Pediatric Acute Myeloid Leukemia

Biology and Therapeutic Implications of Genomic Variants

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KEYWORDS

- Acute myeloid leukemia • Pediatrics • Epigenetic • Genomic • Therapy

KEY POINTS

- Pediatric acute myeloid leukemia (AML) has a genomic and epigenetic profile distinct from that of adult AML.
- Somatic mutations and epigenetic alterations contribute to myeloid leukemogenesis, and can evolve from diagnosis to relapse.
- Next-generation sequencing technologies are providing novel insights into the biology of AML and are highlighting potential targets for therapeutic intervention.
- Cytogenetic alterations, somatic mutations, and response to induction therapy contribute to current risk stratification and appropriate therapy allocation.

INTRODUCTION

Acute myeloid leukemia (AML) is a hematopoietic malignancy that is the culmination of genetic and epigenetic alterations in the hematopoietic stem/progenitor cells, leading to dysregulation of critical signal transduction pathways and resulting in the expansion of undifferentiated myeloid cells. AML can be broadly divided into 2 categories, de novo AML and secondary AML. Secondary AML refers to evolution of AML subsequent to prior exposure to cytotoxic therapy or antecedent hematopoietic insufficiency (eg, myelodysplastic syndrome [MDS], marrow failure), leading to evolution of distinct karyotypic and molecular alterations including *MLL* translocations following exposure to topoisomerase inhibitors. In contrast to secondary AML, AML that evolves without a prior cytotoxic exposure is referred to as de novo AML.

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Numerous somatic, karyotypic and molecular alterations have been identified in de novo AML; however, despite association of many of these alterations with clinical phenotype, most have no prognostic value, nor do they identify a specific target or a distinct pathway that can be readily exploited for therapeutic intervention. The observed age-associated evolution of molecular alterations reveals a profile for younger children with AML distinct from that of older children and adolescents with AML. Furthermore, the landscape of genetic alterations differs markedly from AML in adults and the paucity of potential therapeutic targets is more notable in childhood AML.

EPIDEMIOLOGY OF ACUTE MYELOID LEUKEMIA

AML is diagnosed in very young children and comprises nearly 25% of pediatric leukemias; however, it is far more prevalent in adults, and is generally considered a disease of older adults whereby the median age at diagnosis is nearly 70 years. Incidence of AML can be better appreciated by close evaluation of the most recent SEER (Surveillance, Epidemiology, and End Results) data. Initial evaluation demonstrates low incidence of AML in children and young adults, with a significant increase in older adults (Fig. 1A).[1] However, closer examination of the data demonstrates highest incidence of AML in younger patients (<40 years) to be in infancy with an incidence of 1.6 cases per 100,000, similar to that in the fourth decade of life. After infancy, there is a declining incidence of approximately 0.12 cases per 100,000 per year in the first decade of life (see Fig. 1B) to an incidence of 0.4 per 100,000 by age 10. In the following 3 decades, there is a steady increase in AML incidence of approximately 0.02 cases per 100,000 per year to 1.3 cases per 100,000 per year by age 45 years, nearly equivalent to that seen in infants. A substantial increase in the rate of AML diagnosis occurs in the fifth decade to nearly 10 times the observed rate in the previous 3 decades, to an incidence of 6.2 per 100,000 by age 65. After age 65, AML diagnosis increases again substantially, more than 30-fold higher than that seen in younger patients (age 10–40), likely attributable in part to its evolution from an underlying MDS. This epidemiologic observation parallels the underlying known and emerging karyotypic and genomic makeup of AML, and provides insight into the contribution of genomic alterations to the evolution of AML.

