The Evolution of Allogeneic Stem Cell Transplant for Children and Adolescents With Acute Myeloid Leukemia

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Abstract: Survival rates in subsets of pediatric patients who have acute myeloid leukemia (AML) with favorable risk features are now greater than 90%. However, outcomes for patients with high-risk (HR) features remain unacceptably poor. As novel technologies for the identification of HR biomarkers and the detection of residual disease are developed, risk stratification and the application of allogeneic hematopoietic stem cell transplant (HSCT) are evolving. HSCT has been shown to benefit subpopulations of pediatric patients with AML, including those with HR cytogenetic translocations, genetic mutations, and/or residual disease after induction. Targeted therapies have shown promise for improving outcomes, and their integration into standard therapy and HSCT regimens is a critical area of interest. Also, expansion of the donor pool has led to the successful use of alternative donor sources for those patients without a matched sibling. However, transplant-related morbidity and mortality and late effects are major limiting factors. Reduced-intensity conditioning regimens have resulted in outcomes equivalent to those achieved with myeloablative regimens among patients in complete remission. The limitation of transplant-related morbidity and mortality through reduced-intensity conditioning and supportive care, and improved survival through optimal alloreactivity in combination with targeted therapy, are steps toward advancing outcomes for pediatric patients who have AML with HR features.

Introduction

Pediatric acute myeloid leukemia (AML) constitutes a minor portion of all pediatric cancers but remains a therapeutic challenge, with unacceptably low rates of event-free survival (EFS) and a high incidence of relapse when treated with traditional cytotoxic chemotherapy. The incidence of AML in children younger than 15 years is 7 per 1 million, and the disease accounts for one-fourth of cases of pediatric acute leukemia.[1,2] Recent clinical trials addressing the treatment of pediatric AML have achieved 5-year EFS rates ranging from 49% to 63%, with a relapse rate of 30%.[3,4] Importantly, outcomes in children with high-risk (HR) features remain significantly

inferior to outcomes in those with favorable-risk disease despite intensification of therapy, implementation of hematopoietic stem cell transplant (HSCT), and advances in supportive care.

As cytogenetic markers of HR disease are identified, the stratification of pediatric patients with AML has been updated, and changes have been incorporated into the World Health Organization (WHO) classification of AML. The prevalence of certain cytogenetic mutations seen predominantly in pediatric AML highlights the need for pediatric-specific risk stratification based on known prognostic markers.[2] With the advancement of technology for the detection of minimal residual disease (MRD), including multidimensional flow cytometry, cytogenetics, and next-generation sequencing, MRD positivity at critical points has been a major area of focus for the determination of prognostic significance. The discovery and application of clinically relevant HR features, the use of highly sensitive methods of MRD detection, and the increased sophistication of HSCT strategy—including expansion of the donor pool, human leukocyte antigen (HLA) matching, toxicity management, graft-versus-host disease (GVHD) prophylaxis, supportive care, and integration of targeted therapies—have led to an evolution of the role of HSCT for pediatric patients with AML. HSCT remains a critical area of interest in the effort to improve outcomes for pediatric patients with AML that has HR features. The focus of this article is identification of the subset of pediatric patients with AML who are likely to benefit from HSCT and current practices in HSCT for these patients, including timing, donor selection, conditioning regimens, and incorporation of novel agents.

Risk Stratification in Pediatric Acute Myeloid Leukemia

Clinically relevant cytogenetic markers and treatment response are strongly associated with survival in pediatric patients who have AML.[2] A major factor limiting the interpretation of outcomes in pediatric AML is the variability in the classification of HR patients, which leads to the inconsistent use of HSCT in clinical trials. The risk stratification that has been used for recent clinical trials in pediatric AML reflects the 2008 WHO classification, which is based on prognostically significant cytogenetic markers and disease response according to morphologic evaluation. The role of MRD positivity as detected by flow cytometry, cytogenetics, and real-time polymerase chain reaction (RT-PCR) is under ongoing investigation.

Core binding factor mutations t(8;21) and inv(16) are well-established markers of favorable outcome in response to conventional chemotherapy for pediatric AML patients. Each is associated with an overall survival (OS) rate of greater than 90%.[5,6] Although rare, biallelic mutations of *CEBPA* and *NPM1* with a normal karyotype, and wild-type *FLT3,* also are associated with a favorable prognosis in pediatric AML.[7-9] Other cytogenetic features that have been associated with favorable outcomes in pediatric AML include t(9;11)(p12;q23)/*MLL-AF9* and t(1;11)(q21;q23)/*MLL-AF1q*.[10]

