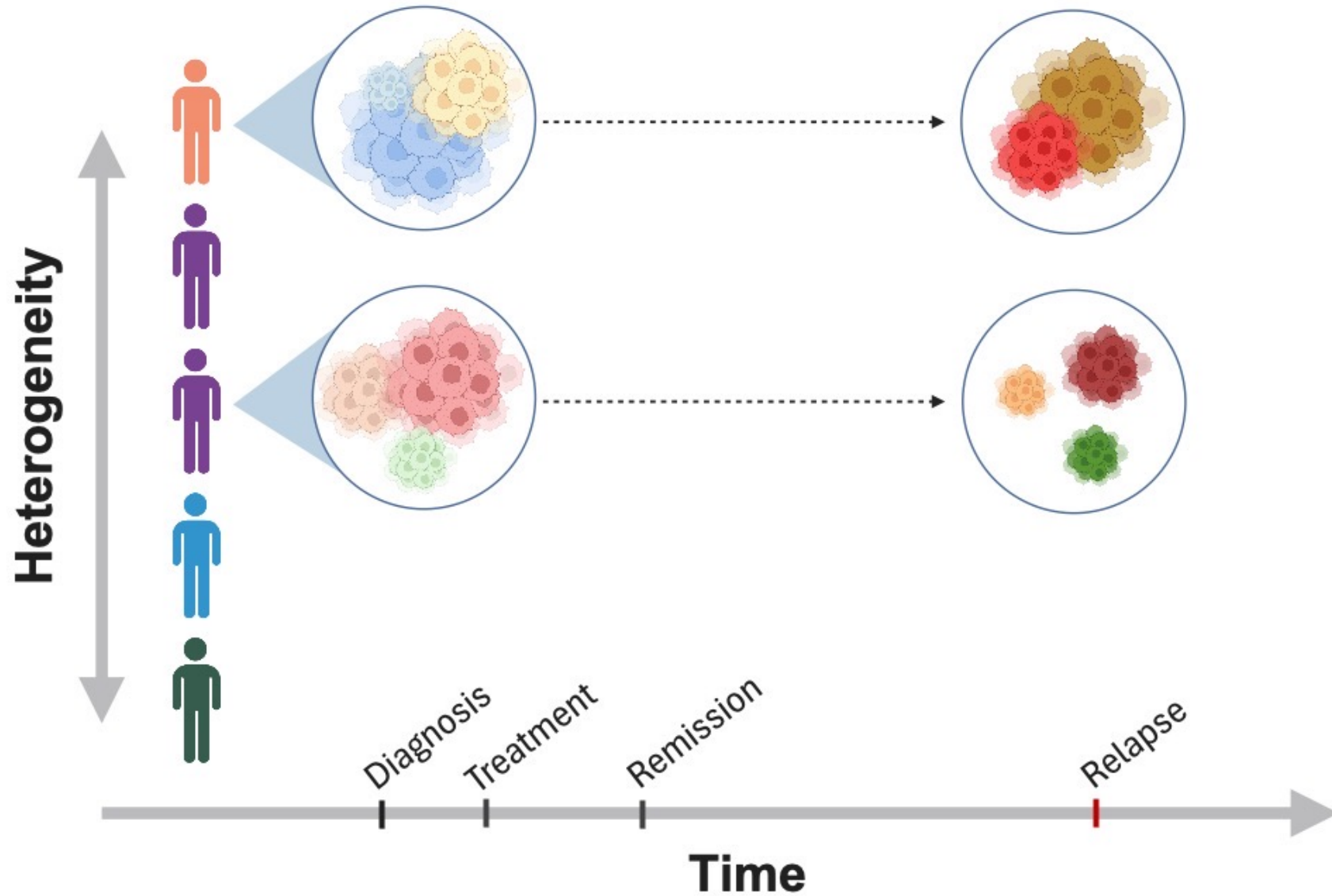
UHN
Toronto General
Toronto Western
Princess Margaret
Toronto Rehab
Michener Institute
University Health Network

56 matched AML bone marrow samples

28 patients

Diagnosis → Relapse

Clinical metadata
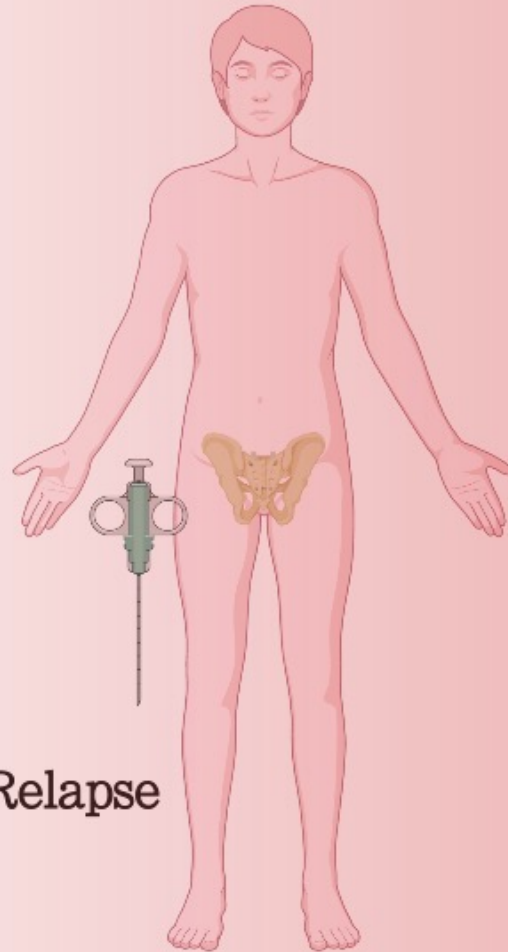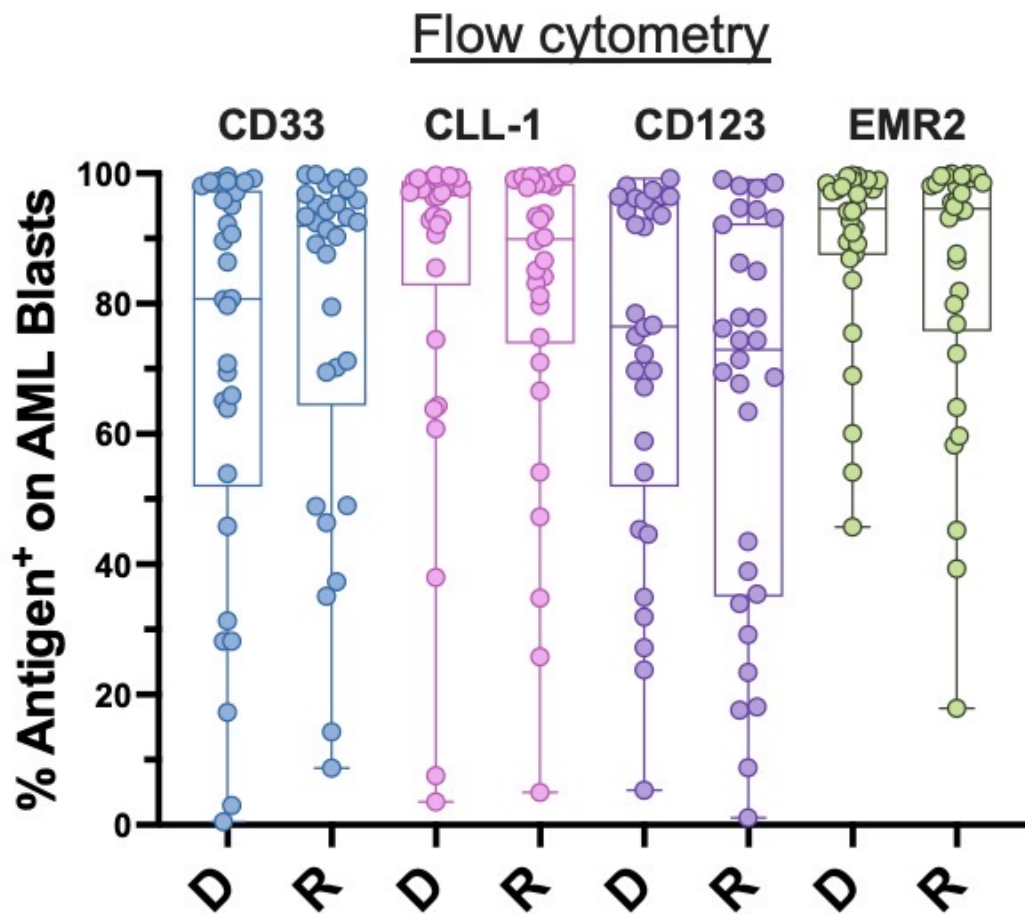
- ✓ Treatment history
- ✓ Sex
- ✓ FAB subtype
- ✓ Cytogenetics
- ✓ Mutation information
- ✓ Vital status
- ✓ Survival information
- ✓ Time to relapse

