How I treat pediatric acute myeloid leukemia

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Acute myeloid leukemia is a heterogeneous disease that accounts for approximately 20% of acute leukemias in children and adolescents. Despite the lack of targeted therapy for most subtypes and a dearth of new agents, survival rates have reached approximately 60% for children treated on clinical trials in developed countries. Most of the advances have been accomplished by better risk classification, the implementation of excellent supportive care measures, adaptation of therapy on the basis of each patient's response to therapy, and improvements in allogeneic hematopoietic stem cell transplantation. However, it is unlikely that further gains can be made through these measures alone. In this regard, high-resolution, genome-wide analyses have led to greater understanding of the pathogenesis of this disease and the identification of molecular abnormalities that are potential targets of new therapies. The development of molecularly targeted agents, some of which are already in clinical trials, holds great promise for the future. (*Blood*. 2012;119(25):5980-5988)

Introduction

Acute myeloid leukemia (AML) is composed of a heterogeneous group of diseases that can be classified by morphology, lineage, and genetics.[1] This heterogeneity reflects the diversity of myeloid precursors that are susceptible to malignant transformation and the assortment of genetic events that can lead to this transformation. Most subtypes of AML are characterized by subpopulations of leukemic stem cells, or leukemia-initiating cells, that have an unlimited self-renewal capacity and a hierarchical organization similar to that of normal hematopoietic cells.[2,3] In addition, different subtypes are characterized by abnormalities in common pathways that regulate proliferation, differentiation, and cell death. These lesions include those that cause constitutive activation of protein kinases that impart proliferative and survival signals, translocations that create fusion proteins that block differentiation, and mutations that lead to abnormalities in self-renewal.[4] Recent analyses of genome-wide DNA copy number alterations, loss of heterozygosity,[5] and the complete DNA sequence of AML genomes[6,7] suggest that AML contains fewer genetic alterations than other malignancies do. Nevertheless, these studies have identified novel lesions, such as mutations in *IDH1* or *IDH2*, which occur in nearly 10% of childhood AML patients with normal karyotypes.[8] Further characterization of the entire spectrum of genetic events involved in AML will lead to a better understanding of the disease and, ultimately, to the development of rationally designed therapy.

Despite the large number of subtypes and the lack of targeted therapy for most subtypes, the treatment outcome has improved markedly for children with AML. Excellent supportive care, adaptation of therapy on the basis of each patient's response, and the use of intensive chemotherapy or hematopoietic stem cell transplantation (HSCT) have led to event-free survival (EFS) rates that are greater than 50% and overall survival (OS) rates greater than 60% on recent trials (Table 1).[9-13] The results of St Jude AML clinical trials conducted since 1980 are shown in Figure 1. The treatment outcome achieved on the multi-institutional AML02 trial is similar to that reported by the Medical Research Council (MRC),[12] the Nordic Society for Paediatric Hematology and Oncology (NOPHO),[13] the Berlin-Frankfurt-Muenster study group (BFM),[11] the Japanese Childhood AML Cooperative Group,[10] and the Children's Oncology Group (COG).[14] However, the cure rates for some subtypes of childhood AML remain unacceptably low, and novel therapies are needed.

In this review, current concepts and future directions in the treatment of childhood AML are discussed. For the purpose of this review, "childhood" or "pediatric" AML is defined as AML occurring in patients who are younger than 22 years. However, biologic and clinical similarities exist among AML in children, adolescents, and young adults, and many of the principles discussed by Rowe and Tallman[15] apply here as well. Because the focus is on treatment of de novo AML, the reader is referred to excellent reviews on the genetics and biology of AML for information about these areas.[2,4] In addition, the treatment of childhood acute promyelocytic leukemia (APL), which is similar to that used in adults with APL,[16] and the treatment of children with Down syndrome and AML[17,18] will not be discussed here. Six cases of children with AML who were treated at our institution during the past few years are presented to demonstrate our approach to the workup, risk classification, and treatment of childhood AML.

Patient 1

A 10-year-old boy presented with a 1-week history of fever, fatigue, dizziness, and leg pain. A complete blood count revealed a leukocyte count of 8800/μL with 78% blasts, many of which contained Auer rods; his hemoglobin concentration was 6.7 g/dL, and his platelet count was 61 000/μL. A review of the bone marrow aspirate revealed dysplastic granulocytes and a population of blasts that were positive for CD13, CD33, CD15, CD11c, CD133, CD34, CD117, HLA-DR, CD71, CD19 (dim), MPO, and Tdt (dim). Cytogenetic analysis revealed t(8;21)(q22;q22), and RT-PCR detected the *RUNX1-RUNX1T1* (formerly *AML1/ETO*) fusion transcript.