Inherited Susceptibility of Acute Myeloid Leukemia

Although AML is a rare event in childhood, there are a wide range of inherited chromosomal and gene defects and marrow failure syndromes that predispose to the development of AML. Some of the more common AML predisposition syndromes include trisomy 21 (Down syndrome [DS]), Fanconi anemia, dyskeratosis congenita (DC), Shwachman-Diamond syndrome (SDS), and Kostmann syndrome (severe congenital neutropenia or SCN). Down syndrome is associated with 10- to 20-fold increased risk of leukemia, as well as high incidence of development of transient myeloproliferative disorder (TMD) in the first 3 months of life that resembles AML, but in most cases the disease undergoes spontaneous resolution.[2] Fanconi anemia is an autosomal recessive disorder caused by mutations in DNA repair genes, and has been estimated to show a cumulative incidence of AML/MDS of approximately 50% by age 40 years.[3] DC is an X-linked disorder caused by mutations in the dyskerin gene, *DKC1*, which is a key subunit of ribosomal RNA processing and the telomerase complex. Autosomal recessive forms of DC exist and are due to mutations in *TERC*, the RNA component of telomerase, or *TERT*, the enzymatic component of telomerase.[4] SDS is an autosomal recessive disorder caused by mutations in the *SBDS*
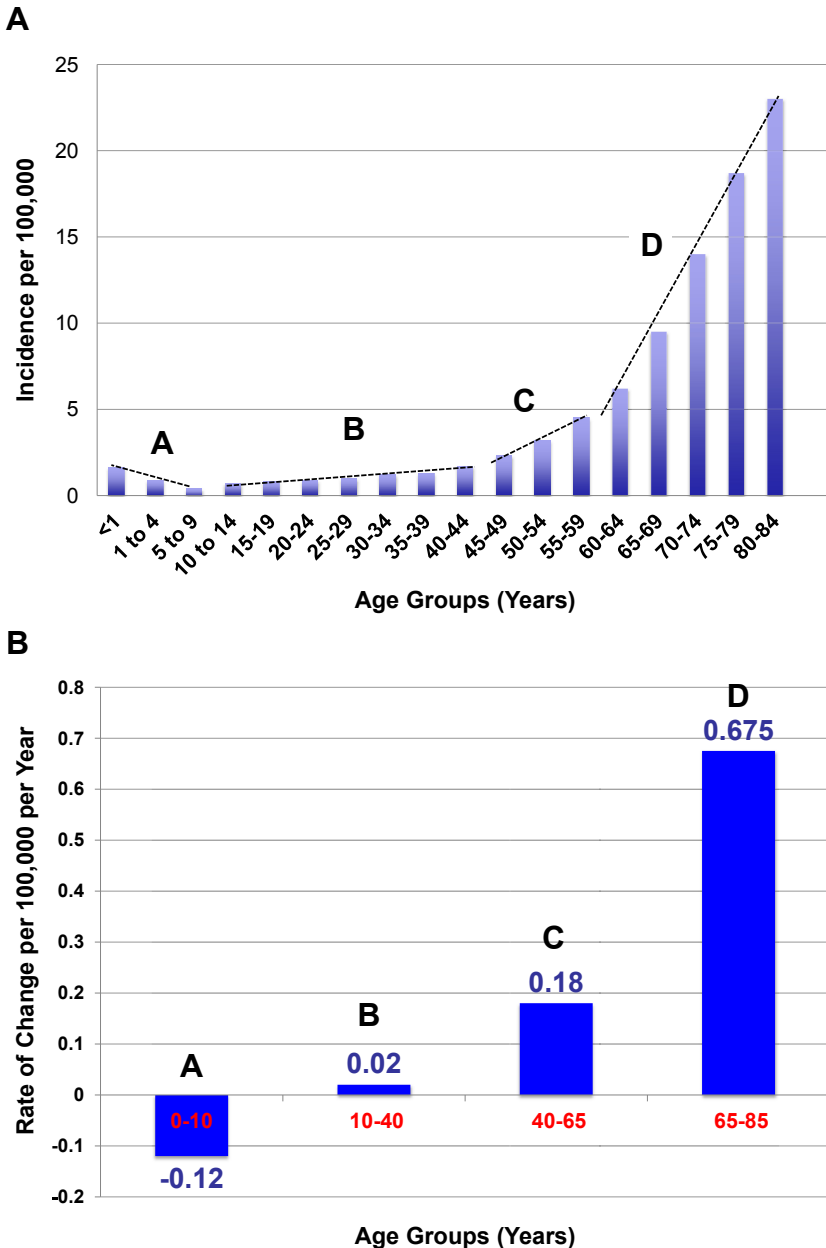
A

B

Fig. 1. Incidence of acute myeloid leukemia (AML) by age (*A*)[1] and rate of change in AML incidence (per 100,000 per year; *B*) in age groups calculated from Fig. 1A.

gene, which functions in ribosome processing and function and is characterized by neutropenia and pancreatic insufficiency.[5] Kostmann syndrome is progressive neutropenia caused by defects in neutrophil elastase (*ELA2*), and is associated with evolution into AML.[6]

GENOMIC LANDSCAPE OF CHILDHOOD ACUTE MYELOID LEUKEMIA

Until recently, most genomic events defined in children were derived from adult studies, thus demonstrating a lower prevalence or lack of such AML-associated genomic lesions in childhood AML.[7] More recently, focused characterization on the somatic genomic changes in childhood AML have provided new insights into the biology of pediatric AML, affording an opportunity to begin to correlate these changes with clinical and biological phenotypes. To date, disease-associated genomic alterations, including translocations, numerical structural alterations (eg, monosomies, trisomies), or sequence variations (mutations, indels), have been used as powerful tools for prediction of response to therapy and outcome in a subset of patients.

Karyotypic Alterations

Cytogenetic alterations have been a cornerstone in the diagnosis of AML, and an invaluable tool for consideration of stratification for treatment based on risk categorization. Although a large number of chromosomal alterations can be identified in AML, most pediatric AML cases separate into distinct cytogenetic categories based on specific chromosomal events: 25% have t(8;21) or Inv(16)/t(16;16), which are collectively referred to as core binding factor AML (CBF AML); 12% have t(15;17); 20% have rearrangements involving the *MLL* gene; and 20% do not have a discernible karyotypic abnormality (normal karyotype). Such cytogenetic alterations have significant age-associated variations (Fig. 2), with some highly prevalent in younger patients (eg, *MLL*) and others more prevalent in teenagers (CBF). In addition to karyotypic alterations, disease-associated mutations have been identified in AML; greater than 90% of pediatric AML cases have at least 1 genomic alteration that can be detected by current methods, with the highest fraction occurring in those with a normal karyotype.

Subkaryotypic (cryptic) chromosomal abnormalities

Cytogenetic abnormalities involving large chromosomal regions can be detected by conventional cytogenetics. However, karyotypic analysis in AML is limited by the ability to culture leukemic cells in vitro before analysis in addition to the size of the region involved. Cryptic translocations, those not amenable to identification by conventional karyotyping, may require more specialized techniques such as polymerase chain reaction or fluorescence in situ hybridization studies (FISH) for identification. Several cryptic translocations, including *NUP98/NSD1*, *CBFA2T3/GLIS2*, and *NUP98/KDM5A*, have been discovered recently and shown to be prevalent in children with AML, and may provide significant insight into the pathogenesis of childhood AML.[8–10] Segmental or whole chromosome deletions and their associated
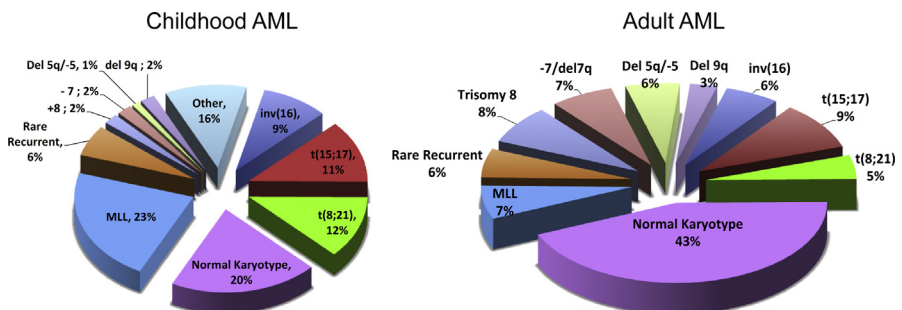

