1ST INTERNATIONAL CONFERENCE ON
Ph+ Leukemias

Bologna, Royal Hotel Carlton **September 29-30, 2025**

Biology of Ph+ ALL: new insights for risk stratification

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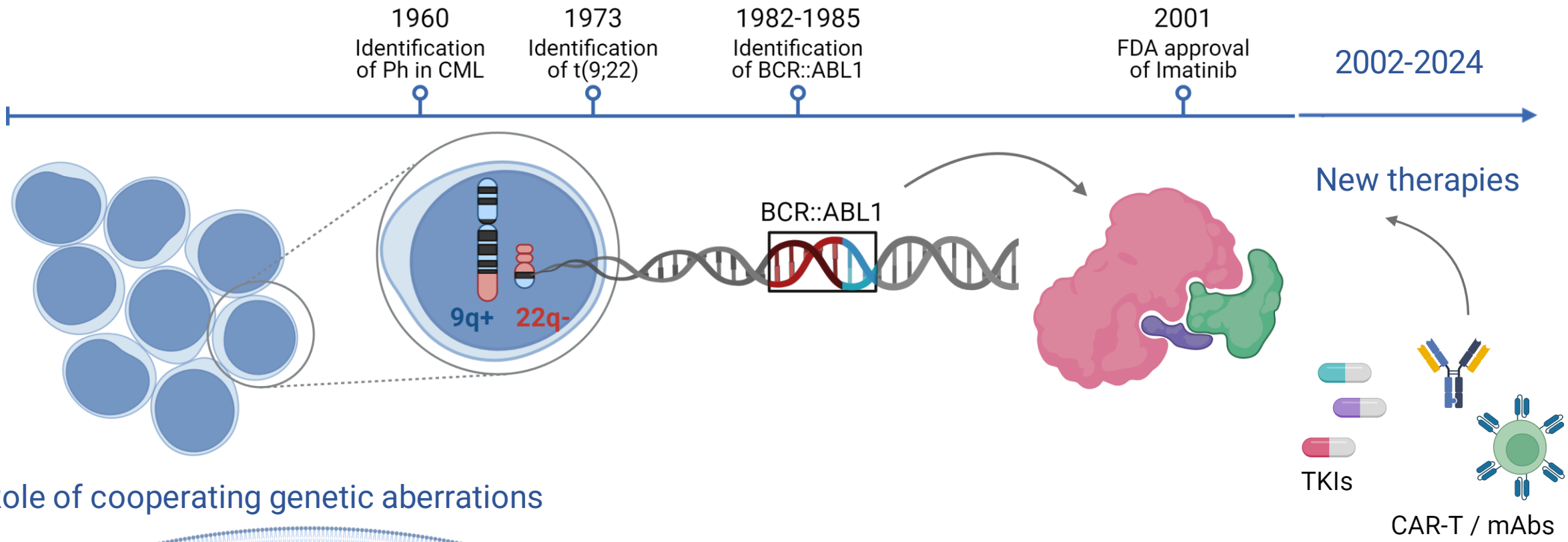
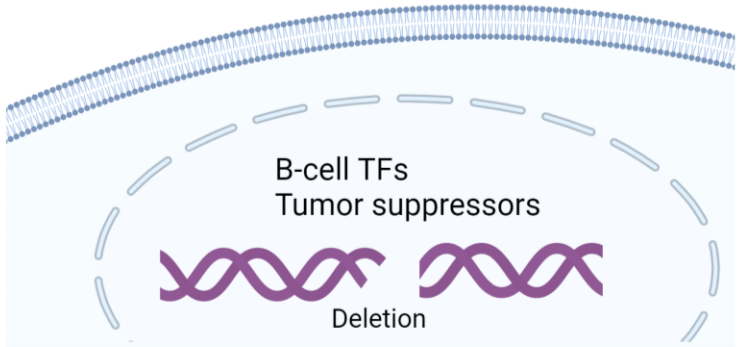
Disclosures ILARIA IACOBUCCI

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Mission Bio							Paid travel for invited talk
Arima			X				
Takara							Paid travel for invited talk

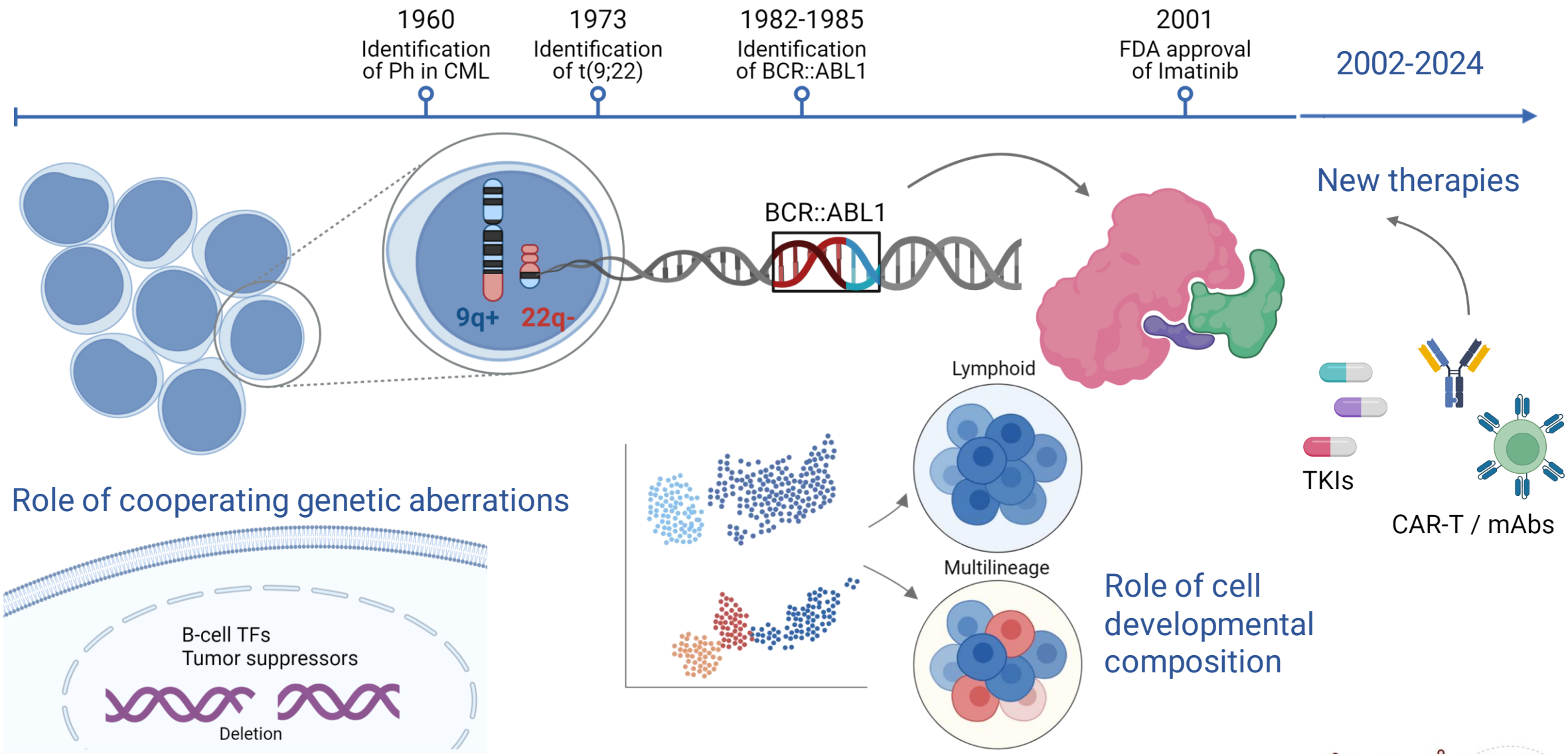
BCR::ABL1: past and ongoing insights



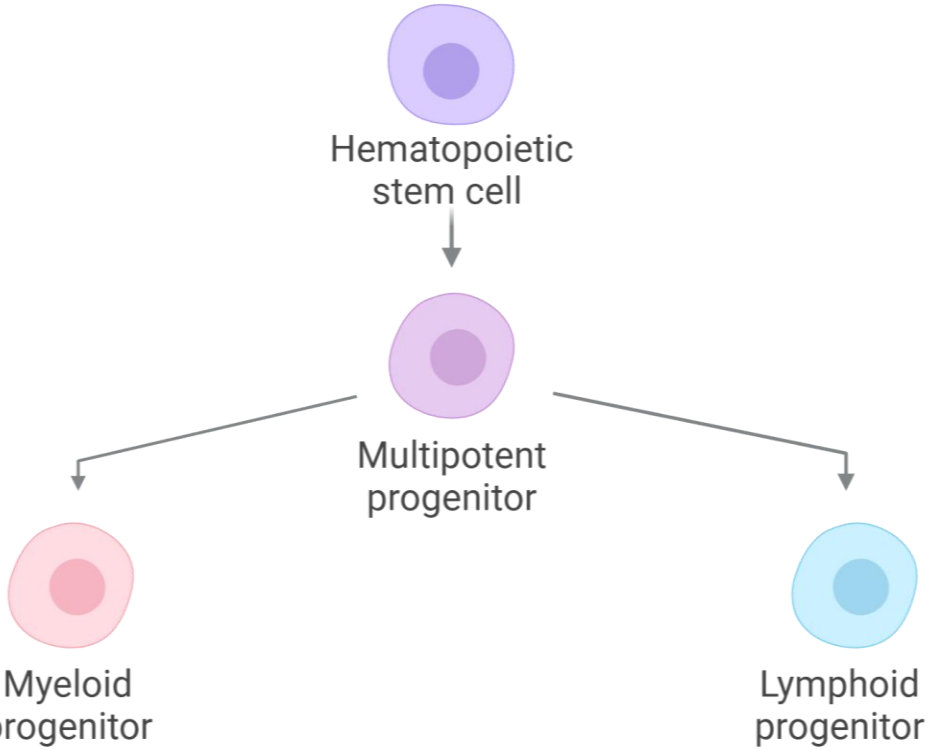
Role of cooperating genetic aberrations



BCR::ABL1: past and ongoing insights

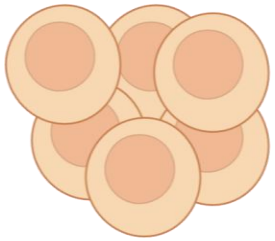


BCR::ABL1 in leukemogenesis

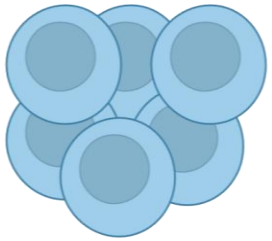


CHRONIC MYELOID LEUKEMIA

- $p210^{BCR::ABL1}$ > $p190^{BCR::ABL1}$
- Excellent prognosis to TKI
- CML-CP → AP → BC My > Ly



ACUTE LYMPHOBLASTIC LEUKEMIA



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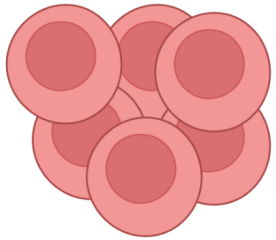
BCR::ABL1 in leukemogenesis

- ~1.5% AML
- Cryptic deletions within the Ig and TCR: differential diagnosis for CML
- Poor prognosis



ACUTE MYELOID LEUKEMIA

Hematopoietic stem cell

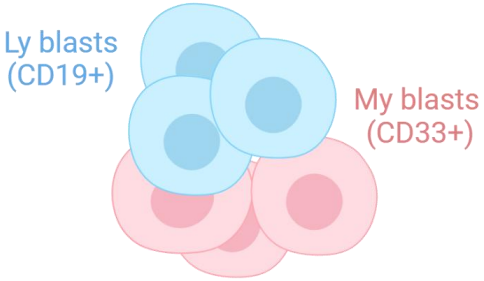
Multipotent progenitor

BCR::ABL1 **transcript type**

Myeloid progenitor

Lymphoid progenitor

- 7%–35% of adult MPAL
- 3% of pediatric MPAL
- No differences in *BCR::ABL1* transcripts
- Treatment outcome is poor, but improved with TKI



MIXED PHENOTYPE ACUTE LEUKEMIA

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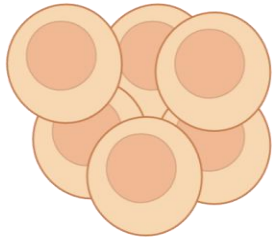

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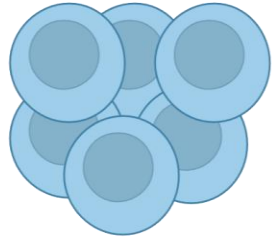

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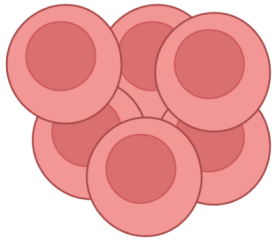

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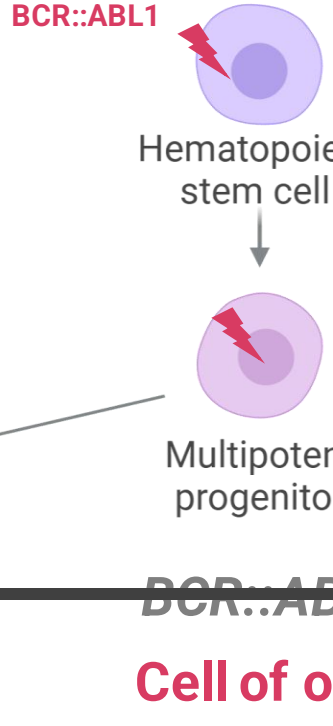

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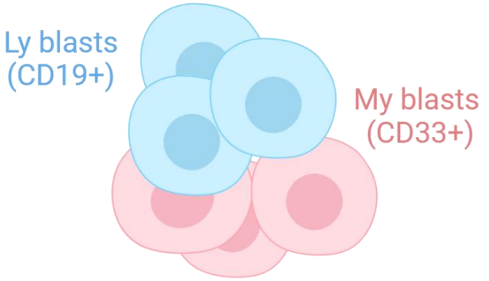

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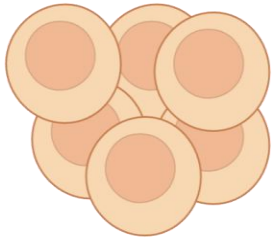

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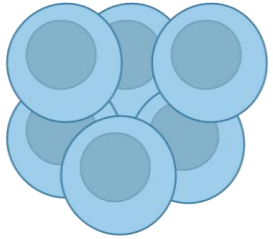

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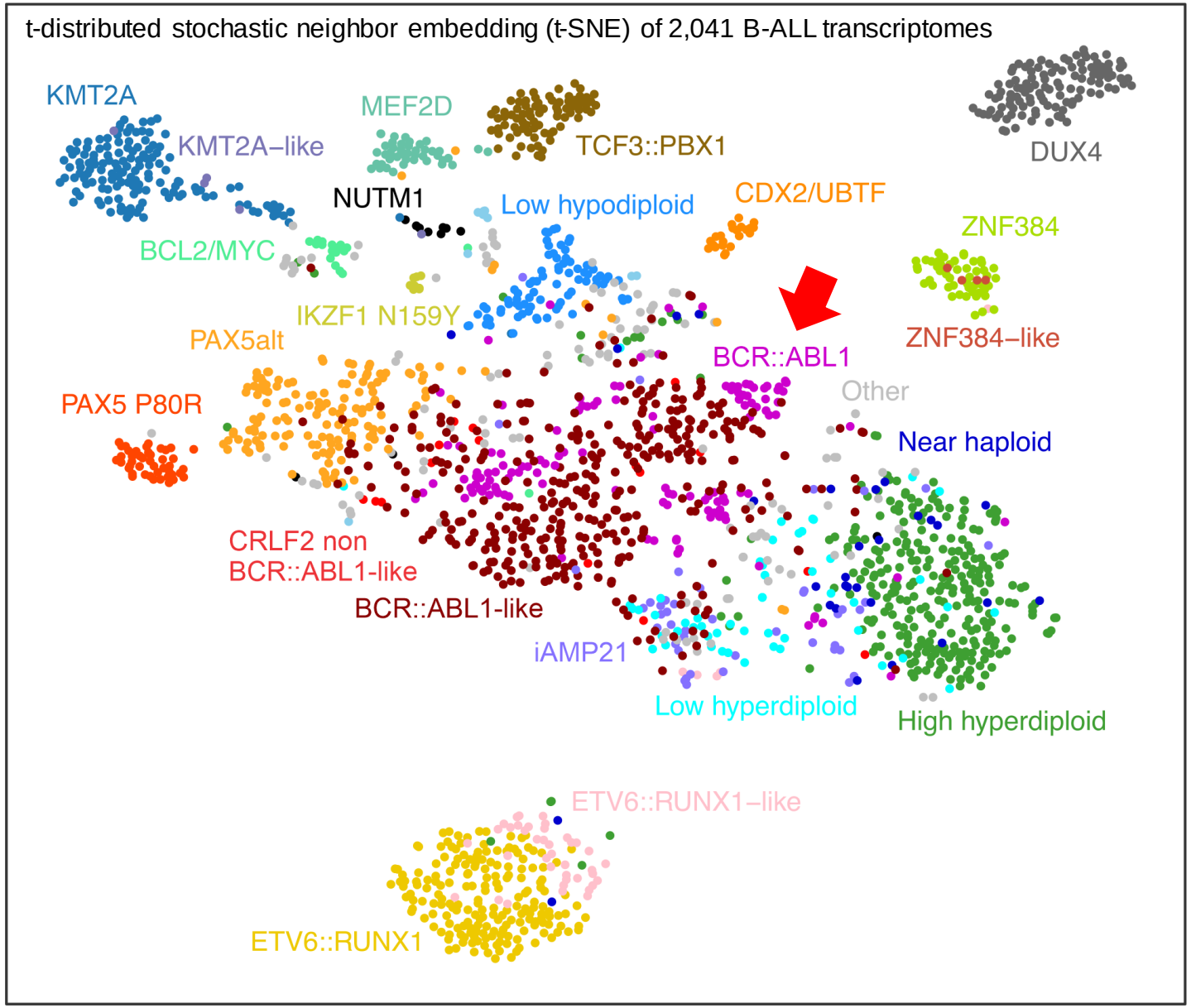
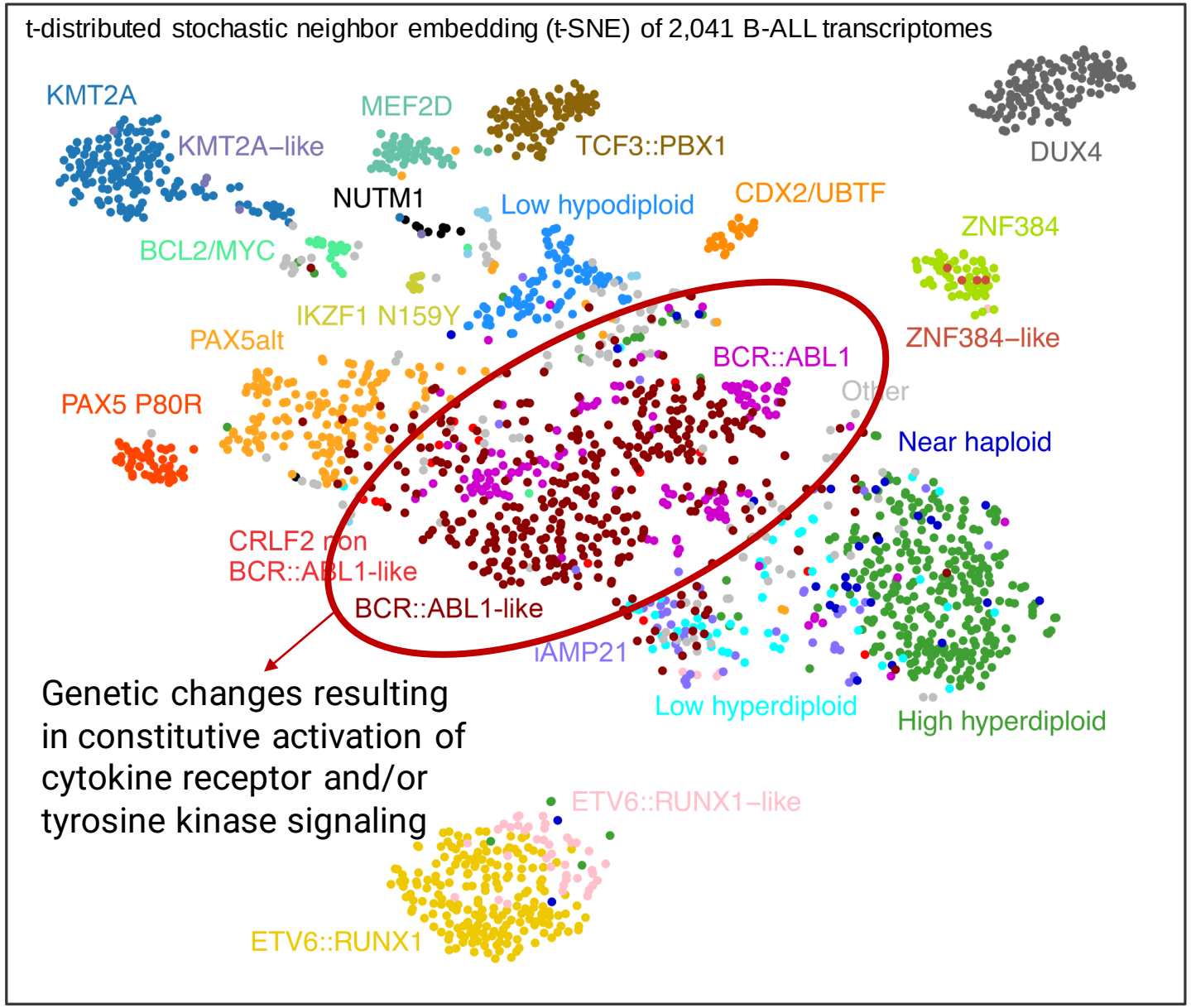
BCR::ABL1 ALL has a distinct gene expression profile



