Le alterazioni genomiche nelle B-ALL: implicazioni prognostiche e terapeutiche

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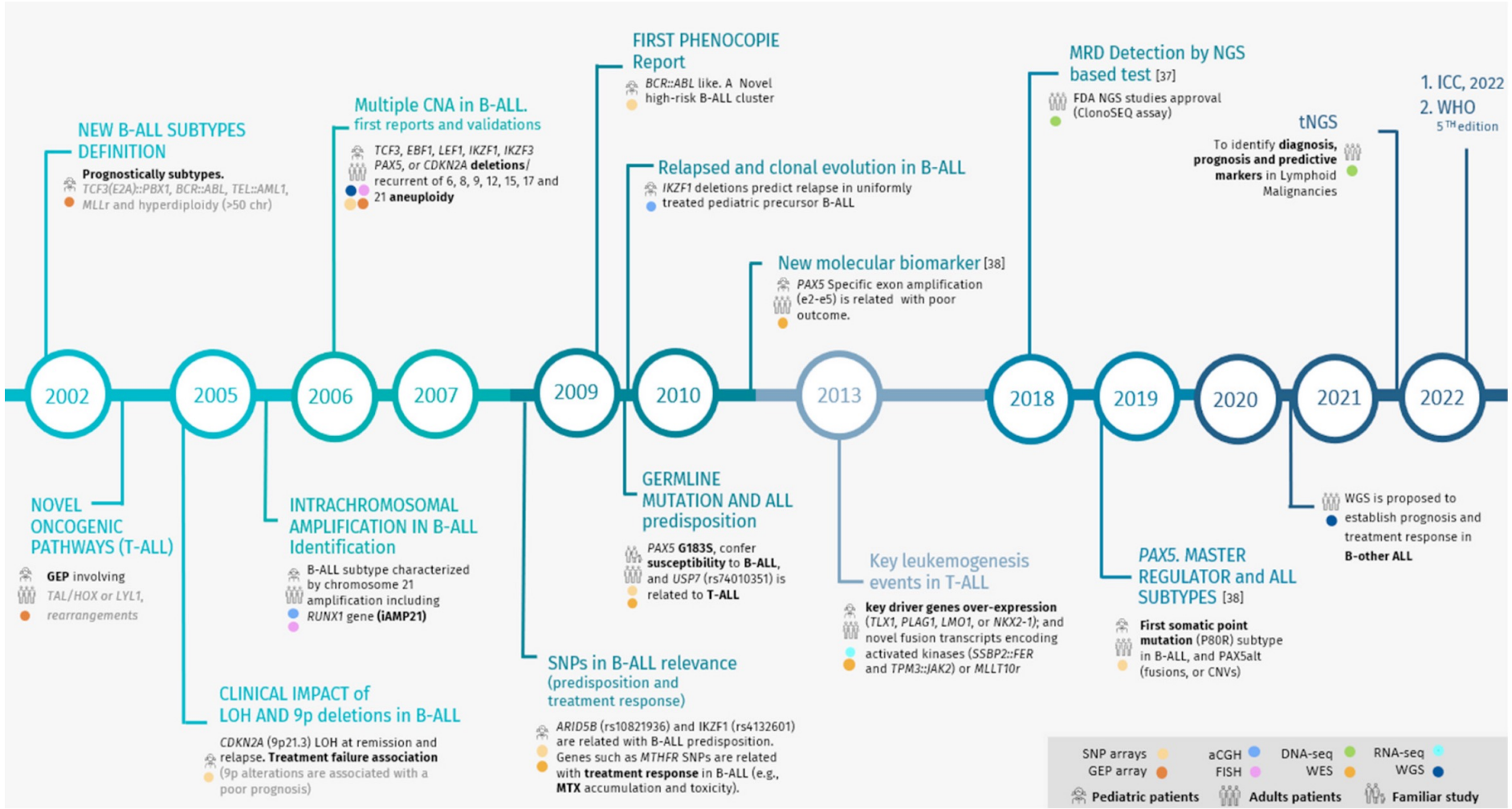
Disclosures of Simona Soverini

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Incyte Biosciences			x				
Novartis			x			x	
Ascentage			x			x	

Milestones in B-ALL molecular characterization



Ramirez Maldonado V, *Cancers* 2024

How ‘omics’ have contributed to refine the molecular subclassification of B-ALL over the past 2 decades

2008

Table 2. WHO classification of myeloid neoplasms and acute leukemia (continued)

Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
Mixed phenotype acute leukemia, B-myeloid, NOS
Mixed phenotype acute leukemia, T-myeloid, NOS
Provisional entity: natural killer (NK) cell lymphoblastic leukemia/lymphoma
B lymphoblastic leukemia/lymphoma
B lymphoblastic leukemia/lymphoma, NOS
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2);*BCR-ABL 1*
B lymphoblastic leukemia/lymphoma with t(v;11q23);*MLL* rearranged
B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) *TEL-AML1* (*ETV6-RUNX1*)
B lymphoblastic leukemia/lymphoma with hyperdiploidy
B lymphoblastic leukemia/lymphoma with hypodiploidy
B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) *IL3-IGH*
B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
T lymphoblastic leukemia/lymphoma

Vardiman JW et al *Blood* 2009

2016

Table 1. (continued)

WHO myeloid neoplasm and acute leukemia classification
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); *BCR-ABL1*
MPAL with t(v;11q23.3); *KMT2A* rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS
B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Arber D et al *Blood* 2016

How ‘omics’ have contributed to refine the molecular subclassification of B-ALL over the past 2 decades

2022

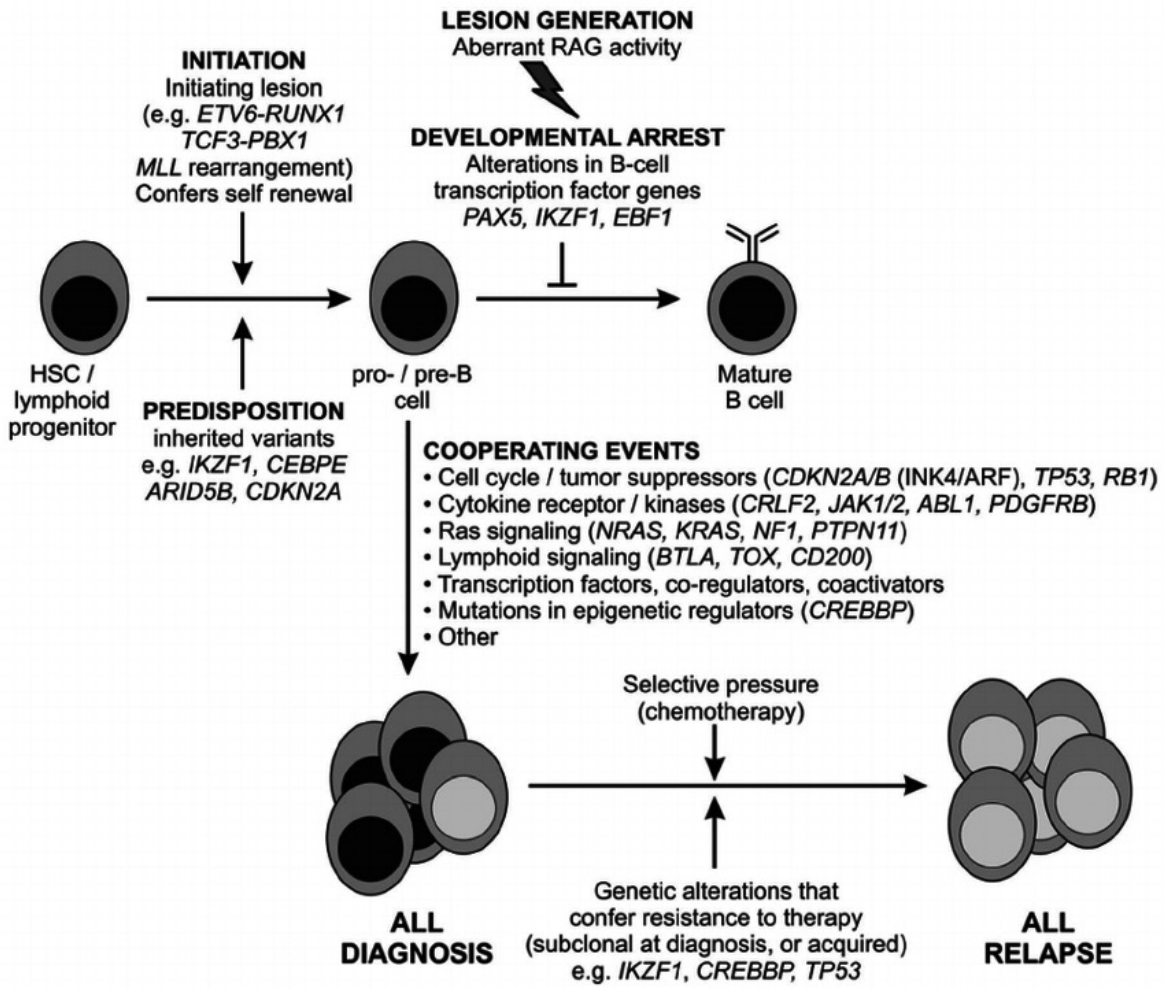
International Consensus Classification (ICC) of B-ALL	
B-ALL with recurrent genetic abnormalities	**Provisional entities:**
B-ALL with t(9;22)(q34.1;q11.2)/*BCR::ABL1*	B-ALL, *ETV6::RUNX1*-like
with lymphoid only involvement	B-ALL, with *PAX5* alteration
with multilineage involvement	B-ALL, with mutated *ZEB2* / *IGH::CEBPE*
B-ALL with t(v;11q23.3)/*KMT2A* rearranged	B-ALL, *ZNF384* rearranged-like
B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1*	B-ALL, *KMT2A* rearranged-like
B-ALL, hyperdiploid	B-ALL, NOS
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	

