# Novel classification system and high-risk categories of pediatric acute myeloid leukemia

by Masayuki Umeda, Yen-Chun Liu, Seth E. Karol, and Jeffery M. Klco

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**Abstract (182/250)**

The prognosis of pediatric acute myeloid leukemia (AML) remains poor compared with pediatric acute lymphoblastic leukemia (ALL); accurate diagnosis and treatment strategies based on the genomic background are strongly needed. Recent advances in sequencing technologies have identified novel pediatric AML subtypes, including *BCL11B* structural variants and *UBTF* tandem duplications (*UBTF*-TD), associated with poor prognosis. In contrast, these novel subtypes do not fit into the diagnostic systems for AML of the 5th edition WHO classification or International Consensus Classifications (ICC) released in 2022. In this review, we describe the current state of pediatric AML classification in the context of a new classification framework based on the findings of updated genomic profiling. Molecular categories in the new classification system are associated with unique transcriptional, mutational, and clinical characteristics, which can be leveraged for predicting clinical outcomes and developing molecular-target therapies based on the initiating driver alterations. We also highlight four high-risk subtypes of pediatric AML, namely *CBFA2T3*::*GLIS2*, *BCL11B*, *UBTF*-TD, and ETS family fusions, focusing on their disease mechanisms, clinical associations, and possible therapeutic strategies to overcome the dismal clinical outcomes associated with these alterations.

**Main text**

*Introduction*

Acute myeloid leukemia (AML) is a disease characterized by uncontrolled growth and differentiation block of hematopoietic cells of myeloid lineage (1). Compared with adult AML, which commonly develops from the somatic accumulation of single nucleotide variants (e.g. *DNMT3A, IDH1-2,* and myelodysplasia-related mutations), often via clonal hematopoiesis or progression from myelodysplastic neoplasm (MDS), pediatric AML has a unique genomic background characterized by frequent chromosomal translocations (2). Traditionally, karyotyping techniques such as G-banding and fluorescence in situ hybridization (FISH) have been utilized in classifying AMLs. Core binding factor (CBF) leukemia with t(8;21) (encoding *RUNX1::RUNX1T1*) or inv(16) (encoding *CBFB::MYH11*) are similarly found both in adult and pediatrics, whereas translocations of chromosome 11 involving *KMT2A* (also known as *MLL*) are enriched in pediatric AML.

Additionally, polymerase chain reaction (PCR)-based and FISH studies revealed gene rearrangements resulting from cryptic karyotype abnormalities such as *NUP98*::*NSD1* (3), which are enriched in pediatric patients and associated with poor prognosis. Recent progress in analytical pipelines and sequencing technologies, including new methodologies like Hi-C, discovered pediatric AML subtypes with complex structural variants involving *BCL11B* (4, 5) or tandem duplication of *UBTF* (*UBTF*-TD) (6, 7). These molecular alterations have been underestimated even in the era of next-generation sequencing. In 2022, updates to the 5th World Health Organization classification (WHO[5th]) recognized *KMT2A* and *NUP98*-rearranged AML as distinct disease entities due to their unique clinical and biological features (8). However, many pediatric-enriched subtypes do not fit in the current diagnostic schema and fall into broad categories of "Acute myeloid leukemia with other defined genetic alterations" or "Acute myeloid leukemia, defined by differentiation" (9). Also, the International Consensus Classification (ICC) (10), an alternative classification system proposed in 2022, included AML with *KMT2A::MLLT3* and other *KMT2A* rearrangements as distinct entities, whereas AML with *NUP98* rearrangements and other subtypes enriched in childhood AML remain categorized as "AML with other rare recurring translocations" or "AML not otherwise specified (NOS)".

From a clinical standpoint, improvements in outcomes for children with AML remain inferior with only a 70% 5-year overall survival (OS) rate from diagnosis, lagging behind the 95% 5-year OS for pediatric acute lymphoblastic leukemia (ALL) (11-13). This is partly because many pediatric-specific AML subtypes are refractory to conventional chemotherapy. Accurate and timely diagnoses that can risk-stratify or nominate targeted therapies based on the genetic background are required to optimize patient management and outcomes (14). European LeukemiaNet (ELN) recommendations based on evidence from clinical trials and expert panels have led to a consensus for adult AML treatment (15), whereas pediatric AML lacks a similar consensus over risk-stratification, and various strategies are currently implemented for pediatric AML according to study groups. Given the changing paradigm of classifications of pediatric AML due to newly characterized subtypes, it is critical for both clinicians and researchers to understand current classification limitations and to provide comprehensive and robust clinical molecular diagnostics for making informed treatment decisions. In this review, we first describe the current and developing classifications of pediatric AML based on the recent advances in pediatric AML genetics. We compare risk-stratification strategies currently used in clinical trials with molecular category-based strategies, which will be a basis for further improvement of patient treatment. We also highlight key genetically defined high-risk subtypes of pediatric AML to better understand them and treat them based on biology.

*New classification framework*

Current classifications of pediatric AML

Pediatric and adult AML are currently classified together in both the ICC and WHO[5th] classifications released in 2022 (8, 10) despite their distinct genetic landscapes. Although the terminologies are slightly different, both the ICC and WHO[5th] divide AMLs into umbrella categories defined by recurrent genetic abnormalities or myelodysplasia-related genetic changes along with phenotypically-defined categories of "AML, defined by differentiation" (WHO[5th]) or "AML not otherwise specified" (ICC). Both ICC and WHO[5th] prioritize the

presence of recurrent driving genetic alterations for classification while incorporating myelodysplasia-related gene mutations or cytogenetic changes, but not morphologic dysplasia, into the definition of myelodysplasia-related changes. Although the new MDS/AML category in ICC acknowledges the continuum between MDS and AML with blasts ranging from 10% to 19%, the category does not apply to pediatric patients< 18 years old (10).

