Review

Indications for transplantation in childhood acute leukemia and the impact of minimal residual disease on relapse: a review

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Practice Points

- Hematopoietic cell transplantation (HCT) in first remission continues to be recommended for select pediatric leukemia subgroups, such as severe hypodiploidy and primary induction failure in B-precursor acute lymphoblastic leukemia (ALL) and high-risk cytogenetic acute myeloid leukemia (AML).
- Patients with B-precursor ALL who relapse early in the bone marrow have greater survival following HCT compared with chemotherapy alone.
- Minimal residual disease (MRD) identified prior to HCT is the strongest predictor of post-HCT relapse in both ALL and AML.
- Whether eliminating pre-HCT MRD in children with ALL or AML will improve their risk for post-HCT relapse and overall survival is presently unknown.
- Clinical trials testing the feasibility and efficacy of novel therapies or combinations that can successfully eliminate pre-HCT MRD and 'bridge' the patient to HCT are needed.

SUMMARY: Allogeneic hematopoietic cell transplantation (HCT) for pediatric acute leukemia continues to offer a cure for some patients with high-risk or relapsed disease. Presently, HCT in first remission is recommended only for patients with predicted leukemia-free survival <50%. Despite recent advances, relapse remains the biggest hurdle in HCT. Minimal residual disease (MRD) identified pre-HCT is one of the strongest predictors for relapse for both acute lymphoblastic leukemia and acute myeloid leukemia. Therefore, novel approaches are needed to eliminate pre-HCT MRD, safely bridging patients to HCT and diminishing relapse. This review highlights the current outcomes for HCT in pediatric acute leukemia, describing the current indications for HCT as well as the significant impact pre-HCT MRD has on relapse for these patients.

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Acute leukemia (acute lymphoblastic leukemia [ALL] and acute myeloid leukemia [AML]) is the most common cancer diagnosis in children and young adults (diagnosed under the age of 20). More than 3600 cases were reported in 2012 according to the National Cancer Institute's (NCI) Surveillance Epidemiology and End Results data. Although 90% of children with ALL and 60–70% with AML are currently cured, 15 and 40–50%, respectively, will relapse [1,2]. Despite aggressive attempts with salvage chemotherapy, the majority of children with relapsed leukemia die, making relapsed leukemia the second leading cause of death in children from a disease [3]. Allogeneic hematopoietic cell transplantation (HCT) using a HLA-matched related [4–6] or unrelated donor [7,8] most often provides the best chance of cure for children with relapsed acute leukemia.

The greatest hurdle in improving survival in children receiving a HCT for ALL or AML, is overcoming post-HCT relapse [9–11]. The identification of minimal residual disease (MRD) immediately prior to HCT may be one of the single greatest prognostic indicators for relapse [12–20]. Whether the presence of pre-HCT MRD is evidence of patient undertreatment or rather a biomarker of more aggressive disease is unknown. Measures incorporated into post-HCT therapy to decrease relapse (e.g., donor lymphocyte infusion [21,22], tyrosine kinase inhibitors [23–26] and epigenetic modifying agents [27–30]) have only partially been successful and therefore interventions to eliminate MRD prior to HCT are needed.

This review will summarize the current outcomes for children with acute leukemia (ALL and AML) who undergo HCT either in first complete remission (CR1) due to very-high-risk features at diagnosis or after relapse has occurred. We will describe patients who may benefit from HCT in CR1, to reduce their chance for later relapse, as well as the critical role pre-HCT MRD plays in predicting relapse for pediatric patients with acute leukemia proceeding to HCT.

