AML Heterogeneity & Chemotherapy Response: Implications for Relapse and MRD

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Disclosures

None

Key studies that have informed this presentation

Data shown is (mostly) derived from these studies:

Zeng *et al*. Nature Medicine 2022

Nat Med. 2022 Jun;28(6):1212-1223. doi: 10.1038/s41591-022-01819-x. Epub 2022 May 26.

A cellular hierarchy framework for understanding heterogeneity and predicting drug response in acute myeloid leukemia

Andy G X Zeng [1 2], Suraj Bansal [# 1], Liqing Jin [# 1], Amanda Mitchell [1], Weihsu Claire Chen [1 3], Hussein A Abbas [4], Michelle Chan-Seng-Yue [1], Veronique Voisin [5], Peter van Galen [6 7 8 9], Anne Tierens [10], Meyling Cheok [11], Claude Preudhomme [11], Hervé Dombret [12], Naval Daver [4], P Andrew Futreal [13], Mark D Minden [1 14 15 16], James A Kennedy [1 17], Jean C Y Wang [1 15 16], John E Dick [18 19]

Zeng *et al*. Blood Cancer Discovery 2025

Blood Cancer Discov. 2025 Jul 1;6(4):307-324. doi: 10.1158/2643-3230.BCD-24-0342.

Single-cell Transcriptional Atlas of Human Hematopoiesis Reveals Genetic and Hierarchy-Based Determinants of Aberrant AML Differentiation

Andy G X Zeng [1 2], Ilaria Iacobucci [# 3], Sayyam Shah [# 1], Amanda Mitchell [1], Gordon Wong [1 2], Suraj Bansal [1], David Chen [1], Qingsong Gao [3], Hyerin Kim [1], James A Kennedy [4], Andrea Arruda [1], Mark D Minden [1 5 6 7], Torsten Haferlach [8], Charles G Mullighan [3 9], John E Dick [1 2]

Additional concepts were borrowed from these studies:

Nuno, Azizi *et al*. Elife 2024

Elife. 2024 Apr 22:13:e93019. doi: 10.7554/eLife.93019.

Convergent epigenetic evolution drives relapse in acute myeloid leukemia

Kevin Nuno [# 1 2 3 4], Armon Azizi [# 2 3 4 5], Thomas Koehnke [2 3 4], Caleb Lareau [6 7], Asiri Ediriwickrema [1 2 3 4], M Ryan Corces [1 2 3 4 8 9 10], Ansuman T Satpathy [6 7 11 12], Ravindra Majeti [2 3 4]

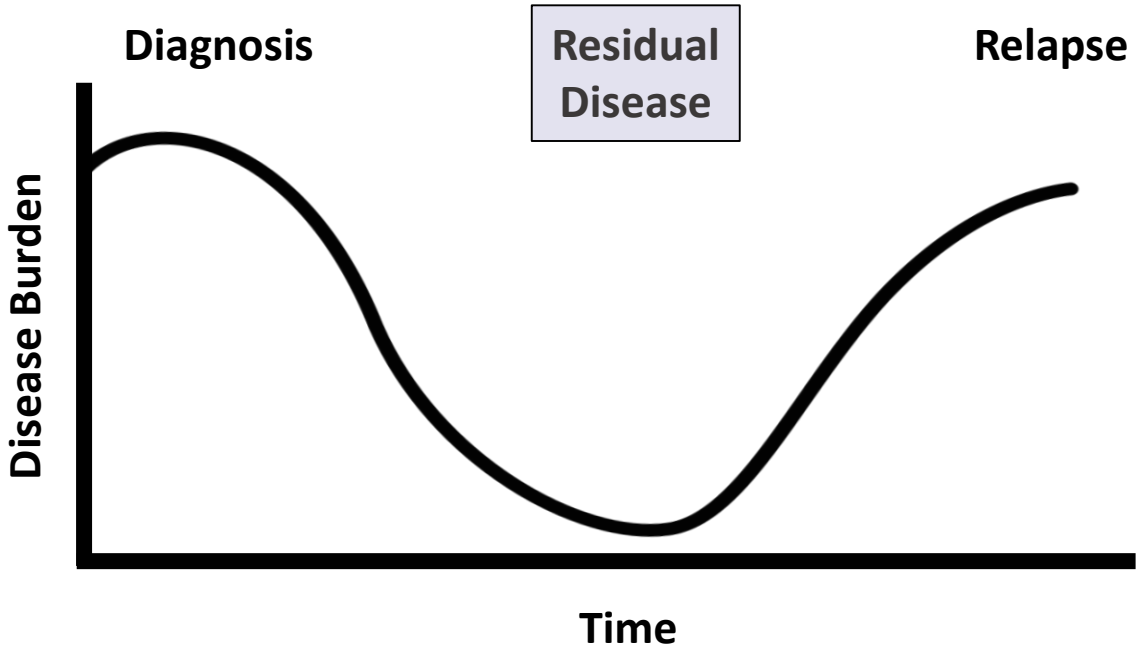
Turkalj, Radtke, Stoilova, Meklenbrauck *et al*. Blood 2026

Blood. 2026 Feb 5;147(6):613-632. doi: 10.1182/blood.2024027948.

Rapid clonal selection within early hematopoietic cell compartments presages the outcome of ivosidenib combination therapy

Sven Turkalj [1], Felix A Radtke [1], Bilyana Stoilova [1], Rabea Mecklenbrauck [1], Angus J Groom [1], Niels Asger Jakobsen [1], Curtis A Lachowiez [2], Marlen Metzner [1], Batchimeg Usukhbayar [1], Mirian Angulo Salazar [1], Zhihong Zeng [2], Sanam Loghavi [3], Jennifer Marvin-Peek [2], Verena Körber [1], Farhad Ravandi [2], Ghayas Issa [2], Tapan Kadia [2], Vasiliki Symeonidou [1], Anne P de Groot [1], Hagop Kantarjian [2], Koichi Takahashi [2], Marina Konopleva [2], Courtney D DiNardo [2], Paresh Vyas [1 4]

Conceptualizing MRD as a Window into Relapse Biology



First-order Questions:

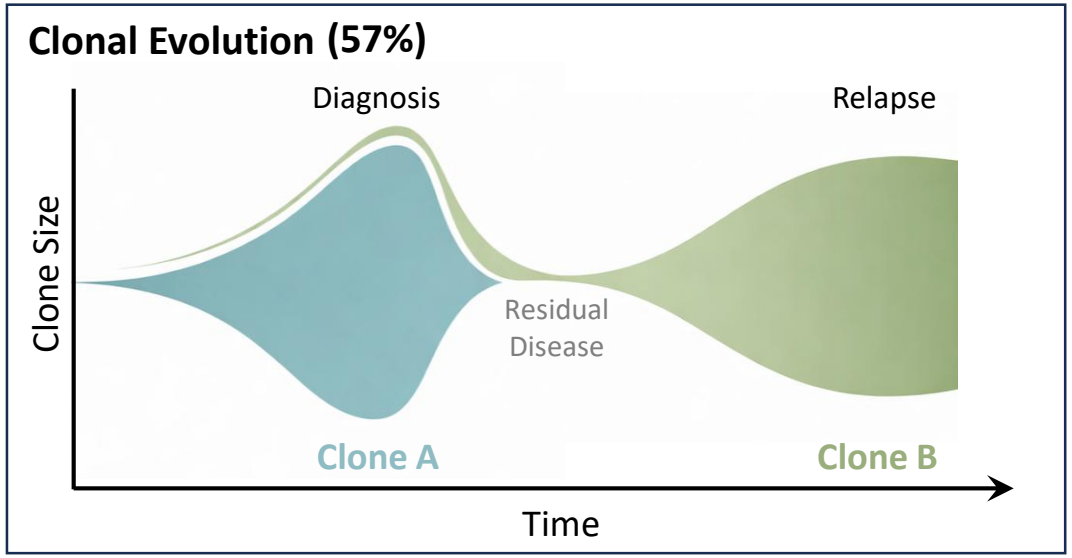
1. Is residual disease present?
2. Does this patient need further therapy?

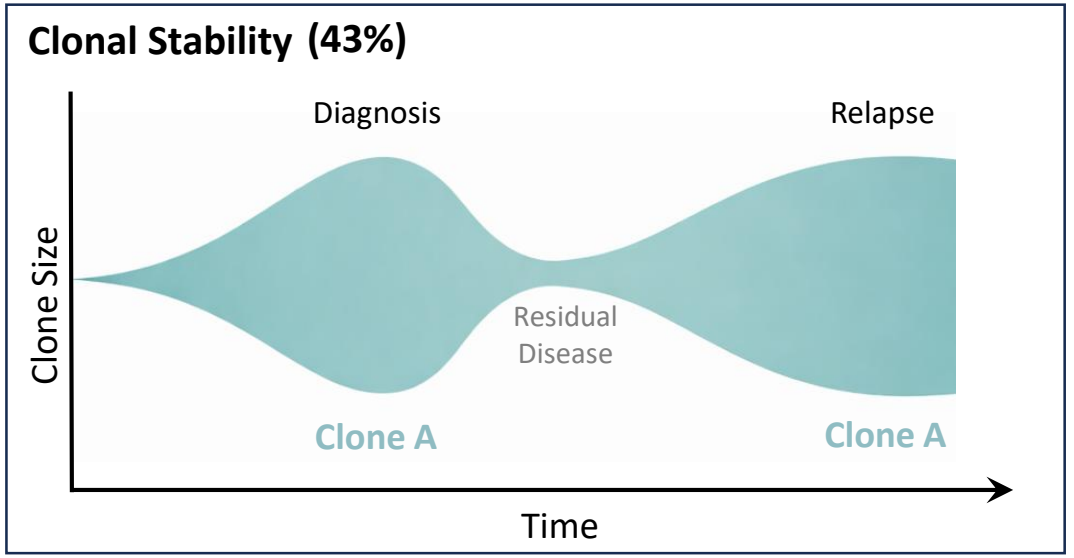
Second-order Questions:

1. What biological changes have already emerged under treatment pressure?
2. What specific therapies might be most effective at this stage?

Addressing second-order questions will require study of the changes to AML biology that occur from diagnosis to relapse.

Evolution of AML Biology from Diagnosis to Relapse





Canonical Framework:

- Minor subclones present at diagnosis (or induced by therapy) may evolve and expand at relapse.
- In some cases, these emerging subclones may be therapeutically targetable (e.g. FLT3-ITD subclone)

Nuno *et al* Elife 2024: Studied 216 AML patients with genome sequencing at diagnosis and disease relapse.

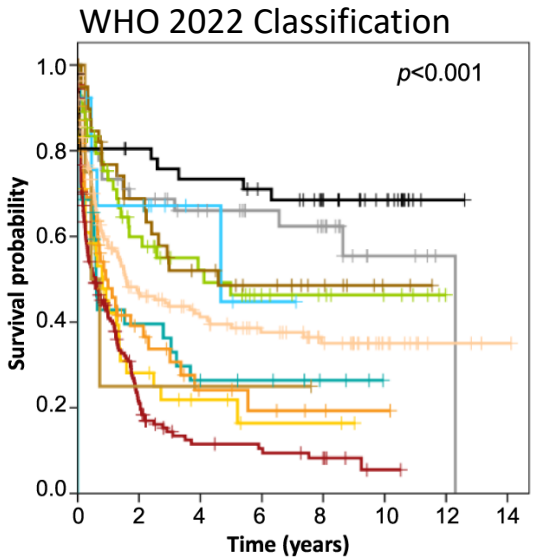
- 57% showed evidence of clonal evolution at relapse (mutation gain, mutation loss, or mutation gain + loss)
- 43% exhibited clonal stability without gain or loss of any mutations from diagnosis to relapse

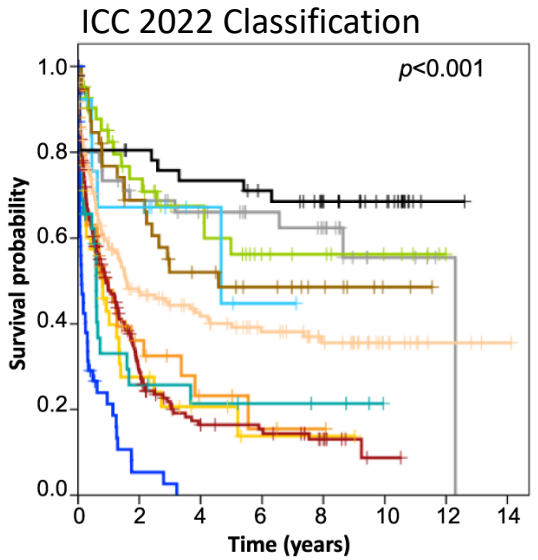
Genetic driver alterations alone are insufficient for explaining relapse biology in ~43% of cases.

To better understand disease progression, we need to consider other models of AML heterogeneity.

