



**XLIX**  
CONGRESSO  
NAZIONALE  
**AIEOP**

# Identificazione tramite RNA-seq di geni di fusione nei pazienti con leucemia acuta linfoblastica T (LAL-T) arruolati in Italia nel protocollo AIEOP-BFM ALL 2017

Grazia Fazio

Centro Tettamanti, Fondazione IRCCS San Gerardo dei Tintori,  
Monza

Bologna, 30 settembre 2024



***Il sottoscritto FAZIO GRAZIA***

*ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-  
Regione del 5 novembre 2009,*

dichiara

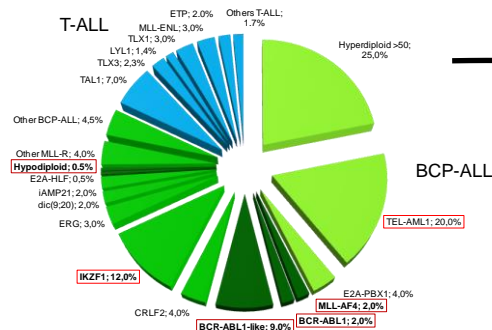
☐ *che negli ultimi due anni NON ha avuto rapporti diretti di finanziamento con soggetti  
portatori di interessi commerciali in campo sanitario*



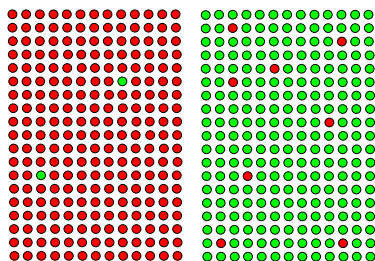
# State of the Art

- T-cell acute lymphoblastic leukemia (T-ALL) :
  - inferior outcome compared to B-cell precursor ALL *poster # AIEOP20485-66*
    - slower treatment response
    - lack of recurrent prognostic cytogenetic alterations.
- With the introduction of **Next-Generation Sequencing** analyses, a deeper characterization of childhood ALL has been achieved.
- However, the frequency and significance of gene fusions in T-ALL still remain poor.