Transplantation for childhood ALL

■ Indications for HCT in first remission

As risk stratification and therapy intensification continue to improve for children with ALL, the role of HCT in CR1 has diminished. Currently, only a few disease subgroups are considered to benefit from transplantation in CR1. Patients with severe hypodiploidy (having <44 chromosomes in the leukemia blasts at diagnosis and/or a DNA index <0.81) and those with primary induction failure (PIF; having >25% blasts in the bone marrow after completing induction therapy) are two subgroups where HCT in CR1 is currently recommended. For patients with severe hypodiploidy, there are limited publications to make a strong recommendation but expert opinion, based on the American Society of Bone Marrow Transplant position statement, would support HCT in first remission [31]. Nachman *et al.* reported the largest study to date investigating the impact of hypodiploidy on prognosis in pediatric ALL [32]. Data were collected from 10 different national ALL study groups and/or institutions. In this analysis of 130 children with hypodiploidy ALL primarily treated with chemotherapy, 8-year event-free survival (EFS) and overall survival (OS) were 38.5 ± 4.4% and 49.8 ± 4.2%, respectively. Patients with severe hypodiploidy defined as <44 chromosomes had an EFS of 30.1% compared with 52.2% for patients having 44 chromosomes (p = 0.01). Whether HCT in CR1 will significantly improve outcomes for children with severe hypodiploidy compared with chemotherapy alone is not clear and this is currently being studied through the Center for International Blood and Marrow Transplant Research (CIBMTR) and Children's Oncology Group (COG).

Another subgroup of patients with ALL that may benefit from HCT in CR1 are those with PIF defined as having >25% blasts in the postinduction bone marrow [31]. Schrappe *et al.* reported outcomes of 705 children with Philadelphia chromosome-negative ALL and PIF from 14 pediatric study groups where the 10-year OS was 35 ± 5% for those treated with chemotherapy alone compared with 59 ± 12% for patients receiving HLA-matched related donor HCT (p = 0.11) [33]. As future improvements continue to be made with upfront chemotherapy for these very-high-risk patients, the role of HCT in CR1 for PIF patients will need to be revisited.

Current data does not support HCT in CR1 for Philadelphia chromosome-positive ALL, infants with ALL or patients with mixed lineage leukemia-rearranged (*MLL-R*) ALL. In Philadelphia chromosome-positive ALL, the COG reported 3-year EFS of 87.7 ± 10.9% with imatinib plus intensive chemotherapy (n = 25)

compared with 56.6 ± 21.5% for HCT with HLA-matched sibling donors (MSDs; n = 21) and 71.6 ± 19% for unrelated donors (MUD; n = 11; p = 0.144) [34]. The recently updated results of the AALL0031 study with a minimum of 6-year follow-up reports 4-year EFS of 75 ± 9% for the imatinib/chemotherapy-only cohort compared with 64 ± 11% for MSD-HCT and 64 ± 16% for MUD HCT (p = 0.77) [M Devidas, Unpublished Data]. The outcomes for infants with *MLL-R* ALL are exceedingly poor with no significant difference in survival between the HCT and chemotherapy alone approaches [35–39], therefore, HCT in CR1 has not typically been recommended for these patients. For noninfant patients with *MLL-R* leukemia, the current data do not support HCT in CR1 when this mutation is the only risk factor present but in cases where patients are at higher risk based on age, white blood cell (WBC) or other cytogenetic abnormalities, then HCT could be considered in first remission for these patients [40–42].

Another subgroup in which to consider HCT in CR1 are patients with persistent MRD after induction or consolidation therapy [31]. Five-year EFS rates are <80% for patients who are end of induction MRD-positive and 52 ± 14% for those who remain MRD-positive after consolidation [43]. As MRD monitoring techniques further evolve and risk stratification continues to identify patients at greater risk of treatment failure, the role of HCT in CR1 for patients with persistent MRD-positivity as well as the other high-risk subgroups listed above will need to be reassessed.

■ HCT for relapsed ALL

Despite the success in treating pediatric ALL [1], 10–20% of patients will have a relapse [44–46]. The majority of relapses occur while on therapy or shortly after completing treatment [47]. Survival of these patients using chemotherapy alone is 10–20% [47]. Features that have been identified as more prevalent in relapsed ALL compared with diagnosis include ages <1 or ≥10 years, male gender, African–American or Hispanic ethnicity, WBC >100,000/µl and specific chromosome abnormalities [47]. As site (bone marrow, extramedullary or combined) and timing of relapse (early vs late) remain two of the most prognostic features in predicting outcomes in pediatric ALL, we will address each of these below.

