



Treatments for children and adolescents with AML

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Abstract

In recent decades, survival rates for childhood acute myeloid leukemia have remarkably improved, owing to chemotherapy intensification, allogeneic hematopoietic stem cell transplantation, and improved supportive care. Furthermore, treatment protocols have evolved and are currently better matched to prognostic factors and treatment responses. Recently, new molecular prognostic factors were discovered via leukemia genomic studies. Moreover, new tumor subtypes with independent gene expression profiles have been characterized. To broaden the therapeutic options for patients with poor prognoses, therapies that target specific candidate mutations are being identified. Additionally, new drugs are undergoing clinical trials, and immunotherapy is attracting significant interest as a treatment option for recurrent or refractory childhood acute myeloid leukemia.

Key Words Childhood, Adolescents, Acute myeloid leukemia, Treatment, Prognosis, Survival

INTRODUCTION

Pediatric acute myeloid leukemia (AML) is a heterogeneous group of diseases classified according to morphology, lineage, and genetics [1]. The overall survival (OS) rates of children with AML have improved over the past three decades, with the current 5-year survival rate ranging from 65% to 75% [2-5]. Overall remission-induction rates are approximately 85% to 95% and event free survival (EFS) rates from the time of diagnosis range from 50% to 65% [2-5]. These improvements were driven by chemotherapy intensification, allogeneic hematopoietic stem cell transplantation (HSCT), and improved supportive care. During this period, most international cooperative study groups have coalesced to derive a treatment strategy that includes four to five courses of intensive myelosuppressive chemotherapy, including two courses of induction, predominantly based on cytarabine and anthracyclines with or without HSCT at the first complete remission (CR).

However, a wide range of outcomes exists for the different biological subtypes of AML. Specific biological factors can shift the predicted outcome for an individual patient to either a much better or a much worse outcome than the overall result of the general population of children with AML. Genomic investigations of AML have led to new genomic

classifications and predictive biomarkers, resulting in better risk stratification and survival outcomes [6, 7].

Despite advancements in chemotherapy and supportive care, primary refractory or relapsed pediatric AML has resulted in significant morbidity and mortality, and long-term survival in patients with these disorders remains poor [8]. As a result, efforts to identify new target therapy and cell therapy to improve OS in these individuals remain under continuous investigation.

RISK-BASED STRATIFICATION

AML prognosis is dependent on a range of cytogenetic and molecular characteristics. The risk factors used for stratification vary according to the pediatric and adult cooperative clinical trial groups, and the prognostic impact of a given risk factor can vary in significance according to the administered therapy. Most protocols warrant treatments based on cytogenetics at diagnosis and the responses after induction (Table 1). According to risk group stratification, survival from CR at 5 years significantly varied and the survival percentages for favorable, standard, and poor risks were 84, 76, and 47% ($P < 0.0001$) in the United Kingdom Medical Research Council's 12nd AML trial (MRC AML12) [3]. The Children's Oncology Group (COG) AAML0531 study strati-

Table 1. Genetic prognostic markers in international prospective clinical trials of pediatric patients with acute myeloid leukemia.

