XIX CONGRESSO NAZIONALE SIES 2026

L'impatto dell'omica nella diagnostica e nella stratificazione delle leucemie pediatriche

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Childhood Leukemia – Profiling

Pediatric acute leukemias (both ALL and AML) are molecularly heterogeneous diseases driven by recurrent cytogenetic and genomic alterations

- *Define biologically distinct subtypes*
- *Guide treatment intensity modulation*
- *Confer prognostic significance*

Therapeutic strategies tailored according to an integrated risk model

- *Cytogenetics and molecular genetics (NGS-based profiling)*
- *Early treatment response (MRD)*
- *Clinical features (age, WBC at diagnosis)*

Childhood ALL: A Success Story With Unfinished Work

Childhood ALL: From 10% to >90% Survival
Most common pediatric cancer (~25% of cases under 15)
Survival improved from ~10% (1960s) to >90% today
A true success story in pediatric oncology

Why?
Risk-adapted multiagent chemotherapy
CNS prophylaxis
International collaborative trials
Enhanced supportive care
Genetic insights + MRD monitoring

23.5% reduction in mortality (U.S., 2000–2010)

Persistent Challenges

- Adolescents and young adults lag behind younger children in outcomes
- Relapsed/refractory ALL carries poor prognosis
- ALL remains a leading cause of cancer-related death in children

Genomic Revolution

- NGS technologies have characterized germline and somatic alterations
- Novel subtype-defined chromosomal alterations identified
- "Others" category reduced from 25% to approximately 5%

Three Facets of Precision Medicine in Pediatric ALL

A decade of genomic discoveries has illuminated three interconnected dimensions of precision medicine that collectively reshape how we diagnose, treat, and monitor childhood ALL.

Inherited Predispositions

Germline variants — from rare high-penetrance mutations in familial ALL to common low-penetrance GWAS risk loci — that define susceptibility across ancestries and phenotypes

Molecularly Targeted Therapies

Precision oncology strategies in genomically-defined subtypes including Ph+ ALL, Ph-like ALL, KMT2A-R infant ALL, and T-ALL

Treatment Response Monitoring

Pharmacogenomics-guided dose adjustment and novel NGS-based MRD biomarkers for refined risk stratification

Inherited Predispositions to ALL

Prior to the NGS era, only a small number of genetic conditions — predominantly **Down syndrome (DS)** — were recognized as predisposing to ALL. Children with DS carry a 10- to 20-fold increased likelihood of developing acute leukemia, with more than half to two-thirds being ALL. DS-ALL exhibits unique biology: lower frequency of favorable cytogenetics (*ETV6-RUNX1*, hyperdiploidy) but 50–60% harboring *CRLF2* rearrangements with concomitant *JAK2* mutations reminiscent of Ph-like ALL.

Rare High-Penetrance Variants

Observed in familial ALL kindreds:

- ***TP53*** — Li-Fraumeni syndrome; found in ~50% of low-hypodiploid ALL
- ***PAX5*** — Two unrelated kindreds, each with 5 affected members
- ***ETV6*** — Familial thrombocytopenia and ALL
- ***IKZF1*** — Variable lymphopenia and ALL

Common Low-Penetrance Variants (GWAS)

Most reproducible genome-wide ALL risk loci:

- ***ARID5B***, ***IKZF1***, ***CEBPE*** — More prevalent in European descent
- ***ARID5B***, ***PIP4K2A*** — Enriched in hyperdiploid ALL
- ***GATA3*** — Increases Ph-like ALL susceptibility; enriched in Hispanic ethnicity
- ***USP7*** — Novel T-ALL risk locus enriched in African descent

Genetic ancestry modulates the frequency and significance of ALL risk alleles, underscoring the importance of studying diverse populations. The first dedicated T-ALL susceptibility GWAS identified USP7, providing genetic insight into the clinical observation that T-ALL is overrepresented among African-American patients.

pB-ALL: Risk group Stratification

Precursor B-cell ALL (pB-ALL) or unknown immunophenotype

Early High Risk (early HR)

