A Phase I Study of Reduced-Intensity Conditioning and Allogeneic Stem Cell Transplantation Followed by Dose Escalation of Targeted Consolidation Immunotherapy with Gemtuzumab Ozogamicin in Children and Adolescents with CD33⁺ Acute Myeloid Leukemia

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ABSTRACT
Myeloablative conditioning and allogeneic hematopoietic stem cell transplant (alloHSCT) in children with acute myeloid leukemia (AML) in first complete remission (CR1) may be associated with significant acute toxicity and late effects. Reduced-intensity conditioning (RIC) and alloHSCT in children is safe, feasible, and may be associated with less adverse effects. Gemtuzumab ozogamicin (GO) induces a response in 30% of patients with CD33⁺ relapsed/refractory AML. The dose of GO is significantly lower when combined with chemotherapy. We examined the feasibility and toxicity of RIC alloHSCT followed by GO targeted immunotherapy in children with CD33⁺ AML in CR1/CR2. Conditioning consisted of fludarabine 30 mg/m² × 6 days, busulfan 3.2 to 4 mg/kg × 2 days ± rabbit antithymocyte globulin 2 mg/kg × 4 days followed by alloHSCT from matched related/unrelated donors. GO was administered 60 days after alloHSCT in 2 doses (8 weeks apart), following a dose-escalation design (4.5, 6, 7.5, and 9 mg/m²). Fourteen patients with average risk AML received RIC alloHSCT and post-GO consolidation: median age 13.5 years at transplant (range, 1 to 21), male-to-female 8:6, and disease status at alloHSCT 11 CR1 and 3 CR2. Eleven patients received alloHSCT from 5-6/6 HLA-matched family donors: 8 received peripheral blood stem cells, 2 received bone marrow, and 1 received related cord blood transplantation. Three patients received an unrelated allograft (two 4-5/6 and one 9/10) from unrelated cord blood unit and bone marrow, respectively. Neutrophil and platelet engraftment was observed in all assessable patients (100%), achieved at median 15.5 days (range, 7 to 31) and 21 days (range, 10 to 52), respectively. Three patients received GO at dose level 1 (4.5 mg/m² per dose), 5 at dose level 2 (6 mg/m² per dose), 3 at dose level 3 (7.5 mg/m² per dose), and 3 at dose level 4 (9 mg/m² per dose). Three of 14 patients received only 1 dose of GO after alloHSCT. One patient experienced grade III transaminitis, which resolved; no grade IV transaminitis, no grade III/IV hyperbilirubinemia, or sinusoidal obstructive syndrome were observed. The second dose of GO was given at median of 143 days (range, 120 to 209) after alloHSCT. Probability of grades II to IV acute and chronic graft-versus-host disease were 21% and 33.5%, respectively. Probability of overall survival after RIC alloHSCT and GO consolidation at 1 and 5 years was 78% and 61%, respectively. Probability of 5-year event-free survival after RIC alloHSCT and GO consolidation in patients in CR1 was 78%. No dose-limiting toxicity was observed. The median duration of follow-up post alloHSCT was 77.9 months (range, 11 to 146).
INTRODUCTION

Despite considerable advances in the treatment in a variety of pediatric hematologic malignancies, particularly in acute lymphoblastic leukemia and non-Hodgkin lymphoma, the 5-year event-free survival (EFS) in pediatric acute myeloid leukemia (AML) is only in the range of 50% to 60% [1-3]. Attempts to improve outcomes in children and adolescents with AML have focused in part on identifying which patients can benefit from allogeneic hematopoietic stem cell transplantation (alloHSCT) as well as improving alloHSCT strategies. Historically, myeloablative conditioning (MAC) regimens have been used for alloHSCT in children with AML [4]. However, significant morbidity and mortality still exist as a result of post-MAC alloHSCT complications [5]. AlloHSCT treatment-related mortality (TRM) after MAC has ranged between 5% and 40% [6-8]. Furthermore, late complications of MAC and alloHSCT include chronic graft-versus-host disease (cGVHD), neurodevelopmental delays, endocrine abnormalities such as growth hormone deficiency, and secondary malignancies [9]. Both acute causes of TRM as well as late complications can offset this survival benefit of alloHSCT, especially after MAC regimens [7,10,11].