## Molecular Basis of Leukemia



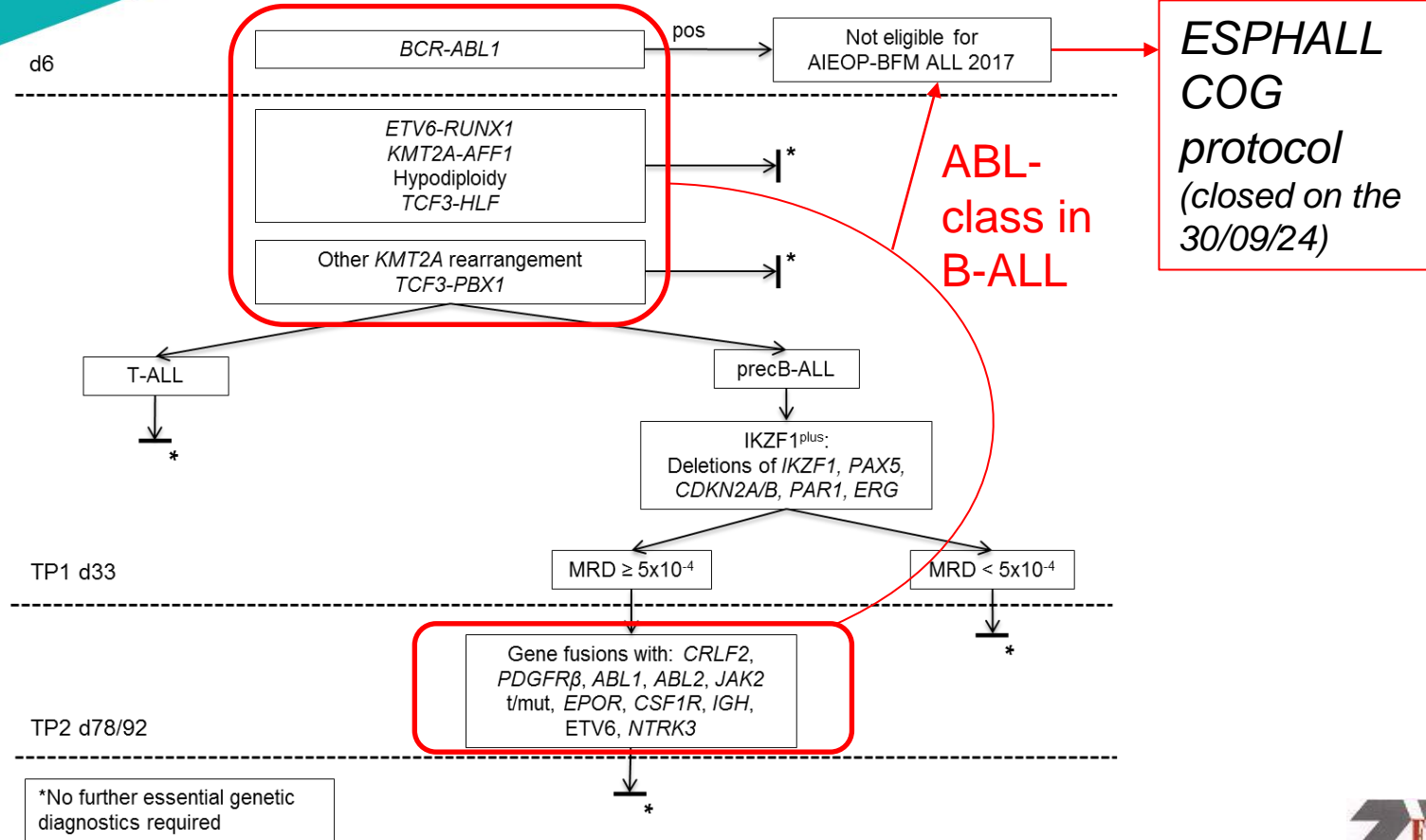
## Minimal Residual Disease



Diagnosis Follow up

- ✓ Pediatric BCP-ALL achieved a **85% cure rate** as a result of a risk adapted therapy, largely based on **Minimal Residual Disease (MRD) monitoring** and biological characterization.
- ✓ During the AIEOP-BFM ALL 2017 protocol, there was the need of **novel fusion genes** identification as, they represent: (i) prognostic value and stratification parameter; (ii) potential target for drugs -> personalized medicine.

Genetic  
diagnostics  
algorithm  
AIEOP-BFM  
ALL 2017





## Aims of the Study

- To describe the incidence of gene-class fusions in T-ALL by transcriptomic analysis (RNA-seq).
- To explore the association with patient characteristics and early treatment response.



# RNA-seq Experimental Strategy

**4 days**

Library  
preparation  
Manual  
**2 days**

NextSeq2000  
2x100  
**18h**

DRAGEN RNA (on Basespace  
cloud) **2h**/12 samples or  
GENOME UP platform for  
automated processing  
+ min **1 day** for manual review

Automated **10h**/8samples

*MAGIC prep  
TECAN GENOMICS*



FISH or RT-PCR  
validation (primer  
design, RT-PCR and  
Sanger sequencing)  
**3-10 days**

**+3-10 days**



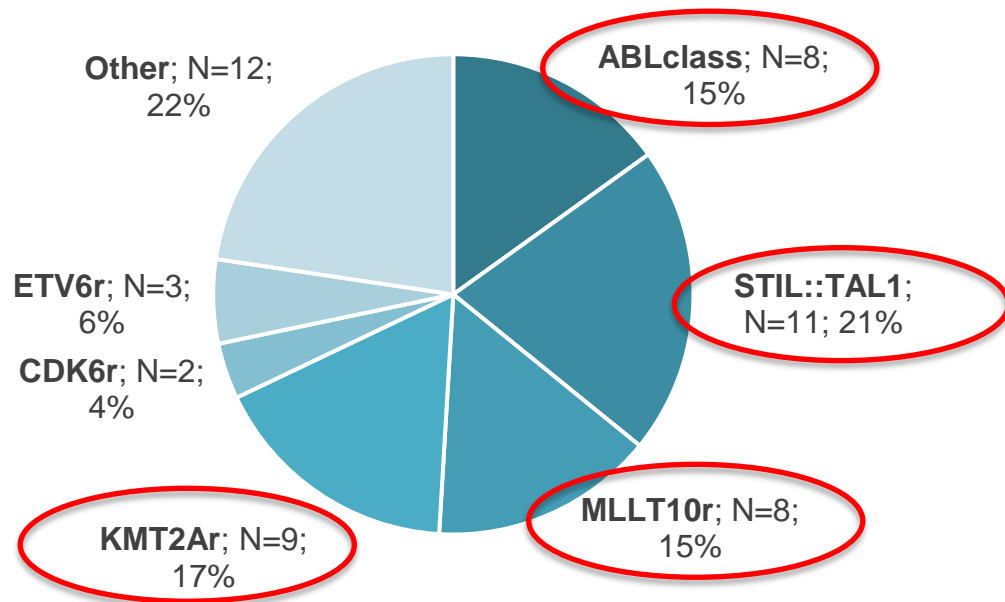
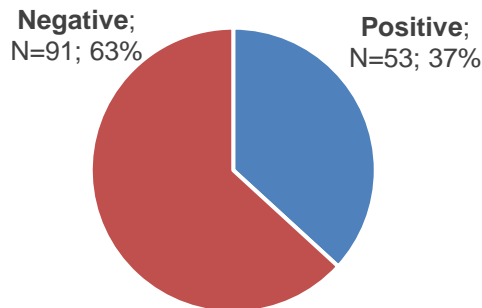




