LEUKEMIA2020-2021

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ASSOCIAZIONE ITALIANA CONTRO LE LEUCEMIE-LINFOMI E MIELOMA ONLUS

New insights in the biology of ALL

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GITMO

SIES

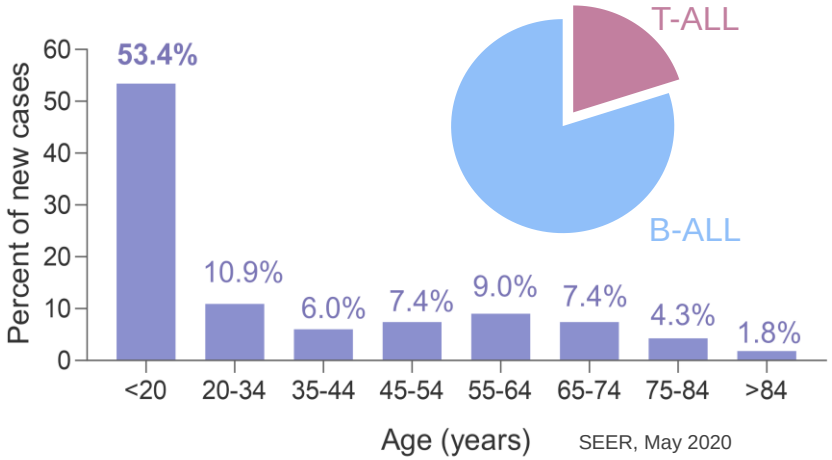
SIE - Società Italiana di Ematologia

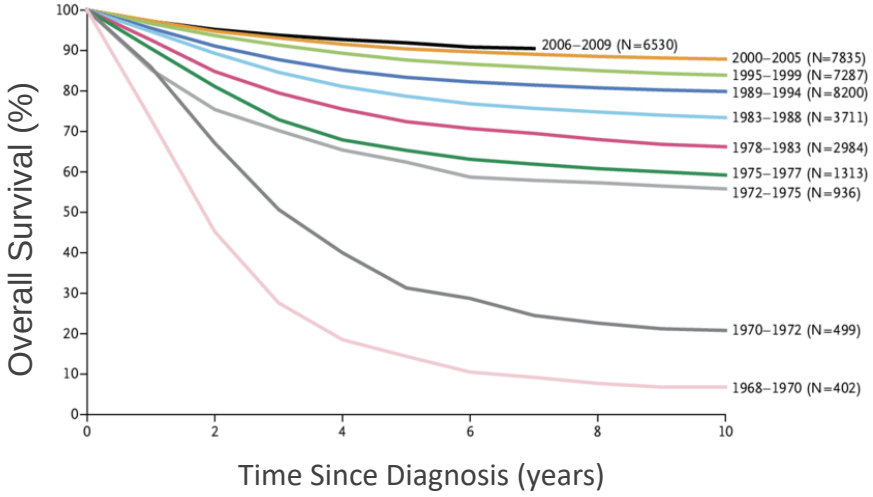
Conflicts of interest

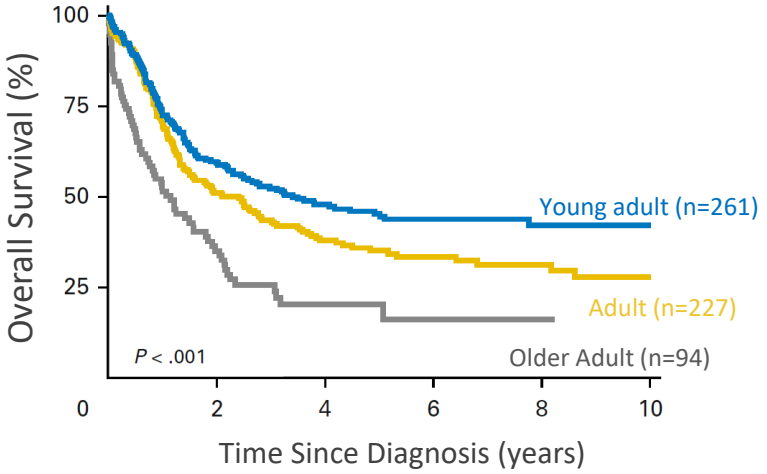
- Honoraria from AMGEN, Mission Bio

Acute lymphoblastic leukemia

- The commonest childhood tumor
- B- or T-lineage (B lineage ~80%)
- Cure rates > 90% in children but < 50% in adults