Reduced-intensity conditioning (RIC) before alloHSCT has transformed the paradigm of conditioning before alloHSCT. We and others have demonstrated the safety and efficacy of RIC alloHSCT in pediatric recipients [12-14]. RIC before alloHSCT in pediatric AML allows for a maximal graft-versus-leukemia effect from allogeneic donor T lymphocytes and/or natural killer cells, while minimizing TRM and late effects after alloHSCT. Various RIC regimens have been tried, including that of fludarabine, busulfan, and rabbit antithymocyte globulin [12,15]. This specific RIC regimen has resulted in excellent overall survival (OS) and provides sustained engraftment in patients undergoing transplant from matched sibling and unrelated donors [12,16].

A more promising approach in the treatment of hematologic malignancies in children, adolescents, and young adults is the use of targeted immunotherapy [17]. Approximately 80% to 90% of children with AML express CD33 on the surface of their leukemia cells [18]. Pollard et al. [19] demonstrated that high CD33 expression in children with AML is associated with high-risk genetic mutations (ie, FLT3/ITD) and inversely associated with low-risk disease. Gemtuzumab ozogamicin (GO, Mylotarg; Pfizer, NY) is a recombinant humanized monoclonal antibody (IgG4) directed against the CD33 antigen on the surface of AML cells. This antibody is coupled to the derivative of the cytotoxic antibacterial calicheamicin [20]. GO was initially used as part of induction or reinduction chemotherapy at varying doses and has been shown to induce a complete remission (CR) or partial CR in 28% to 55% of patients [17,21-23]. This drug has also been used in patients with refractory or relapsed disease and has been shown to be safe and efficacious [24,25].

Pollard et al. [26] reported that children with AML and high CD33 expression had an improved outcome with the use of GO treatment, especially in low- and intermediate-risk patients. Most importantly, Gamis et al. [27] reported on the safety and efficacy of GO in children with de novo AML in the Children’s Oncology Group AAML0531 trial. GO (3 mg/m² per dose) administered with a 5-day cycle of induction and then intensification chemotherapy was associated with a significantly increased EFS and relapse-free survival in children with AML in CR1. Recently, Tarlock et al. [28], reporting for the Children’s Oncology Group, demonstrated that GO reduced the risk of relapse in FLT3/ITD AML, especially in those patients with high-risk ITD allelic ratio who received an allogeneic stem cell transplant in CR1. Furthermore, we have previously reported the safety and efficacy of GO in combination with busulfan and cyclophosphamide as part of a MAC regimen in very poor-risk children with AML in refractory relapse, induction failure, and CR3 [10]. In this previous phase I study, there were no GO-associated dose-limiting toxicities (DLTs) observed at the dose range of 3 to 7.5 mg/m², and no TRM was observed secondary to GO [10].

In the current study we examined the safety and efficacy of dose escalation of GO as consolidation therapy in a phase I study after RIC and alloHSCT in children and adolescents with AML in CR1 or CR2. Our objectives were to determine whether this combination is safe and to identify the tolerable dose or maximum tolerated dose of GO after RIC alloHSCT in children with CD33+ AML in CR1 or CR2.