■ Early bone marrow relapse

Defined by North American groups (e.g., COG) as <36 months from a patient's initial ALL diagnosis or within 6 months of completing therapy by European oncology groups (e.g., Berlin–Frankfurt–Munster [BFM] study group), an early marrow relapse (isolated to the bone marrow or combined with extramedullary relapse) is the most common site of recurrence. The 5-year EFS with chemotherapy for patients who suffer an early marrow relapse is only around 10% [5,48,49]. HCT using HLA-MSD improves the EFS to 31–54% [5,48,49]. As HCT with MUD outcomes for pediatric ALL are similar to results with matched related donors [8,50–52], using the best available donor for early marrow relapse remains the standard approach for these patients. Survey results confirmed this is the general practice, where pediatric oncologists and bone marrow transplant physicians were queried about management of patients with relapsed B-precursor ALL, reporting the decision to use the best available donor for patients with an early marrow relapse by the majority of both groups (67.3 and 81.5%, respectively) [53].

NCI risk stratification (patient age and presenting WBC) is another risk factor affecting survival in these patients [47]. Patients who relapsed early in the bone marrow and were standard risk (SR; age <10 years, WBC <50,000) at their initial diagnosis, had significantly greater survival compared with those who were higher risk (age ≥10 years; WBC ≥50,000; 33.1 ± 3.6 vs 14.9 ± 2.1%; p < 0.0001).

Patients who have a combined bone marrow relapse with an extra medullary site (e.g., CNS, testis, other) have similar survival rates as those with an isolated marrow relapse. In the same analysis by Nguyen *et al.*, 5-year survival rates were nearly identical between patients with an early isolated bone marrow relapse and those with an early combined bone marrow relapse (11.5 ± 1.9 vs 11.6 ± 4.9%, respectively) suggesting that treatment for patients with an early marrow relapse regardless of any extramedullary involvement, should be treated with HCT [47].

■ Late bone marrow relapse

When relapse occurs in the bone marrow at ≥36 months from diagnosis or more than 6 months from completing therapy for ALL, it is considered a late marrow relapse. Patients treated with chemotherapy alone for a late

marrow relapse of B-precursor ALL have EFS in the range of 40% [5,49] compared with 60% for those who receive HCT [49,54]. However, patients who fail HCT in second remission (CR2) are rarely salvaged with further therapy whereas those who relapse after chemotherapy may only enter remission and be salvaged with HCT in third remission [54,55]. This may result in similar OS for patients treated with chemotherapy versus HCT in CR2. Owing to the similarity in survival regardless of treatment strategy, despite the increased EFS observed with HCT in CR2, management for the late relapse patient continues to be debated. As reported in the survey by Burke and colleagues, the majority of pediatric oncology and bone marrow transplant physicians would manage patients with late marrow relapses with intensive chemotherapy alone (59.5 and 56.9%, respectively) rather than recommend HCT using an available matched related donor (35.8 and 30.1%, respectively; p = 0.08) [53]. The recent report by Eckert *et al.* describing results of the ALL-REZ BFM 2002 trial where end re-induction MRD was used to allocate patients to HCT versus chemotherapy alone, supports the approach of chemotherapy only for patients with a late marrow relapse who become MRD-negative after re-induction [56]. Patients with late isolated or combined bone marrow relapse who had end re-induction MRD $<10^{-3}$ leukemic cells and were treated with chemotherapy only had 5-year EFS of 76 ± 5%, which is relatively close to the EFS of newly diagnosed high-risk B-cell acute lymphoblastic leukemia patients (88–92%).

Unlike the late marrow relapses in B-precursor ALL, isolated late marrow relapse for T-cell acute lymphoblastic leukemia (T-ALL) portends equally poor survival as those with T-ALL who relapse early (<5%) [49]. However, due to limited numbers, it is not clear how much, if any, improvement there is with HCT for relapsed T-ALL [10,57].