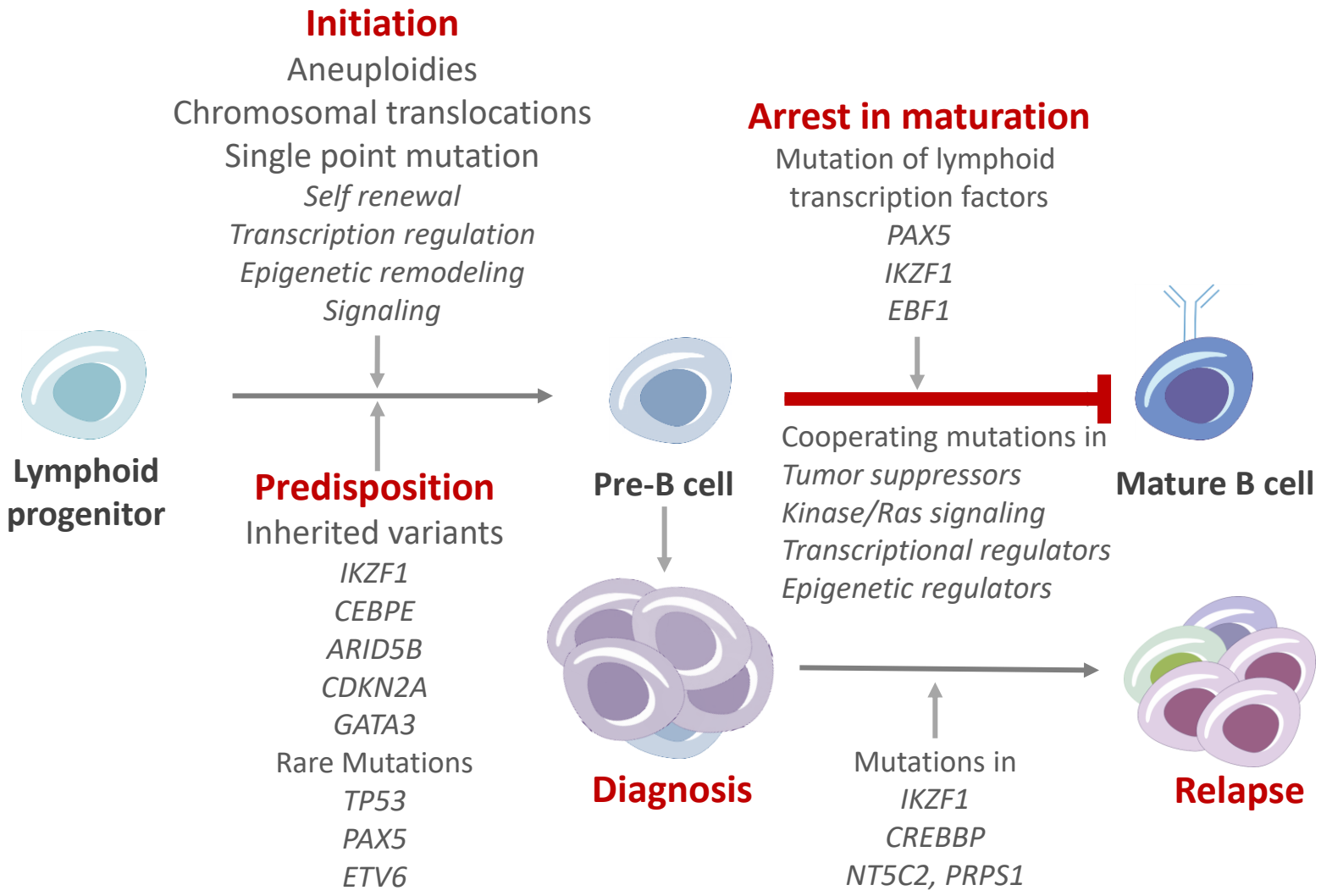






Mullighan, NEJM 2015; Roberts, JCO 2016

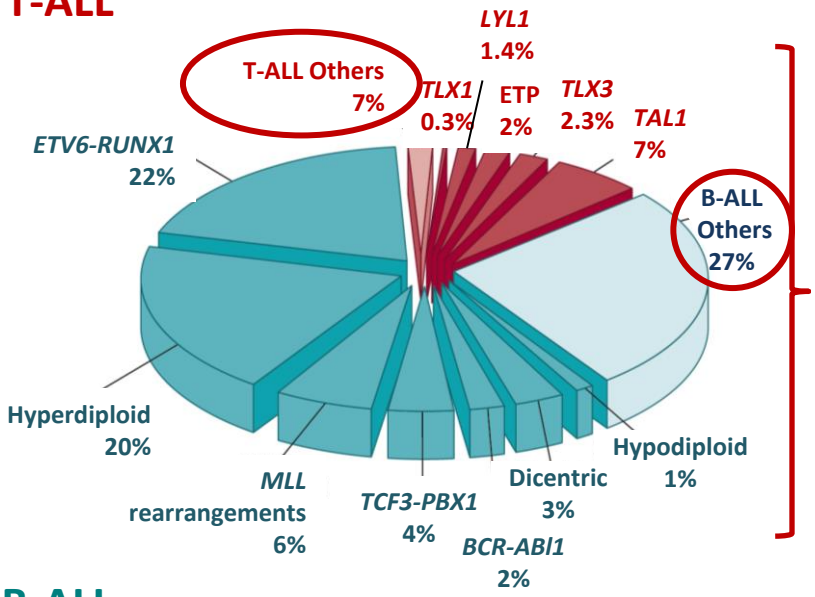
Stepwise genetic evolution of ALL

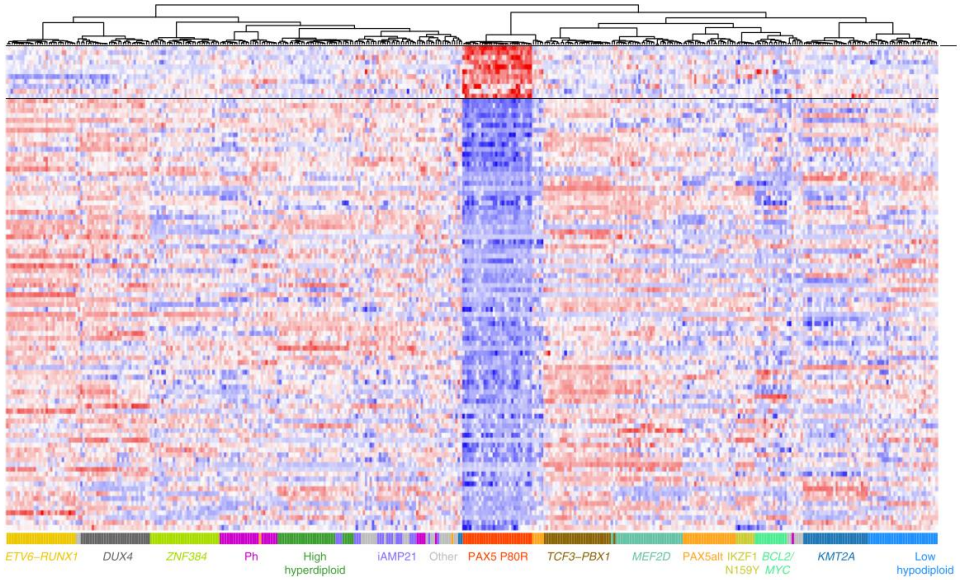


N Engl J Med. 2015,373(16); J Clin Oncol. 2017,35(9); Best Pract Res Clin Haematol. 2019, 32(4)

Classification of ALL post "genomics"

T-ALL





B-ALL

Cytogenetics, FISH, targeted assays

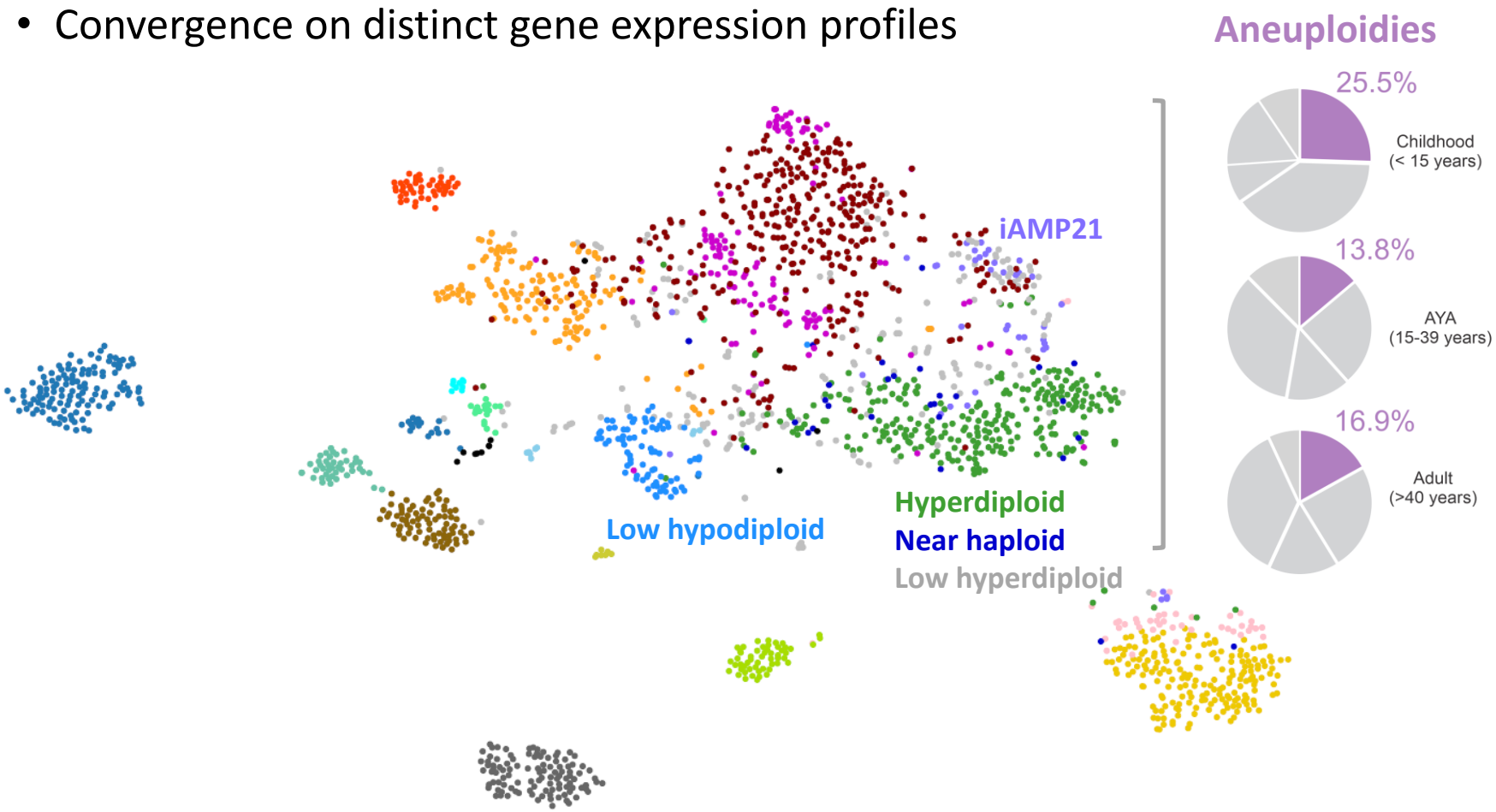
- Aneuploidy
- Chromosomal rearrangements/gene fusions (*ETV6-RUNX1*, *BCR-ABL*, *TCF3-PBX1*)
- *MLL* (*KMT2A*) rearrangements

Genome-wide seq (transcriptome/exome)

- Cryptic rearrangements
- Diverse rearrangement partners to a single gene
- "Phenocopies" of subtypes

Gene expression classification of B-ALL

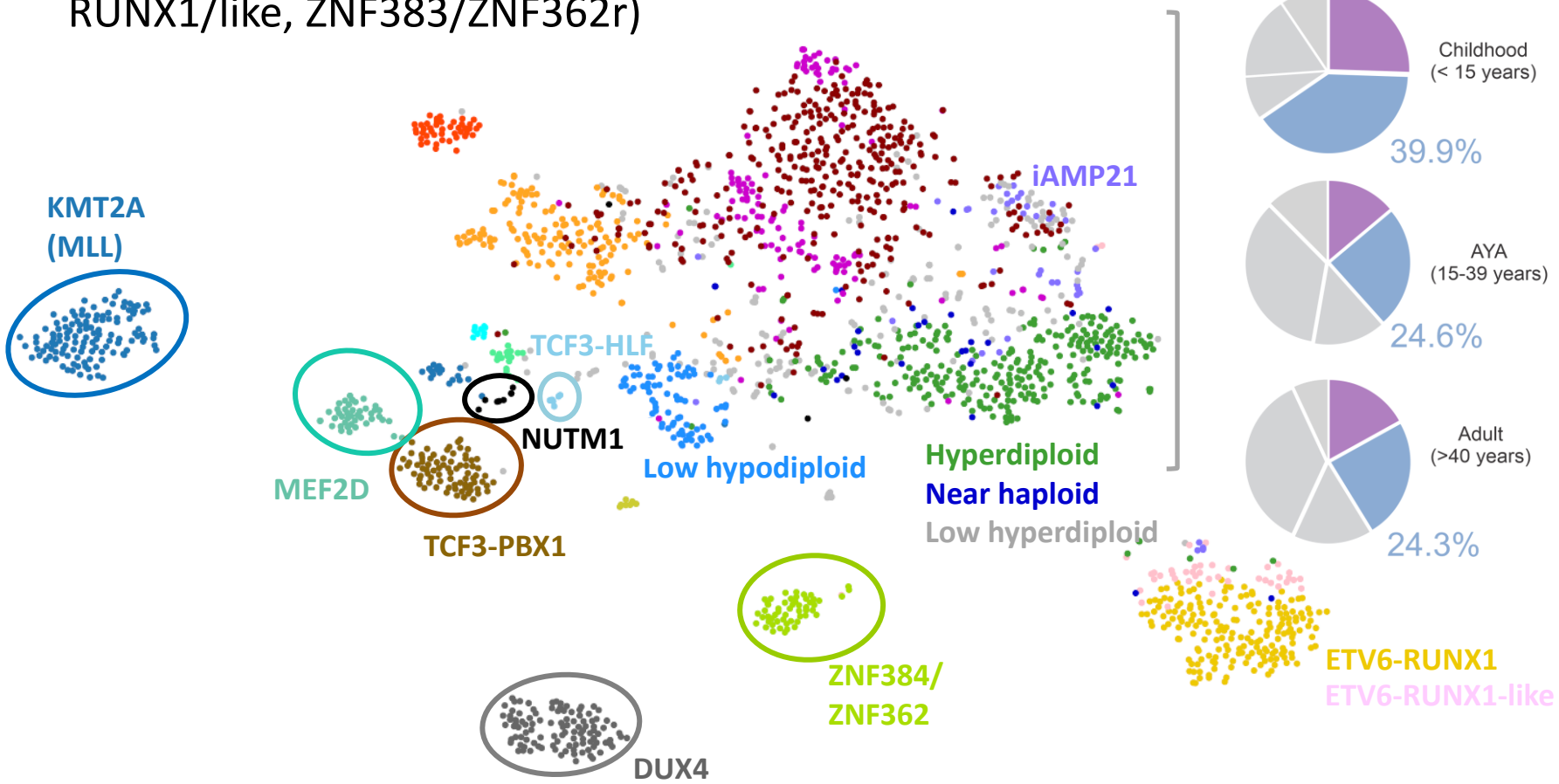
- > 20 subtypes defined by constellations of genetic alterations
- Convergence on distinct gene expression profiles



tSNE analysis of 1988 ALL transcriptomes
1402 childhood, 208 young adult, 378 adult, Gu et al. Nat Genet 2019