METHODS

Eligibility Criteria

Patients were required to be <21 years of age, AML in CR1 or CR2, and with an HLA-matched family donor or an unrelated donor (for the definition of an HLA match, see HLA Typing and Stem Cell Source, below). Patients were excluded if they had acute promyelocytic leukemia in CR1, Down syndrome, or poor cytogenetics (12p, 5q, 7, and FLT3 mutations or duplication t(9;11) and others). Only those patients with favorable or intermediate risk cytogenetics were included. Leukemic cells had to express a minimum >10% CD33 positivity. Patients were also required to have adequate organ function, defined as follows: renal function with serum creatinine <1.5 x normal or creatinine clearance >60 mL/min/1.73m²; liver function with total bilirubin ≤ 2 times the upper limit of normal and aspartate aminotransferase or alanine aminotransferase ≤5 times upper limit of normal; cardiac function with shortening fraction ≥25% or ejection fraction ≥ 45% by echocardiogram; and pulmonary function with diffusing capacity of the lung for carbon monoxide ≥ 40% by pulmonary function tests or, if unable to perform pulmonary function tests, with no evidence of dyspnea at rest, no exercise intolerance, and pulse oximetry > 94% on room air. Patients who achieved CR1 were required to proceed to RIC alloHSCT within 4 months of diagnosis, and patients in CR2 were required to proceed to RIC alloHSCT within 30 days of achieving CR2.

Patients with primary and secondary myelodysplastic syndrome were excluded from this analysis. Patients had to receive at least 1 dose of GO to be assessable for safety and efficacy. Other exclusion criteria included patients with active central nervous system AML disease at time of conditioning therapy, pregnant female patients, human immunodeficiency virus positive patients, and/or those with Karnofsky < 70% or Lansky < 50% (if <10 years old) performance status.

This study was registered at clinicaltrials.gov (NCT01020539). Some patients received GO made available through an investigational new drug approval (IND 111024). All patients signed informed consents approved by the Institutional Review Board of Columbia University Medical Center and New York Medical College, and all research protocols were in compliance with the Declaration of Helsinki.
HLA Typing and Stem Cell Source
HLA typing was determined by hybridization of PCR-amplified DNA with sequence-specific oligonucleotide probes, as previously described [29]. The criteria for graft matching included 4-6/6 loci for umbilical cord blood, 8-10/10 loci for unrelated donor peripheral blood stem cells (PBSC)/bone marrow (BM), and 5-6/6 for matched sibling donor grafts. HLA typing for HLA-A and HLA-B were determined by intermediate-resolution typing, and HLA-C, HLA-DRB1, and HLA-DQB1 were determined by high-resolution typing, as previously described [28].

RIC Regimen
All patients received a RIC regimen, as previously described [12,30,31]. This regimen consisted of fludarabine 30 mg/m² × 6 days (from days -7 to -2) and busulfan 3.2 to 4 mg/kg/day × 2 days (on days -6 and -5). All patients received antiseizure prophylaxis while receiving busulfan. Unrelated donor recipients received rabbit antithymocyte globulin 2 mg/kg/day × 4 days (on days -4 to -1). Premedication for antithymocyte globulin consisted of methylprednisolone 1 mg/kg i.v. (maximum 50 mg), and acetylcarnitine 10 to 15 mg/kg p.o. (maximum 650 mg). Methylprednisolone was given on days -4 to -1.

GO Administration
To receive GO, patients were required to successfully engraft after alloHSCT. GO was administered between days -60 and +110 after alloHSCT when the absolute neutrophil count (ANC) was >1000/mm³ and the platelet count was >40,000/mm³ untransfused × 3 days after alloHSCT. Two separate doses, a minimum of 8 weeks apart, were given following a dose-escalation design. Dose level 1 was 4.5 mg/m² per dose, dose level 2 was 6 mg/m² per dose, dose level 3 was 7.5 mg/m² per dose, and dose level 4 was 9 mg/m² per dose. Premedication included diphendramine 1 mg/kg per dose (maximum 50 mg) and methylprednisolone 1 mg/kg per dose (maximum 60 mg) 1 hour before GO administration. Intravenous fluids were also given at 1.5 × maintenance rate surrounding GO administration. GO was infused over 2 hours through a separate i.v. line equipped with a low protein-binding 1.2-μm terminal filter.