Fig. 2. Karyotypic alterations in childhood versus adult AML.

loss of heterozygosity (LOH) lead to haploinsufficiency of involved genes, and may contribute to malignant transformation. More recent sequencing and array technologies have identified regions of LOH without associated deletions (copy-neutral LOH [CN-LOH]), also referred to as acquired uniparental disomy,[11,12] a homologous recombination-mediated process whereby a homozygous state is achieved after an initial acquisition of a heterozygous mutation. With the use of array and sequencing technologies, regions of CN-LOH have been shown to be prevalent in AML, but their contribution to malignant transformation or progression remains unclear.[13,14]

Somatic Mutations in Childhood Acute Myeloid Leukemia

Somatic mutations in genes known to regulate hematopoiesis have been identified in a significant proportion of AML, and the presence of these mutations has been shown to be associated with clinical outcome. At present, numerous mutations have been implicated in AML pathogenesis, with the number rising with new discovery phase initiatives.[15,16] At present, mutations in 3 genes (*FLT3*, *NPM1*, and *CEBPA*) have been shown to have clinical implications in childhood AML and have been incorporated in clinical trials as prognostic markers, therapeutic targets, or both.

FLT3 mutations

The most commonly mutated gene in childhood AML is FMS-like tyrosine kinase 3 (*FLT3*), which results in constitutive activation of the receptor kinase activity, and can be due to the internal tandem duplication (*FLT3*/ITD) of the juxtamembrane domain coding sequence, or a missense mutation in the activation loop domain (*FLT3*/ALM).[17,18] *FLT3*/ITD is detected in 15% of all children with AML, and has been shown to be highly associated with poor response to induction chemotherapy and high relapse rate.[19,20] Despite biological similarity to *FLT3*/ITD, those with *FLT3*/ALM do not have increased rates of treatment failure.[19] Several studies have shown that patients with *FLT3*/ITD who receive allogeneic stem cell transplantation in complete remission have an improved outcome, thus providing a rationale for therapy allocation in this high-risk cohort of patients.[19,21,22] Furthermore, tyrosine kinase inhibitors (TKI) targeting FLT3 have shown efficacy in inducing high rates of remission in patients with *FLT3*/ITD, although the long-term benefits of such interventions are yet to be determined.[23] In contrast to *FLT3*/ITD, *FLT3*/ALM has not been shown to be similarly amenable to TKI-mediated FLT3 inhibition, which may be in part due to FLT3 conformational changes resulting from the distinct mutations and TKI-binding affinity.[24–26]

Nucleophosmin (NPM1) mutations

NPM1 encodes a ubiquitously expressed molecular chaperone that shuttles rapidly between the nucleus and cytoplasm. Mutations in *NPM1* are common in AML, with a prevalence of 30% in adult and 8% to 10% in pediatric AML.[27–30] *NPM1* mutations appear to be more prevalent in AML with normal karyotype, with a prevalence of nearly 40% to 50% in adults and 20% in pediatric AML. Disease-associated mutations, characterized by 4 base insertions in exon 12 of the *NPM* gene, lead to impaired nuclear localization of the nucleophosmin protein. Presence of *NPM1* mutations portends a favorable outcome with reduced relapse risk and improved survival, similar to the favorable outcome achieved in patients with CBF AML. In pediatric AML, the presence of *NPM1* mutations appears to overlap in a subset of patients with *FLT3*/ITD, and its coexpression seems to partially ameliorate the poor prognosis conferred by *FLT3*/ITD alone.[29]

CEBPA mutations

The *CEBPA* gene encodes CCAAT/enhancer binding protein alpha (C/EBPa), which functions as a transcription factor that regulates granulocytic proliferation and terminal differentiation. Mutations in the *CEBPA* occur in approximately 5% of childhood AML, and most cases with this mutation occur in a biallelic manner whereby two distinct mutations occur, one in the N-terminal domain (NTD) and the second in the opposite allele affecting the bZip domain.[31] Such a biallelic mutation results in the expression of a truncated protein, which has been shown to be sufficient for the development of AML.[32] *CEBPA* mutations tend to occur in patients with normal cytogenetics, and are associated with decreased relapse risk and improved survival.[31]

EPIGENETIC ALTERATIONS

Some of the differences observed in gene and protein expression are regulated by alterations that are not encoded in the genome. Alterations in DNA methylation can lead to silencing of genes, similar to that of a functional mutation. Silencing of the *CEBPA* gene by promoter methylation appears to have a functional consequence similar to that seen with mutations in the *CEBPA* gene[33] and, on a genome-wide scale, altered CpG methylation profiles have been associated with distinct subsets of adult AML.[34] Aberrant methylation can be caused by genomic alterations (mutations, deletions, or translocations) in genes regulating methylation, including a large number of methyltransferase genes (eg, *MLL1*, *DNMT3A*, *TET2*). The strategy of targeting aberrant methylation for therapeutic benefit with demethylating agents is currently under investigation.

AGE-ASSOCIATED GENOMIC VARIATION IN ACUTE MYELOID LEUKEMIA

There is a significant age-associated variation in the incidence of AML, with infants and older adults having an elevated incidence. There is a close association between the biology of AML and its age-based incidence, as evidenced by the significant variability of genomic alterations in AML from infancy to adulthood.