As observed for early marrow relapse, survival differences exist for late marrow relapse patients based on NCI risk stratification. Patients with relapsed B-precursor ALL who were higher risk at their initial diagnosis had significantly inferior survival compared with those who were SR (39.5 ± 7.2 vs 59.6 ± 4.6%; p < 0.0001) [47]. Thus, the decision as to whether or not to recommend HCT in patients with late isolated marrow relapse may incorporate not only lineage (T cell vs B cell) but also NCI risk status (higher risk vs SR). As advancements continue to be made for both chemotherapy and HCT, decisions regarding treatment will need to be revisited.

Extramedullary relapse

Although the majority of relapses in ALL involve the bone marrow, 20% will be isolated to the CNS and 5% to the testes [47]. These extramedullary relapses follow the same risk stratification as medullary recurrence, mainly time to relapse (early: <18 months from diagnosis vs late: ≥18 months), to determine predicted EFS and optimal postrelapse therapy. OS for patients with an isolated CNS (iCNS) relapse in the late 1980s and early 1990s ranged between 46–63% [49,58,59]. Current outcomes have improved through delaying CNS radiation to allow for more intensified chemotherapy earlier in treatment [60]. However, similar to marrow relapses, prognosis with early iCNS recurrences is worse than with late relapses (4-year EFS of 51 vs 80%) [60]. The COG and the CIBMTR compared outcomes for pediatric patients in CR2 after an iCNS relapse: 149 received chemotherapy/irradiation and 60 received HCT between 1990 and 2000 [61]. The 8-year leukemia-free survival for patients treated with chemotherapy/irradiation alone was 67% compared with 65% for MSD HCT (p = not significant). Similar results were seen in OS (67 vs 62%; p = not significant), thus the authors concluded that the treatments were equivalent.

With modern therapy, isolated testicular relapse (iTR) occurs in only 2% of patients. Most of these relapses occur late (≥18 months from diagnosis) [47,62,63]. Either irradiation of the testes or orchiectomy has been used as local treatment for iTR in children with no data to support one being superior to the other [62,64,65]. Patients with iTR treated with intensive chemotherapy with or without irradiation have similar survival to those with iCNS relapse, with iTR patients reporting 6-year OS of 52 and 81% for early and late relapses, respectively [62]. For patients who have an early iTR, HCT is generally accepted as the standard approach following initial intensive chemotherapy/radiotherapy although the data supporting this are limited [66].

Pre-HCT MRD: ALL

Although HCT in children with relapsed ALL has the ability to cure some patients, relapse remains a significant barrier to success. Recent

studies have shown that MRD prior to HCT may be the strongest predictor of relapse [12–18,67–70]. In a study of 83 patients with high-risk ALL receiving autologous HCT at the University of Minnesota (MN, USA), patients with more than 50 leukemia progenitor cells/million mononuclear cells had higher relapse rates compared with those with <51 leukemia progenitor cells/million mononuclear cells (100 vs 41%, respectively; $p < 0.001$) [71]. These findings have since been replicated for allogeneic HCT in high-risk pediatric ALL patients [12–14,16–18,67–69].

Knechtli and colleagues reported their experience using PCR-based MRD testing pre-HCT in 64 children with ALL [14]. Patients had remission bone marrow specimens collected and analyzed for MRD a median of 23 days prior to HCT (range: 6–81 days). Outcomes were considerably worse in patients with detectable MRD pre-HCT compared with those who had no disease detected: 2-year EFS of 0% (MRD $>10^{-3}$ leukemic cells) compared with 73% (MRD $<10^{-5}$ leukemic cells; $p < 0.001$). In patients who had intermediate levels of residual disease ($>10^{-5}$ and $<10^{-3}$ leukemic cells) 2-year EFS was 36%. In one of the larger series investigating the impact of pre-HCT MRD in pediatric ALL, Bader *et al.* reported 91 children with relapsed ALL treated on BFM relapse studies [12]. MRD was quantified using real-time PCR techniques to identify T-cell receptor/immunoglobulin gene rearrangements. For high-risk patients in ≥CR2 who had MRD $\geq 10^{-4}$ leukemic cells, 5-year EFS was 30 ± 9% and relapse 50 ± 9% compared with 53 ± 11% and 18 ± 8% for those $<10^{-4}$ ($p = 0.086$ and $p = 0.012$, respectively).