Study group (protocol no.)	Unfavorable prognostic markers	Favorable prognostic markers
Children's Oncology Group (AAML1831)	inv(3)(q21q26.3)- <i>MECOM-RPN1</i> fusion t(6;9)(p23;q34.1)(<i>DEKDEK-NUP214</i>) Monosomy 7 Monosomy 5/5q Monosomy 5/5q-[<i>EGR1</i> (5q31)deleted] <i>KMT2A(MLL)</i> (11q23.3) -t(4;11)(q21;q23) -t(6;11)(q27;q23) -t(10;11)(p11.2;q23) -t(10;11)(p12;q23) -t(11;19)(q23;p13.3) <i>NUP98</i> (11p15.5) 12p: Rearrangement or loss of <i>ETV6</i> t(16;21)(p11;q22)(<i>FUS-ERG</i>) <i>FLT3/ITD</i> + with allelic ratio > 0.1% <i>CBFA2T3-GLIS2</i> <i>RAM</i> phenotype <i>KAT6A</i> (8p11.21) Fusion (10p12) (for patients who are 90 days or older) Non- <i>KMT2A-MLLT10</i> Fusions	t(8;21)(q22;q22) <i>RUNX1-RUNX1T1</i> inv(16)/t(16;16)(p13.1q22) <i>CBFB-MYH11</i> <i>NPM1</i> mutations Biallelic <i>CEBPA</i> mutation
St. Jude Children's Hospital (AML16)	<i>DEK-NUP214</i> [t(6;9)] <i>KAT6A-CREBBP</i> [t(8;16)] <i>RUNX1-CBFA2T3</i> [t(16;21)] -7, -5, 5q-, <i>KMT2A-MLLT10</i> [t(6;11)] <i>KMT2A-MLLT4</i> [t(10;11)] inv(3)(q21q26.2) <i>CBFA2T3-GLIS2</i> [inv(16)(p13.3q24.3)] <i>NUP98-KDM5A</i> [t(11;12)(p15;p13)] <i>ETV6-HLXB</i> [t(7;12)(q36;p13)] <i>NUP98-HOXA9</i> [t(7;11)(p15.4;p15)] <i>NUP98-NSD1</i> <i>FLT3-ITD</i> in combination with either <i>NUP98-NSD1</i> fusion or <i>WT1</i> mutation Acute megakaryoblastic leukemia with <i>KMT2A</i> rearrangements, <i>CBFA2T3-GLIS2</i> [inv(16)(p13.3q24.3)], or <i>NUP98-KDM5A</i> [t(11;12)(p15;p13)]	Absence of high-risk features
Berlin-Frankfurt-Münster	Complex karyotype -5 del(5q)-7 Abnormalities of 3q <i>FLT3</i> mutation	t(8;21) inv(16)
United Kingdom Medical Research Council (AML MRC 17)	Complex karyotype -5 del(5q)-7 Abnormalities of 3q <i>FLT3</i> mutation	t(8;21)/ <i>AML1-ETO</i> inv(16)/t(16;16)/ <i>CBFB-MYH11</i>

fied treatments according to the initial genetic prognostic markers and responses after induction [9]. These differing approaches yielded significant survival differences between the groups: 3-year EFS of 64.0%, 46%, and 27% in the low risk, intermediate risk, and high risk groups. In the subsequent COG AAML1031 trial, risk groups were defined by integrating the minimal residual disease (MRD) assessed by multiparameter flow cytometry [10]. Patients who were positive for MRD ($\geq 0.1\%$) were allocated to the high-risk group. Most current protocols for pediatric AML patients

recommend transplantation from the most appropriate available donor during the first remission for those at high-risk. Additionally, it is recommended that patients in the low-risk group receive HSCT upon relapse; however, validation of this approach is warranted.

TREATMENTS FOR NEWLY DIAGNOSED PATIENTS

Chemotherapy

Cytarabine and anthracycline are the two most important drugs administered to induce remission. Cytarabine is usually administered for 10 days, followed by daunorubicin for 3 days. Commonly used regimens for the induction phase in pediatric AML patients are cytarabine and anthracycline in combination with etoposide or thioguanine [3]. The MRC AML 10 trial conducted a randomized comparison of the DAT (daunorubicin, cytarabine, thioguanine) and ADE (cytarabine, daunorubicin, etoposide) regimens but found no significant differences in CR rate or induction death. Moreover, the 10-year survival, OS, disease free survival (DFS), and EFS were recognized to be similar between the two regimens [3, 11]. However, the percentages of patients achieving remission after treatment and failing to achieve CR due to disease resistance were slightly better with the ADE regimen. Additionally, a relatively higher CR death rate was found during consolidation chemotherapy with ADE (9%) than with DAT (6%; $P=0.06$) [11]. A subsequent MRC AML15 trial compared DA, ADE, and FLAG-IDA (fludarabine, high-dose cytarabine, filgrastim-idarubicin) with or without gemtuzumab ozogamicin (GO) for the first induction. As a result, induction with DA (daunorubicin, cytarabine) was found to result in survival rates that were equivalent to ADE induction [12]. In addition, FLAG-IDA was demonstrated to be an effective remission induction treatment, with a high CR rate after the first course and reduced relapse, albeit with increased myelosuppression. Currently, etoposide is not used in the COG and MRC trials, resulting in DA regimens with or without targeted agents according to the diagnostic genetic characteristics, such as *GO* or *FLT3* inhibitors.