In addition to the major categories previously defined in WHO[4th] (e.g., *RUNX1::RUNX1T1*, *NPM1*), the AML categories with recurrent genetic abnormalities in both classifications now include AMLs with *KMT2A* rearrangements other than *KMT2A::MLLT3*, variant *MECOM* rearrangements, variant *RARA* fusions, and a list of specified rare recurrent alterations. While *RBM15::MRTFA* fusion and *NUP98* rearrangements are categorized as distinct entities in WHO[5th], ICC classifies them as part of the entity "AML with other rare recurring translocations" under the umbrella category with recurrent genetic abnormalities. The blast percentage requirement is lowered to 10% for all recurrent genetic abnormalities in the umbrella category except for *BCR::ABL1* fusion in ICC, which still requires at least 20% blasts. In WHO[5th], a diagnosis of AML with defining genetic abnormalities can essentially be rendered with any blast count; but the requirement of 20% blasts remains for AMLs with *BCR::ABL1* fusion, *CEBPA* mutation, and AML with rare defined genetic abnormalities.

While many pediatric AMLs fall under the category defined by specific genetic alterations, a significant number of pediatric AMLs demonstrate rare recurring or novel alterations not meeting these definitions. In WHO[5th] released in *Leukemia* in 2022 (8), the broad entity "AML with other defined genetic alterations" allowed for the inclusion of AMLs with ≥20% blasts and initiating alterations including newer entities if recurrent and not overlapping with existing molecularly defined categories, such as *UBTF*-TD or *CBFB*-GDXY (16). In contrast, the version of WHO[5th] currently available online (17) defines the entity as AMLs with ≥20% blasts and *CBFA2T3::GLIS2*, *KAT6A::CREBBP*, *FUS::ERG*, *MNX1::ETV6*, or *NPM1::MLF1*, presumably leaving other new entities as "AML, defined by differentiation". In ICC, AMLs with 12 specific but rare translocations, including the 5 fusions described above that are specified in WHO[5th], as well as *NUP98* rearrangements and *RBM15::MRTFA* fusion, are categorized as "AML with other rare recurring translocations" with a blast percentage requirement of 10% under the umbrella category "AML with recurrent genetic abnormalities". New entities without specified defining alterations are categorized as "AML, NOS", collectively demonstrating challenging situations over appropriately classifying pediatric AMLs according to these systems largely based on adult studies.

Compared with adult AML, a much smaller proportion of pediatric AML is categorized as AML with myelodysplasia-related changes. A study that classified an adult AML cohort (n=746) using the updated WHO and ICC systems showed that the majority of adult AML cases could be classified by somatic mutations of myelodysplasia-related genes or *NPM1*, followed by *CEBPA* mutations, *MECOM* rearrangement or *PML::RARA* fusions (18). Whereas nearly 95.0% of adult AML could be defined by either specific gene alterations (67.1%) or as AML-MR (27.8%) in the WHO classification, only 79.2% of pediatric AML are defined by specific gene alteration (68.5%) or AML-MR (10.7%), respectively as shown in Figure 1 (left-ICC, middle-WHO), leaving 15.8% defined by other recurrent somatic alterations, emphasizing the biological differences between pediatric and adult AML (9). Not only are MR changes uncommon in pediatric AML, but when they do occur they overlap with other defining alterations. For example, myelodysplasia-related cytogenetic changes (WHO[5th] and ICC) or *TP53* mutations (ICC) are frequently observed in AMLs with *PICALM::MLLT10* (9, 19). The inclusion of trisomy 8 as an MDS-defining alteration in ICC is a further complication for pediatric AMLs as this cytogenetic alteration can be recurrent in *UBTF*-TD, *FUS::ERG*, and *HOX* gene rearrangements with AMKL phenotypes. In a classification system designed for pediatric AML, these alterations should be prioritized over trisomy 8.

*New molecular classification of pediatric AML*
Given these gaps in the current classifications of pediatric AML, there has been a critical need for a classification framework based on the unique genetic and biological background of pediatric AML. Using comprehensive sequencing data, 887 unique pediatric AML samples were classified into 23 molecular categories based on mutually exclusive genetic alterations associated with unique expression profiles (9) (Figure 1, right). These molecular categories include recurrent alterations not previously included in

classification systems, such as AML with *UBTF*-TD (7), *PICALM::MLLT10* (19), or *BCL11B* (4, 5), and new subtypes associated with favorable outcomes such as AML with *CBFB*-GDXY mutations (16). Using this detailed genomic profiling, the classification system covered 91.4% of pediatric AML cases by molecularly defined subtypes with unique transcriptional patterns and cooperating mutations, compared with 68.5% by WHO[5th] (Figure 1, middle-right).

It is notable that some of these defining alterations, namely *KMT2A* and *NUP98* rearrangements, *PICALM::MLLT10*, and *BCL11B* structural variations, can be found in leukemias of other lineages, such as mixed phenotype acute leukemia (MPAL), as well as early T-cell precursor ALL (ETP-ALL), or even change lineages during therapy. Whether we should categorize these cases together as part of a disease continuum based on genetics or according to clinical diagnosis using immunophenotyping should be considered in future classifications, as implicated for *BCL11B* in WHO[5th]. Another characteristic of this pediatric-focused classification is the inclusion of functionally redundant alterations with similar transcriptional profiles in a category, such as the ETS family (*FUS::ERG* and *EWSR1::FLI1)* or the GLIS family (*CBFA2T3::GLIS2* and *CBFA2T3*::*GLIS3*). This approach allows for categorizing pediatric AML based on the background mechanism of leukemogenesis, which can have implications for molecular-targeted therapies aimed at specific categories. Many of the remaining pediatric AML cases had structural variations leading to in-frame fusion events (e.g., *MLLT10* fusions not with *KMT2A* or *PICALM* or *RUNX1::USP42*) (20, 21) that likely drive AML, but that are not recurrent enough to warrant designation as a distinct molecular category without interrogating additional datasets. A subset of uncategorized pediatric AML cases also harbored somatic mutations seen in adult AML, such as *DNMT3A* or *TET2* (8). However, these mutations were not associated with consistent expression profiles and often co-occurred with category-defining molecular alterations when present in pediatric AML.