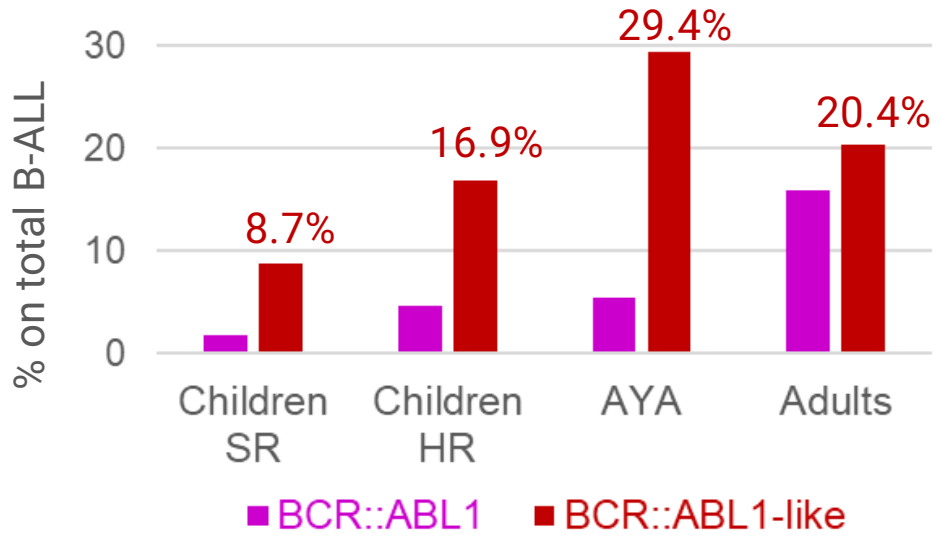
Gu Z et al. *Nat Genet* 2019; Kimura et al *Blood* 2022; Arber D et al *Blood* 2022; Duncavage E et al *Blood* 2022; Alaggio R et al *Leukemia* 2022; Brady S et al. *Nat Genet* 2022

BCR::ABL1/ -like B-ALL



- *BCR::ABL1*-positive and *BCR::ABL1*-like B-ALL share similar gene expression profile

- Prevalence increases with age



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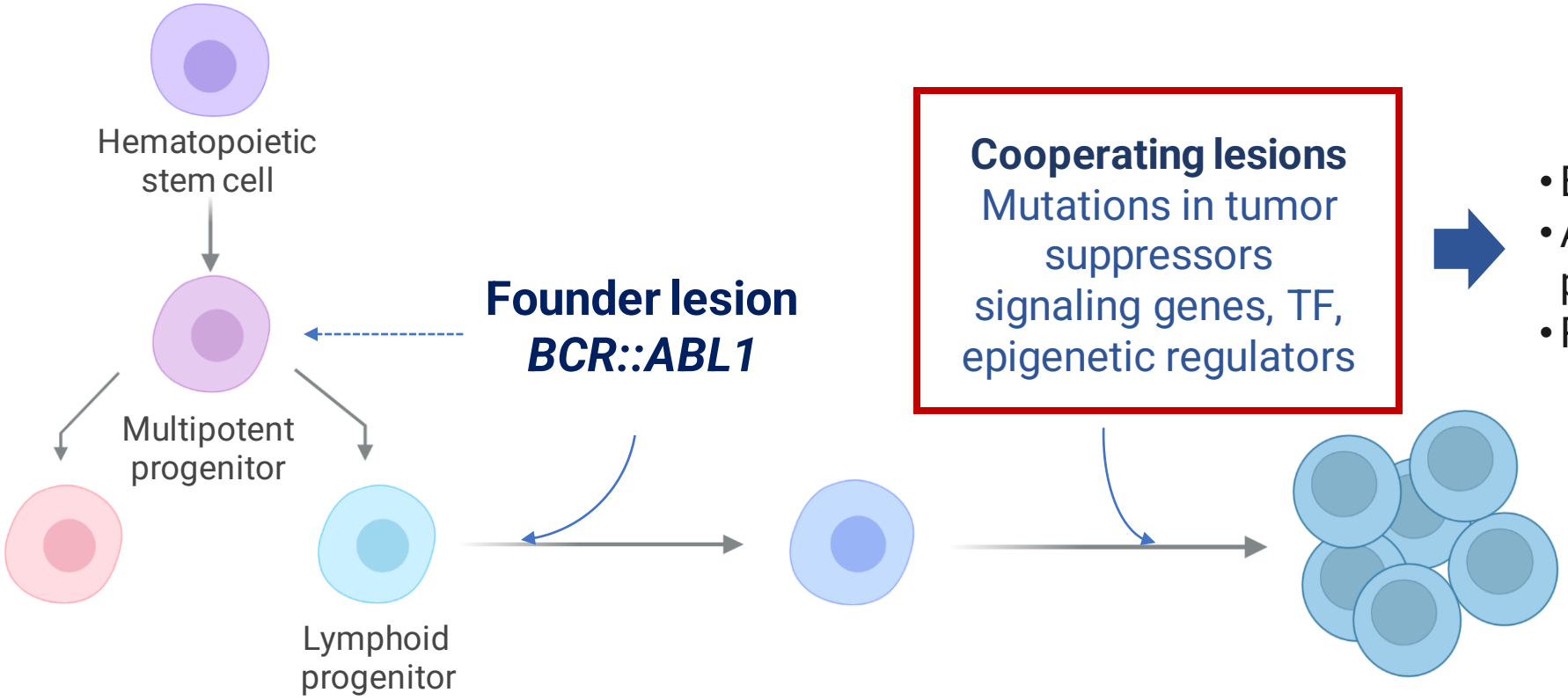
BCR::ABL1 ALL evolution



Founder lesion
BCR::ABL1

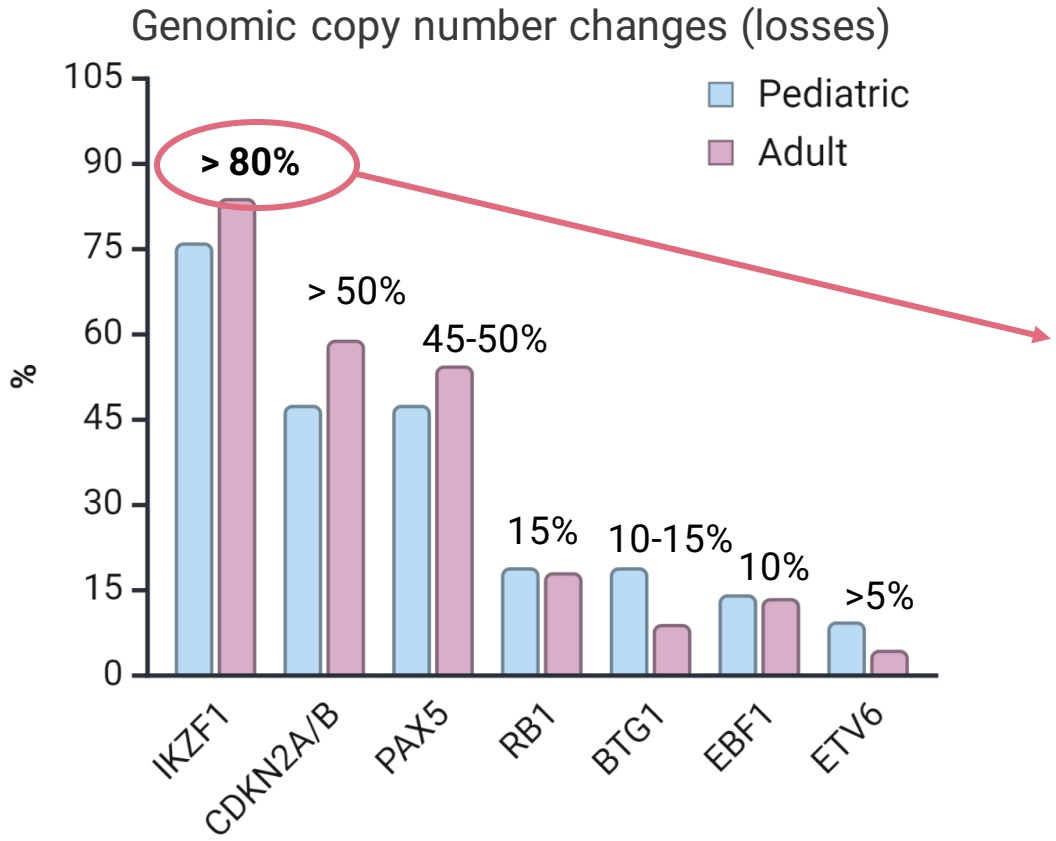
Cooperating lesions
Mutations in tumor suppressors signaling genes, TF, epigenetic regulators

- Block of lymphoid differentiation
- Activation of multiple cellular pathways
- Risk stratification

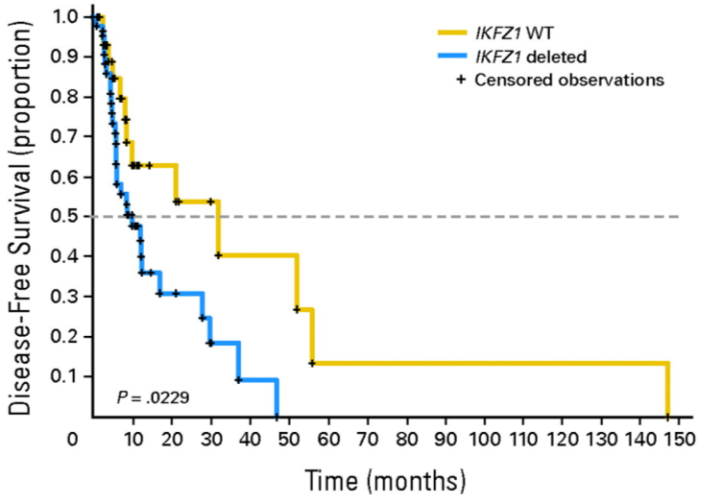
Acute lymphoblastic leukemia

Genomic landscape of *BCR::ABL1* B-ALL

- DNA copy number losses in **lymphoid transcription factors** (*IKZF1*, *PAX5*, *EBF1*) and in **tumor suppressors** (*CDKN2A/B* and *BTG1*) are common
- ***IKZF1* losses** occur in 15% of all B-ALL cases, but **> 80% of Ph+ ALL**

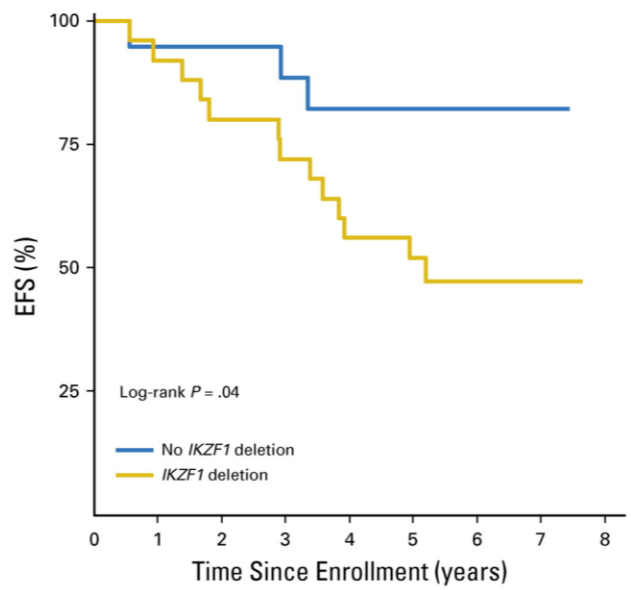


Italian Cohort (GIMEMA) - Adults



Martinelli G, Iacobucci I et al. *JCO* 2009

COG AALL0622 - Pediatric

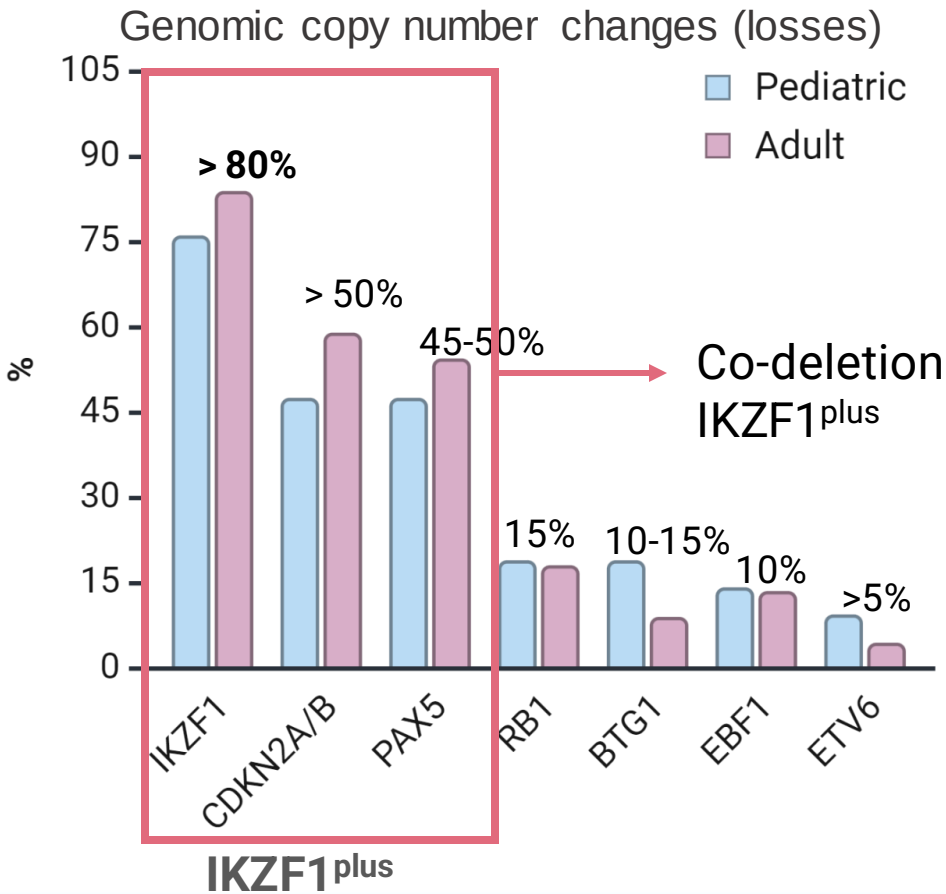


Slayton WB et al. *JCO* 2018

IKZF1 deletions confer poor outcome

Mullighan CG et al. *NEJM* 2008; Iacobucci I et al. *Blood* 2009; Iacobucci I et al. *Hematologica* 2010; Iacobucci I et al. *Clin Cancer Res.* 2011; Brady S et al. *Nature Genetics* 2022

Genomic landscape of *BCR::ABL1* B-ALL

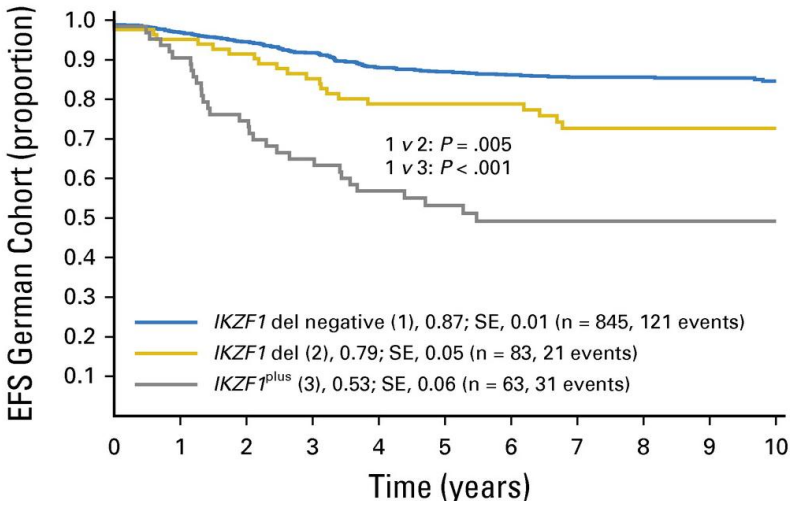


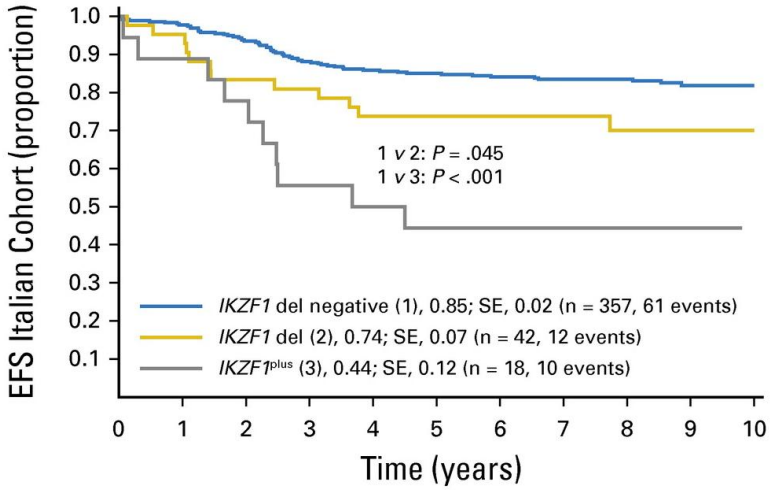
Co-deletion of *IKZF1* with *PAX5* or *CDKN2A/B* defines IKZF1^{plus}

Prognostic implications of *IKZF1*^{plus}

Pediatric *BCR::ABL1* B-ALL

AIEOP-BFM ALL 2000 (Chemotherapy)

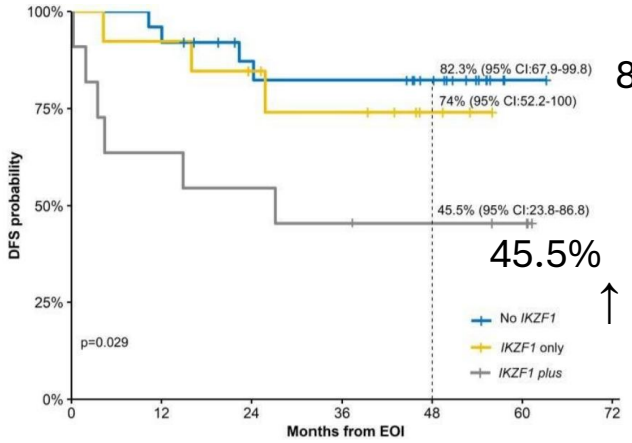




Stanulla M et al. *JCO* 2018

Adult *BCR::ABL1* B-ALL

GIMEMA LAL2116 D-ALBA (Dasatinib-Blinatumomab)



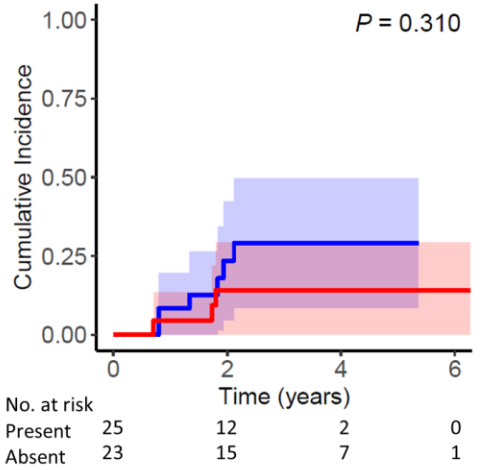
82.3%

45.5%

↑ T315I mutation

Foa R. et al. *N Engl J Med*. 2020: Foa R et al. JCO 2023

Ponatinib-Blinatumomab[1,2]