Dose escalation rules were as follows: 3 subjects were studied at the first dose level: if ≥1 subject (≥1/3) experienced a DLT, 3 more subjects were added at that same dose level, and dose escalation only continued if no further DLTs occurred. Hematologic DLT, possibly, probably, or definitely related to GO, was defined as an ANC <500/mm³ or platelets <25,000/mm³ for ≥21 days and/or grade 5 infection. Nonhematologic DLT, possibly, probably, or definitely related to GO, was defined as any grade 3 or 4 toxicity, excluding grade 3 nausea/vomiting, transaminitis, fever, or infection. The maximum tolerated dose or optimal biologic dose was defined as the dose level at which <1 of 3 patients experienced DLT. The incidence and severity of toxicity was monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

The second dose of GO was given a minimum of 8 weeks after the first dose of GO. To receive the second dose of GO, the ANC was required to be >1000 mm³, platelets >40,000/mm³ without any platelet transfusions for at least 3 days, and the dose had to be administered ≥365 days after alloHSCT. Cytokine support included granulocyte macrophage colonystimulating factor (GM-CSF) at 250 μg/m² per dose, which started on the first day of each GO infusion and continued until ANC was >2500/mm³ after neutrophil nadir.

GVHD Prophylaxis and Gradation
Acute GVHD (aGVHD) prophylaxis consisted of tacrolimus, mycophenolate mofetil (MMF), and, for recipients of grafts from matched unrelated adult donors, methotrexate. Tacrolimus was given via continuous i.v. infusion at 0.03 mg/kg/day, with serum levels monitored daily and dosing adjusted accordingly to maintain therapeutic trough levels, as previously described [32,33]. Tacrolimus was switched to an oral dosage of 12 mg/kg/day divided every 8 to every 12 hours when clinically appropriate. If patients had grade 1 or less aGVHD by day +60, tacrolimus was tapered over 60 days. MMF was started on day +1, as previously described [32,33]. Patients ≥18 years of age weighing >70 kg received MMF 15 mg/kg per dose i.v. every 8 hours, and patients ≤18 years of age weighing <70 kg received 900 mg/m² per dose every 8 hours. For recipients of related donors, MMF was stopped on day +30 if there was grade 1 or less aGVHD. For recipients of unrelated and cord blood donors, MMF was stopped on day +60 if there was grade 1 or less aGVHD. Methotrexate was given to those patients who received HSCT from unrelated adult donors, with a loading dose of 15 mg/m² on day +1 followed by 10 mg/m² on days +3, +6, and +11. Patients who developed at least grade II aGVHD received additional treatment as clinically indicated. aGVHD and GVHD were graded according to the Seattle Criteria [34].

Supportive Care
Hematopoietic growth factor support, blood product support, isolation, and infection prophylaxis (antibacterial, antifungal, and antiviral) was given as previously described [35-37].

Engraftment and Donor Chimerism
Neutrophil engraftment was defined as the first 3 days after the neutrophil nadir with an ANC >500/mm³. Platelet engraftment was defined as the first of 3 consecutive days demonstrating a platelet count >20,000/mm³ after 7 days without any platelet transfusions. Donor myeloid and/or lymphoid chimerism was measured on days +30, +60, +100, +180, and +365 post-transplantation. Percent donor chimerism was determined by quantifying fluorescent-labeled PCR products from donor and recipient alleles at short tandem repeat loci, as previously described [14].

Wilms Tumor Gene 1 Measurements
When feasible, patients’ peripheral blood and/or BM samples were tested for Wilms tumor gene 1 (WT1) levels, as previously described [38]. Samples were examined on days +30, +100, +180, and +365 after alloHSCT.

Statistical Analysis
The probability of time to neutrophil and platelet reconstitution after reduced-intensity alloHSCT, HLA disparity and cell dose, incidence and severity of aGVHD, cGVHD, EFS, and OS were all estimated using the product-limit method of Kaplan-Meier. The continuous variables were summarized as median ± standard deviation, and categorical variables were summarized as percentages.

RESULTS

Demographic Data
Fourteen patients with average risk AML received RIC alloHSCT and at least 1 dose of GO post-HSCT, at a median age of 13.5 years (range, 1 to 21). The gender distribution was 8 males and 6 females. Eleven of 14 patients (78.6%) were in CR1 at the time of enrollment, and 3 of 14 (21.4%) were in CR2. Some of these patients were previously reported in our preliminary analysis [31]. The demographic variables of the patients are summarized in Table 1.

