# Preliminary Results of the Safety of Immunotherapy with Gemtuzumab Ozogamicin following Reduced Intensity Allogeneic Stem Cell Transplant in Children with CD33$^+$ Acute Myeloid Leukemia

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**Abstract**

**Purpose:** Myeloablative allogeneic stem cell transplantation (SCT) has been successful in the treatment of childhood acute myeloid leukemia (AML), but may be associated with significant toxicity and recurrent disease. Reduced-intensity allogeneic SCT may offer a less toxic approach to patients with AML. Targeted immunotherapy with gemtuzumab ozogamicin has been shown to be safe, well tolerated in children, and, as a single agent, gemtuzumab ozogamicin has induced responses in 30% of patients with recurrent CD33$^+$ AML. There are no safety data with gemtuzumab ozogamicin post allogeneic SCT in children. Therefore, we explored the feasibility and toxicity of targeted immunotherapy following reduced-intensity allogeneic SCT in children with CD33$^+$ AML.

**Experimental Design:** Eight patients with CD33$^+$ AML received a reduced-intensity allogeneic SCT following fludarabine 30 mg/m$^2$ for 6 days and busulfan 3.2 mg/kg (<4 years, 4 mg/kg/d) for 2 days. Donor sources included six 6/6 HLA-matched related peripheral blood stem cells, one 6/6 sibling cord blood, and one 4/6 unrelated cord blood.

**Results:** Day 30 and day 60 donor chimerisms in seven of eight evaluable patients were 96 $\pm$ 2% ($n$ = 7) and 94 $\pm$ 3% ($n$ = 6), respectively. Five of six patients (too early for one patient) received two doses of gemtuzumab ozogamicin and one patient received only one dose. After each dose, all patients developed grade 4 neutropenia, with recovery on median days 16 and 13, respectively, after dose 1 and dose 2. Grade 4 thrombocytopenia was only observed in 2 of 11 gemtuzumab ozogamicin courses. No patients have developed dose-limiting toxicity secondary to gemtuzumab ozogamicin.

**Conclusions:** The administration of gemtuzumab ozogamicin post reduced-intensity allogeneic SCT in children with average risk AML is feasible and well tolerated with minimal toxicity. The maximal tolerated dose has yet to be determined for gemtuzumab ozogamicin post reduced-intensity allogeneic SCT in children with CD33$^+$ AML. Additional studies in a larger group of patients will be required to adequately assess the safety of this approach.

CD33 is a sialic acid–dependent adhesion protein on the cell surface, which is present in 80% to 90% of patients with acute myeloid leukemia (AML) (1). Because CD33 is expressed on normal committed myeloid cells but absent from CD34$^+$ cells and nonhematologic tissues, it is an attractive antigen for targeted immunotherapy. Gemtuzumab ozogamicin (Mylotarg) is a recombinant humanized immunoglobulin G4 antibody directed to CD33, conjugated to a cytotoxic antibiotic, calicheamicin. After gemtuzumab ozogamicin binds to the CD33 molecule, the receptor and gemtuzumab ozogamicin are internalized in a lysozyme and hydrolyzed, releasing the gemtuzumab ozogamicin. Gemtuzumab ozogamicin intercalates in the DNA, resulting in cell death. In a phase II study of adults with AML in first relapse, Sievers et al. (2) showed with two doses of gemtuzumab ozogamicin, administered 2 weeks apart, a 30% overall response [complete response (CR) and complete response without platelet recovery (CRp)]. Common side effects secondary to gemtuzumab ozogamicin included infusion-related reactions, myelosuppression, elevations in liver transaminases, infections, and, rarely, sinusoidal obstructive syndrome. Gemtuzumab ozogamicin was Food and Drug Administration approved in May 2000 for patients with CD33$^+$ AML in first relapse (3).

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A pediatric phase I study of gemtuzumab ozogamicin in 18 patients (7 with refractory AML and 11 with relapsed AML) showed that adverse events secondary to gemtuzumab ozogamicin were similar to those in adults and that 4 of 11 patients who received two doses of gemtuzumab ozogamicin at 9 mg/m$^2$ had a reduction in bone marrow blasts to <5% (4). In a report from the compassionate study of gemtuzumab ozogamicin in 15 patients with relapsed/refractory CD33$^+$ AML, there were eight responses, including five CRp (5). A dose escalation of gemtuzumab ozogamicin monotherapy (6-9 mg/m$^2$) was investigated in a phase I open-label, multicenter pediatric study with a 28% response rate (CR + CRp), with no difference in outcome for refractory versus relapsed patients. Arceci et al. showed a dose of 6 mg/m$^2$ to be safe and effective. A dose-limiting toxicity occurred at 9 mg/m$^2$, with one patient developing sinusoidal obstructive syndrome (6). Side effects seen in this study were myelosuppression (100%), transaminase elevation (21%), grade 3 to 4 hyperbilirubinemia, and sepsis (24%) (6). Drug efflux phenotype was shown to be a potential surrogate marker of response for gemtuzumab ozogamicin, as there were no responses in patients whose blasts showed significant levels of drug efflux (6). In a multicenter, phase II pediatric study, 20 patients (13 refractory and 7 second relapses) were given two doses of gemtuzumab ozogamicin at 7.5 mg/m$^2$, 14 days apart, followed by more gemtuzumab ozogamicin courses or transplant, and this regimen had a response rate of 40% (CR + CRp) (7). There was no difference in response rate between the refractory and relapsed patients and the percentage of CD33$^+$ blasts did not correlate with response (7). Patients in this phase II study experienced hematologic toxicity (>90%), infection (38%), and nausea (18%).