- No complete remission on day 33 *or*
- Positivity for *KMT2A-AFF1 or*
- Positivity for *TCF3-HLF*1 *or*
- Hypodiploidy <45 chromosomes *or*
- FCM-MRD in BM on day 15 ≥ 10% *and not ETV6-RUNX1* positive *or*
- *Ikzf1*plus *and* PCR-MRD at TP1 positive or inconclusive *and not* positive for
- *ETV6-RUNX1, TCF3-PBX1* or *KMT2A* rearr. Other than *KMT2A-AFF1 or*
- PCR-MRD at TP1 ≥ 5x10-4 *or*
- Age < 1 year and any *KMT2A* rearrangement

High Risk (HR)

- No complete remission on day 33 *or*
- Positivity for *KMT2A-AFF1 or*
- Positivity for *TCF3-HLF*1 *or*
- Hypodiploidy <45 chromosomes *or*
- FCM-MRD in BM on day 15 ≥ 10% *and not ETV6-RUNX1* positive *or*
- *Ikzf1*plus *and* PCR-MRD at TP1 positive or inconclusive *and not* positive for
- *ETV6-RUNX1, TCF3-PBX1* or *KMT2A* rearr. Other than *KMT2A-AFF1 or*
- PCR-MRD at TP1 ≥ 5x10-4 and positive < 5x10-4 at TP2 (PCR-MRD SER) *or*
- PCR-MRD at TP2 ≥ 5x10-4 *or*
- Age < 1 year and any *KMT2A* rearrangement

Medium Risk (MR)

- No HR criteria and no SR criteria

Standard Risk (SR)

- No HR criteria and
- PCR-MRD at TP1 negative for all investigated markers with at least one marker
- With a quantitative range of ≤ 10-4 or
- Inconclusive PCR-MRD result at TP1 and PCR-MRD not positive at TP2 and FCMMRD
- In BM d15 < 0.1%

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T-ALL: risk group stratification

T cell-ALL (T-ALL)

High Risk (HR)

- No complete remission on day 33 or
- FCM-MRD in BM on day 15 ≥ 10% or
- Prednisone Poor-Response or
- PCR-MRD at TP2 ≥ 5x10-4

Early Standard Risk (early SR)

- Complete remission on day 33 and
- FCM-MRD in BM on day 15 <10% and
- Prednisone good-response and
- PCR-MRD at TP1 negative for all investigated markers with at least one marker
- With a quantitative range of ≤ 10-4 or
- Inconclusive PCR-MRD result at TP1 and FCM-MRD in BM d15 < 0.1%

non-High Risk (non-HR)

- No HR criteria

Experimental groups eligible for possible other treatments (not part of this study)

- Positivity for TCF3-HLF
- Molecular non-response (either ≥ 5x10-3 after 3rd HR block/2nd Blinatumomab cycle [in the R-HR experimental arm] or ≥ 5x10-4 after Myocet/FLA)

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Ph+ ALL: The First Molecularly Targeted Paradigm

•BCR-ABL1 fusion (3–5%) a historically poor prognosis
•Pre-TKI era: HSCT main curative option, <50% survival

•**TKI + chemotherapy changed the paradigm**
~70% of children avoid HSCT in CR1
Imatinib era: EFS ~60–70%, OS ~80%
Dasatinib: improved EFS and OS vs imatinib
Reduced need for HSCT and cranial irradiation

From transplant-dependent disease to targeted precision therapy

Ph-like ALL: Expanding the Precision Oncology Paradigm

Prevalence: ~15% of pediatric acute lymphoblastic leukemia (ALL).
Highest incidence: in young adults aged 21–39 years.
Molecular profile: similar to Ph+ ALL but lacking the BCR-ABL1 fusion.
Targetable lesions: >90% of cases harbor kinase-activating alterations.

Four Main Molecular Pathways

- **JAK-STAT Pathway**

Rearrangements: CRLF2, JAK2, EPOR.
Mutations: IL7R, SH2B3.
Targeted drug: ruxolitinib.

- **ABL-Class Fusions**

Genes: ABL1, ABL2, CSF1R, PDGFRA, PDGFRB, LYN.
Targeted drugs: dasatinib or imatinib.

- **RAS Pathway**

Mutations: NRAS, KRAS, NF1, PTPN11, CBL, FLT3.
Result: activation of MAPK signaling.