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Table 1. Results of recent clinical trials for pediatric AML

Study	Years of enrollment	Eligible age, y	No. of patients	CR rate,* %	Outcome	Key points
St Jude AML02[9]	2002-2008	≤ 21	216	94	3-y EFS: 63% 3-y OS: 71%	Risk-adapted therapy was based on molecular genetics and MRD High-dose cytarabine during induction was not beneficial
AML-BFM 98[11,26]	1998-2003	< 18	473	88	5-y EFS: 49% 5-y OS: 62%	G-CSF did not decrease the incidence of infection or infection-related mortality Shorter, intensive consolidation cycles did not improve outcome
MRC AML12[12]	1995-2002	< 16	529	92	10-y EFS: 54% 10-y OS: 64%	No differences in EFS or OS rates between mitoxantrone- and daunorubicin-based inductions No difference in EFS or OS rates between 4 and 5 courses of therapy
NOPHO-AML 2004[13]	2004-2009	≤ 18	151	92	3-y EFS: 57% 3-y OS: 69%	Intensity of therapy based on early response to therapy
AML99[10]	2000-2002	≤ 18	240	95	5-y EFS: 62% 5-y OS: 76%	Intensive use of high-dose cytarabine Six courses of therapy
COG AAML03P1[79]	2003-2005	≤ 21	350	87	3-y EFS: 53% 3-y OS: 66%	Safe to add gemtuzumab ozogamicin to first and fourth courses of therapy

CR indicates complete remission.
*CR rate after 2 courses of induction therapy.

This patient represents a typical case of t(8;21)–positive AML, which commonly have abundant Auer rods, partial expression of CD19, and dysplastic maturing granulocytes. The t(8;21), inv(16)(p13.1;q22)/*CBF*β-*MYH11*, and t(16;16)(p13.1;q22)/*CBF*β-*MYH11* are the only favorable genetic abnormalities for which there are strong data based on large numbers of pediatric patients. The excellent outcome of patients with these alterations, referred to as core-binding factor leukemia, was established by investigators from the MRC in 1998[19] and recently confirmed by the MRC[20] and by the BFM Study Group,[21] who reported overall OS rates of 91% for children with t(8;21) and 92% for those with inv(16). Similarly, in the St Jude AML02 trial, patients with t(8;21) or inv(16) had a 3-year OS rate of 91% and a 3-year cumulative incidence of relapse of only 3%.[9] Although *KIT* mutations confer an inferior prognosis in adults with core-binding factor leukemia, they appear to have no prognostic significance in children with this subtype of AML.[22] Therefore, we classify all children with core-binding factor leukemia as having low-risk disease (Table 2; Figure 2), regardless of *KIT* mutations or other genetic abnormalities.

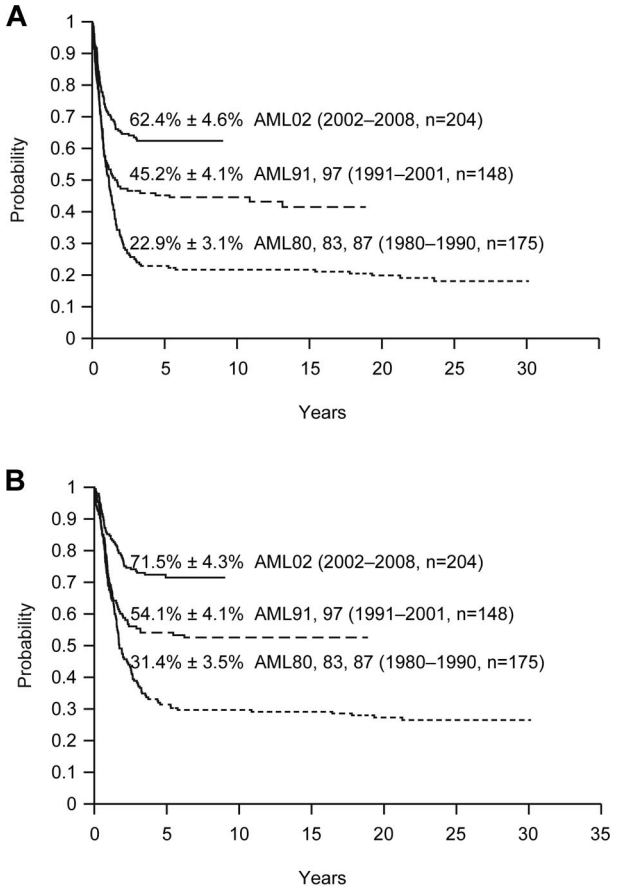

Figure 1. Survival of patients with de novo AML treated on St Jude trials. (A) EFS and (B) OS of patients with de novo AML (excluding those with Down syndrome or APL) treated on St Jude trials during the years indicated. AML02 was multi-institutional, whereas earlier studies were single institution.

How do I treat a patient with low-risk AML? Because he has an excellent chance of cure, this patient should be enrolled on a clinical trial and receive 4 courses of chemotherapy. Even if he has an HLA-matched sibling, he is not a candidate for allogeneic HSCT. Induction therapy should include 2 courses of therapy based on an anthracycline (daunorubicin or idarubicin) and a nucleoside analog (usually cytarabine), unless otherwise specified by the clinical trial.

