Targetable fusions in acute lymphoblastic leukemia/lymphoma

Molecular Diagnostics: Current Roles in Cancer Diagnosis and Management
Harvard Medical School
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DANA-FARBER CANCER INSTITUTE

Boston Children's Hospital
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Acute lymphoblastic leukemia

- Pediatric ALL
  - Most common childhood malignancy: 25% of all cancer diagnoses
  - ~3000 cases annually
  - Approximately 85% B-ALL and 15% T-ALL
- Adult ALL
  - Less common cancer type
  - Slightly less than 3000 cases annually
  - <1% of all adult cancer diagnoses
  - Approximately 75% B-ALL and 25% T-ALL

History

Treatment for pediatric ALL is a success story of modern oncology

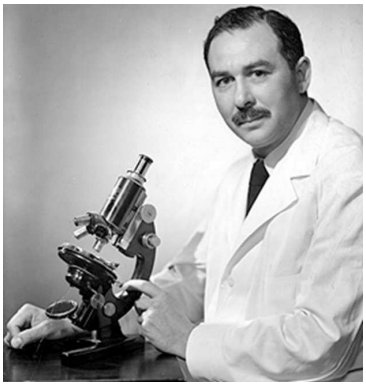
- 1950s: fatal
- Today: ~90% survival for children
  - While overall survival has improved, ALL is still one of the leading causes of cancer deaths in children

Adult survival has also improved

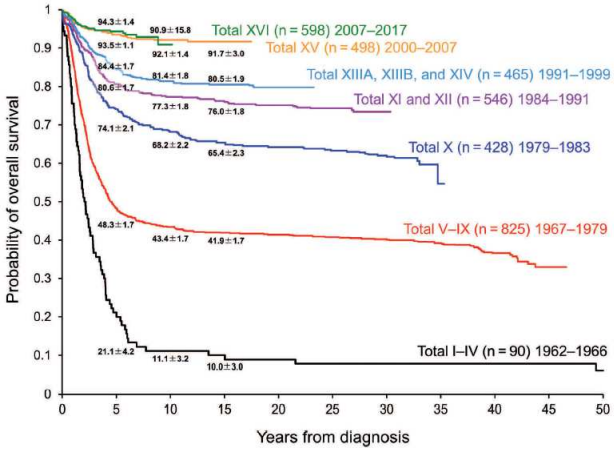
- ~40-50% survival for adults (age dependent)
- AYA patients benefit from pediatric-inspired regimens

Progress through improved understanding of ALL biology

- Advances in diagnosis, prognosis and therapy



Outcome improvement in pediatric lymphoblastic leukemia



Risk-adapted therapy

Intensification and increased duration

CNS directed therapy

Combination chemotherapy

Inaba H, Mullighan CG. Haematologica. 2020 Nov 1;105(11):2524-2539.

What does “targetable” mean?

- Diagnosis
- Prognosis
- Therapy

Diagnosis

Classification of acute leukemias is increasingly complex

- French-American-British classification of 1976
  - Morphology
  - Cytochemistry
- WHO classifications of 2001 and 2008
  - Morphology
  - Clinical features
  - Immunophenotype
  - Genetics
- 2016 revision of WHO classification
  - Includes substantial new genetic knowledge

2016 WHO classification of ALL

13 subtypes of lymphoblastic leukemia, including

- 10 types of B-ALL, including 2 new provisional subtypes
  - B-ALL, BCR-ABL1-like
  - B-ALL with iAMP21
- 1 new provisional subtype of T-ALL
  - Early T-cell precursor lymphoblastic leukemia
- New provisional entity of NK cell lymphoblastic leukemia

9 subtypes, all B-ALL, include specific genetic alterations

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

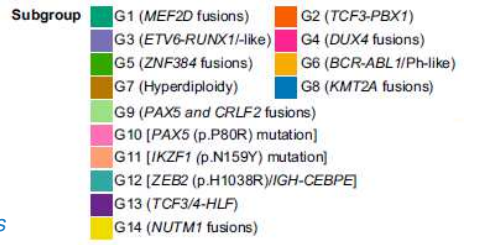
Arber DA et al. Blood. 2016 May 19;127(20):2391-405.

Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases

Jian-Feng Li[a,1], Yu-Ting Dai[a,1], Henrik Lilljebjörn[b,1], Shu-Hong Shen[c], Bo-Wen Cui[a], Ling Bai[a], Yuan-Fang Liu[a], Mao-Xiang Qian[d], Yasuo Kubota[e], Hitoshi Kiyoi[f], Itaru Matsumura[g], Yasushi Miyazaki[h], Linda Olsson[b], Ah Moy Tan[i], Hany Ariffin[j], Jing Chen[c], Junko Takita[k], Takahiko Yasuda[l], Hiroyuki Mano[m], Bertil Johansson[b,n], Jun J. Yang[d,o], Allen Eng-Juh Yeoh[p], Fumihiko Hayakawa[q], Zhu Chen[a,r,s,2], Ching-Hon Pui[o,2], Thoas Fioretos[b,n,2], Sai-Juan Chen[a,r,s,2], and Jin-Yan Huang[a,s,2]

Li JF, et al. Proc Natl Acad Sci U S A. 2018 Dec 11;115(50):E11711-E11720.

1223 cases; 14 subtypes

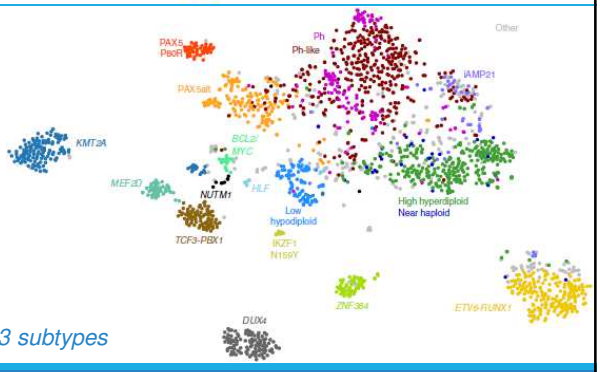


PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia

Zhaohui Gu[1,34], Michelle L. Churchman[1,34], Kathryn G. Roberts[1,34], Ian Moore[1], Xin Zhou[2], Joy Nakitandwe[1], Kohei Hagiwara[2], Stephane Pelletier[3], Sebastien Gingras[4], Hartmut Berns[5], Debbie Payne-Turner[1], Ashley Hill[1], Ilaria Iacobucci[1], Lei Shi[6], Stanley Pounds[6], Cheng Cheng[6], Deqing Pei[6], Chunxu Qu[1], Scott Newman[2], Meenakshi Devidas[7], Yunfeng Dai[7], Shalini C. Reshmi[8], Julie Gastier-Foster[8], Elizabeth A. Raetz[9], Michael J. Borowitz[10], Brent L. Wood[11], William L. Carroll[12], Patrick A. Zweidler-McKay[13], Karen R. Rabin[14], Leonard A. Mattano[15], Kelly W. Maloney[16], Alessandro Rambaldi[17], Orietta Spinelli[17], Jerald P. Radich[18], Mark D. Minden[19], Jacob M. Rowe[20], Selina Luger[21], Mark R. Litzow[22], Martin S. Tallman[23], Janis Racevskis[24], Yanming Zhang[25], Ravi Bhatia[26], Jessica Kohlschmidt[27], Krzysztof Mrózek[27], Clara D. Bloomfield[27], Wendy Stock[28], Steven Kornblau[29], Hagop M. Kantarjian[29], Marina Konopleva[29], Williams E. Evans[30], Sima Jeha[31], Ching-Hon Pui[31], Jun Yang[30], Elisabeth Paietta[24], James R. Downing[1], Mary V. Relling[30], Jinghui Zhang[2], Mignon L. Loh[32], Stephen P. Hunger[33] and Charles G. Mullighan[1*]

Gu Z, et al. Nat Genet. 2019 Feb;51(2):296-307.

1988 cases; 23 subtypes



Recently described ALL rearrangements

DUX4 rearranged
- Often *IGH-DUX4* rearrangement
- Loss of ERG function, either via deletion or expression of dominant negative isoform
- Patients seem to do well with standard therapy despite frequent IKZF1 deletions

MEF2D rearranged

ZNF384 rearranged

Two *PAX5-related* groups:
- PAX5 p.Pro80Arg, typically with loss-of-function of the other allele
- PAX5-altered, with rearrangements, other sequence mutations, or focal intragenic amplifications