The age-based variation in the prevalence of structural and sequence variations is notable in childhood AML (**Figs. 3** and **4**). Examination of the karyotypic alterations demonstrates significant age-based variation, with *MLL* translocations being highly prevalent in infants whereby those younger than 1 year harbor *MLL* alterations in nearly 60% of cases. This rapid evolution of disease, within weeks to months of birth, signifies the impact and high penetrance of *MLL* translocations in causing leukemia. The *MLL* prevalence declines over the following decade of life to less than 10%, similar to what is observed in younger adults (see **Fig. 3**A). In addition, CBF AML is relatively rare in infants, but increases in prevalence to that seen in adults by the second decade of life. It is notable that CBF AML, which is associated with favorable outcome, decreases in prevalence in older adults. Similarly, patients with normal karyotype are rare in younger patients (likely owing to the high prevalence of *MLL* translocations), and their prevalence increases to nearly adult prevalence of nearly 50% in older pediatric patients.

The aforementioned alterations are present in nearly 70% of all childhood AML; however, less common structural alterations are described with variable biology and prognostic significance. Notable examples are monosomy 7 (-7), monosomy 5, and deletion 5q (-5/del5q), which cumulatively account for 2% to 4% of cases of childhood AML and greater than 10% of adult AML. Although specific genes in these chromosomal regions that contribute to disease pathogenesis have not been identified, the
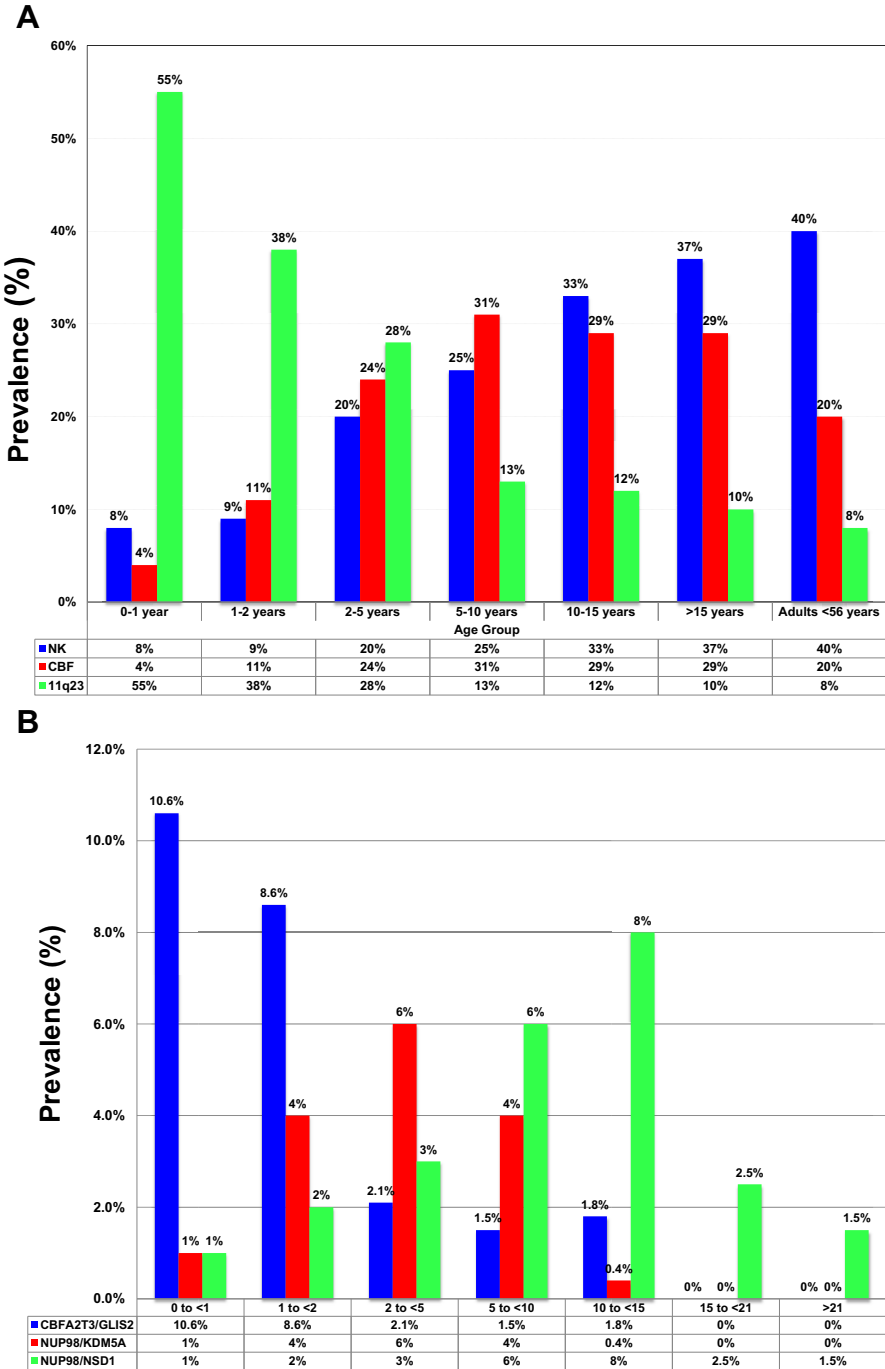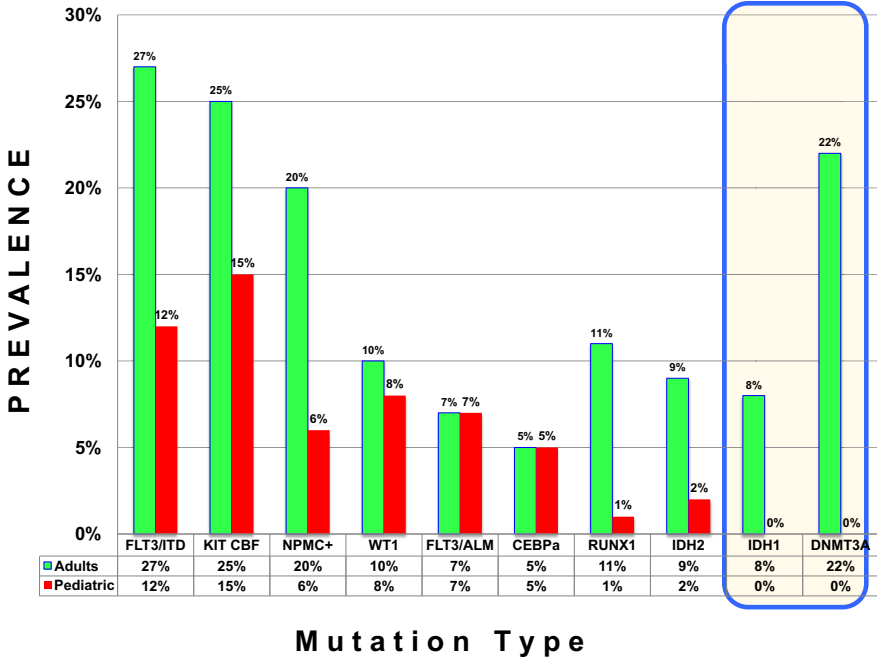
A