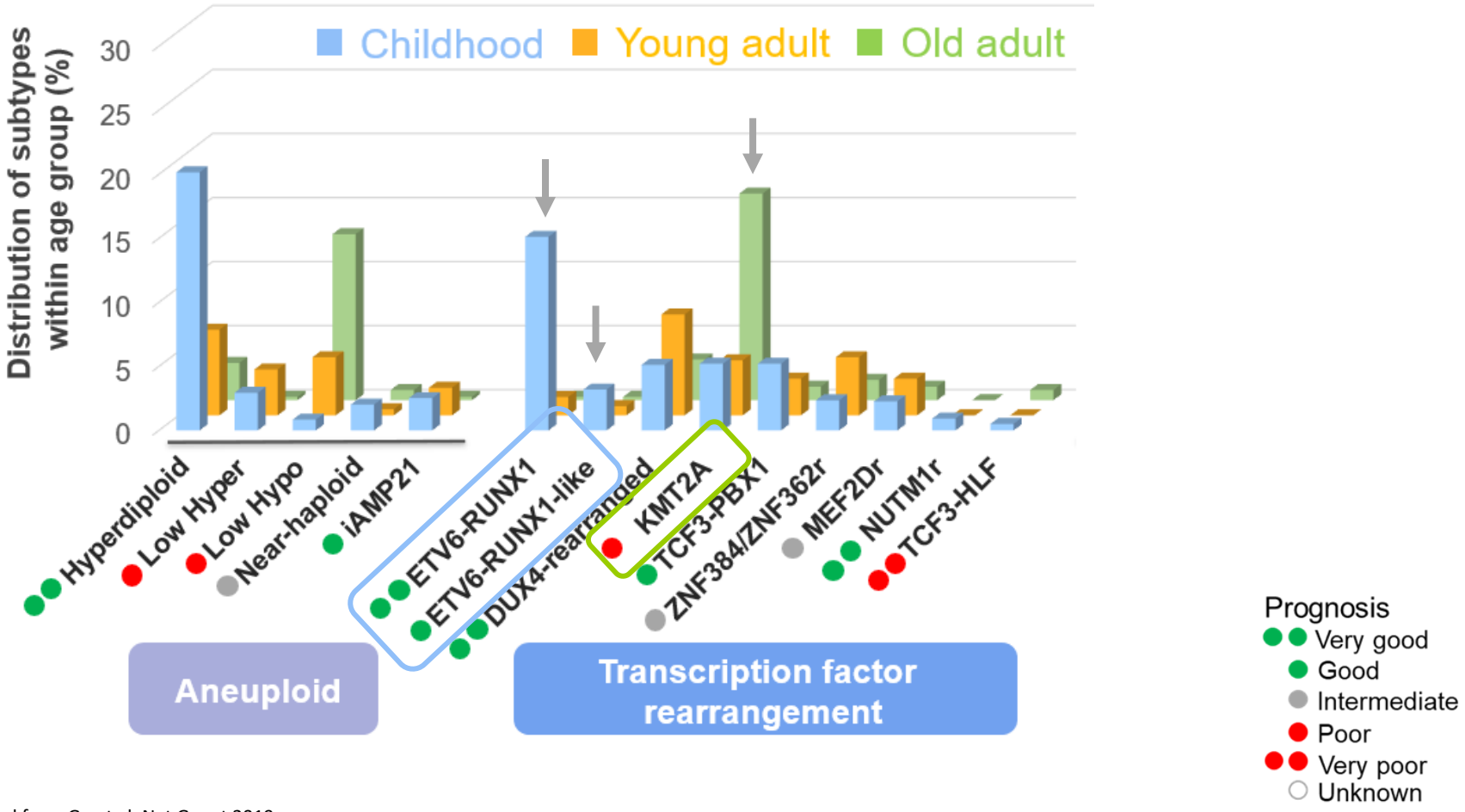
Gene expression classification of B-ALL

- Transcription factor rearrangements (KMT2Ar, MEF2Dr, TCF3-PBX1, TCF3-HLF, NUTM1r, DUX4r, ETV6-RUNX1/like, ZNF383/ZNF362r)



tSNE analysis of 1988 ALL transcriptomes
1402 childhood, 208 young adult, 378 adult, Gu et al. Nat Genet 2019

Molecular classification of B-ALL

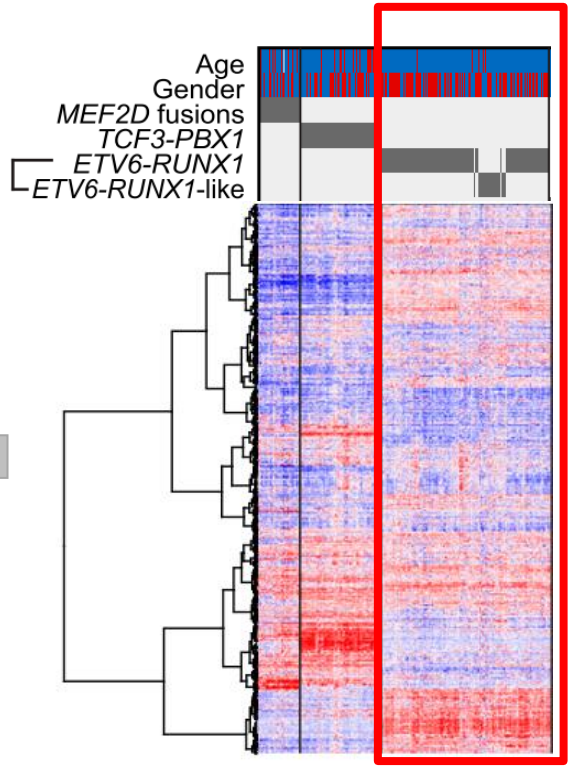


Updated from Gu et al. Nat Genet 2019

ETV6-RUNX1-like ALL subtype

- Similar gene expression profile and immunophenotype to *ETV6-RUNX1+*, but lacking the *ETV6-RUNX1* fusion

- Common *ETV6* and *IKZF1* fusions



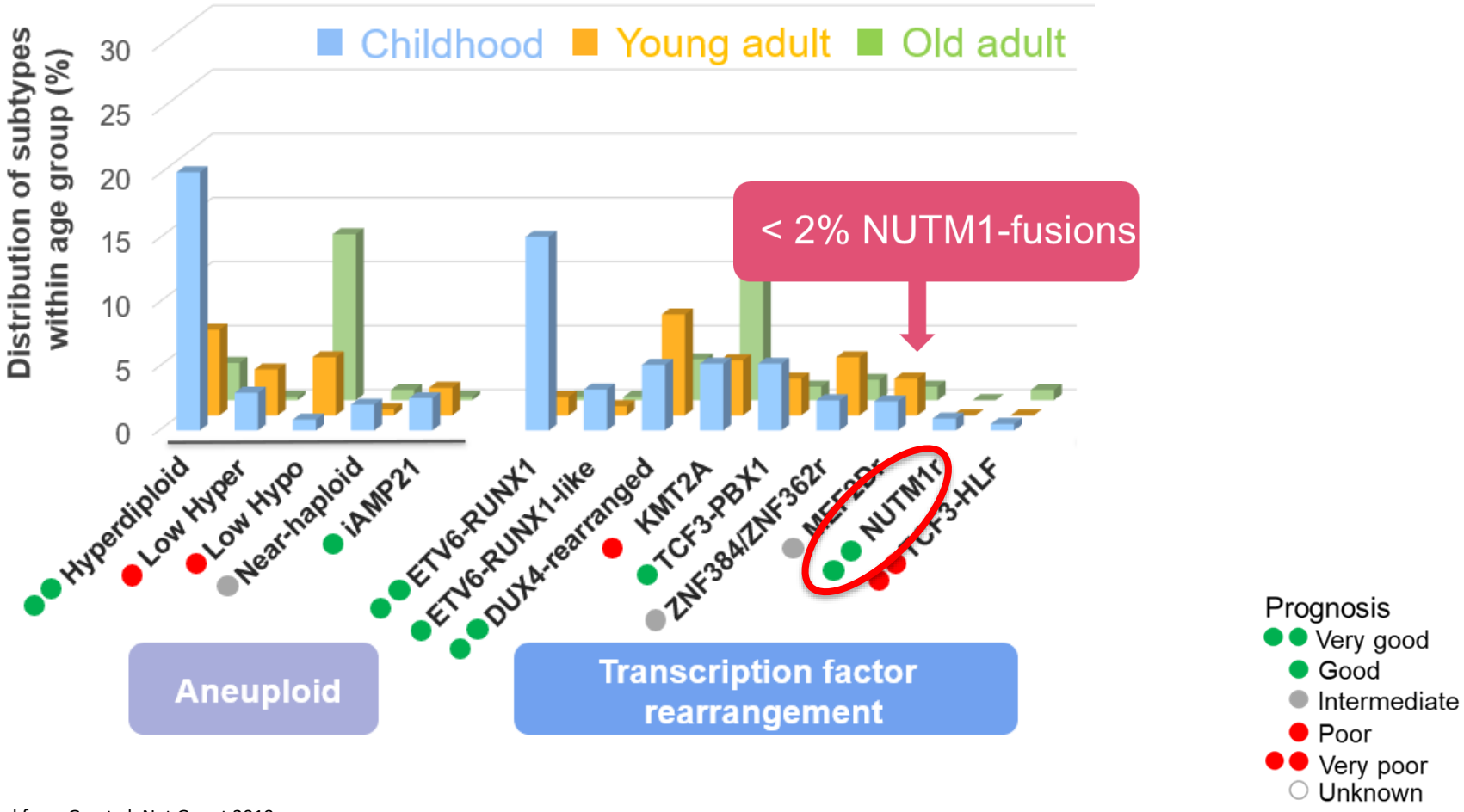
ETV6-RUNX1 like fusions

- *CASC15-ETV6*
- *ETV6-AMPH*
- *ETV6-ELMO1*
- *ETV6-EXTL1*
- *ETV6-LHFPL3-AS2*
- *ETV6-RNU6-19P_locA*
- *ETV6-SLC30A7*
- *ETV6-SRRM1*
- *ETV6-STYK1*
- *IKZF1-CLNK*
- *IKZF1-ETV6*
- *IKZF1-ZPBP*
- *STIM2-IKZF1*
- *TCF3-FLI1*