Arber D et al *Blood* 2022

WHO classification, 5th edition
B-ALL, NOS
B-ALL with *BCR::ABL1*
B-ALL with *KMT2A* rearrangement
B-ALL with *ETV6::RUNX1*
B-ALL with *ETV6::RUNX1*-like features
B-ALL, hyperdiploid
B-ALL, hypodiploid
B-ALL with *IL3::IGH*
B-ALL with *TCF3::PBX1*
B-ALL with *BCR::ABL1*-like features
B-ALL with iAMP21
B-ALL with *HLF* rearrangement
B-ALL with other defined genetic abnormalities

Alaggio R et al *Leukemia* 2022

Role of genetic alterations in the pathogenesis of B-ALL



Subtype-defining lesions
primarily drive aberrant transcriptional programs

+

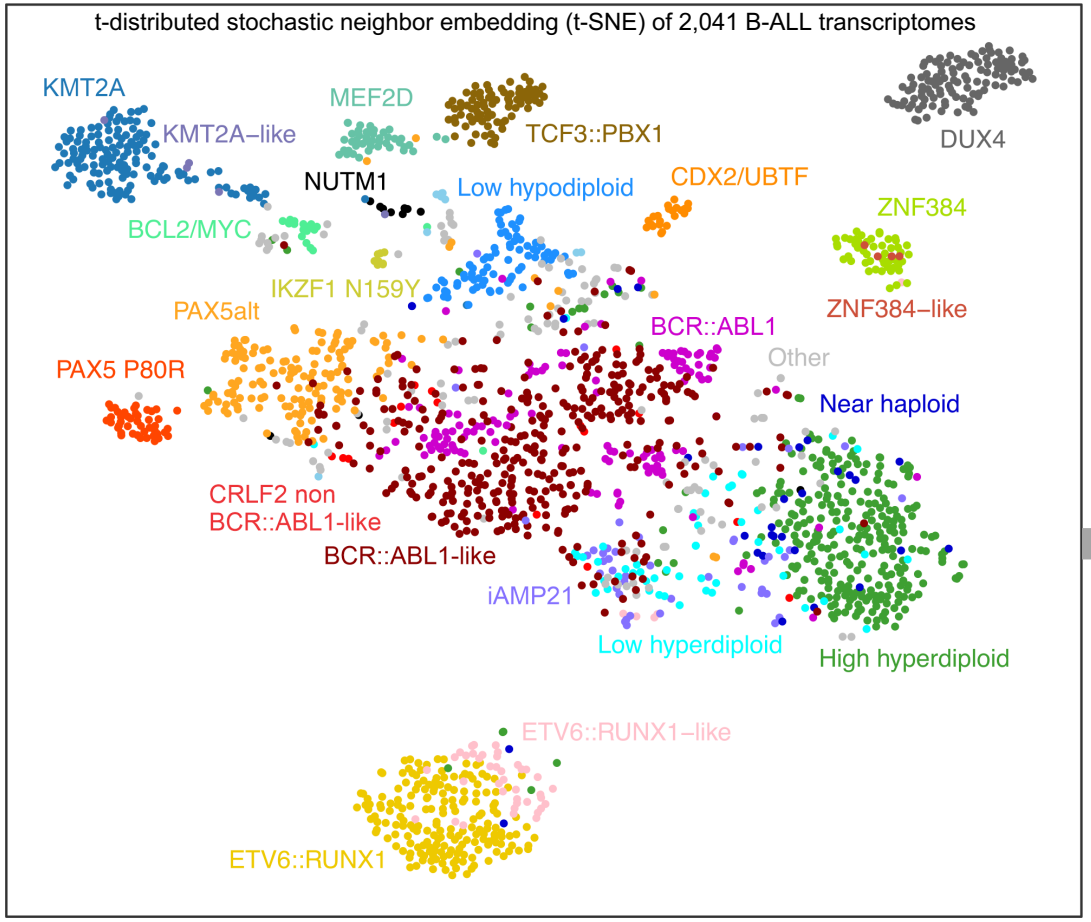
Secondary alterations
CNAs and point mutations affecting B-lymphoid factors (eg, IKZF1, PAX5, and EBF1), cell cycle regulators (eg, CDKN2A and TP53), cell signaling pathways (eg, NRAS, KRAS, FLT3, and JAK2), and epigenetic factors (eg, CREBBP, SETD2, and KMT2D)

