

IL VALORE DELL'RNA-SEQUENCING NELLA DIAGNOSI DELLA LLA PEDIATRICA: CINQUE ANNI DI ESPERIENZA NELLA RETE AIEOP

Claudia Saitta

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AIEOP
ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

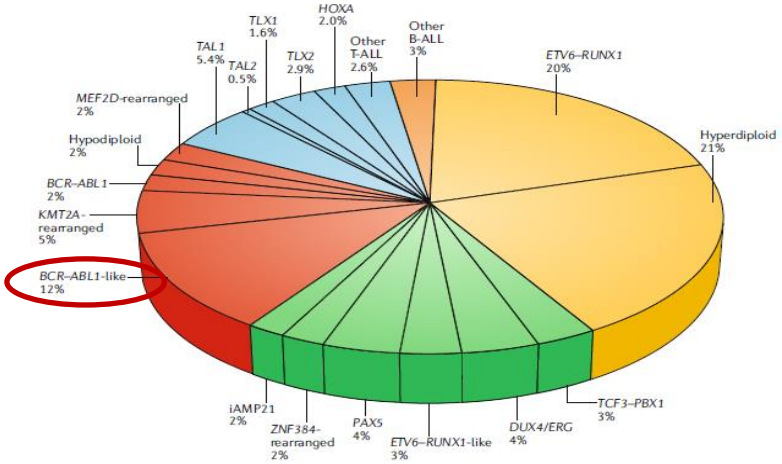
AIEOP MEDICI
LECCE, 12-14 ottobre 2026
HILTON GARDEN INN

Disclosures of Claudia Saitta

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
		NOTHING	TO	DISCLOSE			

From molecular bases of leukemia to personalized medicine

It is now widely acknowledged that leukemia, as other cancers, is a **genetic disease**,
caused largely by the acquisition of mutations in somatic (non-germline) cells after birth - or in some cases during fetal development.

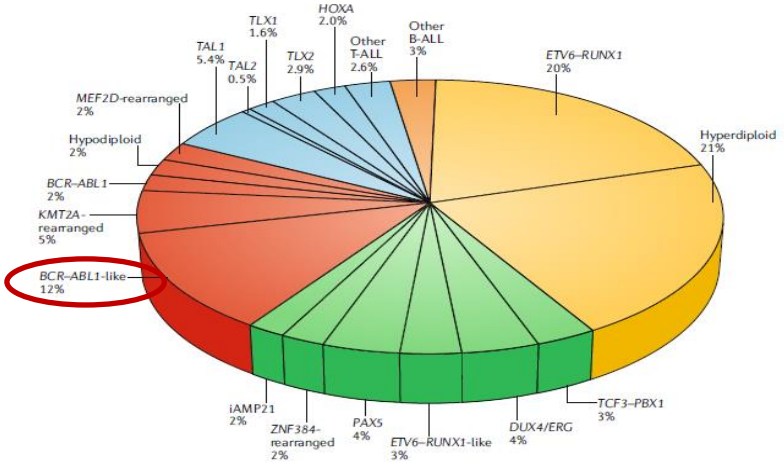


Pui CH, Nichols KE, Yang JJ. Somatic and germline genomics in paediatric acute lymphoblastic leukaemia. Nat Rev Clin Oncol. 2019

Recurrent genomic aberrations allow to classify 75–80% of ALL paediatric patients into prognostically and therapeutically relevant subgroups.

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Table 1. Kinase fusions identified in Ph-like ALL

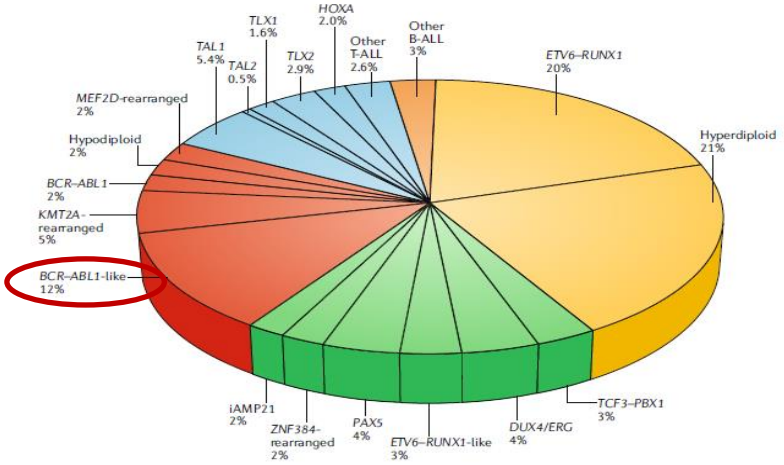
Kinase gene	TKI	Fusion partners identified to date, n	5′ Fusion partners
ABL1	Imatinib/dasatinib	13	*CENPC, ETV6, FOXP1, LSM14A, NUP153, NUP214, RANBP2, RCSD1, SFPQ, SNX1, SNX2, SPTNA1, ZMIZ1*
ABL2	Imatinib/dasatinib	3	*PAG1, RCSD1, ZC3HAV1*
CSF1R	Imatinib/dasatinib	3	*MEF2D, SSBP2, TBL1XR1*
PDGFRA	Imatinib/dasatinib	1	*FIP1L1*
PDGFRB	Imatinib/dasatinib	8	*ATF7IP, EBF1, ETV6, SNX29, SSBP2, TNIP1, ZEB2, ZMYND8*
LYN	Imatinib/dasatinib	2	*GATAD2A, NCOR1*
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, Loh ML, Hunger SP. Philadelphia chromosome-like acute lymphoblastic leukemia. Blood. 2017

ABL-class fusions include rearrangements of the *ABL1*, *ABL2*, *PDGFRB* and *CSF1R* genes, with different fusion partner genes.
They are sensitive to Tyrosine Kinase Inhibitors (TKI), similarly to *BCR::ABL1*.