Although PCR MRD techniques have the ability to identify MRD at a 1–2 log lower detection level compared with flow cytometry ($10^{-5/6}$ vs $10^{-4/5}$ leukemic cells), flow cytometry is less labor-intensive and cheaper to perform. In an analysis of 116 pediatric patients receiving HCT for relapsed or very-high-risk ALL at Seattle Children's Hospital (WA, USA) between 1995 and 2005, flow cytometry was used to assess MRD within 30 days of HCT [15]. Once again the predictive nature of MRD identified prior to HCT was striking with patients identified as MRD-positive (>0.1% leukemia blasts) reporting 5-year EFS of only 11% compared with 58% in MRD-negative patients ($p < 0.001$).

There have been limited reports investigating MRD prior to umbilical cord blood transplant (UCBT). Ruggeri and colleagues reported the Eurocord registry data of 170 pediatric ALL patients who received a single UCBT and had pre-HCT marrow assessments for MRD [17]. The 4-year probability of leukemia-free survival for MRD-positive (MRD $>10^{-4}$ leukemic cells) patients was 29 ± 6% compared with 54 ± 4% for MRD-negative patients ($p = 0.006$). Bachanova *et al.* evaluated 86 pediatric and adult patients with ALL and reported similar results following UCBT [68]. Using multiparameter flow cytometry to measure any detectable MRD (sensitivity ranging between 0.03 and 0.1% leukemia blasts), patients who were MRD-positive pre-HCT had inferior 3-year EFS of 30% compared with 55% for MRD-negative patients ($p = 0.02$) as well as greater relapse among the MRD-positive patients (30 vs 16%; $p = 0.05$).

While the studies referenced above clearly support that pre-HCT MRD identified in patients with ALL is a strong indicator of post-HCT relapse, there is presently no data available to support that the elimination of MRD immediately prior to HCT will improve survival outcomes in these MRD-positive patients. Such an approach aimed to eliminate pre-HCT MRD would not be without risks, as additional chemotherapy in patients who have already achieved a morphologic remission could lead to toxic complications that might preclude proceeding to HCT. Furthermore, any additional cytotoxic therapy given immediately prior to HCT could increase transplant toxicities and overall morbidity. In addition, attempts to 'bridge' patients with pre-HCT MRD to HCT with additional chemotherapy in an attempt to convert them to MRD-negativity will prolong the time to HCT and thus place them at a theoretical increased risk of relapse. Despite these uncertainties, there is a strong sense among the pediatric oncology and transplant community that attempts to eliminate MRD prior to HCT are warranted [53]. The development of clinical trials aimed at safely eliminating MRD, ideally with targeted therapies that may have fewer systemic toxicities such as the immunotoxins (e.g., moxetumomab [anti-CD22] and BU 12-SAPORIN [anti-CD19]) or bispecific T-cell engagers (e.g., blinatumamab; anti-CD19/CD3), will be critical in answering the question of whether or not reducing and/or eliminating pre-HCT MRD can in fact improve survival in pediatric ALL.

