

Eterogeneità genetica della LLA-T pediatrica: implicazioni biologiche e prognostiche.

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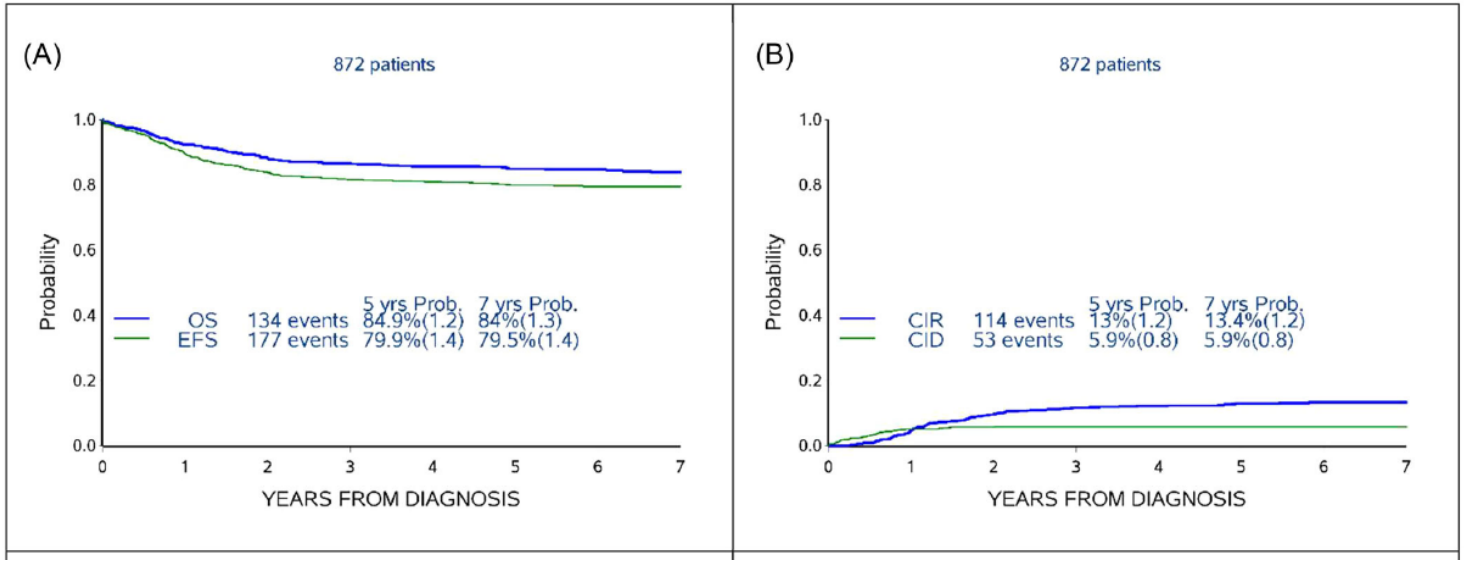
AIEOP MEDICI
LECCE, 12-14 ottobre 2026
HILTON GARDEN INN

Disclosures of Grazia Fazio

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
N.A.							

Background on T-ALL

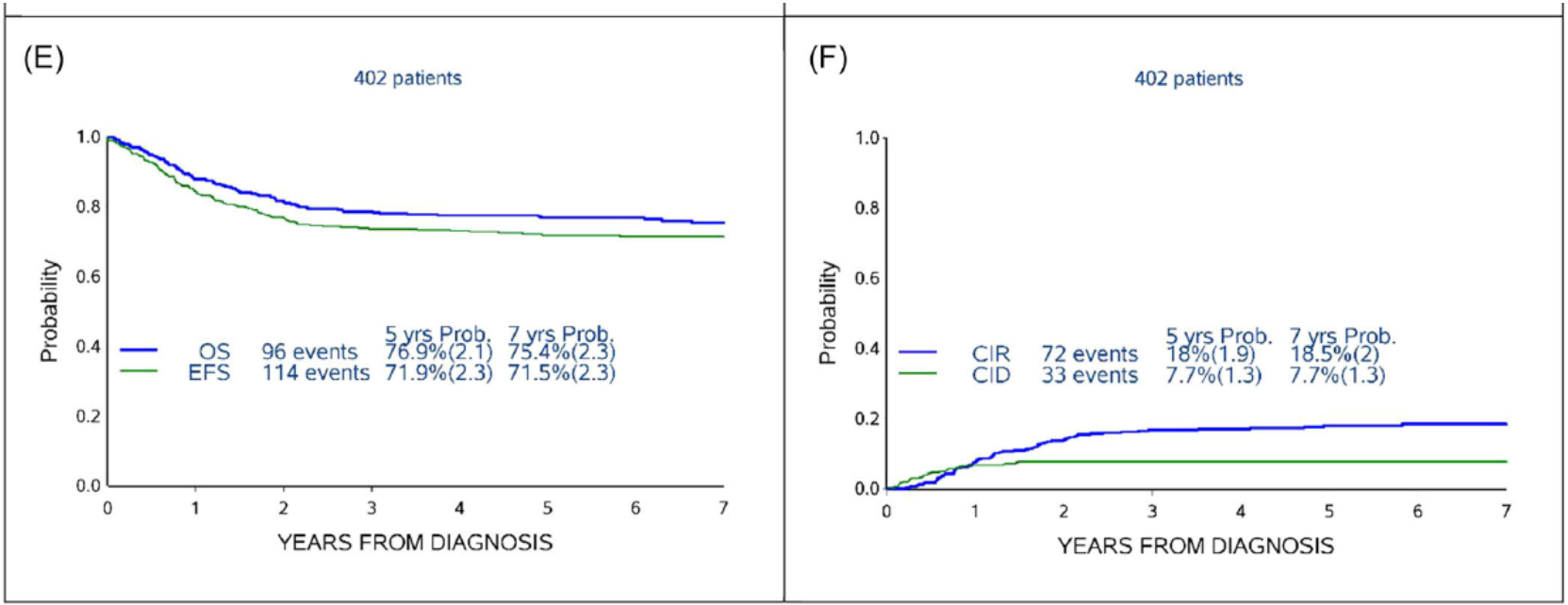
Pediatric T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and biologically heterogeneous hematologic malignancy



Outcome of T-cell acute lymphoblastic leukemia (T-ALL) in AIEOP-BFM ALL 2009 trial.

Cario, … Biondi Hemasphere, 2025

Outcome of High-Risk HR T-cell acute lymphoblastic leukemia (T-ALL) in AIEOP-BFM ALL 2009 trial.



Cario, … Biondi Hemasphere, 2025

Despite therapeutic advances in recent decades, the outcome remains inferior to that of B-ALL, a fact that underscores the urgent need for more advanced prognostic tools.