Donor Sources, Hematopoietic Reconstitution, and Donor Chimerism
A total of 11 patients received allografts from 5-6/6 HLA-matched family donors, 8 of which received PBSCs, 2 BM, and 1 related cord blood. Three patients received a matched unrelated donor allograft (two 4-5/6 HLA match and one 9/10 HLA match) from unrelated cord blood and BM, respectively (Table 1).

Neutrophil and platelet engraftment after alloHSCT were observed in all assessable patients (100%) and were achieved at a median of 15.5 days (range, 7 to 31) and 21 days (range, 10 to 52), respectively (Figure 1A,B). Whole blood donor chimerism was tested on days +30, +60, +100, +180, and +365. Mean donor chimerism at day +30 was 97% (range, 94% to 99%, n = 14), day +60 was 96% (range, 85% to 100%, n = 14), day +100 was 95% (range, 82% to 100%, n = 12), day +180 was 95% (range, 80% to 100%, n = 12), and day +365 was 97% (range, 75% to 100%, n = 8) (Figure 2).

CO-Associated Toxicity
Three patients received GO at dose level 1 (4.5 mg/m² per dose) and 5 at dose level 2 (6 mg/m² per dose). One patient (004) only received 1 dose of GO because of progressive disease before the second dose. The last 2 patients treated at dose level 2 occurred while the third patient was still being evaluated for DLT. Three patients received GO at dose level 3 (7.5 mg/m² per dose) and 3 patients at dose level 4 (9 mg/m² per dose). The second patient at dose level 4 only received 1 dose of GO because of parental refusal. The third patient who received dose level 4 received only 1 dose (this patient
relapsed before administration of the second dose of GO and was thus taken off study.

The first dose of GO was administered a median of 64.5 days after alloHSCT. Twelve of 14 patients experienced grade IV myeloid toxicity after GO administration, and 7 of 14 patients experienced grade IV thrombocytopenia after GO.

The second dose of GO was given at a median of 143 days (range, 120 to 209) after alloHSCT. All 11 patients who received the second dose of GO experienced grade IV myeloid toxicity, and 8 of 11 patients experienced grade IV thrombocytopenia secondary to the second dose of GO. GO was well tolerated, and there were no grade III or IV infusion-related reactions after the first or second doses of GO. One patient experienced grade III transaminitis and grade II renal dysfunction, both of which completely resolved. One patient experienced grade III transaminitis and grade II renal dysfunction, both of which completely resolved. One patient experienced grade III transaminitis and grade II renal dysfunction, both of which completely resolved. One patient experienced grade III transaminitis and grade II renal dysfunction, both of which completely resolved. One patient experienced grade III transaminitis and grade II renal dysfunction, both of which completely resolved.

No patient experienced a DLT after GO administration. Furthermore, there was no significant change in any patients’ percent donor chimerism after GO administration.

### aGVHD and cGVHD

One patient experienced grade III skin aGVHD at day +77, and 1 patient experienced grade III gut aGVHD at day +21. The probability of grades II to IV aGVHD and cGVHD were 21% and 33.5%, respectively (Figure 3A,B, respectively). All 4 patients who experienced cGVHD received a PBSC transplant.

### WT1 Analysis after RIC AlloHSCT

Eight of 14 patients were included in this analysis. WT1 values were considered positive if the levels were ≥ 1 ng/mL, as previously described [38]. The median WT1 level before RIC alloHSCT was 0.02 ng/mL (range, 0.003-0.87 ng/mL). The median WT1 level at day +30 was 0.09 ng/mL (range, 0.006-0.34 ng/mL), at day +100 was 0.07 ng/mL (range, 0.0003-510 ng/mL), at day +180 was 0.04 ng/mL (range, 0.001-0.02 ng/mL), and at day +365 was 0.085 ng/mL (range, 0.01-19 ng/mL). Of the 4 patients who relapsed and had WT1 levels tested, 1 patient had significantly elevated WT1 levels with relapse and progression (patient 504-007). This patient’s WT1 level before RIC alloHSCT was 0.87 ng/mL, and the WT1 level on day +100 was 510 ng/mL and on the day of relapse was 16,000 ng/mL. This patient relapsed on day +109 and died on day +144.