*Molecular categories associated with molecular dependency*
Although these molecular categories are defined by unique driver alterations, some molecular categories also have similar transcriptional profiles and patterns of cooperating mutations that may suggest common mechanisms (9). Notably, categories characterized by *HOXA* cluster gene expression (*KMT2A*-rearranged leukemias) or by *HOXA/B* expression (*UBTF*-TD, *NPM1*, *NUP98::NSD1)* are shown to be dependent on the KMT2A/menin interaction for the development and maintenance of leukemia *in vitro* and *in vivo* (22-26). Clinical trials evaluating the efficacy of various menin inhibitors for *KMT2A*-rearranged and *NPM1*-mutated AML are underway (27) and recently a patient with a *UBTF*-TD myeloid tumor was treated with a menin inhibitor (28). It is intriguing to investigate the menin dependencies in other categories with *HOX* gene expression, namely *DEK*::*NUP214* and *KAT6A*-rearranged AML, through *in vitro* and *in vivo* studies to translate these molecular categories into clinical practice. The clinical impact may be significant as nearly 50% of pediatric AMLs can be assigned to molecular categories characterized by *HOX* gene deregulation, including many high-risk categories.

*Current risk-stratification strategies for pediatric AML*
The basis of current risk stratifications for pediatric AML includes high or low-risk initiating and subtype-defining gene alterations such as high-risk *KMT2A* rearrangements (e.g. *KMT2A::MLLT4*) or CBF AML, whereas many pediatric-specific and novel driver alterations are not yet included in the stratification systems summarized in a recent review (29). This includes state-of-the-art and detailed strategy incorporating various genetic and chromosomal alterations in the ongoing COG AAML1831 trial, a randomized Phase 3 study of CPX-351 in comparison with standard daunorubicin/cytarabine with dexrazoxane for de novo pediatric AML (30). They also include cooperating somatic alterations (e.g., *FLT3* or *KIT* mutations) or chromosomal changes [monosomy 7, del(5q)] associated with poor outcomes. These genetic features are often combined with clinical responses represented by minimal/measurable residual disease (MRD) status at the end of induction to guide subsequent therapies, including allogeneic hematopoietic stem cell transplant (allo-HSCT) in the first complete remission (CR1). Notably, initiating driver alterations are often associated with specific cooperating mutations (e.g., *FLT3*-ITD mutations with *NUP98::NSD1* and *UBTF*, or monosomy 7 with *MECOM* rearrangement) or differential treatment responses. With these systems, conflicting situations may arise where favorable and unfavorable factors co-exist, such as *FLT3*-ITD positive or MRD positive cases in subtypes with molecular alterations associated with favorable outcomes, like *CBF* AML or *NPM1*-mutated leukemia. Risk assignment in these situations can vary depending on the study

groups. For example, CBF AML cases with *FLT3*-ITD allelic ratio > 0.1 could be stratified as high-risk in the COG AAML1831 (30) and NOPHO-DBH AML 2012 (31) studies, whereas they are categorized as standard or intermediate risks in MyeChild 01 and JPLSG AML-20 (32).

*Risk-stratification strategies based on the novel classification framework*
Considering these complexities, a simple risk-stratification framework based on the updated molecular categories of pediatric AML was proposed. Each of the above-mentioned molecular categories was classified into high-intermediate-low-risk categories based on the clinical outcomes, whereas *KMT2A*r was further divided into low or high-risk classes based on fusion partners (9, 33) as summarized in Table 1. The ELN risk-stratification for adult AML and the COG AAML1831 risk-stratification strategies are also shown for comparison. Combined only with MRD positivity, patients can be grouped into 6 strata, whose predictive value was comparable or superior to various risk stratifications that included conventional risk factors of cooperating alterations (e.g., monosomy 7) that can be found in subclones or highly associated with specific molecular categories. High-risk categories were shown to be candidates for allo-HSCT in CR1 independent of MRD status, whereas allo-HSCT for low-risk categories in CR1 could be overtreatment and negatively affect the outcome (9). This framework may require further updates to include newly identified molecular subtypes or heterogeneous treatment responses within one category, as has been shown in *KMT2A*-rearranged AML, and to adapt to the impact of emerging targeted therapies such as venetoclax, 2$^{nd}$ generation FLT3 inhibitors, and menin inhibitors.

*Molecular-targeting therapies*
In the COG AAML1031 study (NCT01371981), which incorporated the multi-kinase inhibitor sorafenib for patients with high-allelic burden *FLT3*-ITD, *FLT3*-ITD+ patients showed comparable outcomes with *FLT3*-ITD negative patients across the cohort (9, 11). The St. Jude AML08 study (12) (NCT00703820), which also included sorafenib for *FLT3*-ITD+ cases, recapitulated results from the AAML1031 study in that the outcomes of *FLT3*-ITD+ patients are comparable to *FLT3*-ITD negative patients. Given the impact of sorafenib in the AAML1031 study and the broad use of selective FLT3-ITD inhibitors (quizartinib and midostaurin) for untreated adult AML patients with *FLT3*-ITD (34, 35), updates of risk-stratification strategies should be considered, along with the inclusion of FLT3-ITD-targeted therapy for untreated pediatric AML.