It is probable that improvements in remission induction rates during the past 20 years have been the result of advances in supportive care and better use of existing chemotherapy rather than the introduction of new agents. For example, efforts to improve the "3 + 7" induction regimen (daunorubicin, 45 mg/m^2 per day for 3 days and cytarabine, 100 mg/m^2 per day for 7 days), which was developed in the 1980s, have included adding etoposide or thioguanine (ADE vs DAT, compared in the MRC AML10 trial[23]) and replacing daunorubicin with either idarubicin (ADE vs AIE, tested in the AML-BFM 93 and the Australian and New Zealand Children's Cancer Study Group AML1 and AML2 trials[24,25]) or mitoxantrone (MRC AML12 trial[12]). However, regimens that included etoposide or thioguanine induced similar complete remission rates, as did those that used daunorubicin, idarubicin, or mitoxantrone. Likewise, in our AML02 trial, complete remission, minimal residual disease (MRD) negativity, and survival rates were similar between patients who received high-dose cytarabine or low-dose cytarabine during the first course of induction therapy.[9] In addition, using G-CSF after induction therapy neither decreased the incidence of infection or treatment-related mortality nor improved survival in the AML-BFM 98 trial.[11,26]

What dose of anthracycline should this patient receive during induction therapy? In adults with AML, the use of high-dose

Table 2. Genetic abnormalities in pediatric AML

Feature	%	Clinical significance
Genetic features with proven prognostic implications		
t(8;21)(q22;q22)/*RUNX1-RUNX1T1*	15	Favorable prognosis
		Not candidates for HSCT
inv(16)(p13.1;q22)/*CBFβ-MYH11* t(16;16)(p13.1;q22)/*CBFβ-MYH11*	10	Favorable prognosis
		Not candidates for HSCT
FLT3-ITD	12	Poor prognosis, especially in cases with a high ratio of mutant to wild-type allele
		May benefit from HSCT or treatment with FLT3 inhibitors
−7	1	Poor prognosis
Genetic features with probable prognostic implications		
11q23; *MLL* rearrangements	20	
t(9;11)(p12;q23)/*MLL-AF9*	8	Favorable prognosis in some studies
t(1;11)(q21;q23)/*MLL-AF1q*	1	Favorable prognosis
t(6;11)(q27;q23)/*MLL-AF6*	1	Poor prognosis
t(10;11)(p12;q23)/*MLL-AF10*	1	Poor prognosis
Others	9	Intermediate prognosis
t(1;22)(p13;q13)/*RBM15-MKL1*	1	Only observed in megakaryoblastic leukemia
		Probably associated with favorable prognosis
NPM1 mutations	8	Seen in 20% of cases with normal karyotype
		Favorable prognosis, except in cases with *FLT3*-ITD
CEBPA mutations	5	Seen in 17% of cases with normal karyotype
		Favorable prognosis, except in cases with *FLT3*-ITD
		Favorable prognosis probably limited to cases with biallelic mutations
t(6;9)(p23;q34)/*DEK-NUP214*	1	Poor prognosis
t(8;16)(p11;p13)/*MYST3-CREBBP*	1	Poor prognosis
t(16;21)(q24;q22)/*RUNX1-CBFA2T3*	1	Poor prognosis
Genetic features with unknown prognostic implications		
WT1		
Mutation	10	Unknown
SNP rs16754	25	May be associated with favorable outcome
IDH1 and *IDH2*		
Mutation	4	Unknown
IDH1 SNP rs11554137	10	Unknown
RUNX1 mutation	Rare	Unknown
TET2 mutation	Rare	Unknown
DNMT3A mutation	Rare	Unknown

SNP indicates single nucleotide polymorphism.

daunorubicin during induction has been evaluated in several randomized trials and has produced mixed results.[27-29] Some studies showed improved remission and OS rates,[27] but others showed improved remission rates only.[28] Another study showed that high-dose daunorubicin was no better than standard-dose idarubicin in terms of remission, relapse, or survival rates.[29] Because of concerns about increased late cardiotoxicity, the use of high-dose anthracycline has not been evaluated in childhood AML.

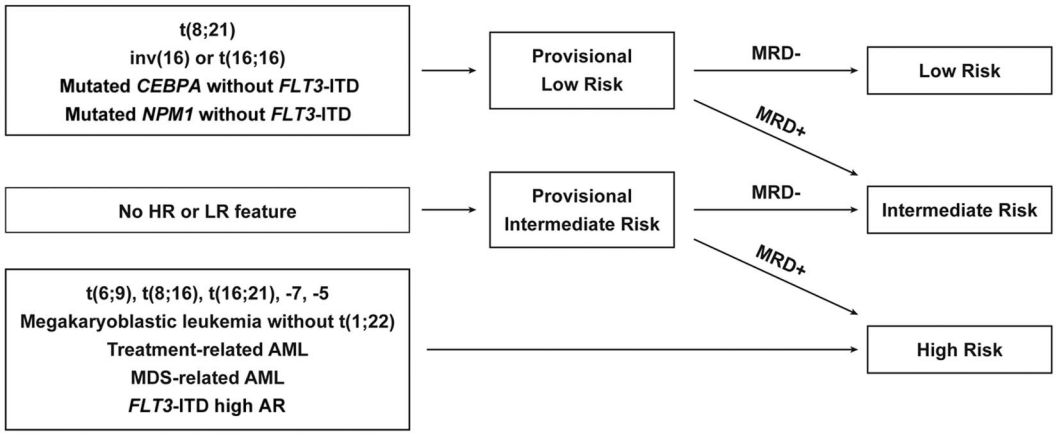

Figure 2. Risk classification of patients with AML. Risk classification scheme based on features at diagnosis and the presence of MRD. LR indicates low-risk; HR, high-risk; and AR, allelic ratio. Patients with t(8;21), inv(16), or t(16;16) are considered to be provisional LR regardless of other genetic alterations. Patients with *NPM1* mutations or biallelic *CEBPA* mutations are provisional LR, except in the presence of *FLT3*-ITD. Provisional LR patients are moved to the intermediate-risk group if they are MRD-positive after one course of induction therapy. HR patients include those with any of the features indicated in the box on the lower left, regardless of response to therapy. Patients who lack LR and HR features are provisionally classified as intermediate risk but are moved to the HR group if they have a poor response to therapy as assessed by MRD.