The *FLT3* internal tandem duplication (*FLT3/ITD*) mutation, and to a greater extent *FLT3/ITD* with an allelic ratio greater than 0.4, is associated with a high risk for relapse and progression-free survival rates as low as 16%.[7,11] The presence of monosomy 7 in pediatric patients with AML has been associated with a complete response (CR) rate of 67% and OS rate of 30%. Although HSCT did not improve outcomes for these patients, 30% of those with persistent disease at the time of HSCT achieved sustained remission.[12] Other rare mutations that have been associated with an adverse prognosis in pediatric AML include monosomy 5 or del(5q), t(6;11)(q27;q23)/*MLL-AF6*, t(10;11)(p12;q23)/*MLL-AF10*, t(6;9)(p23q34)/*DEK-NUP214*, t(8;16)(p11;p13)/*MYST3-CREBBP*, and t(16;21)(q24;q22)/*RUNX1-CBFA2T3*.[10] *KIT, WT1, IDH1, TET2*, and *DNMT3A* mutations currently are under investigation in an ongoing Children's Oncology Group (COG) trial (AAML1031; A Phase III Randomized Trial for Patients With De Novo AML Using Bortezomib and Sorafenib for Patients With High Allelic Ratio FLT3/ITD).[13] The implications of overlap of each of these mutations with independently validated HR markers are of special interest.

The identification of clinically relevant genomic and proteomic markers is of particular importance for the application of targeted therapeutics. A recent international expert panel recommends the evaluation of known prognostically significant genetic markers, including *FLT3/ITD, WT1,* c-*KIT, CEBPA,* and *NPM1*, and of specific mixed lineage leukemia (*MLL*) abnormalities for this purpose.[2] The currently active AAML1031 trial from COG is evaluating the role of bortezomib (Velcade, Millennium/Takeda Oncology) in favorable-risk and HR groups, and of sorafenib (Nexavar, Bayer) for patients positive for *FLT3/ITD* with a high allelic ratio. The use of single-agent sorafenib has led to the successful induction of remission in adult patients with relapsed AML, and this agent can be safely administered to children and young adults in combination with standard chemotherapy.[14,15] In a small cohort of pediatric patients with refractory AML, single-agent sorafenib induced a sustained CR.[16] Sorafenib has a known association with cardiotoxicity, however, and has been shown to cause dose-limiting hematologic and nonhematologic toxicities in children.[17,18] Ongoing research focuses on whole-genome analysis in addition to proteomic profiling of pediatric AML for the purpose

of identifying prognostically significant and targetable biomarkers.[19]

Disease response, traditionally measured by morphology and more recently by flow cytometry, cytogenetics, and RT-PCR, is strongly associated with outcome. Among patients treated with standard chemotherapy, the 3-year relapse rate for those with residual disease after first induction (60%) was significantly higher than that seen in patients without residual disease (29%). Accordingly, relapse-free survival (RFS) was 30% in the residual disease group and 65% in those without residual disease. Similar results were found for patients with residual disease after second induction.[20] These findings have been confirmed in an assessment of leukemic blast count after first induction for pediatric patients with AML, in whom an increasing level of residual disease was correlated with decreased 4-year OS.[21] Patients with persistent disease after first induction who go on to achieve a CR after second induction remain at high risk for relapse, which demonstrates the vital significance of MRD.[22] Patients with greater than 1% MRD after first induction or greater than 0.1% MRD after second induction are considered to be at high risk for relapse.[23] Therefore, MRD can be used to identify HR patients among those without prognostically significant cytogenetic markers. Current methods for the detection of MRD include morphology, flow cytometry, cytogenetics, and RT-PCR.

Rationale for Allogeneic Hematopoietic Stem Cell Transplant During First Complete Remission

The disparity in long-term survival among risk groups justifies the application of alternative treatment strategies in patients with HR cytogenetic markers and MRD positivity after induction. In an attempt to improve survival, postconsolidation maintenance therapy with mercaptopurine and cytarabine was examined in pediatric patients with AML. At 5 years, disease-free survival (DFS) was not significantly increased, and OS was decreased in the maintenance therapy group (5-year OS, 58%) in comparison with the control group (5-year OS, 81%).[24] Alternatively, increased dose intensity and intensively timed therapy have been associated with improved outcome in most AML subtypes and are the current standard of care.[25] However, despite the value of current supportive care practices, these methods result in significant treatment-related toxicity.[2]

An evaluation of autologous HSCT for pediatric patients with AML demonstrated an OS equivalent to that achieved with chemotherapy alone. Although relapse rates were lower in the autologous HSCT group, the rate of treatment-related toxicity was significantly higher for these patients.[26] A clinical trial of pediatric patients with AML that compared chemotherapy, autologous HSCT, and allogeneic HSCT demonstrated superior long-term OS for allogeneic HSCT (60%) vs autologous HSCT (48%) and chemotherapy (53%) for patients in first complete response (CR1) after 2 induction cycles (Figure 1).[27] Therefore, autologous HSCT is not currently indicated for pediatric AML consolidation therapy.