A similar situation can be expected with menin inhibitors. Existing clinical data showing the efficacy of menin inhibitors are limited to relapsed/refractory *NPM1* or *KMT2A*-rearranged AML (36). A Phase I clinical trial addressing the safety and efficacy of revumenib in combination with conventional 7+3 plus midostaurin for *NPM1* AML with *FLT3*-ITD or tyrosine kinase domain (TKD) mutation is set to begin (NCT06313437). Although this clinical trial is intended for adult patients with *NPM1*, there is hope that future clinical trials will explore menin inhibition in untreated pediatric AML either with genetic events known to respond (e.g. *KMT2A, NUP98* rearrangements or *UBTF*-TD) or driven by expression profiles (*e.g. HOXA/B* deregulation) to assess if menin inhibition can alter the clinical course of these high-risk populations.


**High-risk subtypes of pediatric AML**

Advances in the understanding of the molecular and functional consequences of drivers common in high-risk pediatric AML subtypes are expected to alter treatment strategies and improve the overall outcomes of pediatric AML. Classic high-risk categories shared with adult AML (e.g., *MECOM* rearrangements) are discussed elsewhere, while new or pediatric-specific categories have been underappreciated. The following highlights clinical and biological aspects of key high-risk molecular categories and ongoing efforts to develop treatments to overcome the dismal outcomes for patients with these alterations.

*CBFA2T3::GLIS2*
This fusion gene, resulting from a cryptic inversion of chromosome 16, inv(16)(p13q24), had been unrecognized until its discovery in roughly 30% of non-Down syndrome acute megakaryoblastic leukemia (AMKL, French-American-British Classification; FAB M7) pediatric patients (37). The N-terminal CBFA2T3 (also known as ETO2) forms a fusion oncoprotein with the C-terminal GLIS2 or GLIS3, retaining a C2H2 zinc finger domain shared within the family that regulates oncogenic gene expression (Figure 2A) (9, 38, 39). *CBFA2T3::GLIS2* fusions are highly enriched in AMKL patients younger than 3 years old and are strongly

associated with the "RAM" immunophenotype, characterized by bright CD56 expression and dim/absent expression of CD45, CD38, and HLA-DR (40, 41), which is included in a recent clinical trial as an independent high-risk factor (NCT04293562).

Clinical outcomes of this subtype are overall dismal with high rates of MRD at the end of the first induction therapy (EOI1) and only ~15% long-term survival rates in multiple clinical studies (2, 11, 42). Several approaches have been taken to date to overcome the refractoriness of the disease. *CBFA2T3::GLIS2* is now categorized as high-risk in various clinical trials and allo-HSCT in CR1 is universally (e.g., MyeChild01: NCT02724163, JPLSG AML-20: jRCTs041210015, COG AAML1831: NCT04293562, AML16: NCT03164057), despite a lack of direct evidence that allo-HSCT improves outcomes (43).

Other approaches based on the biology of this entity are to target FOLR1 (folate receptor alpha), which is specifically expressed on the cell surface of leukemia cells with *CBFA2T3::GLIS2,* using drug-conjugated antibodies [e.g. luveltamab tazevibulin (44)] or chimeric antigen receptor (CAR)-T cells (45). These treatments demonstrated promising efficacy in preclinical studies using patient-derived xenograft (PDX) mouse models. Lastly, FDA-approved drugs targeting the BCL families, such as navitoclax (a broad BCL-2, BCL-xL, and BCL-W inhibitor) or DT2216 (a selective BCL-xL degrader) are expected to be effective for this subtype (46, 47). Meanwhile, a selective BCL-2 inhibitor commonly used in current AML therapy, venetoclax, may be less effective for this subtype according to *in vitro* assays (46, 47), indicating a selective dependency of AML with *CBFA2T3::GLIS2* on BCL-xL.

*Structural variations deregulating BCL11B*
BCL11B is a zinc-finger protein involved in T-cell development (48). Its role in mature T-cell malignancies has long been acknowledged in various contexts, including the translocation of regulatory elements to T-cell oncogenic genes leading to aberrant expression of *TLX3/NKX2-5* by t(5;14)(q35;q32.2) (49) and recurrent somatic mutations or loss of *BCL11B* resulting in haploinsufficiency of its function as a tumor suppressor (50). Contrarily, chromosomal translocations involving the *BCL11B* locus (14q32), such as t(6;14)(q25;q32) or t(2;14)(q22;q32), were found in AML (51, 52) or mixed phenotype acute leukemia (53) with increased expression of intact *BCL11B*, suggesting BCL11B plays distinct roles in the development of leukemias of different developmental states and cell lineages.

Recent sequencing studies using whole-genome sequencing (WGS) or assays for 3D genomic structure have revealed that genomic rearrangements involving 14q32 result in translocations of active hematopoietic promoters or enhancers, most commonly involving *ARID1B*, *CCDC26,* or *ETV6*, to the proximity of the *BCL11*B gene (4, 5) (Figure 2B). These alterations were found in a series of acute leukemias, including AML (typically FAB M0), mixed phenotype acute leukemia (MPAL), as well as early T-cell precursor ALL (ETP-ALL), suggesting a continuum of these diseases with *BCL11B* alterations. Additionally, focal amplifications of enhancer elements of *BCL11B* have been found in leukemias with similar expression profiles (5). These hijacked regulatory elements led to outlier high expression of *BCL11B* from the affected allele, resulting in leukemias with unique transcriptional profiles that are observed in both adult and pediatric cohorts. These leukemias often harbor internal tandem duplications of *FLT3* (*FLT3*-ITD) and less frequently, mutations in *WT1*, *RUNX1*, and *DNMT3A* (4, 5). Diagnosis of this entity can be challenging because conventional karyotyping will not capture the full spectrum of 14q32 alterations, and translocation or focal amplification of enhancers may likewise not be detected by standard fusion calling or by using panel sequencing strategies.