However, although the adult trials increased the total dose of daunorubicin to 270 mg/m^2 during the first course of induction, no additional anthracyclines were given.[27,28] Thus, the cumulative dose of cardiotoxic drugs was actually lower than that currently given on some pediatric AML trials, which administer 300 mg/m^2 daunorubicin and 36 to 48 mg/m^2 mitoxantrone. Nevertheless, until anthracycline dose intensification is tested in the pediatric population, we cannot recommend it for our patients. This patient should therefore receive standard-dose daunorubicin or idarubicin during each course of induction therapy.

Postremission therapy for this patient should consist of 2 courses of intensive chemotherapy. The results of clinical trials conducted during the 1980s and early 1990s showed that intensive postremission therapy improves outcome, whereas low-dose maintenance therapy may lower the OS rate. Trials performed during the past 15 years (Table 1) have been designed to determine the optimal duration of postremission therapy, the benefit of new agents, the value of MRD monitoring, and the role of HSCT. In the MRC AML12 trial, relapse rates (36%) and OS rates (74%) were the same for children whether randomly assigned to receive 4 or 5 courses of chemotherapy[12]; as a result, a total of 4 courses of chemotherapy is now the standard of care in the United States and is given on the current St Jude and COG trials. The use of idarubicin, fludarabine, and IL-2 in the Children's Cancer Group (CCG) 2961 trial[30] did not improve outcome nor did the addition of cyclosporine in the POG9421 study.[31] In adults with AML, the intensification of cytarabine from 12 g/m^2 to 36 g/m^2 did not improve survival.[32] Thus, we recommend 2 conventional courses of high-dose cytarabine-based postremission therapy for this patient. Additional agents may be included, as specified by the clinical trial. For example, the COG AAML1031 trial is testing, in a randomized fashion, the benefit of adding bortezomib to standard chemotherapy during the induction and intensification phases of therapy.

All contemporary pediatric trials include intrathecal chemotherapy (cytarabine, methotrexate, or both with hydrocortisone) to prevent CNS relapse, which occurs in less than 5% of patients. Although most investigators recommend intrathecal cytarabine alone, the optimal components of intrathecal therapy for children with AML remain unclear. On the basis of the results of AML02,[9] in which the CNS relapse rate decreased from greater than 9% to less than 1% after intrathecal cytarabine was replaced with intrathecal methotrexate, hydrocortisone, and cytarabine (ie, MHA), we recommend 4 monthly doses of intrathecal MHA for patients without CNS leukemia at the time of diagnosis and 8 doses (4 weekly doses followed by 4 monthly doses) for patients with CNS disease.

Supportive care is a crucial component of this patient's treatment because infectious complications remain a major cause of morbidity and mortality in children with AML.[33,34] Randomized, controlled trials of prophylactic antibiotics in adults with cancer have shown that prophylaxis decreases the incidence of fever, infection, hospitalization, and possibly death, leading the National Comprehensive Cancer Network and the Infectious Disease Society of America to recommend the use of fluoroquinolones in patients at high risk of infection.[33] However, data in children with cancer are limited to those from our retrospective study of prophylactic antibiotics in patients treated on the St Jude AML02 trial.[35] In this report, we demonstrated that the use of prophylactic cefepime or prophylactic vancomycin and ciprofloxacin dramatically reduced the odds of bacterial sepsis. Both regimens were administered by parents on an outpatient basis, and both regimens reduced the number of hospital days per course, episodes of febrile neutropenia, and healthcare charges. On the basis of these findings, I recommend prophylactic antibiotics for patient 1 and all other patients with AML. However, I think that a carefully designed and monitored prospective, randomized study should be performed: the COG has recently initiated one such study.

Children with AML are also at risk of invasive fungal infections, most commonly caused by Candida and Aspergillus species.[34] Again, randomized, controlled trials of antifungal prophylaxis in children with cancer are lacking, but the results of multiple studies conducted in adults with cancer support the use of these agents. I think that all children with AML should receive antifungal prophylaxis; voriconazole, posaconazole, micafungin, and caspofungin are all reasonable choices. I do not recommend fluconazole and itraconazole because they are less active against Aspergillus species or other molds. Because of drug interactions and variable pharmacokinetics, voriconazole and posaconazole should be held during courses of chemotherapy, and levels should be checked periodically. I try to maintain trough levels greater than 1 μg/mL for voriconazole and posaconazole.