4 myeloid markers are expressed at targetable levels at diagnosis and relapse

D = Diagnosis R = Relapse
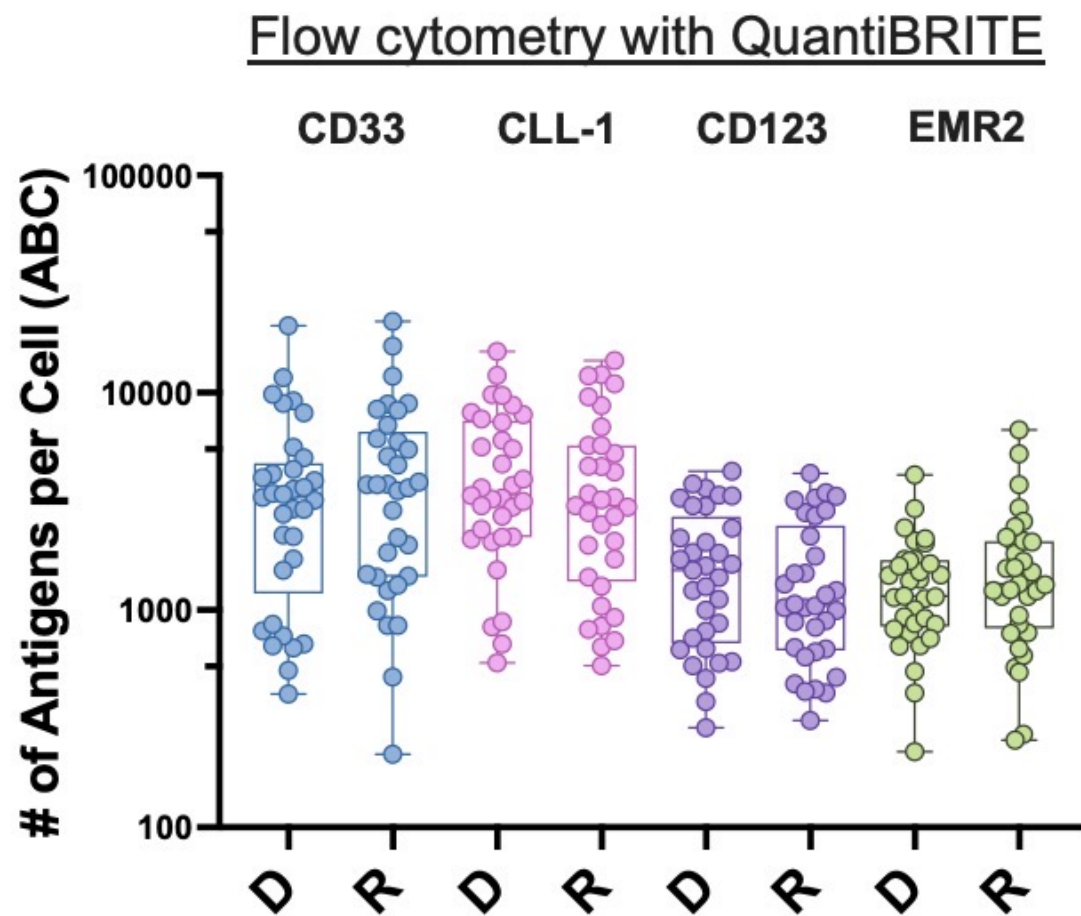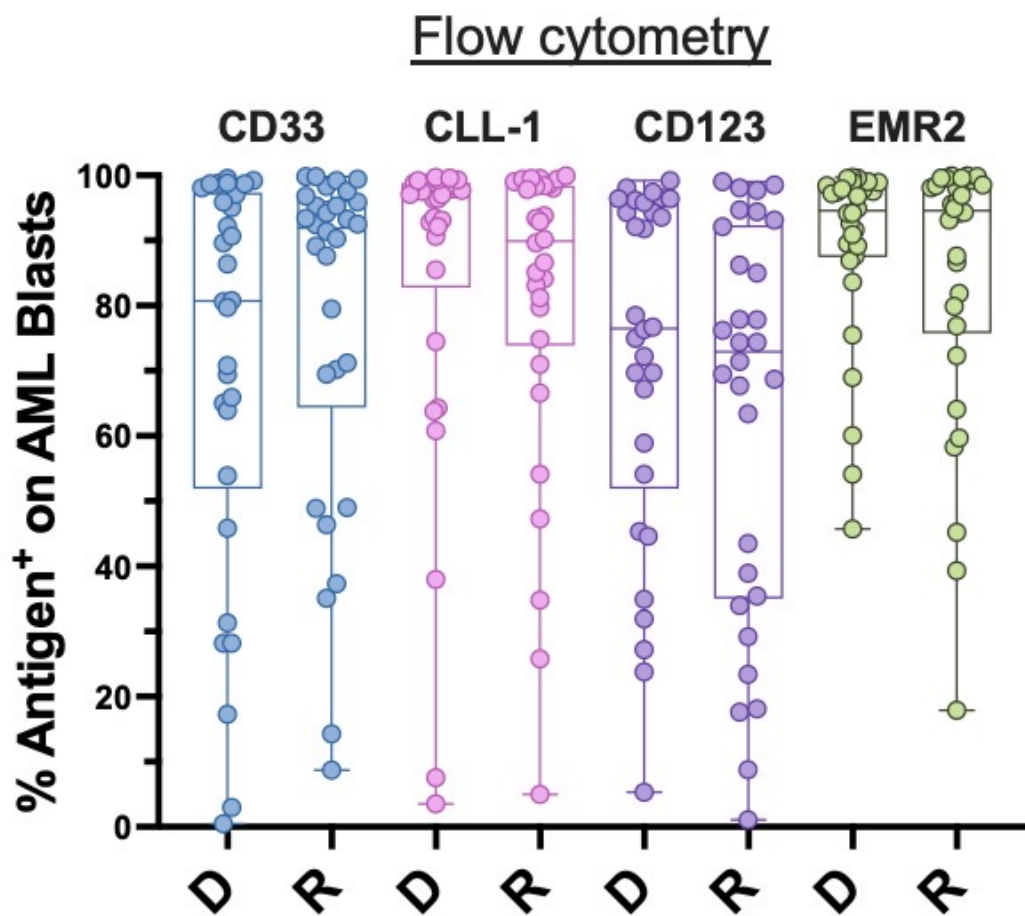
4 myeloid markers are expressed at targetable levels at diagnosis and relapse