The graft-versus-leukemia (GVL) effect of allogeneic HSCT has been proved. In a Children's Cancer Group study of pediatric patients with AML undergoing allogeneic HSCT, grade 1 or 2 GVHD was associated with improved RFS.[28] A pediatric clinical trial that attempted to improve outcomes through enhancement of the GVL effect with reduced dosing of alemtuzumab (Campath, Genzyme), early cessation of GVHD prophylaxis, post-transplant donor lymphocyte infusion, and treatment with interferon alfa demonstrated a 3-year RFS rate of 77%.[29] Although a significant reduction in the relapse rate of pediatric patients with AML after HSCT consolidation therapy in comparison with intensive chemotherapy alone has been shown, OS is limited owing to transplant-related morbidity. For this reason, there is no significant benefit to HSCT consolidation therapy vs nonmyeloablative chemotherapy alone for pediatric patients with favorable-risk AML who are in first remission. However, this approach has been shown to confer a survival benefit for patients with intermediate-risk and HR features.

A high level of variability in risk assignment among recent clinical trials evaluating the survival benefit of HSCT has led to conflicting results in each individual subset. In a COG study, 8-year DFS and OS were significantly improved for intermediate-risk patients without favorable or HR cytogenetic markers who received allogeneic HSCT in CR1 vs chemotherapy alone.[30] A retrospective review of pediatric patients with AML and HR cytogenetics found equivalent 5-year OS rates for those treated with chemotherapy (43%), matched family donor (MFD) HSCT (46%), or matched unrelated donor (MUD) HSCT (50%).[31] In this analysis, assignment to poor risk was limited to those with monosomy 7/del(7q), monosomy 5/del(5q), abnormalities of 3q, t(6;9)(p23;q34), or complex karyotype. The AML02 trial (Treatment of Patients With Newly Diagnosed Acute Myeloid Leukemia or Myelodysplasia) from St Jude Children's Research Hospital assessed MFD HSCT for patients without prognostically significant cytogenetic markers, and MFD or MUD HSCT for HR patients. This trial demonstrated that although outcomes in HR patients were not significantly different for those who underwent HSCT vs those who did not, patients with greater than 1% MRD after first induction in the HSCT

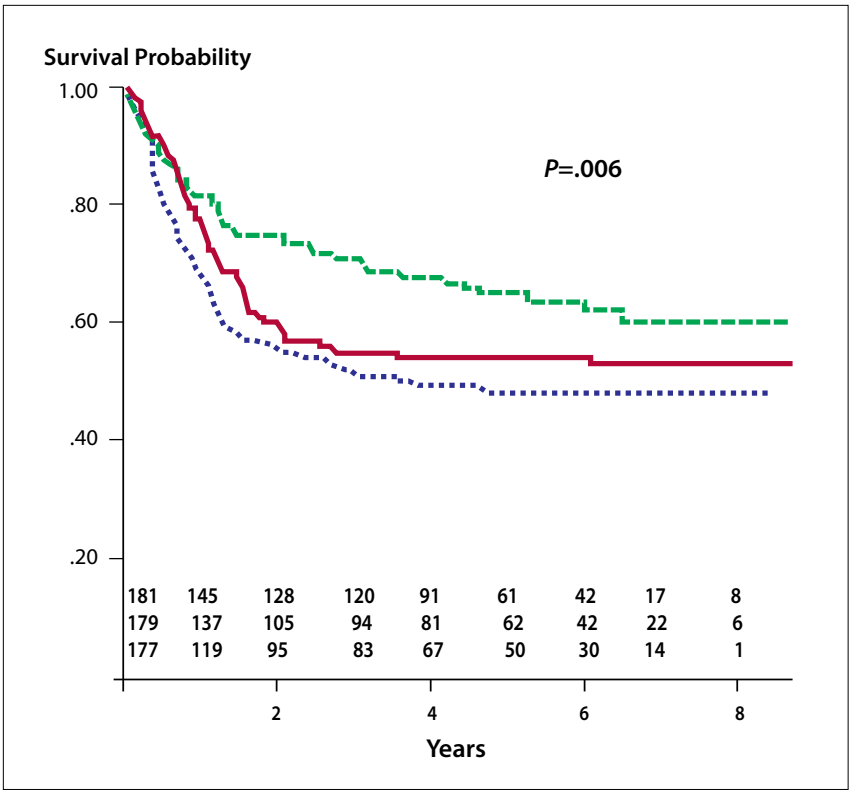

Figure 1. Graph shows actuarial survival from acute myeloid leukemia remission, comparing the 3 post-remission regimens in CCG 2891 of the Children's Cancer Group. The numbers are patients at risk at yearly intervals; the rows are in the same order as curves. The dashed green line indicates allogeneic bone marrow transplant, the solid red line indicates intensive non–marrow-ablative chemotherapy, and the dotted blue line indicates autologous bone marrow transplant.