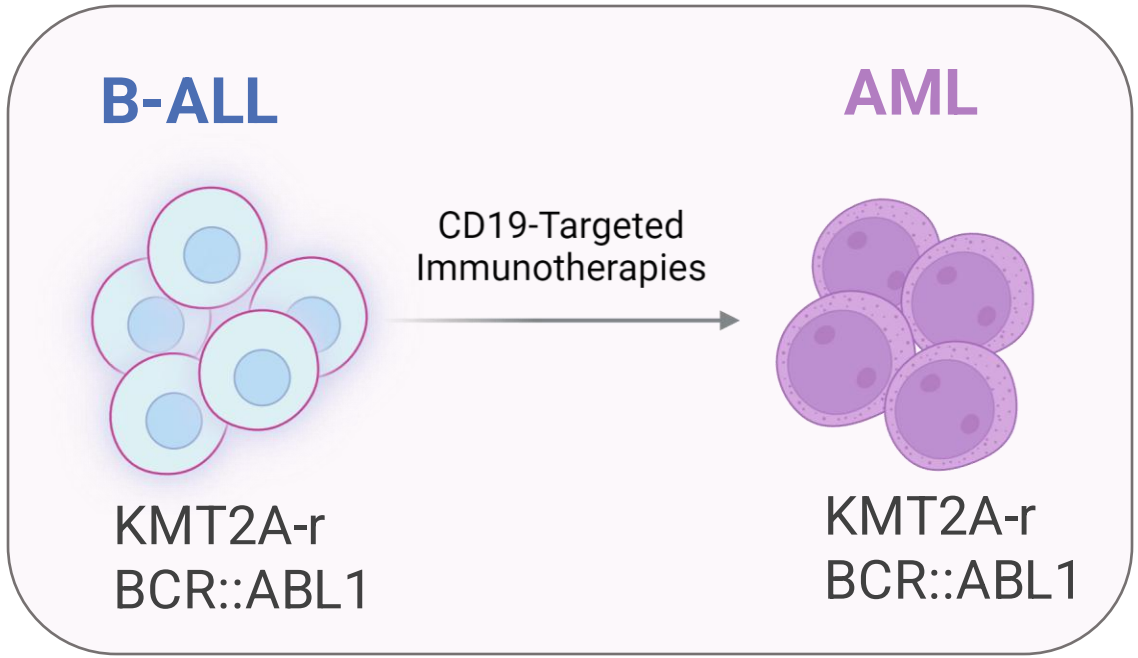


High WBC ($\geq 70 \times 10^9$/L): strongest prognostic factor

[1]Short N.. et al. *J Hematol Oncol* 2025; [2] Plus intrathecal chemotherapy

Lymphoid to myeloid lineage switch

Plasticity & heterogeneity in B-ALL cell states may play a role in determining vulnerability to therapeutic treatments and promote ly → my lineage switch

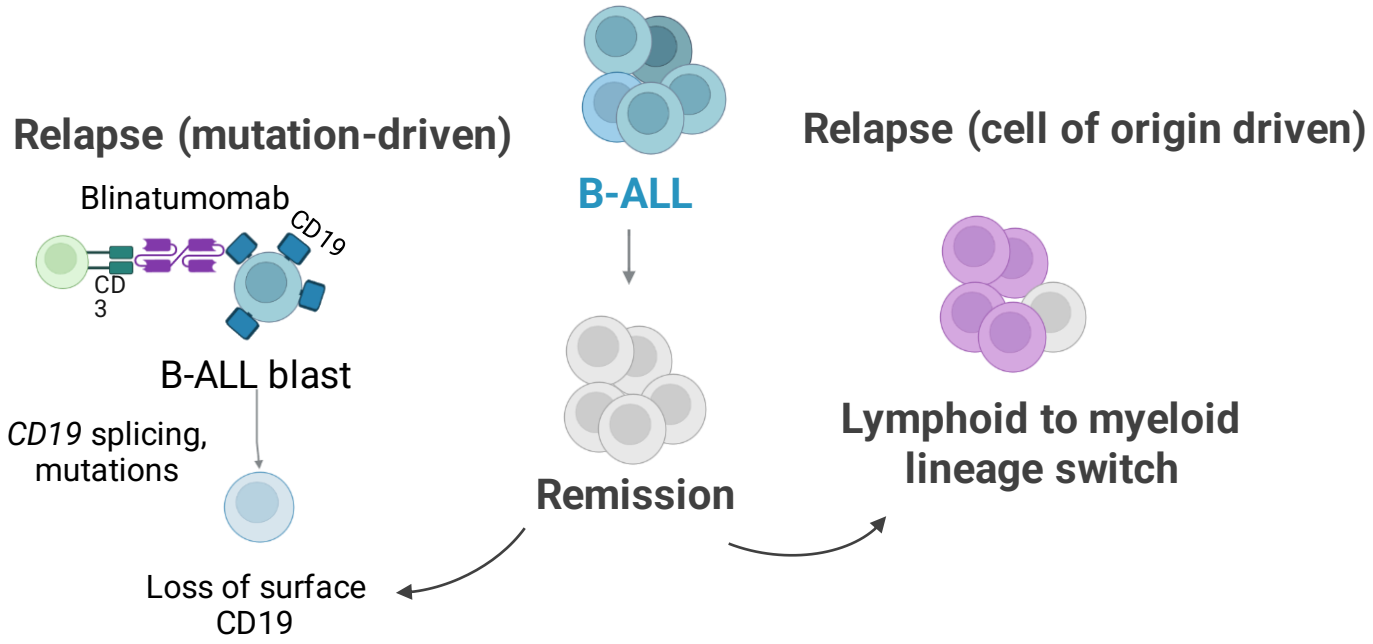


Tirtakusuma R et al. *Blood* 2022; Iacobucci I et al. *Blood* 2022; Lee et al. *Am J Hematol* 2022; Haddox et al. *Blood Cancer J* 2017; Rossi et al. *Am J Hematol* 2012; Novakova M et al. *Haematologica* 2021

Anti-CD19 therapy's resistance

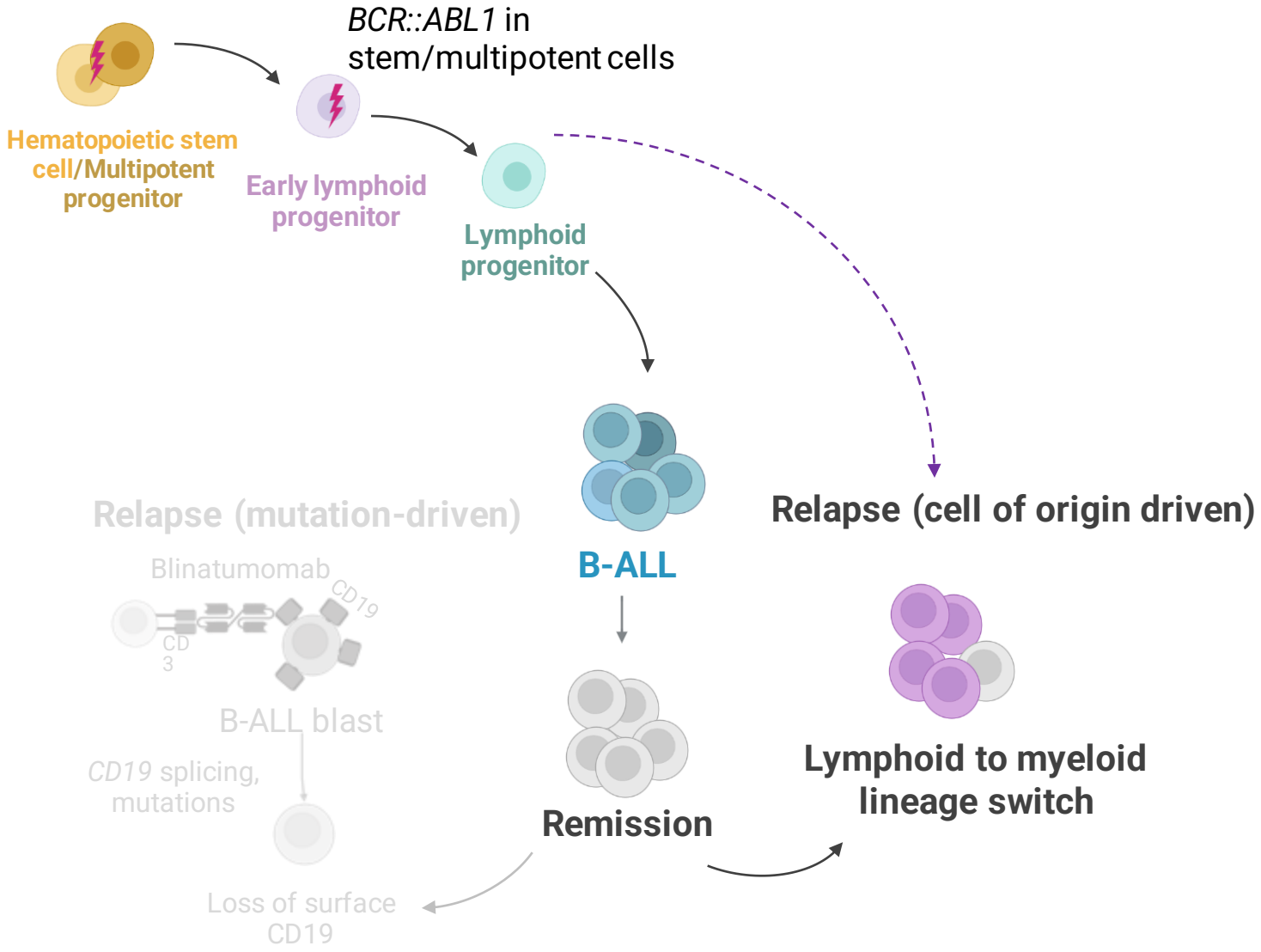
Loss of CD19 expression

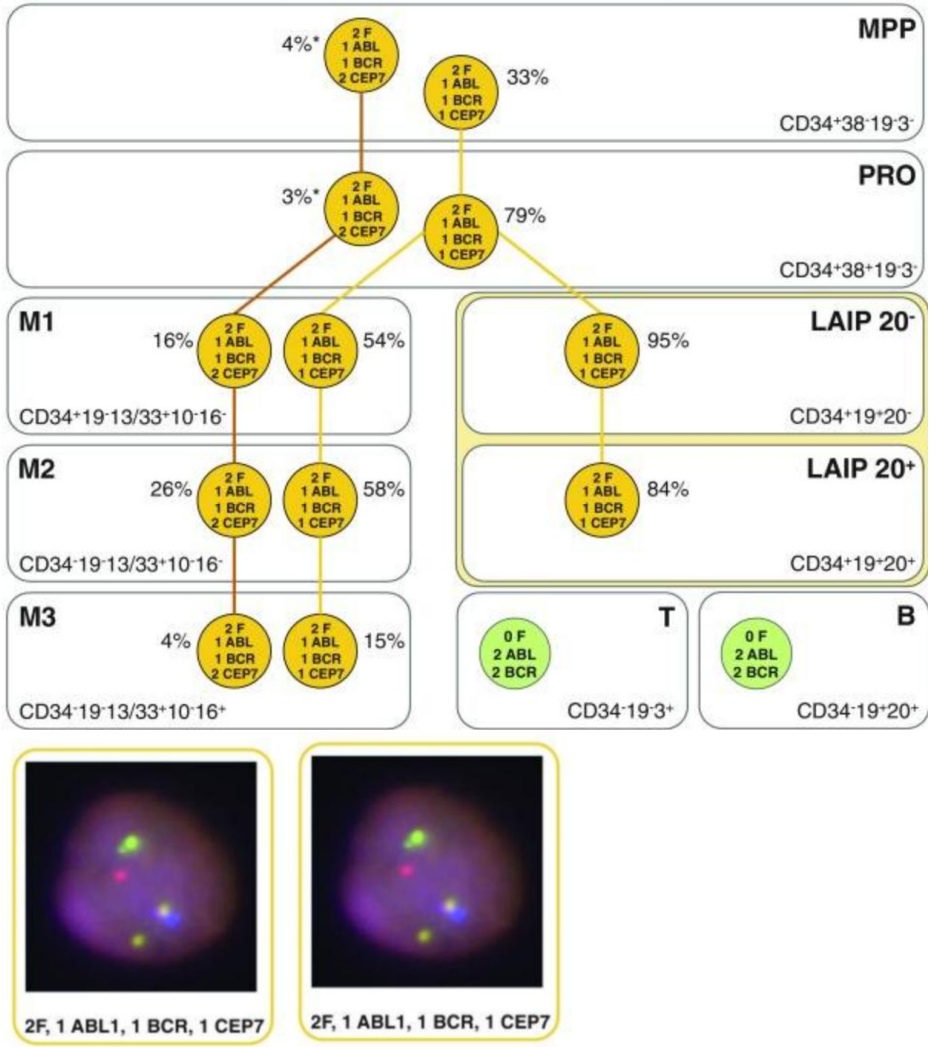
Lineage switch



Zhao et al. *Blood* 2021

Anti-CD19 resistance can “stem” from progenitors





Shah N et al *Blood* 2017; Nagel et al. *Blood* 2017

Zhao et al. *Blood* 2021

Post-Immunotherapy Lineage Switch in ALL

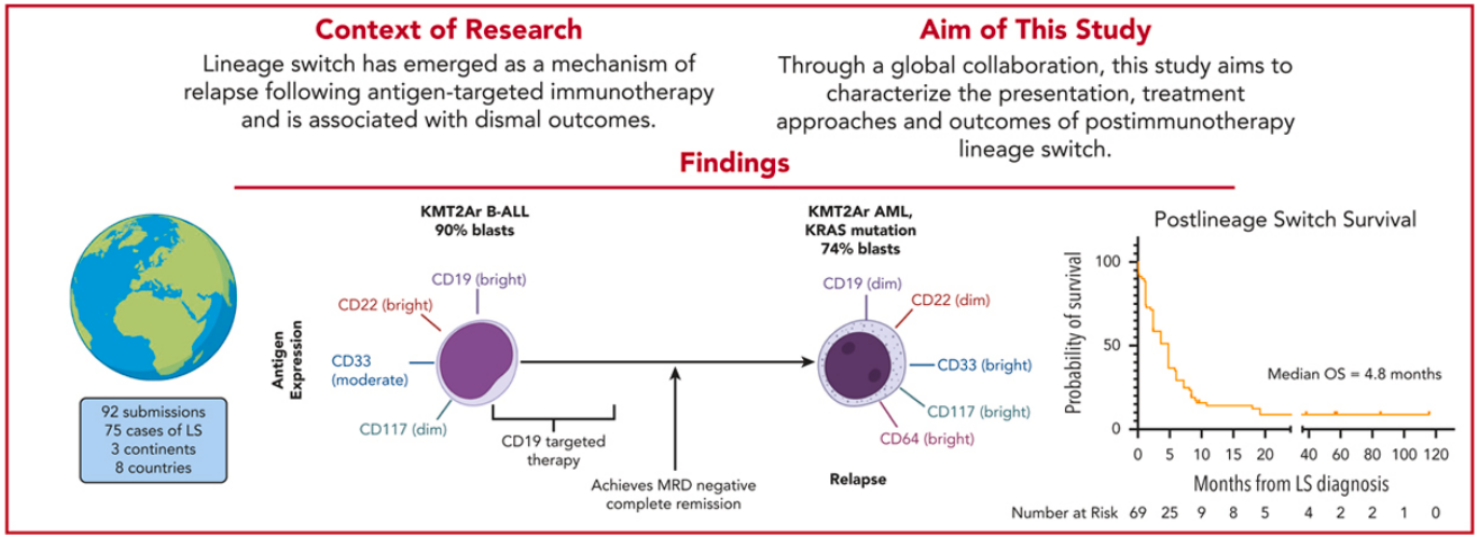
blood — Regular Article

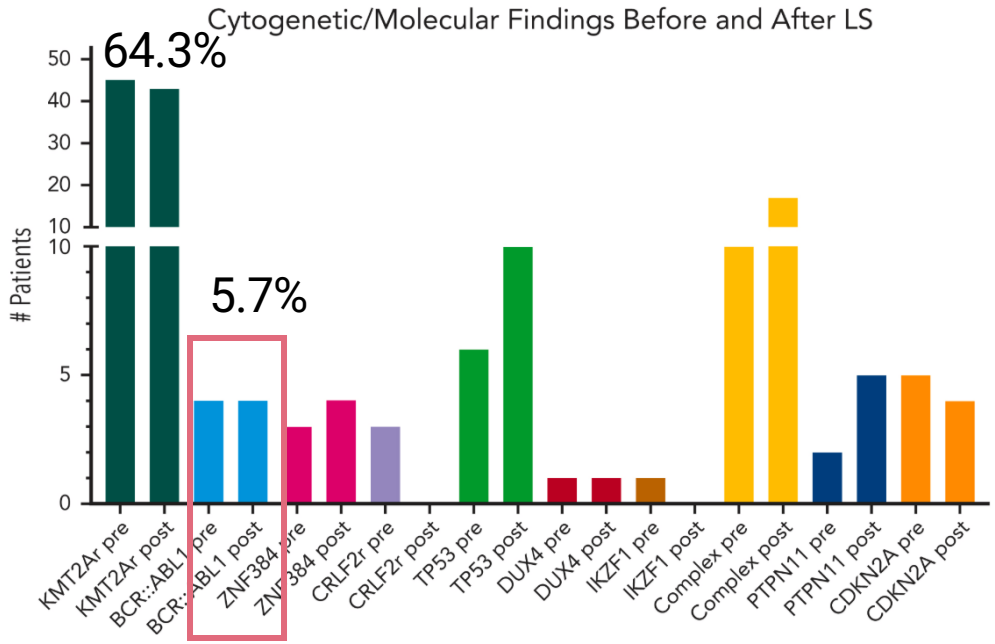
IMMUNOBIOLOGY AND IMMUNOTHERAPY

Project EVOLVE: an international analysis of postimmunotherapy lineage switch, an emergent form of relapse in leukemia

- B-ALL transforming to AML or MPAL
- Outcomes were uniformly poor, with remission rates under 40% and median survival of only 4.8 months
- Need for strategies to address and predict post-immunotherapy relapses