	0-1 year	1-2 years	2-5 years	5-10 years	10-15 years	>15 years	Adults <56 years
■ NK	8%	9%	20%	25%	33%	37%	40%
■ CBF	4%	11%	24%	31%	29%	29%	20%
■ 11q23	55%	38%	28%	13%	12%	10%	8%

B

	0 to <1	1 to <2	2 to <5	5 to <10	10 to <15	15 to <21	>21
■ CBFA2T3/GLIS2	10.6%	8.6%	2.1%	1.5%	1.8%	0%	0%
■ NUP98/KDM5A	1%	4%	6%	4%	0.4%	0%	0%
■ NUP98/NSD1	1%	2%	3%	6%	8%	2.5%	1.5%

Fig. 3. Age-based prevalence of specific karyotypic (*A*) or cryptic (*B*) translocations in AML.

A

	FLT3/ITD	KIT CBF	NPMC+	WT1	FLT3/ALM	CEBPa	RUNX1	IDH2	IDH1	DNMT3A
Adults	27%	25%	20%	10%	7%	5%	11%	9%	8%	22%
Pediatric	12%	15%	6%	8%	7%	5%	1%	2%	0%	0%

Mutation Type

B

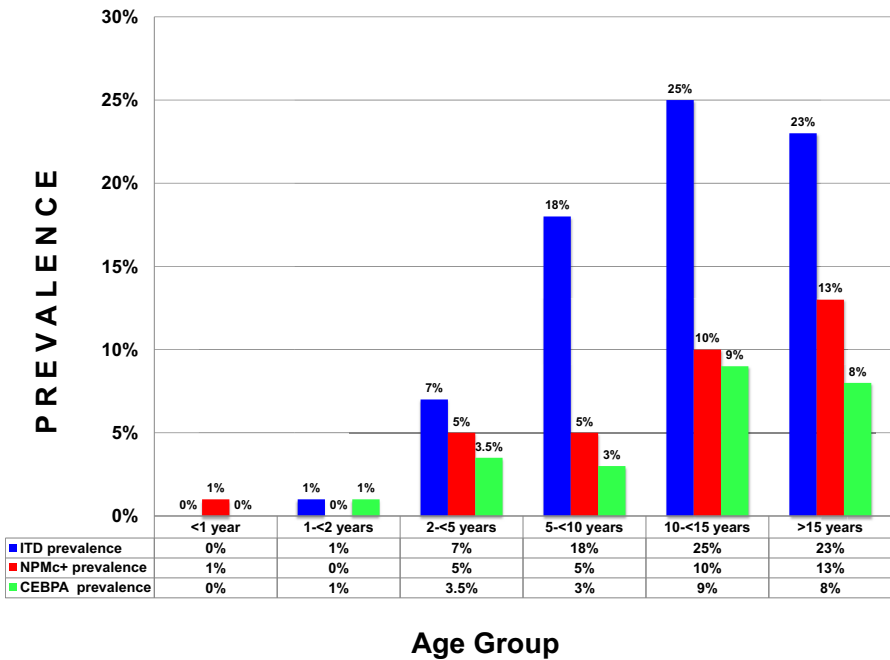|	<1 year	1-<2 years	2-<5 years	5-<10 years	10-<15 years	>15 years
ITD prevalence	0%	1%	7%	18%	25%	23%
NPMc+ prevalence	1%	0%	5%	5%	10%	13%
CEBPA prevalence	0%	1%	3.5%	3%	9%	8%

Age Group

Fig. 4. (*A*) Prevalence of AML-associated mutations in pediatric versus adult AML, demonstrating lower incidence of mutations in pediatric AML. Bordered panel shows 2 newly discovered mutations in adults that are absent in pediatric AML. (*B*) Age-based prevalence of common AML-associated mutations.

presence of these alterations is associated with resistance to chemotherapy and extremely poor survival.[35]

Acute promyelocytic leukemia (APL), whereby normal hematopoiesis is replaced by immature granulocytes (promyelocytes), is the consequence of chromosomal translocation between chromosomes 15 and 17 (t(15;17)), involving the PML gene on chromosome 15 and the retinoic acid receptor alpha (RARα or RARA) gene on chromosome 17 (t(15;17)). The t(15;17) translocation juxtaposes the PML and RARA gene and leads to the expression of a novel fusion product, which is a potent recruiter of transcriptional repressors, resulting in the arrest of leukemic cells in the immature, promyelocyte stage.[36–38]

Cryptic translocations that previously have gone undetected are emerging as significant contributors to childhood AML, with a relatively high prevalence in younger children and significant decline in adults (see **Fig. 3**B). These cryptic structural events include translocations involving methyltransferase genes (NSD1 and KDM5A), which were not previously implicated in AML pathogenesis. Most notable is the fact that somatic mutations affecting the methylation pathway (DNMT3A, IDH, TET2) are highly prevalent in adults but rare or absent in children (see **Fig. 4**A), whereas structural alterations in methyltransferase genes are prevalent in young children but are rare or absent in adults (see **Fig. 3**B). These data highlight the significant contribution of epigenetic derangements to myeloid leukemogenesis that is achieved by different mechanisms in children in comparison with adults.