Genetic alteration	Prevalence in T-LBL	Prevalence in T-ALL	Prognostic effect	References
NOTCH1 signaling				
NOTCH1^{mut}	65%	52%	Favorable prognosis in T-ALL and T-LBL	[33, 42]
FBXW7^{mut}	25%	22%	Favorable prognosis in T-LBL, if *NOTCH1*^{mut} coexist	[33, 43]
NOTCH1 fusions	6%–41%	<1%	Inferior survival and increased CIR in T-LBL	[37, 38]
NOTCH1 intragenic loss	not evaluated	2.8%	Inferior survival in T-ALL	[43]
NOTCH1 intronic SNV	not evaluated	1.6%	Inferior survival in T-ALL	[43]
IL7R/JAK-STAT signaling				
IL7R^{mut}	9%	10%	Treatment resistance and poor outcome in T-ALL	[33, 43, 44]
JAK1^{mut}	6%	7%	Treatment resistance and poor outcome in T-ALL	[40, 44]
NRAS^{mut}/*KRAS*^{mut}	7% / 3%	15%	No impact on survival	[45, 46]
STAT5B^{mut}	6%	6%	Increased CIR in T-ALL	[33, 43, 47]
PI3K-AKT pathway				
PTEN^{mut} /*PTEN* loss	15%	~20%	Inferior outcome in T-LBL and T-ALL	[43, 45, 48]
TP53^{mut}	5%	2%	unclear	[33, 43]
Epigenetic regulators				
KMT2D^{mut}	14%	8%	Increased CIR in T-LBL	[33, 40]
PHF6^{mut}	17%	26%	Favorable outcome in adult T-LBL	[33, 43, 49, 50]
Structural variants				
ABD	23%	44%	Induction failure and inferior outcome in T-ALL, clinical relevance unclear in T-LBL	[33, 51]
CDKN2A/B locus deletion	47%	51%	No statistically significant effects	[52]
LOH6q	19%	13%	Increased CIR in T-LBL, but not in T-ALL	[53]

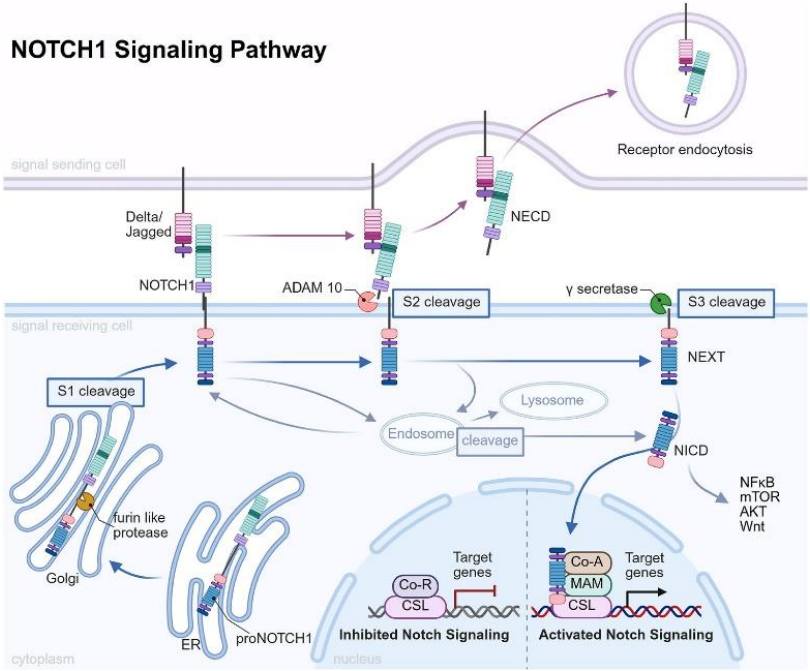
Note: Frequencies and prognostic effects of common genetic variants in pediatric T-ALL and T-LBL. Data concerning *JAK1*^{mut} and *KMT2D*^{mut} are based on a cohort including pediatric and adult patients.

Review, Heider et al., Pediatric Blood and Cancer, 2026

The oncogenic landscape is driven by aberrant chromosomal rearrangements causing the overexpression of transcription factors (primary "drivers"), which cooperate with secondary mutations linked to proliferation, the cell cycle, and epigenetic regulation.

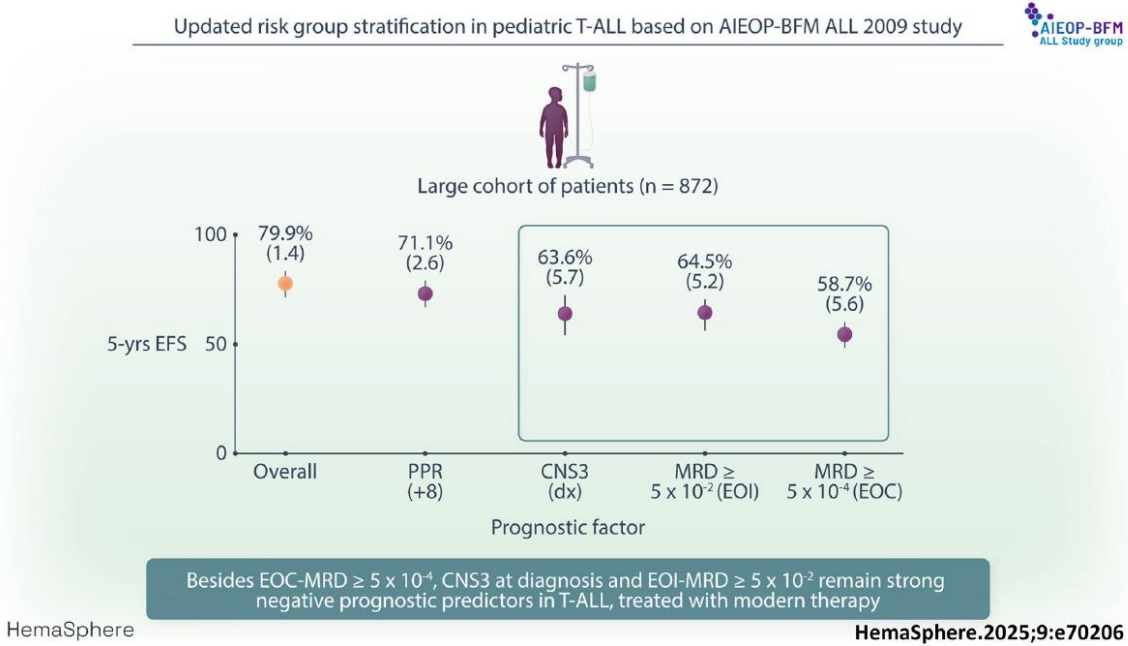
NOTCH1 fusions and NOTCH1 variants in T-ALL resulted in excessive NOTCH1 signaling are associated with increased relapse incidences, suggesting that the intracellular amount of NOTCH1 differentiates between tumor suppressive versus tumor supportive activity.

Future studies may consider incorporating detailed NOTCH1 status to understand disease dynamics and enhance therapeutic strategies.



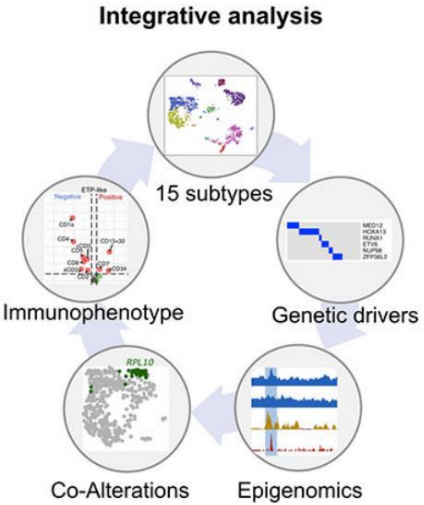
Review, Heider et al., Pediatric Blood and Cancer, 2026

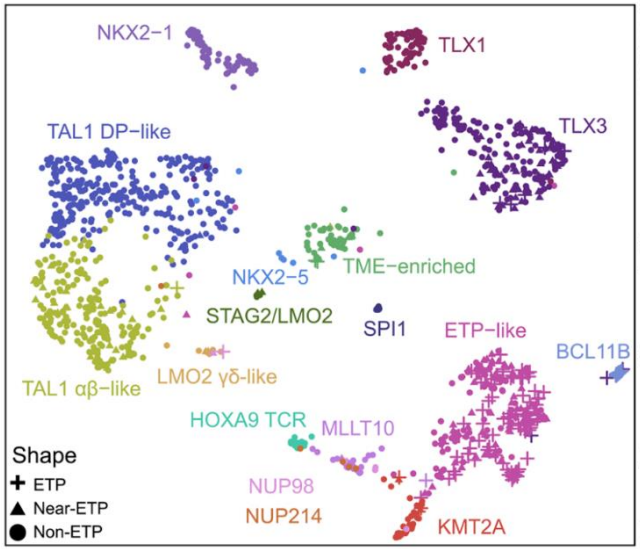
Although individual mutations (such as NOTCH1) hold significant biological importance, the fundamental criterion for risk stratification in modern clinical protocols (e.g., AIEOP-BFM) remains molecular or flow-cytometric Minimal Residual Disease (MRD).