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Enrollment in EsPhALL2022/COGAALL2131 protocol
(only B-ALL patients)

ICC classification

Published in final edited form as:
Virchows Arch. 2023 January ; 482(1): 11–26. doi:10.1007/s00428-022-03448-8.

International Consensus Classification of Acute Lymphoblastic Leukemia/Lymphoma

Amy S. Duffield[1,#], **Charles G. Mullighan**[2], **Michael J. Borowitz**[3,*]

The International Consensus Classification of ALL

- **B-acute lymphoblastic leukemia** (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* rearrangement
    - B-ALL with *NUTM1* rearrangement
    - B-ALL with *HLF* rearrangement
    - B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* ("CDX2/UBTF")
    - B-ALL with IKZF1 N159Y
    - B-ALL with PAX5 P80R
    - Provisional entities (see Table 3):
      - B-ALL, *ETV6::RUNX1*-like
      - B-ALL, with *PAX5* alteration
      - B-ALL, with mutated *ZEB2* (p.H1038R)/IGH::*CEBPE*
      - B-ALL, *ZNF384* rearranged-like
      - B-ALL, *KMT2A* rearranged-like
- **T-acute lymphoblastic leukemia (T-ALL)**
  - Early T-cell precursor ALL, *BCL11B*-activated
  - Early T-cell precursor ALL, NOS
  - T-ALL, NOS
    - Provisional entities (see Table 4)
- **Provisional entity: Natural killer (NK) cell ALL**

Study objectives

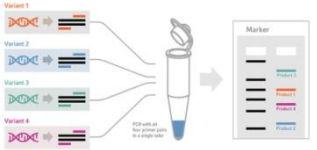
1. CENTRALIZE
Establish a standardized, automated workflow across all Italian AIEOP centers, enabling high-throughput processing and rapid turnaround time.

2. DETECT
Fusion transcripts undetectable by conventional methods (Multiplex-PCR).

3. CHARACTERIZE
Define the prevalence and prognostic impact of fusion genes in Italian pediatric patients with ALL.

4. TARGET
Identify patients who may benefit from alternative targeted therapies, including TKIs and fusion-specific agents.

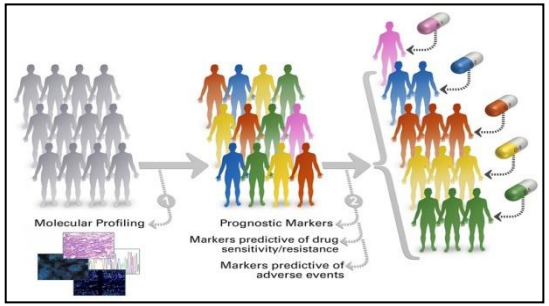
From M-PCR…



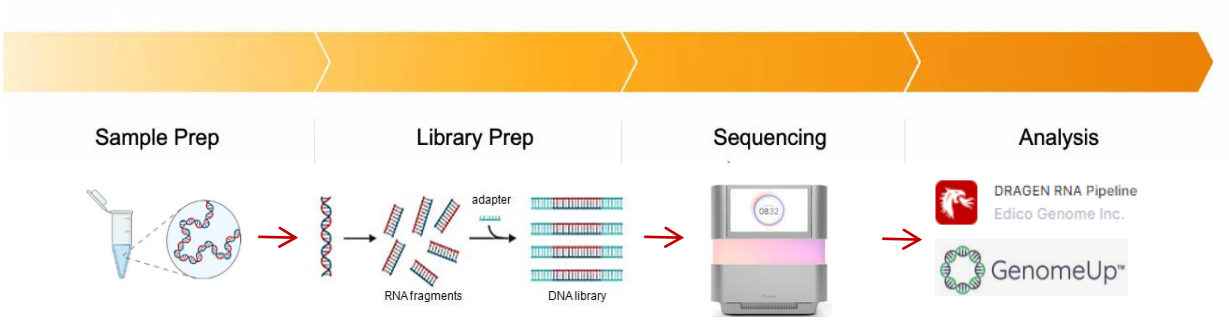
https://handling-solutions.eppendorf.com



…to RNA-SEQ



Workflow – Fusion genes identification



Poly-A mRNA Transcriptome (with ribosomal genes depletion)
– automated library prep