Beyond Genetics: Distinct Models of AML Heterogeneity

Genomic Heterogeneity in AML





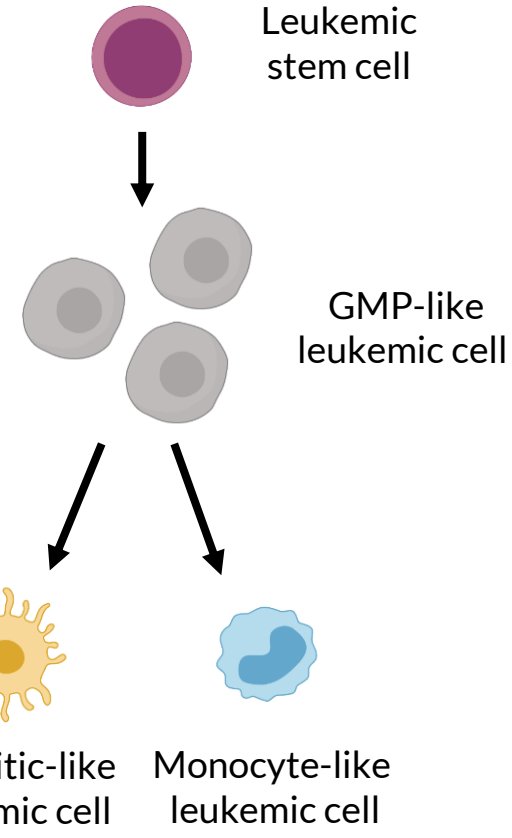
WHO 2022	n	Median OS (years)
PML::RARA	48	Not reached
CBFB::MYH11	45	12.3
DEK::NUP214	15	4.7
RUNX1::RUNX1T1	41	4.6
CEBPA	61	4.1
NPM1	172	1.6
MECOM-r	69	0.9
KMT2A-r	45	0.8
Differentiation	36	0.6
MR	208	0.5
NUP98-r	5	0.4
ODGA	1	0.0
Total	**746**	**1.5**

ICC 2022	n	Median OS (years)
PML::RARA	48	Not reached
CEBPA	47	Not reached
CBFB::MYH11	45	12.3
DEK::NUP214	15	4.7
RUNX1::RUNX1T1	41	4.6
NPM1	170	1.6
MR*	193	1.0
MECOM-r**	57	1.0
KMT2A-r***	40	0.8
NOS	34	0.6
TP53	52	0.1
Total	**742**	**1.5**

Huber *et al.* Leukemia 2023

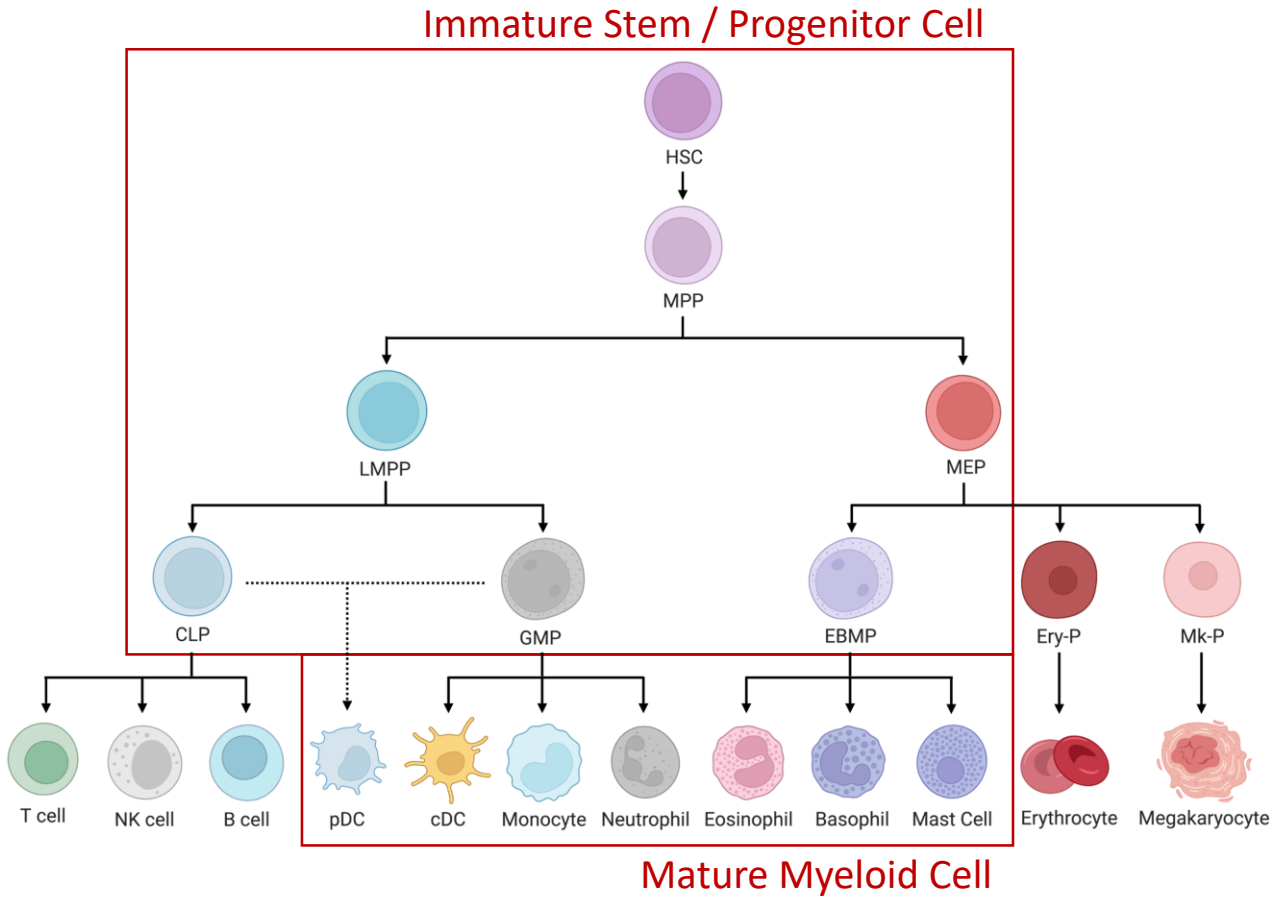
Cellular and Functional Heterogeneity in AML

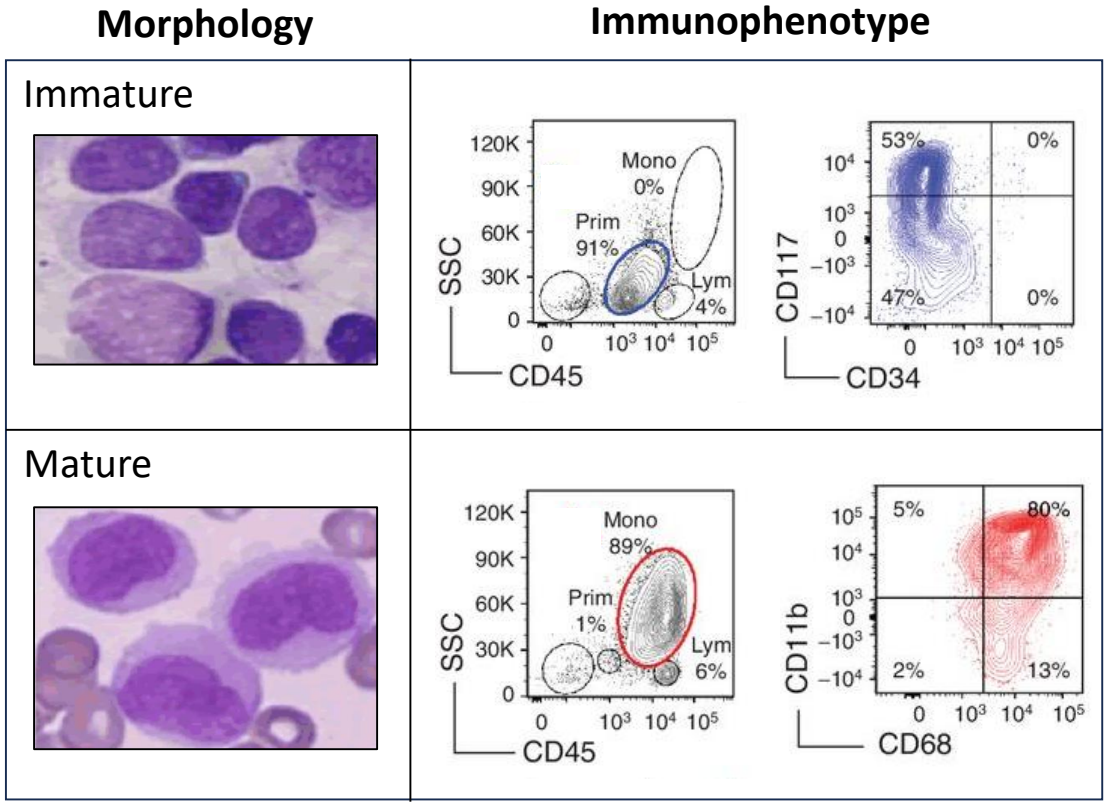
AML cells are arranged within cellular hierarchies



Bonnet & Dick, Nat. Med. 1997; Zeng *et al.* Nat. Med. 2022

Leukemia as a Caricature of Normal Hematopoiesis

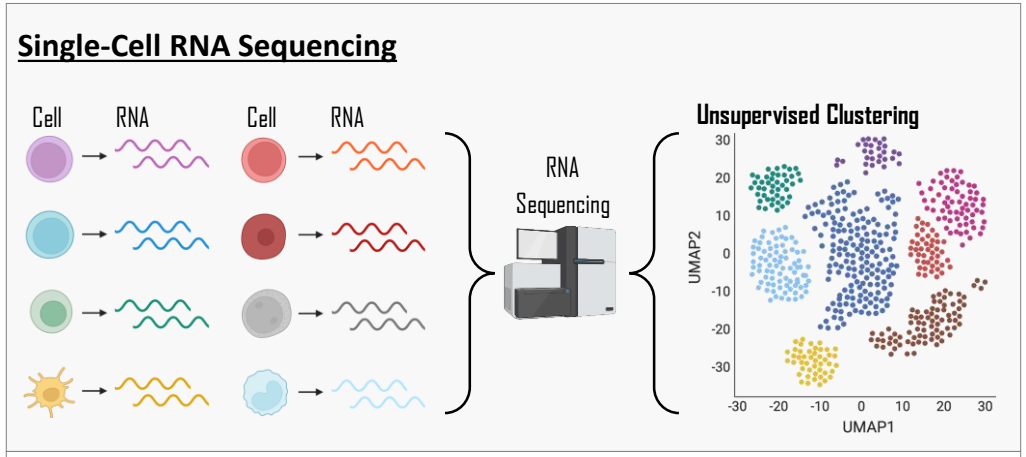


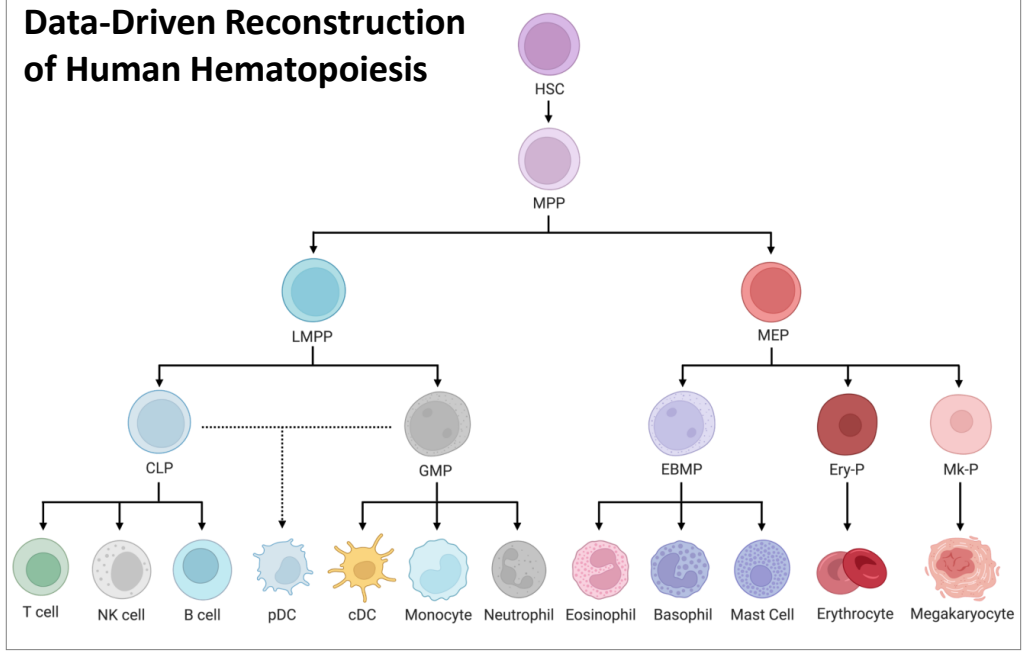


- **Morphology and Immunophenotype reveal broad lineage hematopoietic involvement**
- **These approaches lack specificity in identifying precise cellular states involved in disease**
- **Single cell RNA-sequencing provides thousands of new markers for determining leukemia cell state**

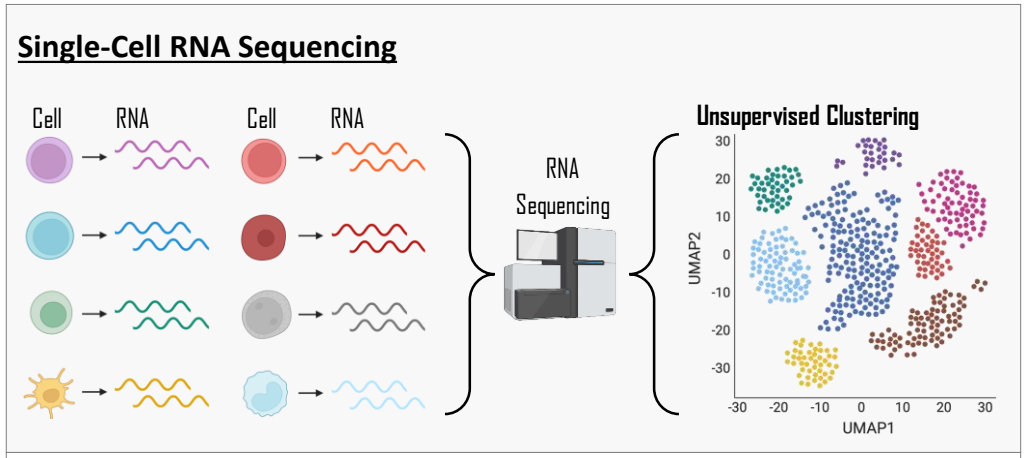
Kumar PS, Vasuki S. J Biomed Imag Bioeng. 2017; Pei et al, Cancer Discovery 2020