Global deregulation of lymphoid development

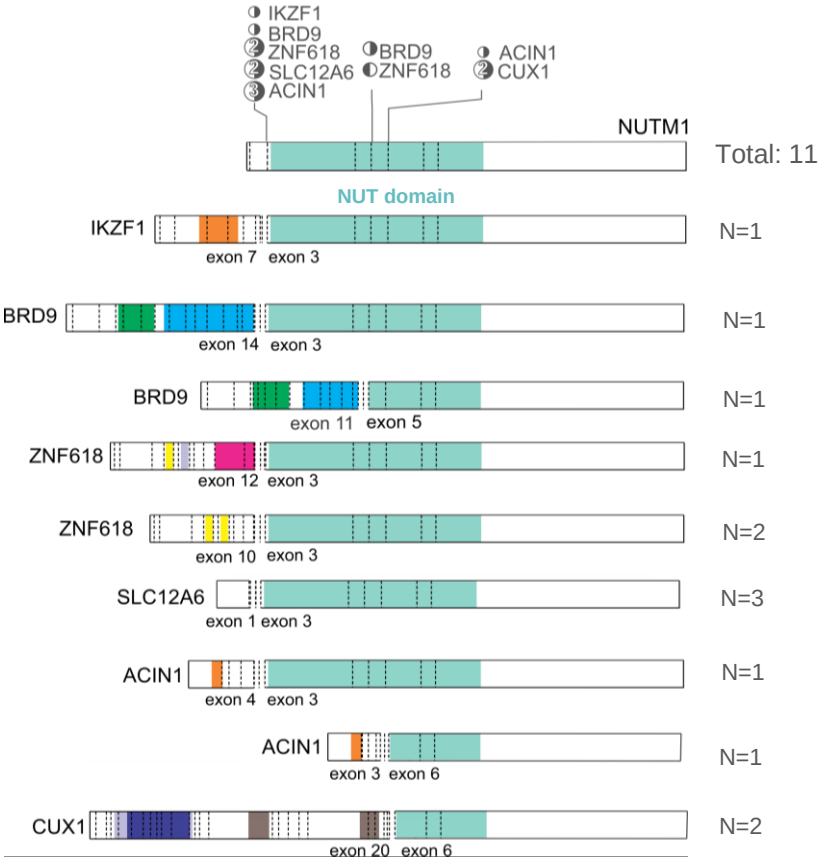
Lilljebjörn H, et al. Nat Commun 2016
Li JF et al. PNAS 2018
Gu et al. Nat Genet 2019

Molecular classification of B-ALL

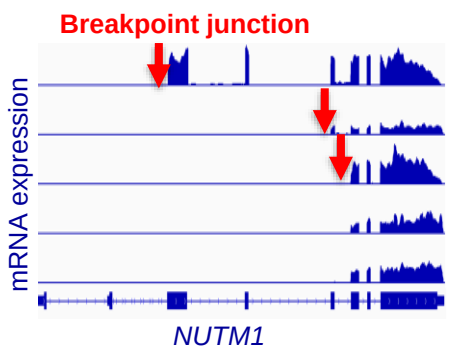


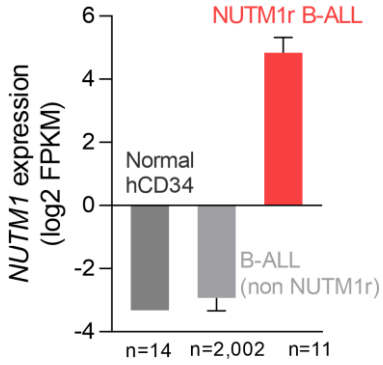
Updated from Gu et al. Nat Genet 2019

NUTM1-rearrangements define a distinct B-ALL subtype



- 3' NUT Family Member 1 (NUTM1) fused to different partner genes that drive its expression



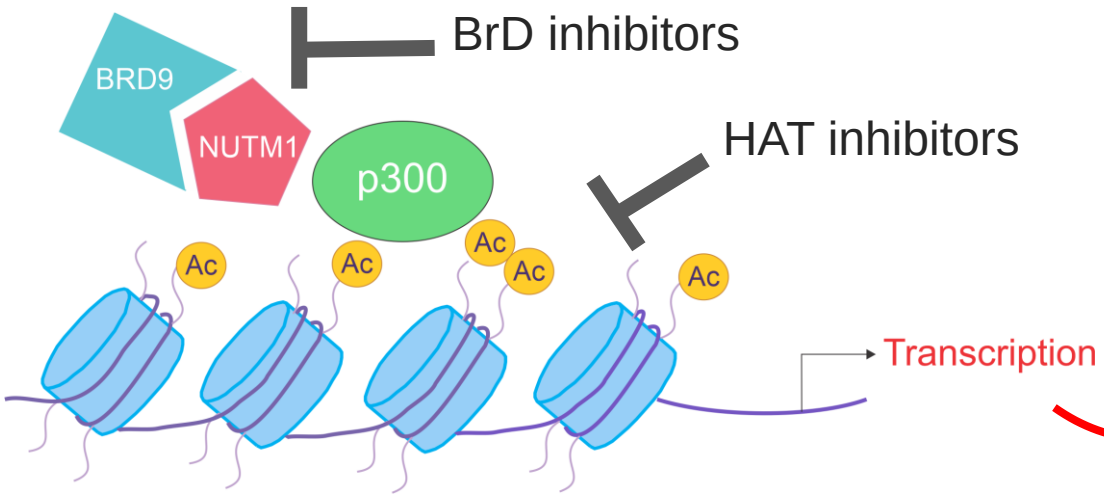


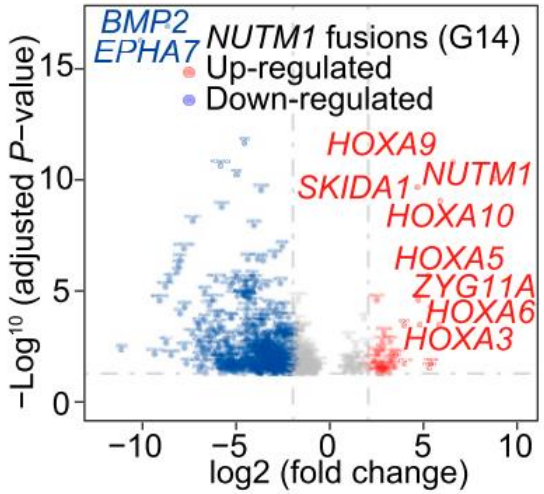
- *NUTM1* is normally NOT expressed in hematopoietic cells nor in other B-ALL cases
- Expression is normally restricted to post-meiotic spermatids
- BRD4-NUTM1 most common (~75%) fusion in NUT midline carcinoma
- Excellent prognosis

Li JF et al. PNAS 2018; Gu et al Nature Genetics 2019; https://pecan.stjude.cloud/proteinpaint/study/PanALL; Hormann FM et al. Haematologica 2019.

NUTM1-rearrangements define a distinct B-ALL subtype

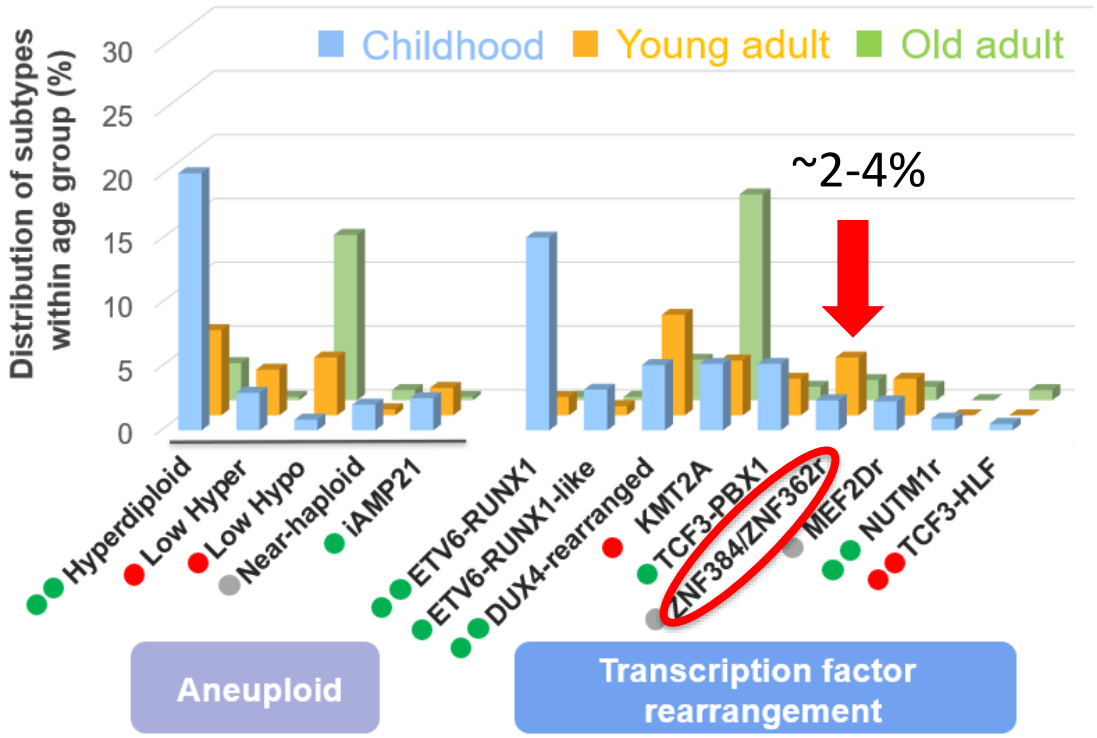
NUTM1 acts as a chromatin modifier by recruiting EP300 to increase local histone acetylation and potently activates transcription