(Nugen, Tecan)
10-500ng total RNA
pooling of 12 samples/run

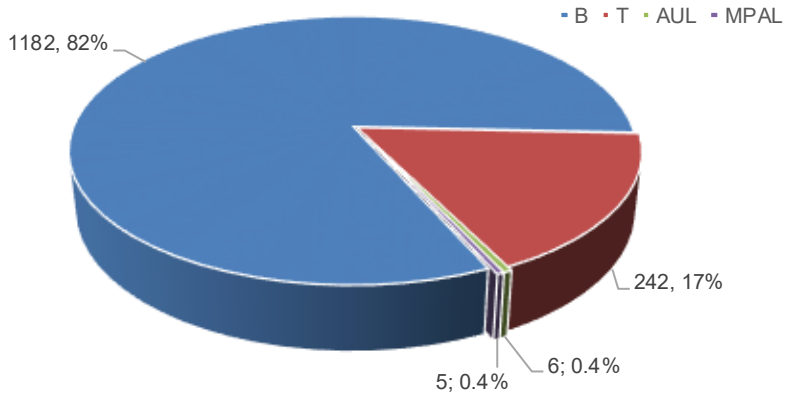
BIOINFORMATIC PLATFORMS

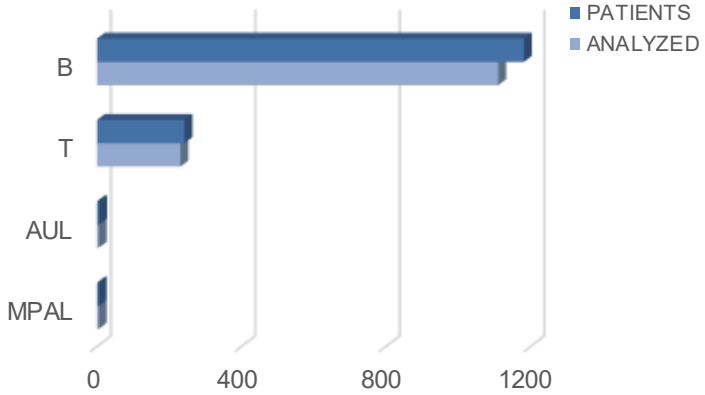
- DRAGEN™ RNA pipeline
- GenomeUP™ platform (FusionCatcher, Arriba, STAR-Fusion)
- AllCatchR – signature profile

IDENTIFICATION of FUSION GENES
Validation by RT-PCR, FISH, digitalMLPA

5 years AIEOP-ALL cohort

1435 pediatric ALL consecutive diagnosis (from 12/2021 to 04/2026)
AIEOP-BFM ALL 2017
Out of protocol





- **B-ALL** 1182/1435 (82.4%)
- **T-ALL** 242/1435 (16.8%)

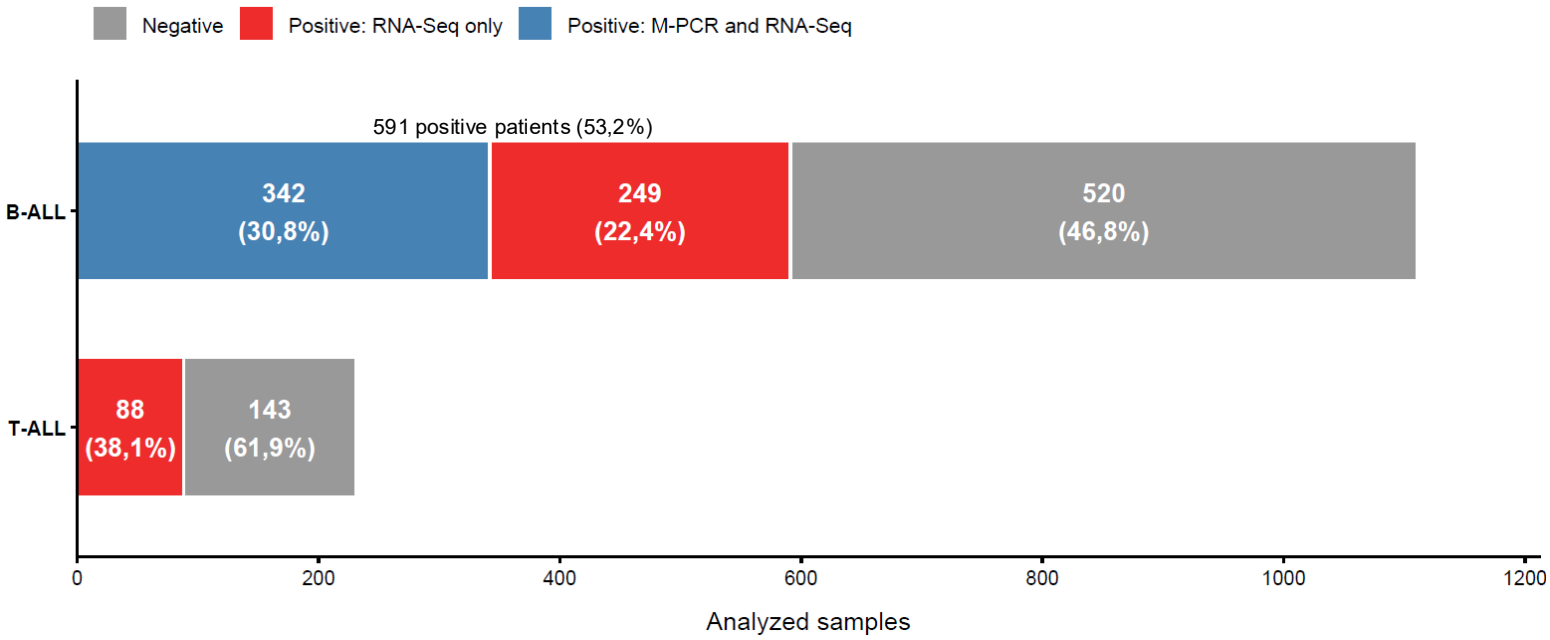
Different phenotipes (data not shown)

- MPAL (Mixed-Phenotype Acute Leukemia) 5/1435 (0.4%)
- AUL (Acute Undifferenciated Leukemia) 6/1435 (0.4%)

RNA-SEQ LIBRARY QUALITY
(coverage, PCR duplicate and reads >Q30)

- **B-ALL** 1111/1182 (94%) ✓
- **T-ALL** 231/242 (95.5%) ✓
- MPAL 5/5 (100%) ✓
- AUL 5/6 (83.3%)

Detected fusions: B-ALL and T-ALL cohorts



679 **fusion genes**/1342 patients - **50.6%** (B-ALL and T-ALL cohorts)

Detected fusions: B-ALL cohort

591 detected fusions / 1111 analyzed patients (**53.2%**)