BCR-ABL1-like
- Multiple subcategories

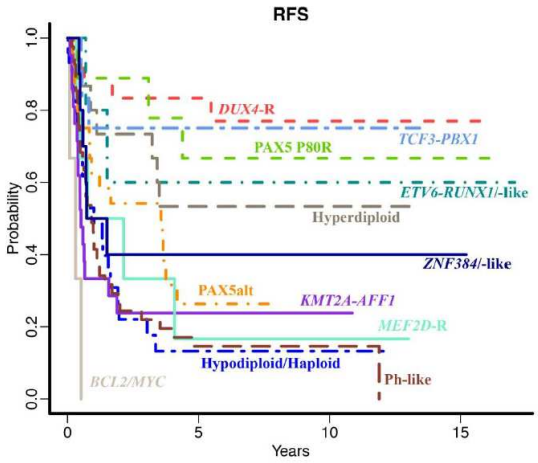
MYC/BCL2/BCL6 rearranged

NUTM1 rearranged

Prognosis

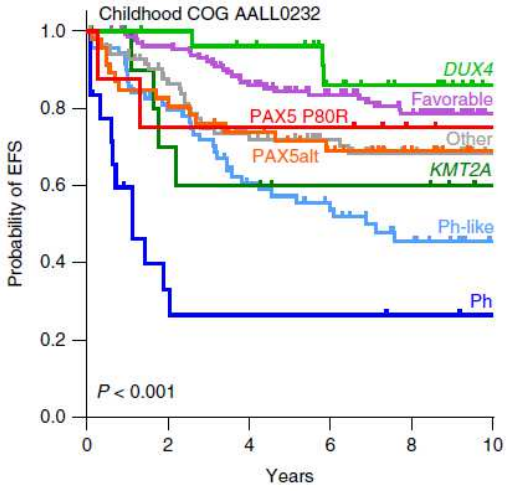
Fusion identification can be used for risk-adapted therapy in ALL

Adult BCR-ABL1-negative B-ALL

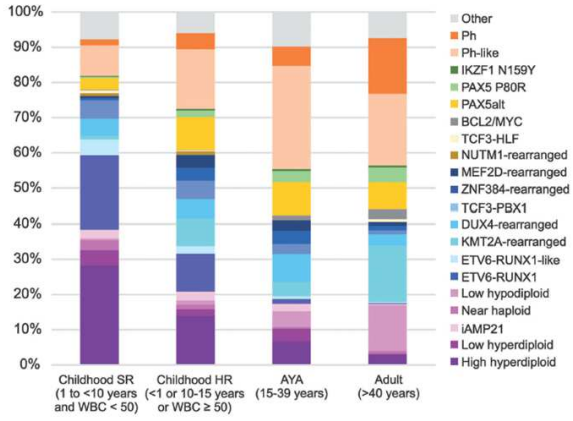


Paietta E, et al. Blood 2021 Apr 25;blood.2020010144.

Pediatric B-ALL



Gu Z, et al. Nat Genet. 2019 Feb;51(2):296-307.



Inaba H, Mullighan CG. Haematologica. 2020 Nov 1;105(11):2524-2539.

BCR-ABL1-like (Ph-like) B-ALL

- Defined by expression pattern similar to BCR-ABL1 rearranged B-ALL, but without BCR-ABL1 rearrangement
  - Originally described in 2009
- Approximately 15% childhood B-ALL overall, with increasing incidence by age
  - From 10% (standard risk children) to ~20% (adolescents) to ~25% (young adults)
- Approximately 20% of adult B-ALL

BCR-ABL1-like B-ALL: biology

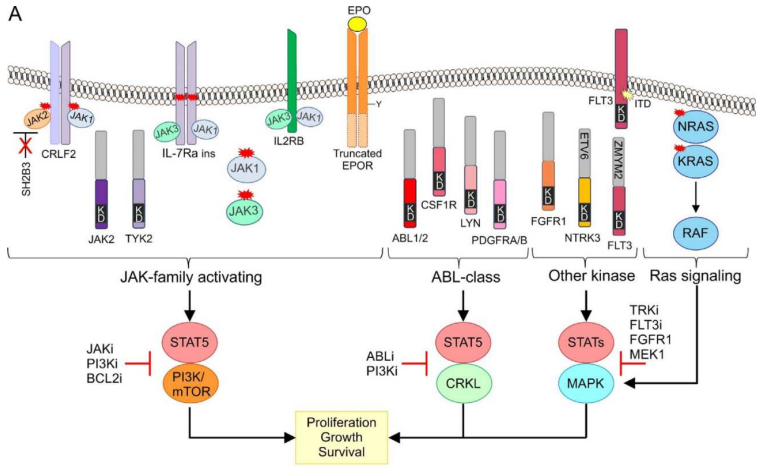
Activation of kinase signaling pathways via many different alterations