# Incidence of gene-class fusions in T-ALL by transcriptomic analysis (RNA-seq)

Fusion positive patients

**144 T-ALL patients** (both  
diagnosis and relapse)





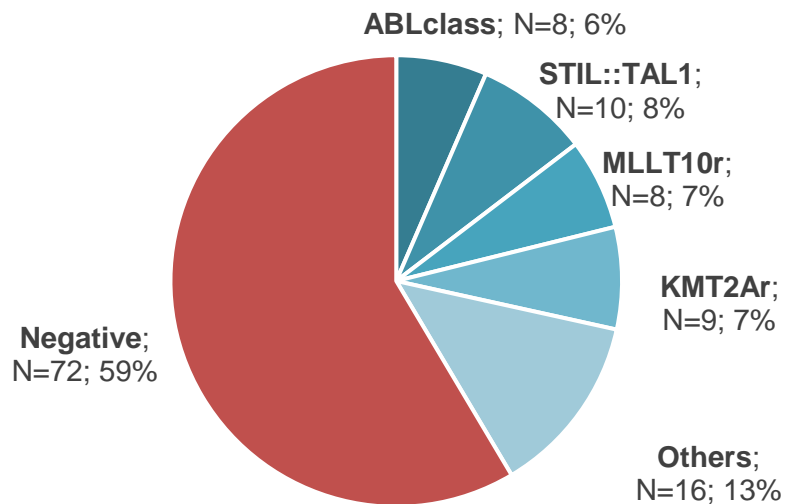


**144** T-ALL patients: both diagnosis and relapse

**132** enrolled into AIEOP-BFM 2017 (01 Dec 2021 – 31 Mar 2024)



**N=123** DX RNA-seq data available



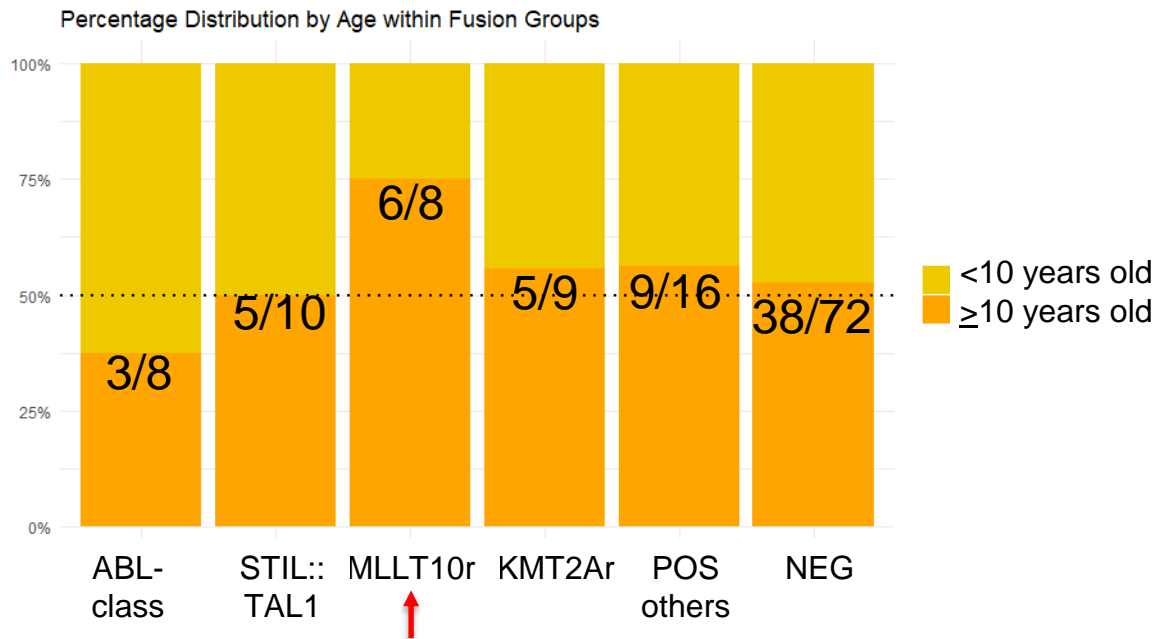