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group had improved OS (43%) compared with those in the chemotherapy-alone group (23%).[23] The Nordic Society for Pediatric Hematology and Oncology (NOPHO)-AML 2004 clinical trial for pediatric AML (Response-Guided Induction Therapy in Pediatric Acute Myeloid Leukemia With Excellent Remission Rate) risk-stratified patients by response after induction and demonstrated that patients with more than 15% blasts who were ultimately treated with HSCT had improved EFS and OS vs patients with less than 5% blasts or with 5% to 15% blasts who were treated with chemotherapy alone.[22] This evidence highlights the importance of cytogenetic and MRD-based risk stratification and enrollment in standardized clinical trials to further define the role of HSCT for pediatric patients with AML in CR1.

Among patients with *FLT3/ITD* mutations, the use of HSCT significantly improves the OS rate and decreased the relapse rate. OS after HSCT for patients with *FLT3/ITD* mutations nears that of patients with wild-type *FLT3*. This advantage demonstrates a clear indication for the use of HSCT in patients with *FLT3/ITD* mutations, particularly those with an allelic ratio higher than 0.4.[32] Outcomes for pediatric myelodysplastic syndrome (MDS) and AML associated with monosomy 7 are poor. However, a 2-year EFS of 69% has been achieved with the use of HSCT.[33]

High levels of MRD at the time of HSCT have been associated with poor outcomes in adult patients.[34] Among pediatric patients with AML, 5-year OS for those with MRD positivity (0.1%-5%) at the time of HSCT was 67%, compared with 80% for MRD-negative patients. MRD positivity, detected by multidimensional flow cytometry at the time of HSCT, is associated with a significantly increased relapse rate and decreased OS in comparison with MRD negativity (Figure 2).[35]

Donor Source, HLA Matching, and Alloreactivity

Over time, donor sources have expanded to include MUDs and umbilical cord blood (UCB) in addition to MFDs. Genomic typing and advances in supportive care have improved outcomes in MUD HSCT recipients to match those in MFD HSCT recipients, so that having a matched sibling is no longer a criterion for HSCT for pediatric patients in CR1 who have AML with HR markers, as it has been historically.[36] In a recent clinical trial, 2-year leukemia-free survival rates in pediatric patients with AML in CR1, CR2, and no CR who received UCB donor HSCT with 1 or 2 HLA mismatches were 59%, 50%, and 21%, respectively. Although these results do not represent an improvement in outcome over MFD HSCT or MUD HSCT, UCB donor HSCT is an option for pediatric patients with HR AML and no alternative