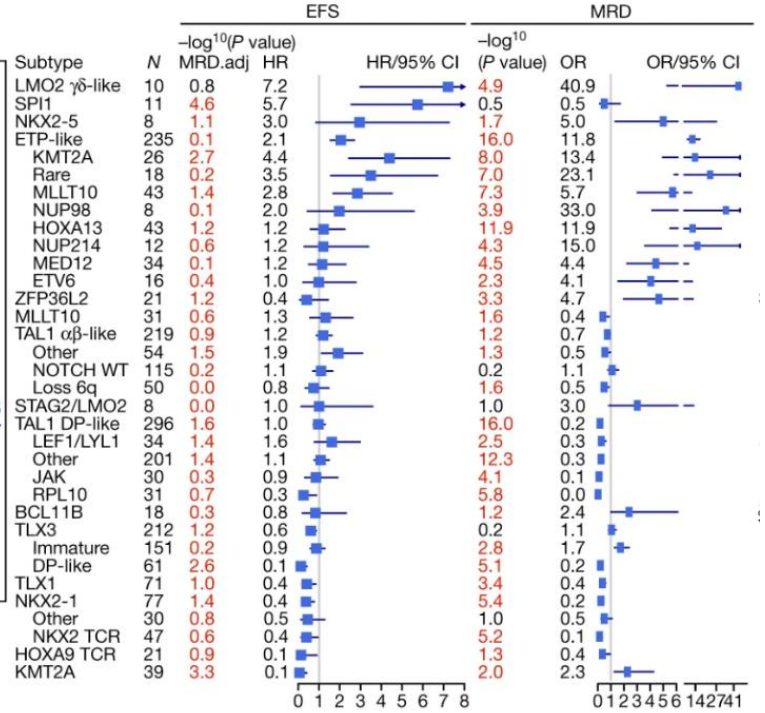


Unlike B-cell ALL, lacks effective risk-stratification strategies due to limited molecular characterization.

- The advent of **Next-Generation Sequencing (NGS)** and the integration of genomic and transcriptomic data have enabled a profound redefinition of the molecular characterization of T-cell acute lymphoblastic leukemia (T-ALL).
- Over 15 molecular subtypes with prognostic significance.
- These reflect the specific stage of differentiation arrest of T-cell progenitors during thymopoiesis.







Pölönen et al., Nature, 2024.

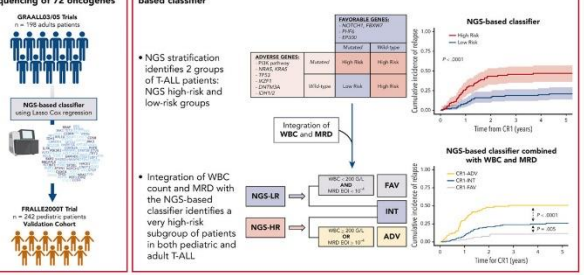
NGS-Based Risk Stratification in T-Cell Acute Lymphoblastic Leukemia (T-ALL)

Context of Research

- Targeted whole-exome sequencing of 72 oncogenes

Main Findings

- Identification of mutant genes associated with poor outcomes and development of NGS-based classifier
- NGS stratification identifies 2 groups of T-ALL patients: NGS high-risk and low-risk groups
- Integration of WBC count and MRD with the NGS-based classifier identifies a very high-risk subgroup of patients in both pediatric and adult T-ALL



Conclusions: The NGS-based approach improves risk stratification in T-ALL. NGS classifier combined with MRD and WBC identifies a group of patients at very high risk of relapse.

*Simonin et al. DOI: 10.1182/**blood**.2023023754*

blood Visual Abstract

CpG Island Methylator Phenotype Classification for Risk Assessment in Pediatric T-Cell Acute Lymphoblastic Leukemia (T-ALL)

Aim of This Study

Firstly, to evaluate whether the classification of pediatric T-ALL cases according to the **CpG Island Methylator Phenotype (CIMP)** at diagnosis, in conjunction with the classification of **measurable residual disease (MRD)** at the end of induction, can improve risk stratification. Secondly, to elucidate the biological basis of the CIMP subgroups.

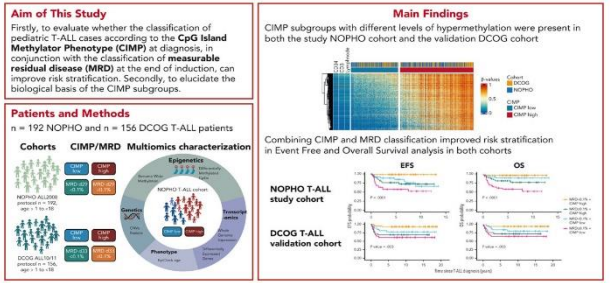
Patients and Methods

n = 192 NOPHO and n = 156 DCOG T-ALL patients

Main Findings

CIMP subgroups with different levels of hypermethylation were present in both the study NOPHO cohort and the validation DCOG cohort

Combining CIMP and MRD classification improved risk stratification in Event Free and Overall Survival analysis in both cohorts



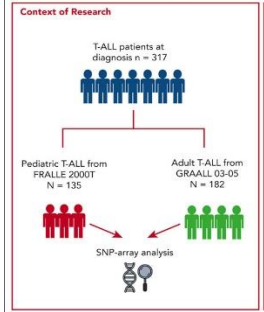
Conclusions: DNA methylation improves measurable residual disease risk group assessment in pediatric T-ALL treated by modern protocols. DNA methylation subgroups in T-ALL are associated with distinct multi-omics profiles, suggesting different routes to leukemia development.

*Schäfer Hackenhaar et al. DOI: 10.1182/**blood**.2024026027*

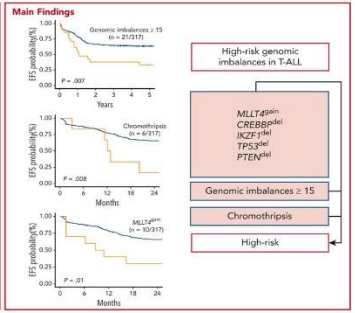
blood Visual Abstract

Analysis of Genomic Imbalances in Adult and Pediatric T-Cell Acute Lymphoblastic Leukemia (T-ALL)

Context of Research



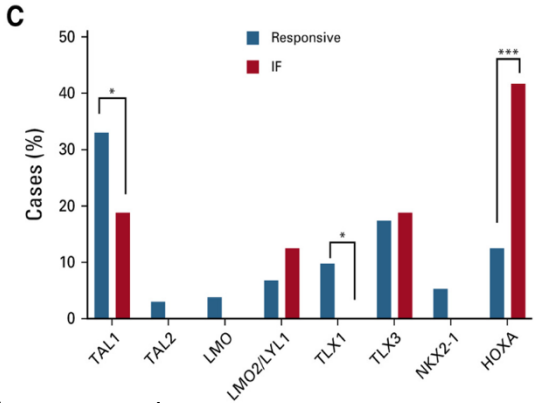
Main Findings



Conclusions: 1) In T-ALL, chromothripsis, genomic complexity, gain at 6q27 (*MLLT4*), and del(16)(p13)/*CREBBP* are associated with poor outcomes. **2)** Gain at 6q27 affecting *MLLT4* is a newly identified recurrent somatic CNV observed in 3% of T-ALL patients.