Transplantation for childhood AML

Indications for HCT in first remission

Although outcomes for pediatric patients with AML have improved over the past few decades, certain subgroups continue to have very poor survival [72]. These observations have led to a risk-stratification model used to guide therapy. There is general agreement (summarized in recommendations from an international expert panel) that HCT in CR1 is of no benefit for pediatric patients with favorable risk factors including the translocation involving chromosomes 8 and 21 (t[8;21]), inversion of chromosome 16 (inv[16]) or a translocation of chromosome 16 (t[16;16]) [72]. Using HCT in CR1 for intermediate or high-risk patients remains controversial [73,74]. As demonstrated by the Medical Research Council AML10 trial and others [73,75], HCT reduces the risk of relapse, but does not always result in an improved OS when compared with chemotherapy alone owing to the increased HCT-related toxicities and lower salvage rates when relapse occurs post-HCT. Conversely, in a meta-analysis that analyzed 1373 pediatric patients treated on four cooperative group trials (through the COG) HCT showed a benefit over chemotherapy alone for patients with intermediate-risk AML (OS: 62 vs 51%; $p = 0.006$) [76]. A significant relapse-free survival benefit of HCT has also been shown in adults with either intermediate (hazard risk [HR]: 0.76; 95% CI: 0.68–0.85; $p < 0.01$) or high-risk (HR: 0.69; 95% CI: 0.57–0.84; $p < 0.01$) disease [77]. Therefore, HCT may be considered for children with intermediate or high-risk AML in CR1, but the potential benefit of a lower relapse rate must be weighed carefully against the risks of graft-versus-host disease (GVHD) and transplant-related mortality (TRM). As both chemotherapy results and transplant-related morbidity and mortality continue to improve, the role and timing of HCT for AML will need to be revisited.

Patients with AML are currently risk-stratified based on cytogenetics, molecular genetics and response to therapy. Cytogenetic abnormalities associated with a poor prognosis have been well-described [72]. More recently, specific mutations identified by molecular genetics are now used to inform risk assignment, such as for patients with *FLT3*-internal tandem duplication (ITD) mutations having significantly greater rates of relapse [74,78–80]. In an analysis of two Children's Cancer Group studies involving 630 pediatric patients, the presence of *FLT3*-ITDs was associated with a progression-free survival of 31 versus 55% for the *FLT3* wild-type ($p < 0.001$) [79]. Progression-free survival dropped to 16% when the *FLT3*-ITD allelic ratio was considered high (>0.4). HCT results in adolescent and young adults (AYA) aged 15–30 years and older adults, all with *FLT3*-ITD AML, have shown improved disease-free survival (DFS) from 8 to 34% in this group when pursued in CR1, and so should be considered in children who harbor this mutation [81,82].

Another AML subgroup identified by cytogenetic or molecular techniques that confers intermediate- to high-risk prognosis in pediatric AML is that of the 11q23 *MLL* rearrangement [83]. In a report by the BFM study group of 247 children (aged 0–18 years) with high-risk AML who achieved CR1 on protocol AML-BFM 98 and were allocated to HCT versus chemotherapy alone based on the availability of an HLA-MSD, 61 children received HCT and 186 received only chemotherapy [84]. Overall there was no significant difference in 5-year DFS between patients receiving HCT versus chemotherapy (49 ± 6 vs 45 ± 4%; $p = 0.44$) or OS (68 ± 6 vs 57 ± 4%; $p = 0.17$), however for patients harboring 11q23 rearrangements ($n = 67$) there was significantly greater DFS with HCT compared with chemotherapy only (67 ± 11 vs 38 ± 7%; $p = 0.04$) and superior OS (94 ± 6 vs 52 ± 7%; $p = 0.01$). Thus, patients with 11q23 rearranged AML, other than the translocation involving chromosome 1 and 11 (t[1;11][q21;q23]) where excellent 5-year EFS (92 ± 5%) and OS (100%) with chemotherapy alone have been reported [83], may be considered for HCT in CR1.

Response to AML therapy has historically been determined by morphologic review, but with the development of more sensitive techniques (e.g., multiparameter flow cytometry and quantitative PCR), assessing for MRD is becoming the new standard. Multiple trials have shown that the presence of MRD after the first induction cycle in AML is an independent predictor of relapse [85–88]. Loken *et al.* showed that patients with high-risk features who were MRD-positive at the end of their first induction cycle had a relapse-free survival

of 0% compared with 45 ± 38% for those who were high-risk and were MRD-negative after their first induction cycle [85]. Rubnitz *et al.* showed that intensifying therapy for MRD-positive patients improved outcomes, and that HCT for high-risk MRD-positive patients (MRD >1% leukemia) after a single induction course trended toward improved OS (43.5 vs 23.1%; p = 0.14) [88]. These retrospective studies, although provocative, are limited by small numbers of patients and varying definitions of MRD positivity. Whether HCT can abrogate the poor prognostic feature of end induction MRD-positivity in pediatric AML will await future prospective trials.