Treatment summary

Patient 1 was enrolled on the St Jude AML08 protocol, in which patients are randomized to receive either clofarabine plus cytarabine or high-dose cytarabine, daunorubicin, and etoposide during induction I. He received clofarabine and cytarabine without complications and was discharged on day 6 of induction. A bone marrow aspirate that was performed on day 22 of induction revealed no evidence of residual leukemia (MRD $< 0.1\%$), and he subsequently received 3 additional courses of chemotherapy. After each course of therapy, he received prophylactic vancomycin, ciprofloxacin, and voriconazole, all administered by his parents in the outpatient setting. He has now completed all therapy and is doing well.

Patient 2

A 16-year-old girl with a history of leg pain, gingival bleeding, and decreased appetite was found to have a leukocyte count of 174 000/μL, consisting predominantly of blasts with monocytic features. Further workup revealed a normal karyotype (46,XX) and 2 mutations in the *CEBPA* gene.

The high leukocyte count in this patient is a medical emergency that requires immediate intervention. Although fewer than 20% of patients with AML have hyperleukocytosis, which is usually defined as leukocyte counts greater than 100 000/μL, these patients are at risk of pulmonary or renal compromise or intracranial hemorrhage secondary to leukostasis.[36] In patients with hyperleukocytosis or with symptoms of hyperviscosity, efforts should be made to reduce the leukemic burden as soon as feasible, even before initiating induction chemotherapy. Leukapheresis, exchange transfusion, hydroxyurea (10-20 mg/kg per day), and cytarabine (100-200 mg/m^2 per day) have all been used successfully. Although these strategies have not been compared and there is no clear advantage of one method over the others, we begin low-dose cytarabine in most cases because of the ease of administration. Despite measures to reduce leukocyte counts, patients with myelomonocytic or monoblastic leukemia are still at risk of severe cardiopulmonary and renal complications associated with rapid cell lysis and systemic inflammatory responses during the initiation of

chemotherapy with nucleoside analogs, including high-dose cytarabine and clofarabine.[37] Because steroids may prevent this inflammatory response, we typically administer methylprednisolone (0.5-1 mg/kg every 12 hours) during the first few days of therapy to patients who are at risk.

What is the appropriate risk classification for this patient? In adults with AML, mutations of *NPM1* and biallelic mutations of *CEBPA* are associated with a favorable prognosis, particularly in patients with normal karyotypes and wild-type *FLT3*.[38] Although these mutations are less common in childhood AML, their prognostic implication appears to be similar to that seen in adults. *NPM1* mutations have been detected in approximately 8% of children with AML and are associated with internal tandem duplication of *FLT3* (*FLT3*-ITD) and normal karyotype.[39-41] Children with *NPM1* mutations, normal karyotypes, wild-type *FLT3* appear to have an outcome similar to that of children with core-binding factor leukemia, with OS rates greater than 80%. Recently, investigators from the COG detected *CEBPA* mutations in 4.5% of children with AML, including 17% of those with normal karyotypes.[42] The presence of a *CEBPA* mutation was an independent favorable prognostic factor: those with mutations had an OS rate of nearly 80%. In contrast, a study of 185 patients treated on NOPHO trials found that *CEBPA* status did not add significant prognostic information.[41] Clearly, the evidence supporting the favorable implication of *CEBPA* and *NPM1* mutations is not as strong as that for t(8;21) and inv(16). Nevertheless, we suggest that patients who have normal karyotypes, wild-type *FLT3*, and mutations of *NPM1* or biallelic mutations of *CEBPA* be considered to have low-risk disease if they are treated in the context of a clinical trial (Table 2; Figure 2). On the current St Jude AML08 and COG AAML1031 trials, such patients are classified as having low-risk disease.

Treatment summary

Because of her elevated leukocyte count, patient 2 was initially treated with cytarabine at a dose of 100 mg/m^2 every 12 hours. Her leukocyte count gradually decreased from 174 000/μL to 65 000/μL after 5 doses of cytarabine, at which time induction with high-dose cytarabine, daunorubicin, and etoposide was started. She also received methylprednisolone, 1 mg/m^2 per day for 5 days. She had no complications related to tumor lysis or cytokine release and was discharged on day 10. MRD was negative at day 22 of induction I, and she began induction II at day 29. She completed all therapy 2 years ago, and her disease remains in first complete remission.

Patient 3

AML was diagnosed in an 18-year-old boy with a normal karyotype. His diagnostic sample was positive for *FLT3*-ITD.

There is clear evidence of an association between *FLT3*-ITD and a high risk of relapse in childhood AML.[41,43,44] In one of the first studies of this association, investigators from the CCG showed that *FLT3*-ITDs were present in 15 of 91 childhood AML cases and were associated with an 8-year EFS estimate of only 7%.[45] In this study, the results of multivariate analysis indicated that *FLT3*-ITD was the most important prognostic factor. Subsequently, Meshinchi et al confirmed the poor outcome of patients with *FLT3*-ITD in a definitive study of 630 patients treated on the CCG-2941 and 2961 studies.[43] Patients with *FLT3*-ITD had a significantly worse progressive-free survival rate than did patients with wild-type *FLT3* (31% vs 55%, $P < .001$). Importantly, Meshinchi et al identified having an *FLT3*-ITD allelic ratio greater than 0.4 as a powerful and independent negative prognostic factor; patients with this feature had a progressive-free survival of only 16%.[43] A preliminary subset analysis[43] as well as a subsequent report[46] suggest that the outcome of these patients can be improved by HSCT. Recently, investigators from the NOPHO confirmed the independent prognostic significance of *FLT3* status.[41] Interestingly, studies of paired diagnosis and relapse samples show that a subset of patients relapses without the mutation, suggesting that the *FLT3*-ITD is not present within the leukemia stem cell but only in a more mature subclone.[44] Because the poor outcome of patients with *FLT3*-ITD has been documented in several large studies, we classify these patients as having high-risk disease (Table 2; Figure 2).