342/591 fusions (57.9%)
both by MULTIPLEX-PCR and RNA-SEQ*

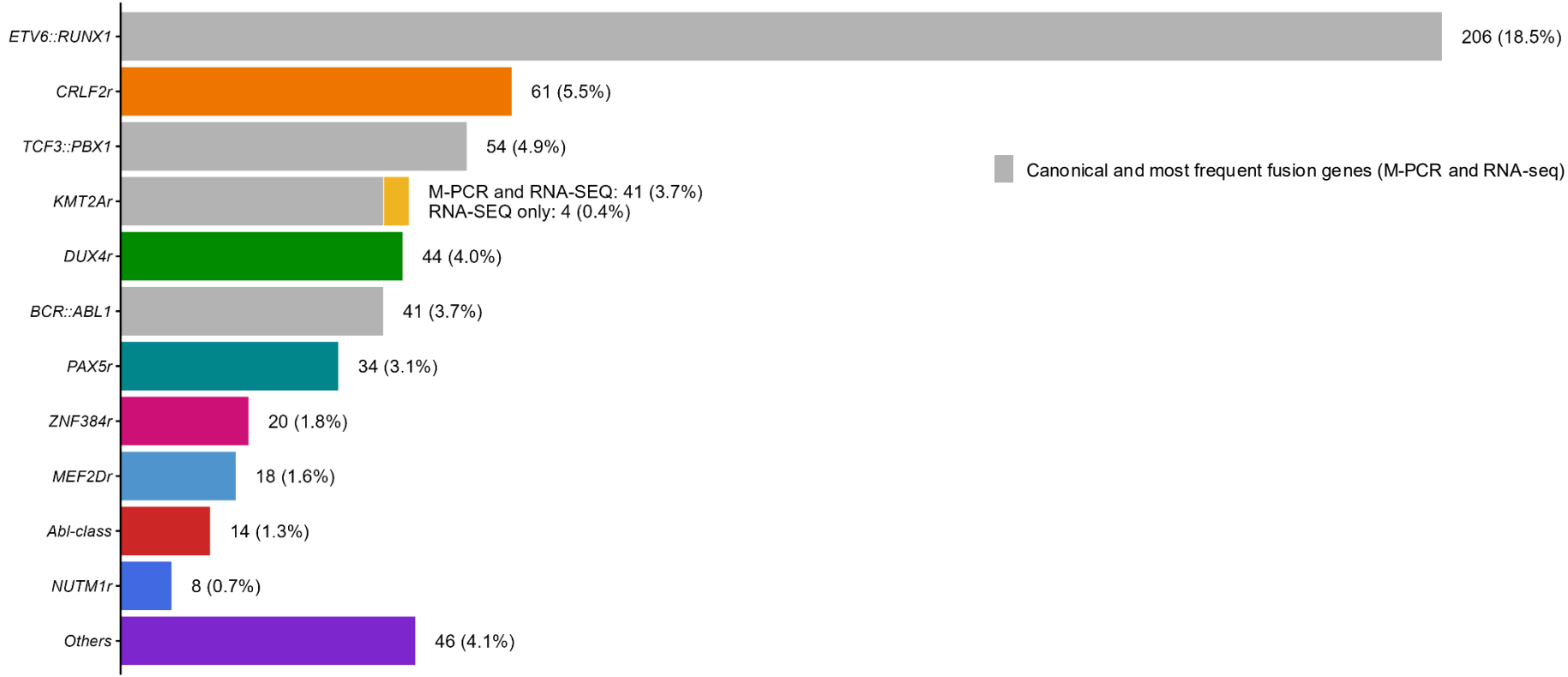
Identified translocation		positive patients	%/1111 pts	
BCR::ABL1	t(9;22)	41	3.70%	
ETV6::RUNX1	t(12;21)	206	18.50%	
TCF3::PBX1	t(1;19)	54	4.90%	
TCF::HLF	t(17;19)	1*	0.10%	
KMT2A::AFF1	t(4;11)	23	2.10%	41; 3.4%
KMT2A::MLLT3	t(9;11)	8	0.70%	
KMT2A::MLLT1	t(11;19)	10	0.90%	
ETV6::ABL1	t(9;12)	0	-	

249/591 (42.1%)
by RNA-SEQ only

Identified translocations	positive patients	% /1111 pts
Abl-class	14	1.30%
CRLF2r	61	5.50%
DUX4r	44	4%
PAX5r	34	3%
ZNF384r	20	1.80%
MEF2Dr	18	1.60%
KMT2Ar	4 (+41)	4%
NUTM1r	8	0.70%
Others	46	4.10%

* Uncoventional breakpoint; one fusion gene identify only by RNA-SEQ.

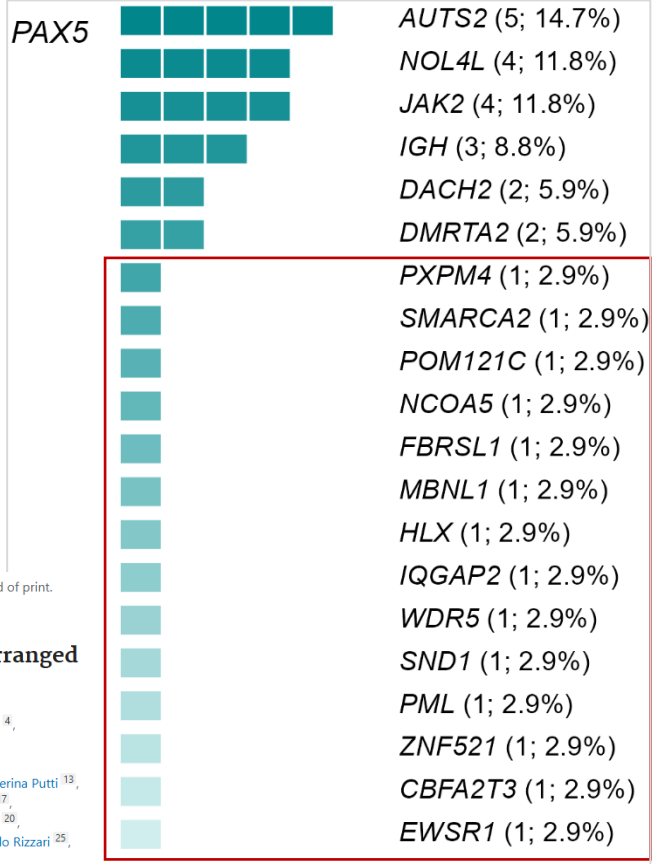
Molecular classification of most frequent rearrangements in B-ALL cohort (N=1111)