HCT for relapsed AML

Despite advancements in the treatment of AML over the past few decades, 40% of children continue to relapse [89]. HCT using the best available donor is generally accepted as the treatment of choice for children with relapsed AML. The Therapeutic Advances in Childhood Leukemia and Lymphoma Consortium retrospectively evaluated outcomes of relapsed/refractory AML patients (1995–2004) to establish current response rates to salvage therapy for children with AML. Complete remission following a first treatment for relapse was observed in only 56 ± 5% of children. Five-year EFS and OS for these patients were 24 ± 5% and 29 ± 5%, respectively [89] indicating a tremendous need for discovery of novel and active therapies in relapsed/refractory AML.

HCT has been shown to be effective consolidation therapy for patients not only after a first relapse (CR2) but also for those with history of PIF or currently active disease/in relapse. In a report by Bunin *et al.* of 268 pediatric AML patients using the National Marrow Donor Program database, they analyzed HCT outcomes for 142 patients in CR2, 90 in relapse and 36 with PIF [11]. Patients in CR2 reported the greatest OS at 5 years (47%) compared with those in relapse (22%) or PIF (17%). Furthermore, relapse post-HCT was lowest for patients transplanted in CR2 (22%) compared with either in relapse (57%) or PIF (51%). These results support the claim that children who undergo HCT for AML have superior outcomes when in remission as well as the ability of HCT to salvage around 20% of children with recurrent or primary refractory disease that are unable to achieve CR2.

Differences in donor graft source (HLA-MUD vs HLA-MSD) for children with AML have not been shown to negatively impact HCT outcomes. Lee *et al.* reported HCT outcomes between MUD and MSD for childhood AML in CR1 between 2002 and 2005 with no differences in neutrophil or platelet engraftment, grade II GVHD or chronic GVHD [90]. Patients had similar EFS at 3 years with MUD recipients reporting 71 (95% CI: 49–93) versus 77% (95% CI: 55–99) for MSD (p = 0.63). Although using MUDs as a graft source for children with AML does not negatively impact outcomes, older age does appear to be associated with inferior survival when compared with younger children. In a study by Rubnitz and colleagues, children with AML receiving HCT between the ages of 10–21 years had significantly worse survival compared with younger children due to a greater toxic death rate (13.2 ± 3.6% vs 4.5 ± 2.0%; p = 0.28) [91]. The deaths in the older patients was primarily due to infection (75%) compared with those younger than 10 years where infection was the cause of death in two out of five (40%). The CIBMTR analyzed AML HCT outcomes for children (<15 years of age) compared with AYA aged 15 to 40 years and older adults (>40 years) [92]. HCT outcomes were assessed over three time periods (1980–1988, 1989–1997 and 1998–2005) and included 900 children, 2,708 AYA and 2728 older adults. Five-year survival over the three time periods for MSD HCT was similar for children and AYA (40, 48 and 53% vs 35, 41 and 42%; p = 0.23) as well as with MUD HCT for the two latter time periods (38 and 37% vs 24 and 28%; p = 0.87). However, TRM was significantly higher in AYA (56 and 39%) compared with children (33 and 26%; p = 0.05) for the two time periods reported (1989–1997 and 1998–2005).