Among anthracyclines, daunorubicin is the most commonly administered drug in induction regimens. Cytarabine and etoposide in combination with either daunorubicin or idarubicin (ADE or AIE) were compared in the Berlin-Frankfurt-Münster (BFM) study, and similar EFS and OS were observed for both regimens [13, 14]. Likewise, a previous MRC trial showed no significant overall survival benefit from cytarabine, mitoxantrone, and etoposide (MAE) relative to ADE [15]. Although idarubicin and mitoxantrone outcomes were not superior to those of daunorubicin, liposomal daunorubicin significantly reduced treatment-related mortality compared to idarubicin [16].

First-line treatments were intensified by the introduction of courses of high-dose cytarabine, new anthracyclines, or anthracycline formulations. High-dose cytarabine with mitoxantrone (HAM) or etoposide (HAE) was administered in the BFM-AML studies. The recent MRC 17 study used high-dose cytarabine containing FLAG-IDA in the poor risk group, and two courses of high-dose cytarabine in the other risk groups.

In the past, five chemotherapy courses served as the standard for frontline treatment. However, comparisons between

the COG AAML0531 and AAML1031 studies showed that MRD level at the end of induction I, and not the removal of a fifth cytarabine-containing course, was the most significant predictor of relapse [17]. Likewise, a fifth course showed no survival benefit in MRC AML15 [12].

CNS treatment, usually with intrathecal medication, is a standard component of most pediatric AML protocols; however, research has yet to directly associate this treatment with improvements in survival. Although CNS involvement (CNS2 or CNS3) at diagnosis was not correlated with OS in most studies, two consecutive COG trials found that CNS involvement, particularly CNS3 status, was associated with worse outcomes, including CR rate, EFS, disease-free survival, and an increased risk of relapse involving the CNS [18]. In contrast to acute lymphoblastic leukemia, traumatic tapping did not increase CNS relapse incidence.

Gemtuzumab ozogamicin

GO (Mylotarg) is a humanized anti-CD33-calicheamicin conjugate developed for the targeted treatment of AML. GO was approved for use by the United States FDA in 2000 [19]; however, in 2010, it was withdrawn from the market due to concerns of increased liver toxicity and myelosuppression. Nonetheless, GO has been incorporated into prospective trials for adults and children with AML. As a result, the manufacturer reapplied for FDA approval in 2017, with support from additional clinical results.

The COG AAML03P1 pilot study administered two doses of GO to 350 children with AML in courses 1 and 4 [20]. Patients achieved a CR rate of 83% after one course and 87% after two courses. Although a mortality rate of 2.6% was obtained after two induction courses, the 3-year EFS and OS percentages were 53% and 66%. Based on these findings, a subsequent COG AAML0531 study randomly administered the standard five-course chemotherapy with or without two doses of GO (3 mg/m²/dose) to 1,022 patients ages 0 to 29 years with newly diagnosed AML [9]. Based on their results, GO significantly reduced relapse risk (3 yr: 32.8% vs. 41.3%; $P=0.006$), albeit with increased post-remission toxic mortality. DFS was thus demonstrated to result in better responses in GO recipients. Based on these results, current COG and MRC trials are using GO in combination with cytarabine and daunorubicin as the initial induction therapy in newly diagnosed AML patients.