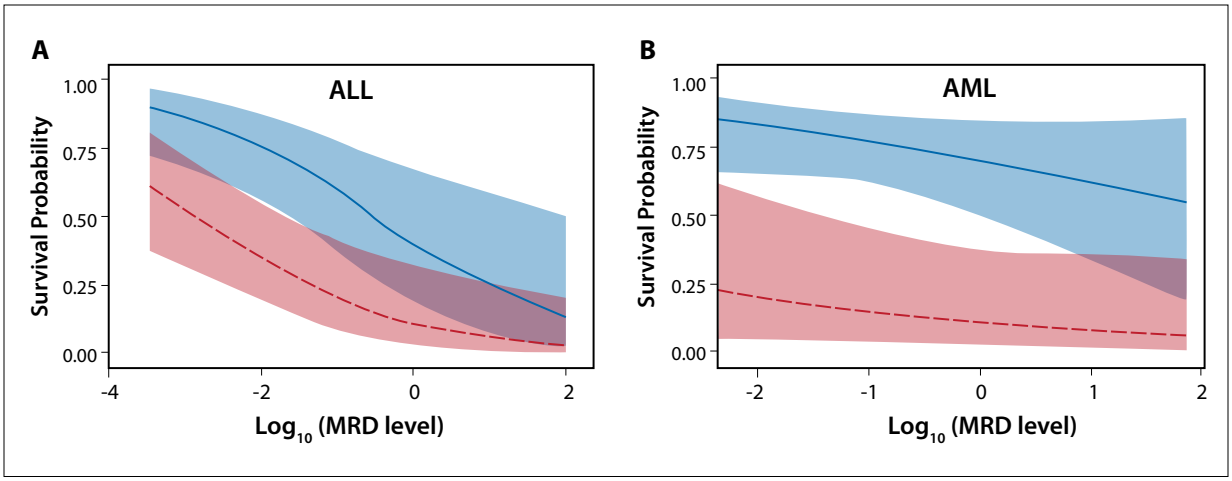

Figure 2. Graphs show probability of survival according to level of MRD, stratified by leukemia type and treatment era. Probability of survival during the observation period in patients with ALL (A) or AML (B) after HSCT in the early era (red) or the recent era cohort (blue). The confidence bands represent 95% confidence interval limits. Both patients with ALL and those with AML treated in the recent era fared significantly better than did those in the early era (P=.005 and P=.007, respectively). The effect of MRD level on survival was significant for ALL (P=.002) but not for AML (P=.18).

ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; HSCT, hematopoietic stem cell transplant; MRD, minimal residual disease.

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donor source. Of note, the incidence of GVHD in this population was 35%, a reduction from rates reported with MFD HSCT or MUD HSCT.[37]

A haploidentical or killer immunoglobulin receptor (KIR)-incompatible donor source confers the advantage of alloreactivity—and ultimately, GVL effect. The role of natural killer (NK) cells in the elimination of AML has been demonstrated in the setting of allogeneic HSCT. The mechanism of this effect is based on NK cell KIR phenotype. KIR-mismatched allogeneic HSCT creates an opportunity for donor NK cell alloreactivity, which contributes to a GVL effect.[38] Certain *KIR2DL1* alleles are known to be associated with stronger cytotoxicity signaling than others. A retrospective analysis of pediatric patients with leukemia undergoing HSCT revealed that patients receiving a *KIR2DL1-R245* graft with an HLA-C receptor ligand mismatch had better survival and a lower risk for progression than did patients receiving *KIR2DL1-C245* homozygous grafts.[39] A recent study evaluating haploidentical HSCT with myeloablative conditioning (MAC) for pediatric AML demonstrated 5-year leukemia-free survival rates of 82%, 59%, and 42% for patients in CR1, in CR2, and with persistent disease at the time of HSCT, respectively. In this study, the 5-year leukemia-free survival for patients with MFDs was similar (at 71%), indicating that this approach is safe in children and may confer benefit through alloreactivity. This approach also widens the donor pool, providing ethnically diverse populations who lack representation in the international donor registries the opportunity for treatment with HSCT when indicated (Table).[40]

Conditioning Regimens for Hematopoietic Stem Cell Transplant in Pediatric Acute Myeloid Leukemia

An international study comparing reduced-intensity conditioning vs MAC HSCT in pediatric patients with AML showed no significant difference in relapse rates or 5-year EFS rates.[41] However, any difference between the incidence of adverse late effects in these cohorts remains to be seen. Outcomes are not significantly different for patients undergoing nonmyeloablative HSCT from an MFD or MUD.[42] The reduced-intensity approach may offer benefit to pediatric patients with AML who are ineligible for myeloablative therapy owing to heavy pretreatment and/or pretransplant comorbidities.

Total-body irradiation (TBI) for pretransplant conditioning in pediatric patients with AML in first remission is associated with an increased incidence of secondary malignancy and adverse late effects in comparison with busulfan/cyclophosphamide alone.[43] Accordingly, outcomes are better with busulfan-based regimens than with TBI-based regimens regardless of donor source,[44] so that chemotherapy-based conditioning regimens are preferred to TBI.[45,46] In the setting of Fanconi anemia–associated

Table. Outcomes of Recent Clinical Trials in Pediatric Patients With Acute Myeloid Leukemia

	Indication	Tome to Follow-up, y	Allogeneic HSCT MAC/RTC	Chemo-therapy	Autologous HSCT	N	Reference
CR1	CR1	3	NR	36% EFS	38% EFS	649	Ravindranath et al,[81] 1996
	CR1	8	60% OS	53% OS	48% OS	652	Woods et al,[27] 2001
	HR CR1	5	46%/50% OS MFD/ MUD	43% OS	NR	233	Kelly et al,[31] 2014
	HR CR1	3	57.5% OS	50.5% OS	23% OS	206	Rubnitz et al,[23] 2010
	<5%/5% to 15%/>15% blasts after induction CR1	3	72.2%/49.8%/81% OS	NA	NA	151	Abrahamsson et al,[22] 2011
	CR1	5	75%	NA	NA	11	Zahler et al,[50] 2016
CR2	CR2	5	62% OS			146	Abrahamsson et al,[55] 2007
	CR2	5	62% OS			109	Beier et al,[58] 2013
	CR2	Long-term	50% OS	24% OS		113	Goemans et al,[56] 2008
	CR2	5	47% OS	35% OS	50% OS	153	Sander et al,[54] 2010
CR2 relapse IF	CR2 or RR	5	45% LFS 20% LFS 12% LFS			268	Zahler et al,[50] 2016
	IF, RR, CR3	1	50% OS/EFS			12	Satwani et al,[59] 2012