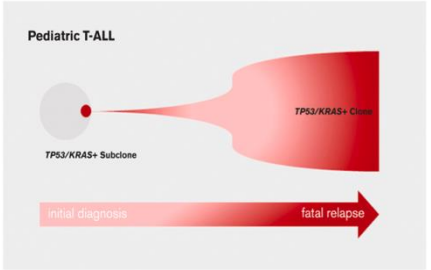
*Balducci et al. DOI: 10.1182/**blood**.2023022154*

blood Visual Abstract

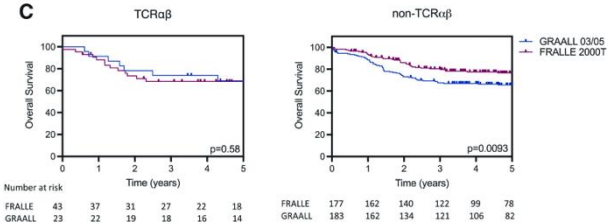


O'Connor et al., JCO 2023

The combination of subclonal *TP53* and *KRAS* variants and poor early response to first line therapy identifies ultra-high-risk patients with pediatric T-lymphoblastic leukemia (T-ALL)



Kempter et al., Blood Advances 2025

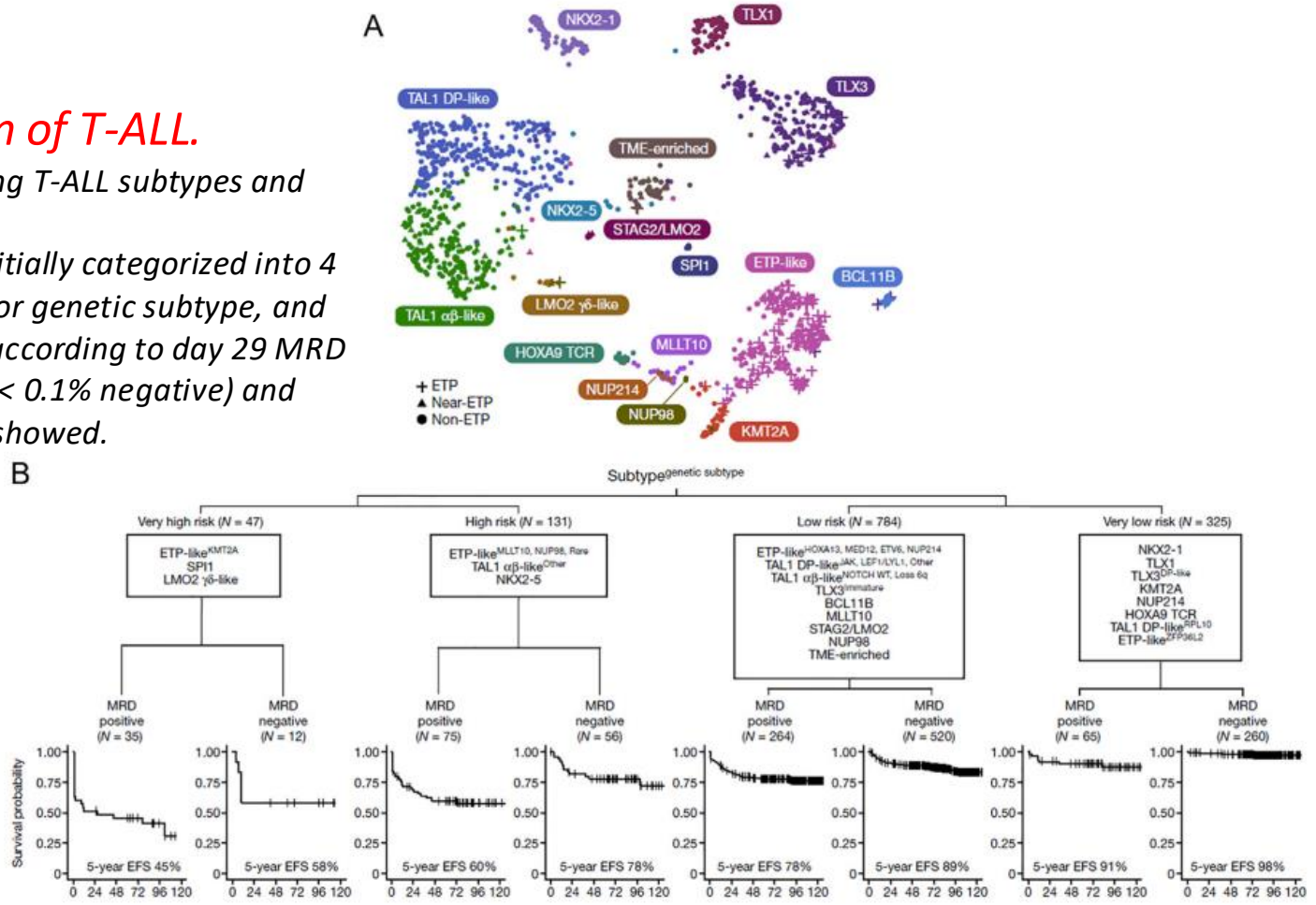


Dourthe et al., Hemasphere 2023

Genomic classification of T-ALL.

(A) UMAP scatterplot depicting T-ALL subtypes and ETP status.

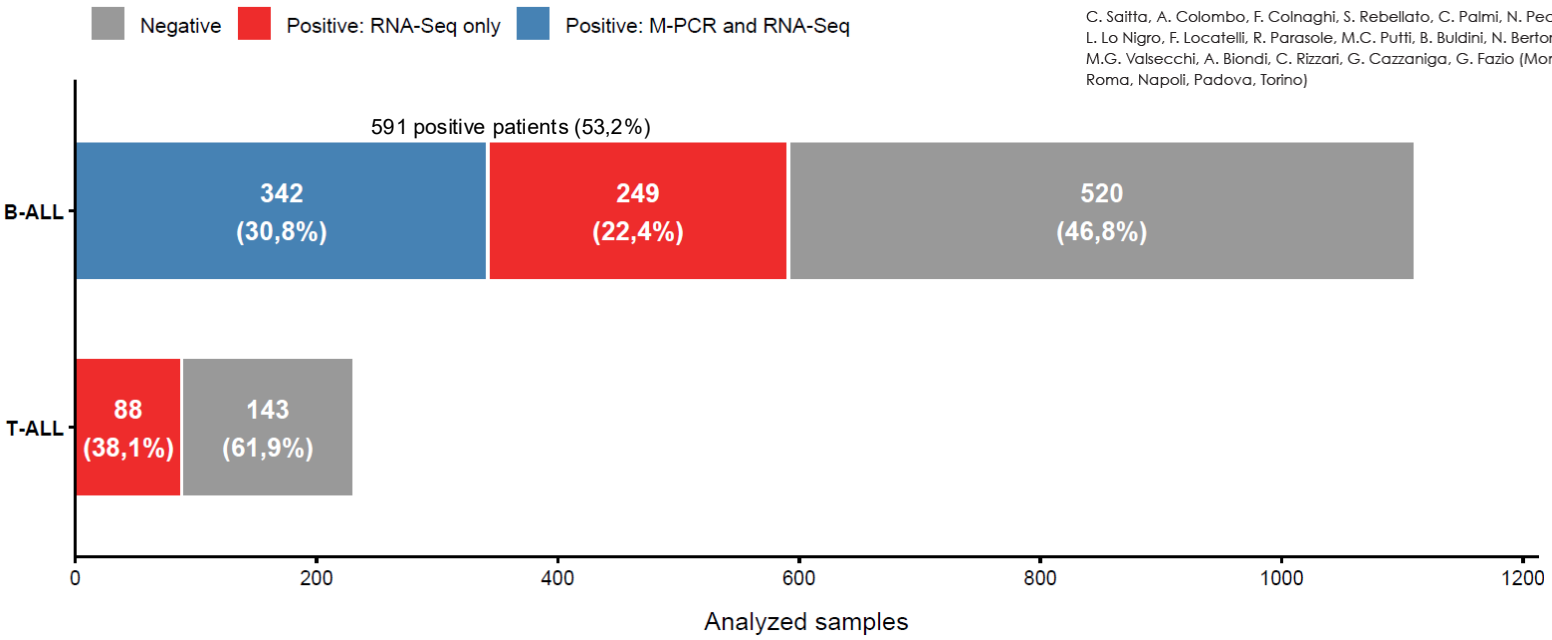
(B) The survival tree model initially categorized into 4 groups based on subtype or genetic subtype, and subsequently subdivided according to day 29 MRD (≥0.1% for MRD positive, < 0.1% negative) and Kaplan–Meier curves are showed.