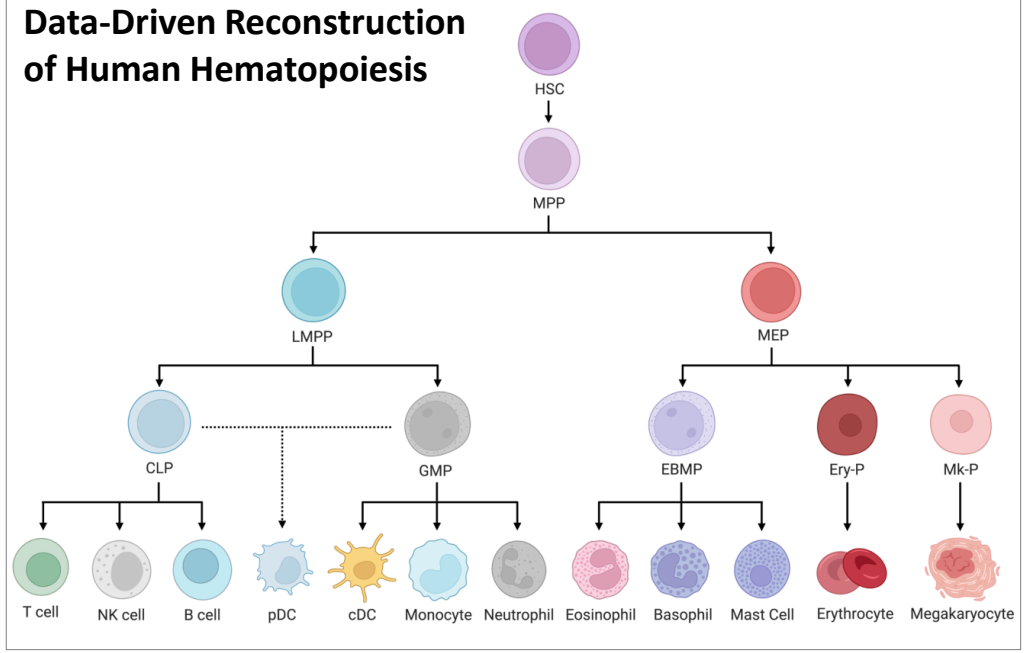
Mapping Human Hematopoiesis at Single-cell Resolution

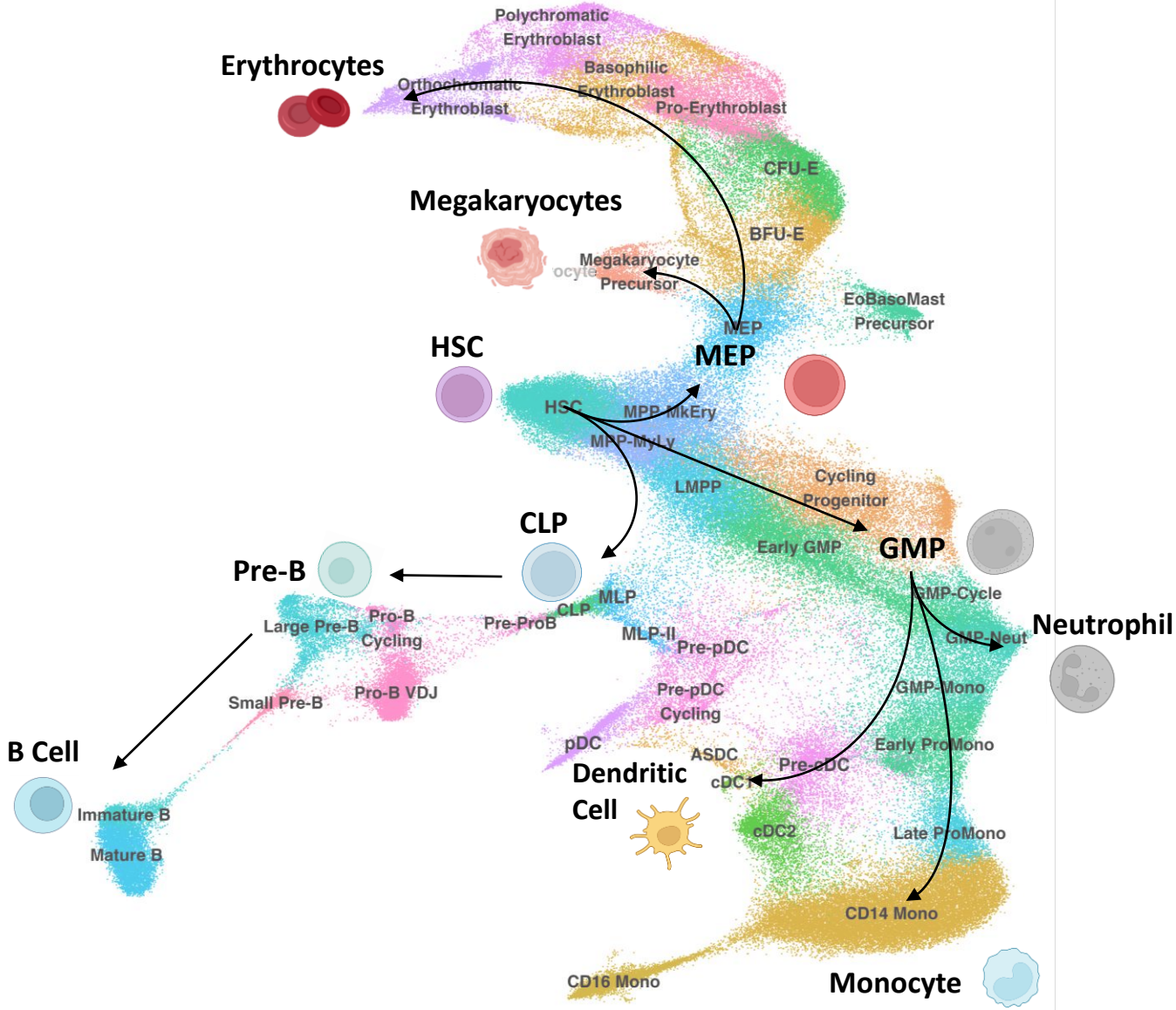




Mapping Human Hematopoiesis at Single-cell Resolution







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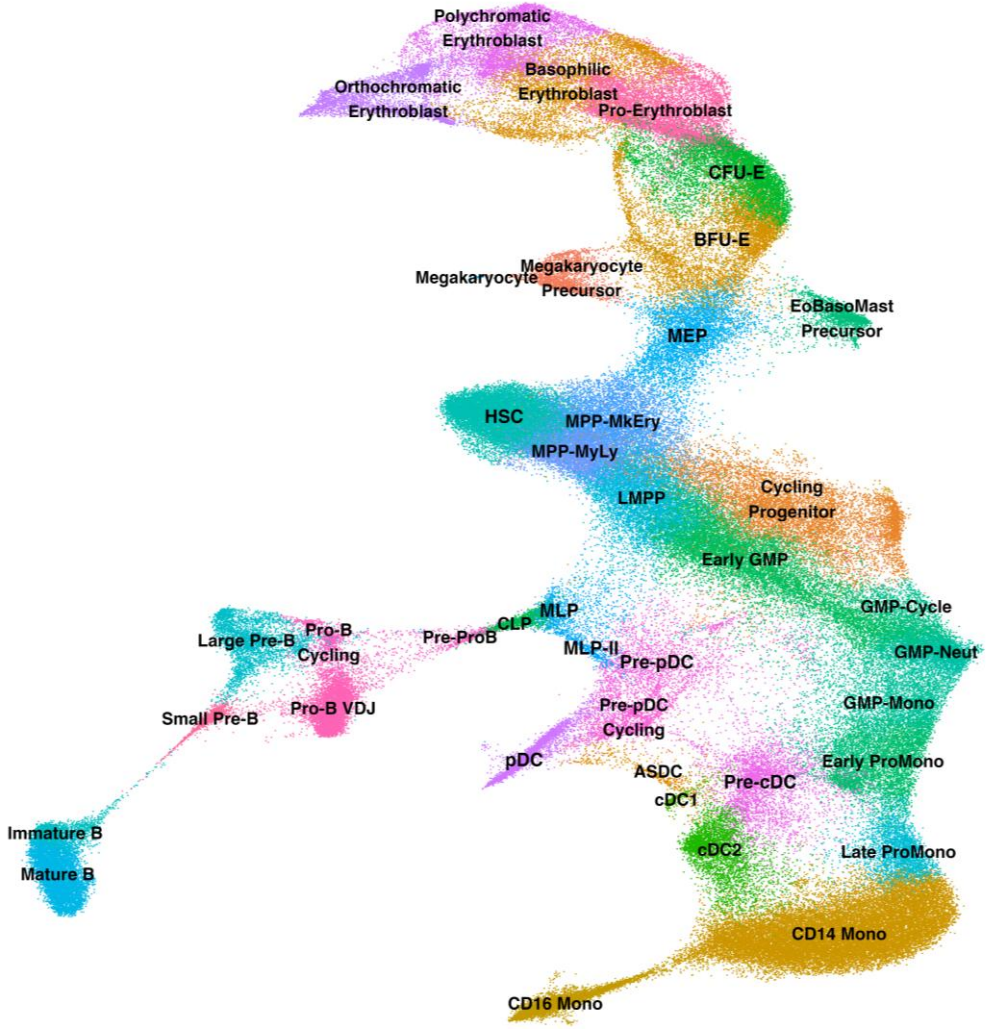
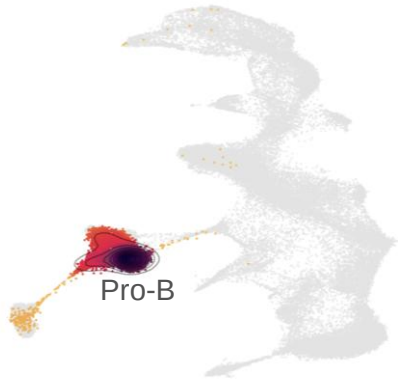
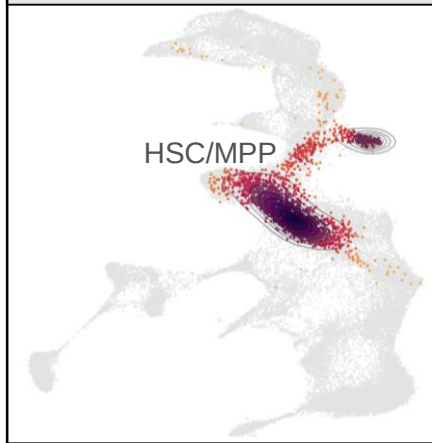
Precise Mapping of Differentiation Patterns in Acute Leukemia



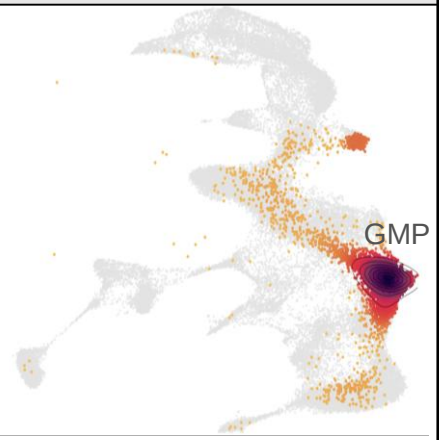
B-Cell Acute Lymphoblastic Leukemia (B-ALL)



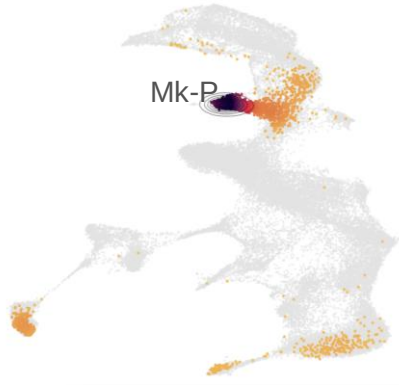
Acute Myeloid Leukemia
Early Differentiation Block



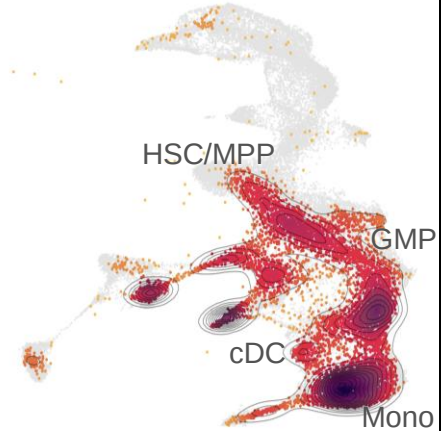
Acute Myeloid Leukemia
Myeloid Progenitor Enriched



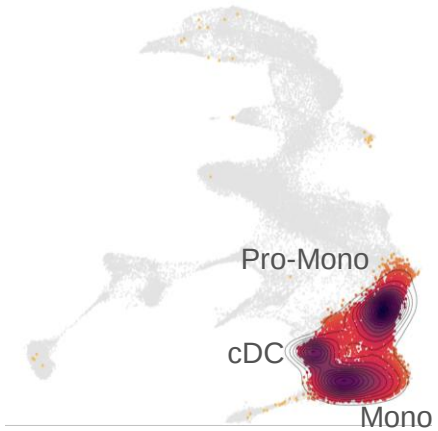
Acute Megakaryoblastic Leukemia (AMKL)



Acute Myeloid Leukemia
Intact Myeloid Differentiation



Acute Myeloid Leukemia
Mature Myeloid Enriched



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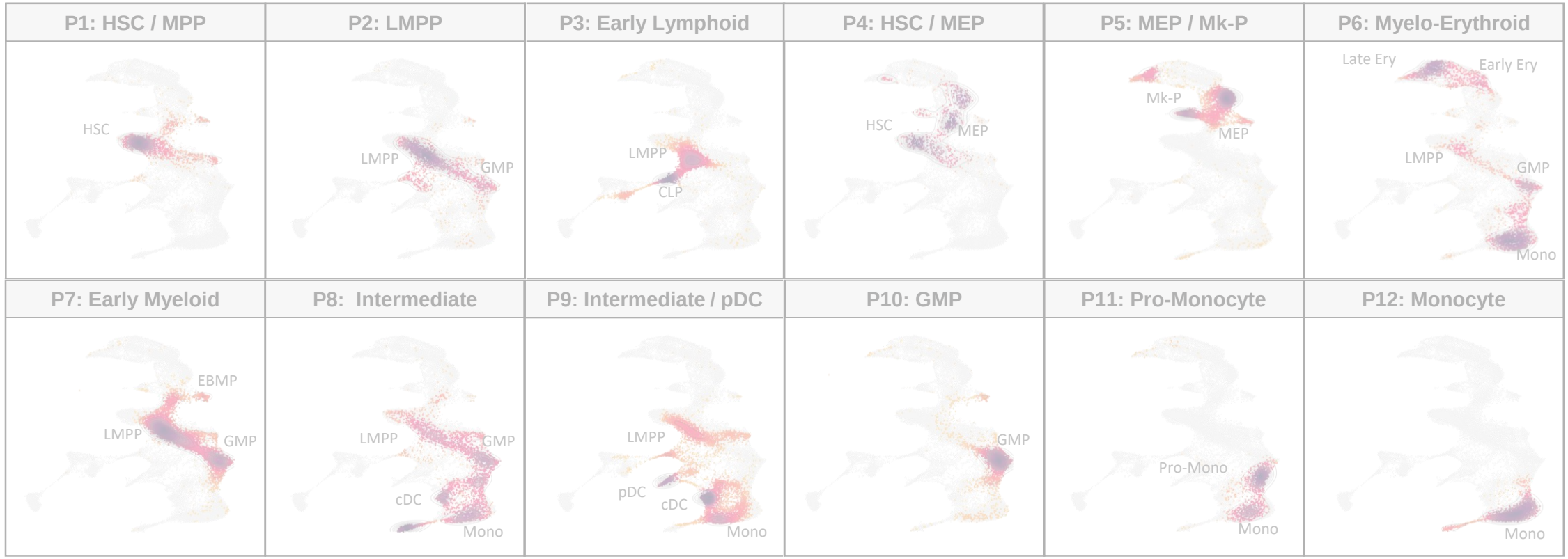
Recurrent Patterns of Aberrant Differentiation in AML

AML cells can be precisely mapped against the hematopoietic hierarchy

- How much variation exists across AML differentiation landscapes and which lineages are involved?