Flow cytometry

CD33   CLL-1   CD123   EMR2

% Antigen+ on AML Blasts

Flow cytometry with QuantiBRITE

CD33   CLL-1   CD123   EMR2

# of Antigens per Cell (ABC)
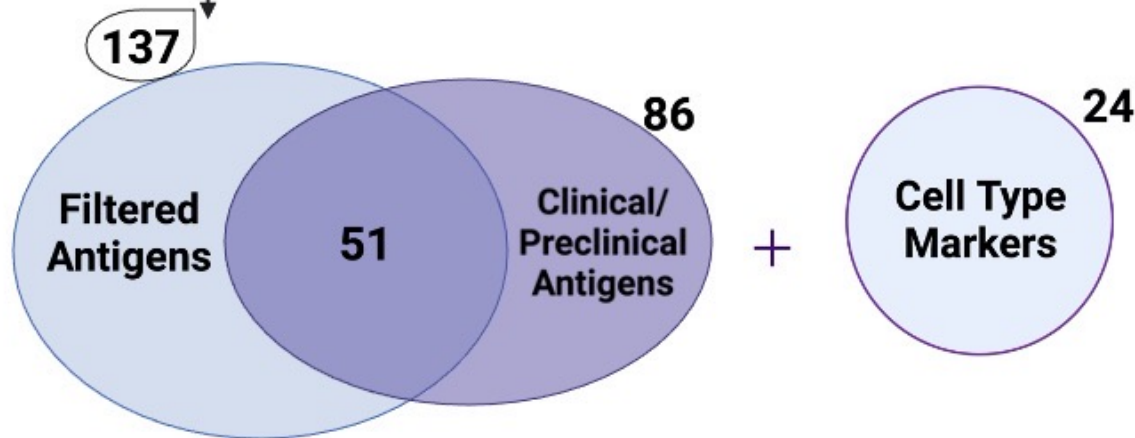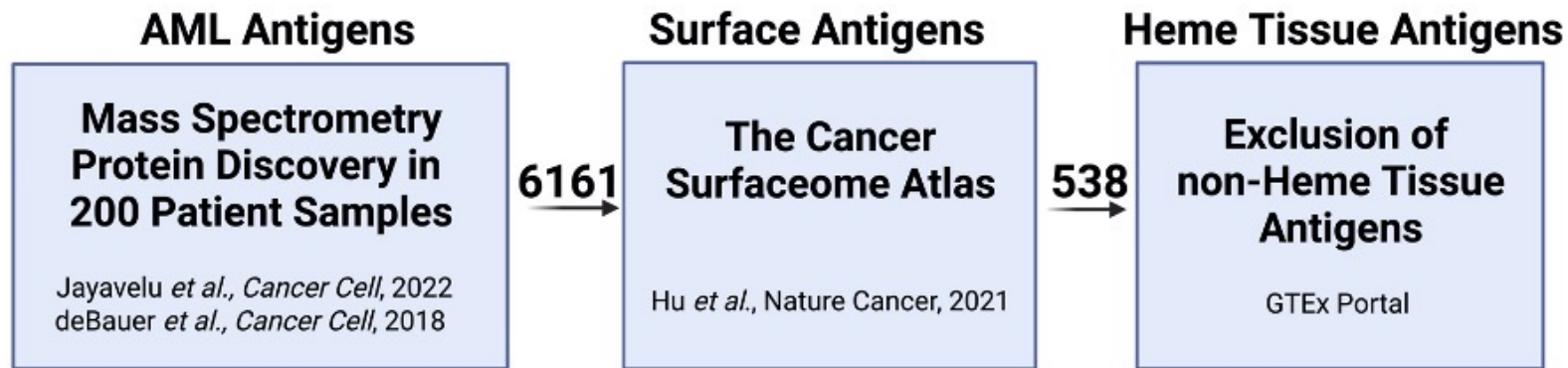
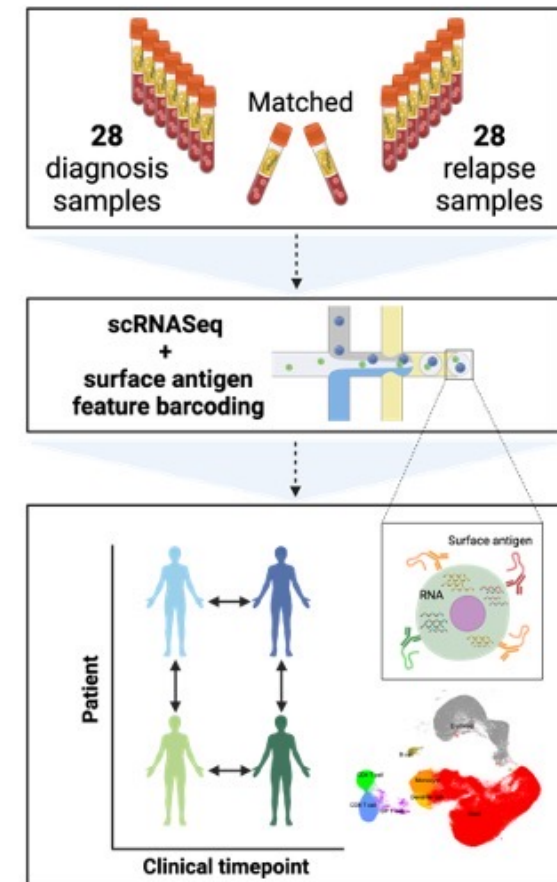Julia Etchin

Amanda Halfond

Julia DiFazio

Yonina Keschner

D = Diagnosis **R** = Relapse

Single cell RNA sequencing of matched AML samples with feature barcoding of 81 surface antigens
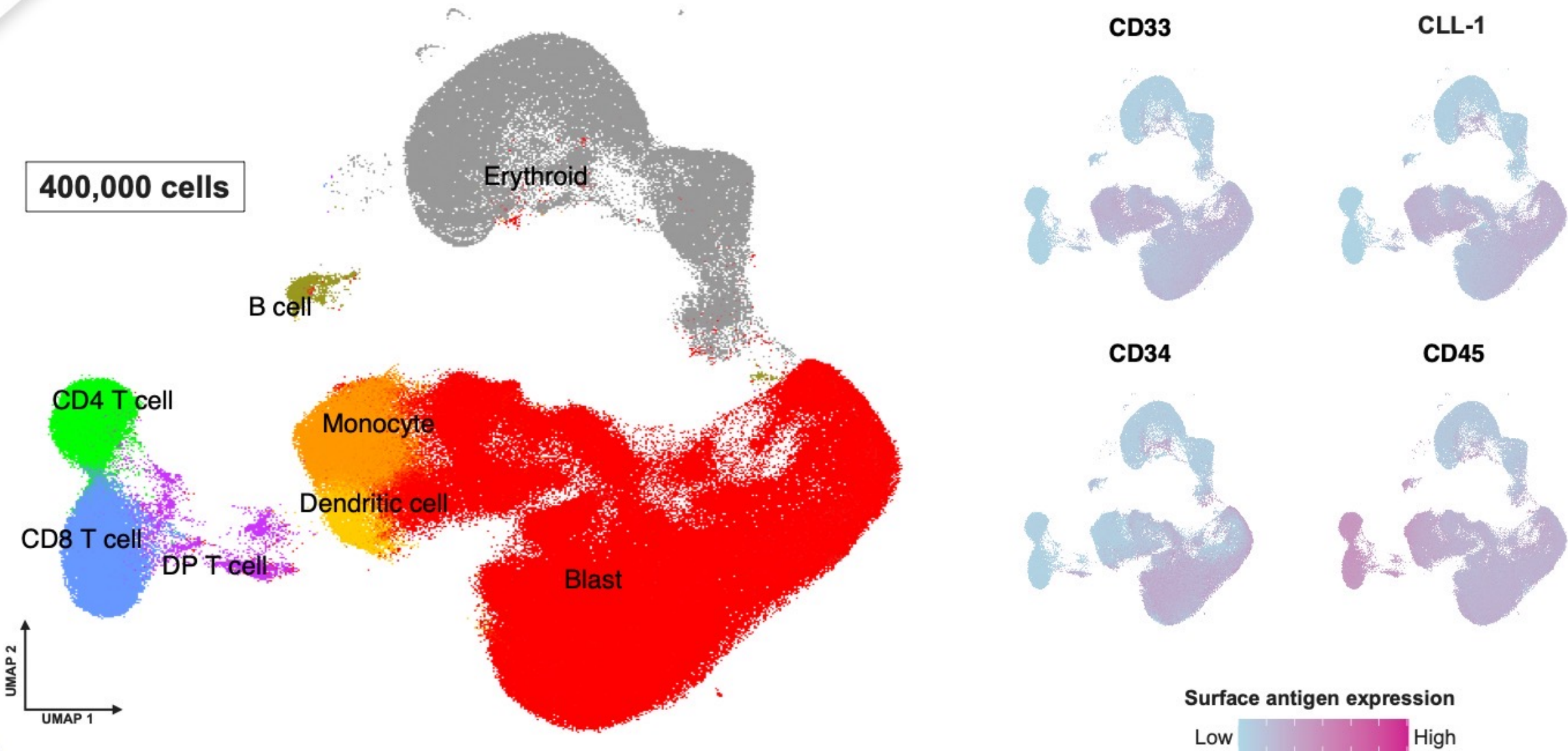