Allogeneic hematopoietic stem cell transplantation

The use of allogeneic HSCT as a consolidation therapy for pediatric patients with AML in the first CR has been continuously evaluated in prospective clinical trials. In an analysis of 1,373 pediatric patients with AML, HLA-matched relative bone marrow transplantation only conferred survival benefits to intermediate cytogenetic risk groups. In prospective trials of allogeneic HSCT compared to chemotherapy and/or autologous HSCT, a superior DFS was observed for patients assigned to received allogeneic HSCT [21, 22]. However, results comparing allogeneic HSCT to chemotherapy remain inconclusive. Allogeneic HSCT has been lim-

ited to intermediate or poor risk groups in the first CR because of improved outcomes in patients with favorable cytogenetics or mutations receiving contemporary myelo-suppressive combination chemotherapy. Conflicting reports on the efficacy of HSCT in intermediate risk groups [23, 24] have led many childhood AML studies to employ upfront allogeneic HSCT for poor risk groups alone; these include patients with unfavorable cytogenetics, genetic mutations, or elevated end-of-induction MRD levels [25]. As the definitions of high-, intermediate-, and low-risk AML are evolving due to ongoing discoveries of the molecular characteristics of AML and more sophisticated assessments of MRD, a further analysis of the subpopulations of patients treated with allogeneic HSCT will be an ongoing need in current and future clinical trials.

The optimal preparative regimen and the source of donor cells have yet to be determined. For approximately 20 years, myeloablative doses of busulfan and high doses of cyclophosphamide were used in many studies. However, ongoing efforts to reduce toxicity- and treatment-related mortalities have led to the replacement of cyclophosphamide with fludarabine to introduce reduced intensity conditioning. There is no prospective randomized study comparing different conditioning regimens; however, previously, two large retrospective studies performed by the Center for International Blood and Marrow Transplant Research (CIBMTR) showed no significant survival difference between myeloablative and reduced-intensity conditioning regimens or busulfan/cyclophosphamide and busulfan/fludarabine regimens [26, 27].

MINIMAL RESIDUAL DISEASE

Early responses to therapy, which are generally measured after the first course of induction therapy, is predictive of outcomes [28, 29]. Molecular approaches using quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to assess MRD in AML has proven challenging owing to the genomic heterogeneity of pediatric AML and the instability of some genomic alterations. Nonetheless, flow cytometric methods have been successfully used for MRD detection and can detect leukemic blasts according to the expression of aberrant surface antigens that differ from patterns observed in normal progenitors.

Quantitative RT-PCR detection of *RUNX1-RUNX1T1* (*AML1-ETO*) fusion transcripts can effectively predict a higher risk of relapse for patients in clinical remission [30–32]. Other molecular alterations, such as *NPM1* mutations [33] and *CBFB-MYH11* fusion transcripts [31], are also used as specific molecular markers in the MRD assays. However, a PCR-based assay for the *FLT3-ITD* mutation loads lack the sensitivity required to quantify MRD [32], and was discordant between diagnosis and relapse, although when it persists, it can be useful pre-transplant prognostic marker [34].

Multiple groups have shown that the level of MRD after one course of induction therapy is an independent predictor

of prognosis. The COG AAML03P1 study prospectively investigated MRD at the end of inductions I and II by flow cytometry and correlated these values with clinical outcomes in 350 patients [29]. In a multivariate analysis that included cytogenetic and molecular risk factors, residual disease was an independent predictor of relapse. According to the St. Jude hospital AML02 study where high-dose or low-dose cytarabine was randomly administered with daunorubicin and etoposide (ADE; induction I) to 232 patients with de novo pediatric AML, an MRD $\geq 1\%$ after induction I was the only significant independent adverse prognostic factor of survival [5].

Next-generation sequencing (NGS) is emerging as an important tool for the molecular analysis of AML at the time of initial diagnosis, especially in cytogenetically normal AML patients [35]. Although more standardization of the methods of testing and interpretation is required, NGS has been used to assess MRD in AML patients.