Review, Summers, Newman, Teachey, Clinical Lymphoma, Myeloma and Leukemia, 2026

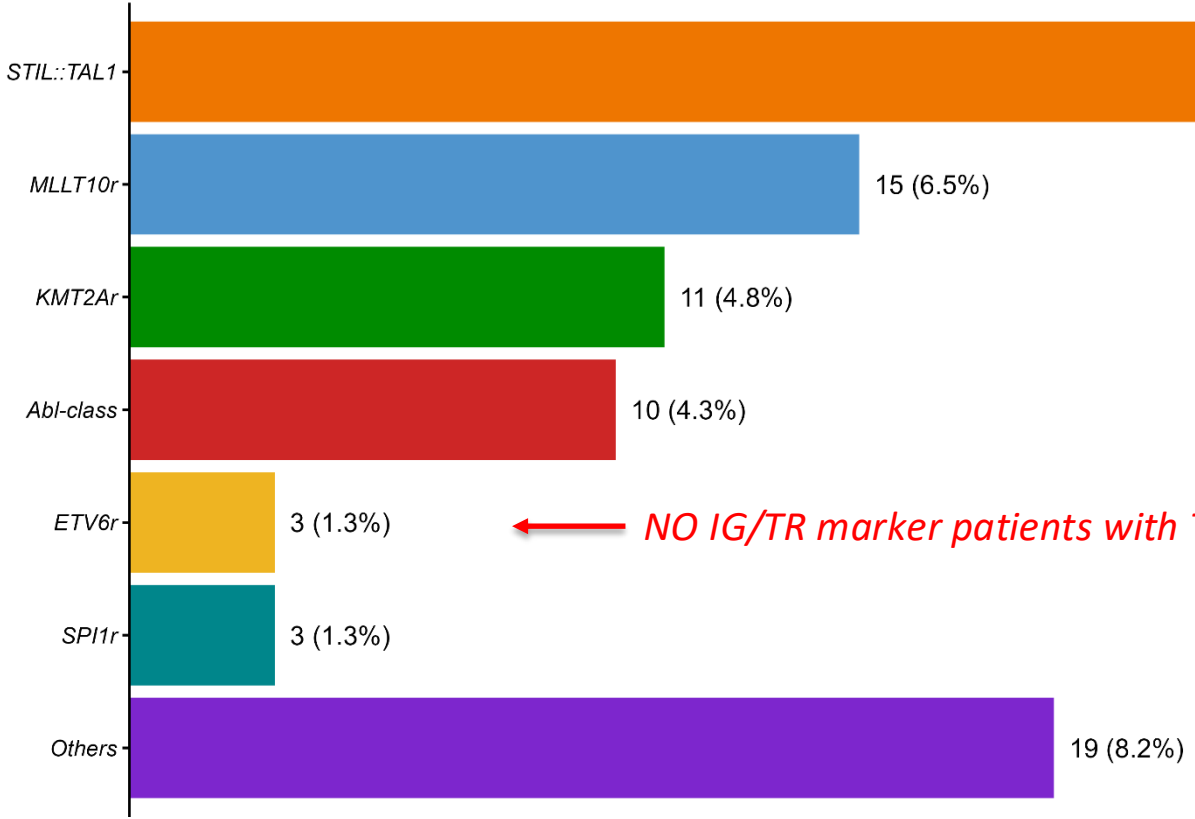
Detected fusions: B-ALL and T-ALL cohorts

B04
IL VALORE DELL'RNA-SEQUENCING NELLA DIAGNOSI DELLA LLA PEDIATRICA: CINQUE ANNI DI ESPERIENZA NELLA RETE AIEOP
C. Saitta, A. Colombo, F. Colnaghi, S. Rebellato, C. Palmi, N. Peccatori, L. Bettini, L. Lo Nigro, F. Locatelli, R. Parasole, M.C. Putti, B. Buldini, N. Bertorello, D. Silvestri, M.G. Valsecchi, A. Biondi, C. Rizzari, G. Cazzaniga, G. Fazio (Monza, Milano, Catania, Roma, Napoli, Padova, Torino)



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

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



NO IG/TR marker patients with TI-TII ALL!!!

campione del :			Marker 1 : ETV6::FOXO1	
			QR :	risultato :
BM	28-01-26	(giorno +15)	1x10-5	**1.8x10-0**
BM	17-02-26	(giorno +33)	1x10-5	**3.9x10-1**
BM	19-03-26	TP1b	NE	**NE**
BM	26-03-26	TP1b 2° prelievo	1x10-4	**pos <1x10-4**
BM	27-04-26	(giorno +78)	1x10-4	**pos <1x10-4**
BM	29-05-26	POST HR1	1x10-4	**neg**
BM	19/06/2026	POST HR2	1x10-4	**neg**
BM	24-07-26	PRE TMO	5x10-4	**neg**

B04

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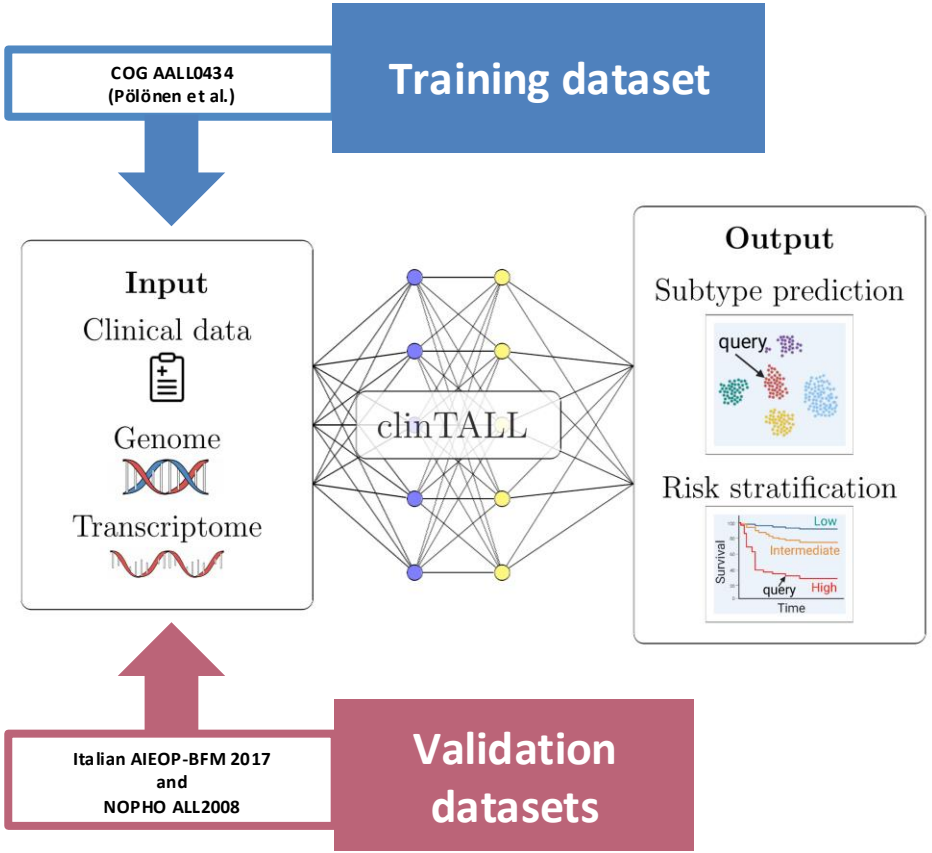
Stoiber *et al. Genome Medicine* (2026) 18:108
https://doi.org/10.1186/s13073-026-01733-8