=

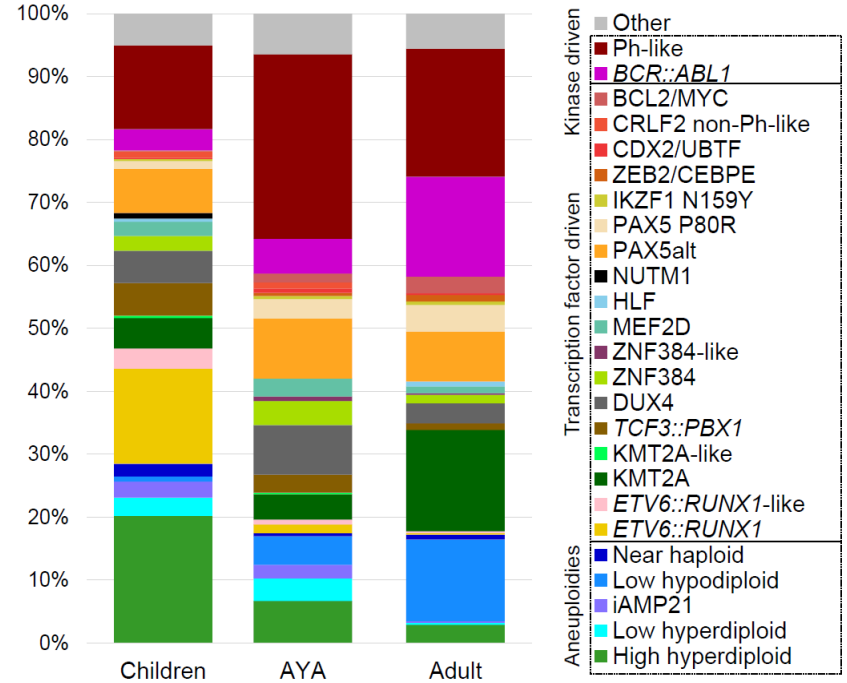
Maturation arrest

Mullighan C, *Hematology (ASH Annual Meeting Proceedings)* 2012

B-ALL genomic subtyping

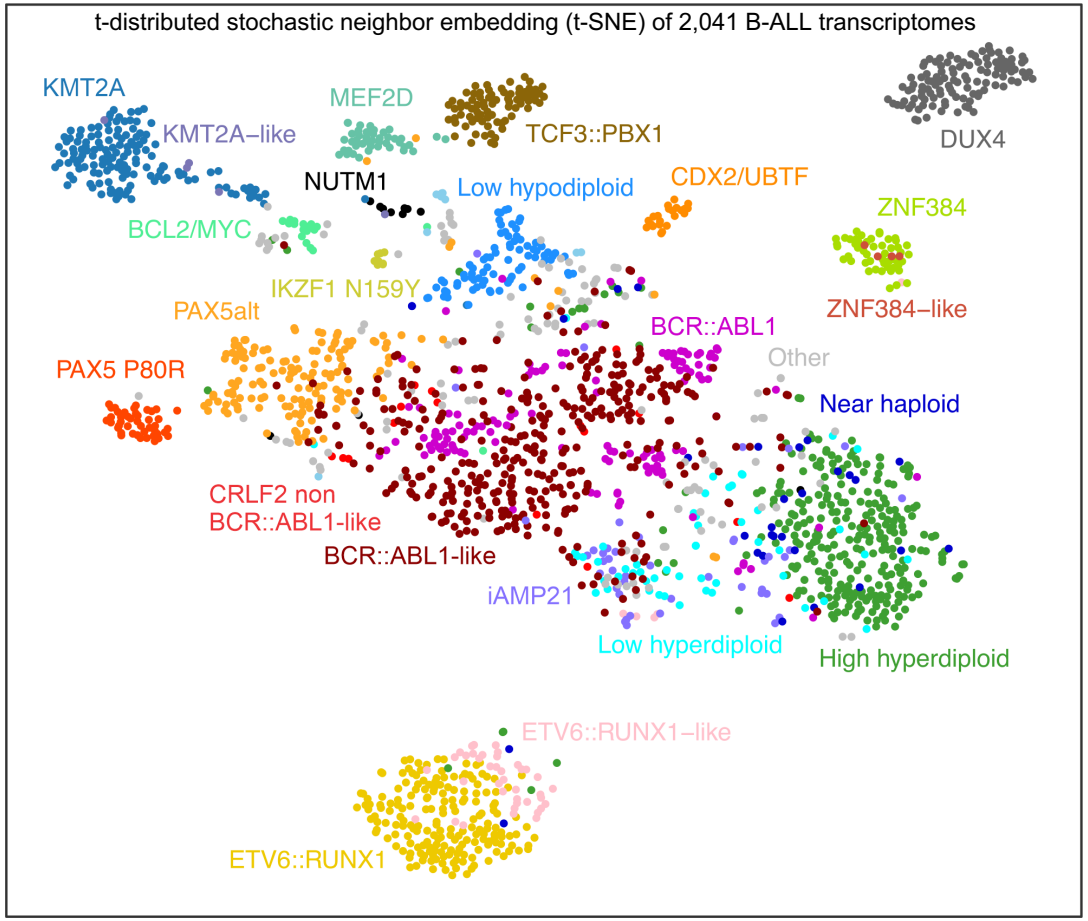


- > 20 B-ALL subtypes by gene expression profiles (WTS), each defined by a specific driver lesion
- Striking diversity in underlying driver alterations



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; Iacobucci I et al. *J Clin Med*. 2021

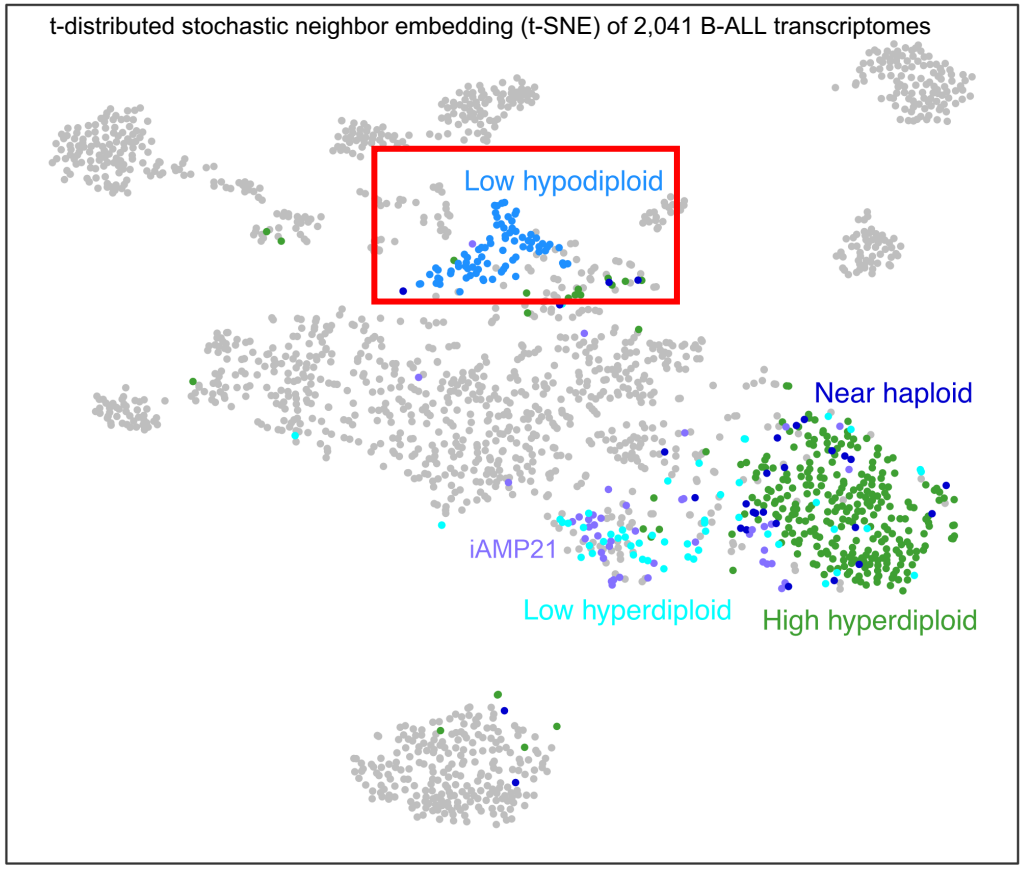
B-ALL genomic subtyping



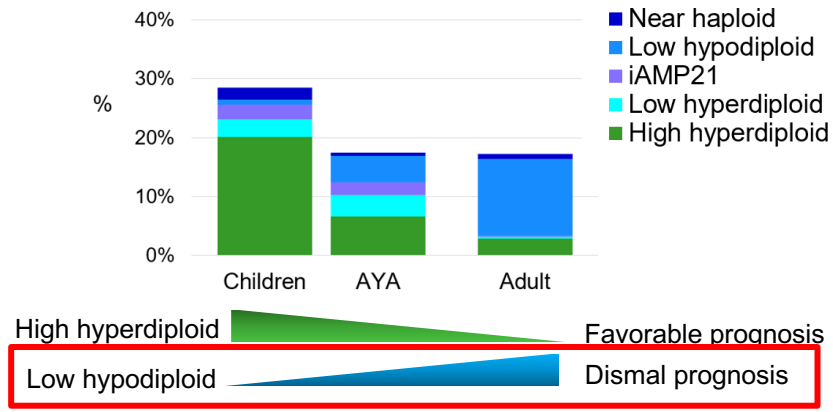
- Aneuploidies
- Chimeric fusion oncoproteins and cryptic rearrangements
- Phenocopies
  - BCR::ABL1-like
  - ETV6::RUNX1-like
  - ZNF384-like
  - KMT2A-like
- Point mutations
  - IKZF1 N159Y
  - PAX5 P80R

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; Iacobucci I et al. *J Clin Med*. 2021

New insights into historical cytogenetic subtypes: aneuploidy



- **Aneuploidy**
  - iAMP21
  - High hyperdiploid (51-67 chrs)
  - Low hyperdiploid (47-50 chrs)
  - Near haploid (24-31 chrs)
  - Low hypodiploid (32-39 chrs)

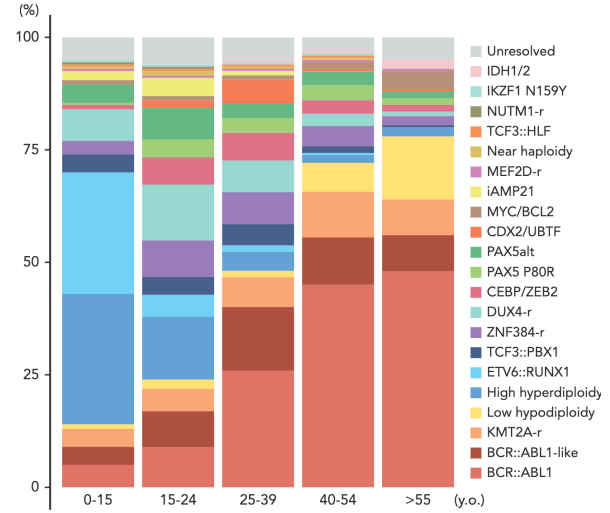


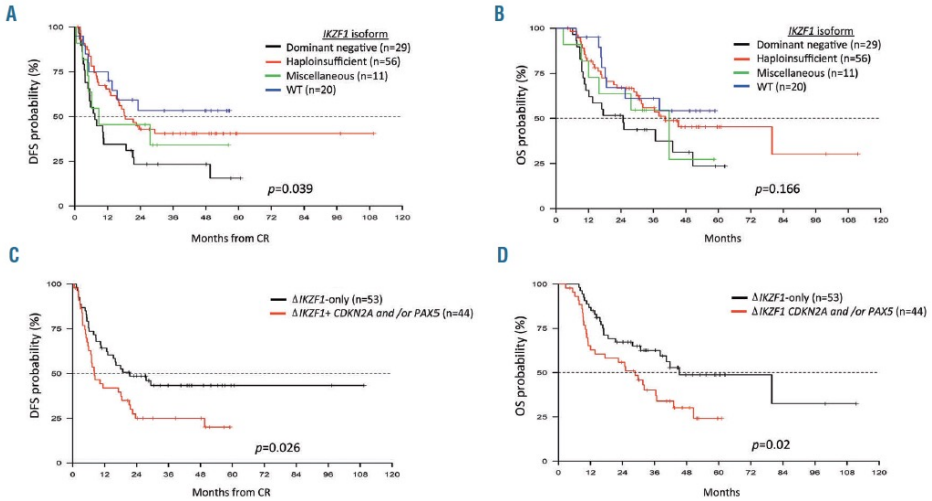
- *TP53* biallelic alterations, typically consisting of 1 mutation and 1 copy loss related to monosomy 17, are the hallmark of low hypodiploidy
- in adults with LH-ALL, TP53 mutations are somatic and thought to originate from age-related CH