CR1, first complete response ; CR2, second complete response; CR3, third complete response; EFS, event-free survival; HR, high-risk; IF, primary induction failure; LFS, leukemia-free survival; MAC, myeloablative conditioning; MFD, matched family donor; MUD, matched unrelated donor; NA, not applicable; NR, not reported; OS, overall survival; RR, refractory relapse; RTC, reduced toxicity conditioning.

pediatric AML, cross-linking chemotherapy and high-dose anthracyclines should be avoided to reduce the risk for secondary malignancy.[47]

The role of targeted therapy in combination with HSCT is under ongoing investigation. The benefits of this approach must be weighed against the potential for treatment-related mortality, and the ideal timing for such therapies is yet to be determined. Gemtuzumab ozogamicin (GO; Mylotarg, Pfizer) has shown promise in pediatric clinical trials. NOPHO-AML 2004, which compared GO as post-consolidation therapy with no further treatment, found no decrease in relapse rates or increase in OS in the GO group.[48] However, a recent COG trial comparing the use of standard 5-course chemotherapy alone vs standard chemotherapy plus GO showed a significant reduction in relapse risk and improvement in EFS for patients with a high level of CD33 expression in all risk groups.[49] In addition, MRD in pediatric patients with AML treated with GO was reduced in comparison with MRD in those treated with chemotherapy alone, suggesting a critical role for this targeted therapy in the peritransplant period. A recent phase 1 clinical trial that evaluated the safety and efficacy of GO before allogeneic HSCT with varying donor sources, in which reduced-intensity conditioning

was used, demonstrated a 61% 5-year EFS for all patients and a 78% EFS for those in CR1 at the time of HSCT.[50] These results suggest that GO plays a role in the clearance of residual MRD after HSCT. A phase 2 clinical trial based on these data is currently active.

In a retrospective review of pediatric patients with *FLT3/ITD*-mutated AML, in which the patients received sorafenib prophylactically or at the time of relapse after HSCT, all of 15 patients achieved a CR at a median of 48 months, and the incidence of GVHD was not increased.[51] The COG AAML1031 study is evaluating the safety and efficacy of combination chemotherapy plus sorafenib followed by HSCT and a post-HSCT sorafenib maintenance phase for *FLT3/ITD*-mutated patients who have a high allelic ratio. As techniques in proteomic and genomic profiling advance and targetable pathways are identified, the development of a personalized approach to HSCT may be warranted. Panobinostat (Farydak, Novartis; NCT01451268), decitabine (NCT01277484), and azacitidine (NCT01995578) are currently under clinical investigation as maintenance therapy for adult patients with AML after HSCT.

The role of early withdrawal of immunosuppression and donor lymphocyte infusion after HSCT as relapse prophylaxis in pediatric patients with leukemia, including AML with mixed chimerism, was examined in limited numbers. A long-term CR was achieved in 7 of 12 patients; however, induction of GVHD and treatment-related mortality was a significant consideration.[52] NK cell infusion has not been shown to increase the incidence of GVHD. Evaluation of a small cohort of adult patients with relapsed and refractory AML treated with third-party alloreactive haploidentical NK cell infusion before allogeneic HSCT demonstrated safe administration without outcome benefit, possibly owing to the small sample size. A phase 1/2 clinical trial based on these data is currently ongoing.[53]

Clinical advances in supportive care, prevention and early treatment of microbial infections, GVHD prophylaxis, veno-occlusive disease prophylaxis, and reduced-intensity conditioning all contribute to the reduction of transplant-related morbidity and mortality. As toxic outcomes of HSCT decrease and HR features are more precisely defined, HSCT for pediatric patients with AML in CR1 may become increasingly advantageous.

The Role of Allogeneic Hematopoietic Stem Cell Transplant in Relapsed and Refractory Disease