What about partner genes? B-ALL cohort





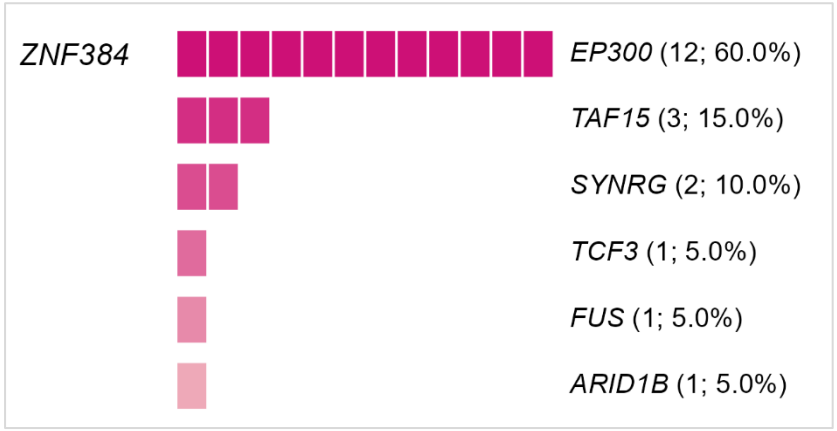


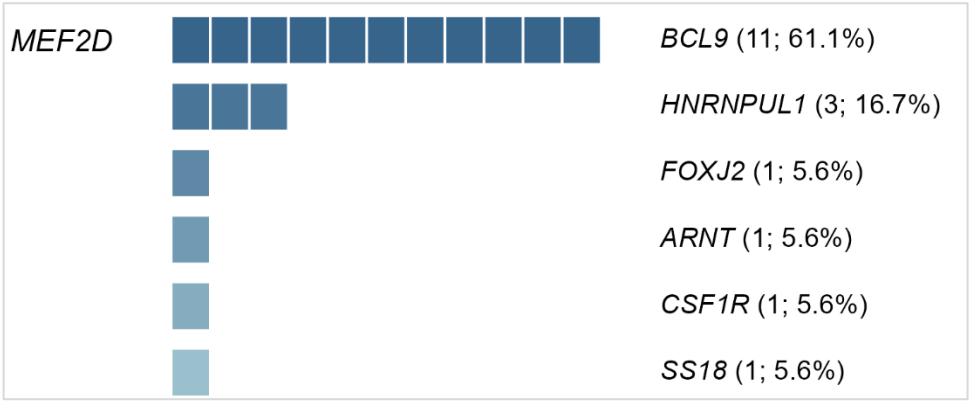
› Blood. 2026 Aug 10:blood.2026033546. doi: 10.1182/blood.2026033546. Online ahead of print.

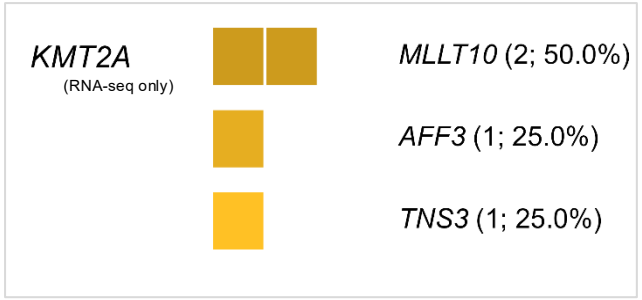
Measurable Residual Disease–Dependent Unfavorable Outcomes in Pediatric PAX5-Rearranged B-Acute Lymphoblastic Leukemia

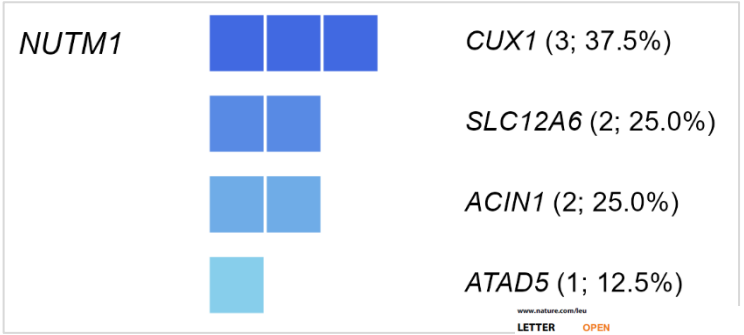
Nicolò Peccatori [1], Alessia Curto [1], Daniela Silvestri [2], Stefano Rebellato [3], Željko Antić [4], Karin Nebral [5], Anke Katharina Bergmann [4], Sabine Strehl [6], Martin Zimmermann [7], Claudia Saitta [3], Chiara Palmi [3], Michela Bardini [3], Sanil Bhatia [8], Arndt Borkhardt [9], Manuel Quadri [3], Daniela Guardo [10], Luca Lo Nigro [11], Rosanna Parasole [12], Maria Caterina Putti [13], Franco Locatelli [14], Julia Alten [15], Martin Stanulla [7], Barbara Buldini [16], Jolanda Sarno [17], Kara L Davis [18], Valentino Conter [3], Adriana Cristina Balduzzi [19], Maria Grazia Valsecchi [20], Andrea Biondi [21], Andishe Attarbaschi [22], Martin Schrappe [23], Gunnar Cario [24], Carmelo Rizzari [25], Giovanni Cazzaniga [20], Grazia Fazio [3]