Similar to the cytogenetic alterations, sequence variations also vary by age, and, as most of these mutations were identified in adults, these AML-associated genes are rare in pediatric AML. However, assessment of prevalence of these mutations has demonstrated paucity of these mutations at a younger age, with rapid evolution of these lesions to adult prevalence by the second decade of life. For example, mutations in FLT3, NPM, and CEBPA are the most prevalent and clinically relevant mutations in childhood AML but are not usually detected in the first year of life, with increasing age-related prevalence to a level commensurate to what is observed in adults by the second decade of life (see **Fig. 4**B).

In the past decade, advances in next-generation sequencing (NGS) technologies have revolutionized the ability to interrogate cancer genomes, culminating in whole-genome sequencing (WGS), whole-exome sequencing (WES), and transcriptome sequencing (RNA Seq). These approaches provide comprehensive coverage of most of the genome at a single base resolution of the genome, exome, or transcriptome, respectively. WGS, in addition to identifying sequence variations (SNVs and indels), provides additional layers of information, including structural alterations such as deletions, duplications, and inter- and intrachromosomal junctions. Transcriptome sequencing provides sequence and fusion data, in addition to mRNA expression levels that are not available by WGS. NGS technologies have rapidly transformed the genomic landscape in cancer by providing high-throughput, comprehensive genomic profiles in a large number of patients.[39] Ley and colleagues[40] identified IDH1 and DNMT3A to be 2 highly prevalent, clinically significant somatic alterations in adult AML.[15] Despite confirmation of the prevalence and clinical utility of these mutations in adult AML, they appear to be rare or absent in childhood AML (see **Fig. 2**),[41,42] highlighting the significant differences between AML in older and younger patients. More recent data from The Cancer Genome Atlas Research Network (TCGA) AML study demonstrated that 23 genes were significantly mutated in this adult cohort. Of the 23 genes, 14 are well-known mutations in AML that have also been well studied in childhood AML and, with the exception of a few (FLT3, NPM, WT1), have been shown to be rare events in childhood AML.

Based on the significant age-associated differences in somatic alterations in AML, a concerted effort has been devoted to the characterization of genomic and epigenomic profiles for the spectrum of childhood AML. Two such projects, the Children's Oncology Group (COG)/National Cancer Institute (NCI) Therapeutically Applicable Research to Generate Effective Treatments (TARGET) AML initiative, and the St Jude/Washington University Pediatric Cancer Genome Project (PCGP), have initiated comprehensive evaluations of the genomic landscape of childhood AML using currently available NGS approaches.[16,43] Early data emerging from these studies has provided new biological insights, and will only further the investigation of the molecular basis of childhood AML.

Already new insights have emerged from these projects. For instance, based on an analysis of RNA Seq, the St Jude PCGP project evaluated the transcriptome profile of a subset of patients with megakaryocytic leukemia (FAB M7), known to be associated with a poor outcome in children. This study identified a cryptic translocation between *CBFA2T3* and *GLIS2* genes in nearly 30% of children with FAB M7 AML, and further demonstrated that the *CBFA2T3/GLIS2* fusion provides enhanced self-renewal capacity in colony-forming assay and that its presence is associated with adverse outcomes in FAB M7 AML.[9] Subsequent studies have demonstrated the presence of this translocation in other AML subsets and its association with adverse outcome.[44,45]

The COG/NCI-sponsored TARGET AML initiative is using WGS and RNA Seq to establish the genomic profile of childhood AML in a large cohort of children with AML and to establish the nature of genomic evolution of childhood AML from diagnosis to relapse. The pilot phase of the TARGET AML initiative used WES to evaluate diagnostic and relapse specimens from 20 patients who lacked karyotypic high-risk features to identify somatic alterations that contribute to AML pathogenesis, and to study genomic evolution from diagnosis to relapse.[16] In this study, of the 195 distinct nonsynonymous mutations present in 180 genes, mutations in 6 genes were detected in more than 1 patient, including *ETV6*, *KIT*, *KRAS*, and *NRAS* in 2 patients and *TET2* or *WT1* in 3 patients, suggesting that, in contrast to adult AML, there may not be a set of highly recurrent mutations in childhood AML. Although most of the identified mutations had been previously described as somatic alterations in pediatric AML, subsequent frequency validation of somatic mutations in *ETV6* showed the mutations to be present in 6% of childhood AML and in less than 1% of adult AML. In this study the presence of *ETV6* mutations, which were primarily observed in those without previously known risk factors, were highly associated with a significantly higher risk of relapse and worse survival.[46] In addition to identification of the somatic mutations in AML at diagnosis, this study surveyed the genomic landscape at relapse compared with that seen at diagnosis, demonstrating significant clonal evolution from diagnosis to relapse, whereby nearly one-third of total mutations identified at diagnosis persisted at relapse with acquisition of a myriad of new mutations at relapse, indicating significant genomic evolution of diagnostic clone en route to relapse. Furthermore, a large number of diagnostic events, primarily minor and subclonal events, resolved from diagnosis to relapse.[16] These findings were in line with observations in 8 matched diagnostic/relapse pairs previously reported by Ding and colleagues[47] in adult AML using WGS, whereby they identified "founding" clones in the primary tumor that gained novel mutations evolving into the relapse clone. This observation signifies that in addition to the genomic alterations that may lead to disease evolution, additional events, whether genomic or epigenomic, may arise or be selected for, and may mediate resistance to therapy and the emergence of new leukemic clones with a genomic profile distinct from that of the diagnostic one.