**Positive N=51; 41%**

**CLINICAL FEATURES**

- Age
- WBC
- Prednisone response
- Flow MRD day+15
- PCR MRD EOI



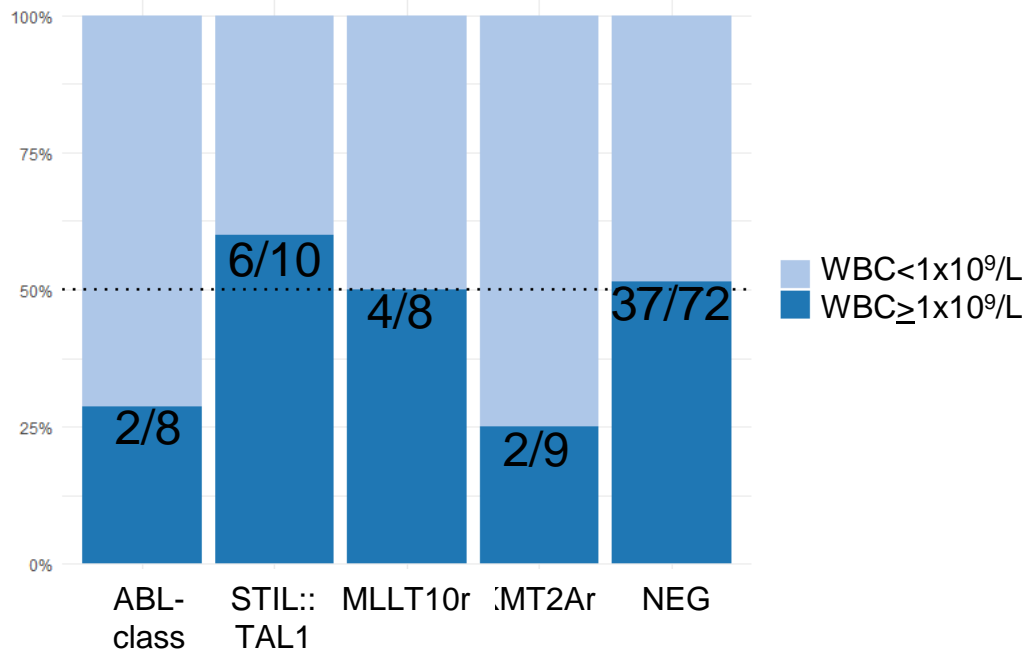
Patients with MLLT10-class fusion were more likely to be older (age  $\geq 10$  years)





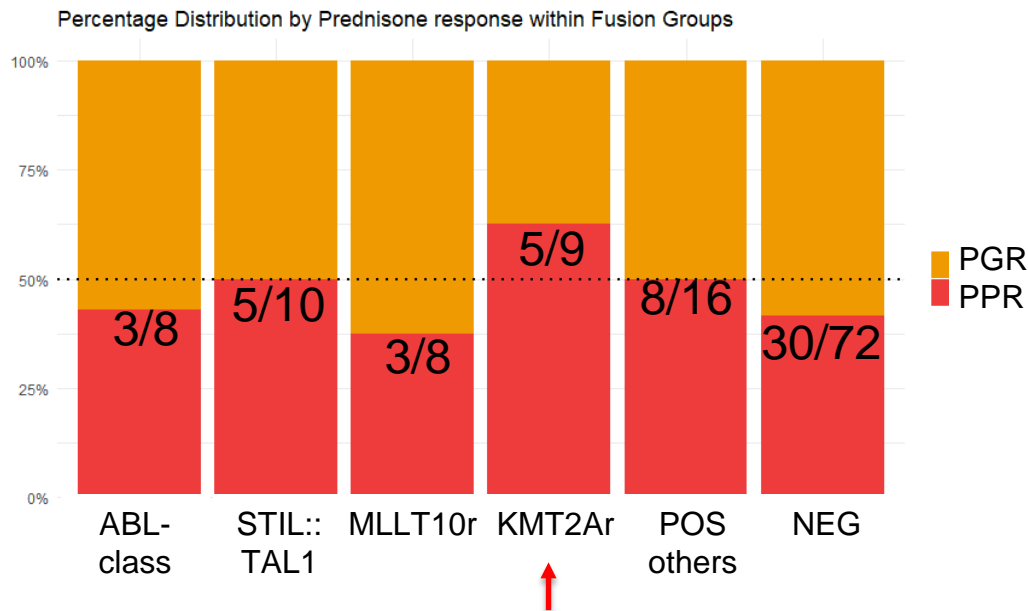
## *STIL::TAL1* fusion cases tended to present with hyperleukocytosis