75 cases of ALL lineage switch post immunotherapy

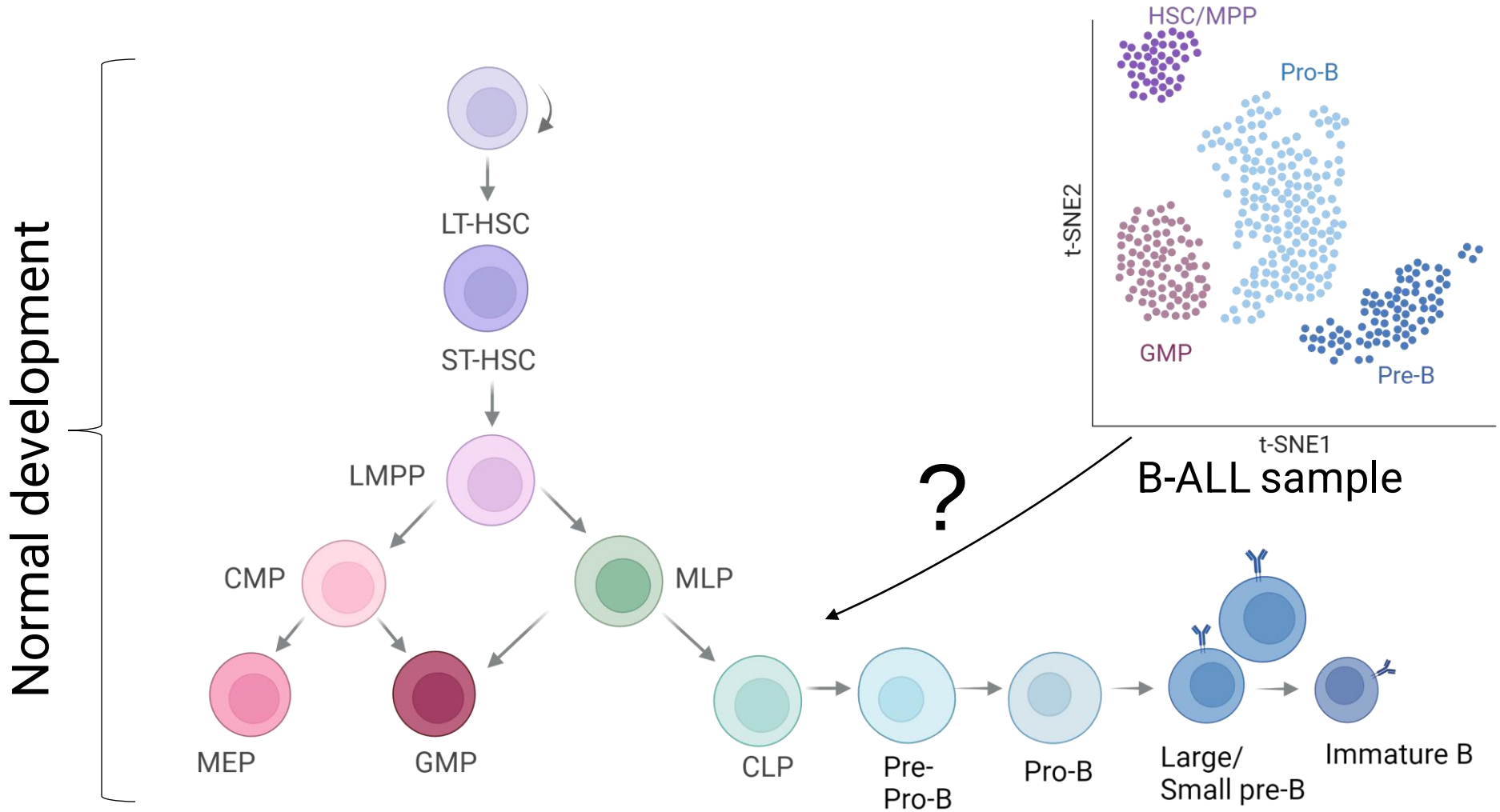




Silbert A et al. *Blood* 2025

Dissection of ALL cell composition

Challenge: inconsistent classification of normal B cells



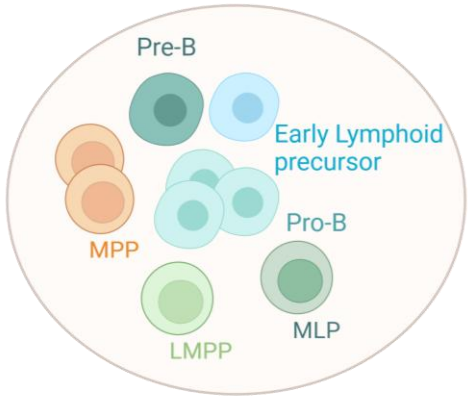
Single-cell cross-ontogeny map of B cell development

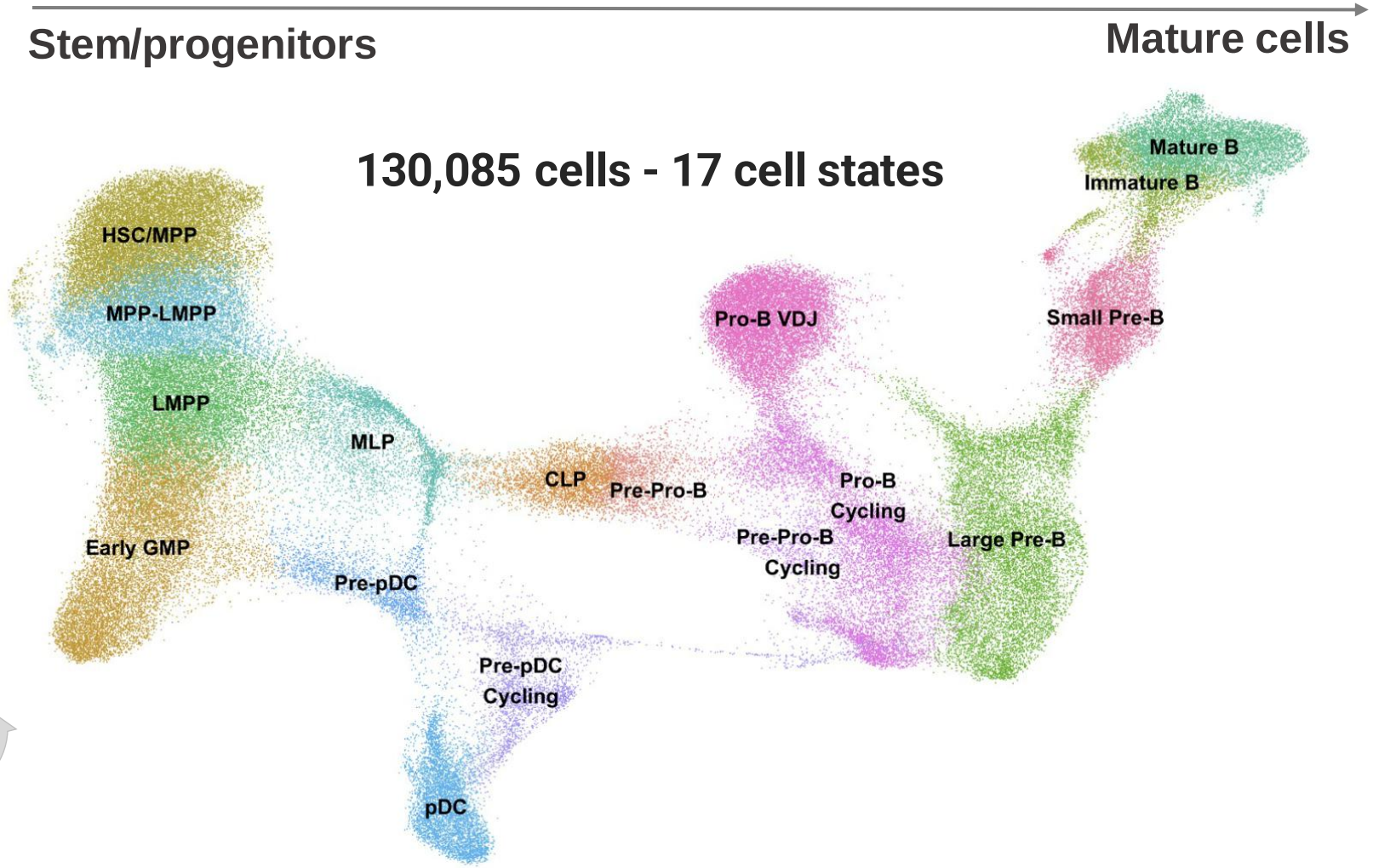
Normal scRNAseq/CITE-seq

Tissue	N cells
Fetal Bone Marrow	39,680
Fetal Liver	20,944
Cord Blood	5,680
Pediatric Bone Marrow	5,870
Adult Bone Marrow	57,911

90 unique donors, **5** tissues

Data from sorted populations

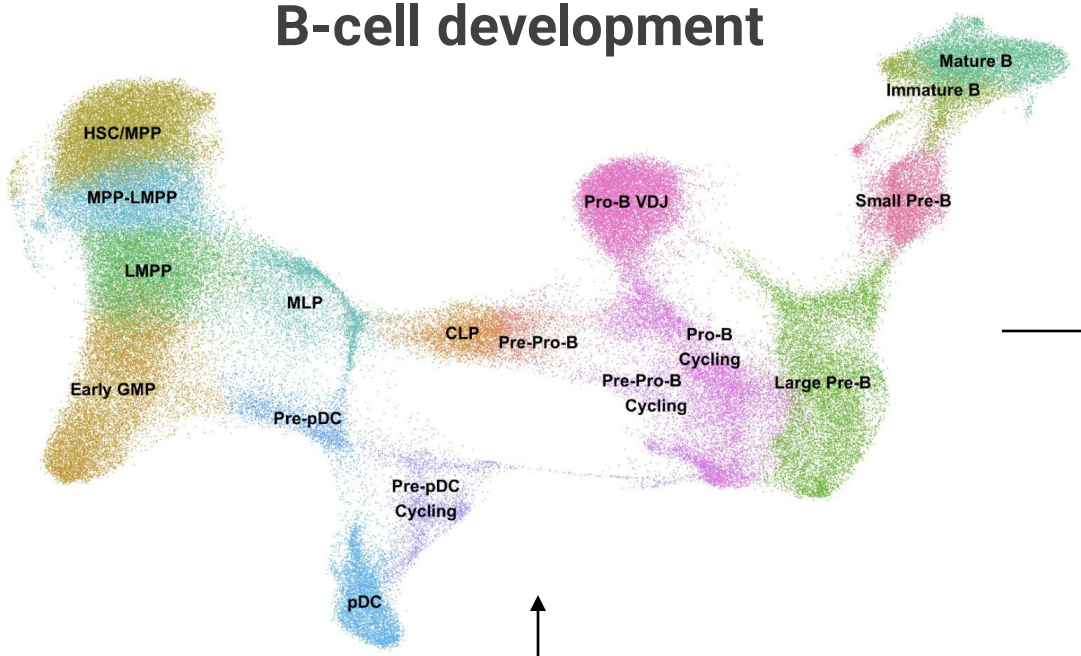




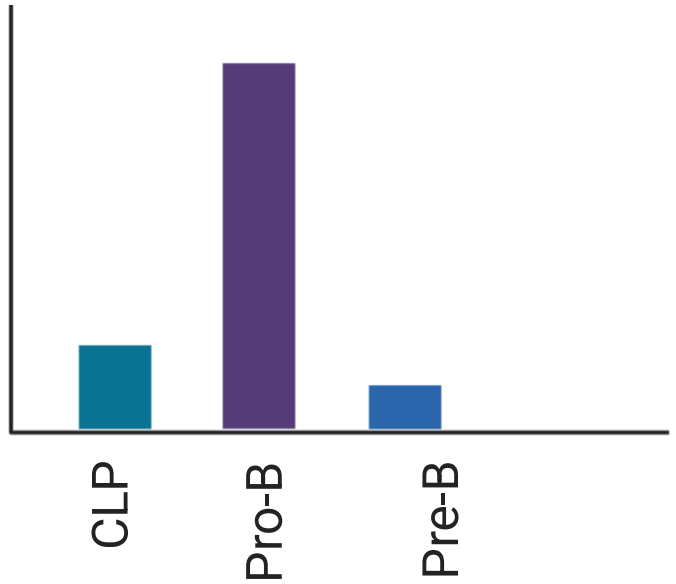
Iacobucci I et al. *Nature Cancer* 2025

Developmental cell states of B-ALL

Normal B-cell development



N=89 B-ALL scRNA-seq samples



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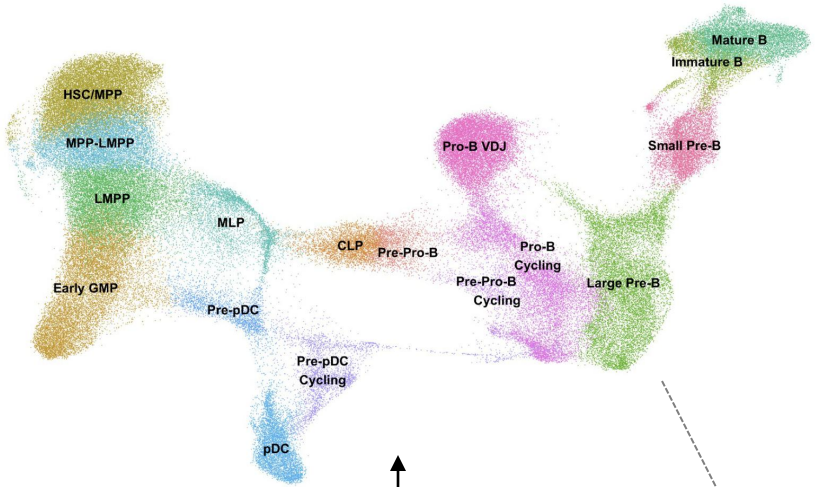
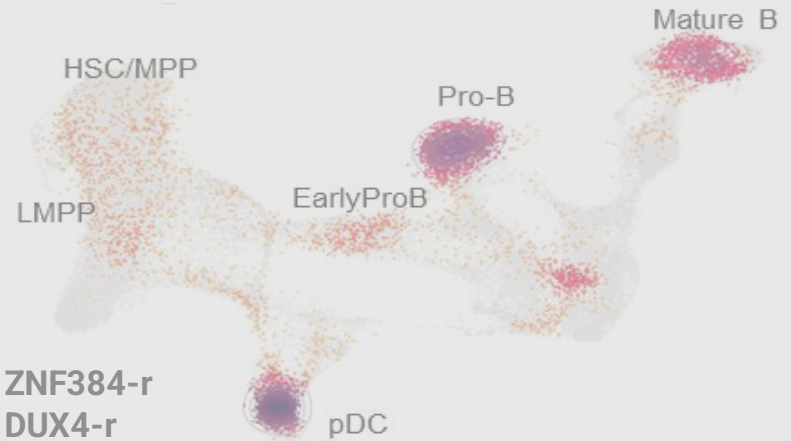


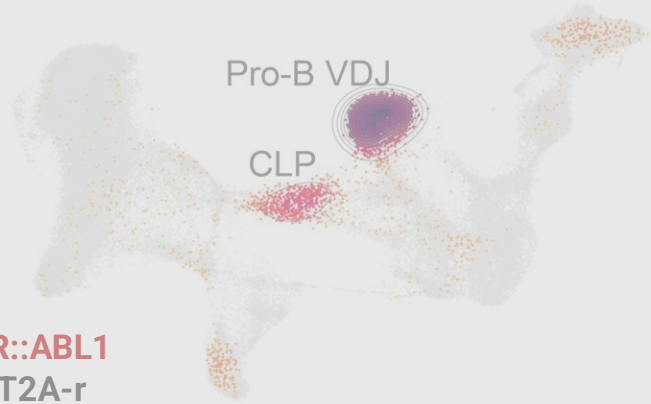
N=89 B-ALL scRNA-seq samples

HSC/MPP/LMPP Enriched



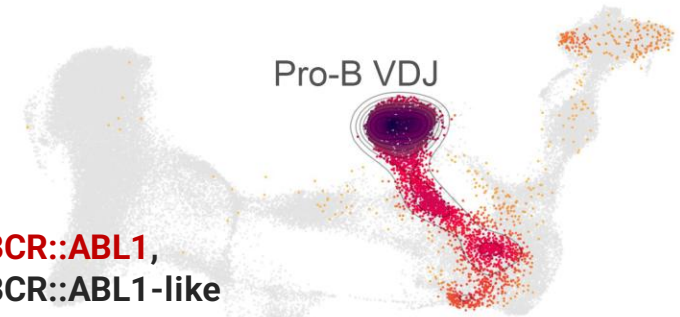
ZNF384-r
DUX4-r

Early Lymphoid Enriched



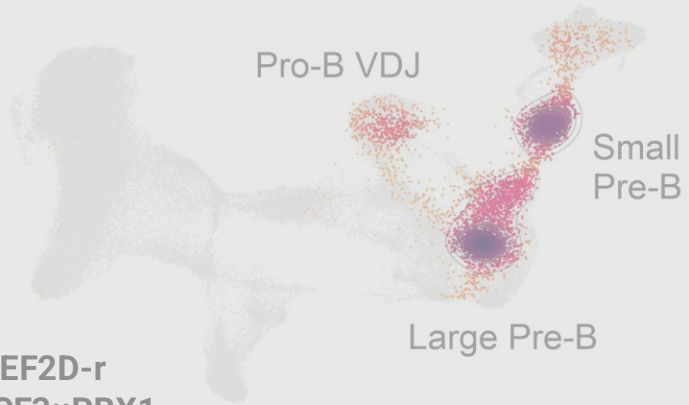
BCR::ABL1
KMT2A-r

Pro-B Enriched



BCR::ABL1,
BCR::ABL1-like
ETV6::RUNX1-like,
hyperdiploid, near-haploid,
iAMP21, PAX5alt

Pre-B Enriched

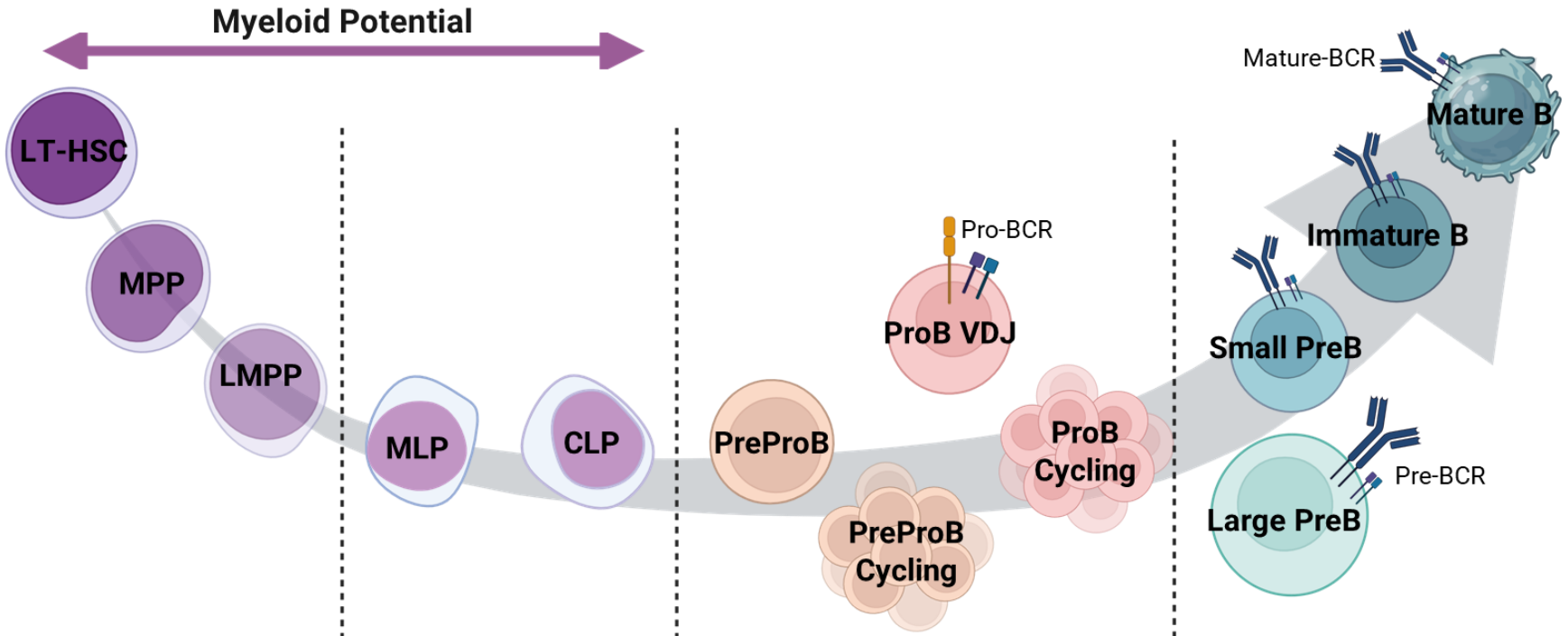


MEF2D-r
TCF3::PBX1

B cell development types of B-ALL

Iacobucci I et al. *Nature Cancer* 2025

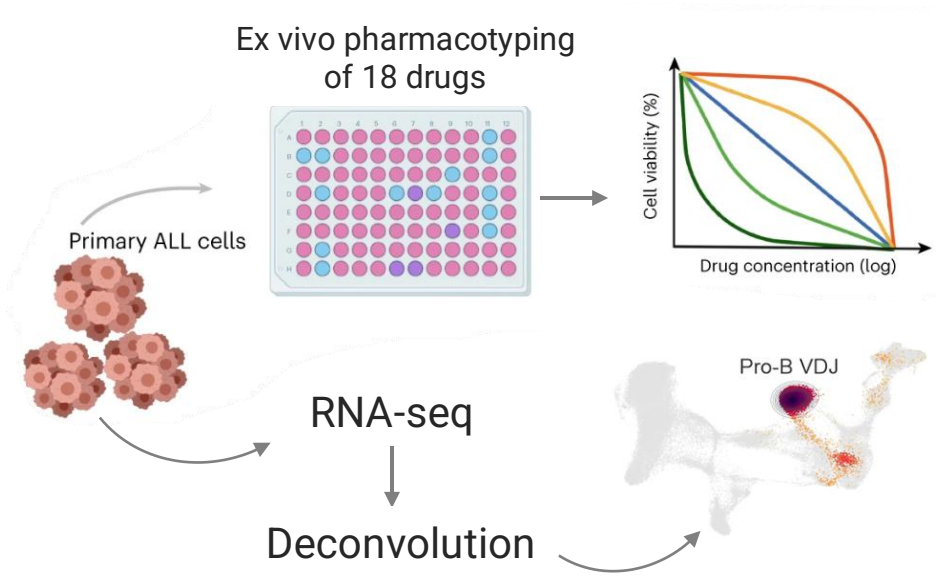
Heterogeneity in B-ALL is driven by developmental states