  → scRNA-seq from >1 million single cells across 318 AML patient samples

Recurrent Differentiation Patterns in AML



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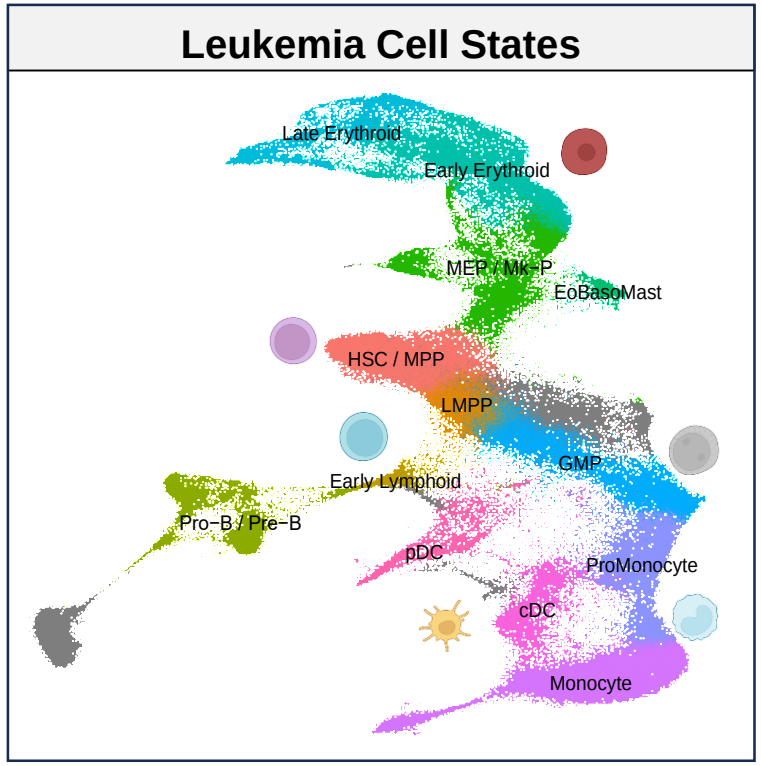
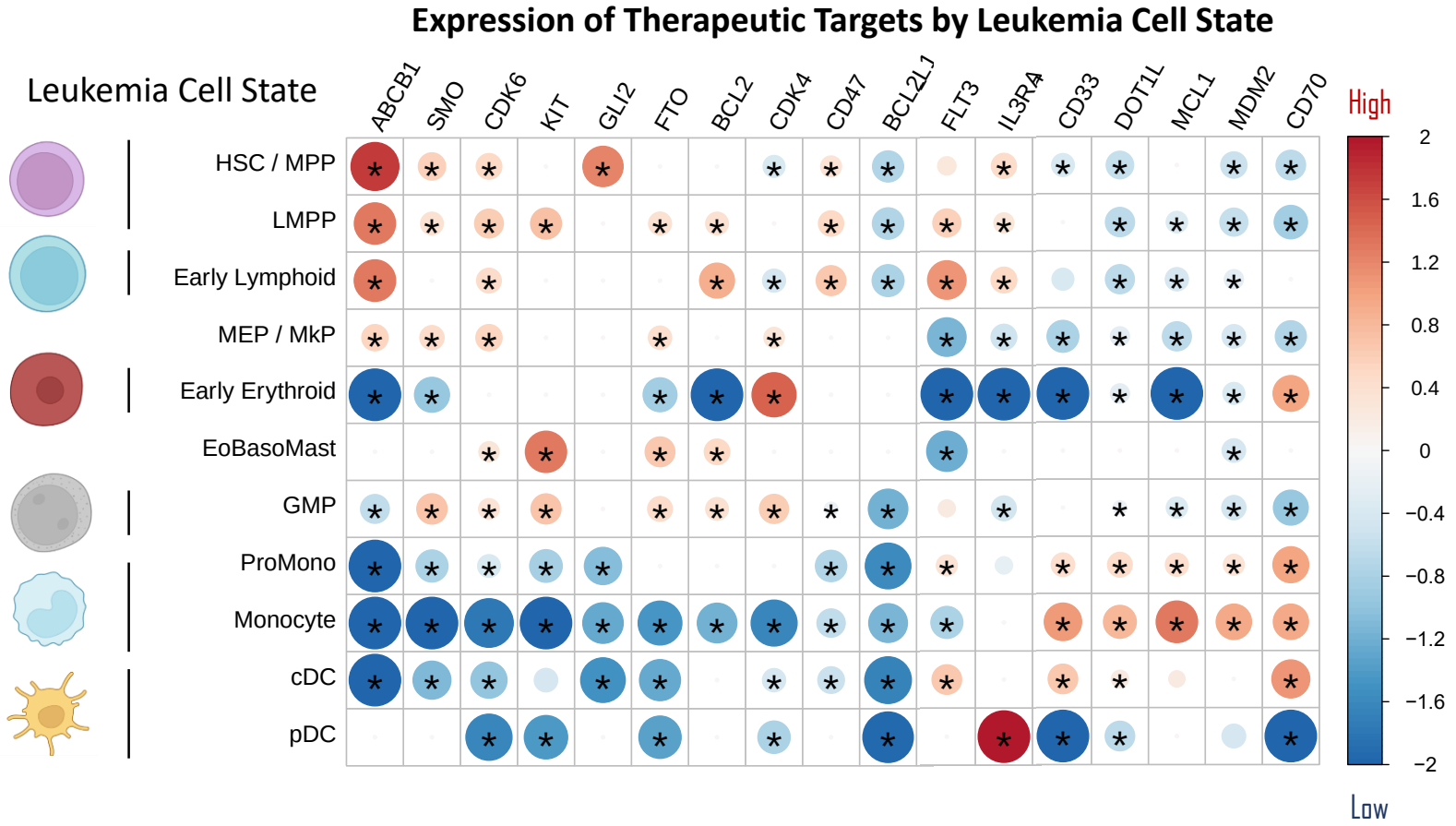
Leukemia Cell States differ in Biology and Therapeutic Target Expression

We mapped leukemia scRNA-seq data from >1 million single cells across 318 myeloid leukemia patients

1. This allowed us to define “leukemia cell states” that were recurrent across patients.
2. Expression of common therapeutic targets in AML differ dramatically across cellular states.

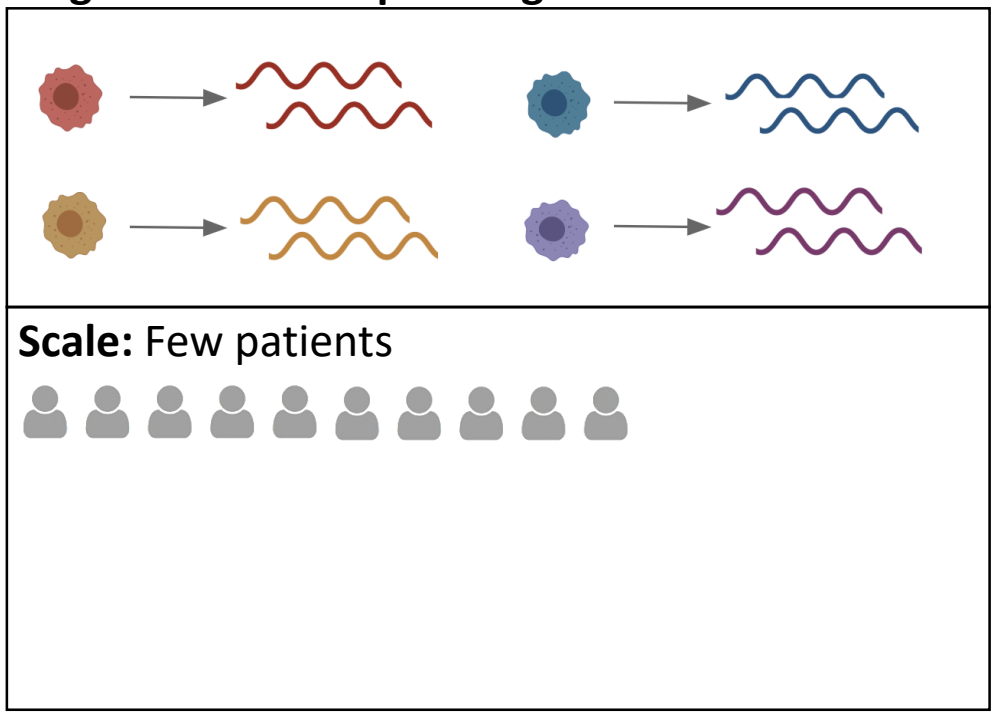




What is the clinical relevance of Leukemia Cell States?

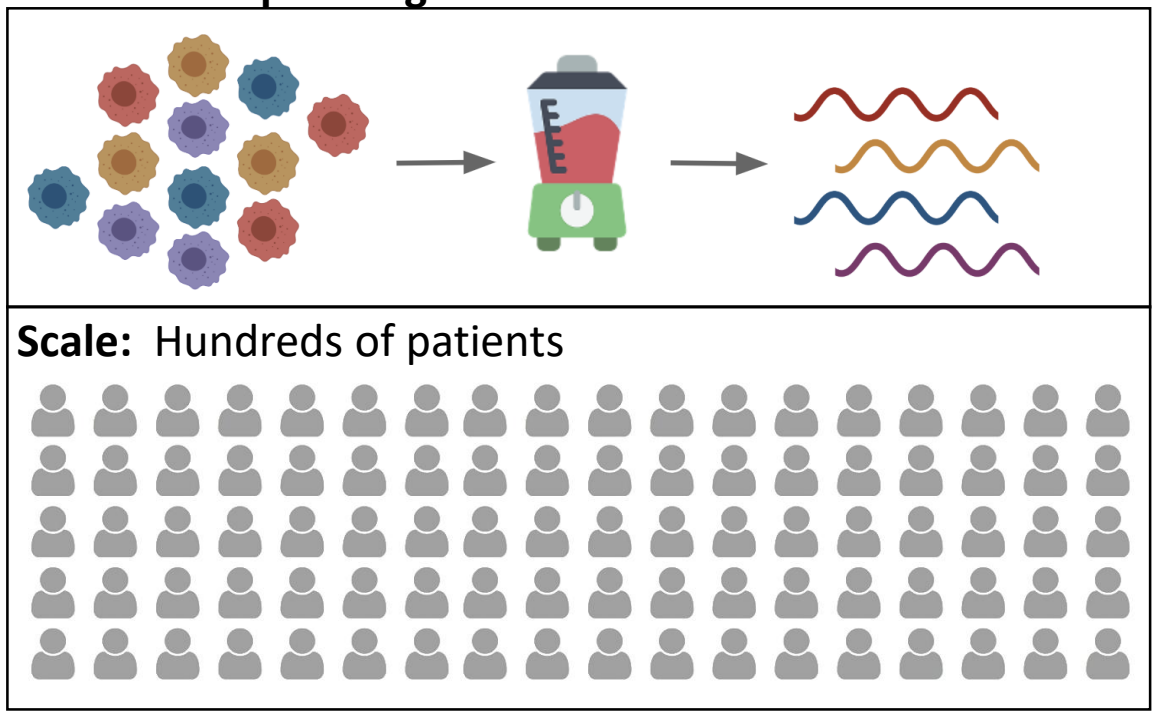
Applying insights from scRNA-seq at the cohort scale

Single-cell RNA sequencing



Scale: Few patients

Bulk RNA sequencing

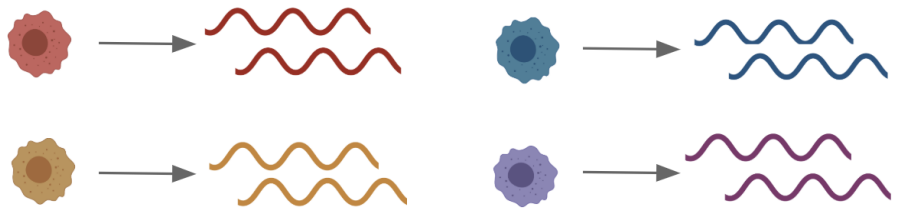


Scale: Hundreds of patients

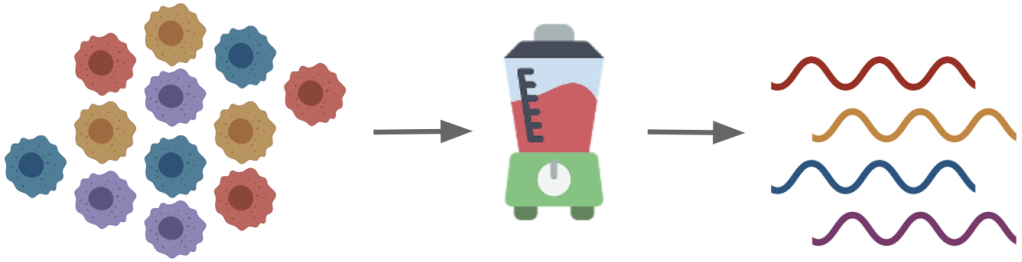
How can we bridge the gap between single-cell RNA-sequencing and bulk RNA-sequencing?