Percentage Distribution of WBC at diagnosis within Fusion Groups



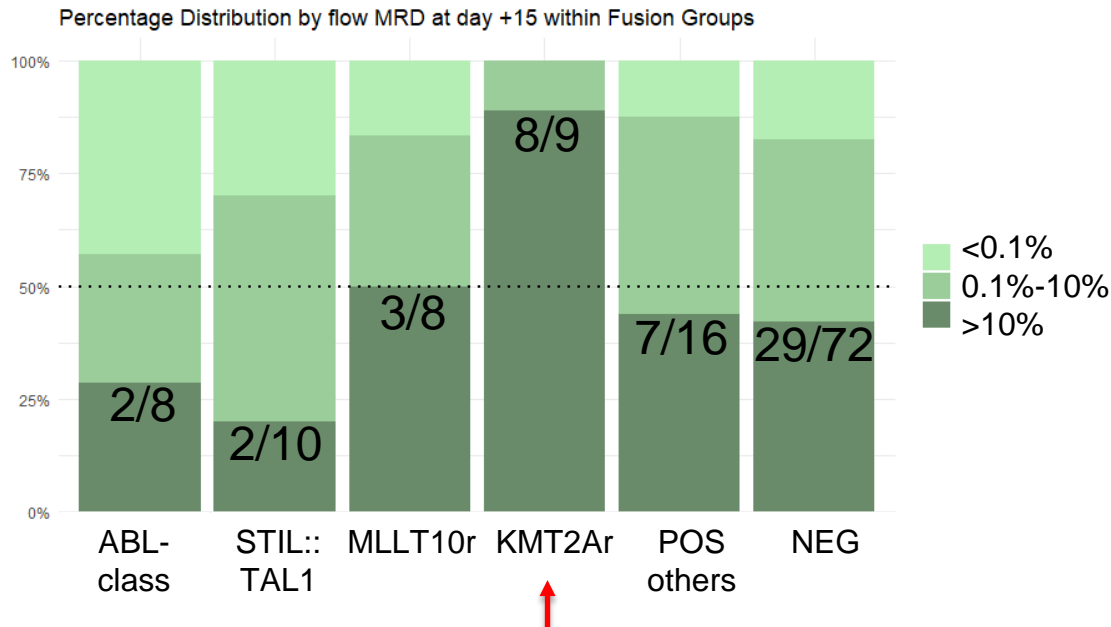


Patients carrying a *KMT2A* rearrangement were characterized by higher proportion of PPR