SPECIFIC SUBGROUPS

Acute promyelocytic leukemia

Acute promyelocytic leukemia (APL) represents ~6% to 11% of children with AML [36]. This distinct patient group has t(15;17) in their blasts and is administered different treatments relative to other AML patient groups. Although the WHO 2016 revision does not include the t(15;17) cytogenetic designation, it references the *PMLRARA* fusion, which may either be cryptic or result from complex karyotypic changes [7]. Anthracycline-based chemotherapy in combination with all-trans-retinoic acid (ATRA) has improved the survival of pediatric patients with AML and is currently the mainstay treatment [37]. In the Gruppo Italiano Malattie Ematologiche dell'Adulto—Associazione Italiana di Ematologia Oncologia Pediatrica AIDA 0493 trial, treatment with ATRA, anthracyclines, and high-dose cytarabine resulted in an OS of approximately 90%. White blood cell (WBC) count at initial diagnosis is used to classify standard and high risk groups in APL. Patients with a WBC count $\geq 10,000/\mu\text{L}$ were assigned to a high risk group, and these patients have an increased risk of differentiation syndrome and early death [38]. In the COG AAML0631 study, the high risk group received earlier idarubicin, one additional consolidation (high-dose cytarabine and idarubicin), and ATRA [37].

Maintenance therapy for APL consisting of ATRA and chemotherapy has been shown to improve EFS and OS [39]. More recently, APL clinical trials have evaluated treatment with arsenic trioxide (ATO) [40]. In a pediatric study (AAML0631), all patients received two cycles of ATO therapy during consolidation 1, an additional two (standard risk) or three (high risk) consolidation courses that included high-dose cytarabine and anthracycline, and maintenance therapy comprising of ATRA, oral methotrexate, and mercaptopurine [37]. As a result, the ATO consolidation cycles were well tolerated and allowed a significant reduction in cumulative anthracycline doses while maintaining excellent survival and

a low relapse risk for both risk groups. ATO is currently being used in clinical trials for newly diagnosed and relapsed APL.

AML in down syndrome

Approximately 10% of neonates with Down syndrome develop transient abnormal myelopoiesis (TAM). The subsequent myeloid leukemia associated with Down syndrome also occurs in 10% to 30% of children with a spontaneous resolution of TAM when reported at a mean age of 16 months (range, 1–30 mo) [41, 42]. Survival outcomes of patients with Down syndrome and AML are superior to those of children with AML but without Down syndrome. Current treatment for children with Down syndrome and AML is less intensive than the standard chemotherapy for those with AML, and HSCT is not indicated at the first remission [43]. In this group of patients, reduced doses of anthracyclines and treatment duration are recommended because of toxicity. According to a recent COG trial (AAML0431), substituting anthracycline with high-dose cytarabine-based regimens reduced the cumulative anthracycline dose. Moreover, the reduced doses of intrathecal chemotherapy did not negatively affect the final survival outcomes and relapse rates of patients compared to previous studies [44]. Instead, intensification included two cycles of cytarabine and etoposide. Currently, the first prospective trial based on risk stratification in this patient group is being conducted through an assessment of MRD levels at the end of induction I [45].

FLT3-mutated AML

The presence of the *FLT3-ITD* mutation is associated with poor prognosis in AML. In pediatric patients with AML, its prevalence is approximately 15% lower than that in adults [46]. A retrospective report in children and young adults

with AML found that only patients with high allelic ratios of *FLT3-ITD* benefited from allogeneic HSCT (i.e., those with low allelic ratios did not benefit from this treatment) [47], according to a subset analysis of the COG phase 3 trial, which evaluated GO during induction therapy in children with newly diagnosed AML [48].