Applying insights from scRNA-seq at the cohort scale

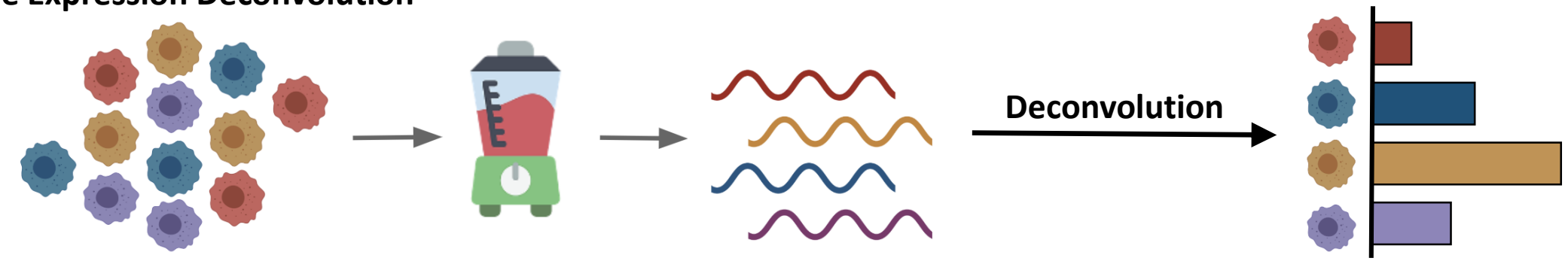
Single-cell RNA sequencing



Bulk RNA sequencing

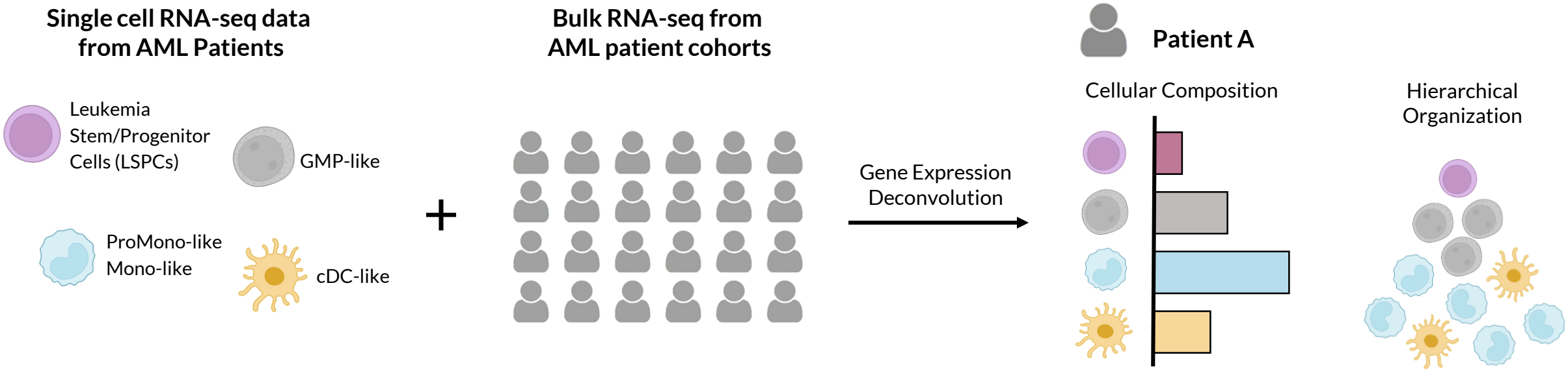


Gene Expression Deconvolution



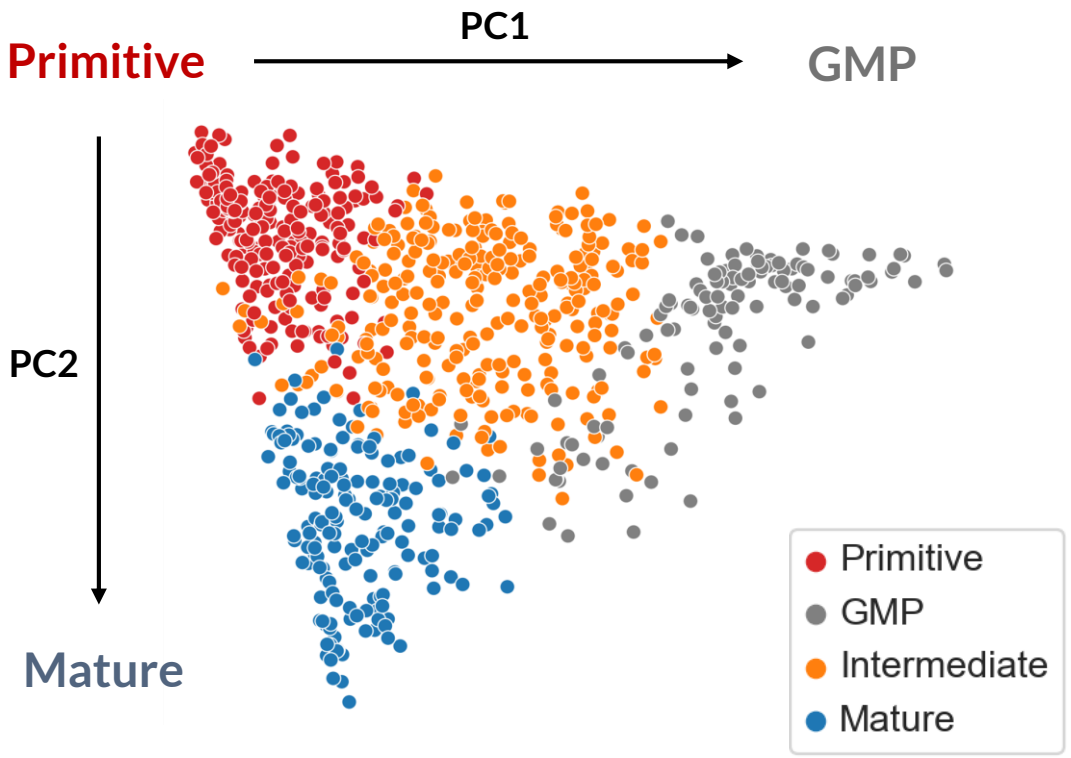
Analogy – inferring the recipe of a smoothie

Inferring Leukemia Cell Composition in AML Patient Cohorts

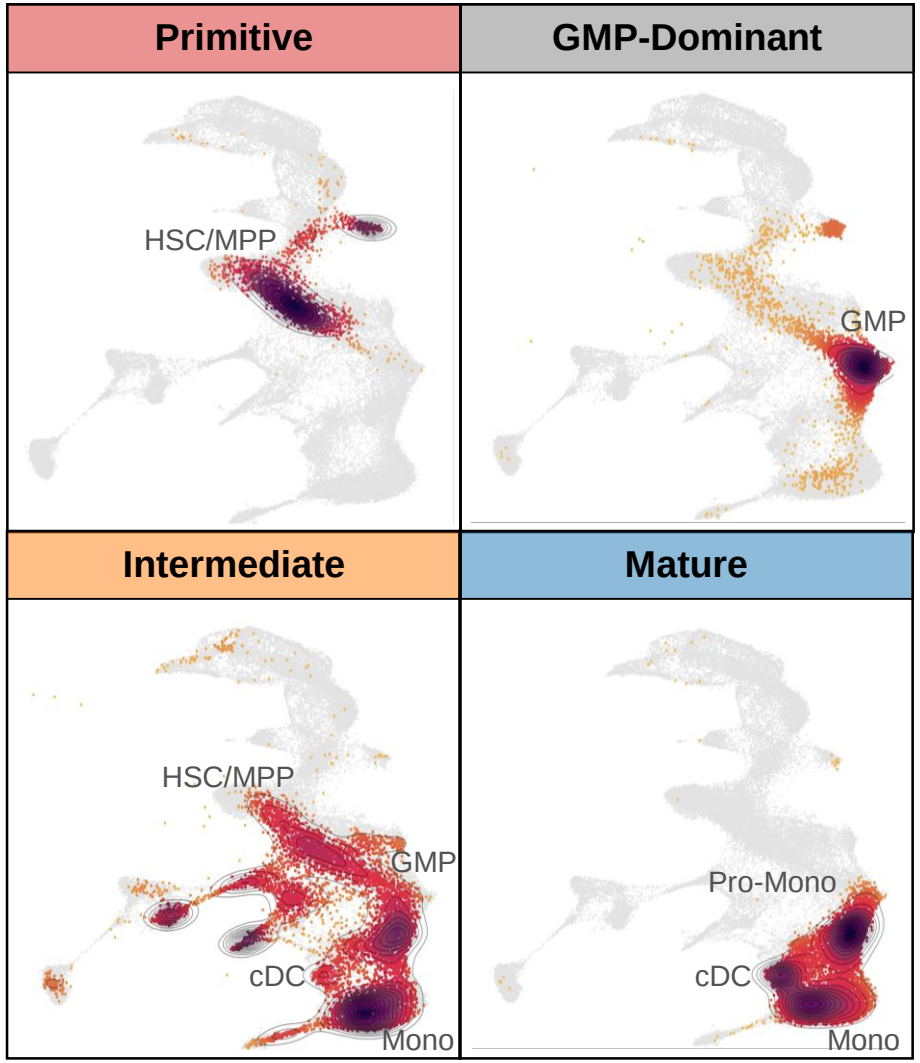


Zeng *et al.* Nature Medicine 2022

AML Subtypes defined by Leukemia Cell Composition



864 patients from three independent cohorts
(TCGA, BeatAML, Leucegene)



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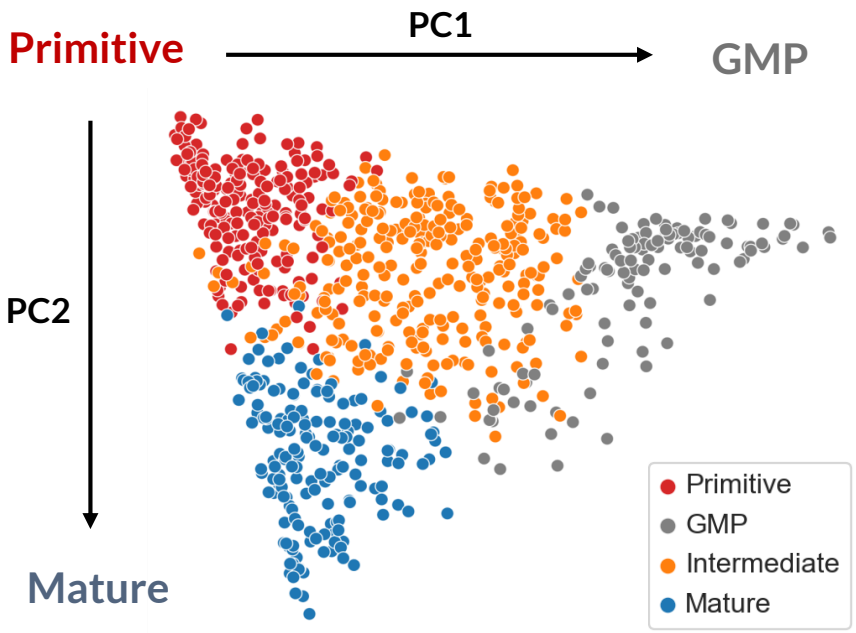
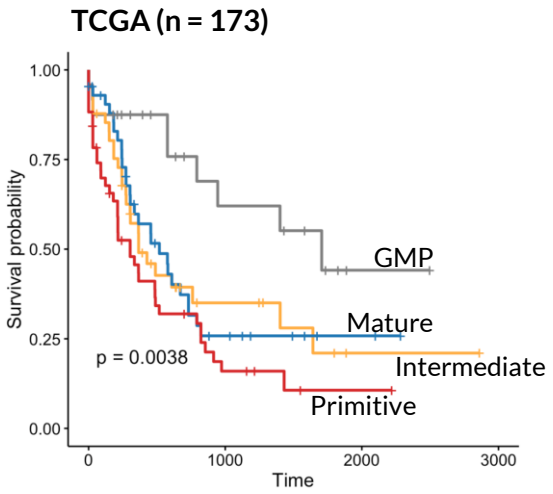
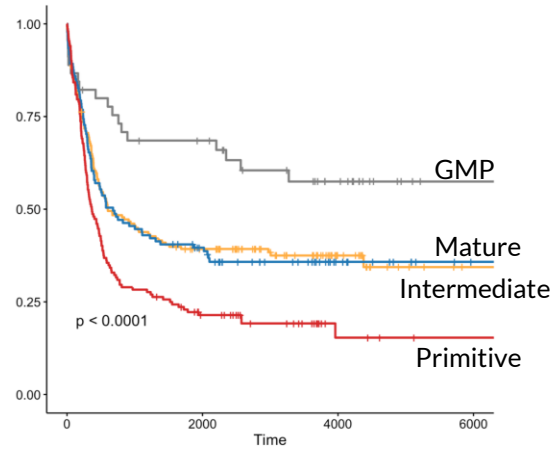
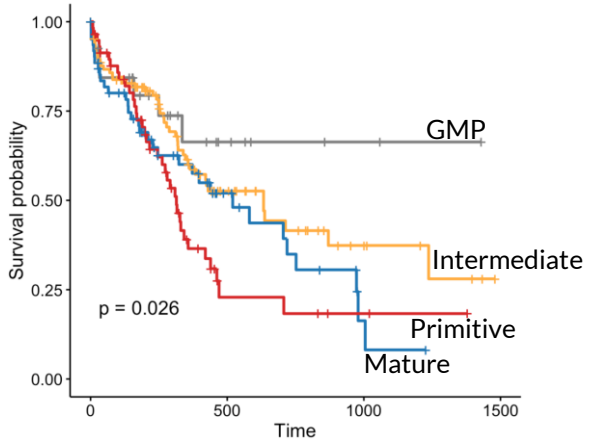
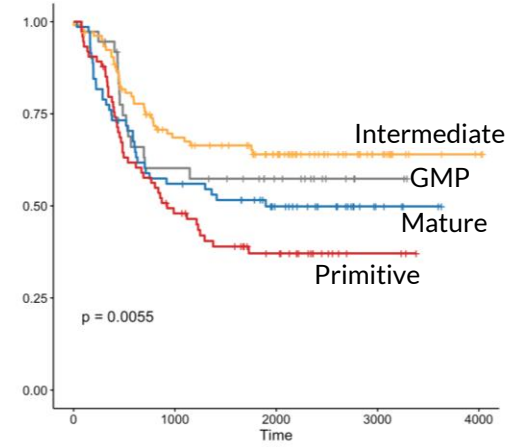
AML Subtypes have Distinct Survival Outcomes