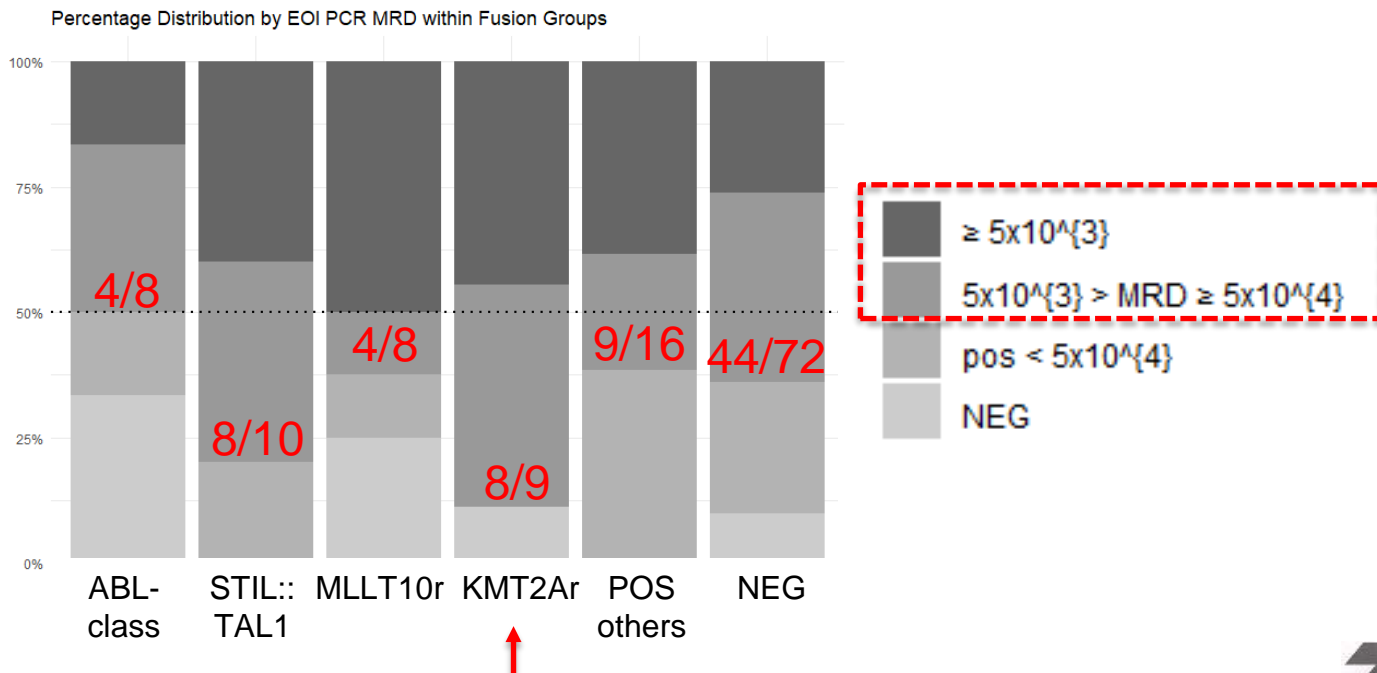


Patients carrying a *KMT2A* rearrangement were characterized by higher proportion of FCM-MRD with  $\geq 10\%$  of blasts.





Patients carrying a *KMT2A* rearrangement were characterized by higher EOI MRD levels





# Conclusions/1

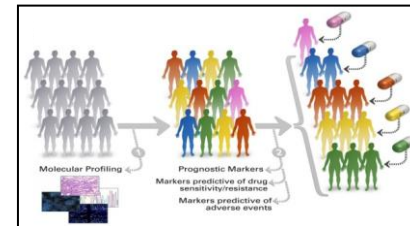
- ✓ High incidence of gene-class fusions in children and adolescents with newly diagnosed T-ALL.
- ✓ Identification of ABL-class patients among T-ALL, who may be responsive to targeted therapies (TKI).
- ✓ Despite our findings need to be validated in larger prospective cohorts, we found that KMT2Ar are associated with higher proportion of PPR, FCM-MRD with  $\geq 10\%$  of blasts and higher MRD levels at EOI, qualifying these patients for further early interventional studies.





## Conclusions/2

- ✓ **Molecular Biology** strategies allowed to achieve information in a short time, accordingly to protocol needs.
- ✓ Molecular Biology tests are **standardized** across Italian and International Laboratories.
- ✓ **NGS** implementation (when possible, e.g. marker screening /**fusion detection**) allowed the improvement of information rate, especially for difficult patients.
- ✓ Unexpectedly **high rate of fusion genes** in pediatric ALL patients, both B- and **T**-phenotype.
- ✓ **ABL-CLASS ALL**



These results could contribute to a **redefinition of ALL patients based on genetic profiles**, a better patient **stratification**, and potentially to a more tailored treatment approach with new **targeted drugs**.



## AIEOP Centers

**Valentino Conter**  
**Antonella Colombini**  
**Carmelo Rizzari**



**Laboratorio Ultraspecialistico di Patologia Clinica-Ematologia**  
**Pediatria - Centro M. Tettamanti**

**Fondazione IRCCS San Gerardo dei Tintori, Monza**

**Claudia Saitta**

**Nicolò Peccatori**

**Federica Colnaghi**

**Arianna Colombo**

**Giacomo Gotti**

**Andrea Biondi**

**Gianni Cazzaniga**



**Comitato**  
**Maria Letizia Verga**  
— ODV —

**CORS**

**Daniela Silvestri**

**Maria Grazia Valsecchi**



**Paediatric Haematology, Oncology and Stem Cell**  
**Transplant**

**Division, Women and Child Health Department**

**Elena Varotto**

**Barbara Buldini**

**Alessandra Biffi**