Several *FLT3* tyrosine kinase inhibitors that vary in kinase selectivity, potency, and clinical activity are either being developed or have been approved for the treatment of AML [49]. In preliminary studies, sorafenib displayed promising clinical activity in patients with *FLT3-ITD*-mutated AML; however, randomized assessments were rarely conducted. Another multitargeted agent, midostaurin, was approved in combination with standard cytarabine and daunorubicin-based chemotherapy for patients with newly diagnosed *FLT3*-mutated AML [50]. Currently, it is undergoing trials to elucidate its efficacy in pediatric patients *FLT3-ITD*-mutated AML. A more recent inhibitor, gilteritinib, is a highly selective oral *FLT3* inhibitor that exhibits activity against both *FLT3* mutation subtypes (ITD and TKD) and weak activity against c-Kit [50]. Gilteritinib also inhibits the tyrosine kinase, AXL, which is implicated in *FLT3* inhibitor resistance. Gilteritinib was recently approved by the FDA for the treatment of adults with relapsed or refractory AML. Currently, the COG AML study (AAML1831) is assessing randomized treatment with or without gilteritinib in newly diagnosed pediatric AML patients with the *FLT3* mutation.

Relapsed or refractory pediatric patients with AML

Survival in children with relapsed or refractory AML remains unsatisfactory. Despite the induction of second remission in 60% to 70% of children with AML treated with similar drugs at their initial induction, their prognosis for recurrent or progressive AML is generally poor [51, 52].

Table 2. Recent publications on relapsed or refractory pediatric patients with acute myeloid leukemia.

Reference	Drugs	Response (No. of patients)
Niktoreh <i>et al.</i> 2019 [59]	Gemtuzumab ozogamicin±other chemotherapeutic agents	51% (36/71)
Cooper <i>et al.</i> 2019 [60]	CPX-351 (a liposomal preparation of cytarabine and daunorubicin)	81% (30/37) CR/CRp/CRi
van Eijkelenburg <i>et al.</i> 2018 [61]	Clofarabine, liposomal daunorubicin, high-dose cytarabine	68% (21/31) CR/CRi/PR
Messinger <i>et al.</i> 2017 [62]	Clofarabine, cyclophosphamide, and etoposide	51% (9/17) CR/CRp/CRi
Cooper <i>et al.</i> 2017 [63]	Plerixafor, high dose cytarabine, etoposide	23% (3/13) CR/CRp/CRi
Horton <i>et al.</i> 2014 [64]	Bortezomib, low-dose cytarabine, idarubicin	57.1% (8/14) CR/CRp/CRi (cycle 1)
	Bortezomib, high-dose cytarabine, etoposide	47.8% (11/23) CR/CRp/CRi (cycle 1)
Cooper <i>et al.</i> 2014 [57]	Clofarabine, cytarabine	45% (21/47) CR/CRp
Shukla <i>et al.</i> 2014 [56]	Clofarabine, topotecan, vinorelbine, thiotepa	67% (8/12)
Kaspers <i>et al.</i> 2013 [55]	FLAG	59% (117/197)
	FLAG+liposomal daunorubicin	69% (135/197)
Miano <i>et al.</i> 2012 [58]	Clofarabine, etoposide, cyclophosphamide	44% (7/16) CR/CRi
Inaba <i>et al.</i> 2011 [65]	Clofarabine, cytarabine, daily sorafenib	72% (8/11) CR/CRi
Inaba <i>et al.</i> 2010 [66]	Cladribine, topotecan	35% (9/26)

Abbreviations: CR, complete remission; CRi, complete remission with incomplete count recovery; CRp, notable CR with incomplete platelet recovery; FLAG, fludarabine, high dose cytarabine, filgrastim.

For relapsed patients, the most significant prognostic factors associated with a favorable outcome include the achievement of a second CR, early response after salvage, late relapse, no previous allogeneic HSCT, and initial favorable cytogenetics [53, 54]. Consolidation chemotherapy followed by HSCT is generally recommended for these individuals, despite the lack of controlled prospective data.