Incorporating gemtuzumab ozogamicin into standard induction has been shown to be safe and effective in a phase II study of elderly patients (>60 years old), treated with two doses of gemtuzumab ozogamicin at 9 mg/m$^2$ on days 1 and 15, followed by mitoxantrone, cytarabine, and etoposide. This sequential induction therapy had an overall response rate of 54% and a failure rate secondary to treatment-related mortality or resistant disease of 14% and 30%, respectively (8). Pediatric data on the use of gemtuzumab ozogamicin in combination with induction or consolidative chemotherapy are not yet available. Gemtuzumab ozogamicin is incorporated into frontline AML therapy in the recently opened Children's Oncology Group phase I study (COG AAML03P1, J. Franklin, Study Chair) using cytarabine, daunorubicin, and etoposide followed by gemtuzumab ozogamicin as part of induction therapy.

There is no experience yet on the use of gemtuzumab ozogamicin as consolidative immunotherapy after allogeneic stem cell transplantation (SCT); however, a recent study by van der Velden et al. (9) showed that with high levels of peripheral blood CD33$^+$ cells, saturation of bone marrow CD33$^+$ cells by gemtuzumab ozogamicin is drastically reduced and cell kill is impaired. Additionally, they suggest that gemtuzumab ozogamicin may be administered at a higher or more frequent dose, or "preferably, after reduction of the leukemic cell burden" (state of minimal residual disease).

Reduced-intensity allogeneic SCT offers several advantages over myeloablative conditioning regimens, including decreased acute morbidity and mortality and potentially reduced late effects, but it still maintains the benefits of the graft-versus-leukemia effect. The increased risk of relapse with T-cell depleted grafts compared with unmanipulated grafts (10), the success of donor lymphocyte infusion to induce remission (11–16), and the improved disease-free survival rates for patients who develop graft-versus-host disease (GVHD) (17–22) support the concept of the graft-versus-leukemia effect. These reduced-intensity conditioning regimens provide early immunosuppression, optimizing leukemia control as well as allowing successful engraftment. Ho et al. (23) showed that bone marrow status before reduced-intensity allogeneic SCT significantly affects outcome. Ho et al. (23), following fludarabine, busulfan, and alemtuzumab for reduced-intensity conditioning in adults with AML/myelodysplastic syndrome, showed that the patients with advanced disease (relapsed disease, progressive disease, and partial remission) had a significantly decreased survival rate (≤20%) when compared with patients in complete remission (76%) or complete morphologic remission (46%). Other disadvantages of reduced-intensity allogeneic SCT are a higher incidence of graft failure, delayed time to graft-versus-leukemia effects, and unknown long-term disease-free survival. In a pilot study in children with hematologic and solid tumor malignancies, Del Toro et al. from our group reported that reduced-intensity allogeneic SCT is feasible and tolerable, with ≥90% patients achieving ≥98% donor chimerism (24).

Woods et al. (25) have shown a relapse-free survival advantage of myeloablative allogeneic SCT in children with AML in first remission, 60 ± 9% at 8 years, when compared with myeloablative autologous SCT (48 ± 8%) and systemic chemotherapy alone (53 ± 8%). However, the nonleukemic mortality rate (regimen-related toxicity) following each of these two myeloablative allogeneic SCT approaches in children with AML in CR1 and CR2 remains high, 14% and 22%, respectively (25, 26). Another disadvantage to the myeloablative approach is the late effects, which can be quite detrimental to the growing child or adolescent, including growth retardation (27–29), infertility (27, 30–32), and secondary malignancy (33–36).

Therefore, we decided to conduct a pilot study in pediatric patients with CD33$^+$ AML in CR1 or CR2 of targeted immunotherapy with gemtuzumab ozogamicin, at a time of potential minimal residual disease following reduced-intensity allogeneic SCT, to determine whether this combination would be safe and well tolerated.

## Materials and Methods

***Eligibility.*** Patients <30 years of age with AML first CR with a matched family donor (excluding Down syndrome and acute promyelocytic leukemia), AML first CR with an unrelated family donor [excluding Down syndrome, acute promyelocytic leukemia, and translocation (8;21) or inversion (16)], or AML second CR were eligible for reduced-intensity allogeneic SCT followed by a dose escalation of targeted consolidative immunotherapy with gemtuzumab ozogamicin if they had ≥10% CD33 positivity and met specific allogeneic SCT protocol eligibility requirements. Patients were eligible if they had a fully HLA-matched family donor, a one-antigen HLA-mismatched family donor, or a one- or two-antigen mismatched unrelated cord blood unit with a minimum of 2.0 × 10$^7$ nucleated cells/kg. All patients required a >50% Lansky (≤16 years) or a >70% Karnofsky (>16 years) performance status before study entry. All



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patients signed informed consents approved by the Institutional Review Board of Columbia University Medical Center and all research protocols were in compliance with the Declaration of Helsinki.