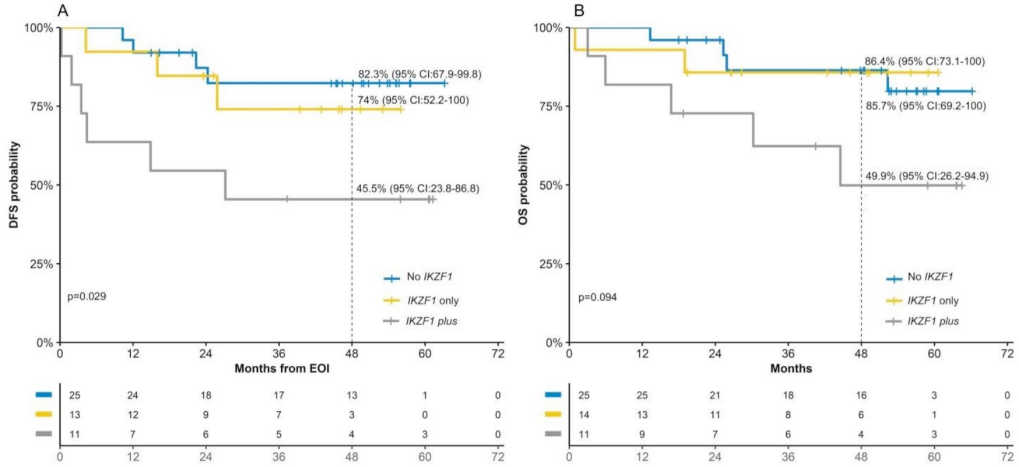
Gu Z et al. *Nat Genet* 2019; Kim R, Blood Cancer Discov 2023; Kitadze G, *Blood* 2023; Saygin C, Blood Cancer Discov 2024

New insights into historical cytogenetic subtypes: Ph+ ALL

- Most common genetic subtype of B-ALL in adults
- Incidence increasing with age, reaching up to half of B-ALL in individuals aged >55 years
- Comutations: IKZF1 intragenic deletions, monosomy 7, CDKN2A and PAX5 deletions, HBS1L deletions
- Ikaros+ pattern: IKZF1 deletion + PAX5 or CDKN2A/B deletion; confers dismal outcome



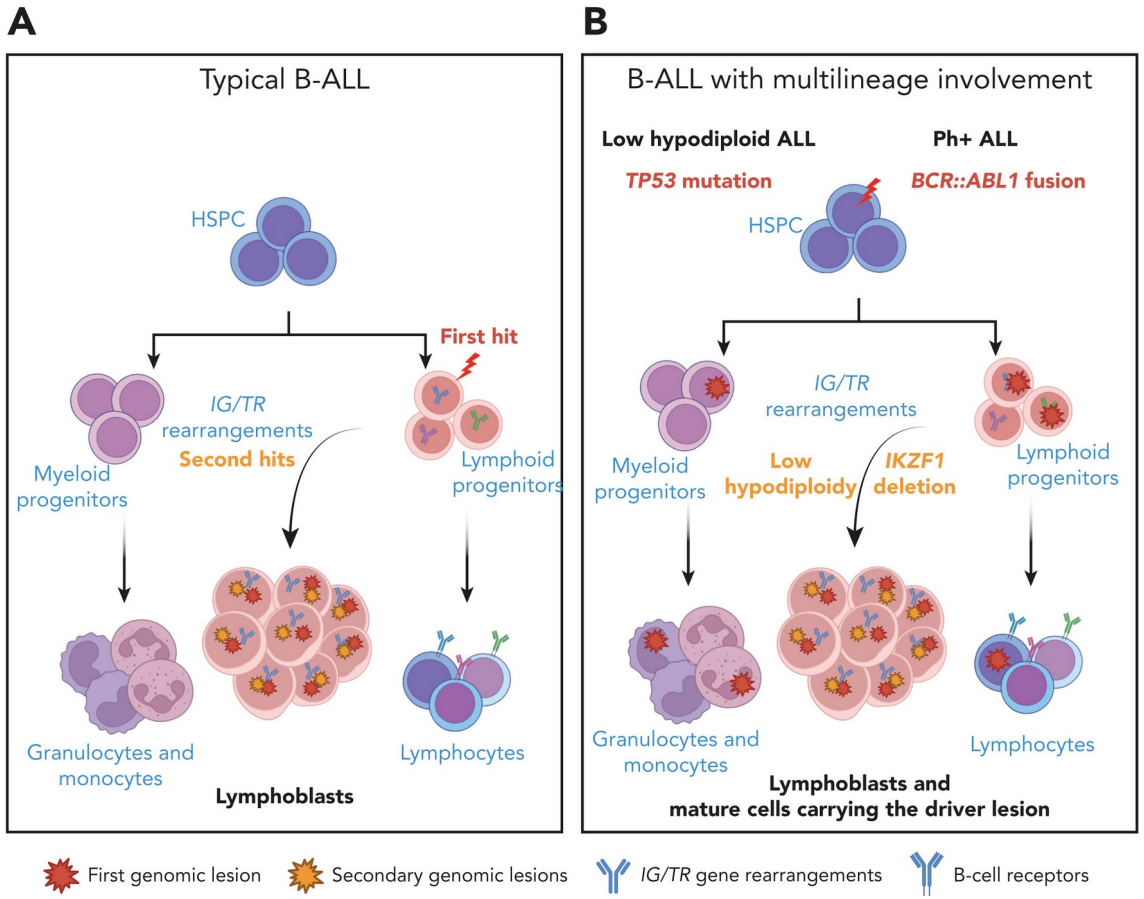




Fedullo C, *Haematologica* 2019; Foà R *J Clin Oncol* 2023; Passet M, Kim R and Clappier E, *Blood* 2025

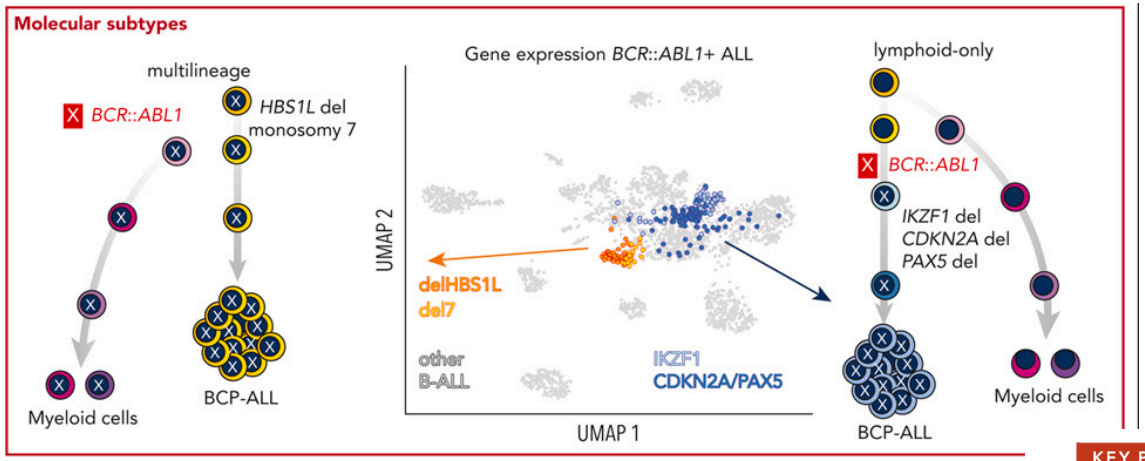
‘Multilineage’ or ’CML-like’ Ph+ ALL

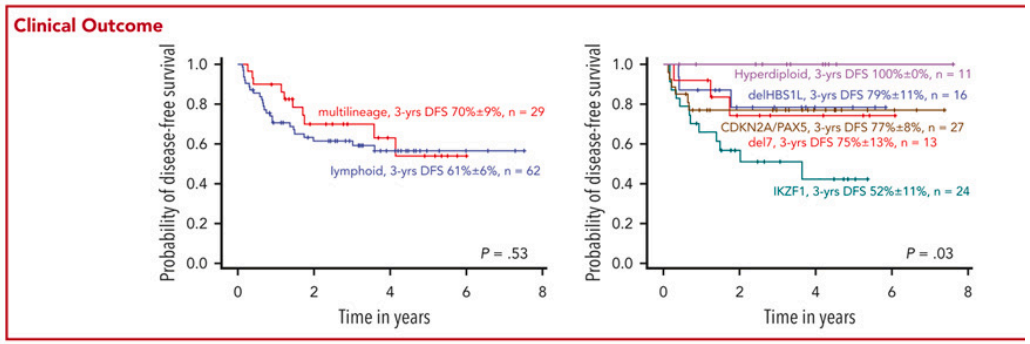
- Multilineage involvement (‘CML-LIKE’) vs lymphoid involvement (‘TYPICAL ALL’)
- Accounts for 32% to 37% of patients with de novo Ph + ALL
- Patients with multilineage BCR::ABL1 B-ALL are commonly older with higher white blood cell count, neutrophil, and immature myeloid cell counts
- Patients with multilineage BCR::ABL1 B-ALL often experience lineage switch following CD19-targeted immunotherapy
- MRD monitoring using immunoglobulin or T-cell receptor (IG/TR) genes rearrangements, rather than BCR::ABL1 quantification, better reflects the residual lymphoblast burden and more accurately predicts outcomes