What about partner genes? B-ALL cohort









www.nature.com/leu
Leukemia
LETTER OPEN
ACUTE LYMPHOBLASTIC LEUKEMIA

Favorable outcome of *NUTM1*-rearranged infant and pediatric B cell precursor acute lymphoblastic leukemia in a collaborative international study

What about partner genes? B-ALL cohort

ABL1	*NUP214* (1; 7.1%)
	SSBP2 (1; 7.1%)
	SNX2 (1; 7.1%)
	ZMIZ1 (1; 7.1%)
ABL2	*RASAL2* (1; 7.1%)
PDGFRB	*EBF1* (6; 42.9%)
	CD74 (1; 7.1%)
CSF1R	*SSBP2* (2; 14.3%)

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EJC Paediatr Oncol. 2025 December ; 6: . doi:10.1016/j.ejcped.2025.100304.

From the bench of molecular understanding to the bedside of optimal therapy for *BCR::ABL1* and ABL-class acute lymphoblastic leukemia in children and adolescents

An International Pilot Study of Chemotherapy and Tyrosine Kinase Inhibitor with Blinatumomab in Patients with Newly-Diagnosed Philadelphia Chromosome-Positive or ABL-class Philadelphia Chromosome-Like B-cell Acute Lymphoblastic Leukemia