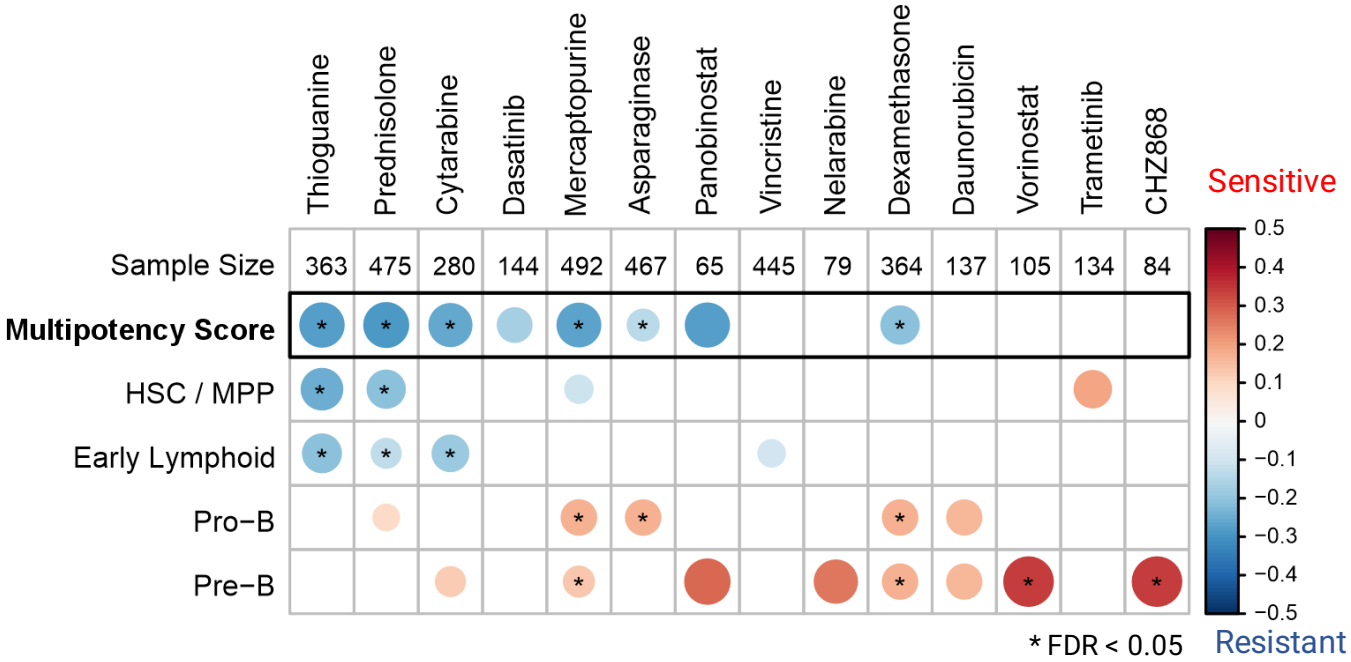


Iacobucci I et al. *Nature Cancer* 2025

Developmental state can influence drug sensitivity

- B-leukemic cells may have different chemosensitivity
- *Ex vivo* drug sensitivity to 18 therapeutic agents in 595 B-ALL samples profiled by bulk RNA-seq[1]





- Chemosensitivity of Pro-B and Pre-B cells and chemo-resistance of Early Lymphoid cells

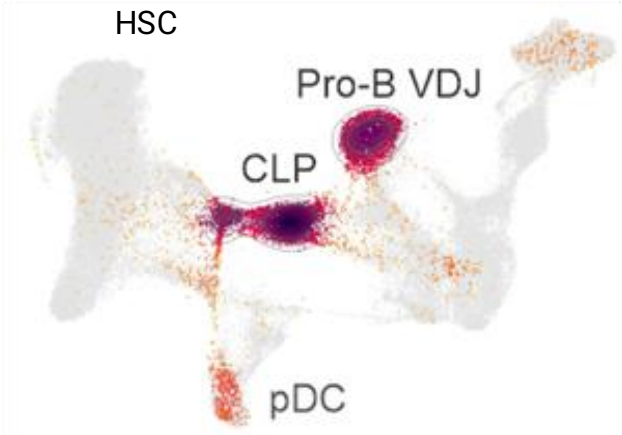
[1] Lee, S.H.R., *et al. Nat Med* 2023

Developmental state refines *BCR::ABL1* ALL

Myeloid potential

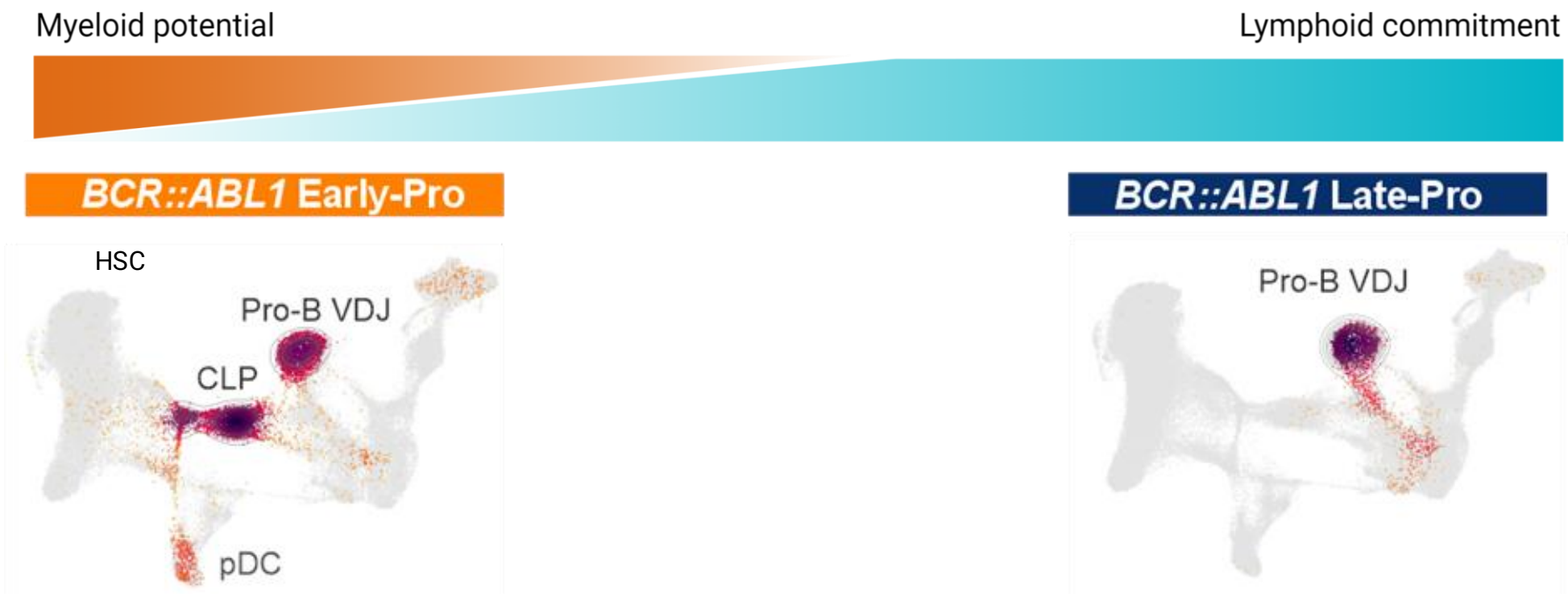
Lymphoid commitment

***BCR::ABL1* Early-Pro**



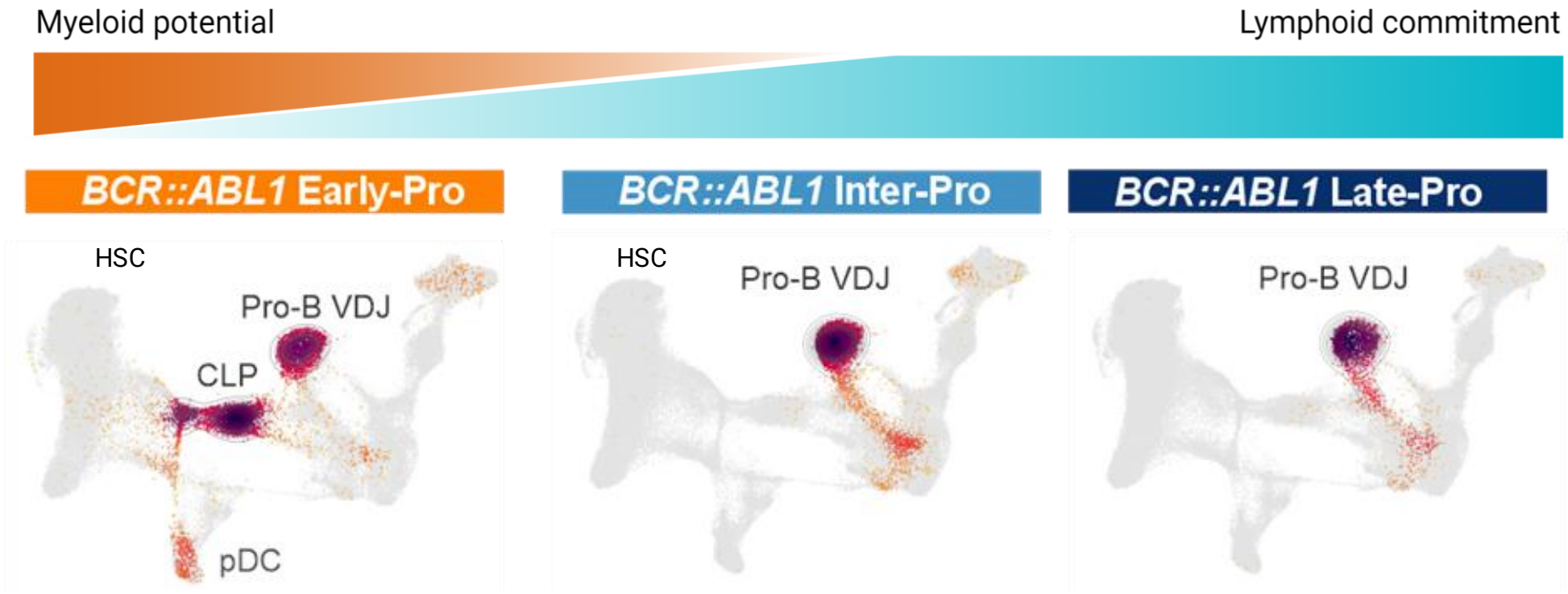
Iacobucci I et al. *Nature Cancer* 2025

Developmental state refines *BCR::ABL1* ALL



Iacobucci I et al. *Nature Cancer* 2025

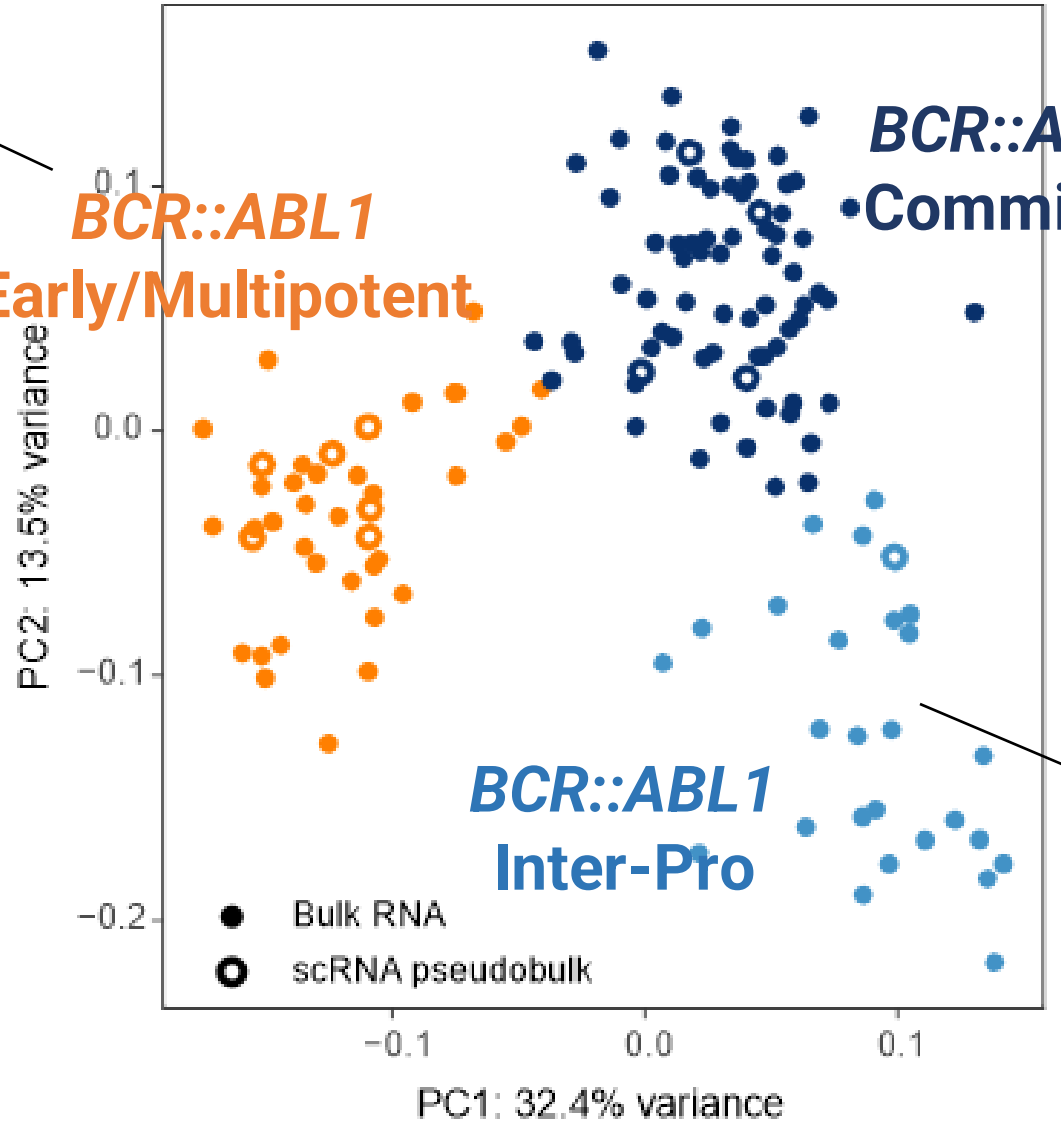
Developmental state refines *BCR::ABL1* ALL



Iacobucci I et al. *Nature Cancer* 2025

Transcriptomic classes of *BCR::ABL1* ALL

Aberrant expression of stem and myeloid lineage genes (e.g. *KIT*, *MECOM*, *CEBPA*)



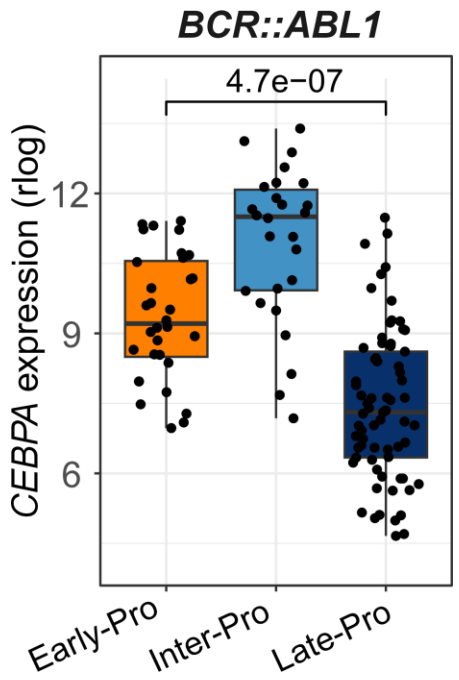
Highest expression of genes associated with B-cell differentiation, such as *IL7R*, *MS4A1*, *BACH2* and *TCL1A*

Expression of both myeloid (*CSF2RA* and *CSF1R*) and lymphoid genes (*MS4A1/CD20* and *IL7R*)

Kim et al. *Nat Genetics* 2023

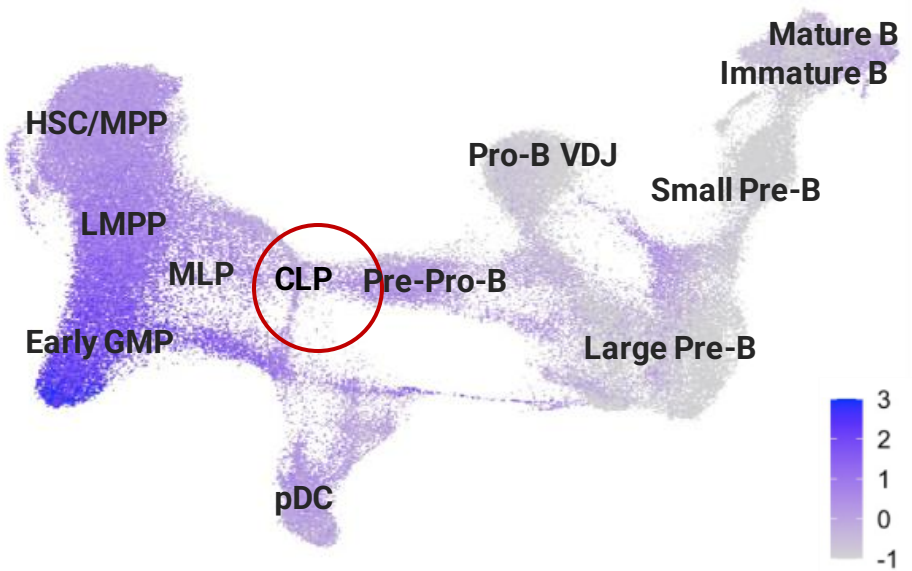
CEBPA expression in Early Lymphoid samples

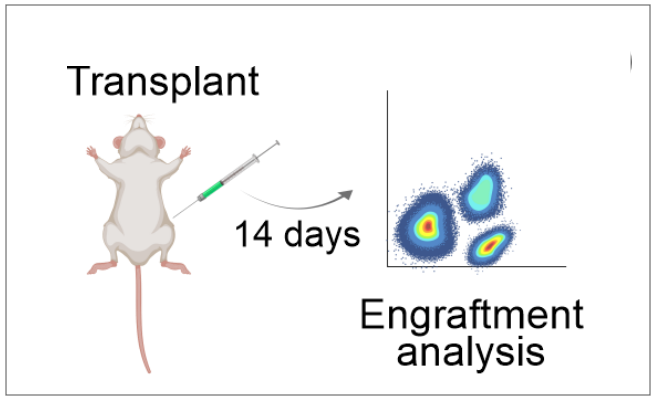
B-ALL with early lymphoid abundance expresses myeloid signatures ⟶ latent myeloid potential