Li JF et al. PNAS 2018
Gu et al. Nat Genet 2019

ZNF384-rearrangements



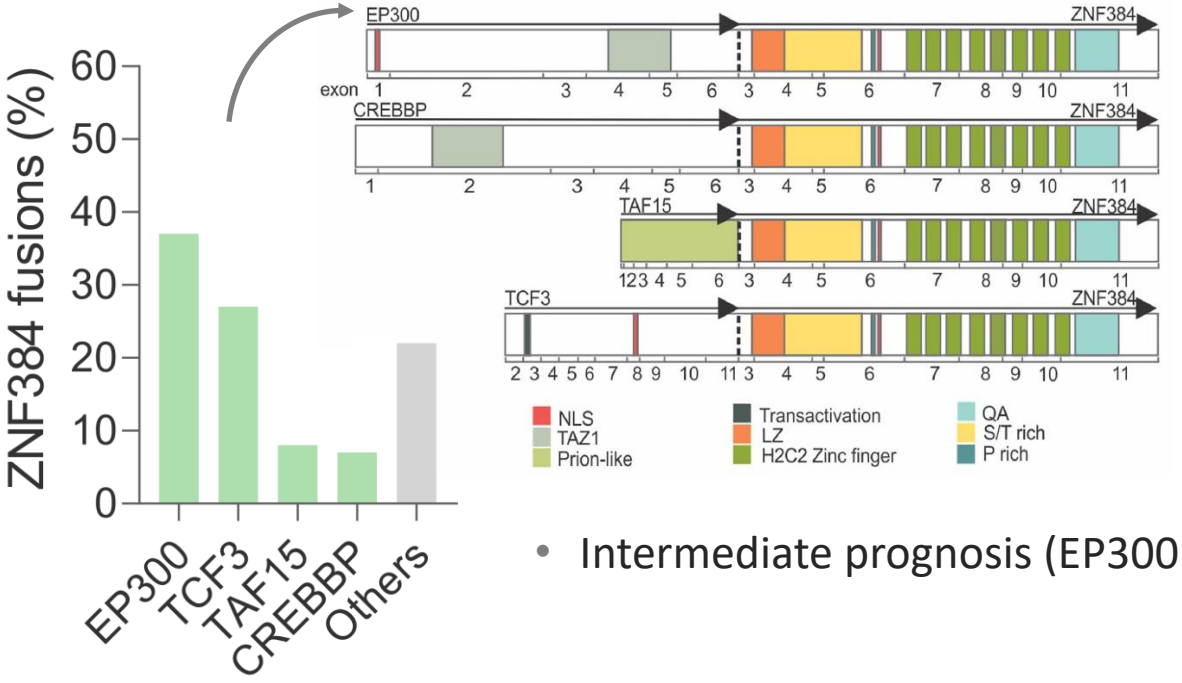
- Median age of presentation 9 years old (range, 1 - 25 years)
- Immunophenotype:
  classical pre-B ALL
  > 70% CD13 expression
  > 80% has CD33 expression
- 48% B/M mixed phenotype acute leukemia (MPAL)
- ZNF384/ZNF362 are C2H2-type transcription factors containing six zinc fingers

Updated from Gu et al. Nat Genet 2019

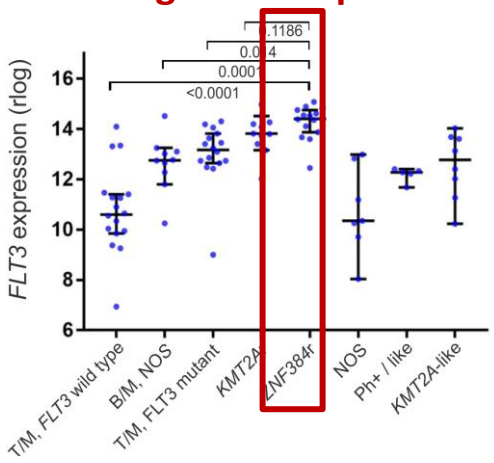
Li JF et al. PNAS 2018
Alexander T, Zhaohui G., Iacobucci I et al. Nature 2018
Gu et al Nature Genetics 2019
Ponte Di Legno Childhood ALL Working Group, Leukemia 2019

ZNF384-rearrangements

- Fusion partners include transcription factors and epigenetic regulators
- The zinc-finger domains are retained in all fusion proteins



High *FLT3* expression



- Intermediate prognosis (EP300 fusion better prognosis)

Alexander T, Zhaohui G., Iacobucci I et al. Nature 2018
Li JF et al. PNAS 2018
Gu et al Nature Genetics 2019
Ponte Di Legno Childhood ALL Working Group. Leukemia 2019

B-ALL with *PAX5* aberrations

PAX5-altered (**PAX5alt**)

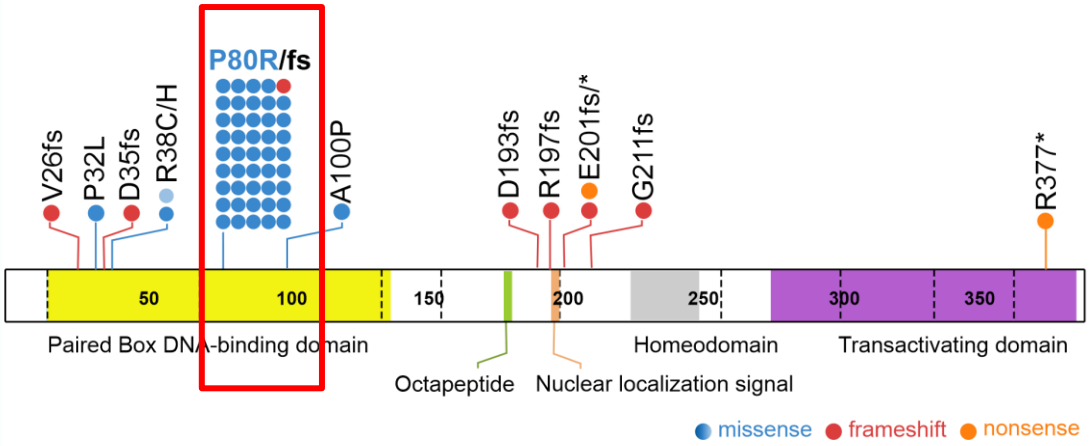
- ~7% B-ALL
- rearrangements, sequence mutations and focal intragenic amplifications
- > 20 **fusion partner genes** (most frequent *PAX5-ETV6*)
- high risk > standard risk

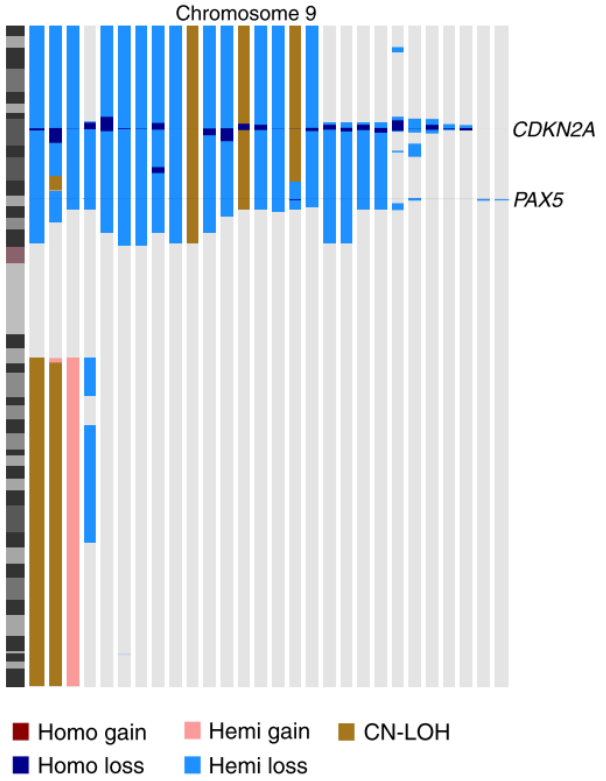
PAX5 P80R

- ~3% B-ALL
- distinct gene expression profile

PAX5 alterations: PAX5 P80R

- 68% have hemizygous or homozygous PAX5 p.Pro80Arg
- 32% harbor a second frameshift, nonsense or deleterious missense *PAX5* mutation