Between 3% and 19% of pediatric patients with AML do not achieve CR after induction therapy, and 30% to 40% of all pediatric patients with AML relapse.[4] Although more than 60% of patients with relapsed disease achieve a second CR (CR2), only 29% achieve long-term remission. Key factors that are independently associated with survival include age younger than 10 years, favorable cytogenetics, duration of CR1, and no HSCT in CR1.[54] OS for patients with relapse more than 12 months after diagnosis (48%) is superior to that for patients with relapse during the first 12 months (21%).[55] For those achieving CR2 with cytarabine, etoposide, and an anthracycline, OS is significantly improved after HSCT (50%) vs chemotherapy alone (24%).[56] An international study in which fludarabine, cytarabine, and granulocyte-colony stimulating factor (FLAG) or FLAG plus liposomal daunorubicin followed by HSCT was used for those achieving a CR reported a 64% CR rate and 38% OS.[57] Others have reported OS of 62% for patients in CR2 before HSCT.[55,58] A phase 1 study of pediatric patients with refractory or relapsed AML undergoing allogeneic HSCT with myeloablative conditioning consisting of GO, busulfan, and cyclophosphamide reported 1-year EFS and OS rates of 50%.[59] There is a consensus that HSCT should be offered to all children with relapsed AML in CR2.[2] In this setting, MFD transplant has not been associated with a long-term survival benefit vs alternative donor sources.[60] Outcomes for patients with persistent disease before HSCT are poor. OS for patients not achieving CR2 before HSCT has been reported to be as low as 3%.[54] Others have reported a 5-year leukemia-free survival rate for pediatric patients not achieving CR2 at the time of HSCT of 20%.[61]

Given the proven GVL effect of HSCT for pediatric AML, donor lymphocyte infusion for patients with relapse after MFD or MUD HSCT is a consideration but has a limited effect. Donor lymphocyte infusion as sole therapy for pediatric patients with hematologic malignancy relapsing after HSCT rarely induces remission and has not shown benefit over no donor lymphocyte infusion. However, donor lymphocyte infusion in combination with chemotherapy has resulted in 1-year DFS of 30%.[62] Single-agent sorafenib and other novel targeted therapies, including antibody- and cell-based therapy, for patients with *FLT3/ITD*-mutated AML are under investigation for this purpose in the adult population.

AML cell surface antigens CD33 and CD123 have been investigated as immunotherapeutic targets. Chimeric antigen receptor (CAR)–modified cytokine-induced killer cells induced significant cytotoxicity against AML blasts and normal hematopoietic stem cells in preclinical models, although regenerative capacity was preserved.[63,64] Virally transduced anti-CD123 CAR T cells have also shown efficacy in reducing CD123-positive AML blast and leukemia stem cell burden with a low hematopoietic toxicity profile, and they are currently under investigation in an open clinical trial (NCT02159495).[65] Several

novel targeted antibodies are under clinical investigation in the adult AML population, including an anti-CD33 pyrrolobenzodiazepine-conjugated monoclonal antibody that has shown increased apoptosis vs GO in preclinical models (NCT02326584, NCT01902329). Bispecific T-cell engager CD33 x CD3 (AMG 330), bispecific killer cell engager CD33 x CD16, and trispecific engager CD33 x CD16 x CD123 have shown evidence of successful clearance of AML blasts in preclinical models and are candidates for clinical investigation.[66-70]

Central Nervous System Therapy

Patients with central nervous system (CNS) involvement at the time of diagnosis are at increased risk for isolated CNS relapse and require intensified CNS-directed therapy.[71] An evaluation of adult patients who had AML with CNS disease at diagnosis and underwent HSCT with intrathecal therapy (ITT) vs cranial or craniospinal irradiation boost demonstrated a significant advantage in 5-year relapse-free survival and OS in the radiation therapy group. However, a retrospective study of adults with CNS disease at diagnosis demonstrated successful clearance with or without radiation therapy, and no association between CNS disease at diagnosis and outcome after HSCT.[72] The role of post-HSCT ITT and pre-HSCT irradiation boost in pediatric patients with acute lymphoblastic leukemia or AML has been evaluated. No significant difference in outcomes was found between those who received intrathecal chemotherapy and those who received no ITT. In addition, there was no significant difference in outcomes between patients who underwent pre-HSCT CNS irradiation boost and those who underwent ITT alone.[73]

Subgroups

Several reports have distinguished acute erythroid leukemia (FAB M6 in the French-American-British classification system) and acute megakaryoblastic leukemia (AMKL; FAB M7) from FAB M0-M5. A comparison of pediatric AML subtypes and MDS in patients without constitutional trisomy 21 (T21) found that those with MDS, M6 AML, or M7 AML had a higher frequency of monosomy 7 and –7q and EFS and OS significantly inferior to that of other AML subtypes.[74] AMKL is the most common subtype of AML in children with T21 and accounts for up to 15% of cases of pediatric AML. In a single-center study, the remission induction rate for pediatric AMKL with or without T21 was reported at 60%, with a 48% relapse rate. The 2-year EFS for patients who had M7 AML without T21 (14%) was significantly inferior to the 2-year EFS for those who had M7 AML with T21 (83%). The 2-year EFS for patients without T21 was significantly improved after allogeneic HSCT, and those undergoing allogeneic HSCT in CR had a significantly higher 2-year EFS (46%) than did those not in CR at the time of HSCT (0%). Successful induction of remission was the prominent prognostic feature. The 5-year EFS was significantly lower for M7 AML without T21 than for other subtypes.[75] These data clearly indicate that allogeneic HSCT in CR1 confers optimal survival for pediatric patients who have M7 AML without T21.