ABL1-class rearrangements
- ~15-20% BCR-ABL1-like
- Rearrangements of ABL1, ABL2, CSF1R, PDGFRB ("ABL1-class")
- Diverse partners with common result of kinase signaling activation

CRLF2 rearrangements leading to CRLF2 overexpression
- ~50% of BCR-ABL1-like
- Cytokine receptor-like factor 2 located in pseudo-autosomal region of the X and Y chromosomes
- Rearrangement with IGH (chromosome 14) or P2RY8 (Xp22/Yp11)
- Many of these cases also have activating alterations in the JAK/STAT pathway, particularly JAK2 point mutations

Activating rearrangements of JAK2 or EPOR
- ~10-15% BCR-ABL1-like
- Diverse partners with common result of signaling pathway activation

BCR-ABL1-like B-ALL pathways



Mullighan CG. Best Pract Res Clin Haematol. 2019 Dec;32(4):101095.

BCR-ABL1-like B-ALL: biology/therapy

	Kinase gene	TKI	Fusion partners identified to date, n	5′ Fusion partners
	ABL1	Imatinib/dasatinib	13	*CENPC, ETV6, FOXP1, LSM14A, NUP153, NUP214, RANBP2, RCSD1, SFPQ, SNX1, SNX2, SPTNA1, ZMIZ1*
ABL-class fusions	*ABL2*	Imatinib/dasatinib	3	*PAG1, RCSD1, ZC3HAV1*
	CSF1R	Imatinib/dasatinib	3	*MEF2D, SSBP2, TBL1XR1*
	PDGFRA	Imatinib/dasatinib	1	*FIP1L1*
	PDGFRB	Imatinib/dasatinib	8	*ATF7IP, EBF1, ETV6, SNX29, SSBP2, TNIP1, ZEB2, ZMYND8*
	LYN	Imatinib/dasatinib	2	*GATAD2A, NCOR1*
JAK/STAT activating fusions	*CRLF2*	JAK2 inhibitor	3	*CSF2RA, IGH, P2RY8*
	JAK2	JAK2 inhibitor	21	*ATF7IP, BCR, EBF1, ETV6, GOLGA5, HMBOX1, OFD1, PAX5, PCM1, PPFIBP1, RFX3, SMU1, SNX29, SSBP2, STRN3, TERF2, TPR, USP25, ZBTB46, ZNF274, ZNF340*
	EPOR	JAK2 inhibitor	4	*IGH, IGK, LAIR1, THADA*
	TSLP	JAK2 inhibitor	1	*IQGAP2*
	TYK2	TYK2 inhibitor	3	*MYB, SMARCA4, ZNF340*
	IL2RB	JAK1/JAK3 inhibitor	1	*MYH9*
	NTRK3	TRK inhibitor	1	*ETV6*
	PTK2B	FAK inhibitor	3	*KDM6A, STAG2, TMEM2*
	FGFR1	Ponatinib	1	*BCR*
	FLT3	FLT3 inhibitor	1	*ZMYM2*
	DGKH		1	*ZFAND3*
	BLNK		1	*DNTT*
	CBL		1	*KANK1*

Tasian SK et al. Blood. 2017 Nov 9;130(19):2064-2072.

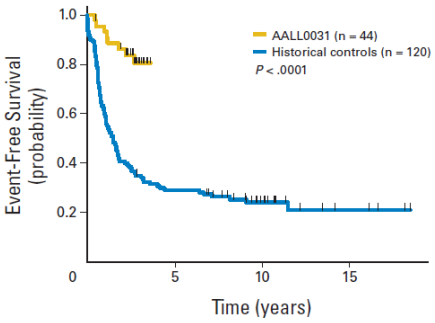
Therapy: targetable fusions

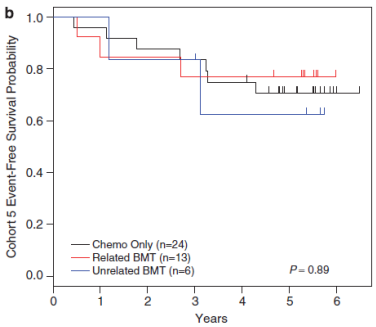
B-ALL with BCR-ABL1 (Ph+)

Investigation of imatinib in Ph+ B-ALL began shortly after its activity was shown in CML

Unlike CML, monotherapy with imatinib was only transiently effective in ALL

→ investigation of imatinib with combination chemotherapy





Schultz KR. J Clin Oncol. 2009 Nov 1;27(31):5175-81.