Other genetic alterations associated with a poor outcome include monosomy 7, the effect of which was confirmed in children and adolescents with AML by the results of a large international collaborative study.[47] Less common genetic abnormalities that probably confer a poor prognosis include t(6;9)(p23;q34)/*DEK-NUP214*, t(8;16)(p11;p13)/*MYST3-CREBBP*, and t(16;21)(q24;q22)/*RUNX1-CBFA2T3*.[48-50] The t(6;9), for example, occurs in approximately 1% of children with AML and is associated with poor prognosis and a high frequency of *FLT3*-ITD.[48] However, because children with t(6;9)–positive AML and wild-type *FLT3* are rare, the independent prognostic significance of t(6;9) is unknown.

Treatment issues relevant to patient 3, whose disease is classified as being high risk and whose leukemia cells contain *FLT3*-ITD, include the use of tyrosine kinase inhibitors and the application of HSCT. Several genetic alterations in AML, including *FLT3*-ITD, are associated with constitutive activation of tyrosine kinases, aberrations in downstream signaling pathways, and a poor prognosis.[51,52] Therefore, tyrosine kinase inhibitors are a potentially attractive therapeutic approach: lestaurtinib, midostaurin, quizartinib, and sorafenib have been tested for this purpose in AML. We recently evaluated sorafenib, which inhibits multiple intracellular kinases, including FLT3, alone or in combination with cytarabine and clofarabine, in 12 children with refractory or relapsed leukemia.[53] In this study, 7 days of treatment with single-agent sorafenib decreased blast percentages in 10 of 11 patients with AML. After combination chemotherapy, 8 patients (5 *FLT3*-ITD and 3 wild-type *FLT*3) experienced either complete remission or complete remission with incomplete blood count recovery, and one (*FLT*3 wild-type AML) experienced partial remission. Sorafenib is currently being evaluated in newly diagnosed patients with AML and *FLT3*-ITD in the St Jude AML08 and the COG AAML1031 trials.

The use of sorafenib, in combination with conventional chemotherapy, may help to induce complete remission in this patient. Should this patient then undergo HSCT in first remission? Although most investigators now agree that patients with low-risk AML are not candidates for HSCT, the role of HSCT for other patients in first remission remains controversial.[54] In some studies, such as the MRC AML10 trial, HSCT reduced the risk of relapse but did not lead to an OS advantage.[55] In the CCG 2891 trial, however, survival probability was significantly higher for patients in the HSCT group than for those in the chemotherapy group.[56] In an analysis of 1373 children with AML treated with HSCT or chemotherapy on cooperative group trials, investigators from the COG showed that, compared with chemotherapy, HSCT was associated with a lower incidence of relapse (47% vs 28%; $P < .001$) but a higher incidence of treatment-related mortality (7% vs 16%; $P < .001$).[57] When stratified by risk group, only

patients with intermediate-risk AML benefited from HSCT; within this group, a large reduction in relapse rates and a slight increase in treatment-related mortality led to a superior OS rate (62% vs 51%; $P = .006$) in the HSCT group. The results of a meta-analysis of more than 6000 adults with AML show that HSCT provides a significant survival advantage for both intermediate- and poor-risk patients.[58]

The comprehensive review of clinical trials by Niewerth et al shows that the OS rates of patients who underwent HSCT and those who received chemotherapy are similar in most studies.[54] The authors show that, in general, a reduction in the risk of relapse is offset by increased treatment-related mortality, more severe late effects, and decreased salvage rates after HSCT. However, advances in the field of HSCT will probably lead to fewer short- and long-term side effects and greater benefit.[59,60]

Treatment summary

Patient 3 was enrolled on AML08 and randomly assigned to the high-dose cytarabine, daunorubicin, and etoposide arm. He tolerated induction well, but his MRD was 52% on day 22. Because of the presence of the *FLT3*-ITD, he then received low-dose cytarabine, daunorubicin, and etoposide, followed by sorafenib at a planned dose of 200 mg/m^2 given twice daily for 21 days. At approximately day 15 of sorafenib, a diffuse maculopapular, erythematous rash developed, and his sorafenib dose was decreased by 50%. A bone marrow aspirate collected after completion of sorafenib was hypocellular, with no morphologic evidence of leukemia and negative MRD. A repeat examination 2 weeks later showed signs of marrow recovery, with no detectable MRD, and he then underwent matched-sibling donor HSCT as specified in the AML08 protocol. His disease remained in remission for approximately 4 months, but he then had a hematologic relapse. Although the patient then had a transient response to sorafenib, his disease ultimately relapsed again, and he died of progressive leukemia.