- **Rare Kinase Fusions**

Genes: NTRK3, PTK2B, FGFR1, TYK2.
Targeted drug: larotrectinib (for NTRK) and other agent
Therapeutic implications: robust preclinical and growing clinical data indicate durable remissions with appropriate TKIs, ongoing trials are testing TKI–chemotherapy combinations, and frequent IKZF1 lesions offer additional targets for retinoids and

CHAPTER 2 — CONTINUED

Relapsed ALL & the Immunotherapy Revolution

Relapse occurs in **10–20%** of childhood ALL cases and carries substantially worse survival. Genome-wide studies reveal that approximately 75% of relapsed ALL mutations derive from minor subclones present at diagnosis, while relapse-specific mutations enriched in drug-resistance genes occur in **65% of on-therapy relapses**. Therapy-induced mutations in *NT5C2* and *PRPS1* — undetectable at diagnosis — mediate acquired purine resistance.

While molecularly targeted approaches (Ras, PI3K/mTOR, epigenetic modifiers) have yielded promising preclinical results, none has translated into meaningful survival improvement for relapsed ALL. In contrast, **immunotherapies have transformed this landscape** by operating independently of sentinel genetic aberrations, thus overcoming chemoresistance mutations.

Blinatumomab

CD3/CD19 bispecific T-cell engager. Superior efficacy and fewer toxicities vs. standard chemotherapy as post-reinduction consolidation for first B-ALL relapse (COG AALL1331).

Inotuzumab Ozogamicin

Anti-CD22 antibody-drug conjugate. Achieved **58% CR rate** after one cycle in heavily pretreated R/R B-ALL; 65% of responders achieved MRD <0.01%.

CAR-T Cell Therapy

CD19-directed CAR-T cells. **90% CR rate**, 6-month EFS 67%, OS 78%. Potentially definitive salvage therapy rather than merely a bridge to transplant.

Relapsed ALL: Risk Stratification

- Classification from IntReALL 2010

vs

- Revised risk-group stratification schema from IntReALL 2020 for BCP-ALL

A. Definition of IntReALL 2010 Risk Group.

	Immunophenotype B-cell precursor ALL		
Time point vs Site of relapse	**Isolated EM**	**Combined BM/EM**	**Isolated BM**
Very early < 18 months after initial diagnosis	HR	HR	HR
Early ≥ 18 months after initial diagnosis and < 6 months after completion of 1L therapy	SR	SR	HR
Late ≥ 6 months after completion of 1L therapy	SR	SR	SR

B. Definition of IntReALL 2020 Risk Group.

	Immunophenotype B-cell precursor ALL		
Time point and cytogenetic characteristics vs Site of relapse	**Isolated EM relapse**	**Combined BM/EM**	**Bone marrow isolated**
Very early < 18 months after initial diagnosis	HR	VHR	VHR
Early ≥ 18 months after initial diagnosis and < 6 months after completion of initial therapy	SR	SR	HR
Late ≥ 6 months after completion of initial therapy	SR	SR	SR
Presence of • **TP53 mutation and/or deletion** • **Hypodiploidy(< 40 chromosomes)** • **t(1;19) TCF3-PBX1 or (17;19) TCF3-HLF** • **KTM2A/AF4**	Refer to time point of relapse	**VHR Independently from timing**	**VHR Independently from timing**

Abbreviations: HR: High-Risk; SR: Standard-Risk; VHR: Very-High-Risk; EM=extramedullary; BM=bone marrow; 1L=first line therapy

C. Subgroup analysis of VHR patients obtained from pooled data of ALL-R3 and ALL-REZ BFM 2002.

EFS at 10 years	PFS at 10 years	OS at 10 years	Induction failure*
20.6% (SE ±3.4%)	32.6% (SE ±5.0%)	25.4% (SE ±3.7%)	36.9%

Pharmacogenomics, Novel MRD Biomarkers & Future Directions

Inherited gene polymorphisms influencing drug response represent the most immediately actionable facet of precision medicine in ALL. Pre-emptive ***TPMT*** and ***NUDT15*** genotyping is now recommended at diagnosis: homozygous patients tolerate <10% of intended mercaptopurine dose, while heterozygous patients tolerate 60–70% of dose intensity. Risk allele frequencies vary substantially by ancestry — *NUDT15* is most common in East Asians (10%), while *TPMT* variants predominate in Africans.

1

Pharmacogenomics Integration

TPMT/NUDT15 genotyping for thiopurine dosing. Additional variants identified for asparaginase hypersensitivity (*HLA*), methotrexate toxicity (*SLCO1B1*), vincristine neuropathy (*CEP72*), and anthracycline cardiomyopathy (*CBR*).