Clinical associations of this category are limited to studies involving single cohorts. Whereas adult T-ALL cases in this category showed relatively favorable outcomes with overall survival of 9.9 years (5), pediatric AML cases exhibited high MRD after induction I and a low overall survival rate (4-year OS <60%) compared with other subtypes (9). Given the nature of this entity found in leukemias of various lineages, AML in this category could benefit from ALL/AML combined regimens as reported in MPAL (54); further accumulation of outcome data associated with molecular categories and treatment regimens is required. Another consideration is the use of FLT3 inhibitors given the high frequency of *FLT3*-ITD or D835Y mutations (80%) and high expression of the *FLT3* gene (5), which suggests its involvement in leukemogenesis and cellular identity.

*UBTF tandem duplications (UBTF-TD)*

UBTF is a transcription factor that regulates ribosomal RNA expression by recruiting polymerase I (PolI) to ribosomal DNA. Recently, our group and others reported recurrent tandem duplications in exon 13 of *UBTF* in approximately 10% of relapsed pediatric AML samples without other defining alterations (7, 55) (Figure 2C). The high frequencies of this subtype, despite a scarcity of previous reports, indicate that standard analytical pipelines may not efficiently identify *UBTF*-TD, possibly due to heterogeneity of the duplications (7). Updated pipelines and studies using PCR-based screening identified *UBTF*-TD in about 4% of newly diagnosed pediatric AML and 3% of adult AML aged 18-60 years (7, 56, 57). Similar alterations have also been reported in high-grade pediatric MDS lacking known germline predispositions or monosomy 7, suggesting that *UBTF*-TD is a driver alteration across a spectrum of myeloid tumors (58, 59). Also, rare but recurrent *UBTF*-TD in exon 9 in pediatric AML samples with similar expression profiles have been described (58). These data highlight that accurate diagnosis of this subtype will require an unbiased approach with RNA or whole genome sequencing and careful inspection of sequencing data, whereas many commonly used commercial or academic clinical sequencing panels currently lack coverage for *UBTF*. PCR-based screening of exon 13 in *UBTD* could be an alternative approach with the limitation that this strategy could underestimate larger duplications extending beyond exon 13 or the rare exon 9 duplications.

*UBTF*-TD AML is prevalent in adolescent or young adult patients who exhibit AMLs with normal karyotypes or trisomy 8. *UBTF*-TD is mutually exclusive with other category-defining driver alterations, suggesting *UBTD*-TD is an initiating event, while it frequently co-occurs with *WT1* or *FLT3*-ITD mutations (7, 60). *UBTF*-TD itself is an independent risk factor for high MRD positivity at end of induction 1 (EOI1) and low survival rates both in pediatric (44% 5-year OS) (7) and adult AML patients aged 18-60 years (57% 3-year OS) (56). Due to the high rate of *FLT3*-ITD and MRD positivity at EOI1, a subset of *UBTF*-TD cases underwent allo-HSCT in CR1 in clinical trials (11, 57). While allo-HSCT status at CR1 was associated with prolonged event-free or relapse-free survival, its benefit for overall survival and whether salvage HSCT can rescue relapsed patients without HSCT at CR1 requires additional study.

Another strategy could involve molecular targeted therapies. Using experimental models with cord blood CD34+ cells and patient-derived xenografts, the UBTF-TD protein was found to co-localize with and depend on KMT2A/menin complex to activate leukemic genes (26). The menin inhibitor SNDX-5613 (revumenib) can suppress tumor growth both *in vitro* and *in vivo.* These preclinical studies have allowed for patients with *UBTF*-TD AML to be treated with menin inhibitors (28) and for *UBTF*-TD status to be an enrollment criterion for a phase I clinical trial of revumenib, azacytidine, and venetoclax in relapsed/refractory pediatric AML (NCT06177067).

*ETS family fusions*
*FUS::ERG,* resulting from t(16;21)(p11;q22), is a rare but well-recognized alteration associated with poor outcomes (61, 62). The N-terminal FUS (FET family) and the C-terminal ERG (ETS family) form fusion oncoproteins that activate downstream genes (63) (Figure 2D). While *FUS::ERG* is the most common fusion in this AML family, recent studies have shown fusion oncoproteins of another FET family, EWS encoded by *EWSR1*, or structurally similar HNRNPH1 and other ETS families such as FLI1 or ELF5 in transcriptionally similar pediatric AML, indicating the shared biological features among this entity (9, 64). It is also notable that among Ewing sarcoma cases, the *EWSR1::FLI1* fusion gene is found in 80-85% and *EWSR1::ERG* in 5-10% (and rarely *FUS::ERG*) (65), suggesting a potential biological relationship between AML in this category and Ewing sarcoma in respect to gene regulation.

Clinical outcomes of pediatric AML with *FUS::ERG* were shown to be dismal with 4-year event-free survival of 51% and 4-year OS of 68% (62), while the clinical benefit of allo-HSCT in CR1 for *FUS::ERG* patients still needs larger cohorts to assess. Cases in this category of alterations other than *FUS::ERG* may also have aggressive clinical courses and poor outcomes in previous studies. Another study assessing the outcomes of pediatric AML with ETS fusions also showed unfavorable outcomes (66). However, this study included *ETV6* rearrangements, which typically result in out-of-frame fusion events leading to loss of the ETS domain unlike *FUS::ERG* and other ETS family fusions, thus fusions other than *FUS::ERG* need further evaluation based on accurate diagnosis.