*Reduced-intensity conditioning regimen.* All patients received a fludarabine/busulfan-based reduced-intensity conditioning regimen as we have previously described (37). Six patients received fludarabine 180 mg/m$^2$ (30 mg/m$^2$/d i.v. × 6 days, starting on day −7 and ending on day −2) and busulfan i.v., 4 mg/kg ≤4 years or 3.2 mg/kg >4 years, divided Q12h i.v. on day −6 and day −5. As busulfan has been associated with seizures, prophylaxis with phenytoin was initiated on day −7 with a loading dose (15 mg/kg), and then continued with maintenance at 5 mg/kg/d divided Q8h for children >6 years old (9 mg/kg/d divided Q8h for children ≤6 years) for 48 hours after the last dose of the busulfan. Phenytoin levels were monitored and desired levels were 10 to 20 mg/L. Patient 504-006 with an unrelated cord blood donor received additional immunosuppression with thymoglobulin 8 mg/kg (2 mg/kg × 4 days) starting on day −4 and ending on day −1. Premedication for thymoglobulin included methylprednisolone, diphenhydramine, and acetaminophen. Meperidine (0.2 mg/kg) was given for rigors.

*Allogeneic stem cell transplantation.* Recipients of HLA-matched family donor stem cells received filgrastim- (10 μg/kg/d × 4 days) stimulated peripheral blood stem cells using standard apheresis procedures for a minimum target dose of 5 × 10$^6$ CD34$^+$ cells/kg/recipient body weight. Recipients of unrelated cord blood donors (2 × 10$^7$ total nucleated cells/kg) received thawed, unrelated cord blood progenitor cells following our previously described methods (38).

*Gemtuzumab ozogamicin administration.* Two doses of gemtuzumab ozogamicin were administered following reduced-intensity allogeneic SCT as targeted consolidative immunotherapy. The first dose of gemtuzumab ozogamicin was administered between day +60 and day +180 when the absolute neutrophil count (ANC) recovered, ≥1,000/mm$^3$ and platelet count reached ≥40,000/mm$^3$ untransfused × 3 days after allogeneic SCT. The second dose of gemtuzumab ozogamicin was administered post ANC and platelet recovery after the first dose of gemtuzumab ozogamicin, approximately 8 weeks after the first gemtuzumab ozogamicin infusion but no later than day +365. Gemtuzumab ozogamicin was administered as a 2-hour i.v. infusion through a separate i.v. line (peripheral or central) equipped with a low protein-binding 1.2 μm terminal filter. Patients received prehydration with i.v. fluids at 1.5 × maintenance for 24 hours before gemtuzumab ozogamicin administration. Premedication before gemtuzumab ozogamicin consisted of diphenhydramine 1 mg/kg/dose i.v. (maximum dose, 50 mg) and methylprednisolone 1 mg/kg/dose i.v. (maximum dose, 60 mg). Acetaminophen was avoided for 48 hours after gemtuzumab ozogamicin administration. Although the gemtuzumab ozogamicin package insert recommends acetaminophen as a premedication, we were concerned about the theoretical risk of glutathione depletion in the setting of localized pro-oxidant state generated by gemtuzumab ozogamicin and its possible contribution to sinusoidal obstructive syndrome (39). Two patients with a prior history of elevated transaminases and hyperbilirubinemia received enoxaparin prophylaxis at 1 mg/kg s.c. with each dose of gemtuzumab ozogamicin starting on the day of infusion and continuing for 21 days. Sarmograstim (GM-CSF; 250 μg/m$^2$/d) was started on the first day after each gemtuzumab ozogamicin infusion and continued until the ANC ≥ 2,500/mm$^3$ after the neutrophil nadir.

Interim monitoring is done for each stem cell source (related versus unrelated cord versus unrelated adult donor). A formal interim analysis has been executed after the first five patients in each group. If two or more of the first five patients experience graft failure, the study will be discontinued secondary to futility. At this point, the seven patients who received related donor stem cells have all been successfully engrafted. Additionally, within each stem cell source (related versus unrelated cord versus unrelated adult donor), a formal interim analysis is executed after the first five patients in each group have recovered their ANC and platelet count after receiving gemtuzumab ozogamicin. If two or more of the first five patients experience secondary graft failure after gemtuzumab ozogamicin, the study will be discontinued secondary to futility. Off-study criteria included death, patient/parent withdrawal from protocol, and progressive disease with initiation of new therapy. One patient (#504-007) has been removed from the study secondary to leukemic relapse and initiation of new therapy. One patient (#504-006) who had a primary graft failure and went on to receive a fully ablative allogeneic SCT was also taken off the study when he relapsed on day +100 from the second transplant (day +190 from the reduced-intensity allogeneic SCT).