Genome Medicine

RESEARCH Open Access

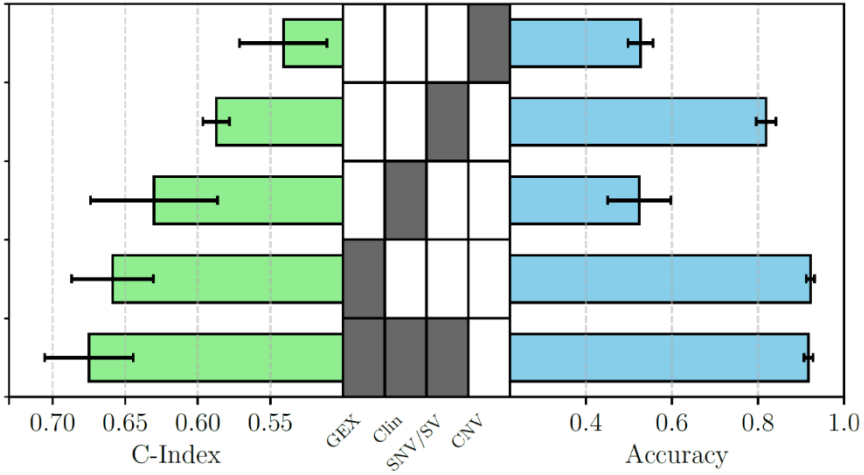
clinTALL: machine learning-driven multimodal subtype classification and treatment outcome prediction in pediatric T-ALL

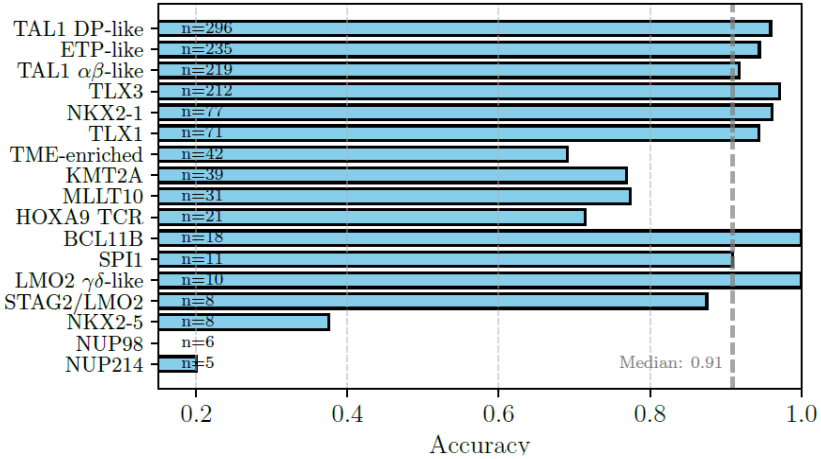
Lukas Stoiber[1], Željko Antić[1], Stefano Rebellato[2], Grazia Fazio[2,3], Annika Rademacher[4], Lennart Lenk[4], Franco Locatelli[5,6], Adriana Balduzzi[3,7], Gunnar Cario[4], Carmelo Rizzari[7], Giovanni Cazzaniga[2,3], Jiangyan Yu[1,8*] and Anke Katharina Bergmann[1,8,9,10]



Performance comparison across different data modalities

- Multimodal data outperforms unimodal data
- High per subtype accuracy for the majority of the tested subtypes

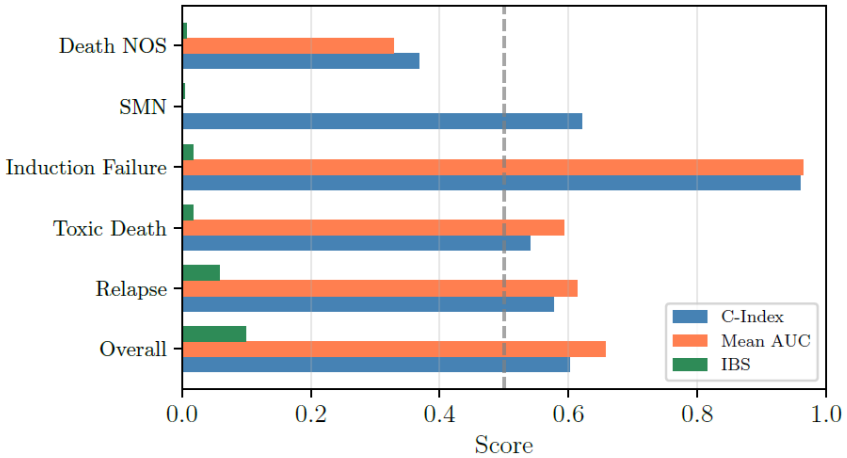




Stoiber et al., Genome Medicine 2026

Outcomes and limitations of a single risk score

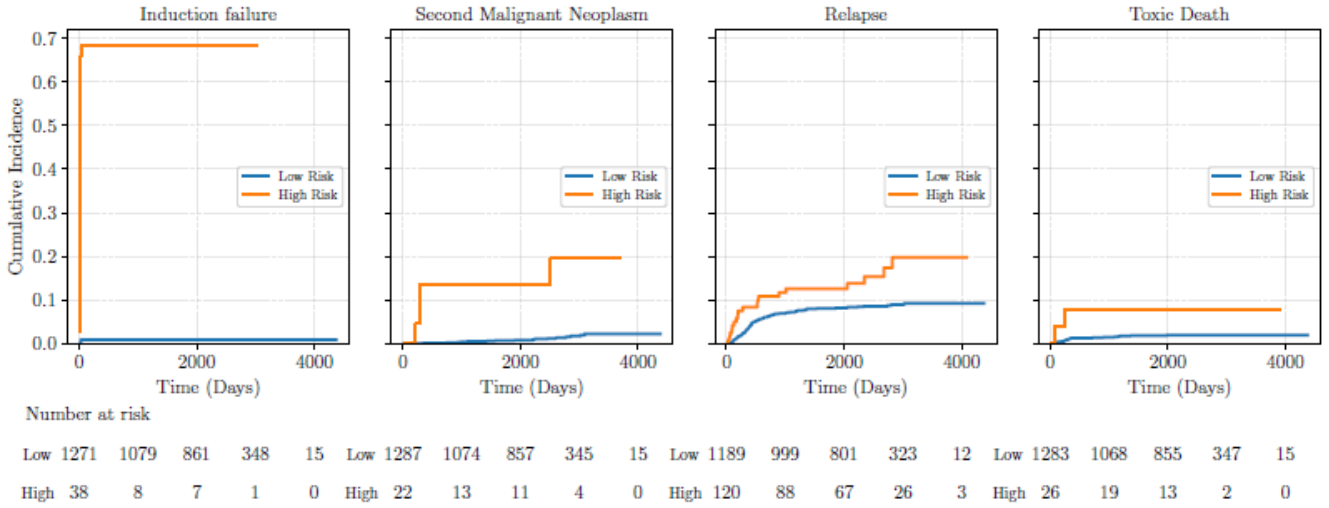
- Standard model successfully identifies patients with unfavorable outcome
- Can we move from a single „unfavorable“ outcome prediction model?