***Discussion***

Our understanding of the genetic background of pediatric AML has been rapidly broadening in large part due to the progress of sequencing technologies and analytical pipelines, highlighting fundamental differences between pediatric and adult AML genetics (2). However, this situation also poses challenges at various levels in investigating and treating pediatric AML.

First, many newly identified pediatric AML subtypes require unbiased diagnostic sequencing or need bioinformatic expertise to accurately diagnose from RNA sequencing or WGS data (4, 5, 7, 37). To translate information on AML subtypes into clinical decisions, all the steps from sampling to reporting the genomic alterations need to be completed by the end of induction therapies (14). However, each institute, area, or country has different access to clinical sequencing technologies and approaches, hampering treatments based on genetic status. Updates in clinical targeted sequencing panels reflecting newly identified molecular subtypes like *UBTF*-TD would more broadly benefit patients in routine clinical treatment outside academic institutes or clinical trials. Although rare, DNA-based sequencing panels will likely be unable to consistently detect structural variations that lead to aberrant expression through enhancer hijacking, such as those involving *BCL11B* (4, 5) or *MNX1* (67). While not yet universally available, transcriptome sequencing approaches can likely predict these events since they are associated with outlier-high expression of the involved genes and unique global expression signatures (68).

Second, these high-risk subtypes lack enough outcome data to formulate data-driven treatment strategies since they have only recently been recognized by sequencing approaches. It is a commonly accepted idea that allo-HSCT at CR1 is intended for these high-risk subtypes in current clinical trials (29), whereas the benefits of allo-HSCT at CR1 for all high-risk subtypes without positive MRD are still in debate with various outcome data being reported in the literature (69). Also, a benefit of allo-HSCT for each subtype will need to be re-assessed with new genetic profiling and current clinical standards, including broadened choices of haploidentical donors using posttransplant cyclophosphamide (PTCy) (70) and improved supportive care. In addition to the impact on survival, the impact of allo-HSCT on quality of life (QOL) affected by graft-versus-host disease (GVHD), immunosuppression, and possible infertility (71) must be considered.

Lastly, targeted therapies based on new and evolving high-risk genomic subtypes are required, while recognizing that continued division of AML into unique categories will result in fewer patients in each category, especially newer entities. For example, 60% of pediatric AML cases consist of *KMT2A* or *NUP98* rearrangements, *RUNX1::RUNX1T1*, *CBFB::MYH11*, and *NPM1* cases, and the remaining 40% are accounted for by ~20 other molecular categories (9). Despite various new treatments targeting cooperating partners [menin (29) or DOT1L (72)], signaling dependencies [*FLT3* or *KIT* mutations (73)], molecular dependencies [the BCL2 family (46, 47)], and immunotherapies [antibodies (44) and CAR-T cells (45) targeting FOLR1] showing promising results in preclinical tests or phase 1 clinical trials, the scarcity of each subtype complicates the execution of clinical trials aimed at newly identified high-risk subtypes. Also, with the relative rarity of pediatric AML, with approximately 800 cases annually in the United States, there is a critical need for an international collaborative effort to identify patients eligible for clinical trials to efficiently test various treatments. Such collaborations are exemplified in the PedAL/EuPAL project (74) and the Leukemia & Lymphoma Society and Children's Oncology Group's APAL2020SC Pediatric Screening Trial (NCT04726241)(75), including the ITCC-101/APAL2020D subtrial (NCT05183035)(76) and ITCC-101/APAL 2020K (NCT06376162) subtrials. These efforts will be crucial in designing, recruiting patients, analyzing data, and ultimately pushing these promising therapies toward becoming new clinical standards. Importantly, these efforts also need to include state-of-the-art sequencing strategies to appropriately identify the full range of genomic alterations to maximize the potential. We remain hopeful that with sustained international cooperation, we can overcome these challenges and significantly improve outcomes for children with high-risk pediatric AML.