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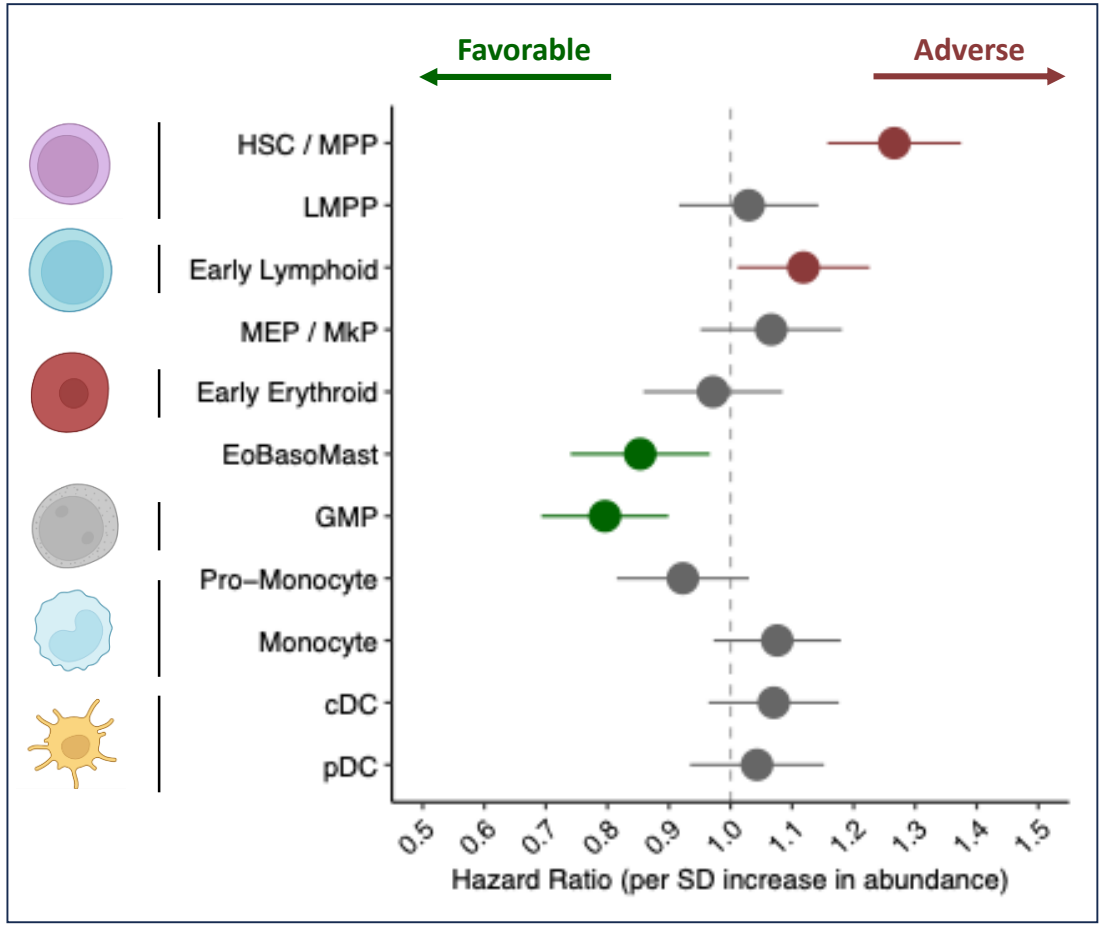
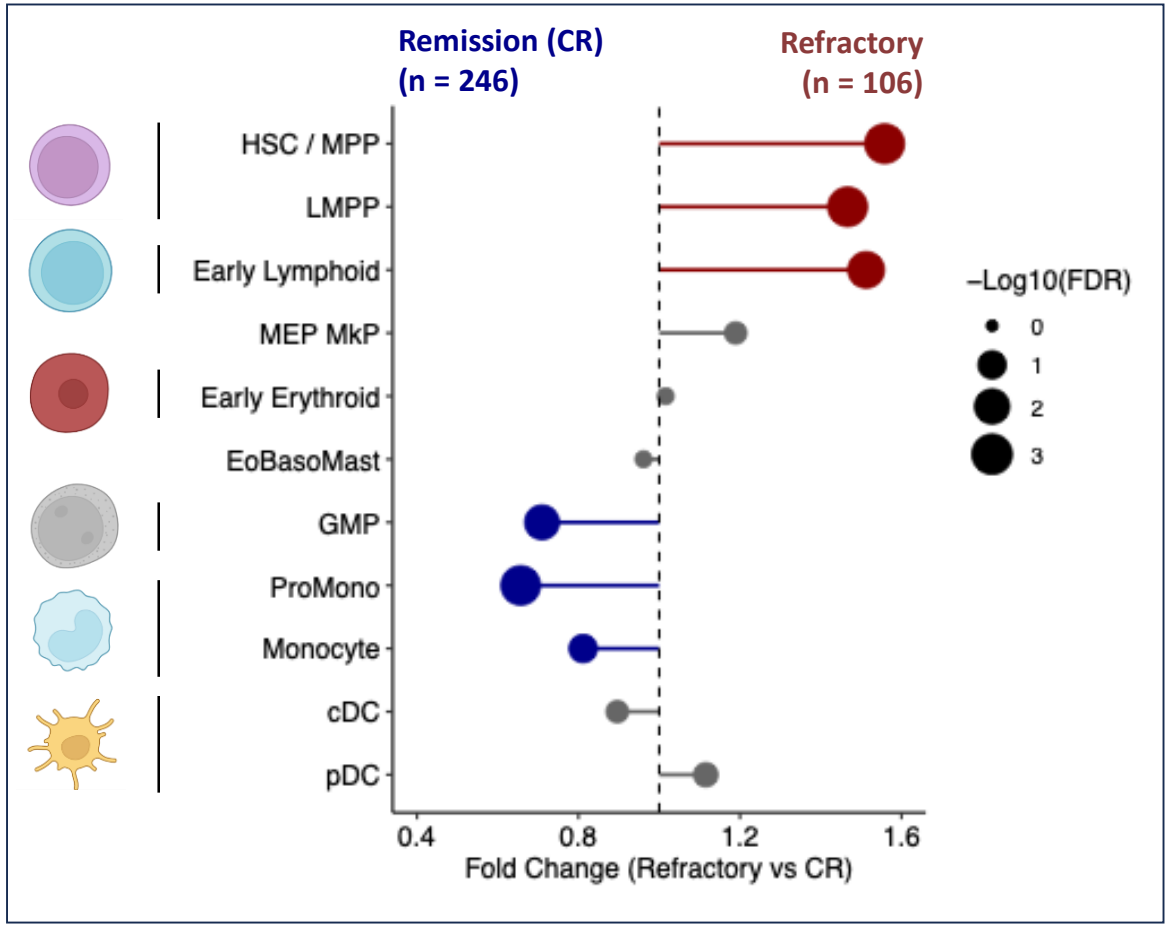
AML Cell States associate with Survival & Chemotherapy Response

Association with Overall Survival (n = 582)



Association with Induction Chemotherapy Response (n = 352)



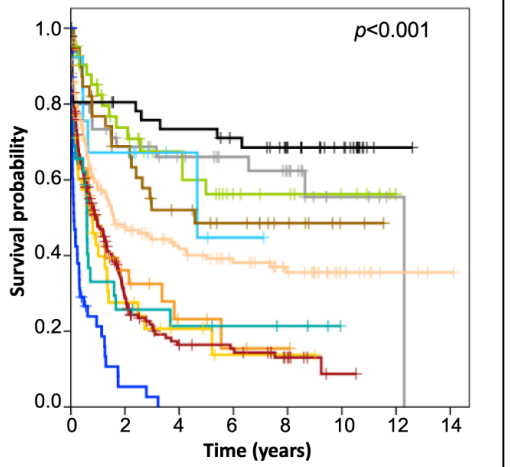
Convergence of Genetic Driver Alterations on Cellular States in AML

What are the determinants of variation between aberrant differentiation landscapes in AML?

- Infer leukemia cell state abundance in bulk RNA-seq from 1,224 AML patients

  → Map associations between >45 genetic drivers and abundance of each leukemia cell state

Genetic Subtypes



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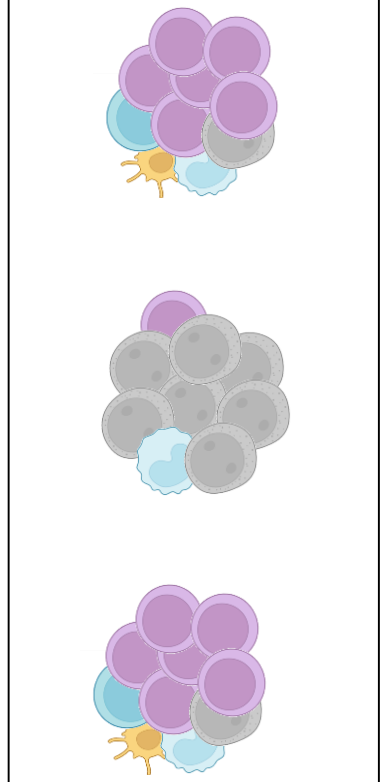
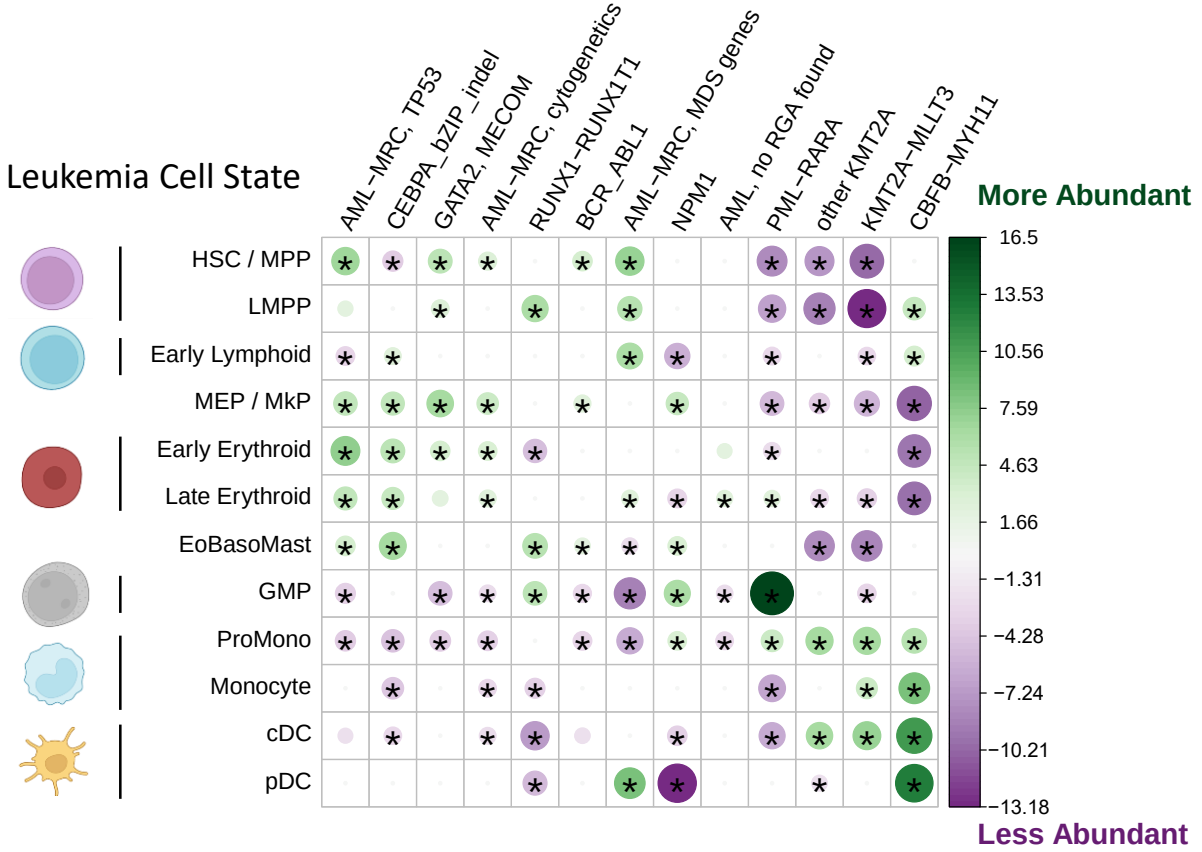
Cellular Phenotype



Genotype-to-Phenotype Associations (n=1,224)