Stoiber et al., Genome Medicine 2026

Cumulative incidence of events

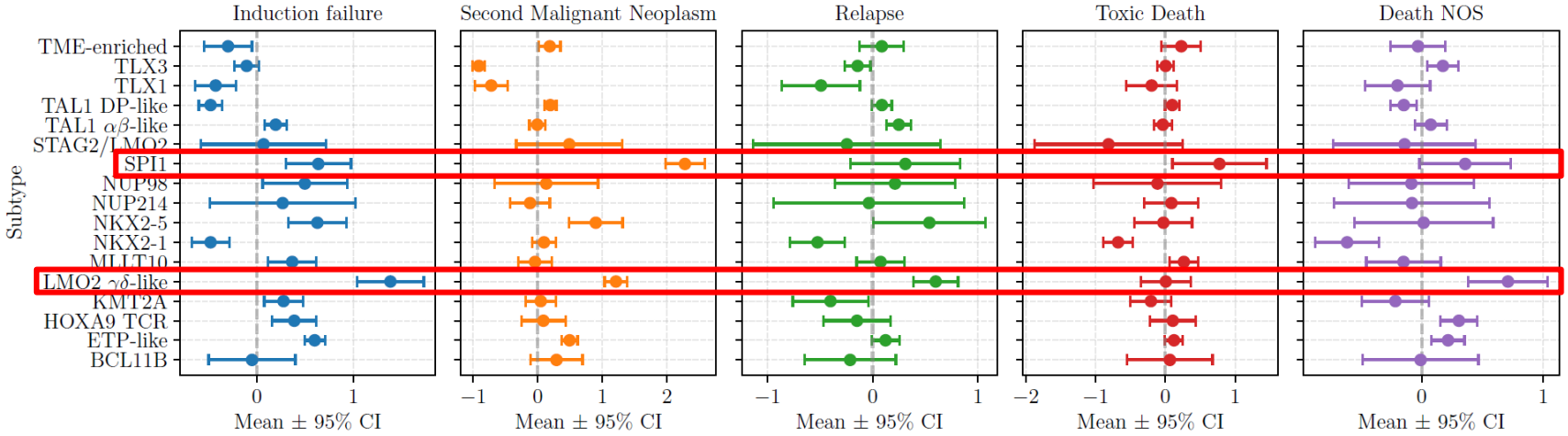
- Competing risk model
- Risk group stratification defined based on the third percentile of the observed scores of each event



Stoiber et al., Genome Medicine 2026

Cause-specific hazard coefficients per molecular subtype

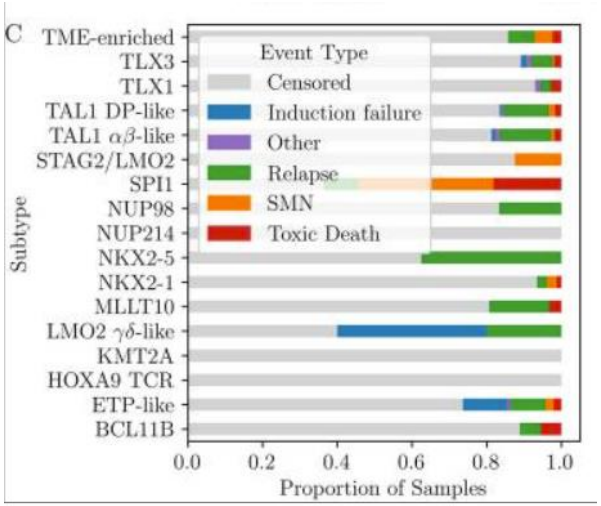
- LMO2 γδ-like – induction failure and relapse
- SPI1 – second malignancy and toxic death

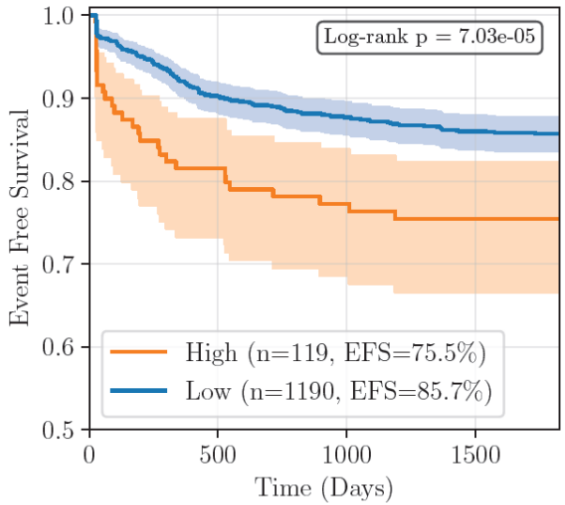


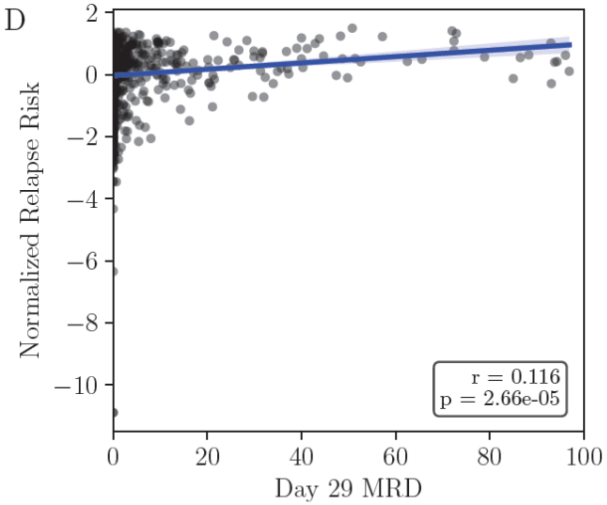
Stoiber et al., Genome Medicine 2026

High-risk patients have lower 5-year EFS

- Relapse-specific risk score stratification
- Lower 5-year EFS in high-risk group
- Score correlates with day 29 MRD