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| Risk | ELN for adult AML | COG AAML1831 | Molecular category |
|---|---|---|---|
| **Low-Favorable** | t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*<br>inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*<br>Mutated *NPM1* without *FLT3*-ITD<br>bZIP in-frame mutated *CEBPA* | CBF-AML without MRD*@EOI1>0.05%, *KIT* exon17 mutations, or other HR factors<br>Mutated *NPM1/CEBPA*-bZip without MRD*@EOI1>0.05% or other HR factors | *RUNX1::RUNX1T1, CBFB::MYH11, NPM1, CEBPA, RUNX1::RUNX1T1*-like, *CBFB*-GDXY, *MNX1, DEK::NUP214,* and Low-risk *KMT2A*r (non-*MLLT3, MLLT10, MLLT4, MLLT1*) fusions |
| **Intermediate** | Mutated *NPM1* with *FLT3*-ITD<br>Wild-type *NPM1* with *FLT3*-ITD<br>t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*<br>Cytogenetic and/or molecular abnormalities<br>not classified as favorable or adverse | | *BCL11B, KMT2A*-PTD, *GATA1, RBM15::MRTFA,* ETS family, or no defining alteration |
| **High-Unfavorable** | t(6;9)(p23;q34.1)/*DEK::NUP214*<br>t(v;11q23.3)/*KMT2A*-rearranged<br>t(9;22)(q34.1;q11.2)/*BCR::ABL1*<br>t(8;16)(p11;p13)/*KAT6A::CREBBP*<br>inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)*<br>t(3q26.2;v)/*MECOM(EVI1)*-rearranged<br>-5 or del(5q); -7; -17/abn(17p)<br>Complex karyotype, monosomal karyotype<br>Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* or *ZRSR2*<br>Mutated *TP53* | *FLT3*-ITD allelic ratio > 0.1 with no *NPM1/CEBPA*-bZip mutation<br>*FLT3*-ITD allelic ratio > 0.1 with Mutated *NPM1/CEBPA*-bZip and MRD@EOI1 ≥ 0.05%<br>Mutated non-ITD *FLT3* and MRD@EOI1 ≥ 0.05%<br>RAM phenotype<br>inv(3)/t(3;3): *RPN1::MECOM,* t(3;21): *RUNX1::MECOM,* t(3;5): *NPM1::MLF1,*<br>t(6;9): *DEK::NUP214,* t(8;16): *KAT6A::CREBBP* (≥90 days old)<br>t(16;21)(p11;q22): *FUS::ERG,* inv(16)(p13q24): *CBFA2T3::GLIS2*<br>High-risk *KMT2A*r<br>t(4;11): *KMT2A::AFF1,* t(6;11): *KMT2A::AFDN,* t(10;11): *KMT2A::MLLT10*<br>t(10;11): *KMT2A::ABI1,* t(11;19): *KMT2A::MLLT1*<br>11p15-r: any *NUP98* fusion, 12p13-r: any *ETV6* fusion, 12p deletion: *ETV6* loss<br>–5/del(5q): *EGR1* loss, Monosomy 7, 10p12.3-r: any *MLLT10* fusion<br>No favorable/unfavorable abnormalities with MRD@EOI1 ≥ 0.05% | *KAT6A*r, *NUP98*r, *HOX*r, *MECOM, UBTF, GLIS*r, *PICALM::MLLT10,* and High-risk *KMT2A*r (*MLLT3, MLLT10, MLLT4, MLLT1*) fusions |

**Table 1**. Comparison of risk-stratification systems. Abbreviations: ELN; The European LeukemiaNet, ITD; internal tandem duplication, HR; high risk, MRD; measurable/minimal residual disease, EOI1, End of Induction 1, PTD; partial tandem duplication.
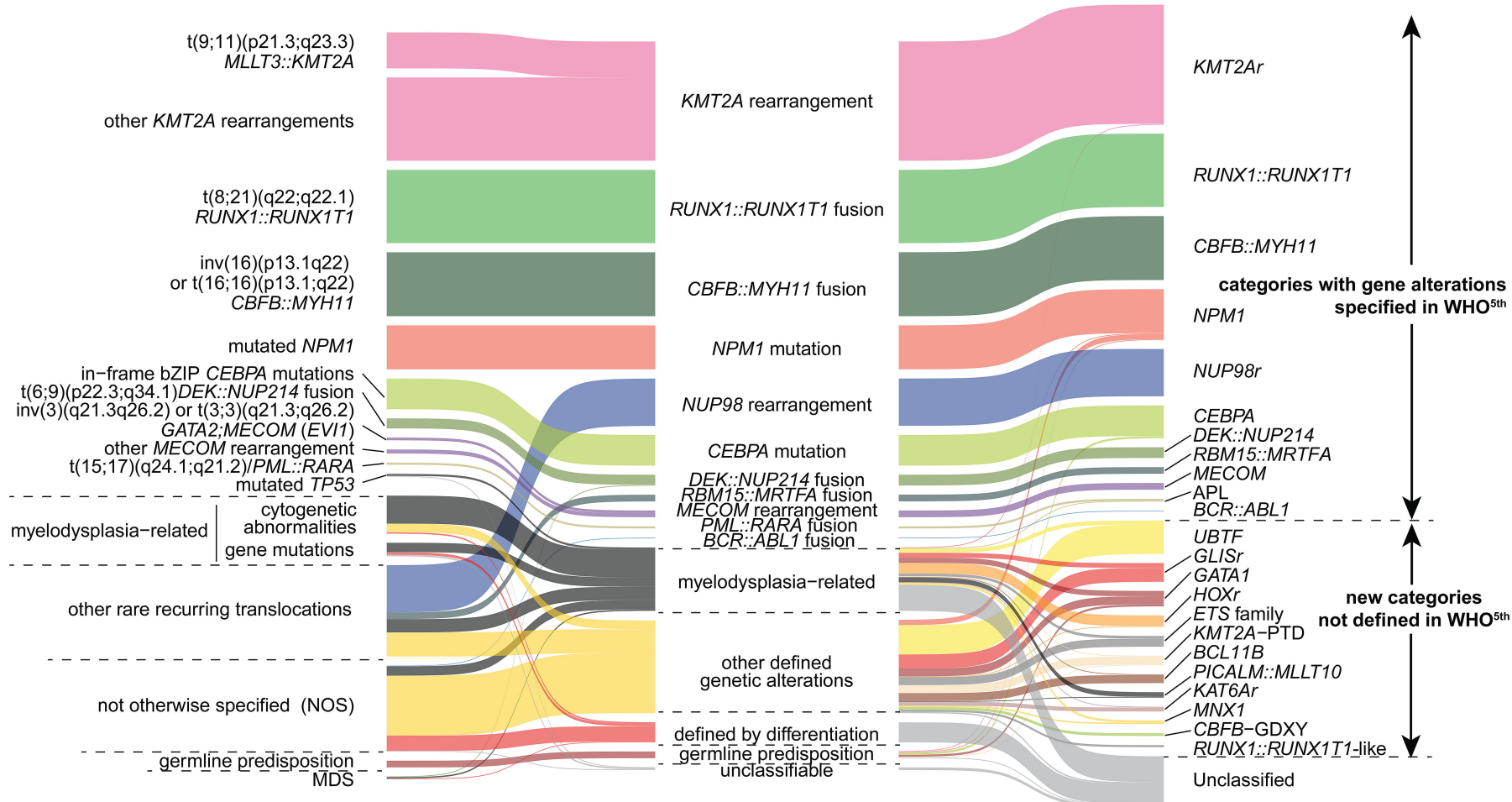
**Figure 1.** Comparisons of the International Consensus Classification (ICC-**left**), The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours (WHO[5th]-**middle**), and a new classification system (**right**) of pediatric AML. Classifications or categories of each patient are connected by ribbons, with the colors of ribbons representing WHO[5th] (**left-mid**) and molecular categories (**middle-right**) and the width representing the numbers of patients in the reference study cohort (9) (total 887 patients). Adapted from Umeda et al (9). Abbreviations: MDS; myelodysplastic syndrome, APL; acute promyelocytic leukemia, PTD; partial tandem duplication.