### Survival after RIC AlloSCT and GO Consolidation

Eight of 14 patients are alive with a median follow-up of 10 years (range, 4-12). Probability of OS after RIC alloHSCT...
and GO consolidation at 1 year was 77.9% and at 5 years was 61.4% (Figure 4A). The probability of EFS after RIC alloHSCT and GO consolidation at 1 year and 5 years was 61.4% (Figure 4A). Four patients died as a result of progressive disease, and 2 patients died of cGVHD and pulmonary failure (1 at 7.5 years after alloHSCT). Of the 4 patients who relapsed, 2 were in CR2, 1 had a secondary AML, and 1 patient in CR1 had an M7 phenotype. The 1- and 5-year OS and EFS rate of patients who were in CR1 before RIC alloHSCT and GO consolidation was 72.8% (Figure 4B). One patient (CR2) was registered on study but progressed prior to receiving any doses of GO. An intent-to-treat analysis that includes this patient yields a probability of OS at one and 5 years of 72.3% and 59.3%, respectively.

**DISCUSSION**

We have demonstrated in a preliminary study that GO is a safe and tolerable consolidative targeted immunotherapy in children, adolescents, and young adults with AML in CR1 or CR2 after RIC and alloHSCT. GO appears to be tolerable when used in consolidation post-RIC alloHSCT. In the present study there were no graft failures and no deaths secondary to GO. Most importantly, there was no reduction in donor chimerism after GO in consolidation after RIC alloHSCT. The toxicities that patients did have as a result of GO were expected: grade 4 neutropenia and thrombocytopenia, which resolved in all patients. Only 1 patient experienced grade III transaminitis and grade II renal dysfunction, both of which resolved. Most importantly, we did not observe any sinusoidal obstructive syndrome in our cohort. This is a significant finding, especially because several other studies have found a higher incidence of sinusoidal obstructive syndrome related to the administration of GO before alloHSCT [21,39,40]. The maximum tolerated dose of GO was never reached at dose level 4. We are currently using dose level 4 (9 mg/m² per dose) as the optimal tolerable dose in a multicenter phase II study after RIC and alloHSCT.

In addition to evaluating the safety and efficacy of GO, this study also supports the use of RIC before alloHSCT in children and adolescents with AML in CR1 or CR2. Although our cohort is small, these pilot data are encouraging and need to be substantiated in a larger cohort of patients. Children and adolescents who undergo MAC compared with RIC before alloHSCT have reported 5-year OS rates of 48% and 45%, respectively, and relapse rates of patients who receive RIC alloHSCT do not appear to be higher than those who receive MAC alloHSCT [41].

MAC alloHSCT recipients also have increased morbidity and mortality from long-term effects [5]. Socie et al. [5] reported long-term survival and late effects data on 6691 patients who underwent MAC alloHSCT and were in CR for at least 2 years after alloHSCT. They reported that 31% of patients died of GVHD-related complications, 6% died of infection, 6% of secondary malignancies, and 6% of organ failure [5]. Another study reported a 59% incidence of a chronic health condition 10 years after MAC alloHSCT, as well as a 35% incidence of severe life-threatening illness or death at 10 years after MAC alloHSCT in these patients [42]. TRM after MAC alloHSCT has been shown to be considerably higher than that of RIC alloHSCT. We have previously described a TRM of only 3% at day +100 in a moderately sized cohort of 100 children and adolescents after reduced-toxicity conditioning and alloHSCT in pediatric alloHSCT recipients [12]. One multicenter retrospective study analyzed the utility of an alloHSCT comorbidity index on TRM and found that 36% of the 189 patients who underwent MAC alloHSCT had nonrelapse mortality and index scores of 3+, whereas 19% of the 61 patients who underwent RIC alloHSCT had nonrelapse mortality and index scores of 3+ [43]. It is this recurring evidence of toxicities and long-term effects resulting from MAC alloHSCT that has led to the development of reduced-toxicity conditioning and alloHSCT in children with high-risk myeloid leukemias in first CR. Indeed, the Children’s Oncology Group is now investigating a reduced-toxicity conditioning regimen and alloHSCT in children with AML in CR1 with high-risk features (Children’s Oncology Group AAML1031).