Pre-HCT MRD: AML

Similar to ALL, post-HCT outcomes for relapsed AML are related to the amount of disease present prior to HCT, with MRD-negative patients faring better than patients with measurable leukemia [20]. MRD identified prior to HCT for AML portends a poor prognosis in both pediatric and adult patients [19,20,93–103]. In a report by Walter *et al.* 99 pediatric and adult patients with

AML receiving a HCT in CR1 were evaluated for pre-HCT MRD using multiparameter flow cytometry with any detectable MRD defined as being MRD-positive [19]. The 2-year EFS for MRD-positive patients was 9% with a relapse rate of 64.9% compared with 74.8 and 17.6% in MRD-negative patients, respectively. The multivariate analysis identified pre-HCT MRD as a significant risk factor for both overall mortality (HR: 4.05; 95% CI: 1.90–8.62; $p < 0.001$) and relapse (HR: 8.49; 95% CI: 3.67–19.65; $p < 0.001$). In another analysis reporting a larger group of pediatric and adult patients with AML (n = 253) receiving HCT in CR1 or CR2, OS (HR: 2.61; 95% CI: 1.62–4.20; $p < 0.001$), DFS (HR: 3.74; 95% CI: 2.38–5.87; $p < 0.001$) and relapse (HR: 4.90; 95% CI: 2.87–8.37; $p < 0.001$) were all significantly worse for patients identified as MRD-positive by flow cytometry (≥0.1% blasts), compared with MRD-negative patients in a multivariate cox regression model [20].

Combining methods of MRD detection using flow cytometry and *WT1* analysis is another means to identify MRD-positive patients in AML. A recent report used this combined approach where MRD-positive patients, using a lower level of disease detection, were defined as >0.001% by flow cytometry and *WT1* expression >0.6% by PCR in pediatric and adult patients with AML undergoing HCT [97]. The 3-year leukemia-free survival for 20 MRD combined positive patients was 52% compared with 76% for 110 MRD-negative patients ($p = 0.41$) with greater relapse reported in the MRD-positive group (81 vs 47%; $p = 0.013$).

Although data regarding the presence of pre-HCT MRD in children with AML continue to correlate with an increased risk of relapse and poorer post-HCT survival, merely having detectable disease prior to HCT does not necessarily indicate an inability to cure the disease [98]. As shown in the report by Leung *et al.* from the St. Jude Children's Research Hospital (TN, USA), detectable MRD prior to HCT should not be regarded as a contraindication for HCT [99]. In this study, 57 children, AYA (ages 15–25 years) with high-risk or relapsed AML received haplo-identical HCT with flow-based MRD measurements performed pre-HCT. MRD was identified as an independent prognostic factor of poor outcomes in the multivariate model ($p = 0.0035$). OS in patients with MRD <0.01% was 80.4% compared with 66.7% for patients with high MRD (>0.01% but <5.0%). The survival reported for these MRD-positive AML patients was strikingly high which suggests that HCT should continue to be considered for children, AYA with high-risk or relapsed AML.

The idea of eliminating pre-HCT MRD through 'bridging' therapy in AML to improve survival is enticing. The same concerns for bridging approaches in ALL would apply to AML, in that there is currently no data to suggest that converting an MRD-positive patient to MRD-negative immediately prior to HCT will improve survival. It is also unknown whether successfully eliminating MRD in AML would provide HCT outcomes similar to those patients that were MRD-negative pre-HCT. Furthermore, any additional therapy given with a bridging approach may introduce more toxicities and increase HCT related morbidities and TRM. Despite these risks, pursuing such an approach with targeted, relatively nontoxic therapies to successfully bridge the MRD-positive patient with AML to HCT are needed.

Conclusion & future perspective

Allogeneic transplantation continues to offer durable cures for many children with high-risk or relapsed ALL or AML. Despite increasing survival for children with ALL or AML receiving HCT for their disease, relapse remains the number one cause of morbidity and death. The identification of MRD present prior to HCT has been one of the most important discoveries in the recent era as to why post-HCT relapse occurs in our patients. Thus identifying ways to safely eliminate pre-HCT MRD in hopes of improving post-HCT outcomes are desperately needed, but await prospective clinical trials in pediatrics investigating such an approach.

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- ▪ of interest
- ▪▪ of considerable interest

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