*Dose escalation of gemtuzumab ozogamicin.* Dose escalation was planned in groups of three subjects with an additional three subjects to be added at the first indication of dose-limiting toxicity. The four potential dose levels of gemtuzumab ozogamicin were as follows: dose level one, 4.5 mg/m$^2$/dose; dose level two, 6.0 mg/m$^2$/dose; dose level three, 7.5 mg/m$^2$/dose; and dose level four, 9.0 mg/m$^2$/dose. The maximal tolerable dose was defined as the dose level at which <1/3 of the subjects experience dose-limiting toxicity (<2/6 doses).

*Definition of dose-limiting toxicity of gemtuzumab ozogamicin.* Dose-limiting hematologic toxicity was defined as follows: neutrophil toxicity was defined as grade 4 neutropenia lasting ≥21 days and/or any incidence of grade 5 infection following gemtuzumab ozogamicin administration. Platelet toxicity was defined as grade 4 thrombocytopenia lasting ≥21 days and/or any incidence of grade 5 hemorrhage following gemtuzumab ozogamicin administration. Dose-limiting nonhematologic toxicity included any grade 3 or 4 nonhematologic toxicity attributable to gemtuzumab ozogamicin with the specific exclusion of grade 3 nausea and vomiting, grade 3 transaminase (aspartate aminotransferase/alanine aminotransferase) elevation which returned to grade <1 before the time for the next treatment course, or grade 3 fever or infection. All toxicities after reduced-intensity allogeneic SCT and after gemtuzumab ozogamicin were graded according to the National Cancer Institute Common Toxicity Criteria for bone marrow transplantation recipients (CTC 3.0) (40).

*Graft-versus-host disease prophylaxis.* Acute GVHD prophylaxis consisted of tacrolimus starting at 0.03 mg/kg/d as a continuous i.v. infusion or at 0.12 mg/kg/d given orally divided Q12h with dosage adjustment to maintain blood levels between 5 and 20 ng/mL and mycophenolate mofetil at 15 mg/kg or 900 mg/m$^2$ every 6 to 12 hours either orally or i.v. as we have previously described (41). Tacrolimus was started on day −7 and mycophenolate mofetil was begun on day +1. GVHD prophylactic medications were maintained for a minimum of 30 days post allogeneic SCT. Mycophenolate mofetil was stopped on day +30 if patient had grade ≤2 acute GVHD. Tacrolimus was tapered over 60 days beginning on day +60 if patient had grade ≤1 acute GVHD. Acute and chronic GVHDs were scored according to previously published guidelines (42, 43).

*Supportive care.* Hematopoietic growth factor support, isolation, and antibacterial, antifungal, and antiviral prophylaxes were done according to the institutional protocol. Briefly, all patients received GM-CSF (250 μg/m$^2$/d) i.v. daily from day 0 until the ANC ≥ 2,500/mm$^3$ for 2 consecutive days and then tapered every 2 days by 50% to maintain the ANC ≥ 1,000/mm$^3$. Once the ANC remained ≥ 1,000/mm$^3$ for 2 days at 75 μg/m$^2$/d, then GM-CSF was discontinued. Packed RBCs were transfused to maintain hemoglobin levels of 8.0 g/dL or more and platelets were transfused to keep the platelet count at 10,000/mm$^3$ or greater. All blood products were filtered and irradiated to 1,500 cGy. Herpes simplex prophylaxis consisted of acyclovir (250 mg/m$^2$) i.v. every 8 hours from day −5 until engraftment and grade ≤2 mucositis. *Pneumocystis carinii* prophylaxis consisted of trimethoprim/sulfamethoxazole until day −1 and then resumed thrice weekly after myeloid engraftment. Patients unable to tolerate trimethoprim/sulfamethoxazole received pentamidine i.v. every 2 to 4 weeks. Fungal prophylaxis consisted of liposomal



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**Table 1.** Patient demographics ($n$ = 8)

| Patient no. | Age (y) | Sex | Diagnosis | FAB | Cytogenetic abnormalities | Donor | TNC/kg ($\times 10^7$) | CD34/kg ($\times 10^5$) |
|---|---|---|---|---|---|---|---|---|
| 504-002 | 15 | M | AML CR1 | $M_{4/5}$ | No abnormalities | RPBSC | 98.56 | 50.27 |
| 504-004 | 0.5 | M | AML CR1 | $M_5$ | t(11;19)q23p13 | RCB | 5.64 | 2.66 |
| 504-005 | 14 | M | AML CR1 | $M_4$ | t(9;11)p22q23 | RPBSC | 112.92 | 50.31 |
| 504-006 | 3 | M | AML CR2 | $M_0$ | t(4;7) | UCB | 3.96 | 2.68 |
| 504-007 | 14 | F | AML CR2 | $M_4$ | 16q22 | RPBSC | 110.2 | 51.14 |
| 504-008 | 11 | M | AML CR1 | $M_5$ | t(3;5)(q21;q33), del(9)(q22q33) | RPBSC | 89.8 | 49.9 |
| 504-009 | 7 | M | APL CR2 | $M_3$ | t(15;17)(q22;21) | RPBSC | 33.7 | 50.2 |
| 504-010 | 7 | F | AML CR1 | $M_4$ | Not done | RPBSC | 106.1 | 75.2 |