Regimens for salvage chemotherapy in relapsed or refractory patients include new agents with conventional chemotherapeutics, such as cytarabine or daunorubicin (Table 2) [55-66]. Liposomal daunorubicin yields a favorable pharmacokinetic profile that causes a ten-fold increase in concentration compared to that achieved with conventional preparations [67]. In a study by the BFM group, FLAG was compared to FLAG plus liposomal daunorubicin [55]. Based on their findings, no significant survival differences were found; however, the addition of liposomal daunorubicin increased response rates by 10% and led to significant improvements in OS in patients with CBF mutations (82% vs. 58%; $P=0.04$).

Clofarabine is a purine nucleoside antimetabolite that is used to treat relapsed or refractory AML either as a single agent or in combination therapy [56-58, 61, 62, 65, 68, 69]. The COG AAML0532 trial evaluated the combination of clofarabine and high dose cytarabine in relapsed pediatric patients with AML. Based on their findings, response rate was 48% and OS was 46% [57].

CPX-351 is a fixed liposomal formulation of daunorubicin and cytarabine approved by the FDA as Vyxeos (Jazz Pharmaceuticals) for the treatment of adults with newly diagnosed, therapy-related AML or AML with myelodysplasia-related changes. In adult AML patients, Vyxeos improved OS (estimated median OS, 9.6 mo) compared to the "7+3" control arm (5.9 mo) [60, 70]. Furthermore, Vyxeos was administered to relapsed pediatric patients with AML as a Phase I trial; this study comprised of 37 children and adolescents with relapsed or refractory AML [58]. One treatment cycle consisted of three doses of Vyxeos on days 1, 3, and 5, and after up to two cycles of therapy (Cycle 1, CPX-351; Cycle 2, FLAG), response rates were assessed. The overall response rates (CR+CRp+Cri) were relatively high (81%; 20 CR, 5 CRp, and 5 Cri), with 70% of these responses being achieved after cycle 1. In this study, 23/29 responders (79%) received HSCT at follow-up. These promising results in relapsed or refractory pediatric patients with AML led to the adoption of a randomized study arm and GO compared to DA+GO in de novo pediatric patients with AML (AAML1831).

NEW AGENTS

Epigenetic modifiers

Epigenetics is defined as alterations in the transcriptional potential of genes owing to modifications of histones and chromatin rather than DNA itself. DNA methylation signatures have been used to identify biologically distinct subtypes of AML. By sequencing the DNA in AML cells, re-

current mutations in genes that encode proteins that alter DNA methylation patterns, such as *DNMT3A*, *IDHI*, and *TET2*, were observed. Azacitidine is a DNA methylation inhibitor (DMTi) and the first clinically adopted epigenetic modifier. Azacitidine was found to exert in vitro therapeutic efficacy in cell lines of AML [71]. Recent trials comparing single agent azacitidine in older patients with AML or relapsed or refractory pediatric patients with AML to conventional care regimens revealed a trend toward increased survival [72, 73]. Moreover, an ongoing study of newly diagnosed pediatric patients with AML at St. Jude Hospital incorporates 5 days of DMTi into each chemotherapy block, with DMTi being randomly selected between azacitidine and decitabine.

CAR-T cell

In AML, current developments of CAR-T cells aim to express CD33 or CD123 on the surface of AML blasts [74]. Furthermore, researchers are targeting cell surface proteins that are often overexpressed or mutated in AML blasts, such as FLT3 [74]. However, these strategies are confounded by the lack of specific markers for AML blasts and the significant adverse events caused by off-tumor effects.

CONCLUSION

Because of the heterogeneity of AML, an intervention or experimental agent that is beneficial to a subgroup of patients might not be suitable for the overall population. As a result, current and future trials of pediatric patients with AML are aiming or will aim to examine new agents that are designed for specific subgroups of patients. However, there may also be developments of new immunotherapeutic approaches that may be applicable to a wider range of AML subtypes [74]. By gaining new insights into the genetics of AML and the biology and microenvironment of leukemia stem cells, new avenues could be established to aid in the future treatment of childhood AML.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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