THERAPEUTIC APPROACHES IN ACUTE MYELOID LEUKEMIA

Survival in childhood AML has improved from less than 10% to nearly 60% over the last several decades.[48] In the process, several unique genomic subsets of patients have been identified to receive specific therapies based on their unique susceptibilities as a result of their genomic makeup. In particular, the therapeutic approach for AML in patients with DS-AML and those with APL have been tailored to meet the unique susceptibilities to conventional therapy (DS AML) or response to molecularly directed therapy (APL).[2,49]

In APL, identification of its underlying genomic etiology led the discovery that pharmacologic doses of retinoic acid overcome the transcription repression induced by the *PML/RARA* fusion product, allowing for forced differentiation of the leukemic cells along the granulocytic pathway. As a result, APL became the first disease to be treated by a molecularly targeted therapy. With the incorporation of all-*trans* retinoic acid (ATRA) into the conventional therapeutic regimen, survival for APL dramatically improved to 75% to 85%, leading to treatment of APL differently from those having other types of AML.[36] More recently, arsenic trioxide (ATO) was shown to have significant efficacy in the management of APL.[37] Lo-Coco and colleagues[38] demonstrated that in combining ATO with ATRA, all other cytotoxic agents can be safely eliminated from APL therapeutic regimens, maintaining the outstanding survival in APL while sparing the patient the short-term and long-term toxicities of cytotoxic therapies. Understanding the biological basis and genomic alterations of APL allowed for the development of a rational, targeted therapy for this subtype of AML. The resultant fusion transcript can also be used for monitoring response to therapy and predicting relapse as emergence of the fusion transcript can be detected in advance of morphologic relapse, allowing for initiation of treatment at the time of submorphologic disease, thus permitting intervention and improved survival for those with impending relapse.

In those with de novo AML, therapeutic modalities have been based on demonstrated efficacy of anthracycline and the nucleoside analogue class of chemotherapies, whereby combination of daunorubicin and cytarabine remains the mainstay of induction therapy in AML. The current approach to remission induction at diagnosis includes treatment with daunorubicin and cytarabine with or without etoposide at varying doses and schedules. Although this therapy leads to an 80% or higher remission rate, it is also associated with significant cytopenias, leading to toxicities and life-threatening events that may lead to treatment-related mortality (TRM) in 3% to 6% of patients. Use of the 3 + 7 combination of daunorubicin and cytarabine for induction of remission, and postinduction therapy with multiagent drug combinations and high-dose cytarabine, improved the results, however cure rates remained at less than 40% in the early 1980s.[50,51]

Emerging data about the resistance of "dormant" leukemia stem cells (LSC) to chemotherapy (not in cell cycle during exposure) led to attempts at targeting the dormant LSC. Trials led by Woods and colleagues[52] demonstrated that intensifying the induction chemotherapy (time sequential induction), though leading to elevated induction TRM, significantly improved survival by decreasing the postinduction relapse rate. A similar strategy at targeting the LSC using prolonged exposure to cytarabine was conducted by investigators in the United Kingdom,[53] demonstrating overall survival rates similar to those achieved by Woods and colleagues.[54] These studies also demonstrated the efficacy of allogeneic hematopoietic cell transplantation (allo HCT) as a postinduction consolidation strategy in AML, in which those who received postinduction consolidation with allo HCT had a significantly improved

survival in comparison with those who received conventional chemotherapy. As a result, postinduction consolidation with allo HCT became the standard of care in those with a matched sibling donors in the United States. Although initial trials limited allo HCT to those with matched sibling donors, evolving improvements in molecular human leukocyte antigen typing, graft-versus-host disease management, and supportive care has led to similar survival in allo HCT from matched unrelated donors and has expanded the population who may benefit from allo HCT. The routine use of autologous stem cell transplantation as a means of consolidation, however, did not show improvement in results over intensified chemotherapy.[55] Current postinduction consolidation uses relapse risk as a determining factor for allocation to the high-risk consolidation arm of the therapy, whereby those deemed to be at a high risk of relapse receive more intensive postinduction chemotherapy followed by allo HCT from the most suitable related or unrelated donor. The clinical trials that led to the current general strategy of therapy for childhood AML were recently reviewed.[56–59] Thus a standard protocol includes cytarabine (10 days), daunorubicin (3 days), and etoposide (3 days), followed by 3 to 4 courses of intensive drug combinations consisting of high-dose cytarabine by itself or with other agents. Unlike in childhood acute lymphoblastic leukemia, central nervous system relapse is uncommon and is well controlled with a single dose of intrathecal cytarabine with each of the drug courses. Prolonged neutropenia is common with standard AML therapy, and requires intensive supportive care to minimize morbidity from bacterial and fungal infections. Because of the unique gram-positive infections from the central lines and cytarabine, special precautions are needed, and some investigators have used antibiotic prophylaxis with some success.[60] With the current improved overall results and better understanding of the risk factors (see the following discussion), allo HCT in first remission is now recommended only to patients at high risk for relapse.[61,62] Therapeutic innovations used for improving the results in high-risk patients include use during induction of high-dose cytarabine (1–3 g/m^2/dose × 6 doses), and use of idarubicin or targeted therapy with gemtuzumab (anti-CD33 antibody) conjugated with ozogamicin.[63–66] Postremission strategies used for modulation of multidrug-resistance gene (MDR1) include cyclosporine and the use of the FLT3 inhibitor, lestaurtinib.[63] These strategies have yielded mixed results. Most promising of these was the unexpected finding that gemtuzumab ozogamicin may improve outcome further in the CBF leukemias.[65]