Abbreviations: RPBSC, related peripheral blood stem cells; RCB, related cord blood; UCB, unrelated cord blood; TNC, total nucleated cells; FAB, French-American-British classification.

amphotericin B (3 mg/kg/d) i.v. starting at day 0 through day +100 as we have previously described (44). Cytomegalovirus prophylaxis for cytomegalovirus-negative donor and recipients consisted of cytomegalovirus-negative blood products only. Cytomegalovirus prophylaxis for recipients that were either serologically cytomegalovirus positive and or donor positive was alternating daily doses of ganciclovir (5 mg/kg/d) i.v. and foscarnet (90 mg/kg/d) i.v. starting at ANC $\geq$ 750/mm$^3$ $\times$ 2 days through day +100 as we have previously described (45).

***Engraftment, chimerism, and HLA typing.*** Myeloid engraftment (neutrophil recovery) was defined as an ANC $\geq$ 500/mm$^3$ for 2 consecutive days after the nadir. Platelet recovery was defined as a platelet count $\geq$ 20,000/mm$^3$ independent of platelet transfusions for at least 7 consecutive days. Whole-blood WBC donor chimerism was done using quantitative fluorescent-labeled variable number of tandem repeats utilizing loci *D1580*, *D1757*, *D15111*, and APO B–specific loci utilizing fluorescent-labeled oligonucleotide primers analyzed by Visible Genetics Open Gene System (Visible Genetics, Toronto, Ontario, Canada; ref. 46). Subset chimerism of specific WBC subsets was not done. HLA typing of donor and recipient was done by low-resolution serology for class I (HLA A and B) and high-resolution DNA typing for class II (HLA DRB1).

***Statistical Analysis.*** Results are presented as medians with specified ranges of data sets. Probabilities of myeloid and platelet engraftment after reduced-intensity allogeneic SCT and gemtuzumab ozogamicin were calculated with the Kaplan-Meier method done with the Prism statistical program (GraphPad, San Diego, CA).

## Results

***Patient demographics and disease status.*** From March 2003 to July 2005, eight patients with AML in either CR1 ($n$ = 5) or CR2 ($n$ = 3) received a reduced-intensity allogeneic SCT [matched family donor ($n$ = 6), unrelated cord blood ($n$ = 1), related cord blood ($n$ = 1)] at the Morgan Stanley Children's Hospital of NewYork-Presbyterian. Patients were evaluated for disease status immediately before reduced-intensity allogeneic SCT. Characteristics of the patients are summarized in Table 1. The median age was 9 years (0.5-15 years). The gender distribution was 6 M: 2 F. Cytogenetic abnormalities were present in six of seven patients (Table 1) and one patient did not have a cytogenetic analysis at initial diagnosis. Donor sources included six 6/6 HLA-matched related peripheral blood stem cells, one 6/6 sibling cord blood, and one 4/6 unrelated cord blood. The total nucleated cells per kilogram ranged from 3.96 to 112.92 $\times$ 10$^7$. The CD34$^+$ cell dose ranged from 2.66 to 75.2 $\times$ 10$^5$. The median total nucleated cells per kilogram and the median CD34$^+$ cell dose per kilogram inthe peripheral blood stem cell group were 102.3 $\times$ 10$^7$ and 50.29 $\times$ 10$^5$, respectively. The median total nucleated cells per kilogram and the median CD34$^+$ cell dose per kilogram in the cord blood group ($n$ = 2) were 4.8 $\times$ 10$^7$ (range 3.96-5.64 $\times$ 10$^7$) and 2.67 $\times$ 10$^5$ (range 2.66-2.88 $\times$ 10$^5$), respectively.

***Hematologic reconstitution after reduced-intensity allogeneic stem cell transplantation.*** The median time to myeloid engraftment after the reduced-intensity allogeneic SCT was 15 days (range 14-18 days) and platelet engraftment was 16 days (range 0-45 days; Fig. 1). Patient #504-006 had autologous reconstitution and was censored for both myeloid and platelet engraftment. Patient 504-008 had two episodes of dyspnea and chest tightness after receiving GM-CSF and was changed to filgrastim.

***Toxicity after reduced-intensity allogeneic stem cell transplantation.*** One patient developed grade 3 transaminase elevation but this patient had experienced previous grade 4 transaminase elevation during prior induction chemotherapy and subsequent pseudomonal necrotizing fasciitis. Infectious complications after reduced-intensity allogeneic SCT were three episodes of bacterial sepsis (resolved).

***Gemtuzumab ozogamicin and toxicity.*** Three patients received two doses of gemtuzumab ozogamicin at dose level one (4.5 mg/m$^2$/dose) following reduced-intensity allogeneic SCT. The next three patients received dose level two (6 mg/m$^2$/dose) but one of these patients had only one dose as she relapsed before the second dose was given. One patient was ineligible to receive gemtuzumab ozogamicin due to primary graft failure. The last patient is currently less than day +60 but will be given gemtuzumab ozogamicin at dose level two, when appropriate. Gemtuzumab ozogamicin was well tolerated with no grade 3 or 4 infusion-related reactions.