AML Antigens

Mass Spectrometry Protein Discovery in 200 Patient Samples

Jayavelu *et al., Cancer Cell*, 2022
deBauer *et al., Cancer Cell*, 2018

6161 →

Surface Antigens

The Cancer Surfaceome Atlas

Hu *et al.*, Nature Cancer, 2021

538 →

Heme Tissue Antigens

Exclusion of non-Heme Tissue Antigens

GTEx Portal

137

Filtered Antigens — 51 — Clinical/ Preclinical Antigens — 86

+ Cell Type Markers — 24

Total: 196

Total Antibody-Derived Tags (ADT) in CITE-seq: 81*

*Commercially Available or Custom Conjugated ADT

28 diagnosis samples — Matched — 28 relapse samples

scRNASeq + surface antigen feature barcoding
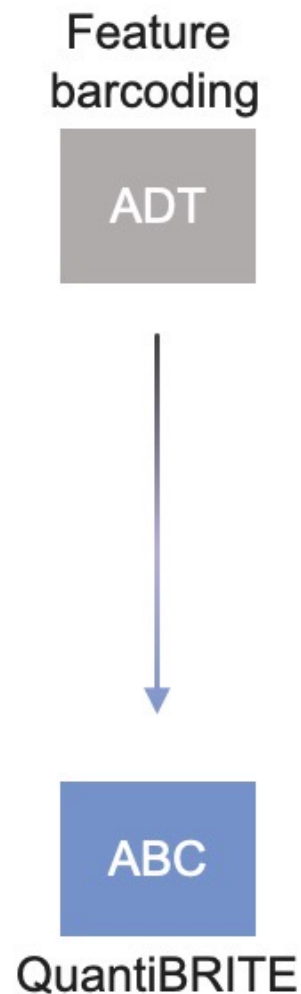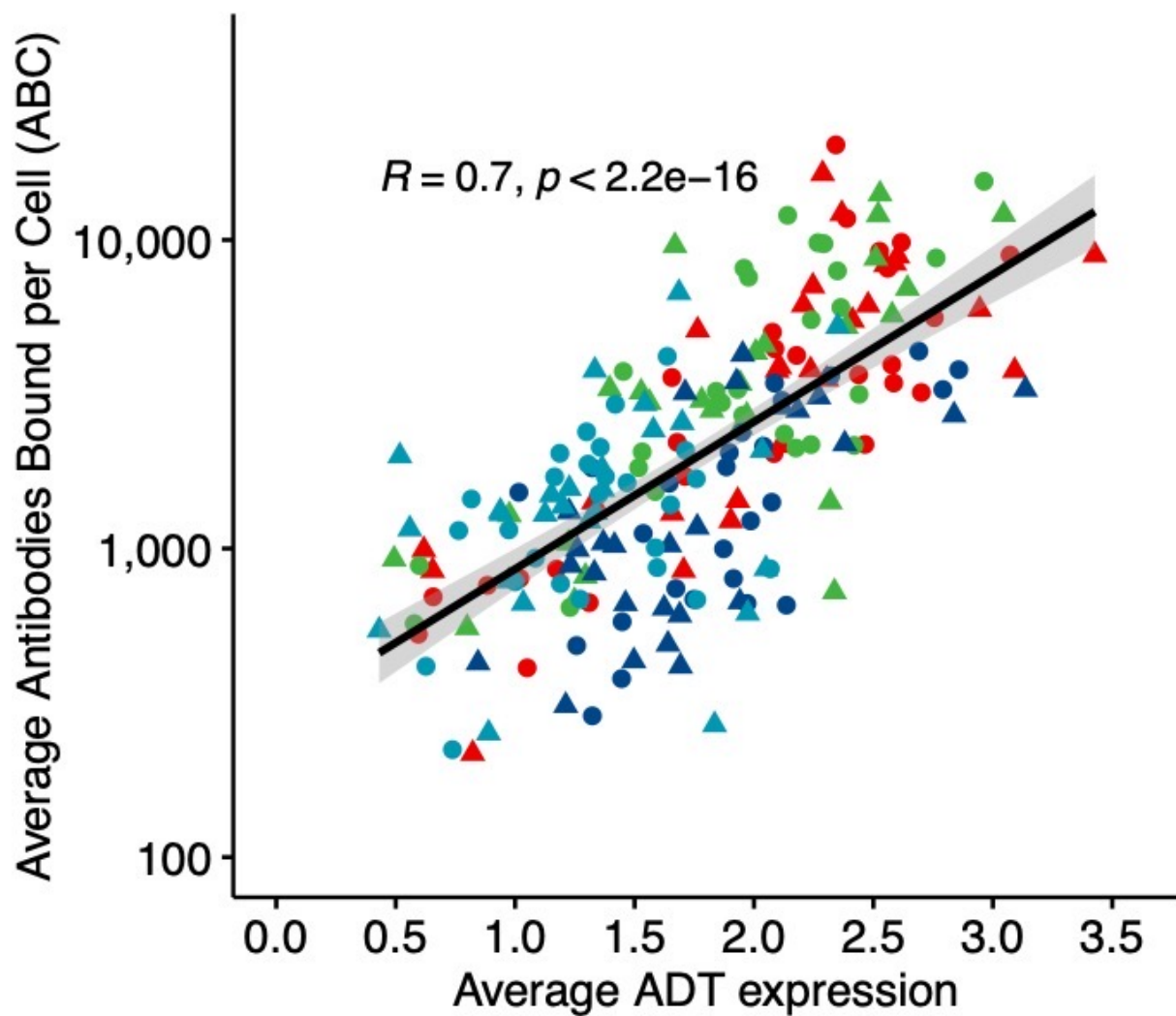
Patient / Clinical timepoint