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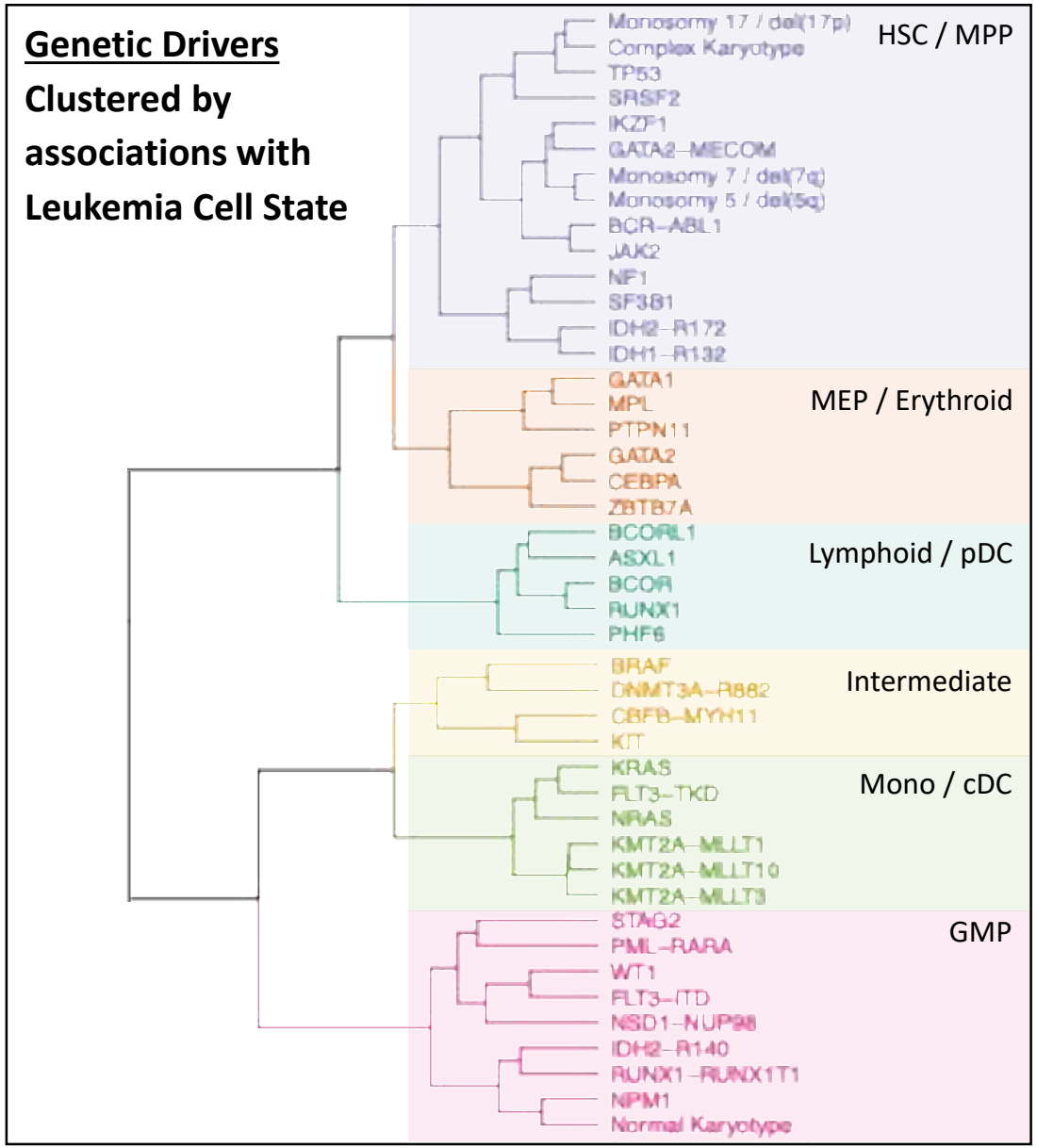
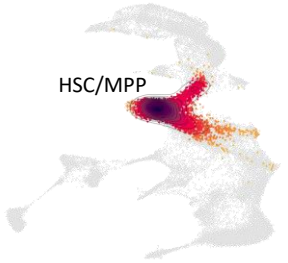
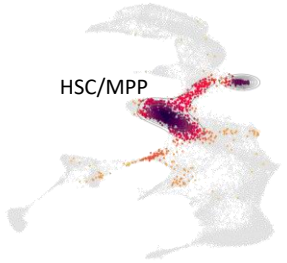
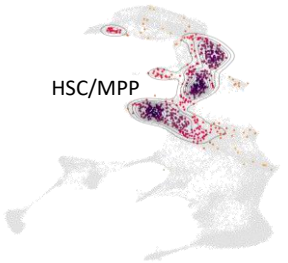
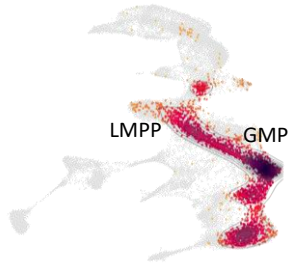
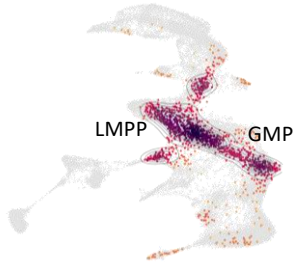
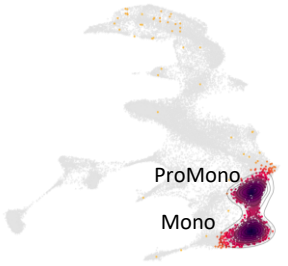
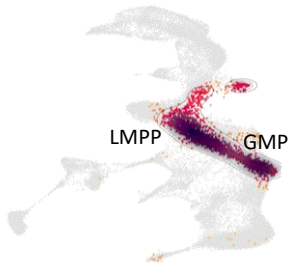
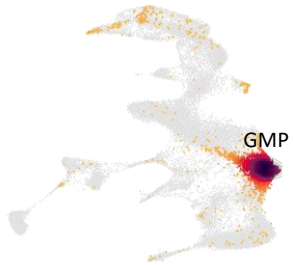
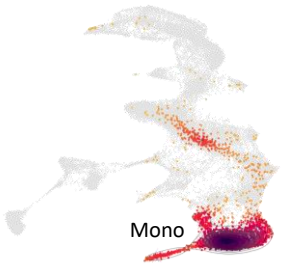
Convergence of Genetic Driver Alterations on Cellular States in AML



Impact of genetic driver mutations on differentiation:

Chr7 deletion	Chr5 deletion	*TP53* mutation
		

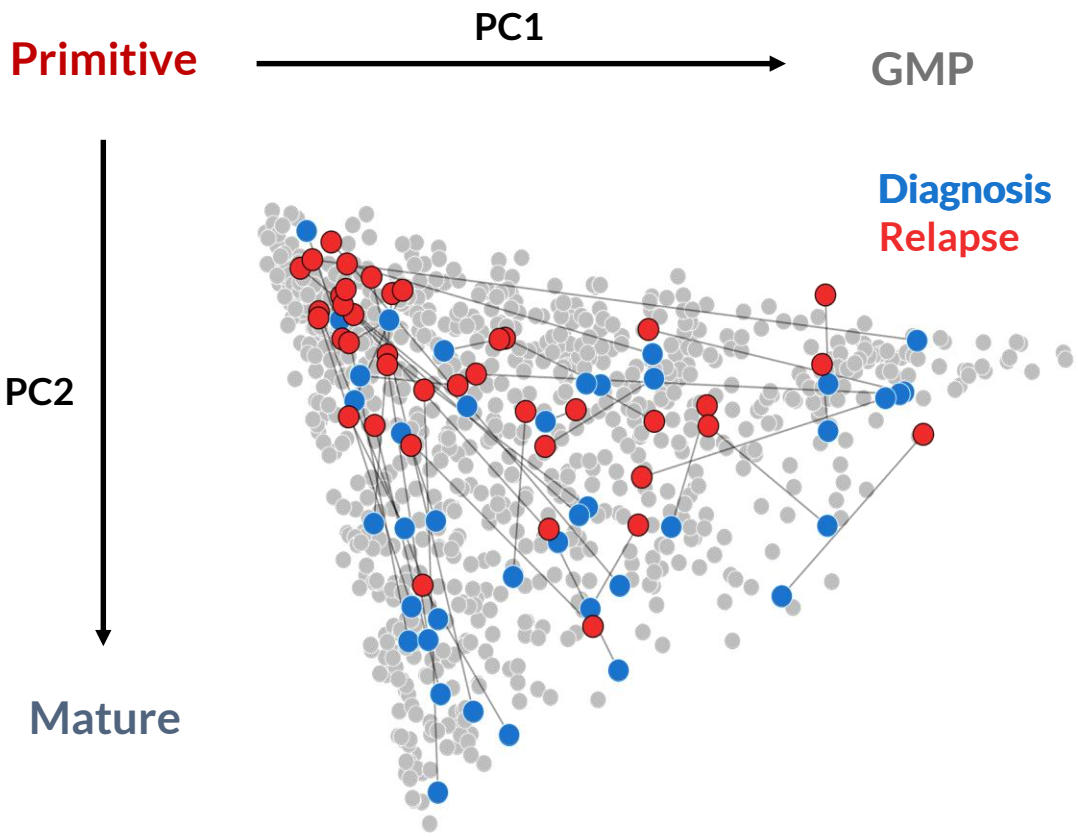
NPM1	*NPM1* + *FLT3*-ITD	*NPM1* + *NRAS*
		

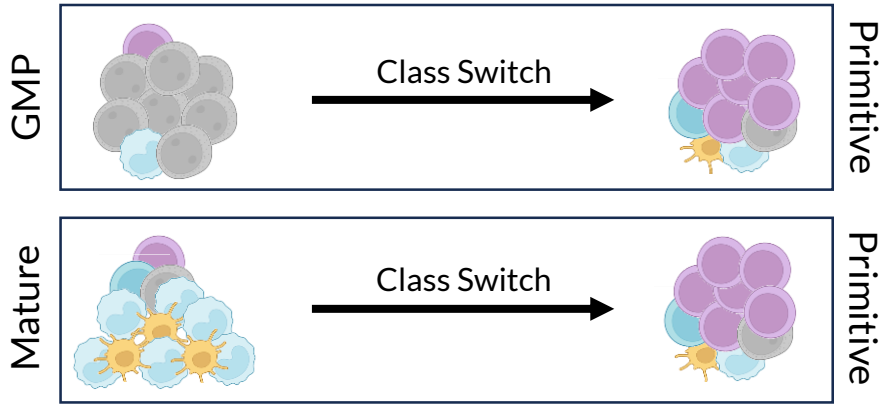
RUNX1::RUNX1T1	*PML::RARA*	*CBFB::MYH11*
		

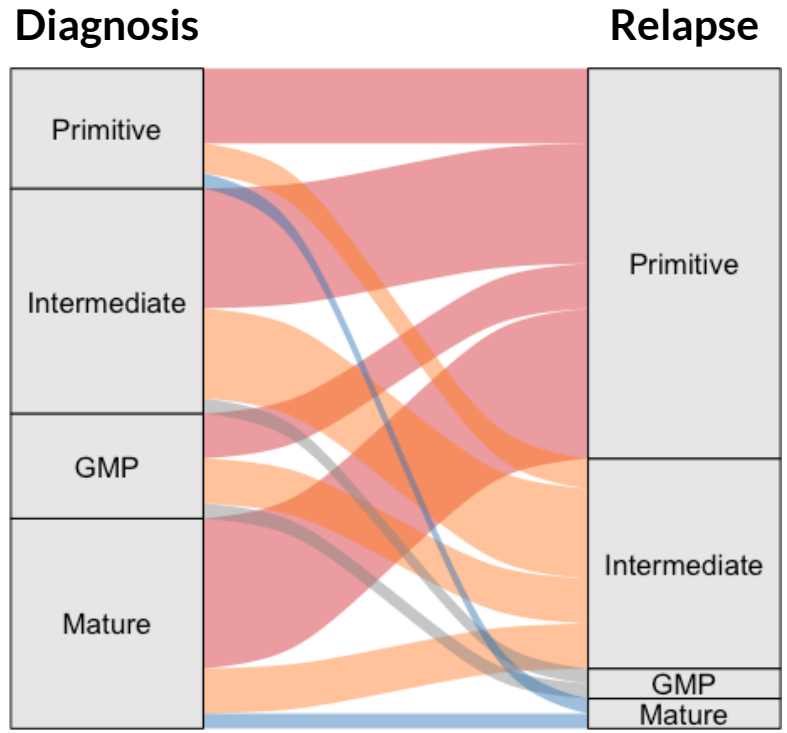
Defining a convergent Relapse Phenotype in the post-chemotherapy setting

Convergent Cellular Phenotypes at AML Relapse

RNA-seq from 44 Diagnosis-Relapse Pairs (4 Cohorts)







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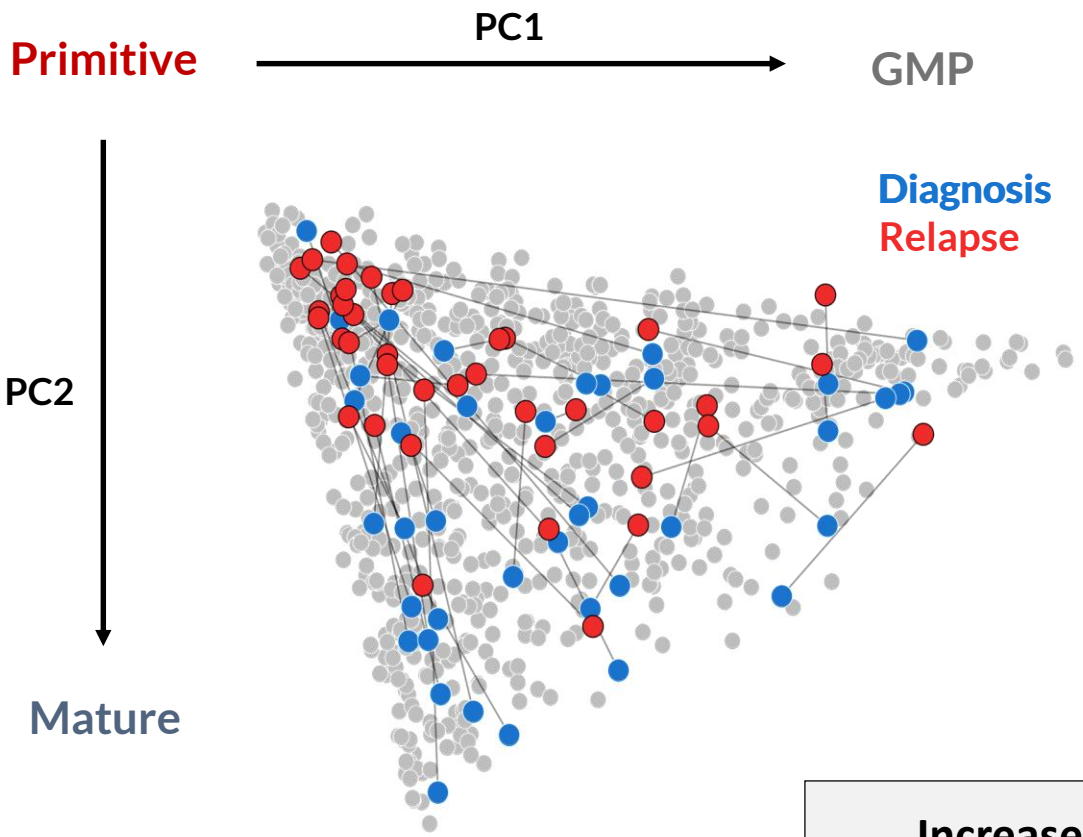
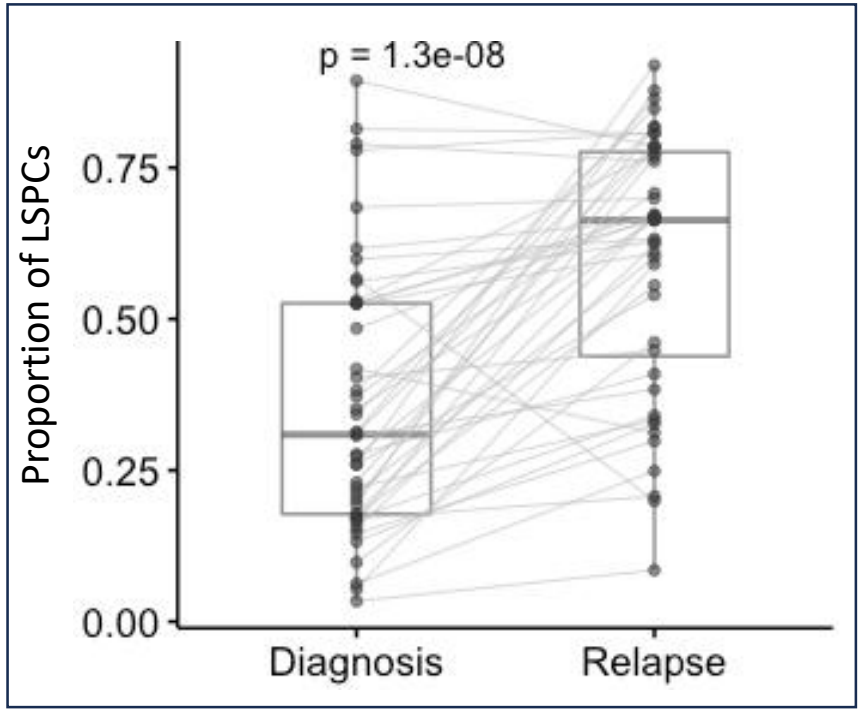
Convergent Cellular Phenotypes at AML Relapse