Schultz KR. Leukemia. 2014 Jul;28(7):1467-71

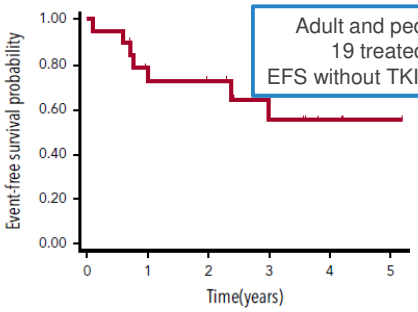
BCR-ABL1-like B-ALL: ABL class fusions

Fusions of *ABL1*, *ABL2*, *CSF1R*, *LYN*, *PDGFRA*, and *PDGFRB* with various partners

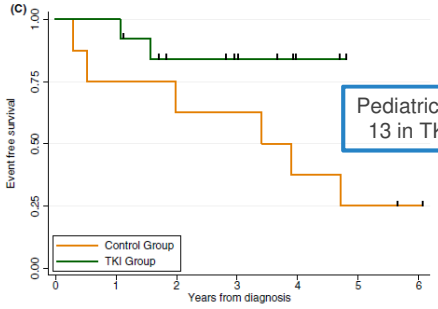
In vitro and in vivo xenograft studies have demonstrated sensitivity to imatinib and/or dasatinib

Case reports and now small cohorts combining TKIs with combination chemotherapy in patients

Larger studies ongoing



Adult and pediatric patients;
19 treated with TKI;
EFS without TKI historically ~25%



Pediatric patients;
13 in TKI group

Tanasi I. Blood. 2019 Oct 17;134(16):1351-1355

Moorman AV. Br J Haematol. 2020 Dec;191(5):844-851.

BCR-ABL1-like B-ALL: JAK/STAT activating

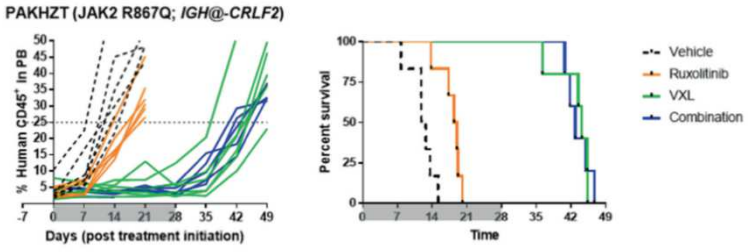
Fusions of *CRLF2*, *JAK2*, *EPOR, TYK2, TLSP,* and *IL2RB* with various partners

Possible sensitivity to JAK inhibitor ruxolitinib

Some promising results in vitro, in patient-derived xenografts, and scattered case studies

Clinical studies ongoing

Patient-derived mouse xenograft

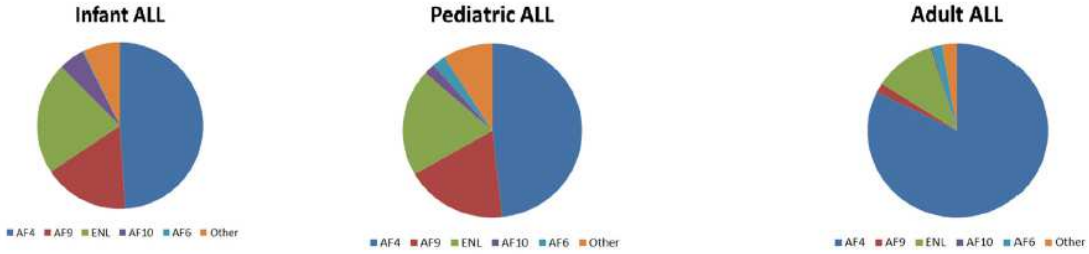


Bohm JW. Leukemia. 2021 Apr 24. doi: 10.1038/s41375-021-01248-8. Online ahead of print

KMT2A-rearranged ALL

KMT2A (previously known as *MLL*) can rearrange with numerous different partner genes

- Most commonly in B-ALL, but also T-ALL as well as de novo AML, therapy-related AML, and mixed phenotype acute leukemia
- Most common in infants, but second peak in adults
- KMT2A rearrangement leads to epigenetic dysregulation through activity of multi-protein complex
- Inhibitors in development for multiple proteins in this complex: Menin inhibitors, DOT1L inhibitors, bromodomain inhibitors, lysine-specific demethylase-1 inhibitors, polycomb repressive complex inhibitors



Winters AC and Bernt KM. Front Pediatr. 2017 Feb 9;5:4.