Created with BioRender.com

Multimodal atlas of AML reveals distinct cell populations

Multimodal atlas of AML reveals distinct cell populations

400,000 cells

Erythroid

B cell

CD4 T cell

Monocyte

Dendritic cell

CD8 T cell

DP T cell

Blast

UMAP 2

UMAP 1

Colored by patient
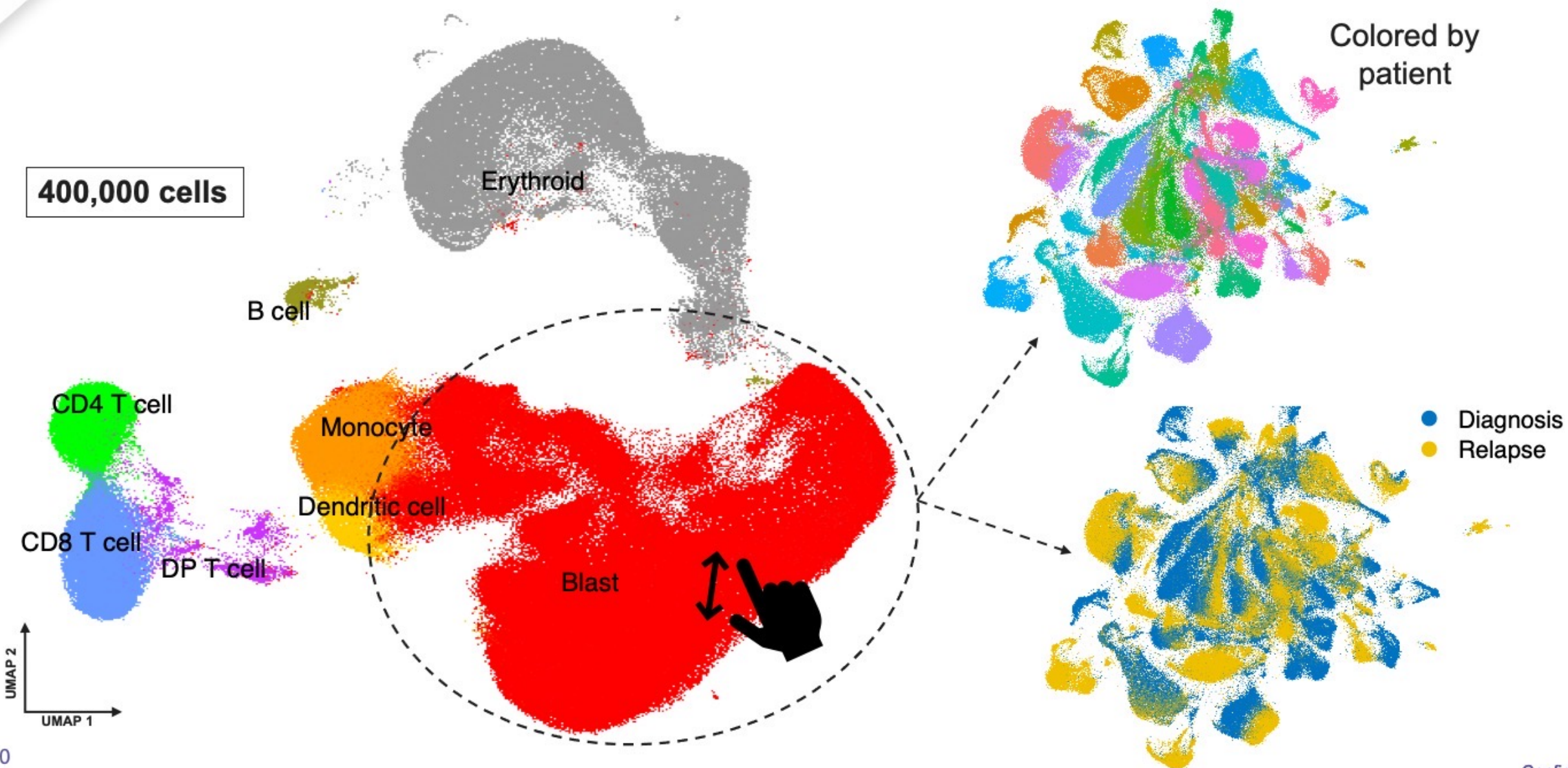
Diagnosis
Relapse

Cell surface antigen density can be estimated for each blast by incorporating QuantiBRITE information

$R = 0.7$, $p < 2.2e{-}16$

Antigen
- CD33
- CLL-1
- CD123
- EMR2

Timepoint
- Diagnosis
- Relapse

Feature barcoding

ADT

ABC

QuantiBRITE

Estimated blast surface antigen density reveals targetable antigens across patients at diagnosis and relapse

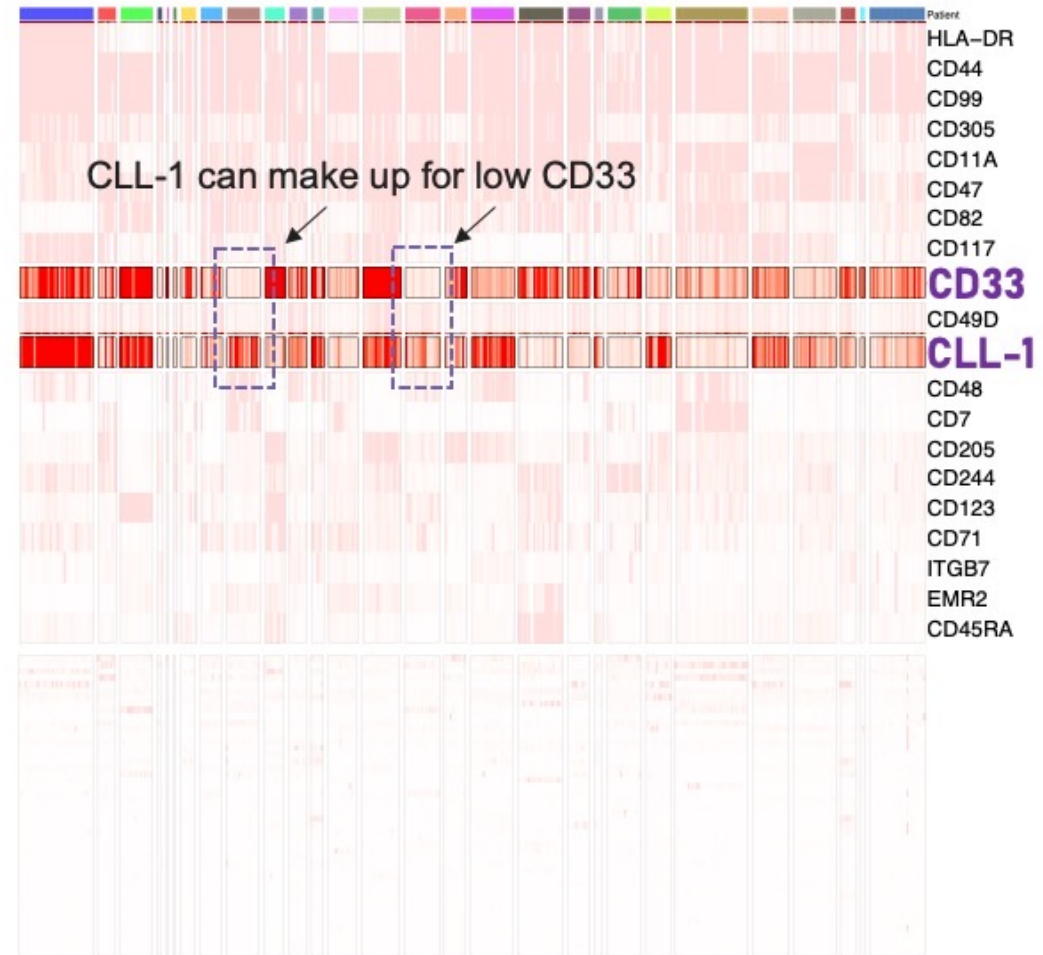*2000 antigens corresponds to a normalized ADT value of 1.8