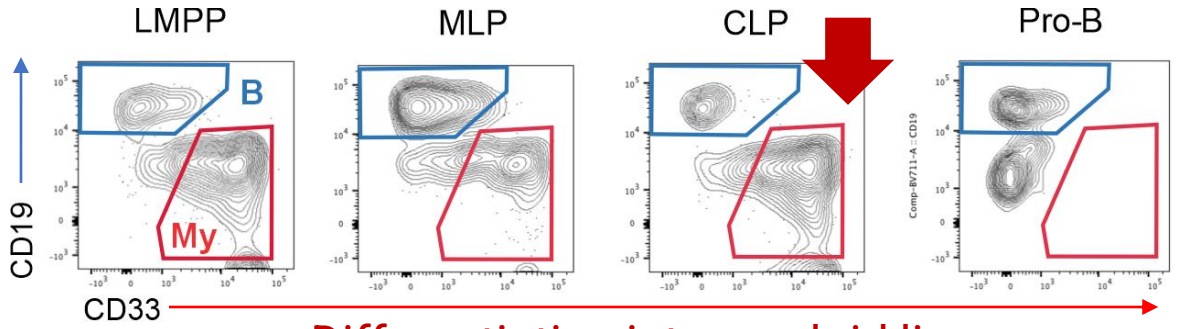
CEBPA regulon activity

Persistent expression and activity of CEBPA into CLP stage





In vivo xenotransplantation assay of sorted population

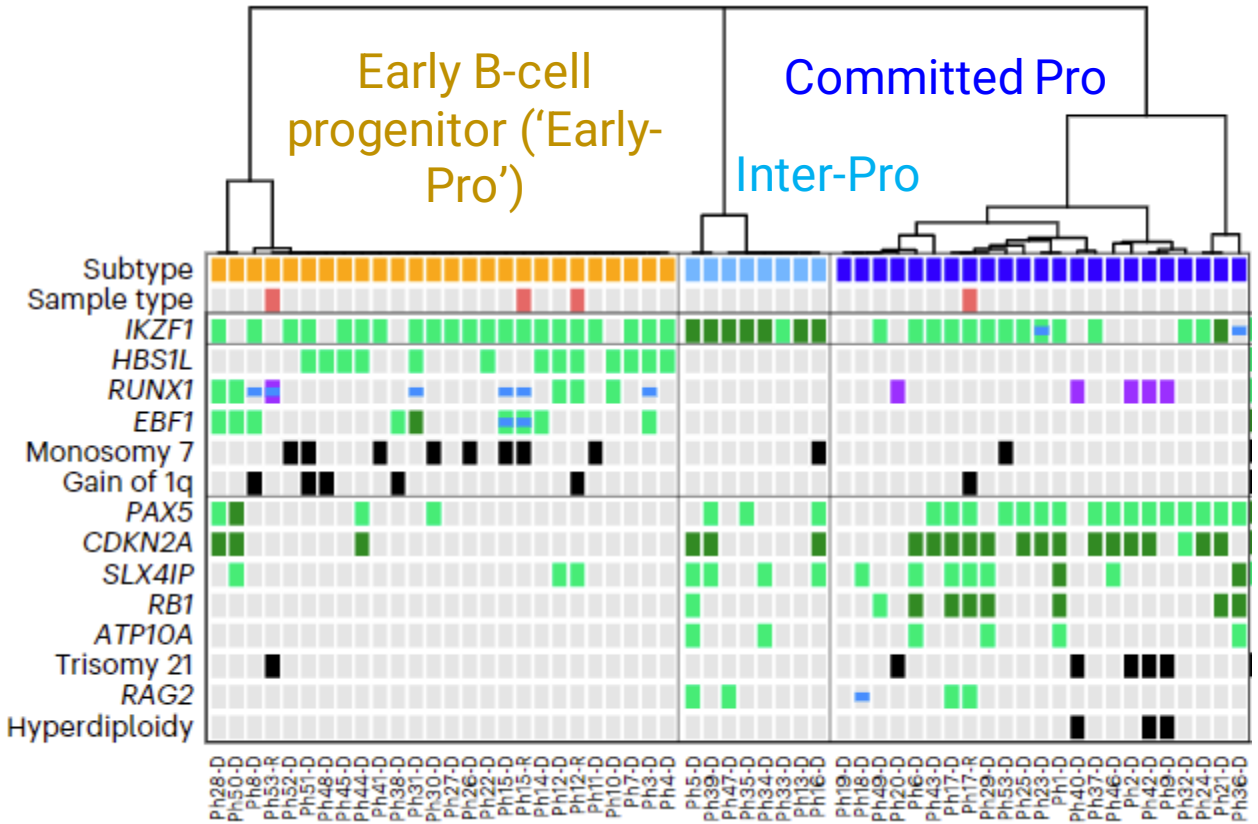


Differentiation into myeloid lineage

Iacobucci I et al. *Nature Cancer* 2025

Transcriptomic classes of *BCR::ABL1* ALL

- 96.2% of leukemias harbored alterations in genes that regulate normal lymphoid differentiation
- Distinct genetic alterations define each molecular subtype



EBF1 del; *IKZF1 het* del; *HBS1L deletions; RUNX1* mut; monosomy 7

IKZF1 bi-allelic del

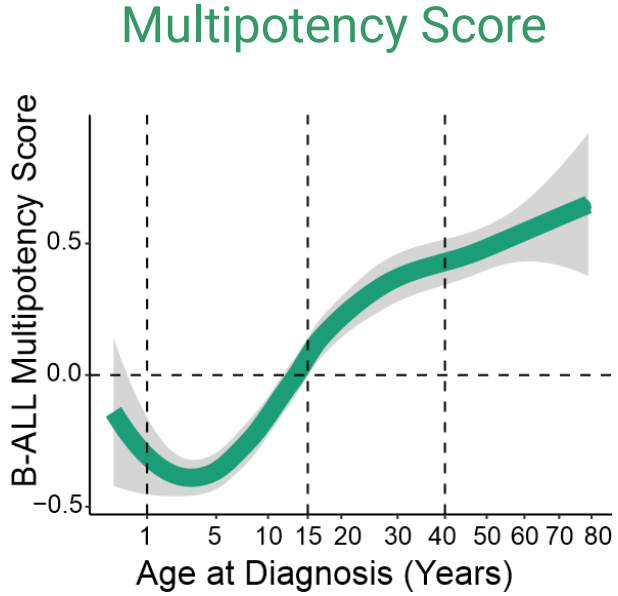
IKZF1 monoallelic; *CDKN2A/B* del; *PAX5* del; *RB1* del

Kim et al. *Nat Genetics* 2023; Iacobucci I et al. *Nature Cancer* 2025

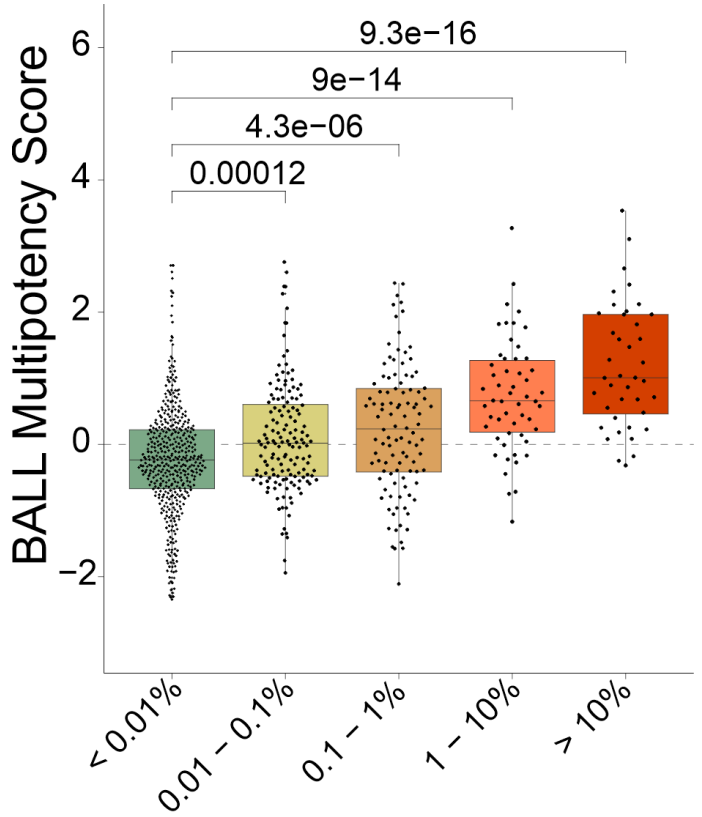
Multipotency and clinical correlations with outcome

↑ Multipotency Score

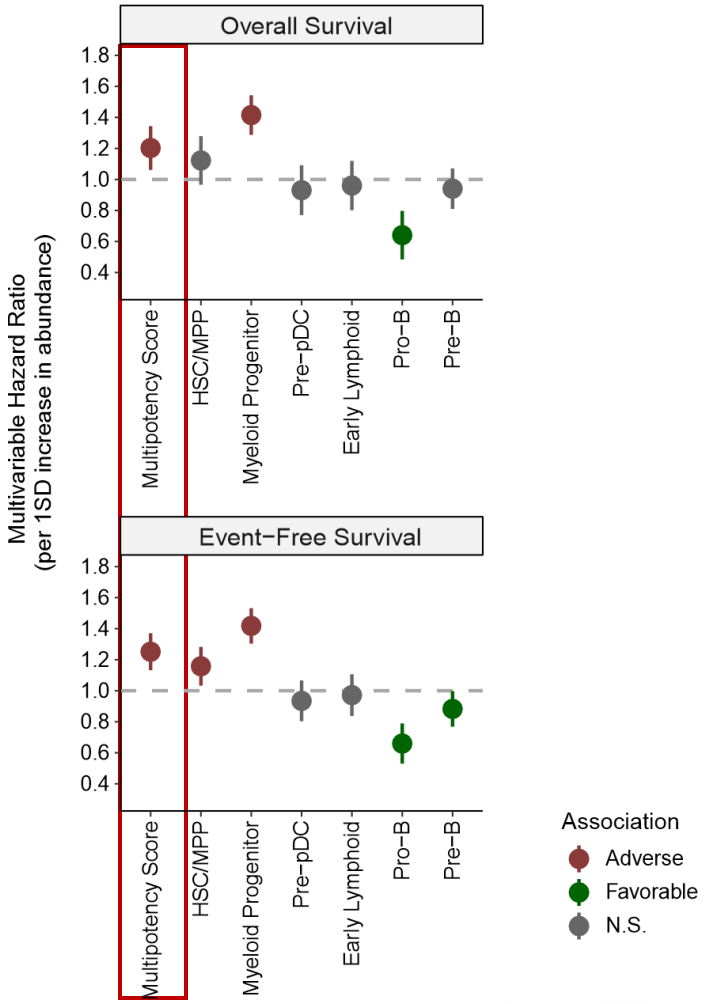
Age at diagnosis:
infancy/adulthood



MRD at day 29:
High MRD levels

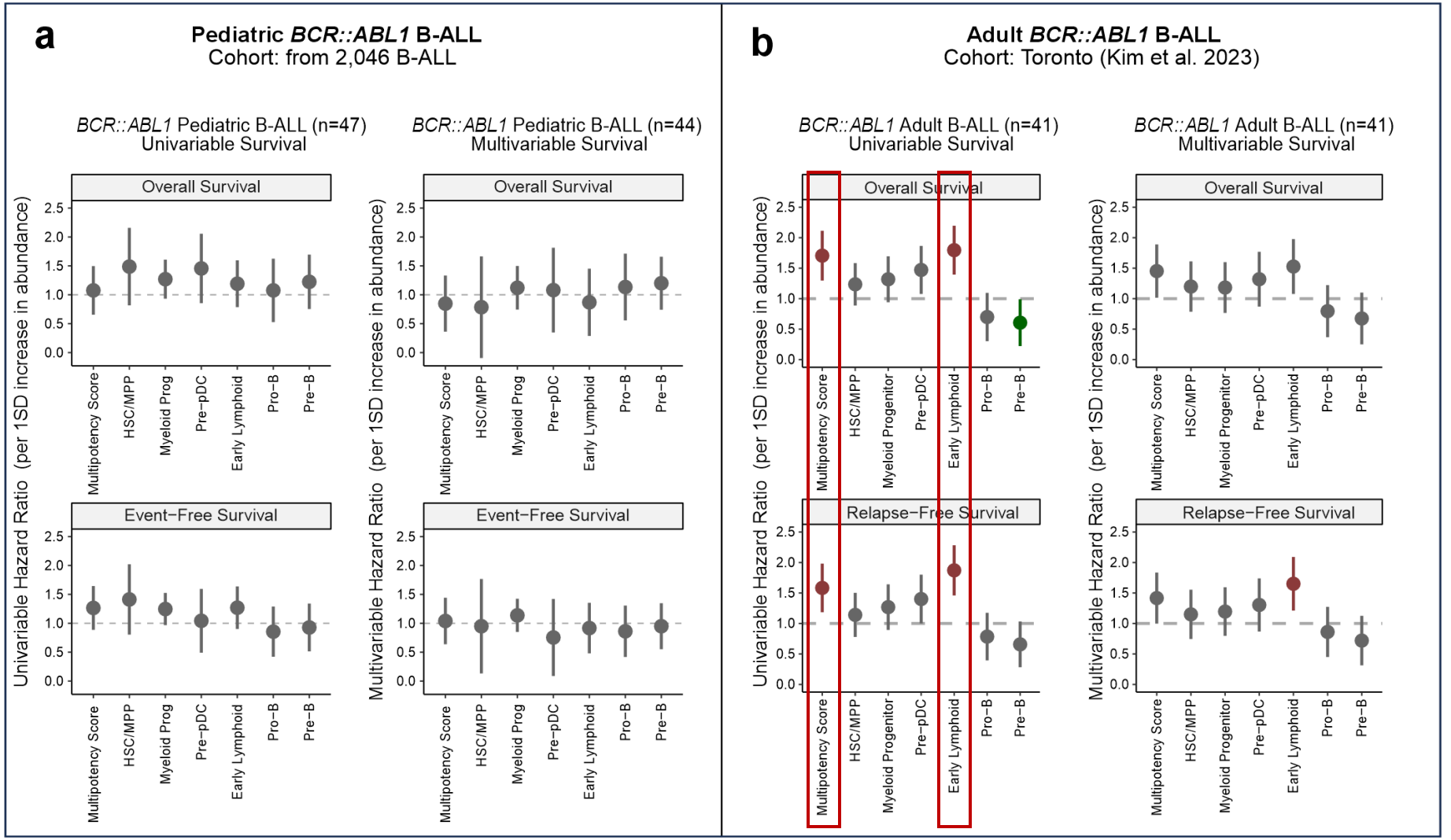


Clinical outcome:
Worse OS and EFS



Multivariable analysis; N= 1,010 pediatric B-ALL patients
Covariates: age, sex, WBC, clinical risk group and genomic subtype

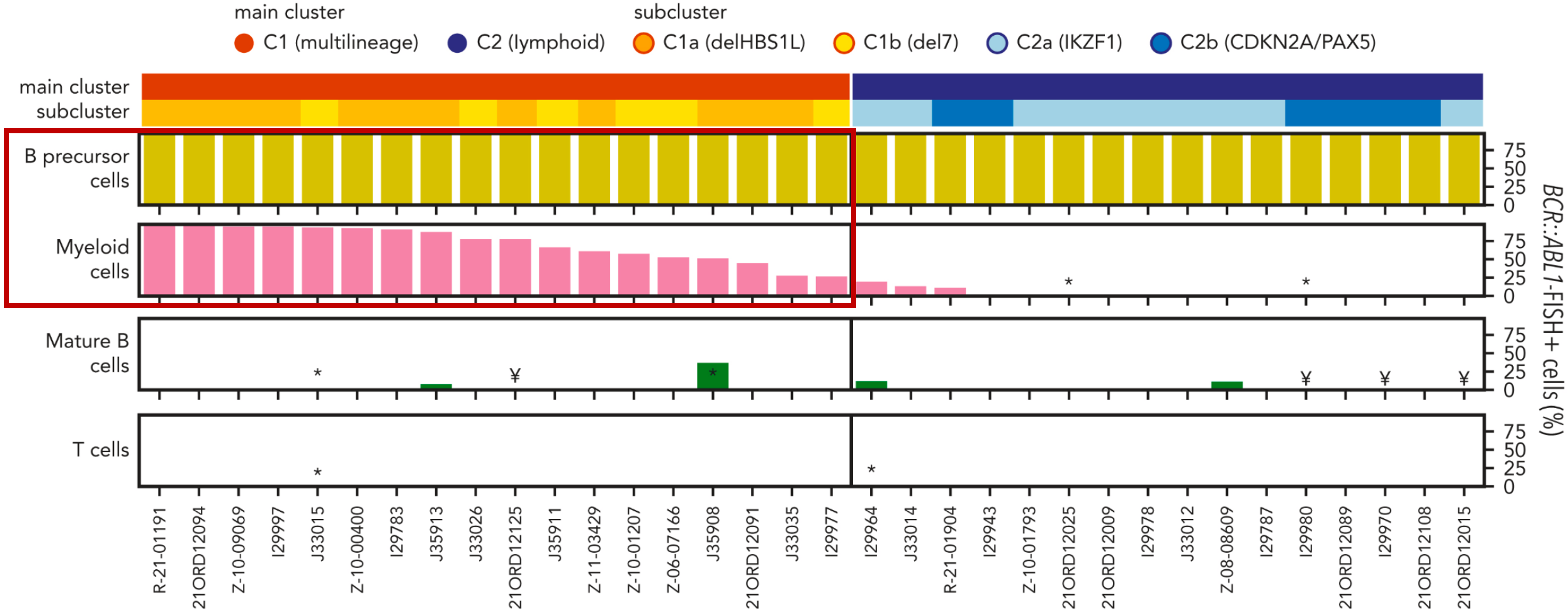
BCR::ABL1 subgroups and outcome



Chemotherapy regimen + imatinib

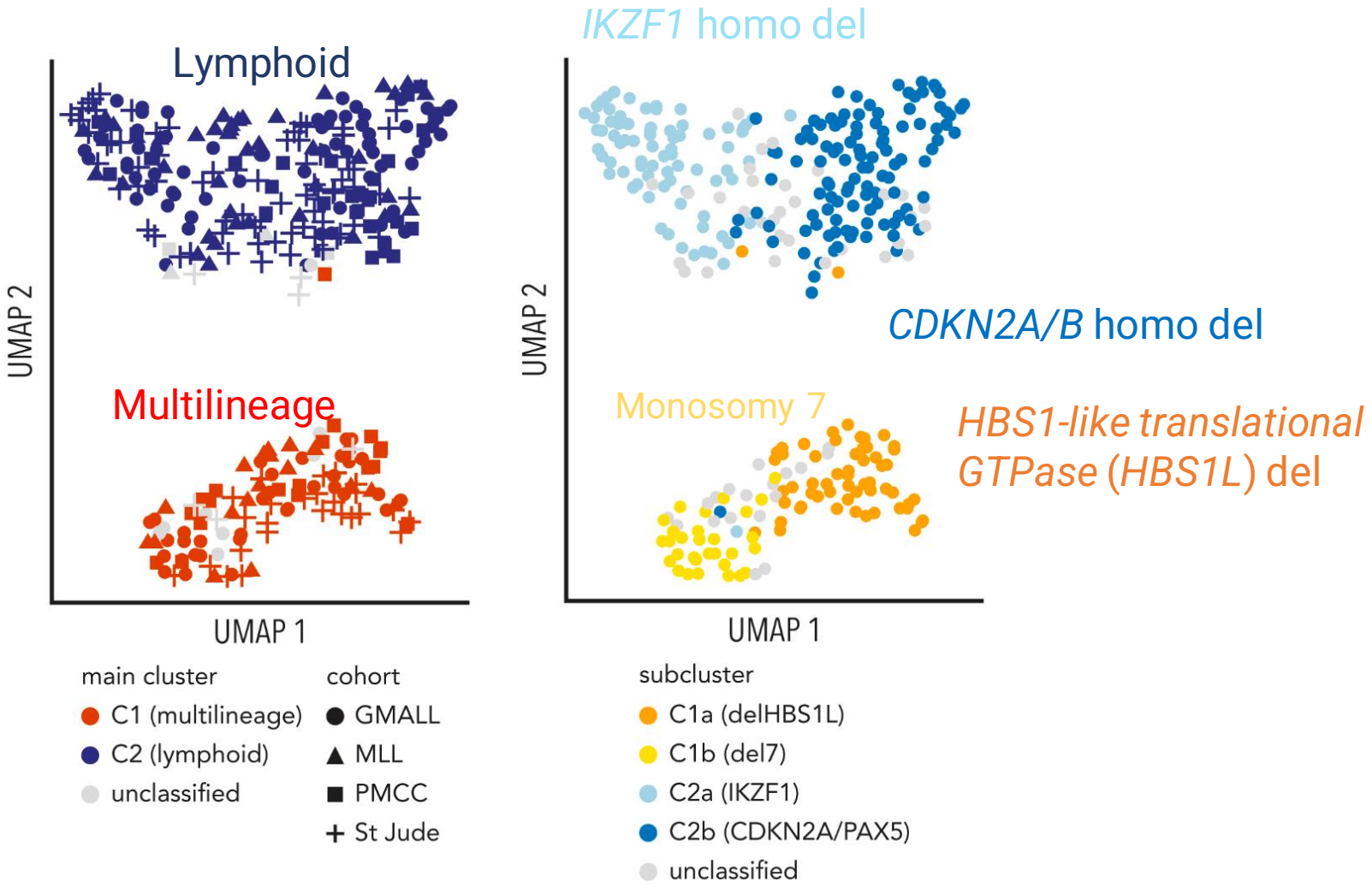
Transcriptomic classes of *BCR::ABL1* ALL (GMALL)

Bulk RNA-seq from 327 *BCR*::*ABL1*-positive patients with ALL (age, 2-84 years; median, 46 years)