2

NGS-Based MRD

NGS MRD offers superior sensitivity to PCR, identifies 95.4% trackable index sequences, and reclassifies 55 MFC-negative patients as positive with significantly poorer EFS. Identified 20% of SR B-ALL with undetectable MRD and outstanding 5-year EFS of **98.1%**.

3

The Road Ahead

Implement NGS platforms in clinical labs with cost-effective diagnostic algorithms. Design international collaborative trials for rare subtypes. Investigate TKI-era resistance mechanisms. Prioritize combinatorial strategies synergizing pathway inhibitors with immunotherapy.

> *"The landscape of ALL holds exciting therapeutic opportunities and a new ensemble of challenges that the pediatric ALL community will endeavor together in the quest for finding the needle in the haystack."* — Tran & Hunger

Infant KMT2A-R ALL & T-ALL: Urgent Therapeutic Frontiers

KMT2A-Rearranged Infant ALL

Infant ALL remains one of the most challenging subtypes, with outcomes essentially unchanged over decades. *KMT2A* rearrangements occur in **70–75%** of cases, and the genome is remarkably silent with one of the lowest somatic mutation rates in human cancers.

- **Lestaurtinib** (FLT3 inhibitor): No added benefit (3-year EFS 37% in both arms)
- **Menin inhibitors**: Profound preclinical antileukemic activity; Phase 1 trials in adults (SNDX-5613, KO-539)
- **DOT1L inhibitors**: Pinometostat showed limited single-agent efficacy; next-generation agents show potent activity in PDX models
- **Epigenetic priming**: Azacitidine (COG pilot) and vorinostat/bortezomib (SJCRH Phase 1B) under investigation
- **Venetoclax**: Enhanced sensitivity in *KMT2A-R* xenografts; prioritized for high-risk pipeline

T-Cell ALL

Sentinel genetic alterations in T-ALL do not significantly refine MRD-based risk stratification but highlight multiple precision medicine opportunities across dysregulated functional pathways.

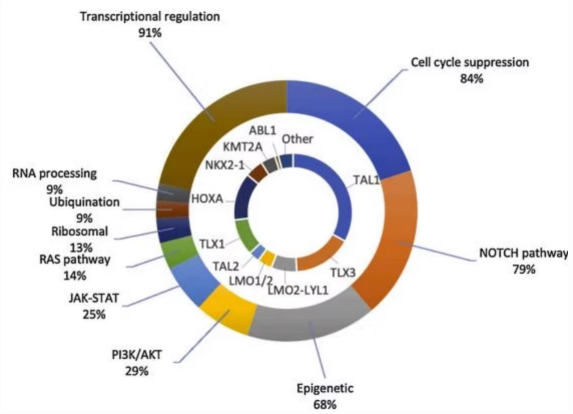
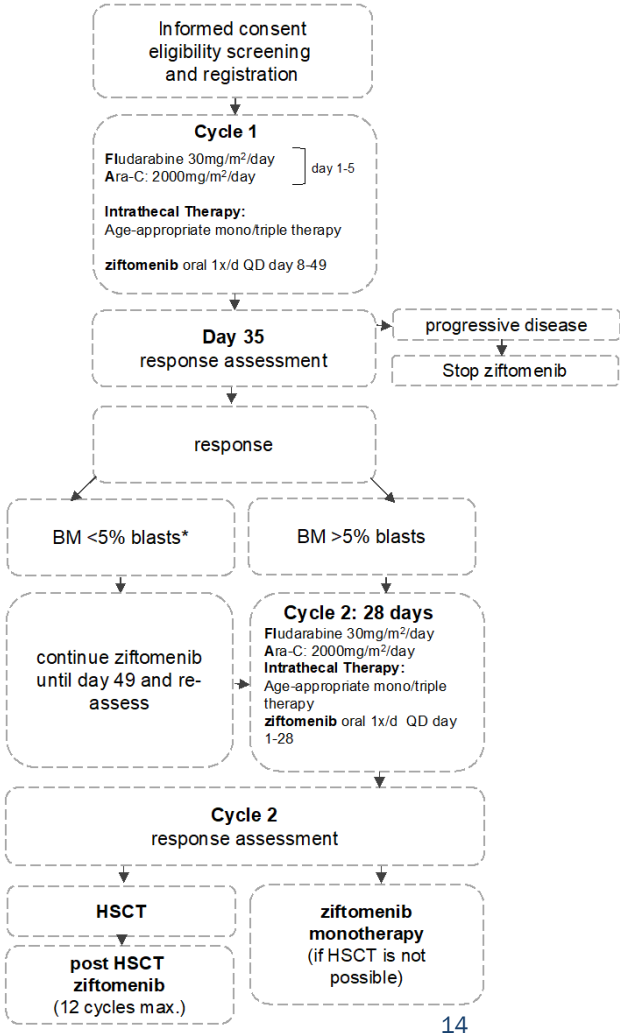