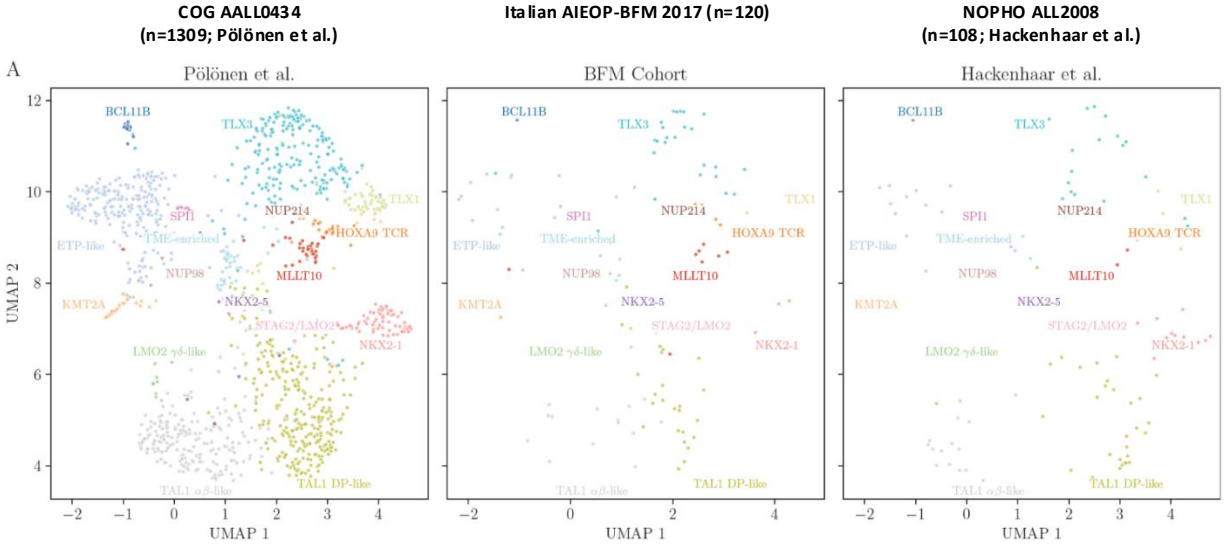


Stoiber et al., Genome Medicine 2026

Stoiber et al., Genome Medicine 2026

Performance in validation cohorts

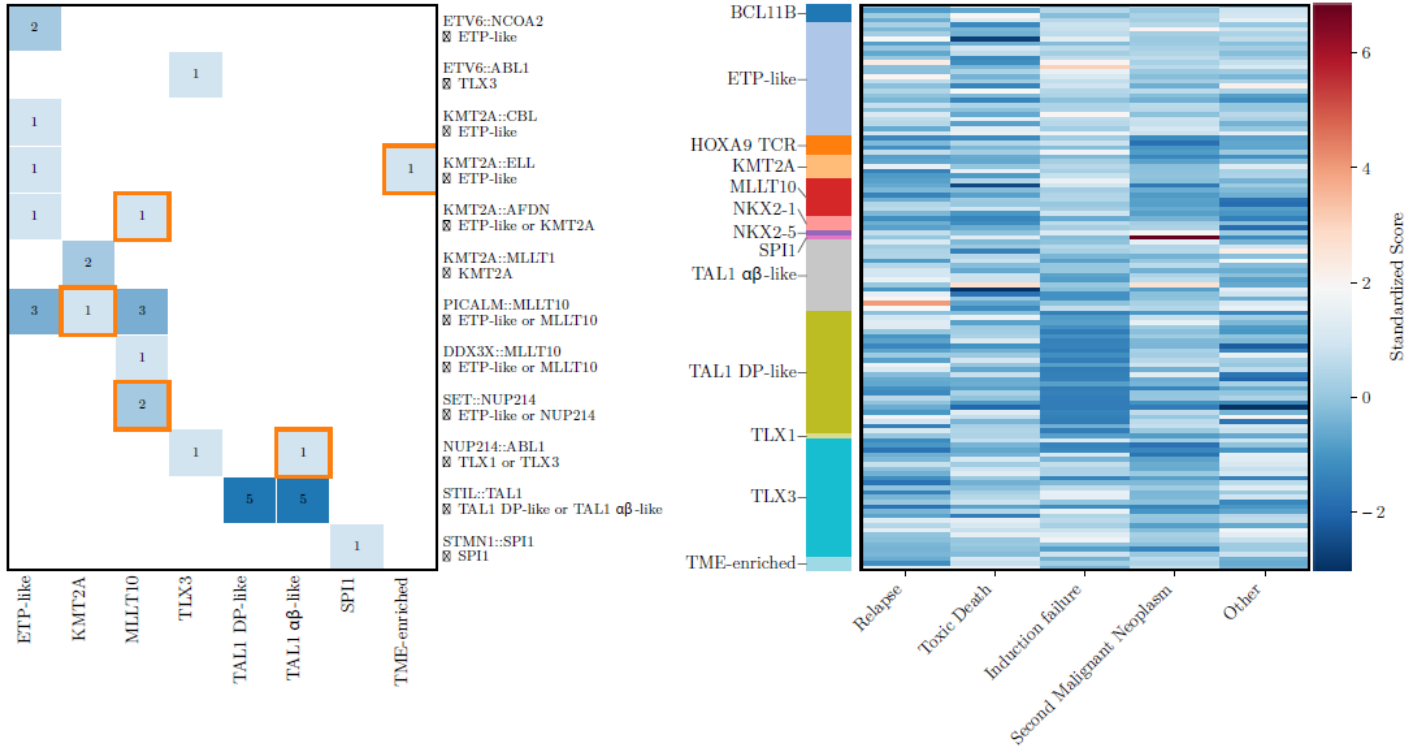
- Correct assignment based on the subtypes
- Consistent molecular subtype distribution across transcriptomics space in two independent validation cohorts



Performance in validation cohorts

- In total 8 predicted events in Italian AIEOP-BFM 2017:

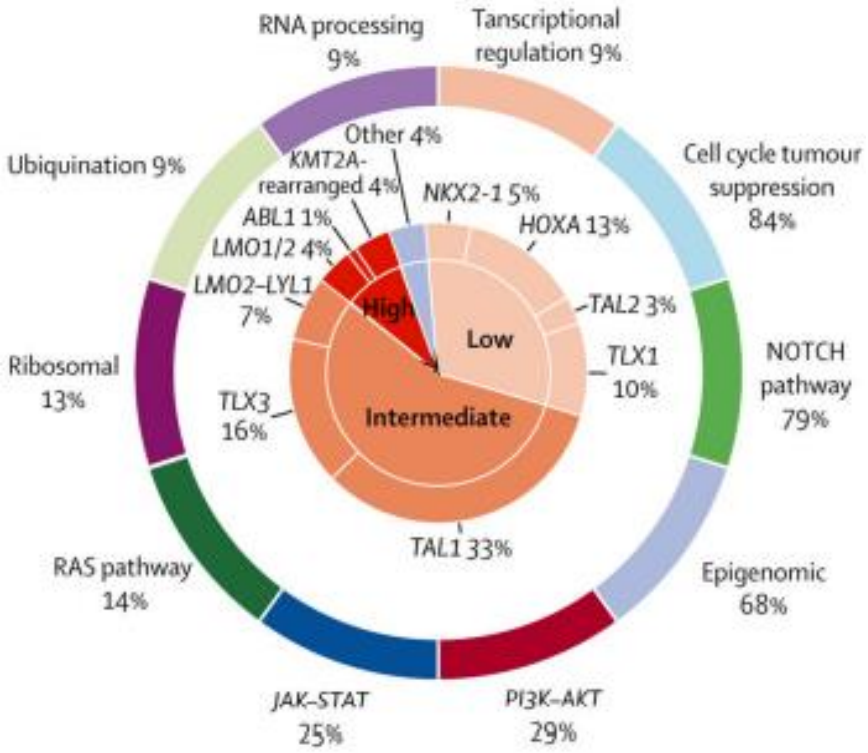
1) 5 relapses
2) 2 SMN
3) 1 induction failure



Stoiber et al., Genome Medicine 2026

Potential targeted agents for T-ALL

- Jak/Stat inhibitors
- LCK/SRC inhibitors
- BCL2 family inhibitors
- CDK4/6 inhibitors
- PARP inhibitors
- AKR1C3 inhibitors
- Menin inhibitors
- TKs inhibitors



Teachey and Pui, Genome Medicine 2026

Conclusions and future directions

- clinTALL, among other tools and studies, provides a unified multimodal, multitask framework
  1) molecular subtype
  2) outcome prediction
- Longitudinal data might be needed to improve prediction for later timepoints
- Exploratory studies in European cohorts

However, genetics is already crucial today for guiding strategies in patients with refractory or relapsed disease and for determining eligibility for hematopoietic stem cell transplantation in high-risk patients.

In conclusion, deciphering the genetic heterogeneity of pediatric T-ALL is no longer merely an exercise in biological classification; rather, it represents a fundamental prerequisite for the transition toward precision medicine.

Acknowledgements

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