After each dose of gemtuzumab ozogamicin, all patients developed grade 3 or 4 neutropenia. Grade 3 or 4 thrombocytopenia was observed in four patients after dose 1 and in three patients after dose 2. Grade 3 or 4 infection was observed in four patients; one patient had bacteremia with *Agrobacterium radiobacter* and *Staphylococcus hominis* after the first dose of gemtuzumab ozogamicin and one patient had



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bacteremia with *Klebsiella pneumoniae* after the second dose. One patient developed bacteremia with three organisms after each dose of gemtuzumab ozogamicin. After the first dose, this patient became infected with *Acinetobacter lwoffi*, *Rhodococcus*, and *Pantoea agglomerans*, and after the second dose, *Acinetobacter baumannii*, *Acenitobacter lwoffi*, and diphtheroids. One patient had Gram-positive rods after the second dose of gemtuzumab ozogamicin. All episodes were treated with appropriate i.v. antibiotics and none of the patients required blood pressure support or admission to the intensive care unit. Central lines were removed for persistent positive blood cultures. All patients recovered without sequelae following each of these episodes of bacteremia.

Sinusoidal obstructive syndrome was not observed following gemtuzumab ozogamicin (0/11 doses). Two patients developed transient grade 3 aspartate aminotransferase and/or alanine aminotransferase elevation after the first dose of gemtuzumab ozogamicin; both of these patients had a preexisting history of elevated transaminases. There has been no hyperbilirubinemia. The maximal tolerable dose of gemtuzumab ozogamicin following reduced-intensity allogeneic SCT has not yet been determined. No patient has experienced dose-limiting toxicity following gemtuzumab ozogamicin. The study is currently accruing patients at gemtuzumab ozogamicin dose level two.

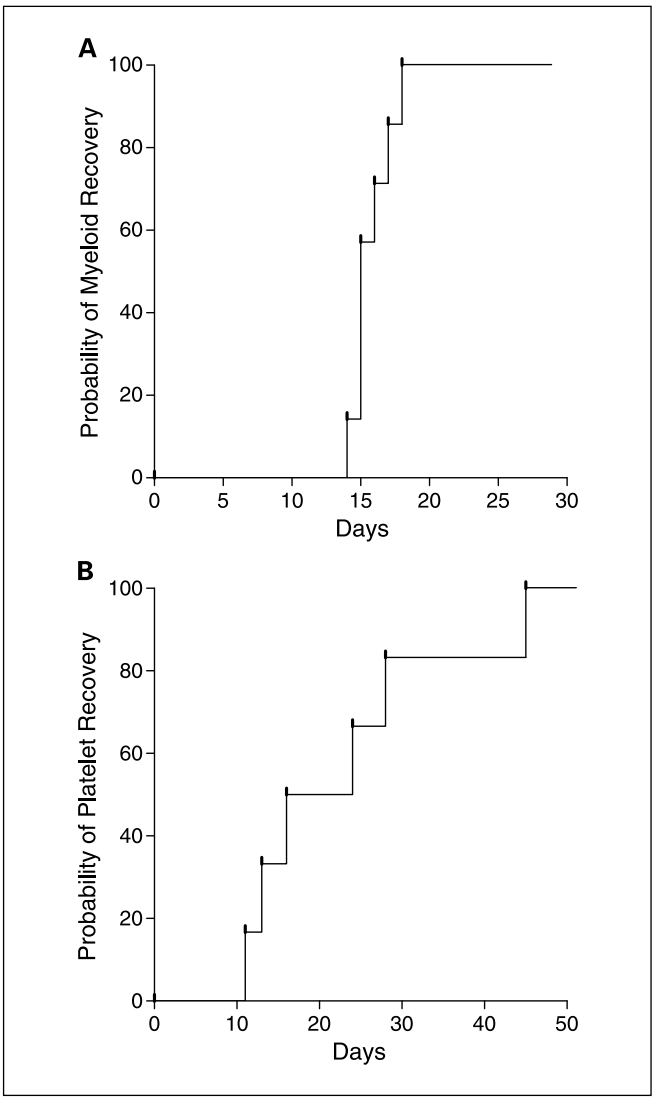



**Fig. 1.** *A,* probability of myeloid engraftment following reduced-intensity allogeneic SCT, determined by Kaplan-Meier method. *B,* probability of platelet engraftment following reduced-intensity allogeneic SCT, determined by Kaplan-Meier method.