AALL2131/EsPhALL2022

EsPhALL network I-BFM Study Group Children's Oncology Group

Date 02 December 2025 – Version 1.2

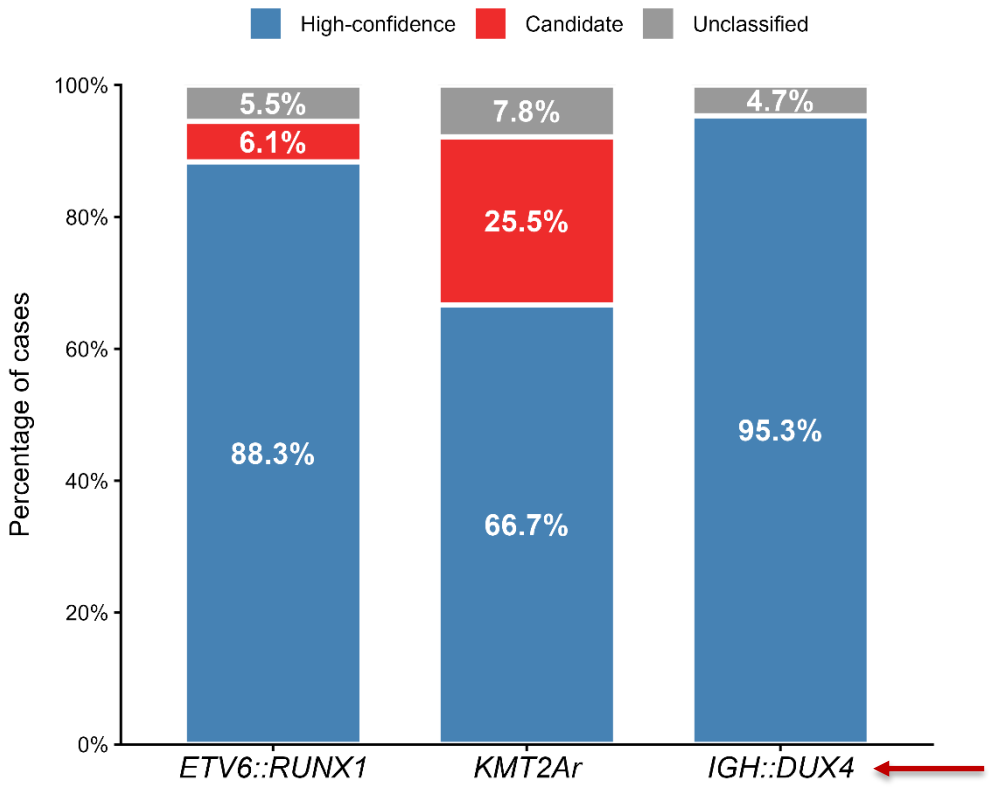
CTIS No.: 2025-520982-39-00



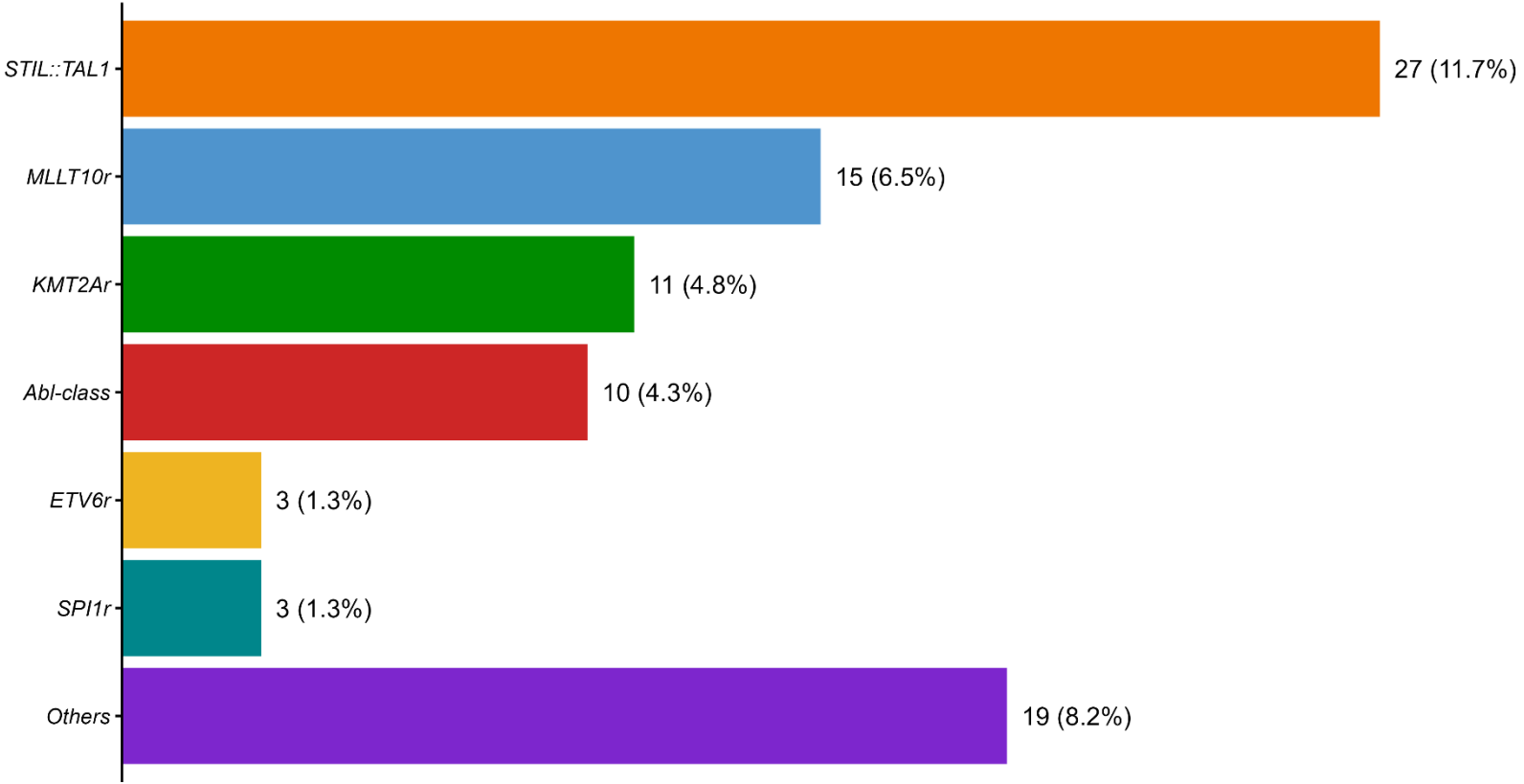
HemaSphere
EHA
Article
Open Access

The Gene Expression Classifier ALLCatchR Identifies B-cell Precursor ALL Subtypes and Underlying Developmental Trajectories Across Age

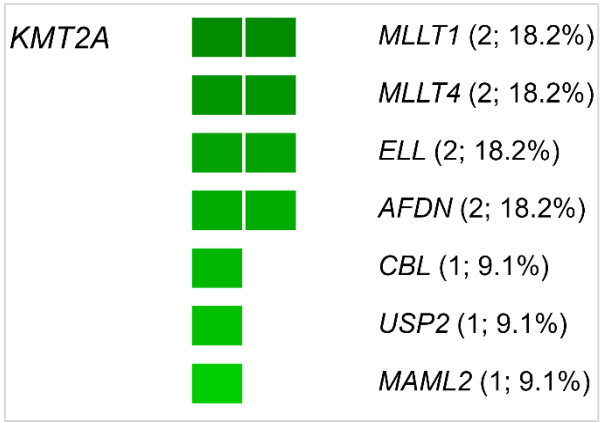
ALLCatchR classification confidence (B-ALL cohort)



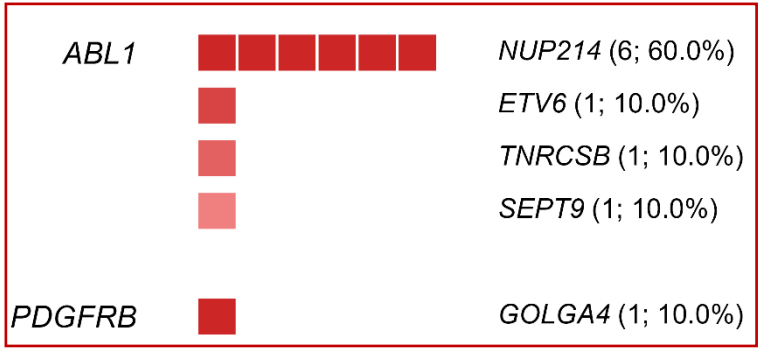
Molecular classification of most frequent rearrangements in T-ALL cohort (N=231)