**Figure 2.** Disease mechanisms of high-risk subtypes of pediatric AML. **A.** Schematics of CBFA2T3::GLIS2 proteins (**top**) and characteristics of surface marker expression of acute megakaryocytic leukemia (AMKL) with *CBFA2T3::GLIS2* fusion and targeted therapies (**bottom**). **B.** Disease mechanisms of the *BCL11B* subtype (enhancer hijacking, promoter translocation, or enhancer duplication) that lead to outlier expressions of *BCL11B*. **C.** Duplicated genetic regions in *UBTF* tandem duplications (*UBTF*-TD, **top**), and resulting proteins that regulate ribosomal DNA (*rDNA)* and leukemic gene expression, represented by *HOXA/B* cluster genes (**bottom**). Recurrently duplicated regions are highlighted in red. **D.** Schematic illustrations of fusion proteins of the N-terminal FET or hnRNP family proteins and the C-terminal ETS family proteins retaining ETS domains (**top**) and representative ETS family fusion proteins in pediatric AML (**bottom**). Abbreviations: CAR-T cell; Chimeric antigen receptor T-cell, IDR; intrinsically disordered region. These images were created with BioRender.com.

the International Consensus Classification (ICC, ref.10)

the 5th edition of the WHO classification (WHO⁵ᵗʰ, ref.8)

a new pediatric AML classification (ref.9)

t(9;11)(p21.3;q23.3) *MLLT3::KMT2A*

other *KMT2A* rearrangements

t(8;21)(q22;q22.1) *RUNX1::RUNX1T1*

inv(16)(p13.1q22) or t(16;16)(p13.1;q22) *CBFB::MYH11*

mutated *NPM1*

in-frame bZIP *CEBPA* mutations
t(6;9)(p22.3;q34.1)*DEK::NUP214* fusion
inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)
*GATA2;MECOM (EVI1)*
other *MECOM* rearrangement
t(15;17)(q24.1;q21.2)/*PML::RARA*
mutated *TP53*

myelodysplasia-related ─ cytogenetic abnormalities gene mutations

other rare recurring translocations

not otherwise specified (NOS)

germline predisposition
MDS

*KMT2A* rearrangement

*RUNX1::RUNX1T1* fusion

*CBFB::MYH11* fusion

*NPM1* mutation

*NUP98* rearrangement

*CEBPA* mutation

*DEK::NUP214* fusion
*RBM15::MRTFA* fusion
*MECOM* rearrangement
*PML::RARA* fusion
*BCR::ABL1* fusion

myelodysplasia-related

other defined genetic alterations

defined by differentiation
germline predisposition
unclassifiable

*KMT2Ar*

*RUNX1::RUNX1T1*

*CBFB::MYH11*

*NPM1*

*NUP98r*

*CEBPA*
*DEK::NUP214*
*RBM15::MRTFA*
*MECOM*
APL
*BCR::ABL1*

**categories with gene alterations specified in WHO⁵ᵗʰ**

*UBTF*
*GLISr*
*GATA1*
*HOXr*
*ETS* family
*KMT2A*-PTD
*BCL11B*
*PICALM::MLLT10*
*KAT6Ar*
*MNX1*
*CBFB*-GDXY
*RUNX1::RUNX1T1*-like

Unclassified

**new categories not defined in WHO⁵ᵗʰ**

**A** *CBFA2T3::GLIS2*

CBFA2T3

☐ C2H2 domain

the N-terminus | GLIS2 | the C-terminus

CBFA2T3 | GLIS2

RAM phenotype · high FOLR1 expression

CD45 · CD38 · HLA-DR · luveltamab tazevibulin

CAR-T

CD56 · FOLR1

*CBFA2T3::GLIS2 AMKL*

**B** *BCL11B*

active genes in progenitors (e.g., *ETV6*)

*BCL11B* inactive in hematopoietic progenitors

enhancer · promoter · *ETV6*

*BCL11B*

structural variation · focal amplification

enhancer hijacking · promoter translocation · enhancer duplication

outlier expression of *BCL11B*

**C** *UBTF*-TD

Exon13

*UBTF*

duplicated sequences

recurrently duplicated region

UBTF

focal duplication

UBTF-TD

UBTF wildtype
regulation of ribosomal genes

*rDNA* · *HOXA/B*

UBTF-TD
regulation of ribosomal genes +
leukemia genes with KMT2A/menin

KMT2A

*rDNA* · *HOXA/B*

menin

**D** ETS family

EWS · FUS · } FET family

HNRNPH1 · } hnRNP family

the N-terminus ✕ the C-terminus

ERG · FLI1 · FEV etc. · } ETS family

☐ IDR (SYGQ-rich) · ☐ ETS domain · ☐ RNA-binding domain

ETS fusions in pediatric AML

FUS::ERG (50%)

FUS::FEV (19%)

HNRNPH1::ERG (13%)