RISK-BASED THERAPY IN CHILDHOOD ACUTE MYELOID LEUKEMIA

Until recently, cytogenetic alterations were the only means of risk identification in AML, whereby those with CBF AML were understood to have a lower risk of relapse, whereas those with -7 or 5/del5q were at high risk of treatment failure. However, most children with AML lack clinically informative karyotypes and were not amenable to risk-based therapy allocation based solely on cytogenetic subgroups. More recently, somatic mutations in *FLT3*, *NPM1*, and *CEBPA* have been shown to correlate with outcome,[19,30,33] and were incorporated into clinical practice for treatment allocation. Cumulatively, clinically significant cytogenetic alterations and somatic mutations account for nearly 35% of AML in children; however, most children with AML still lack a prognostic biomarker. Although other clinically significant mutations have been demonstrated in adult AML, such mutations are either not seen in childhood AML (eg, *IDH1*, *DNMT3A* mutations)[41,42] or are not independently prognostic (*WT1* mutations).[67] In patients without an informative genomic biomarker, response to therapy as defined by the presence or absence of minimal residual disease (MRD) based on
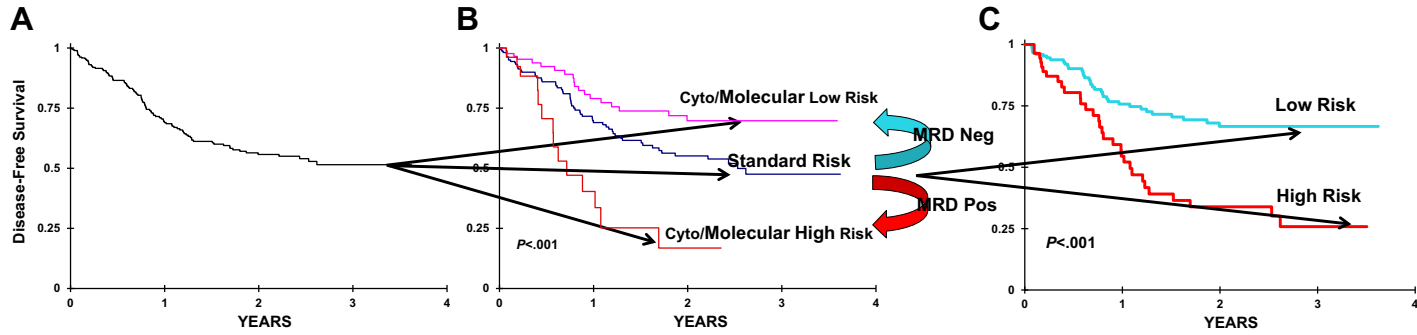
Fig. 5. Newly devised risk stratification for AML. Overall disease-free survival for all patients is presented in panel *A*. Incorporation of known cytogenetic and molecular risk factors creates a 3-tier risk-stratification schema (*B*), allocating 35% of patients to cyto/molecular high risk (10%) or low risk (25%), with 65% of patients without informative cyto/molecular markers remaining in the standard-risk group. Incorporation of disease assessment by multidimensional flow cytometry after the initial induction allows for identification of risk groups within the standard-risk cohort, providing a 2-tier risk stratification schema by combining the cyto/molecular and minimal residual disease (MRD) data (*C*).

detection by multidimensional flow cytometry (MDF) has been used to inform relapse risk.[67,68]

In the ongoing COG de novo AML trial, the combination of diagnostic molecular risk factors with the postinduction response assessment by MRD detection has allowed risk assessment in all patients with AML. This strategy allows for patients with informative molecular markers to be assigned to the appropriate risk class and those lacking any prognostic biomarkers to be risk stratified based on the presence or absence of MRD at the end of induction therapy. Such an approach allows appropriate risk-based therapy allocation for nearly all patients by creating a 2-tier risk allocation system whereby patients are assigned to either a high-risk (cyto/molecular high risk or standard risk with MRD) or low-risk (cyto/molecular low risk or standard risk without MRD) arm based on the most comprehensive set of prognostic data available (Fig. 5). This trial is also allocating patients with high-risk *FLT3*/ITD to receive targeted therapy with the addition of sorafenib, a second-generation TKI with FLT3 inhibitory activity, to conventional chemotherapy. Emergence of novel biomarkers will further enhance the existing risk-based therapy schema, whereby those with either high risk of relapse or those with appropriate targets for directed therapy can be allocated to an alternative therapy to optimize outcome and minimize undue toxicity.

SUMMARY

AML is an enormously complex disease characterized by a myriad of genomic and epigenomic alterations that interact to create a wide variety of distinct subtypes with significantly different outcomes. This level of heterogeneity has precluded uniform outcome, given the uniform approach to AML therapy for all patients. Genomic alterations, whether karyotypic abnormalities or specific disease-associated mutations, have been essential in informing clinical decision making. Until recently, genomic profiling only provided clinical prognostic information based on intensity-modulated therapy and, with the exception of *PML-RARA*, there has been a paucity of potential therapeutic targets. More recently, *FLT3*/ITD has emerged as another lesion to potentially benefit from therapeutic targeting. Comprehensive genomic profiling will not only enhance the ability of physicians to provide more individualized prognosis and treatment information to patients with AML, but has the potential to identify lesions that could be targeted for more personalized and directed therapy, negating the need for highly intensive, cytotoxic therapies with their short-term and long-term side effects. New discovery phase initiatives including the NCI-sponsored TARGET AML initiative are aimed at discovering novel biomarkers to be used for more precise risk identification and identify potential therapeutic targets. With the increase in the number of tools for interrogation of the AML genome and epigenome, the ability to allocate patients to individualized therapy directed by the underlying biology of their disease is anticipated. Researchers are emerging from one the most exciting eras in cancer biology in which NGS has provided the tools for comprehensive interrogation of the cancer genome, whose data will be mined for years to come in identifying new biomarkers for risk and target identification, leading to more appropriate risk-based and target-based therapeutic interventions that can make personalized medicine a reality in the near future.

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