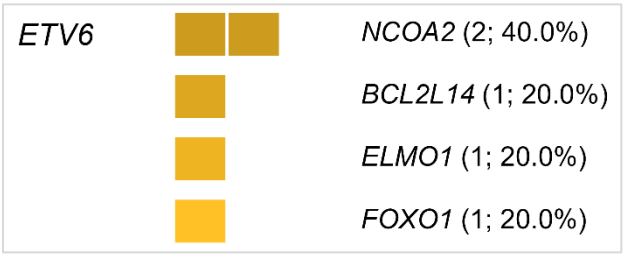


What about partner genes? T-ALL cohort









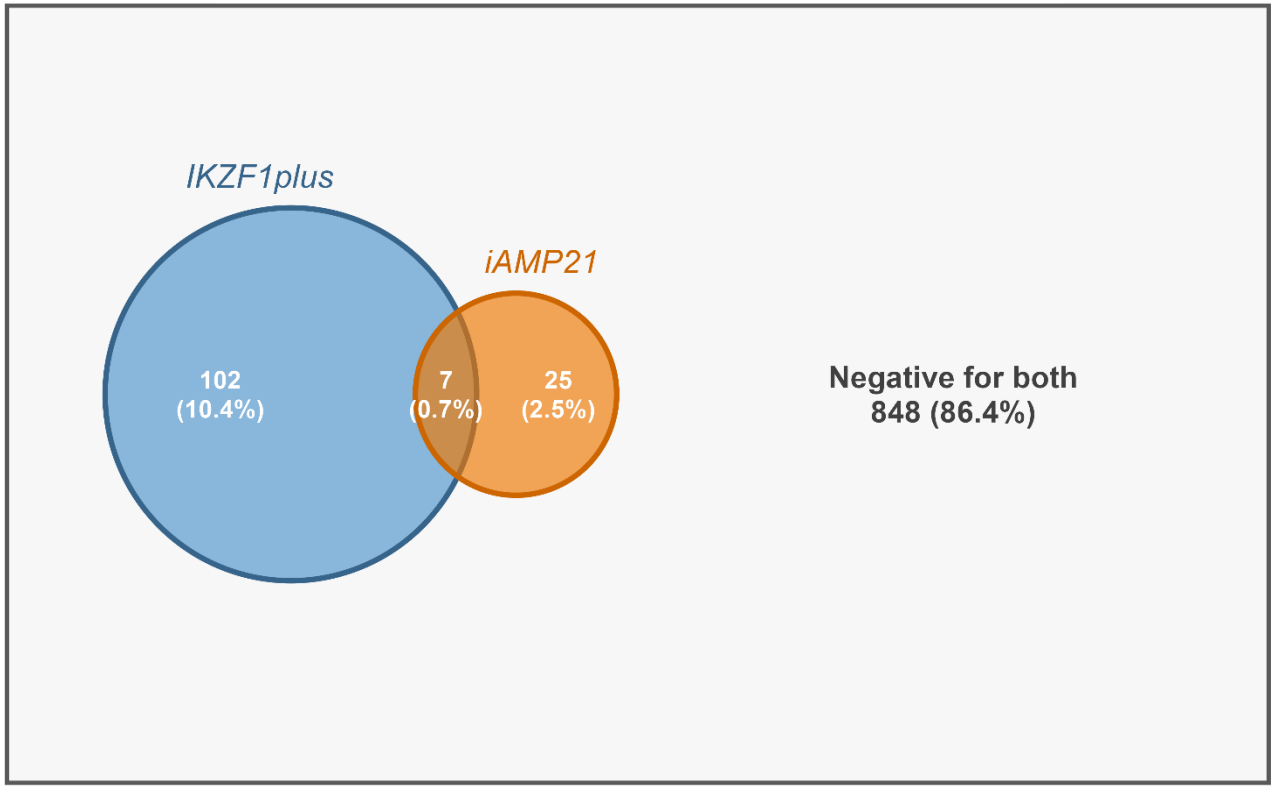


nature genetics

Recurrent *SPI1* (PU.1) fusions in high-risk pediatric T cell acute lymphoblastic leukemia

IKZF1plus and iAMP21 subgroups (dMLPA)

B-ALL cohort (N = 982)



Conclusion - Take home message

- HIGH CONCORDANCE
  Strong correlation with the gold-standard Multiplex RT-PCR.

- INCREASED DETECTION RATE
  RNA-seq identified significantly more fusion events in both B-ALL and T-ALL (from 25,5% to 50,6%)

- UNEXPECTED FINDINGS IN T-ALL
  A high frequency of fusion genes was detected in T-ALL (38%), including ABL-class fusions, which are not currently addressed by TKI treatment protocols (4,3%).



✓ Refined genetic classification of pedatric ALL patients

✓ Improved patient stratification

✓ Personalized treatment strategies based on novel targeted therapies

FROM GENETIC PROFILING TO TARGETED THERAPY

Acknowledgments

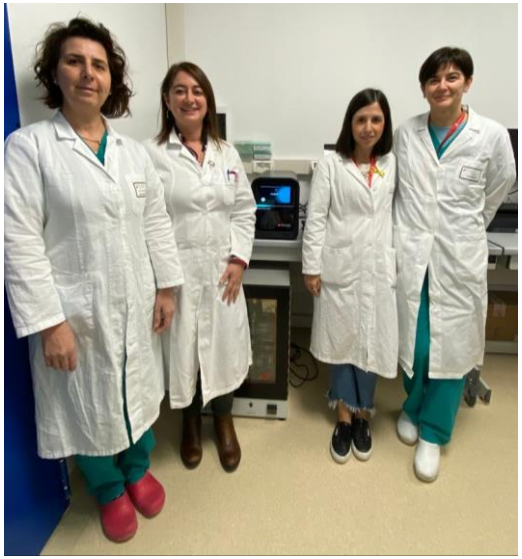
Laboratorio ultraspecialistico di Patologia clinica-Ematologia
Tettamanti Research Center, Clinica Pediatrica
Univ. Milano Bicocca, Monza, Italy

Genetics of Leukemia Unit

Arianna Colombo
Federica Colnaghi
Stefano Rebellato
Nicolò Peccatori
Chiara Palmi

Daniela Silvestri
Maria Grazia Valsecchi

Gianni Cazzaniga
Grazia Fazio
Andrea Biondi





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