RNA-seq from 44 Diagnosis-Relapse Pairs (4 Cohorts)



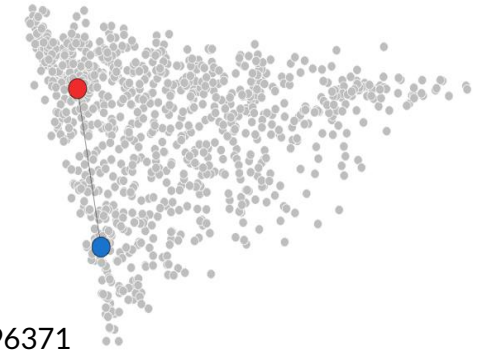
Proportion of Leukemia Stem/Progenitor Cells



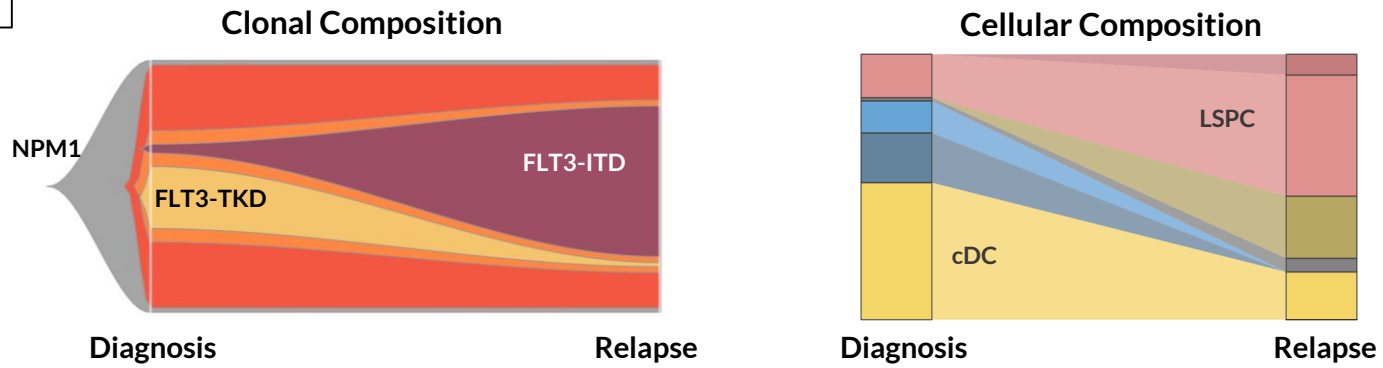
Increase in Primitive Leukemia Stem/Progenitor Cells (LSPCs) at Relapse was observed in 39/44 (89%) of Diagnosis-Relapse pairs

Clonal Composition vs Cellular Composition at Relapse

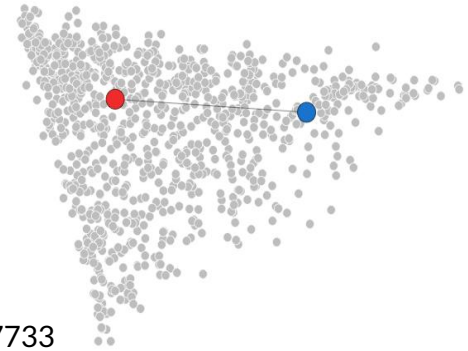
Clonal Evolution with Phenotypic Evolution



Pt. 196371



Clonal Stability with Phenotypic Evolution



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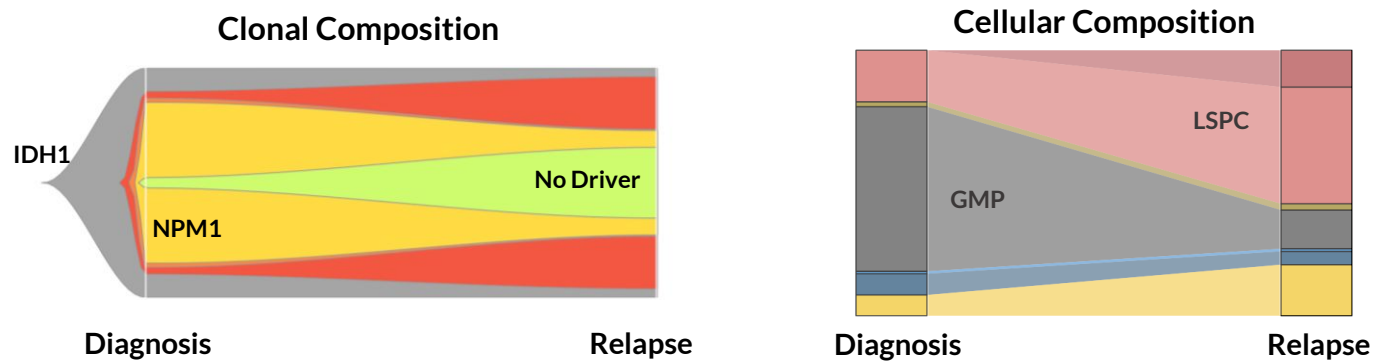

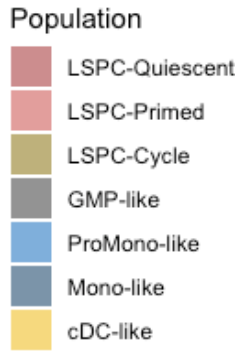

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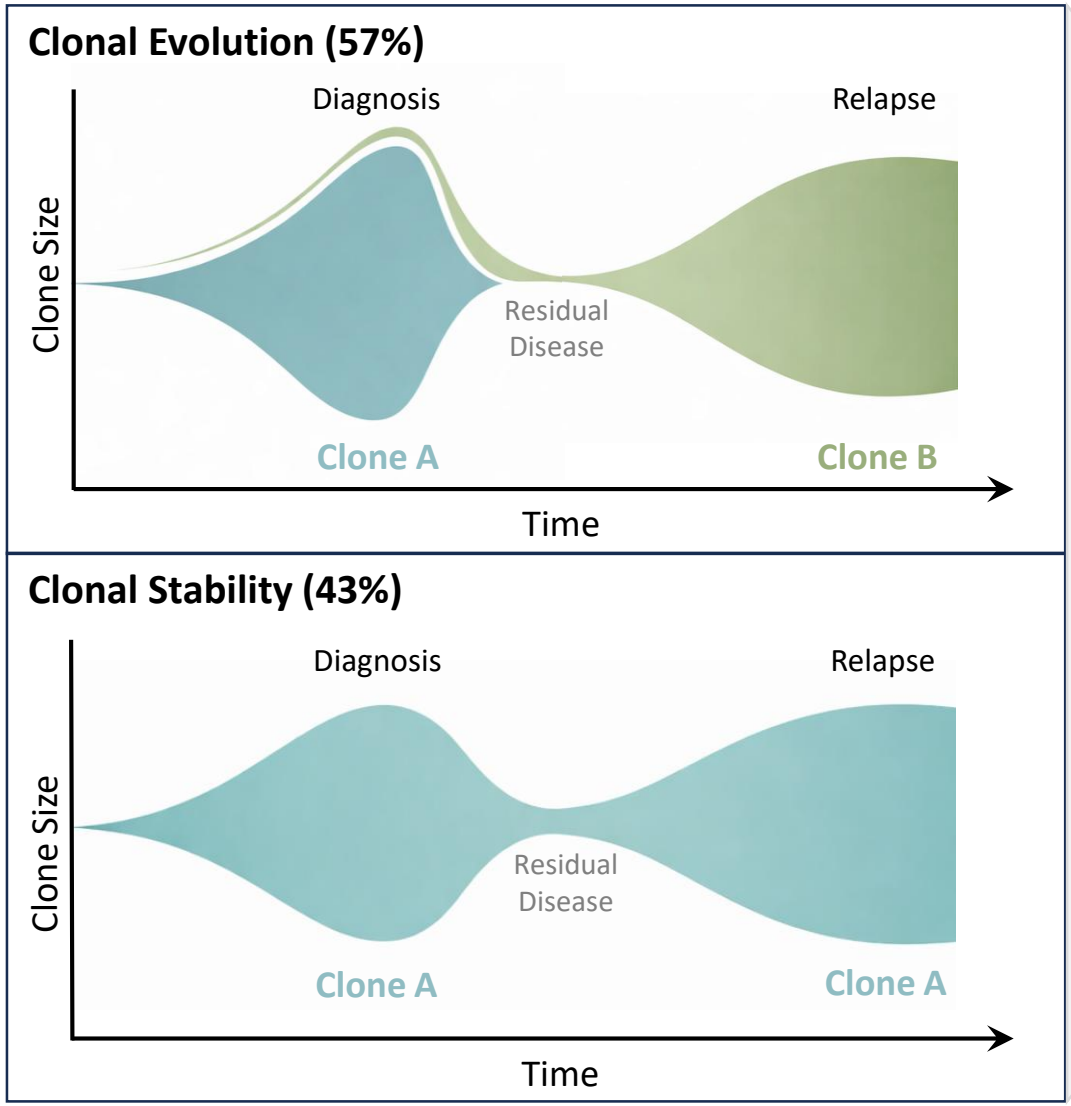
Genetic Pathways Converge upon a Common Phenotypic Endpoint



Convergent Relapse Phenotype (89%):

- Near-universal shift towards primitive LSPC-enriched cellular composition*

*Applies specifically to post-chemotherapy relapse.
Relapse following other treatments (e.g. Allo-BMT, Aza-Ven) will differ.

Is the Relapse Phenotype present at the time of MRD assessment?

Turkalj*, Radtke*, Mecklenbrauck*, Stoilova*, Groom*, *et al.* Blood 2026:

Ivosidenib + Venetoclax ± Azacitidine in IDH1-mutated AML (n = 8 patients)

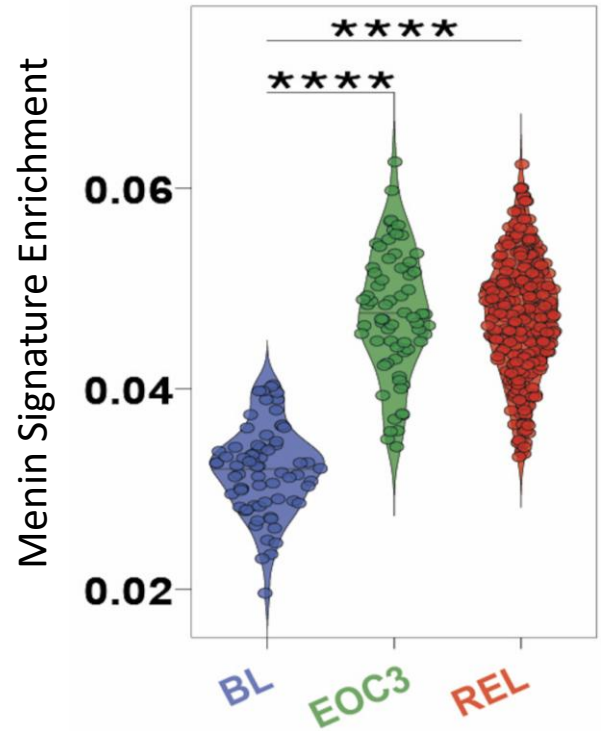
Diagnosis → End of Cycle 3 → Relapse

Single-cell Multi-omic Profiling (Genotype, Immunophenotype, Transcriptome)

Findings:

1. Relapse-specific clones were present by the End of Cycle 3.
2. Relapse-specific clones were enriched within primitive and immature leukemia cell states (e.g. LMPP, GMP).
3. Relapse-specific clones from diverse genetic backgrounds shared common gene expression programs
   - In this study, clones resistant to Ivo + Ven ± Aza were enriched for a Menin-related gene signature

Upregulation of a Menin Signature within resistant clones at EOC3



New Directions for MRD-Directed Therapies

Key Takeaways - AML Heterogeneity and Chemotherapy Response

1. AML heterogeneity extends beyond genetics alone.
2. Leukemia cell states are linked to distinct survival outcomes and therapy responses.
3. Post-chemotherapy relapse often converges upon a primitive LSPC-enriched phenotype.
4. This relapse-associated phenotype may already be present at timing of MRD assessment.
5. MRD provides an early window into relapse biology and may guide **what therapy to use**:

   e.g. enabling MRD-directed therapies targeting convergent relapse phenotypes.

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