Bastian L et al. *Blood* 2024

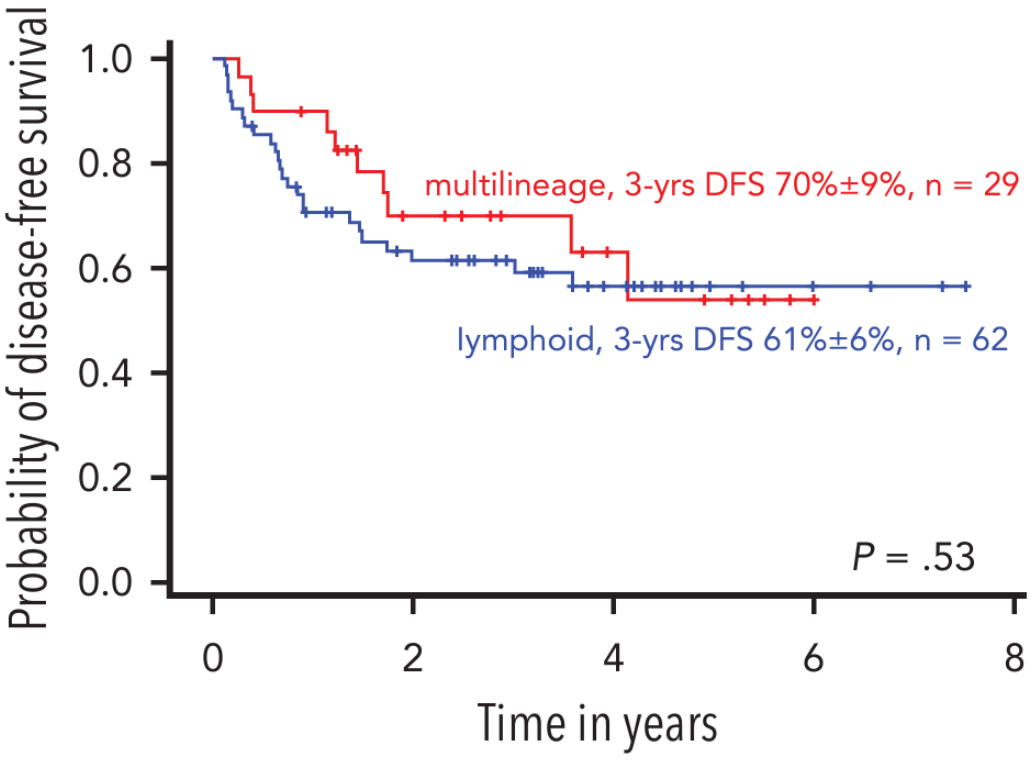
Transcriptomic classes of *BCR::ABL1* ALL (GMALL)

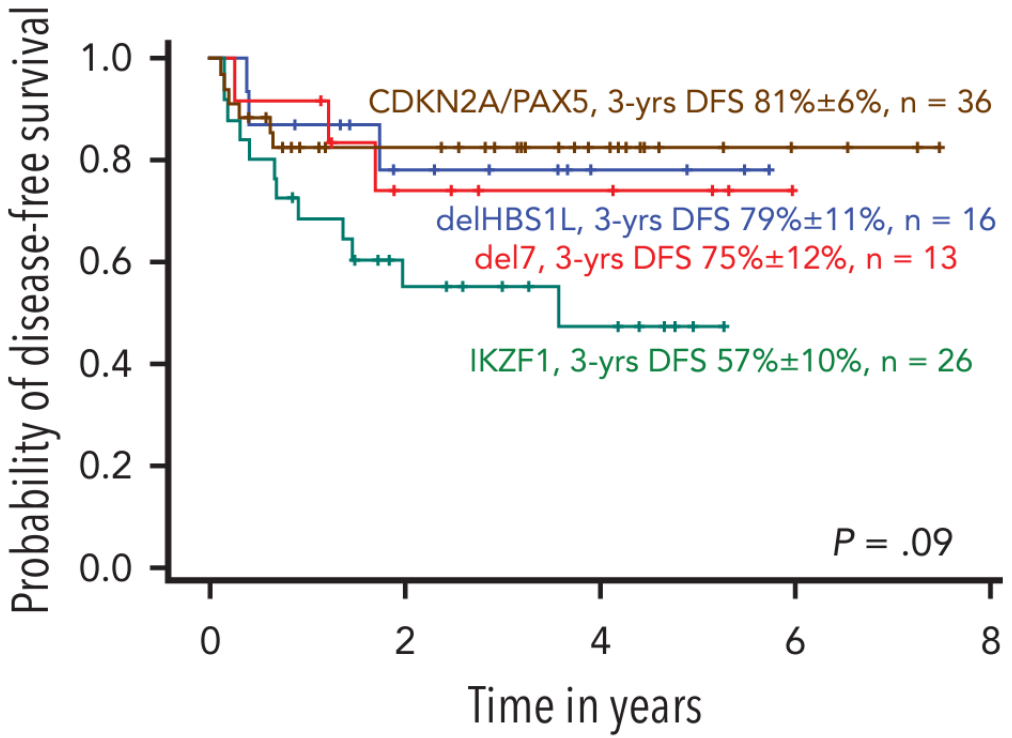


Bastian L et al. *Blood* 2024

Transcriptomic classes of *BCR::ABL1* ALL (GMALL)

- Adult Ph+ ALL (n = 98)
- Imatinib combined with adapted chemotherapy
- Disease-free survival probabilities were uniformly high in both Ph+ subtypes
- Inferior outcome for *IKZF1* deleted cluster



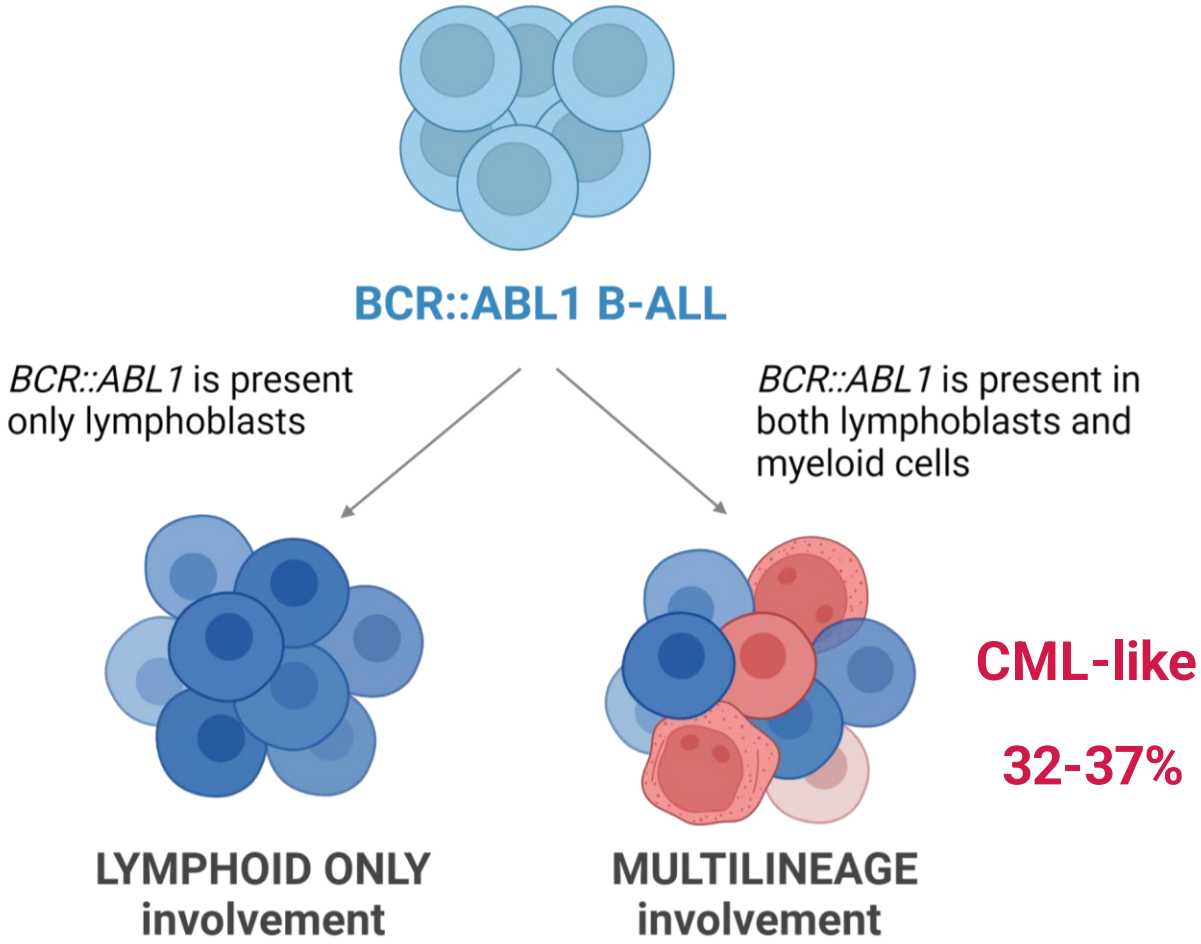


Bastian L et al. *Blood* 2024

Developmental state refines *BCR::ABL1* B-ALL

International Consensus Classification (ICC) of B-ALL

B-ALL with recurrent genetic abnormalities
B-ALL with t(9;22)(q34.1;q11.2)/*BCR::ABL1*
with lymphoid only involvement
with multilineage involvement
B-ALL with t(v;11q23.3)/*KMT2A* rearranged
B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1*
B-ALL, hyperdiploid
B-ALL, low hypodiploid
B-ALL, near haploid
B-ALL with t(5;14)(q31.1;q32.3)/*IL3::IGH*
B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1*
B-ALL, *BCR::ABL1*–like, ABL-1 class rearranged
B-ALL, *BCR::ABL1*–like, JAK-STAT activated
B-ALL, *BCR::ABL1*–like, NOS
B-ALL with iAMP21
B-ALL with *MYC* rearrangement
B-ALL with *DUX4* rearrangement
B-ALL with *MEF2D* rearrangement
B-ALL with *ZNF384*(362) rearrangement
B-ALL with *NUTM1* rearrangement
B-ALL with *HLF* rearrangement
B-ALL with *UBTF::ATXN7L3/PAN3*,CDX2 ("CDX2/UBTF")
B-ALL with mutated IKZF1 N159Y
B-ALL with mutated PAX5 P80R

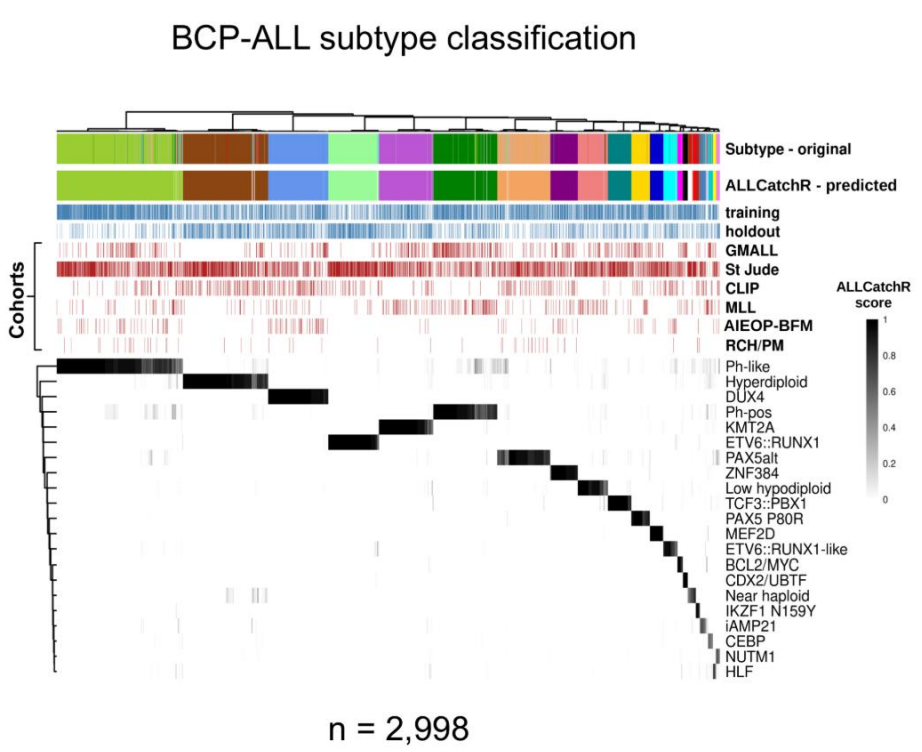


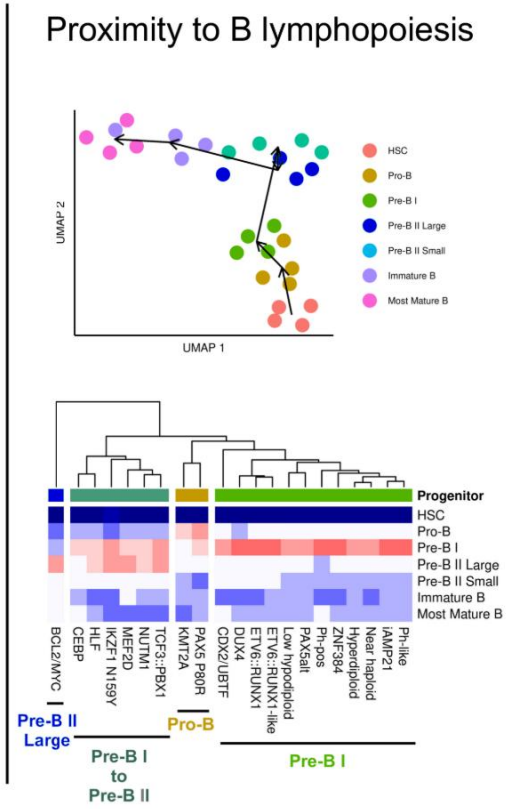
- Older patients
- Higher WBC, neutrophil, and immature myeloid cell counts
- **Lineage switch** following CD19-targeted immunotherapy

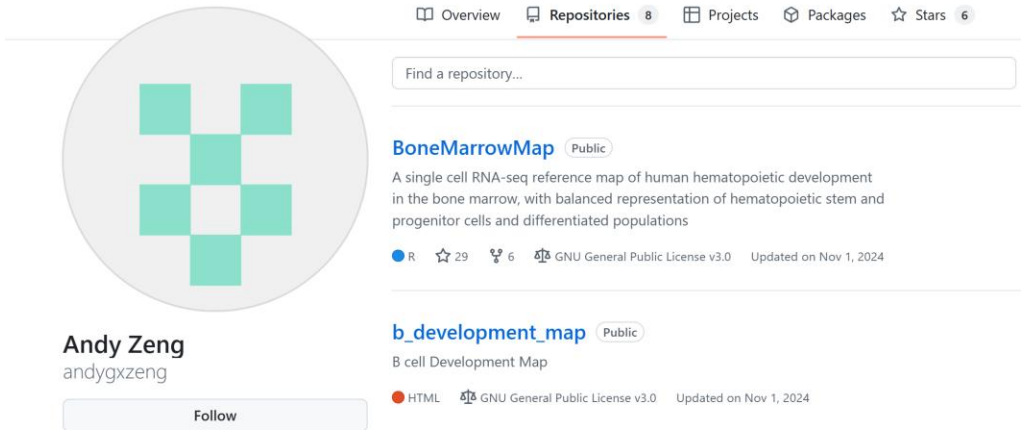
Hovorkova L. et al. *Blood* 2016; Kamoda Y et al. *Acta Haematol*. 2016; Cazzaninga G et al. *Haematologica* 2018; Chen Z et al. *Leukemia & Lymphoma* 2020; Arber D et al *Blood* 2022; Kim R et al *JCO* 2024

ALLCatchR

Machine learning-based classifiers can allocate B-ALL gene expression samples to all expression-defined molecular subtypes of the WHO-HAEM5 and ICC classifications.





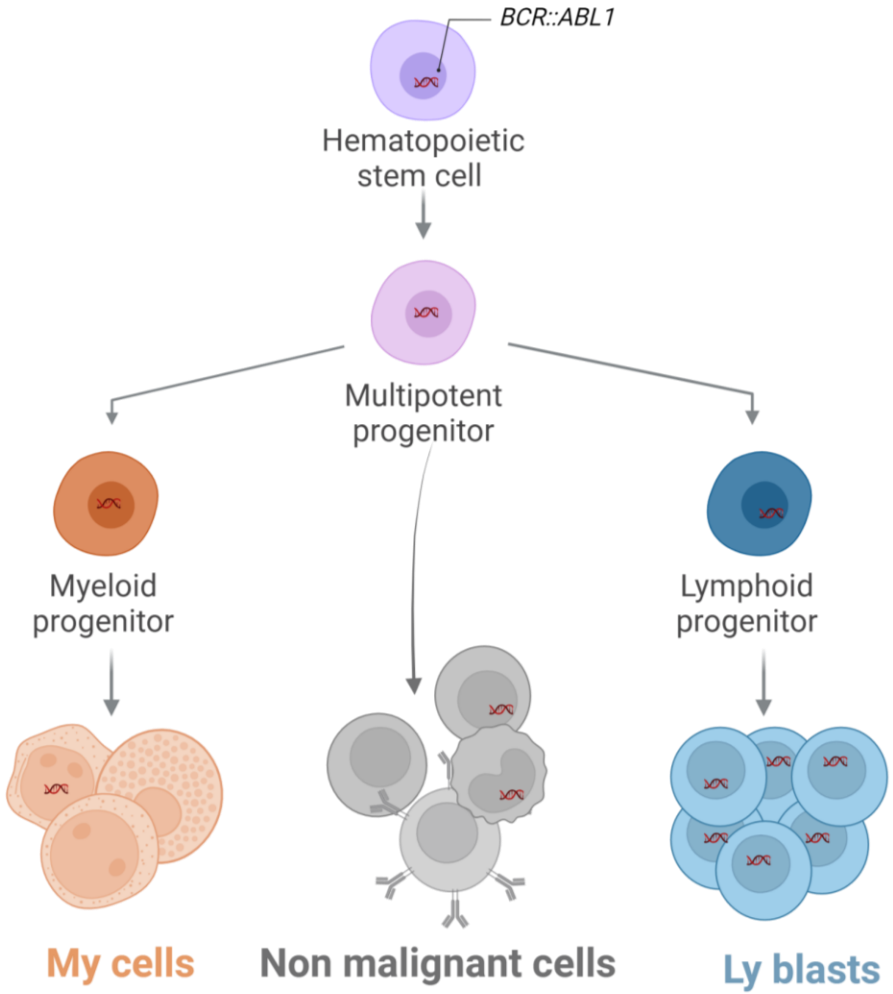


https://github.com/andygxzeng?tab=repositories
Iacobucci I et al. *Nat Cancer* 2025

https://github.com/ThomasBeder/ALLCatchR_bcrabl1; Beder et al. HemaSphere 2023.