Hovorkova L, *Blood* 2017; Zuna J, Leukemia 2022; Short NJ, *Am J Hematol* 2023; Kim R, J Clin Oncol 2024; Passet M, Kim R and Clappier E, *Blood 2025*

Molecular subtyping of Ph+ ALL





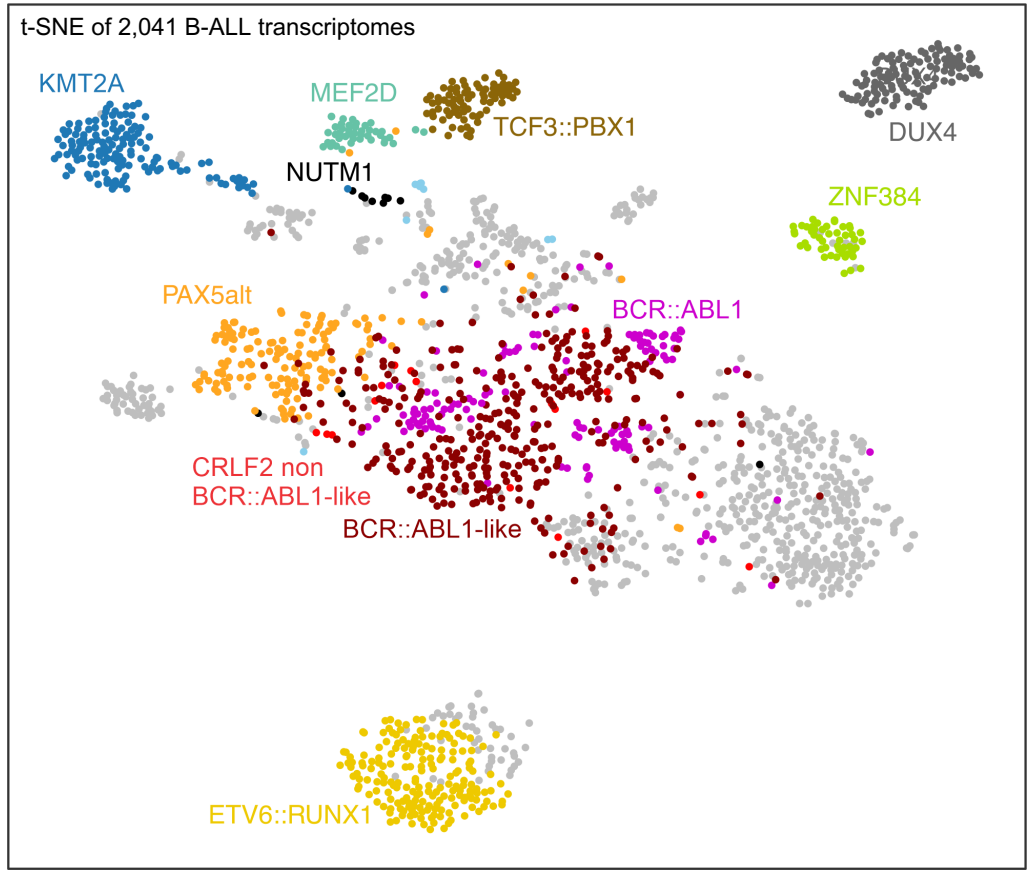
Bastian L, *Blood* 2024

KEY POINTS

- **"Multilineage" vs "lymphoid-only" *BCR::ABL1* involvement and distinct cooperating events determine gene expression in *BCR::ABL1*-positive ALL.**
- **Outcome with recent GMALL protocols is similar for *BCR::ABL1* lineage clusters, but inferior for an *IKZF1*$^{-/-}$ enriched "lymphoid" subcluster.**

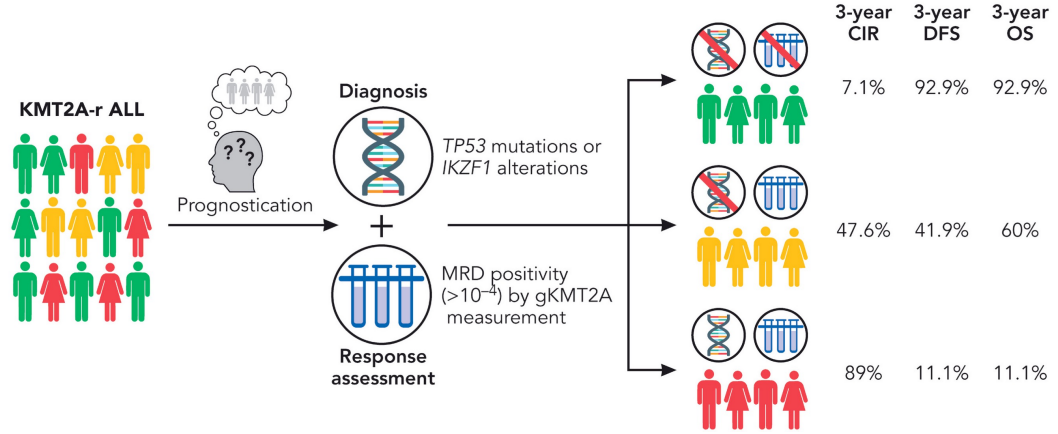
Distinct diagnostic entities within *BCR::ABL1*-positive acute lymphoblastic leukemia (ALL) are currently defined by the International Consensus Classification of myeloid neoplasms and acute leukemias (ICC): "lymphoid only", with *BCR::ABL1* observed exclusively in lymphatic precursors, vs "multilineage", where *BCR::ABL1* is also present in other hematopoietic lineages. Here, we analyzed transcriptomes of 327 *BCR::ABL1*-positive patients with ALL (age, 2-84 years; median, 46 years) and identified 2 main gene expression clusters reproducible across 4 independent patient cohorts. Fluorescence in situ hybridization analysis of fluorescence-activated cell-sorted hematopoietic compartments showed distinct *BCR::ABL1* involvement in myeloid cells for these clusters (n = 18/18 vs n = 3/16 patients; *P* < .001), indicating that a multilineage or lymphoid *BCR::ABL1* subtype can be inferred from gene expression. Further subclusters grouped samples according to cooperating genomic events (multilineage: *HBS1L* deletion or monosomy 7; lymphoid: *IKZF1*$^{-/-}$ or *CDKN2A/PAX5* deletions/hyperdiploidy). A novel *HSB1L* transcript was highly specific for *BCR::ABL1* multilineage cases independent of *HBS1L* genomic aberrations. Treatment on current German Multicenter Study Group for Adult ALL (GMALL) protocols resulted in comparable disease-free survival (DFS) for multilineage vs lymphoid cluster patients (3-year DFS: 70% vs 61%; *P* = .530; n = 91). However, the *IKZF1*$^{-/-}$ enriched lymphoid subcluster was associated with inferior DFS, whereas hyperdiploid cases showed a superior outcome. Thus, gene expression clusters define underlying developmental trajectories and distinct patterns of cooperating events in *BCR::ABL1*-positive ALL with prognostic relevance.