Figure 2. Targetable functional pathways in T-ALL: Notch (79%), PI3K/AKT (29%), JAK-STAT (25%), Ras (14%). Bortezomib is the only targeted agent incorporated into frontline Phase III T-ALL trials (COG AALL1231), with benefit in MRD-defined favorable-risk patients.

ITCC-101/APAL2020K: eligibility criteria

- Age: 0-21 years (and at least 5 kg BW), with a minimum of 80% of patients under 18 years of age
- Diagnosis: KMT2A-r, NPM1-m, or NUP98-r acute leukemia in first or greater relapse or refractory to standard (re-) induction treatment (including HSCT).
- Eligible patients also must fulfill one of the following conditions:
  - *First or subsequent relapse:*
    - a) Bone marrow relapse is defined as:
      - *i) a single bone marrow sample or bone biopsy showing ≥ 5% leukemic blasts by local morphology*
    - b) Patients with combined extramedullary and bone marrow relapse (defined as above) including CNS disease are eligible.
  - *Refractory disease/induction failure:*
    - Bone marrow ≥ 5% leukemic blasts by local morphology at the end of 2 cycles of induction therapy



AML in Children: The Challenge

Clinical Landscape

•~20% of childhood leukemias
•Responsible for >50% of adverse outcomes
•Survival improved from ~30% (1970s) to ~70% today
→ Chemotherapy intensification
→ Better supportive care
→ Expanded HSCT use
⚠ Further progress requires targeted therapies

Genetic Landscape: Highly Heterogeneous
Leukemogenesis follows a "two-hit" model

➢ Type I aberrations – ↑ proliferation/survival
•FLT3, KIT
•NRAS, KRAS

➢ Type II aberrations – Impaired differentiation/self-renewal
•KMT2A rearrangements (11q23)
•RUNX1–RUNX1T1 t(8;21)
•CBFB–MYH11 inv(16

Pediatric vs Adult AML

Abnormal karyotype: 76% pediatric vs 55% adult
•Normal karyotype: 20–25% pediatric vs ~50% adult

•Pediatric-specific fusion
NUP98-KDM5A, CBFA2T3-GLIS2, MNX1-ETV6

AML: risk stratification

RISK GROUP	GENETIC RISK CRITERIA	RESPONSE CRITERIA

STANDARD RISK (SR)	• **CBFβ abnormalities**
	  • **t(8;21)(q22;q22) with adequate (≥ 2 log) reduction by qPCR at IND 2**
	  • **inv(16)(p13q22)/t(16;16)(p13;q22)**
	• **Biallelic CEBPα aberrations**
	• **NPM1 mutations**
	• **t(16;21) *CBFA2T3/RUNX1***
	
	and FLT3-ITD negative
		Genetic standard risk and
		• **MRD <0.1% at IND 2**
		
		t(8;21) and
		• **MRD ≥2 log reduction at IND 2 (qPCR)**

INTERMEDIATE RISK (IR)	• **NON SR and NON HR patients**	**Genetic standard or intermediate risk and** • **MRD at IND 1 ≥ 0.1% and <1% and MRD at IND 2 < 0.1%**