Patient 4

An 8-year-old boy was scheduled to undergo tonsillectomy and adenoidectomy for a history of throat infections and sleep apnea. However, preoperative laboratory results revealed pancytopenia, and the patient was referred for examination. Analysis of the bone marrow showed acute myeloid leukemia with maturation. The karyotype was normal, and mutations of *FLT3*, *NPM1*, and *CEBPA* were not detected.

Risk classification of leukemias with cells that lack favorable or unfavorable genetic features is best determined by careful assessment of each patient's response to therapy. Indeed, we think that assessing response to therapy is essential for the treatment of all patients with AML, including those with favorable or unfavorable genetic features. In contrast to morphologic examinations, which can be imprecise and insensitive, MRD assays provide specific and sensitive measurements of low levels of leukemic cells. MRD detection methods rely on leukemia-specific features that distinguish residual leukemia cells from normal hematopoietic precursors. These methods include DNA-based PCR analysis of clonal antigen-receptor gene rearrangements, RNA-based PCR analysis of leukemia-specific gene fusions, and flow cytometric detection of aberrant immunophenotypes.[61] Because antigen-receptor gene rearrangements rarely occur in AML and RT-PCR detection of fusion transcripts can be used in less than 50% of cases, we rely primarily on the detection of abnormal phenotypes, which can be identified in more than 90% of cases.[62] However, it should be noted that phenotypic shifts or the emergence of a clone that was present only at low levels at the time of diagnosis may occasionally lead to false-negative results.[61] Flow-based MRD detection has been used successfully by investigators from the BFM study group,[63] the COG,[64,65] and St Jude.[9,66]

In one of the first studies reported, Sievers et al demonstrated that immunophenotypic evidence of leukemic blasts at the time of morphologic remission was predictive of more rapid relapse.[64] Subsequently, Sievers et al evaluated the effect of MRD among 252 patients treated on the CCG-2941 and 2961 trials.[65] At the end of induction therapy, 16% of patients had MRD (defined as $\geq 5\%$ blasts) and were 4.8 times more likely to experience relapse than were patients without MRD ($P < .0001$). Similarly, in the St Jude AML97 trial, children with MRD levels of at least 0.1% after one course of induction had a 2-year survival rate of only 33%; however, it was 72% for those with undetectable MRD levels.[66] In the St Jude AML02 trial,[9] the presence of MRD after the first course of induction was significantly associated with an adverse outcome: the 3-year cumulative incidence of relapse was only 16.9% for the 128 patients without MRD but 38.6% for the 74 with MRD ($P < .0001$).

On the basis of these results, we consider patients who have greater than 1% MRD after one course of therapy or greater than 0.1% after 2 courses of therapy to be at high risk of relapse.[9] Thus, we use a combination of conventional cytogenetic studies, molecular genetic studies, and response to therapy for comprehensive risk classification (Figure 2).

Treatment summary

Patient 4 was enrolled on AML08, and his disease was provisionally classified as being intermediate risk. Because his MRD was negative at day 22 of induction I, his final risk classification remained intermediate. After completing 4 courses of chemotherapy, he was eligible to participate in a phase 2 trial of haploidentical NK cells to evaluate their efficacy at reducing the risk of relapse. On the basis of our previous study,[67] in which we demonstrated that it is safe and feasible to administer mild immunosuppression followed by KIR-mismatched NK cells to patients with AML in remission, patient 4 received cyclophosphamide, fludarabine, and 4×10^7 purified NK cells/kg, which were obtained from his mother. His disease remains in remission 6 months after the completion of therapy.

Patient 5

A 10-year-old boy presented with a history of headaches, fatigue, weight loss, malaise, intermittent fevers, and leg pain. Initial evaluation revealed diffuse adenopathy and a peripheral blood smear that was notable for monocytic blasts. Examination of his bone marrow aspirate led to a preliminary diagnosis of acute monoblastic leukemia (AML M5a). Additional testing showed the presence of t(6;11)(q27;q23), splitting of the *MLL* gene, and expression of the *MLL-AF-6* fusion transcript, thereby confirming a diagnosis of AML with t(6;11)(q27;q23)/*AF6-MLL*.

AML with rearrangements of the *MLL* gene may be the most heterogeneous of all genetic subgroups.[68,69] Although our results suggested that t(9;11)(p12;q23) confers a favorable outcome, this finding has not been confirmed in other trials.[70] Among pediatric

patients treated on the MRC AML10 and AML12 trials, the outcome of patients with *MLL* gene rearrangements was intermediate (10-year OS, 62%), and the outcome of patients with t(9;11) was not different from that of patients with other 11q23 translocations.[20] However, the outcome of patients with t(9;11) and additional aberrations, as well as that of patients with *MLL* rearrangements other than t(9;11) and t(11;19), was unfavorable on the AML-BFM 98 trial.[21] A collaborative study of 756 children with 11q23/*MLL* rearrangements, which was designed to further clarify the importance of specific 11q23 translocations, showed that prognosis depended largely on the translocation partner.[68] Whereas the t(1;11)(q21;q23) was associated with an excellent outcome, the t(6;11)(q27;q23), t(10;11)(p12;q23), and t(10;11)(p11.2;q23) were associated with poor outcomes, suggesting that specific *MLL* subgroups should be classified separately.