Other chimeric fusion oncoproteins and cryptic rearrangements



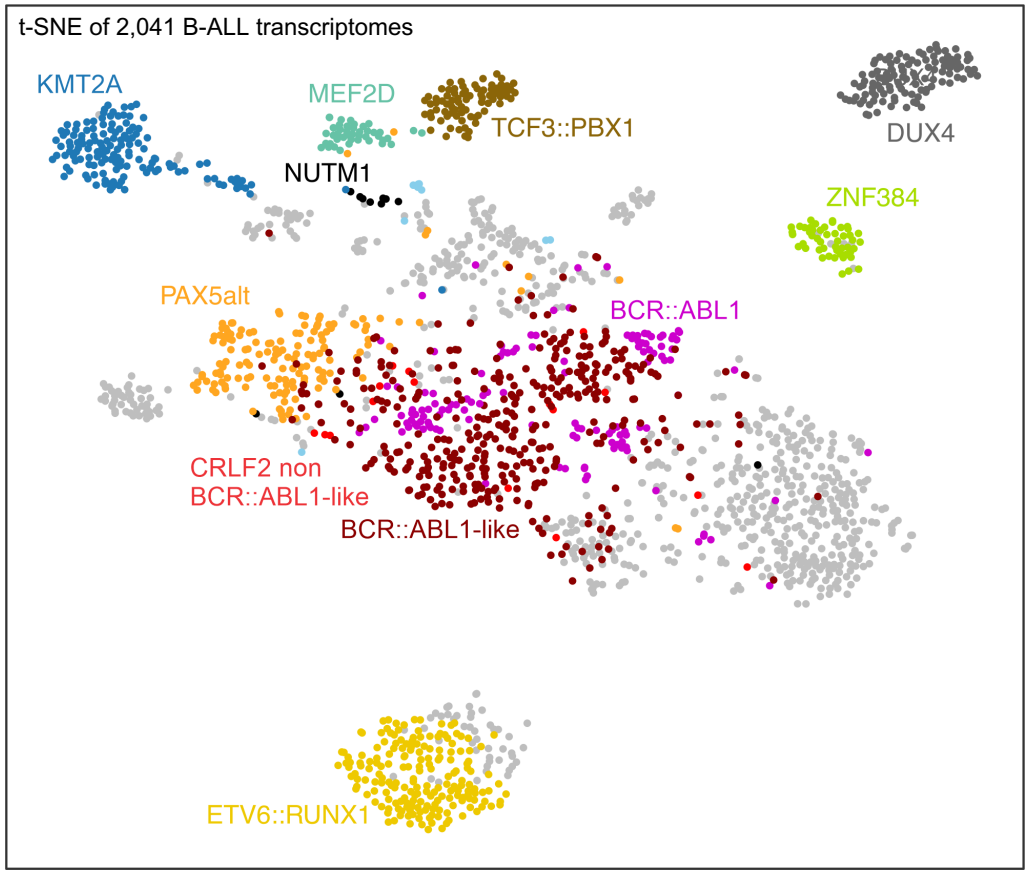
B-ALL with t(v;11q23.3)/KMT2A-Rearrangements

- 5% to 10% of adult BCP-ALL
- Historically dismal outcomes in infants and adults
- display lineage ambiguity and retain myeloid potential
- prognostic importance of specific comutations, namely TP53 point mutations and IKZF1 deletions, as well as postinduction MRD, enabling the identification of distinct KMT2A-r ALL subgroups with varying outcomes



Kim R, *Blood* 2023

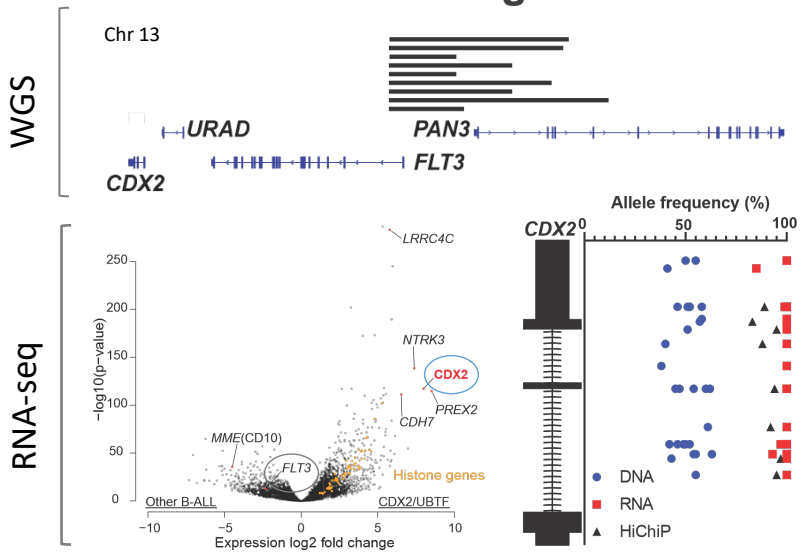
Other chimeric fusion oncoproteins and cryptic rearrangements



- B-ALL with t(v;11q23.3)/KMT2A-rearrangements
- B-ALL with t(12;21)(p13.2;q22.1)/ETV6::RUNX1
- B-ALL with t(1;19)(q23;q13)/TCF3::PBX1
- B-ALL with t(5;14)(q31.1;q32.3)/*IL3::IGH*
- ZNF384-r
- DUX4-r
- MEF2D-r
- NUTM1-r
- MYC-r
- CDX2/UBTF

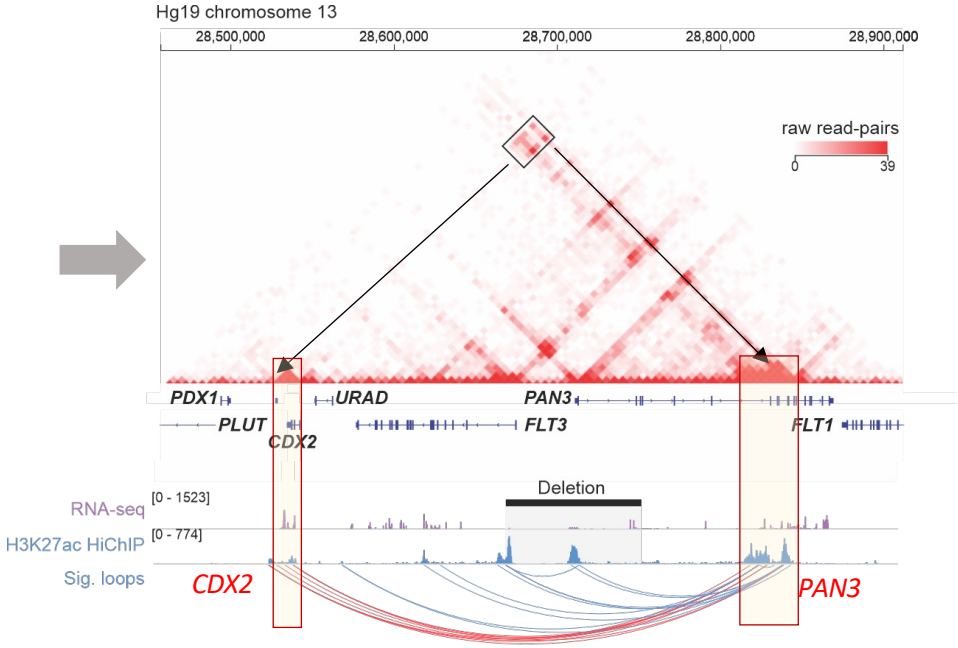
PAN3/CDX2, UBTF::ATXN7L3 (‘CDX2/UBTF’) B-ALL: enhancer hijacking

1. *CDX2* Deregulation



- **Focal deletion** at chr **13q12.2** including the PAN3 gene and the promoter region and exon 1 of *FLT3*
- Low *FLT3* expression
- *CDX2* upregulation

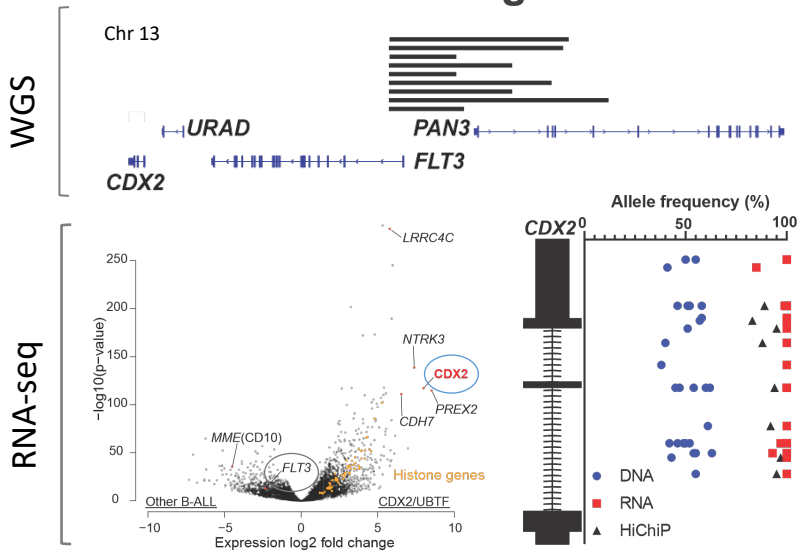
Kimura et al Blood 2022; Bastian et al. Leukemia 2022; Passet et al. Blood 2022