Estimated blast surface antigen density reveals targetable antigens across patients at diagnosis and relapse

Estimated blast surface antigen density reveals targetable antigens across patients at diagnosis and relapse
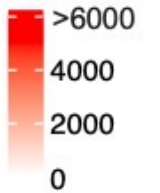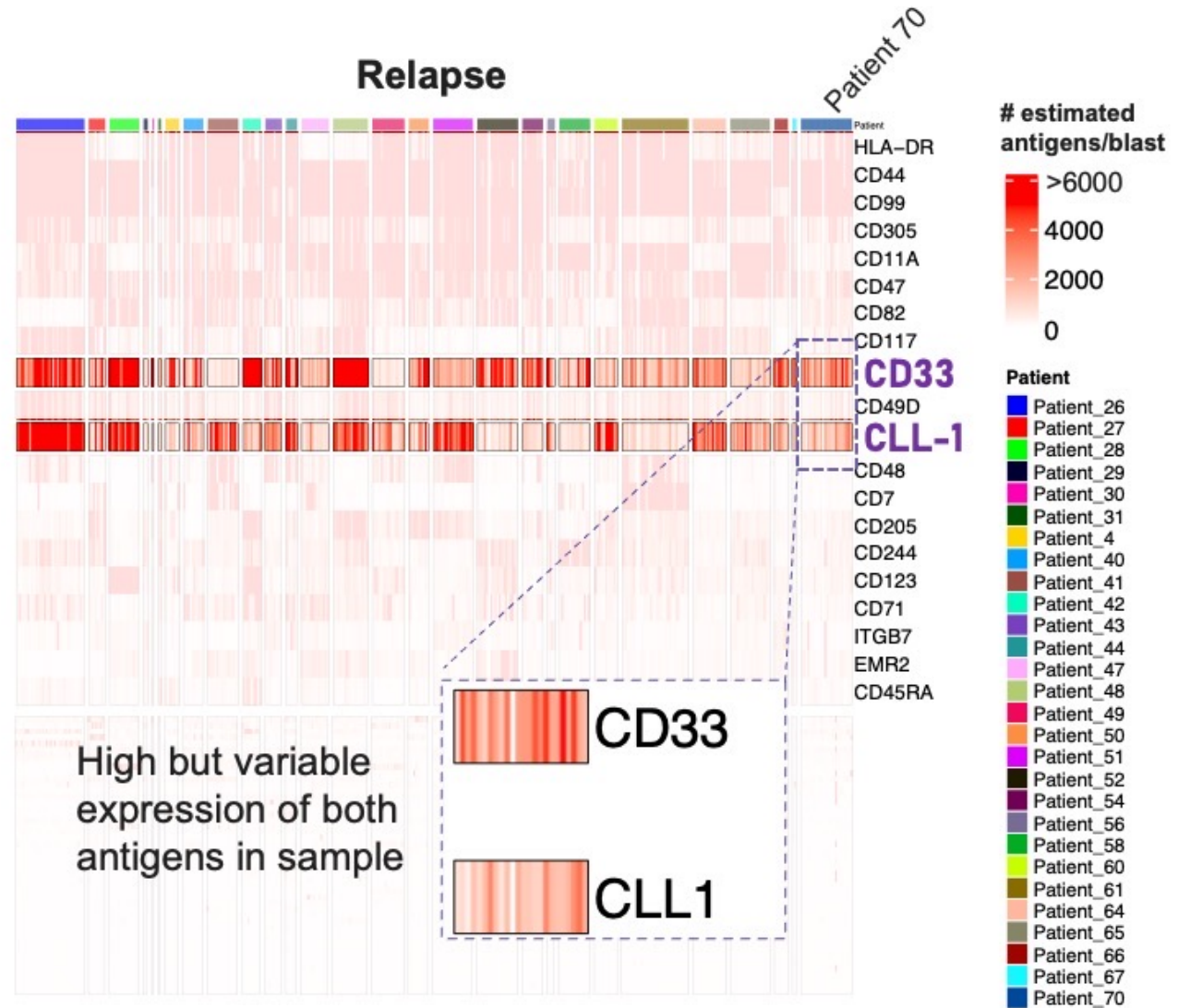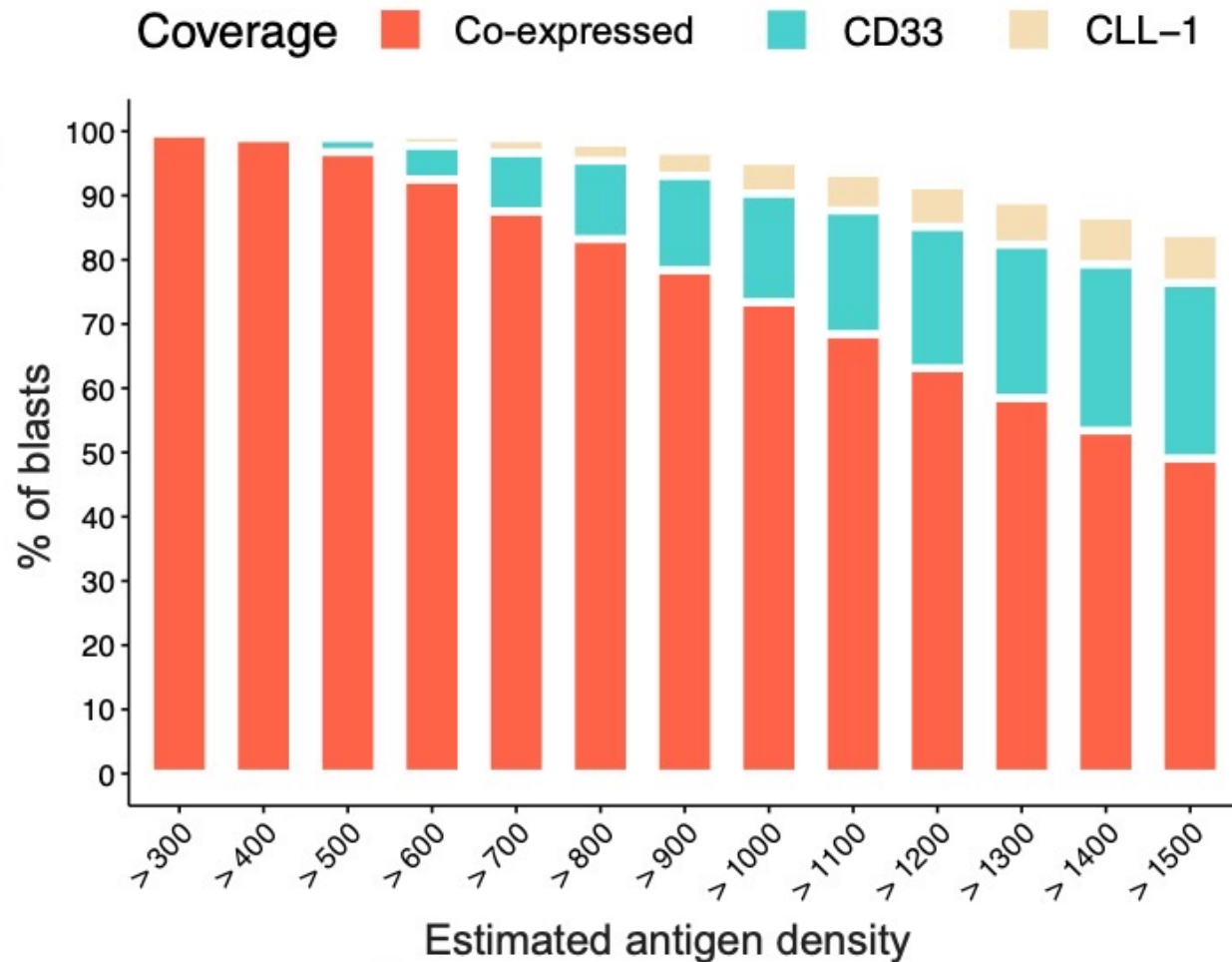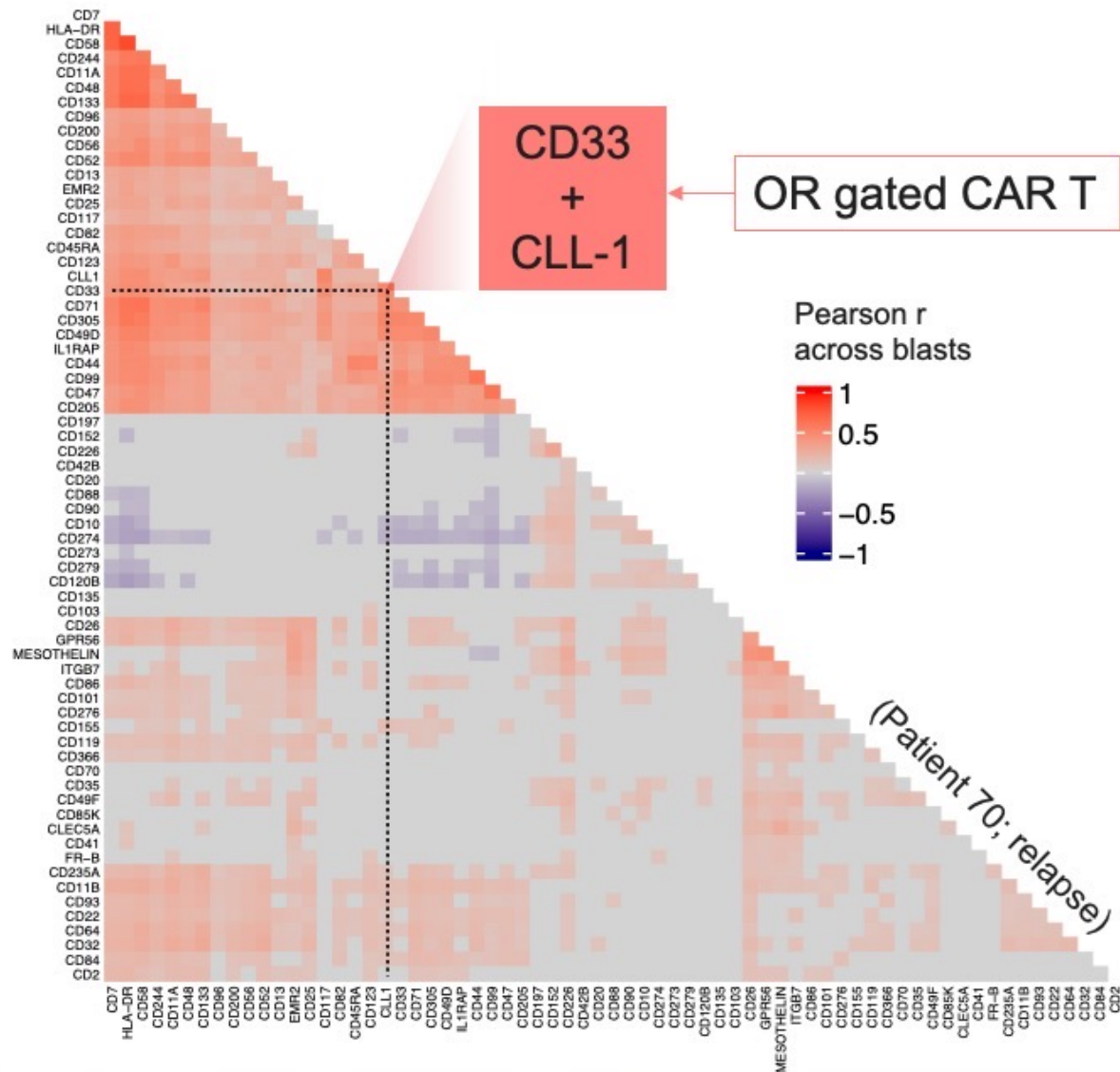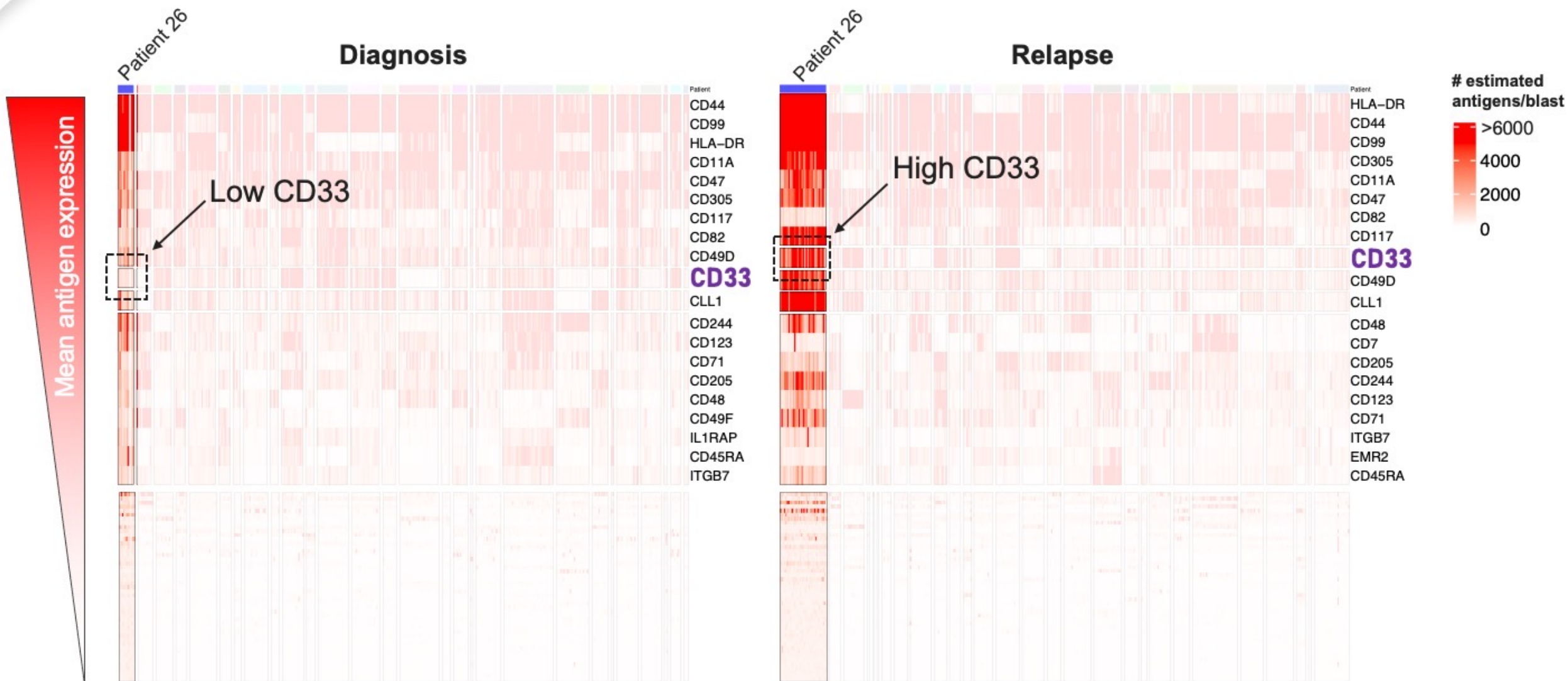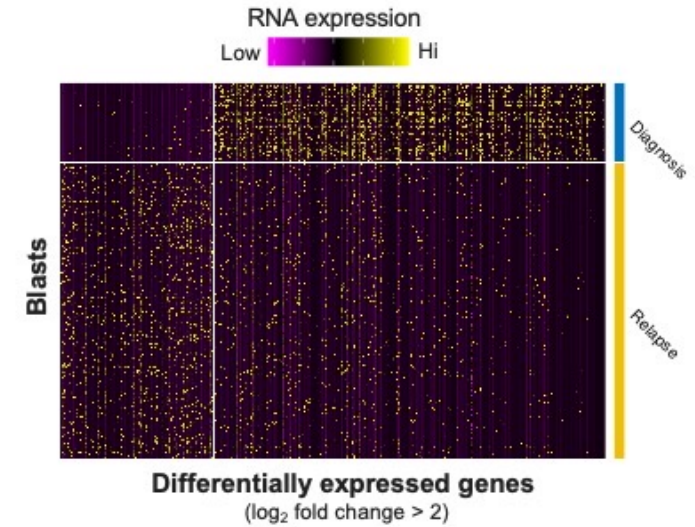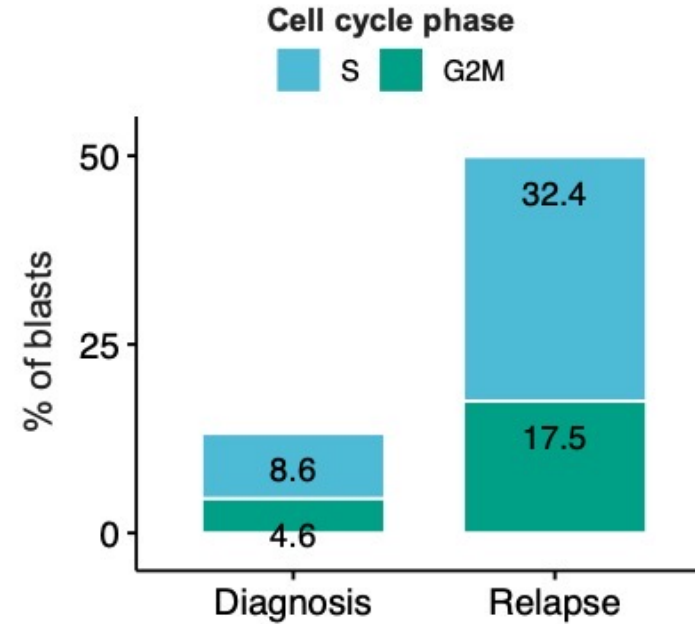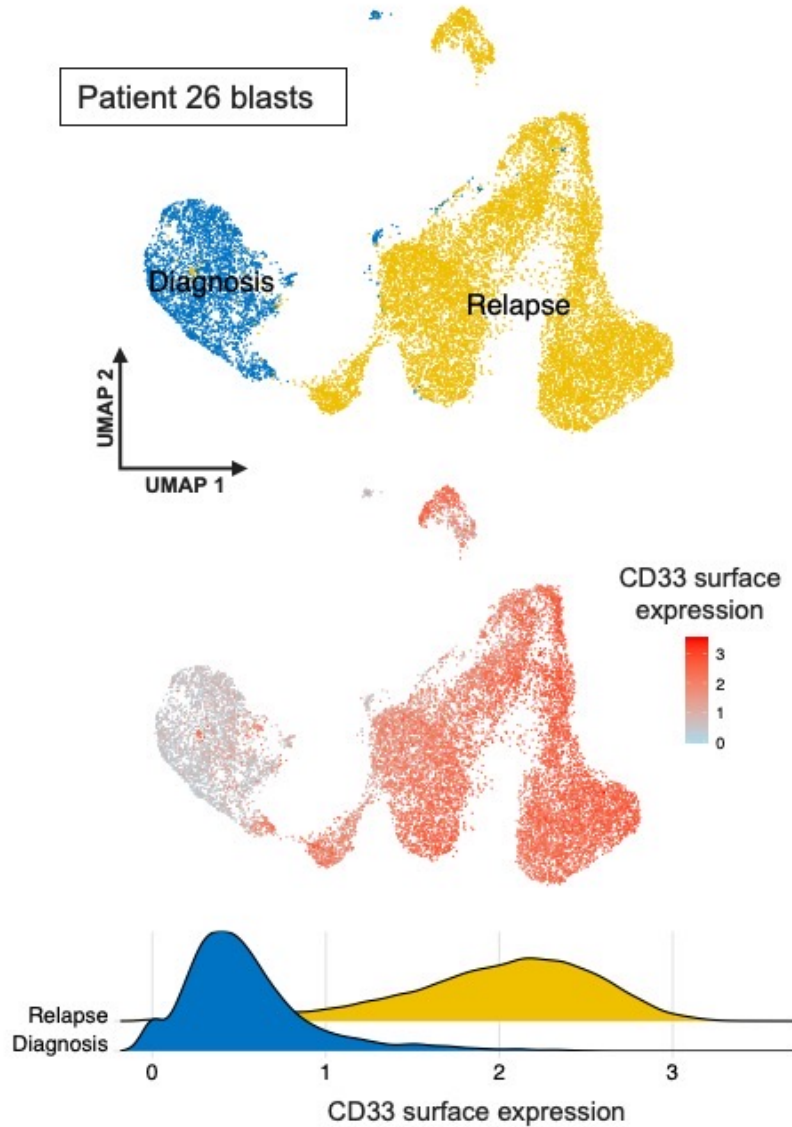