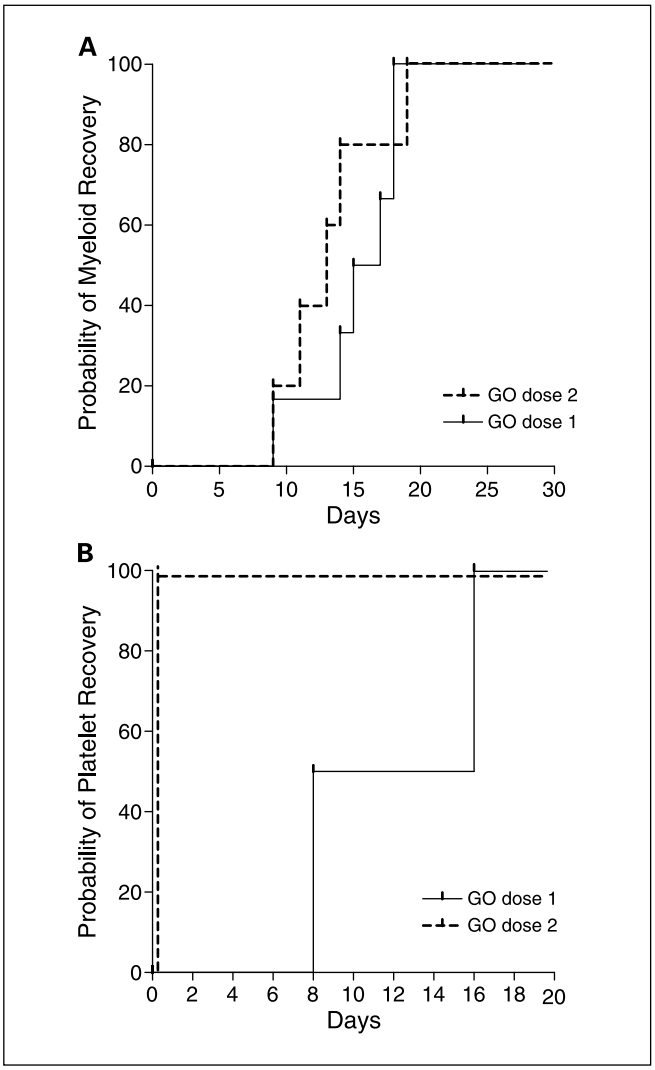



**Fig. 2.** *A,* probability of myeloid recovery following gemtuzumab ozogamicin dose 1 and 2, determined by Kaplan-Meier method. *B,* probability of platelet recovery following gemtuzumab ozogamicin dose 1 and 2, determined by Kaplan-Meier method.

***Hematologic reconstitution after gemtuzumab ozogamicin.*** Myeloid engraftment after gemtuzumab ozogamicin dose 1 and 2 occurred at median day 16 (range 9-18 days) and median day 13 (range 9-19 days), respectively (Fig. 2A). Platelet engraftment after gemtuzumab ozogamicin dose 1 occurred at median day 0 (range 0-16 days), and after dose 2, none of the patients had a platelet count < 20,000/mm$^3$ (Fig. 2B).

***Acute and chronic graft-versus-host disease.*** Patient #504-010 developed grade 3 gut acute GVHD on day +20, which responded to treatment with solumedrol (2 mg/kg/d). Patient #504-005 developed extensive *de novo* chronic GVHD on day +225 with skin, liver, mouth, and joint involvement.



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***Chimerism.*** Chimerism was determined on days +30, +60, +180, and +270. The median mixed donor chimerism for patients who achieved myeloid engraftment was 96 ± 2% at day +30 ($n$ = 7), 94 ± 3% at day +60 ($n$ = 3), 97 ± 9% at day +180 ($n$ = 4), and 95 ± 7% at day +270 ($n$ = 2; Fig. 3).

***Survival.*** Five patients are alive with a median follow-up of 231 days (range 39-838) and have an $M_1$ marrow. Patient #504-006, who never engrafted, received a fully ablative unrelated cord blood transplant and obtained full donor chimerism. However, on day +100 after the second unrelated cord blood transplant, the patient relapsed and died on day +238 from the reduced-intensity allogeneic SCT. Patient #504-007, who had received only one dose of gemtuzumab ozogamicin, relapsed on day +111; this patient was then treated with mitoxantrone, etoposide, and cyclosporine, but died on day +144. Patient #504-005 died on day +325 from complications of extensive chronic GVHD.

## Discussion

Our use of gemtuzumab ozogamicin as immunotherapy during a time of minimal residual disease, to date, has been safe and well tolerated. We have observed the expected grade 4 myeloid suppression following gemtuzumab ozogamicin and, to some extent, less than expected grade 4 thrombopoietic toxicity. Nine of eleven cycles of gemtuzumab ozogamicin have been given without grade 4 thrombocytopenia. There have been no cases of sinusoidal obstructive syndrome in our pilot study. Two patients had transient grade 3 aspartate aminotransferase/alanine aminotransferase elevation. The five patients who have received both doses of gemtuzumab ozogamicin have had complete recovery of their platelet counts and have maintained stable donor chimerism. The maximal tolerable dose of gemtuzumab ozogamicin following reduced-intensity allogeneic SCT in children with AML in CR1 or CR2 has yet to be determined in this study. Patient #504-010 is to receive gemtuzumab ozogamicin at dose level two (6 mg/m$^2$/dose), and if this dose is tolerated, the next patient will receive dose level three (7.5 mg/m$^2$/dose).