Although the probability of developing grades II to IV aGVHD was 21% (all cases of which were transient and resolved), the probability of developing cGVHD was 33.5%. Of those patients who developed cGVHD, all had related PBSCs as the donor source. An increased incidence of cGVHD has been demonstrated in patients whose donor source is PBSCs compared with those whose allogeneic donor source is BM.
Because of the small sample size, we cannot rule out the possibility that GO may have contributed to the development of cGVHD. However, our cohort of patients had complete persistence of donor chimerism after GO consolidation. The median donor chimerism on day +365 was 97%.

Establishing risk stratification criteria in children with de novo AML is important in determining the risk of relapse in those treated with standard induction and consolidation chemotherapy. It is important to identify which patients in CR1 should undergo alloHSCT, as this would certainly improve long-term outcomes. In the last several years, minimal residual disease (MRD) has been validated as a risk factor for disease progression or relapse in childhood AML. Loken et al. [49] demonstrated the use of multidimensional flow cytometry in MRD detection at the end of induction as a means for predicting outcome in children and adolescents with AML in CR1. Those children and adolescents who had detectable MRD at the end of first induction had a 3-year relapse risk of 60% compared with a risk of 29% in those patients with no MRD at the end of first induction [49]. Rubnitz et al. [50] demonstrated that MRDs of 1% or higher after induction was the only significant independent adverse prognostic factor for both EFS and OS in children with newly diagnosed AML in CR1. Furthermore, our group and others have demonstrated that WT1 gene expression is a useful marker in detecting MRD in children with AML. Jacobsohn et al. [38] demonstrated that 76% of children with AML and high WT1 expression relapsed after alloHSCT, whereas relapse did not occur in patients with low WT1 expression. Those patients with high WT1 expression had a significantly lower 5-year EFS rate of 18% compared with those with a low WT1 expression, whose 5-year EFS rate was 68%.

In the present study, all patients were MRD negative on day +100 after RIC alloHSCT with GO consolidation, although 1 patient’s WT1 level on day +100 was high, and this patient did relapse on day +109. The WT1 levels before RIC alloHSCT were all negative, and of the 8 assessable patients, only 1 patient who died of progressive disease had progressively increasing WT1 levels upon relapse. Thus, we cannot conclude from our small cohort whether or not WT1 levels are significantly related to disease relapse or progression. Currently, the Pediatric Blood and Marrow Transplantation Consortium has completed a study determining the importance of MRD both by flow cytometry and WT1 by PCR as a prognostic factor after alloHSCT in children with AML in CR1 and CR2. Those results should be forthcoming (David Jacobsohn, MD, personal communication).

In summary, we have demonstrated that GO, administered after RIC alloHSCT as consolidative immunotherapy, is a safe and tolerable treatment for children, adolescents, and young adults with average-risk AML in CR1 or CR2. Furthermore, there was no incidence of secondary graft failure or reduction of donor chimerism. Thus, consolidation with a targeted therapy such as GO can potentially eradicate any existing MRD after RIC alloHSCT, thereby reducing risk of relapse and increasing disease-free survival. One of the major limitations of this study is the small size of our patient phase I study cohort. Clearly, a larger phase II cohort with longer follow-up is needed to determine the efficacy of GO consolidative therapy after RIC and alloHSCT in children with AML in CR1 and CR2. We are in the process of conducting a multicenter phase II trial (NCT02117297) to investigate this question in a large cohort using dose level 4 of GO.

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