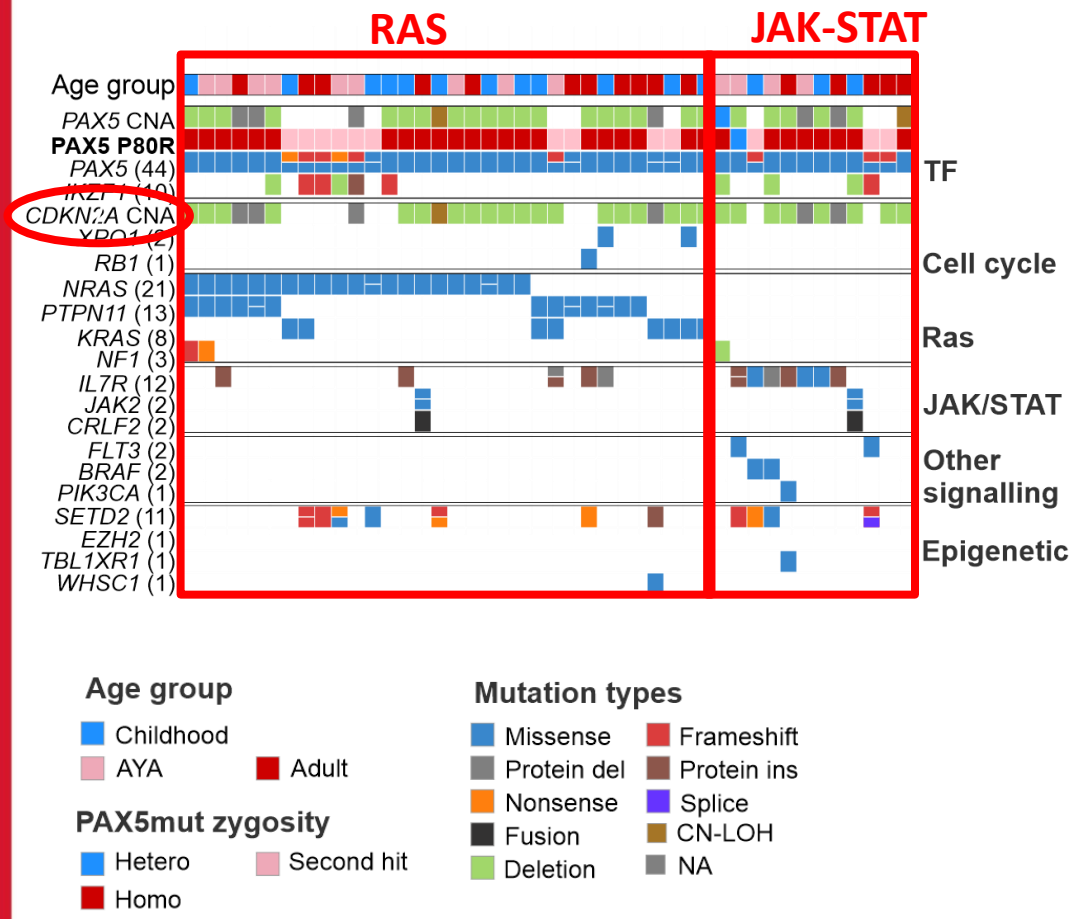


Biallelic PAX5 alterations are a hallmark of this subtype

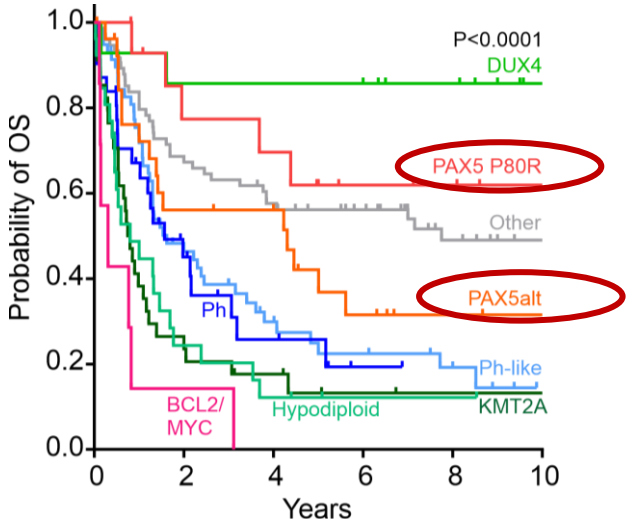
Passet M Blood 2018
Li JF et al. PNAS 2018
Gu et al. Nat Genet 2019
Bastian L. et al. Leukemia 2019

PAX5 alterations: PAX5 P80R

- > 95% cases harbor *CDKN2A* loss and signaling pathway mutations



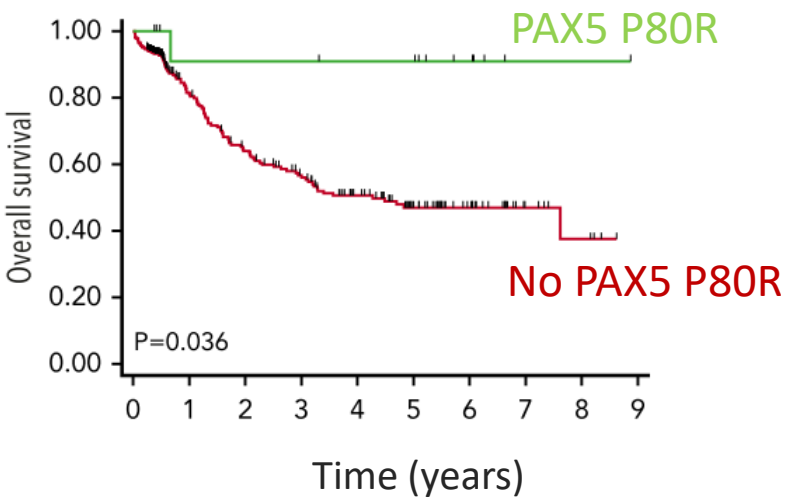
- PAX5 P80R and PAX5alt confer intermediate/favorable outcome in children and adults with B-ALL



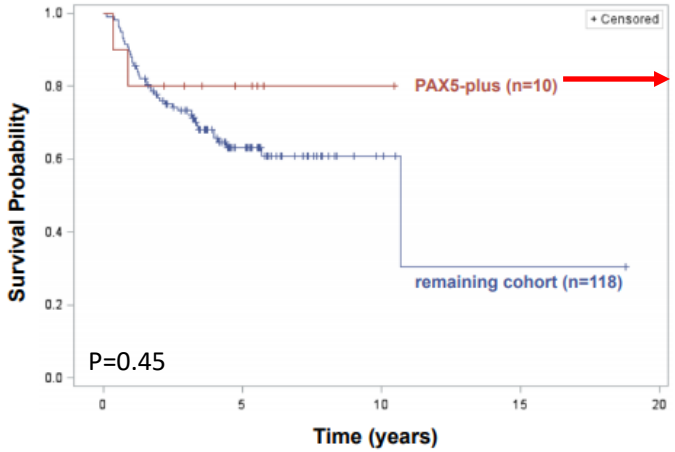
Gu et al. Nat Genet 2019

PAX5^{P80R} outcome in adult with Ph-negative BCP-ALL (GRALL and GMALL)

- ~5-7% PAX5 P80R in adult patients with newly diagnosed Ph-negative B-ALL
- **Favorable prognosis** with high-intensity, pediatric-inspired chemotherapy regimen



N=312 patients with Ph-negative BCP-ALL enrolled in the GRAALL-2003 and GRAALL-2005 trials

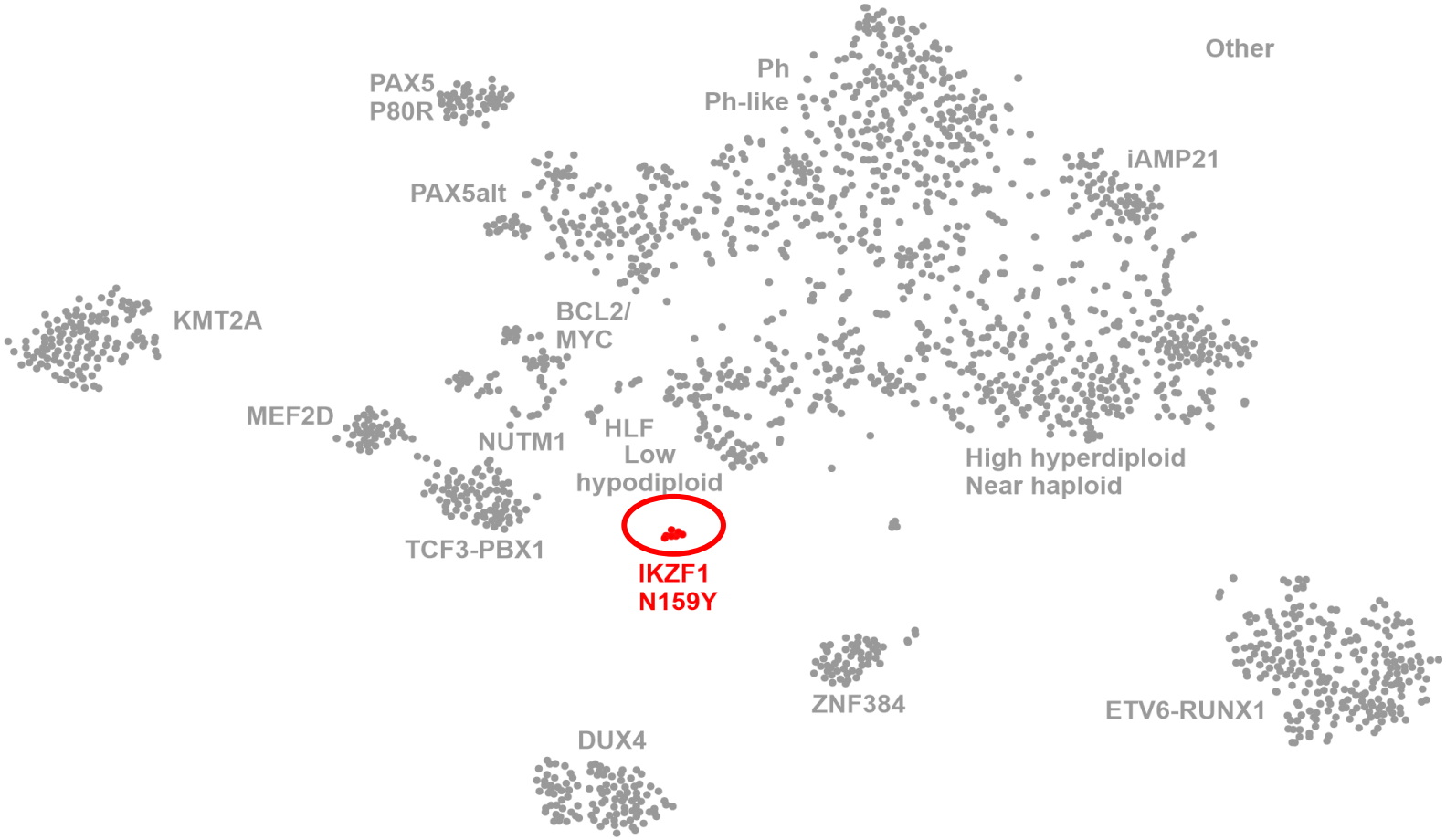


PAX5 plus
PAX5 P80R
NRAS/KRAS mut
CDKN2A deletion

N= 128 adult patients with B-ALL who received intensive treatment on subsequent GMALL trials