MRD by PCR for *BCR::ABL1* and NGS for IG/TR

Multilineage BCR::ABL1 ALL questions the significance of MRD → Discordant MRD results

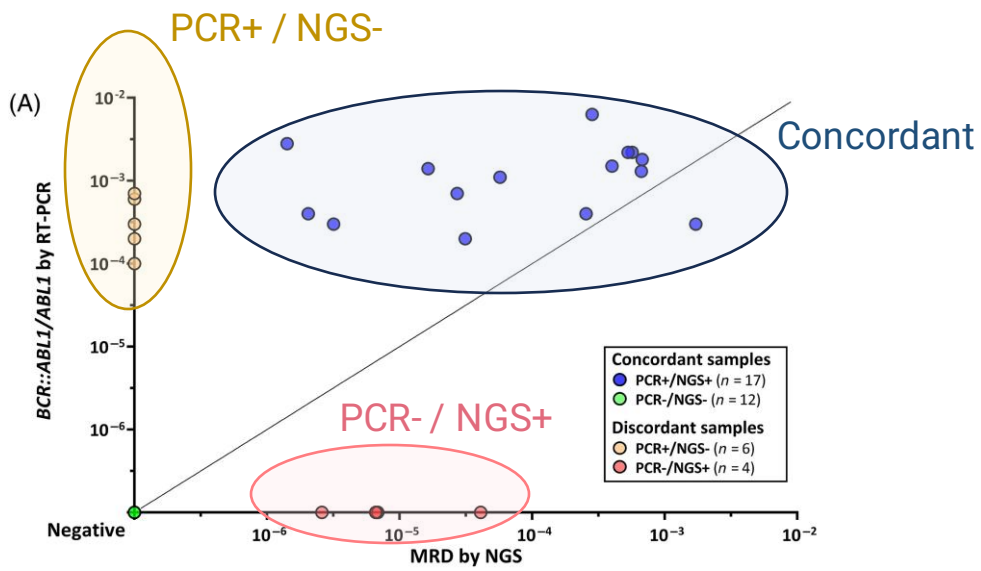


→

Comparison between MRD assessed by PCR for *BCR::ABL1* and NGS for IG/TR

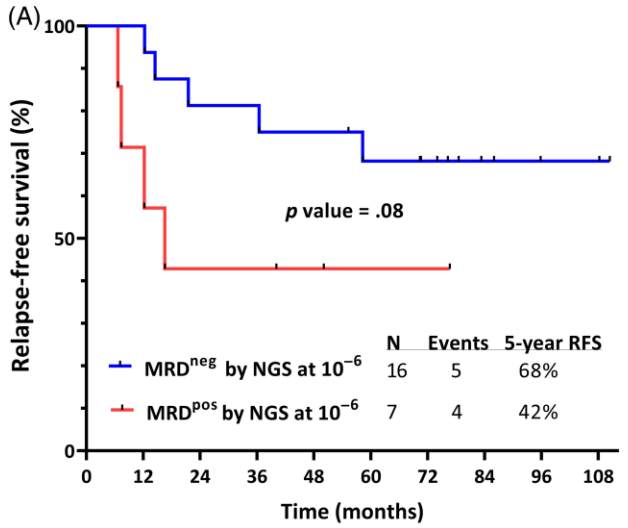
MRD by PCR for *BCR::ABL1* vs NGS for IG/TR

Adults with Ph+ ALL undergoing frontline therapy[1]
(retrospective study, N=44
validation study, N=74)

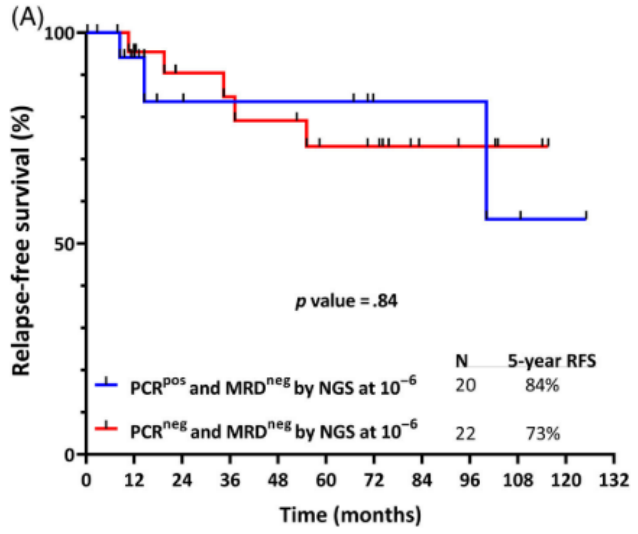


32% discordant PCR / NGS results

NGS MRD in first 6 months of therapy is prognostic for RFS and OS



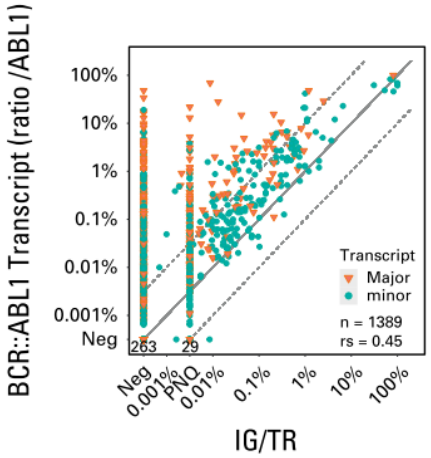
PCR for *BCR::ABL1* is not prognostic in patients with NGS MRD negativity

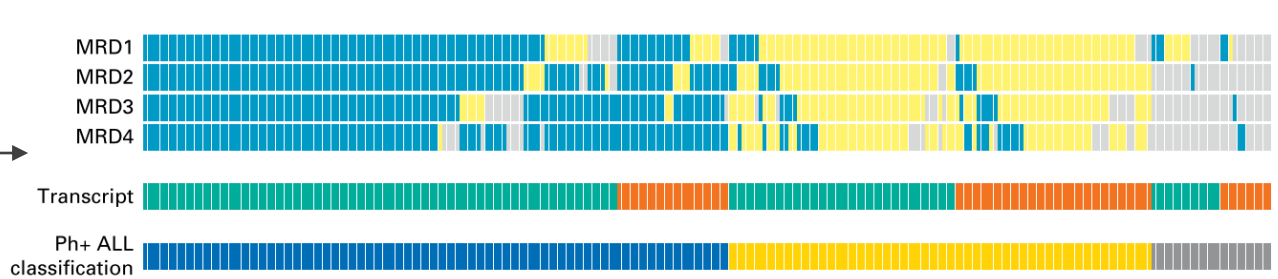


Short NJ et al *American Journal of Hematology* 2023; [1]Hyper-CVAD-based therapy plus a BCR::ABL1 TKI

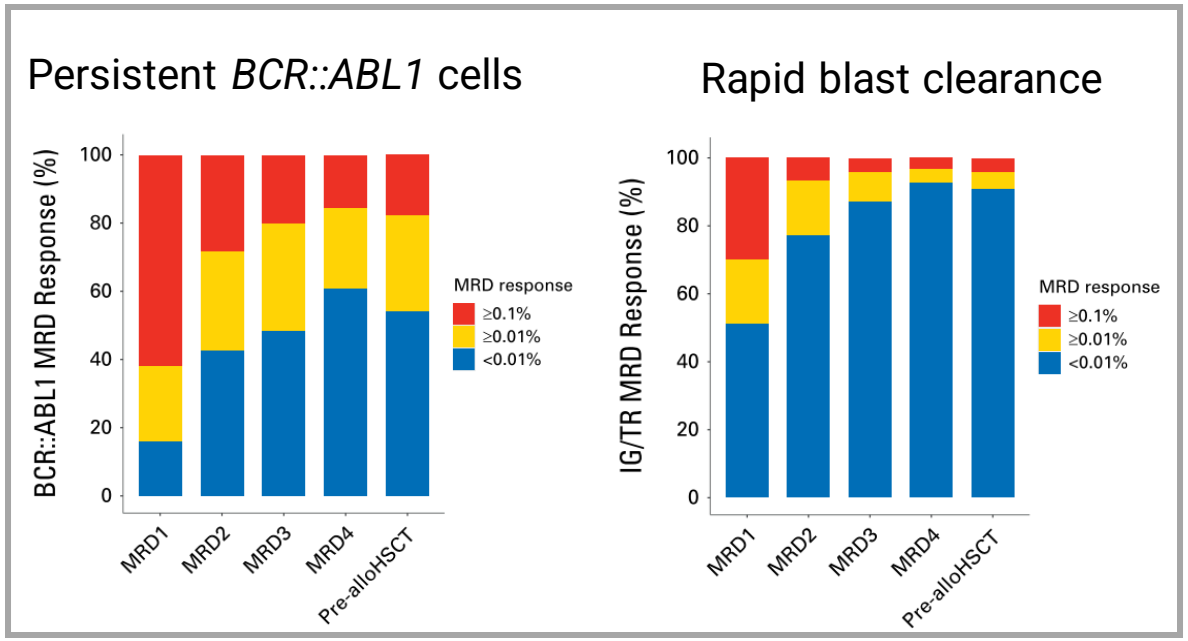
Adult Ph+ ALL: MRD in the GRAAPH-2014 Study

- GRAAPH-2014: four cycles of reduced-intensity chemotherapy with nilotinib, followed by HSCT
- 1,389 samples, 259 patients
- Significant disparity between the two types of markers, with higher *BCR::ABL1* levels in a subset of samples

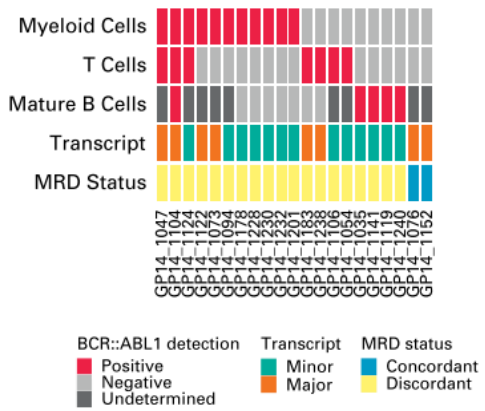




Discordant MRD kinetics in **43% of cases**



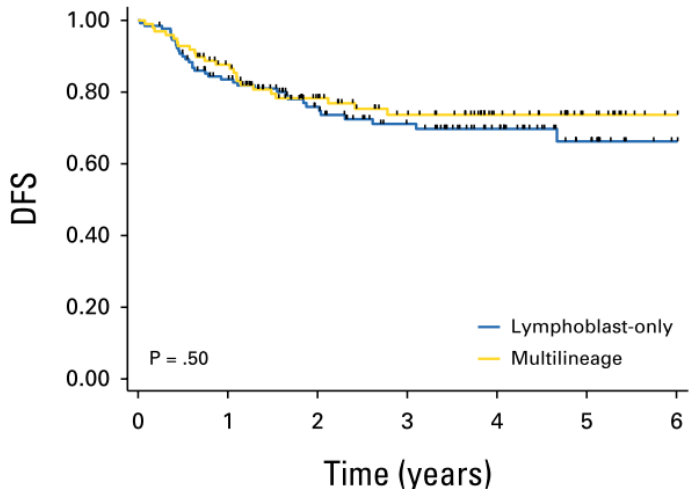
BCR::ABL1 in flow-sorted mature blood cells

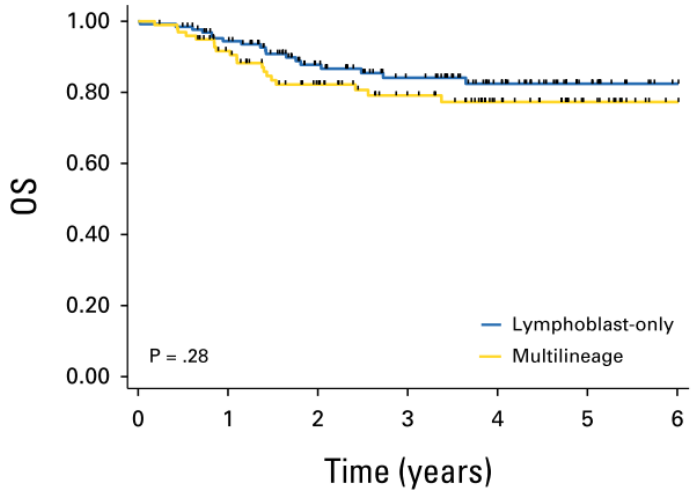


Kim R et al *JCO* 2024

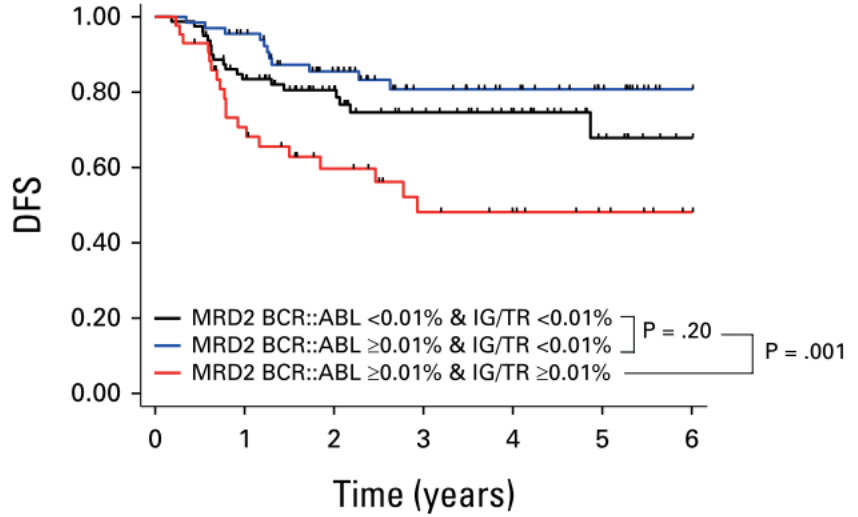
Adult Ph+ ALL: MRD in the GRAAPH-2014 Study

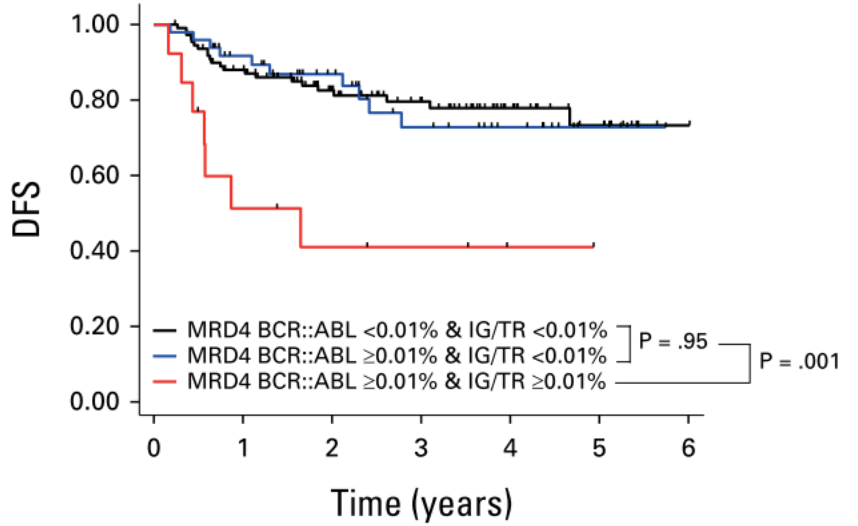
Multilineage and lymphoid only Ph ALL have similar DFS and OS





No difference in DFS according to *BCR::ABL1* response; IG/TR MRD ≥0.01% after cycle 2/4 is associated with lower DFS





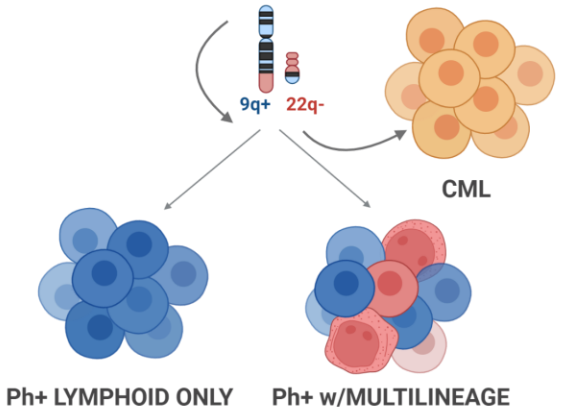
Kim R et al *JCO* 2024

Take home messages

- *BCR::ABL1* transcripts can persist in non-lymphoid cells, so transcript-based MRD alone can be misleading
- Prioritize lymphoid-specific MRD (IG/TR) over *BCR::ABL1* alone for risk stratification
- Future trials need to evaluate the role of *BCR::ABL1* MRD and *BCR::ABL1* subgroups (early vs committed) in the context of chemotherapy-free treatments relying on immunotherapy targets

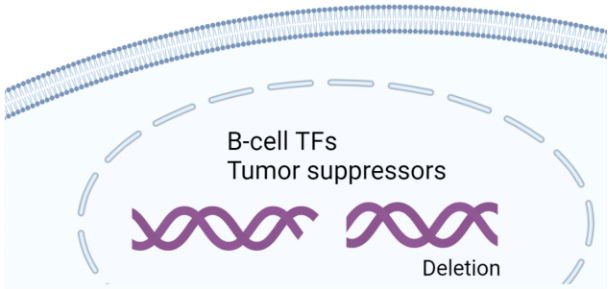
Conclusions

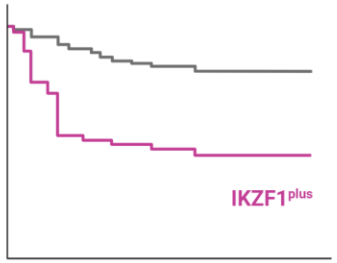
BCR::ABL1 and leukemia phenotype



BCR::ABL1 fusion gene is the key initiator of different phenotypes of leukemia with diverse prognoses

Role of cooperating lesions

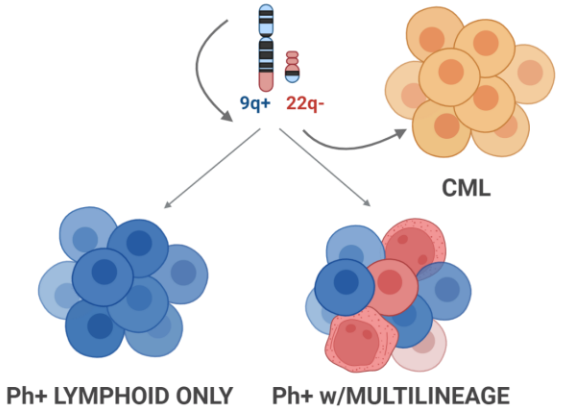




DNA copy number losses in lymphoid transcription factors (*IKZF1, PAX5, EBF1*) and in tumor suppressors (*CDKN2A/B*) are common and confer poor outcome irrespective of TKI exposure

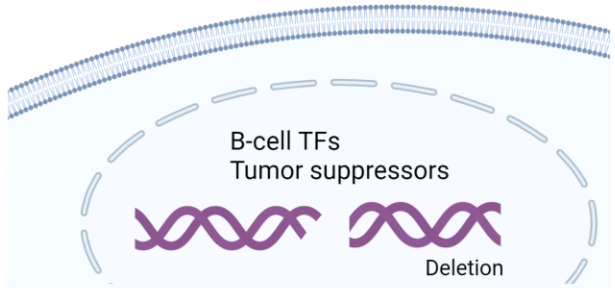
Conclusions

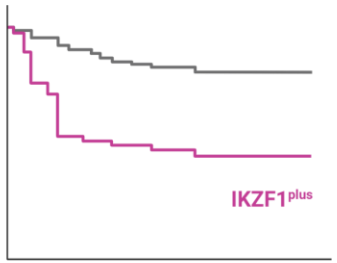
BCR::ABL1 and leukemia phenotype



BCR::ABL1 fusion gene is the key initiator of different phenotypes of leukemia with diverse prognoses

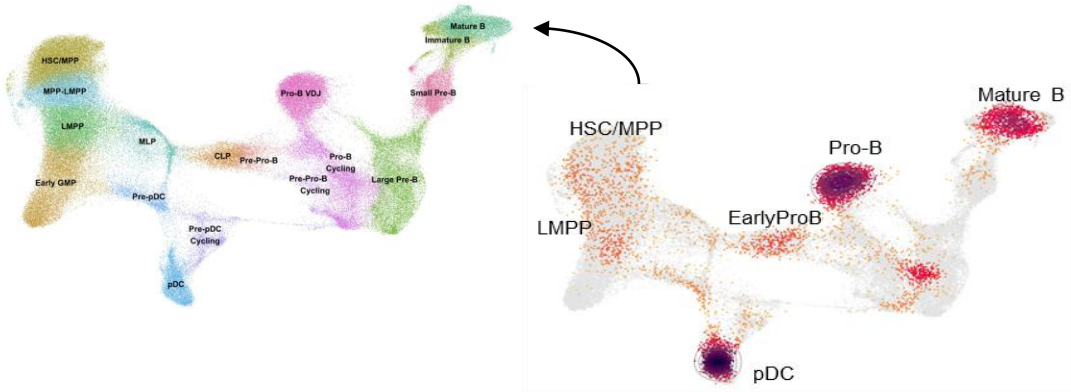
Role of cooperating lesions





DNA copy number losses in lymphoid transcription factors (*IKZF1, PAX5, EBF1*) and in tumor suppressors (*CDKN2A/B*) are common and confer poor outcome irrespective of TKI exposure

scRNA-seq B-cell Developmental Reference Map



Development of scRNASeq B-cell reference map which can be used to identify the transcriptomic subtypes of *BCR-ABL1* ALL

Acknowledgments

St. Jude Children's Research Hospital (Memphis)

Charles Mullighan
Qingsong Gao
Pradyumma Baviskar
Emily Backhaus
Francesca Benini
Yunchao Chang
Danika Di Giacomo
Pankaj Ghate
Tanya Khan
Shunsuke Kimura
Nicole Michmerhuizen
Vibhor Mishra
Lindsey Montefiori
Chun-Shik Park
Petri Polonen
Chunxu Qu (Former)
Kasia Szoltysek
Ruth Wang'ondu
Xin Zhou
Airen Zaldivar Peraza
Karishma Gangwani

Princess Margaret Cancer Centre (Toronto, ON, Canada)

Andy Zeng
Laura Garcia-Prat
Sayyam Shah
Alex Murison
Veronique Voisin
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Figures were created with **BioRender.com**

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