HIGH RISK (HR)	• **Complex karyotype (≥3 aberrations including at least one structural aberration) *excluding those with recurrent translocations***
	• **Monosomal Karyotype, i.e. -7, -5/del(5q)**
	• **11q23/*KMT2A* rearrangements involving:**
	  • **t(4;11)(q21;q23) KMT2A/AFF1**
	  • **t(6;11)(q27;q23) KMT2A/AFDN**
	  • **t(10;11)(p12;q23) KMT2A/MLLT10**
	• **t(16;21)(p11;q22) FUS/ERG**
	• **t(9;22)(q34;q11.2) BCR/ABL1**
	• **t(6;9)(p22;q34) DEK/NUP214**
	• **t(7;12)(q36;p13) MNX1/ETV6**
	• **inv3(q21q26)/t(3;3)(q21;q26) RPN1/MECOM**
	• **12p abnormalities**
	• ***FLT3-ITD* with AR ≥ 0.5 not in combination with other recurrent abnormalities or NPM1 mutations**
	• ***WT1* mutation and *FLT3-ITD***
	• ***inv(16)(p13q24) CBFA2T3/GLIS2***
	• ***t(5;11)(q35;p15.5) NUP98/NSD1 and t(11;12)(p15;p13) NUP98/KDM5A***
	• **Pure Erythroid leukemia**
	• **UBTF-TD**
		• **MRD ≥ 1% at IND 1 or ≥0.1 at IND 2 or (only if FLOW-result not available/informative) blast count ≥5% at IND 1**

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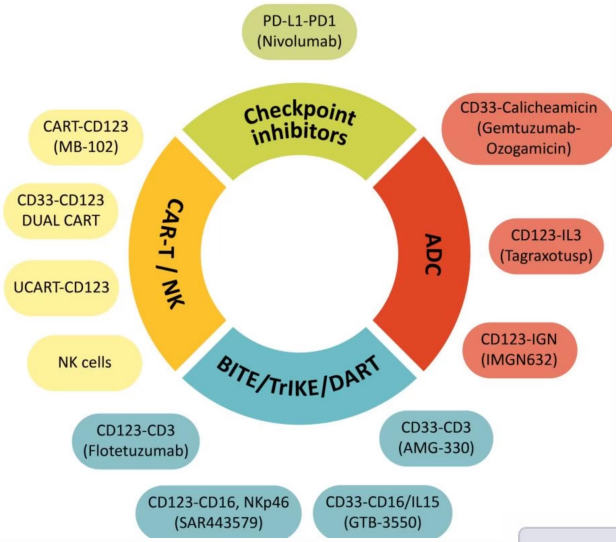
Immunotherapy Approaches in Pediatric AML



Immunotherapy aims to stimulate anti-tumor immune responses by targeting molecules highly expressed on leukemic cells but absent or minimal on normal cells. The two primary targets in pediatric AML are CD33 (present in >80% of cases) and CD123 (present in >90% of cases).

CD33: Gemtuzumab Ozogamicin

ADC targeting CD33 — FDA-approved for pediatric R/R AML (≥2 years). COG Phase III showed improved event-free survival. Patients with *FLT3-ITD*, *KMT2A*, and high CD33 expression benefit most.

CD123: Multiple Modalities

Tagraxofusp (SL-401) — FDA-approved for BPDCN. **IMGN632** — ADC showing 33% CR in R/R AML; pediatric trial in preparation (NCT05320380). **Flotetuzumab** — CD123/CD3 DART with 30% ORR; pediatric trial planned.

CAR-T & NK Cells

CD123-CAR-T trials underway at St. Jude (NCT04318678) and CHOP (NCT04678336). Cytokine-pretreated memory-like NK cells show **robust, persistent antileukemic effect** in pediatric patients.

Checkpoint inhibitors (nivolumab) are also being explored, with a pediatric R/R AML trial in preparation (NCT03825367), particularly in combination with hypomethylating agents to enhance PD-1 expression on tumor cells.

Looking Ahead: The Path Forward

Comprehensive genetic profiling has revealed the great molecular heterogeneity of pediatric AML, making laboratory research clinically relevant and enabling causal-directed treatment design. However, the rarity of pediatric AML limits clinical trial enrollment, and heterogeneity contributes to a **survival plateau not exceeding 70%**.

1 Pediatric-Specific Biology

Simply adopting adult therapy is insufficient — pediatric AML has distinct genetic profiles requiring tailored approaches.

2 Targeted + Immunotherapy Synergy

Combining molecular inhibitors (venetoclax, FLT3 inhibitors, menin inhibitors) with immunotherapy (GO, CAR-T, NK cells) holds the greatest promise.

3 International Collaboration

Improved personalized treatment demands intensive, broad international collaboration through large randomized studies — including adolescents and young adults.

The development of novel targeted therapies, guided by precise molecular characterization and risk stratification, is essential for breaking through the current survival plateau in pediatric AML.

Thank you!

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