Bastian L et al. Leukemia 2019
Passet M Blood 2018

B-ALL Subtype defined by a single TF alteration

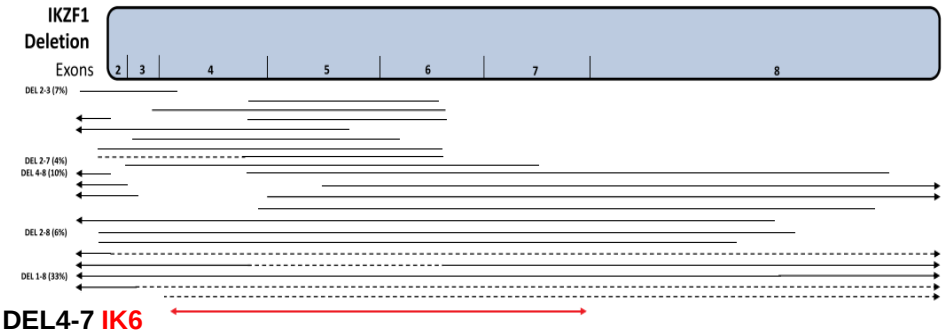


tSNE analysis of 1988 ALL transcriptomes
1402 childhood, 208 young adult, 378 adult, Gu et al. Nat Genet 2019

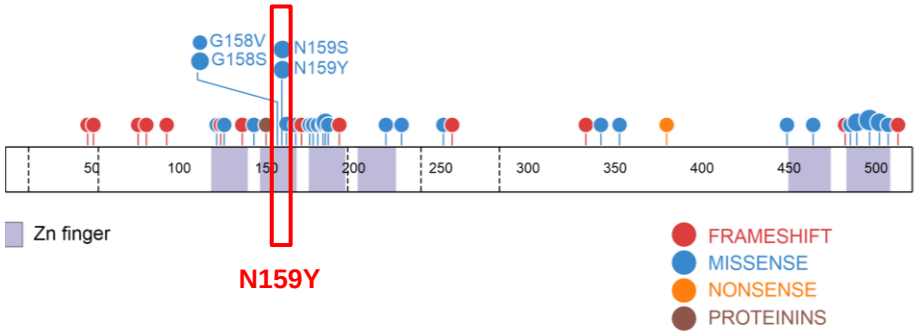
IKZF1 N159Y (~1% ALL)

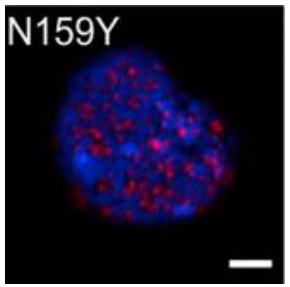
15% ALL *IKZF1* deletions (>50% high risk ALL)

Deletions

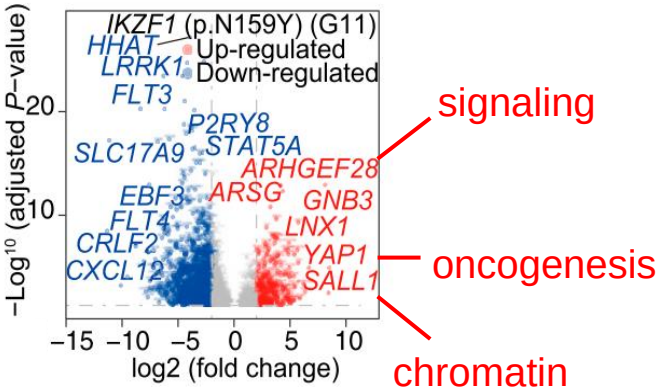


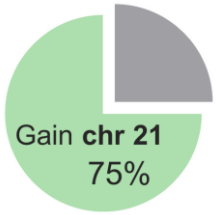
Mutations





Distinctive **nuclear mislocalization** and induction of aberrant intercellular adhesion

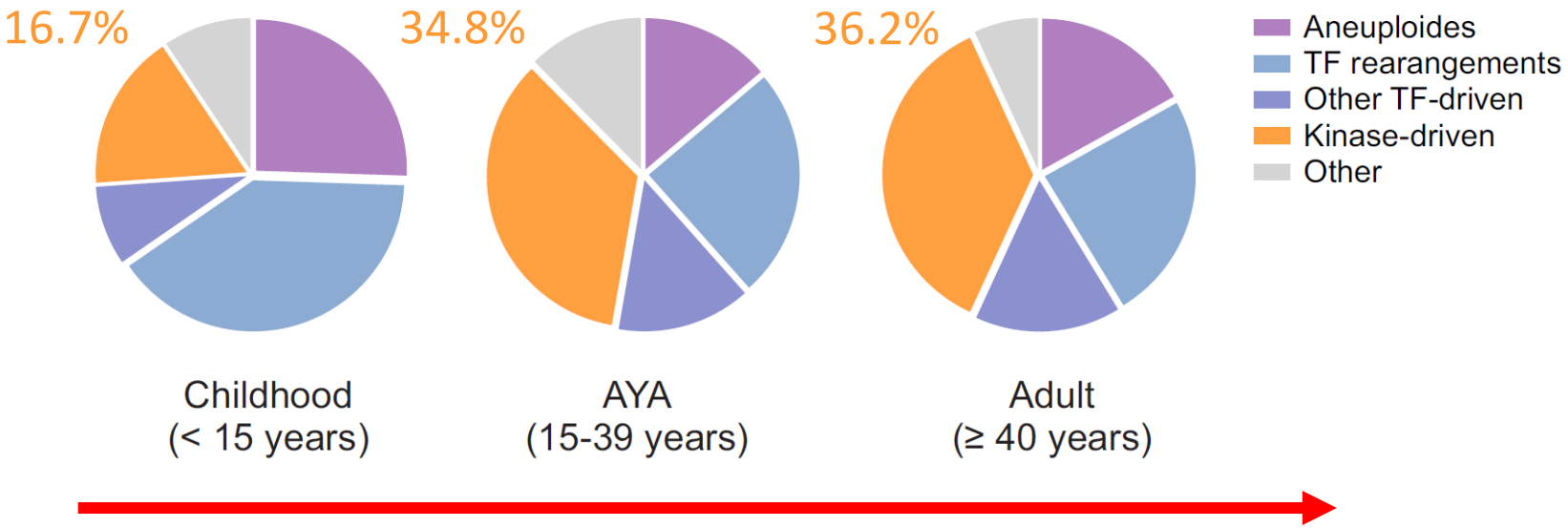




Churchman ML et al. Cancer Cell 2015
Li JF et al. PNAS 2018
Gu et al. Nat Genet 2019

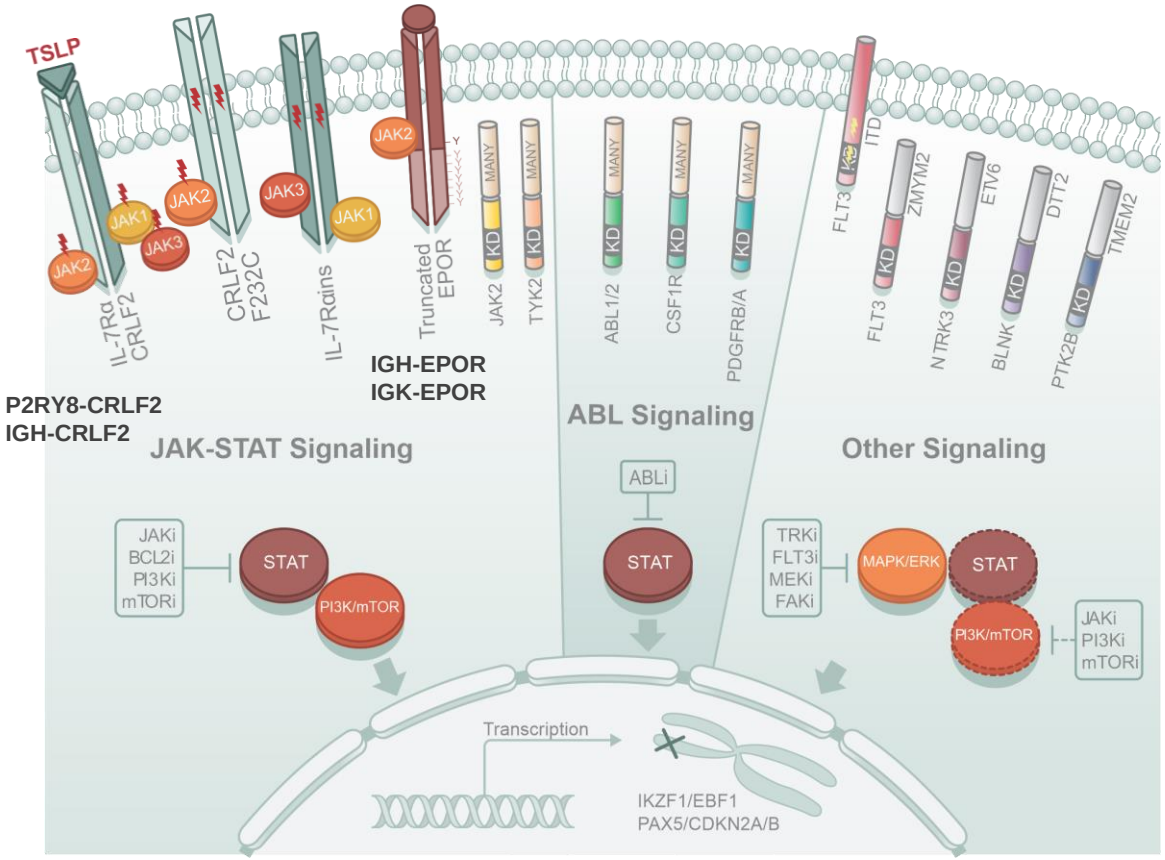
Mullighan CG et al NEJM 2009, Iacobucci I et al. Blood 2009, Vairy S et. al Blood Reviews 2020,
https://proteinpaint.stjude.org/