Chromatin looping between the enhancer in *PAN3* and *CDX2*

PAN3/CDX2, UBTF::ATXN7L3 (‘CDX2/UBTF) B-ALL: enhancer hijacking

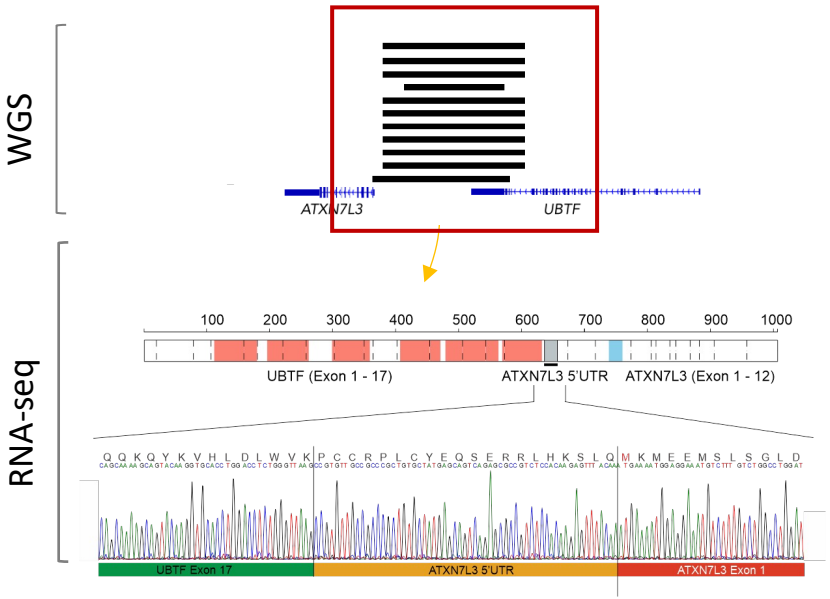
1. *CDX2* Deregulation



- **Focal deletion** at chr **13q12.2** including the promoter region and exon 1 of *FLT3*
- Low *FLT3* expression
- *CDX2* upregulation (homeobox gene)

Kimura et al Blood 2022; Bastian et al. Leukemia 2022; Passet et al. Blood 2022

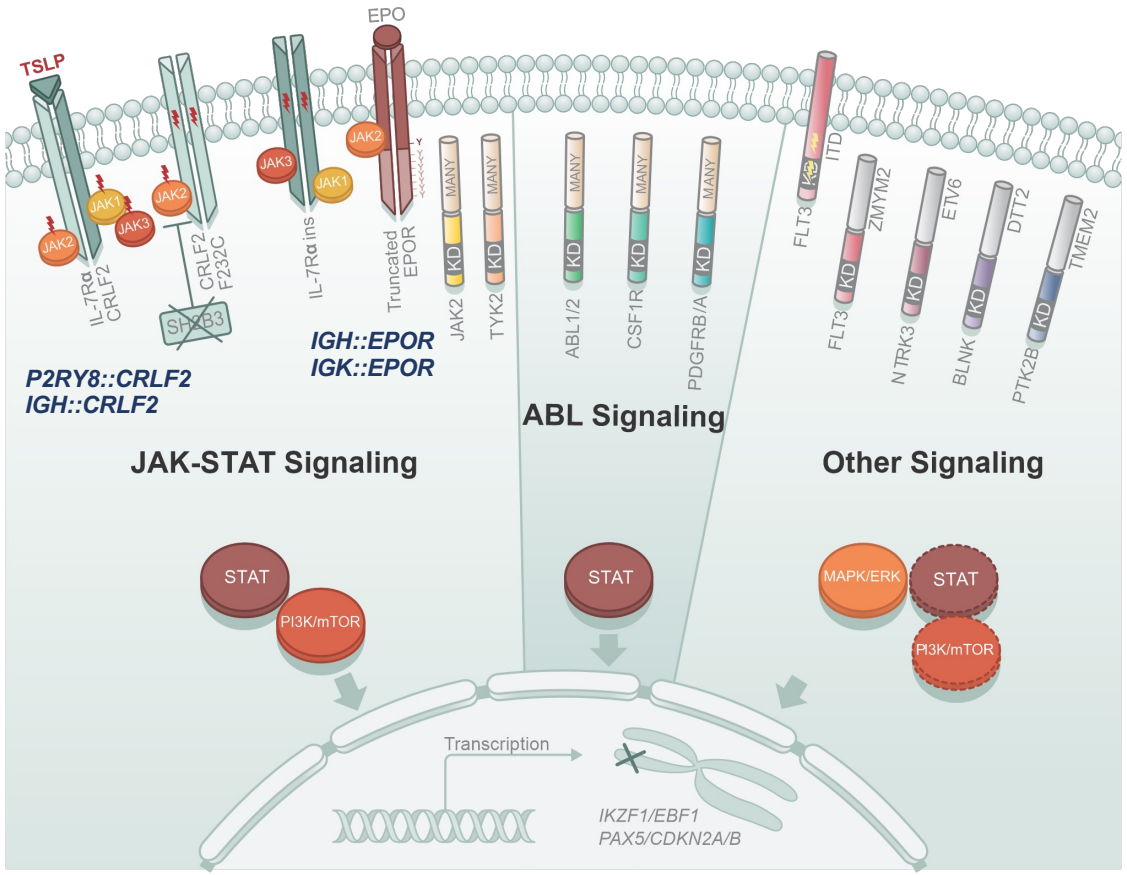
2. *UBTF::ATXN7L3*



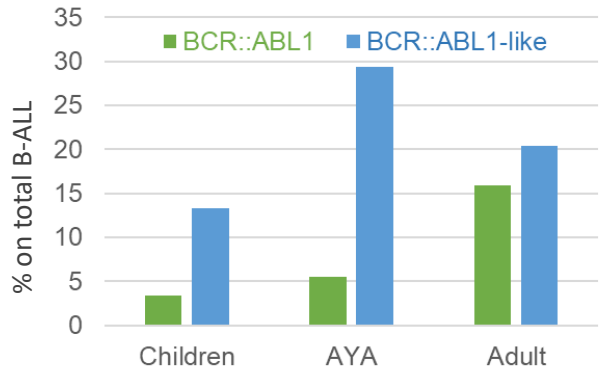
- Focal deletion at chr 17q21.3
- Chimeric fusion *UBTF::ATXN7L3*
- UBTF: ribosomal DNA transcription; ATXN7L3 transcriptional regulator

- Distinct pattern of additional alterations, including 1q duplications and CXCR4 mutations
- Very poor response to treatment

Phenocopies: Ph-like ALL

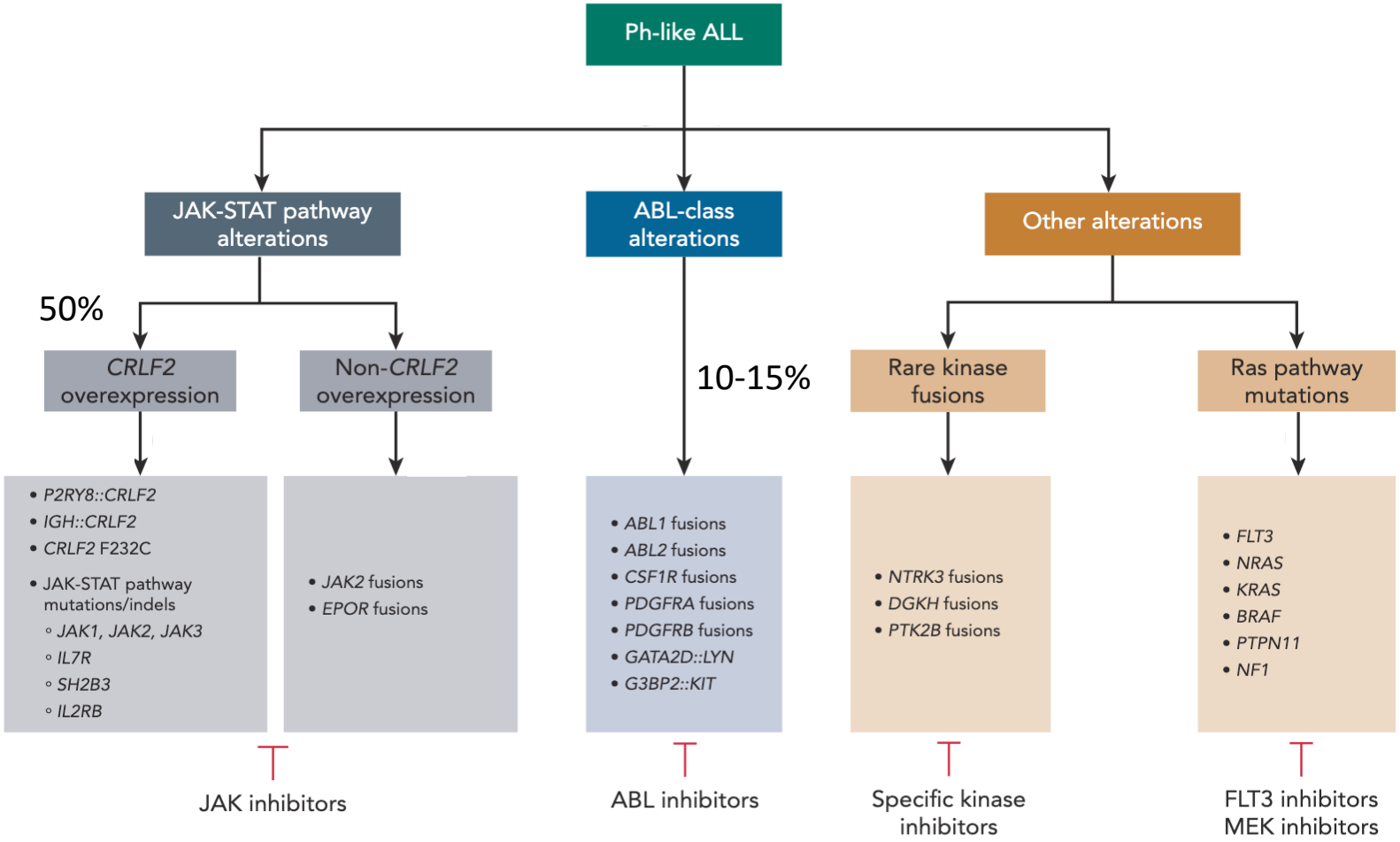


- Similar gene expression profile of BCR::ABL1 ALL but lacking the pathognomonic fusion
- > 60 genetic alterations in kinases and cytokine receptors driving constitutively active kinase signaling
- *IKZF1* deletions, poor outcome (but some variability based on the exact genetic lesion)