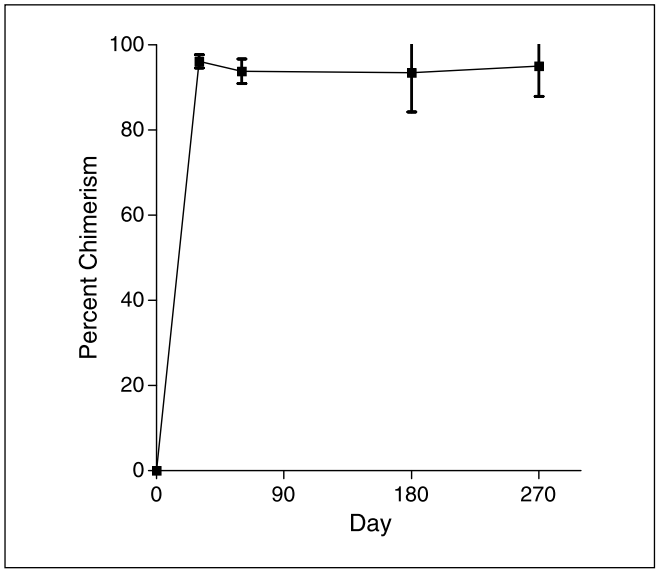



**Fig. 3.** Percent of mixed donor chimerism after reduced-intensity allogeneic SCT and gemtuzumab ozogamicin (excluding one patient with primary graft failure); day +30 ($n$ = 7), day +60 ($n$ = 6), day +180 ($n$ = 4), day +270 ($n$ = 2).

The study design included several features to protect the allograft following allogeneic SCT and to avoid sinusoidal obstructive syndrome, including initiation of gemtuzumab ozogamicin therapy at a minimum of day +60, starting at a dose of 4.5 mg/m$^2$/dose, which is half the dose used in adult phase II trials, and a minimum of 8 weeks before the second dose of gemtuzumab ozogamicin. Arceci et al. (6) showed the overall incidence of sinusoidal obstructive syndrome to be 24% (7 of 29 patients) with six of these seven patients receiving a hematopoietic stem cell transplant after exposure to gemtuzumab ozogamicin. Arceci et al.'s study also showed an increased risk of sinusoidal obstructive syndrome (40%) in patients who underwent hematopoietic stem cell transplantation <3.5 months after the last dose of gemtuzumab ozogamicin (6). Therefore, as there is a substantial risk of sinusoidal obstructive syndrome when gemtuzumab ozogamicin is given either before or after allogeneic SCT, we decided to give the two doses of gemtuzumab ozogamicin after the transplant as consolidative immunotherapy with the intention to eradicate any minimal residual disease at a minimum of day +60 and to provide a second dose of targeted immunotherapy at a minimum of day +116. Because one of the disadvantages of reduced-intensity allogeneic SCT is a risk of relapse, we hypothesized that we could reduce the risk of relapse by giving gemtuzumab ozogamicin as consolidative immunotherapy. Alternatively, the study design could have included one dose of gemtuzumab ozogamicin with the conditioning regimen and a second dose as consolidative therapy. Because the use of gemtuzumab ozogamicin after allogeneic SCT has been limited to relapsed AML, it is difficult to compare our results with others, as we have used it in the setting of potential minimal residual disease. Cohen et al. showed that in a study of eight relapsed AML adult patients receiving gemtuzumab ozogamicin at a median of 9 months after transplant (six autologous, two allogeneic), none had sinusoidal obstructive syndrome after the SCT; however, one of the eight (12.5%) developed sinusoidal obstructive syndrome and expired 14 days after gemtuzumab ozogamicin (47). This patient had several risk factors for developing sinusoidal obstructive syndrome, including a history of abnormal liver function tests and a history of two transplants, one autologous SCT, and one allogeneic SCT. Although our cohort was limited in size, there has been no sinusoidal obstructive syndrome and patients at increased risk for sinusoidal obstructive syndrome were successfully prophylaxed with enoxaparin.

In summary, we have shown in this pilot study that reduced-intensity allogeneic SCT followed by gemtuzumab ozogamicin is safe and well tolerated for pediatric patients with AML in CR1 or CR2. Patients who engrafted have maintained stable donor chimerism. The maximal tolerable dose of gemtuzumab ozogamicin following reduced-intensity allogeneic SCT in children with AML has yet to be determined. Limitations of our study, however, include both a small number of patients and variability in donor and stem cell sources within this pilot. In addition, the duration of follow-up is short, prohibiting conclusions about long-term complications or disease outcome after reduced-intensity allogeneic SCT followed by gemtuzumab ozogamicin. We speculate that gemtuzumab ozogamicin,



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as immunotherapy to eradicate any minimal residual disease, may decrease the incidence of relapse, which occurs more frequently in reduced-intensity allogeneic SCT. A larger cohort and longer follow-up period are required to appropriately address these issues.

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# Preliminary Results of the Safety of Immunotherapy with Gemtuzumab Ozogamicin following Reduced Intensity Allogeneic Stem Cell Transplant in Children with CD33$^+$ Acute Myeloid Leukemia

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