Multi-targeting strategies can leverage antigen combinations: Example from a single patient sample
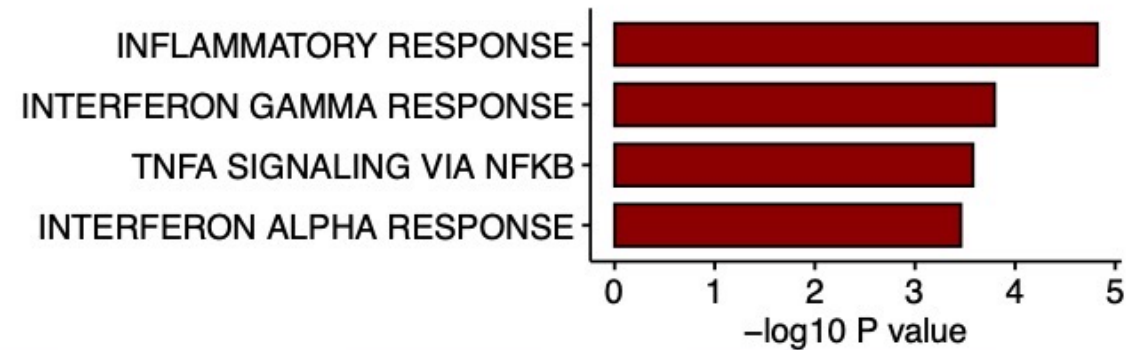
CD33 expression is higher in relapse blasts: Example from another patient

CD33 upregulation on cell surface is associated with proliferation and downregulation of cell-intrinsic inflammation in the patient

Summary

✓ Vor's AML atlas enables extensive characterization of inter- and intra-patient blast heterogeneity

✓ CD33 and CLL-1 are among the highest expressed antigens across entire cohort

✓ Transcriptomic analysis of a single patient identified downregulation of inflammatory pathways in CD33-high relapse blasts

✓ Analysis of surface antigen density supports multi-targeting strategies that can leverage antigen combinations and guard against antigen escape

Acknowledgement

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The patients and their families and caregivers