Treatment summary

Because only limited data based on small numbers of patients are available, most cooperative group trials do not consider t(6;11) to be a high-risk feature. However, in the retrospective study just described, the 35 patients with t(6;11) had the worst outcome of any 11q23 subgroup, with a 5-year EFS rate of only 11%.[68] Patient 5 received clofarabine plus cytarabine and had 0.03% MRD, a level for which existing data are insufficient to determine the risk of relapse. After induction II (cytarabine, daunorubicin, etoposide), his MRD was 0.07%, below the 0.1% level that is associated with an increased risk of relapse.[9] Although this patient did not meet proven criteria for HSCT, the presence of the t(6;11), the persistence of detectable disease after 2 courses of therapy, and the availability of a matched sibling donor led us to recommend HSCT. The patient, therefore, received one course of consolidation therapy (mitoxantrone and cytarabine) followed by HSCT.

Patient 6

A 19-month-old girl with decreased appetite and activity, epistaxis, and pancytopenia was referred by her pediatrician for examination. Her blasts, which compromised approximately 20% of her bone marrow, were negative for cMPO but positive for CD33, CD13, CD133, CD7, CD117 (dim), CD61, CD41, and CD42B. The morphologic findings and immunophenotype were, therefore, consistent with those of acute megakaryoblastic leukemia. However, cytogenetic analysis revealed that 18 of 20 metaphases contained a del(13)(q12q14); 7 of the abnormal metaphases contained an additional del(11q), and 5 contained an additional +X, +4, +6, (inv)(12), +21, and a +22. Because of the complex karyotype, this case was classified as being AML with myelodysplasia-related changes according to the 2008 World Health Organization classification system.[1]

This patient has 2 features with potential high-risk implications: a complex karyotype and megakaryoblastic differentiation. In a combined analysis of children and adults treated on the MRC AML10 trial, Grimwade et al found that a complex karyotype (defined as 3 or more abnormalities) was associated with an inferior outcome and suggested that this feature be classified as high-risk on the subsequent AML12 trial.[19] Grimwade et al later confirmed this finding in a large study of adults treated on the MRC AML10, AML12, and AML15 trials.[71] But what is the effect of complex karyotypes in children with AML? An analysis of more than 700 children with AML treated on the MRC AML10 and AML12 trials showed that a complex karyotype was associated with a 10-year OS rate of only 46% but was not an independent predictor of adverse outcome.[20] However, among pediatric patients treated on the AML-BFM 98 trial, a complex karyotype was significantly associated with inferior EFS and OS rates and was, therefore, considered to be a high-risk feature.[21]

The prognostic implication of megakaryoblastic differentiation, similar to that of complex karyotypes, is unknown.[72-75] For example, the EFS rate of patients with megakaryoblastic leukemia but without Down syndrome who were treated at St Jude from 1985 to 1998 was only 14%, whereas that of patients treated on the AML-BFM 87, 93, and 98 trials was 35%.[72,76] Moreover, increased intensity of therapy probably led this subgroup of patients treated on AML-BFM 93 and 98 to have an EFS rate superior to that of patients treated on AML-BFM 87 (42% vs 12%).[76] Analysis of a study of 21 patients with megakaryoblastic leukemia who were treated in Japan showed even better outcomes, with a 10-year EFS rate of 57%.[75] In contrast, the 3-year EFS rate of patients with megakaryoblastic leukemia who lacked the t(1;22) was only 36% on our AML02 trial.[9]

The limited and, at times, conflicting data about the outcome of patients with complex karyotypes or megakaryoblastic leukemia make risk classification of patient 6's disease problematic. In addition, the number of patients with both features (ie, complex karyotype and megakaryoblastic differentiation) is too small to inform clinical decisions. Nevertheless, I consider this patient to have high-risk disease and acknowledge that this classification is not universally accepted.

Treatment summary

Patient 6 was assigned to the clofarabine/cytarabine arm of AML08 and experienced a poor response, with 35% MRD at day 22. After receiving induction II (ADE), she still had refractory disease, with 8% MRD, confirming our belief that she had high-risk leukemia. She then received mitoxantrone/cytarabine; MRD was no longer detected, and she subsequently underwent a matched, unrelated-donor HSCT.

Conclusions

One of the main limitations of previous clinical trials is that, with the exception of APL, AML was treated as a homogeneous disease. Because of the tremendous heterogeneity of AML, it is possible that an intervention or experimental agent that was beneficial for a subgroup of patients was not beneficial in the overall study population. Therefore, current and future trials must test new agents only in the subgroup of patients for whom they are designed. Alternatively, new approaches to immunotherapy may be applicable to a wider range of AML subtypes.[77] We think that new insights into the genetics of AML and the biology of the elusive AML stem cell, along with the development of targeted therapy and immunotherapy, will transform the future of childhood AML treatment. Moreover, knowledge uncovered by studying pediatric AML may provide insights to improve treatment and outcome of childhood malignancies in general. We hope that our success in treating AML will someday rival that achieved for acute lymphoblastic leukemia.[78]

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Authorship

Contribution: J.E.R. wrote the manuscript.

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