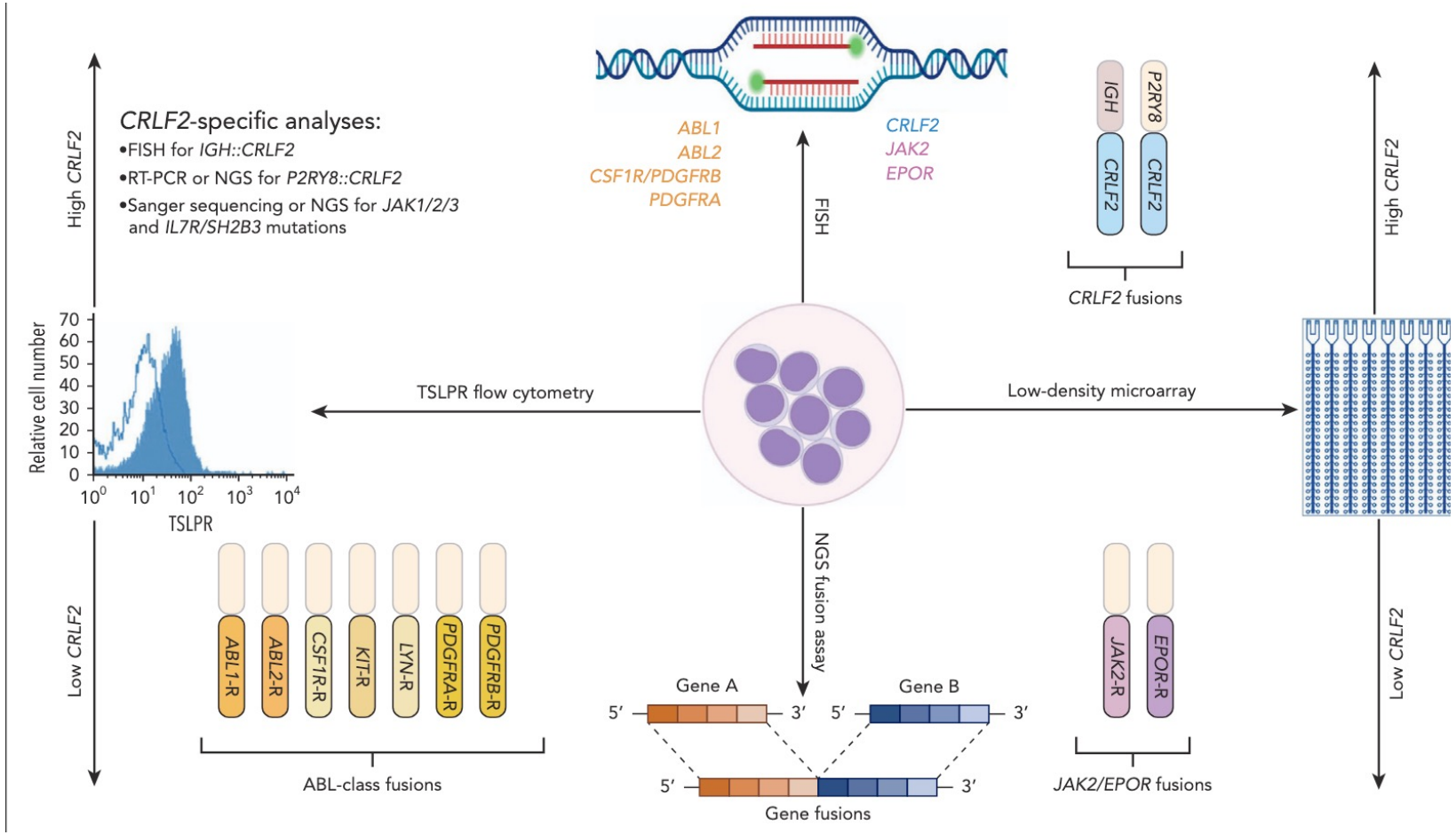
Modified from Iacobucci I, Mullighan CG. *JCO* 2017; Mullighan *NEJM* 2009; Den Boer ML, et al. *Lancet Oncol* 2009; Roberts *Cancer Cell* 2012; Roberts *NEJM* 2014; Iacobucci *Cancer Cell* 2016; Roberts *JCO* 2017; Iacobucci *Genes* 2021

Phenocopies: Ph-like ALL



Tran TH and Tasian SK, Blood 2025

Diagnostics of Ph-like ALL



Tran TH and Tasian SK, Blood 2025

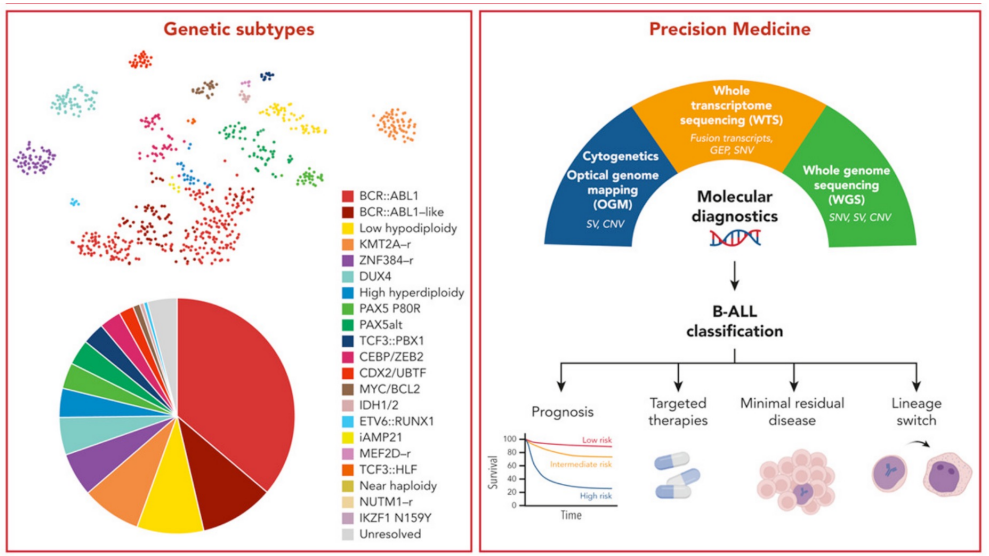
Risk stratification of B-ALL

	FAVORABLE	INTERMEDIATE	UNFAVORABLE
PEDIATRIC	ETV6::RUNX1 High hyperdiploid DUX4-rearranged NUTM1-rearranged	TCF3::PBX1 PAX5alt iAMP21 Hypodiploid ZNF384-rearranged PAX5 P80R	BCR::ABL1 BCR::ABL1-like ETV6::RUNX1-like KMT2A-rearranged MEF2D-rearranged
ADOLESCENTS and ADULTS	ETV6::RUNX1**/-like** **TCF3::PBX1** High hyperdiploid DUX4-rearranged **PAX5 P80R** ZNF384-rearranged	PAX5alt **MEF2D-rearranged**	BCR::ABL1 BCR::ABL1-like KMT2A-rearranged **Hypodiploid** **BCL2/MYC** **CDX2/UBTF**

Courtesy of Ilaria Iacobucci

Conclusions

- Bulk WTS and WGS and, lately, single cell (proteo)genomics approaches have greatly advanced our undestanding of the molecular heterogeneity of B-ALL, providing key information regarding pathogenesis, prognosis, susceptibility to therapeutic targeting, mechanisms of therapeutic escape
- This has transformed the classification and will transform the management of B-ALL, underscoring the integral role of molecular profiling
- Due to the heterogeneous genomic landscape of ALL, genome-wide sequencing, either as WTS, WGS or both, is optimal to identify all alterations with diagnostic and prognostic significance at once



Passet M, Kim R and Clappier E, *Blood* 2025