Activated kinase signaling-driven B-ALL



Ph-like acute lymphoblastic leukemia

- Gene expression profile similar to Ph+ (*BCR-ABL1*) ALL, but negative for *BCR-ABL1*
- Frequent alterations of *IKZF1*; poor outcome



- > 60 rearrangements in kinases and cytokine receptors
- Gene fusions activating kinases
- Mutations activating cytokine receptors
- Gene fusions hijacking cytokine receptor expression (*CRLF2*)
- Gene fusions hijacking and truncating cytokine receptor expression (*EPOR*)

Iacobucci I, Mullighan CG. JCO 2017

Mullighan NEJM 2009; Roberts Cancer Cell 2012; Roberts NEJM 2014; Iacobucci Cancer Cell 2016; Roberts JCO 2017

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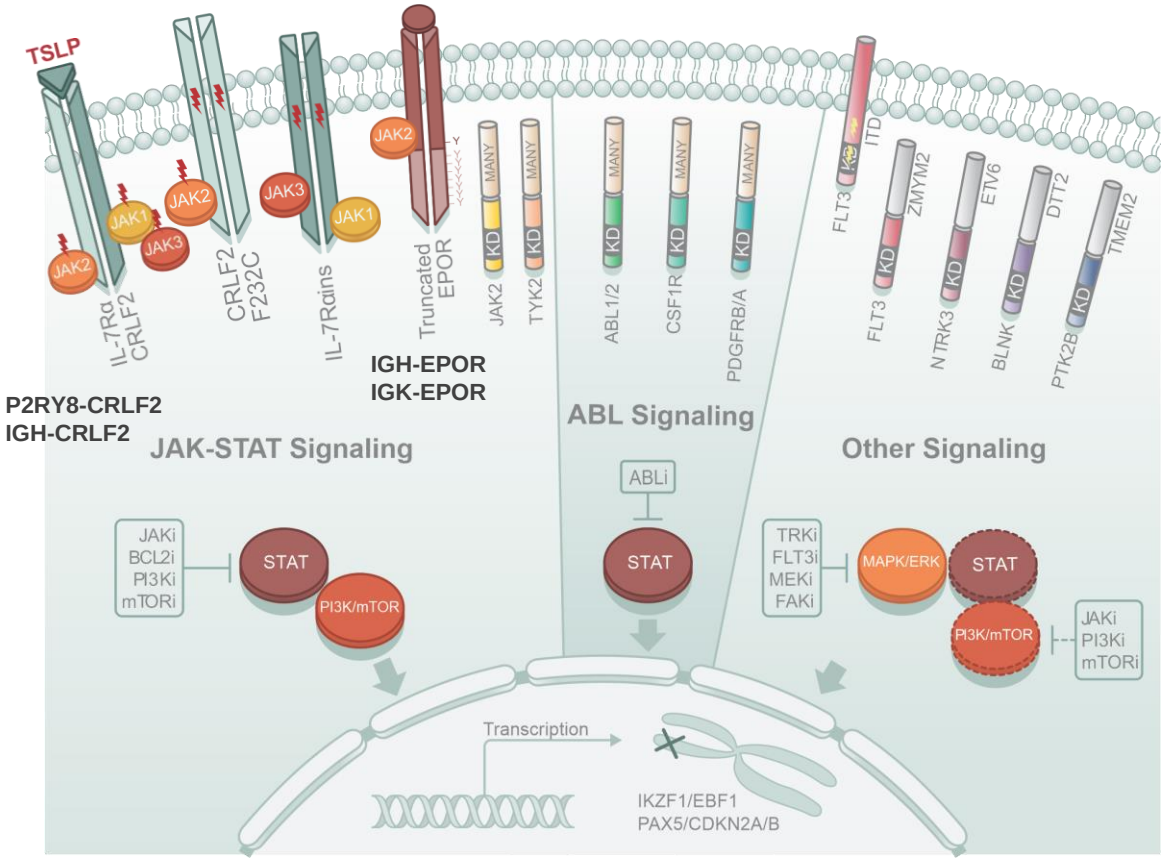


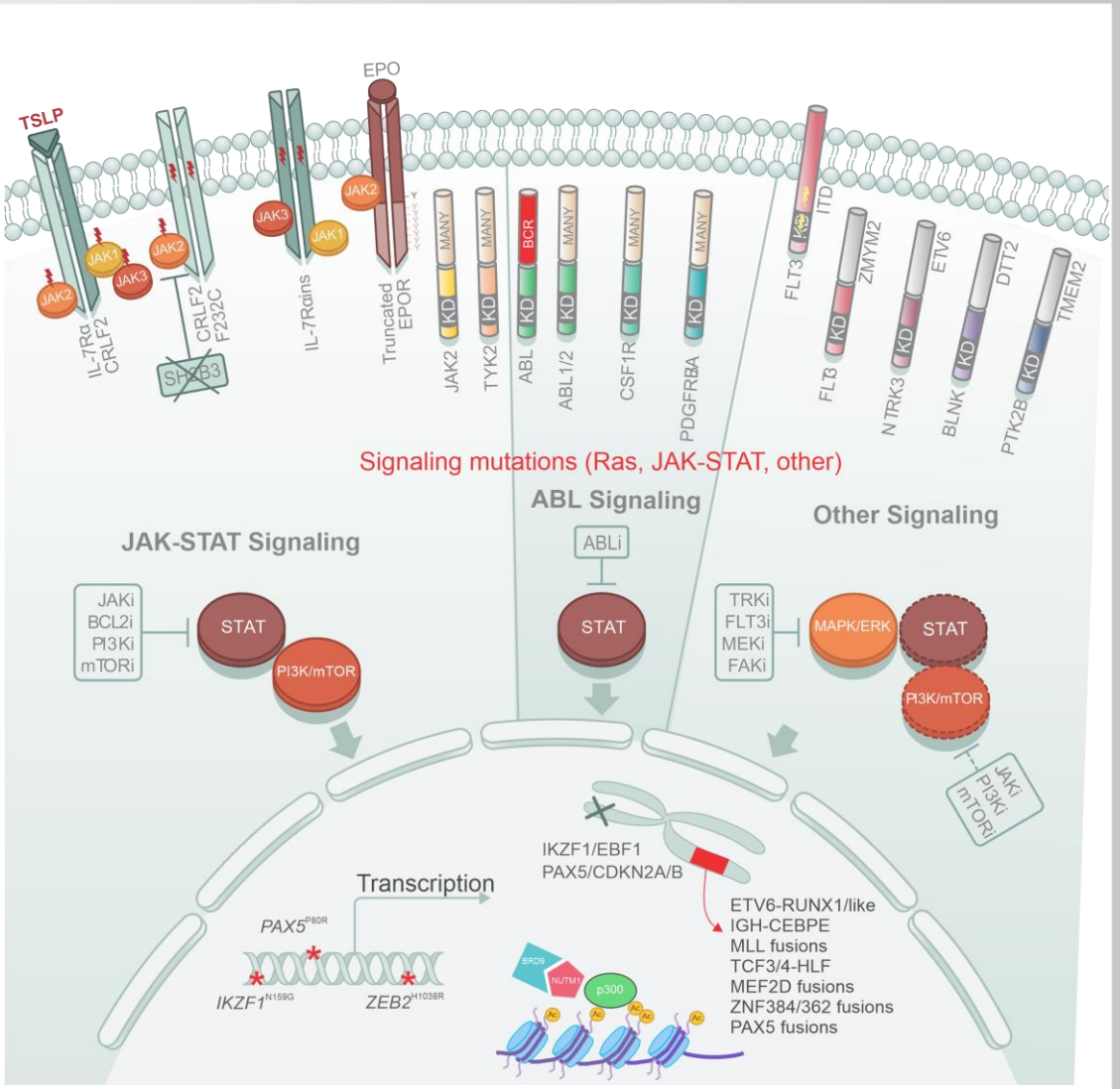
Iacobucci I, Mullighan CG. JCO 2017

Blinatumomab
Inotuzumab
CAR-T cells

Mullighan NEJM 2009; Roberts Cancer Cell 2012; Roberts NEJM 2014; Iacobucci Cancer Cell 2016; Roberts JCO 2017

Conclusions

- >20 age related B-ALL subtypes defined by constellations of mutations (disease-initiating + secondary cooperating lesions)
- Distinct gene expression profiles with prognostic and therapeutic significance
- Implementation of gene expression approaches into the clinical diagnostic workup of ALL



Modified from Iacobucci I, Mullighan CG. JCO 2017

Acknowledgments

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Charles G. Mullighan
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Yunchao Chang
Debbie Payne-Turner
Ashley Hill
Yaqi Zhao
Kirsten Dickerson
Thomas Alexander

St. Jude Children's Research Hospital - Shared Resources

Animal Resource Center (ARC), Chemical Biology & Therapeutics (CBT)
Flow Cytometry & Cell Sorting, Hartwell Center, Preclinical Pharmacokinetics

Genomic data portal: https://www.stjude.cloud/

Xenograft portal: PROPEL (Public Resource of Patient-derived and Expanded Leukemias)
https://www.stjude.org/research/resources-data/propel-public-resource-patient-derived-expanded-leukemias.html