Acute promyelocytic leukemia, Down syndrome–associated AML, therapy-related secondary AML, and familial cancer syndrome–associated AML should be considered separately owing to their vastly different biology and sensitivity to therapy. Because of the excellent outcomes achieved in pediatric acute promyelocytic leukemia when all-trans retinoic acid is give in combination with cytotoxic chemotherapy, HSCT should be considered only for patients with relapse. In a comparison of allogeneic vs autologous HSCT for the treatment of relapsed or refractory childhood acute promyelocytic leukemia, 5-year EFS and OS were not significantly different. Although lower rates of relapse were observed in the allogeneic HSCT group, treatment-related mortality was significantly higher.[76] Treatment-related mortality and morbidity are also a barrier to HSCT for patients with Down syndrome who have refractory or relapsed AML. The 3-year OS for pediatric patients who have Down syndrome–associated AML has been reported at 19%, and treatment-related mortality and relapse risk are significantly higher than in pediatric patients without Down syndrome who undergo HSCT for AML. Pediatric patients with chemotherapy-related and radiation therapy–related secondary AML can be salvaged. The 2-year OS for therapy-related secondary AML in pediatric patients undergoing HSCT while in CR after reinduction chemotherapy has been reported at 40%.[77] There are few reported cases of familial cancer syndrome–associated pediatric AML; therefore, the role of HSCT in this population is unclear.

Late Effects

Late toxicity associated with HSCT for pediatric malignancies is an important consideration. A review of pediatric patients receiving HSCT for hematologic malignancy demonstrated a significantly increased relative risk for severe or life-threatening infections, 2 or more chronic health conditions, functional impairment, and activity limitation in comparison with matched sibling controls. Recipients of MUD HSCT were at greatest risk.[78] The incidence of late adverse effects is significantly higher among pediatric patients with AML who receive HSCT (72%) than among those who receive chemotherapy

alone (31%).[79] Younger age and cranial irradiation or TBI are independent risk factors for the development of late effects.[80] The risk for late effects and their impact on long-term survival, chronic comorbidities, and quality of life must be weighed against the significant risk for relapse in pediatric patients who have AML with HR features. All patients who have undergone HSCT in childhood are encouraged to participate when possible in organized survivorship programs developed for the early detection and prevention of late transplant-related morbidities, and for the improvement of long-term outcomes among survivors of pediatric AML.

Conclusions

The interpretation of outcomes for pediatric patients with AML based on previous clinical trials is limited by variations in the definition of high risk and the indications for allogeneic HSCT. The identification of genetic markers of HR disease and the detection of MRD are critical to risk stratification in pediatric AML and the identification of patients who might benefit from intensified therapy. The emergence of additional proteomic and genomic markers is expected, and these must be integrated into risk stratification and the treatment strategy to further define the HR population.

It is clear that patients with favorable features, such as core-binding factor mutations, biallelic *CEBPA* mutations, and *NPM1* mutations without other adverse factors are unlikely to benefit from allogeneic HSCT. However, allogeneic HSCT has resulted in improved outcomes for specific subsets of pediatric patients with AML—including those who have *ITD/FLT3*-mutated disease with a high allelic ratio, monosomy 7, or AMKL and those who have MRD positivity at the end of induction—and may be of benefit for patients with newly identified HR features.

Outcomes for patients with refractory and relapsed disease are improved by allogeneic HSCT. Clearance of residual disease, however, is essential to long-term survival. The objective of a cytogenetic marker and an MRD-driven, pediatric-specific risk stratification system is to limit toxicity for patients without a high risk for relapse while intensifying therapy for those known to be at increased risk for relapse. The treatment strategy should be focused on MRD clearance and eradication of the preleukemic stem cell clone, which may be achieved through the GVL effect of allogeneic HSCT in combination with targeted therapies.

Advances in supportive care; expansion of the donor pool to include MFDs, MUDs, UCB, and haploidentical donors; high-resolution HLA typing; graft manipulation; and GVHD prophylaxis have all contributed to improved HSCT strategy. In addition, reductions in toxicity and conditioning and the limited use of TBI may result in a decrease in long-term toxicity. Ultimately, the identification of patients with known adverse features and the benefits of alloreactivity in combination with targeted therapy may reduce relapse rates and close the gap in OS for those pediatric patients with HR AML disease.

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