T-ALL

The only WHO subtype of T-ALL is a provisional entity: early T precursor T-ALL

- Defined by immunophenotype
  - positive for CD3 (usually cytoplasmic) and CD7, negative for CD8 and CD1a, co-expression of myeloid/stem cell markers, and expression of CD5 on less than 75% of blasts
- Mutational pattern – not diagnostic
  - Less frequent alterations of *NOTCH1* and *CDKN2A*
  - More frequent alterations in signaling, transcriptional, and epigenetic pathways

Common genetic findings in T-ALL

- Rearrangements between T cell receptor loci and various partner genes are most common
- Alterations in *NOTCH1* and/or *FBXW7*, both of which result in *NOTCH1* pathway activation
- Deletions of *CDKN2A/B*
- JAK-STAT, PI3K-AKT, RAS-MAPK pathway activation

Recurrent T-ALL rearrangements

Rearrangements resulting in deregulated expression of a target gene

Target gene	*n*	Rearrangement partner (number of cases)
TLX1	17	*TRB* (7), *TRA* (5), *TLX1*-upstream (4), *LINC00502–TLX1* (1)
TLX3	13	*BCL11B–TLX3* (8), *CDK6–TLX3* (2), *TRB* (1), *TLX3–CASC15* (1), *TLX3–TARBP1* (1)
TAL1	58	*STIL* (50), *TRA* (5), *DHX9–TAL1* (1), *GNPAT–TAL1* (1), *TAL1–TARP* (1)
TAL2	6	*TRB* (6)
LMO1	3	*TRB* (2), *TRA* (1)
LMO2	13	*TRA* (6), *TRB* (5), *CSTF3–LMO2* (1), *FOXJ3–LMO2* (1)
LYL1	1	*TRB* (1)
NKX2-1	9	*TRA* (6), *NKX2-1–BCL11B* (1), *CDK6–NKX2-1* (1), *NKX2-1–DIO2* (1)
HOXA	7	*TRB* (4), *HOXA* insertion (1), *LINC01260–HOXA* (1), *POLR2E–HOXA* (1)
MYB	11	*TRB* (2), *SLC12A9–MYB* (1), *MYB–PLAGL1* (1), *MYB–BDP1* (1), *MYB–CHMP1A* (1) *AHI1–MYB* (5)

Rearrangements resulting in chimeric fusions

Gene	*n*	Partner gene
MLLT10	15	*PICALM* (9), *DDX3X* (2), *KMT2A* (1), *FAM171A1* (1), *NAP1L1* (1), *CAPS2* (1)
KMT2A	12	*MLLT1* (7), *ELL* (1), *MLLT10* (1), *AFDN* (1), *MLLT6* (1), *CT45A3* (1)
ABL1	7	*NUP214* (4), *SLC9A3R1* (1), *ETV6* (1), *MBNL1* (1)
NUP98	5	*RAP1GDS1* (2), *CCDC28A* (1), *LNP1* (1), *PSIP1* (1)
TFG	3	*ADGRG7* (3)
JAK2	2	*PCM1* (1), *CD99* (1)
ETV6	2	*ABL1* (1), *CTNNB1* (1)
ZC3HAV1	2	*ABL2* (1), *AKAP11* (1)
LMAN2	2	*NSD1* (1), *PAPOLA* (1)

Liu Y et al, Nat Genet. 2017 Aug;49(8):1211-1218.

Fusion detection strategies

Diversity of underlying genetic alterations makes detection complex!

Expression analysis
- Can identify category as a whole, but not specific alteration
- Possible screening tool
  - Children's Oncology Group (COG) uses a low density array (LDA) for *BCR-ABL1*-like screening

Targeted fusion analysis
- Multiplex FISH
- Multiplex RT-PCR
- Targeted RNA sequencing panels

Comprehensive analysis
- Whole transcriptome sequencing
- Whole genome sequencing

Increasing role for molecular testing