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ORIGINAL ARTICLE

Phase I/II study of gemtuzumab ozogamicin added to fludarabine, melphalan and allogeneic hematopoietic stem cell transplantation for high-risk CD33 positive myeloid leukemias and myelodysplastic syndrome

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We investigated the hypothesis that gemtuzumab ozogamicin (GO), an anti-CD33 immunotoxin would improve the efficacy of fludarabine/melphalan as a preparative regimen for allogeneic hematopoietic stem cell transplantation (HSCT) in a phase I/II trial. Toxicity was defined as grades III–IV organ damage, engraftment failure or death within 30 days. 'Response' was engraftment and remission (CR) on day +30. We sought to determine the GO dose (2, 4 or 6 mg m^{-2}) giving the best trade-off between toxicity and response. All patients were not candidates for myeloablative regimens. Treatment plan: GO (day −12), fludarabine 30 mg m^{-2} (days −5 to −2), melphalan 140 mg m^{-2} (day −2) and HSCT (day 0). GVHD prophylaxis was tacrolimus and mini-methotrexate. Diagnoses were AML ($n=47$), MDS ($n=4$) or CML ($n=1$). Median age was 53 years (range, 13–72). All but three patients were not in CR. Donors were related ($n=33$) or unrelated ($n=19$). Toxicity and response rates at 4 mg m^{-2} were 50% ($n=4$) and 50% ($n=4$). GO dose was de-escalated to 2 mg m^{-2}: 18% had toxicity ($n=8$) and 82% responded ($n=36$). 100-day TRM was 15%; one patient had reversible hepatic VOD. Median follow-up was 37 months. Median event-free and overall survival was 6 and 11 months. GO 2 mg m^{-2} can be safely added to fludarabine/melphalan, and this regimen merits further evaluation.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS) and other myeloid leukemias. HSCT has generally utilized high-dose chemotherapy and/or radiation as the preparative regimen, but because of the associated toxicities, use of this modality largely has been limited to younger patients without major comorbidities. However, AML is a disease of the elderly with a median age of 68 years. Older patients with this disease have a high rate of poor prognosis characteristics and most cannot achieve complete remission with available chemotherapy.[1]

There are two components to anti-leukemia effects of allogeneic transplants: cytoreduction by the conditioning regimen and the immune-mediated graft-versus-leukemia effect (GVL). Recently, reduced intensity preparative regimens have been developed with the goal of achieving engraftment with acceptable toxicity, allowing the GVL effect to occur. This approach has allowed successful extension of HSCT to patients in the 6th and 7th decades of life.

The optimal reduced intensity regimen for older patients with AML is unknown. We previously have reported that a more intensive preparative regimen reduces the risk of relapse in patients with advanced AML.[2] Thus, patients with active disease may benefit from greater cytoreduction if it can be achieved without excessive toxicity.

Disease status is the major determinant of treatment success. Reported long-term event-free survival (EFS) rates for patients with relapsed/refractory disease is <30%, and outcome of AML or MDS relapsing after allogeneic transplantation is usually dismal.[3,4] A significant fraction of failures are potentially related to poor disease control with the conditioning regimen. Unfortunately, this patient population is unlikely to benefit from 'classic' dose escalation approaches, given prohibitive increases in non-relapse mortality. Clearly, innovative strategies are needed in order to increase the anti-leukemic activity of allogeneic transplantation in these patients. One potential approach is the incorporation of targeted monoclonal antibodies or immunotoxins in the preparative regimen, as has been done successfully in lymphomas.[5]

Gemtuzumab ozogamicin (GO; Wyeth Pharmaceuticals, Radnor, PA, USA) is an immunoconjugate that targets the CD33 antigen expressed by myeloid leukemias. The humanized murine IgG4 monoclonal antibody is conjugated with calicheamicin, a cytotoxic enediyene antibiotic that causes DNA double-strand DNA breaks and ultimately apoptosis. A response rate of 26% (13% CR) was observed in a cohort of 277 patients with AML in first relapse (median age of 61 years) treated with single agent GO 9 mg m^{-2} in two doses separated by 2 weeks,[6] which provided significant however short-lived cytoreduction in a substantial fraction of patients in that high-risk cohort. The major toxicity of GO is myelosuppression, although hepatic veno-occlusive disease may also occur.

We hypothesized that GO could be added to fludarabine/melphalan (FM) in order to improve efficacy of this reduced-intensity preparative regimen while also controlling toxicity. Our primary objective was to determine the safety and optimal dose of GO, when given in combination with fixed doses of FM for patients undergoing allogeneic HSCT.

Eligibility criteria

Patients were required to be aged 12–75 years and ineligible for higher dose myeloablative preparative regimens because of

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concurrent medical conditions or older age. Minimal physiologic parameters were to be met as follows: ECOG performance status $\leqslant 2$, serum creatinine $\leqslant 2.0\,mg\,dl^{-1}$, direct bilirubin $\leqslant 2$, serum GPT $\leqslant$ three times the upper normal limit, left ventricular ejection fraction $\geqslant 35\%$ and DLCO $\geqslant 40\%$.

Disease-specific requirements included AML not in first remission, MDS with an international prognostic score[7] of intermediate-2 or high-risk disease, or Gleevec resistant-CML in accelerated phase or blast crisis, with leukemia cells expressing CD33 as evaluated by flow cytometry (positivity defined at $\geqslant 20\%$). Patients who received prior HSCT were eligible. Patients were required to have a human leukocyte antigen (HLA)-compatible related (6/6 or 5/6 HLA-match at HLA-A, -B, -drb1)) or a 6/6 HLA-matched unrelated donor (MUD). Peripheral blood was the preferred source of stem cells. Exclusion criteria included uncontrolled active infection, HIV disease, pregnancy, nursing and active central nervous system disease. The MD Anderson Cancer Center IRB approved and monitored this study. All patients signed an informed consent.

Treatment plan

Patients received GO 12 days before HSCT to allow clearance of the immunotoxin in order to minimize possible interference with engraftment. Fludarabine $30\,mg\,m^{-2}$ was given intravenously daily on days -5, -4, -3, -2. Melphalan $140\,mg\,m^{-2}$ was administered intravenously on day -2, and HSCT on day 0. Patients with a MUD or mismatched related donor received rabbit anti-thymocyte globulin $0.5\,mg\,kg^{-1}$ (day -3) and $1.25\,mg\,kg^{-1}$ (days -2, -1). Initially, we planned to study GO doses 4, 6, 9 and $16\,mg\,m^{-2}$. The study was initiated at $4\,mg\,m^{-2}$, but when dose-limiting toxicity occurred at this dose, the trial was re-designed to evaluate the three doses 2, 4, $6\,mg\,m^{-2}$. Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and mini-dose methotrexate $5\,mg\,m^{-2}$ on days 1, 3, 6 and 11 post-transplant.[8] Tacrolimus was to be continued for 6 months or as indicated to control GVHD.

Antiemetics, prophylactic antibiotics, CMV prophylaxis, blood products and other supportive measures were utilized according to institutional practices. All patients received filgrastim (Neupogen, Amgen Inc, Thousand Oaks, CA, USA) $5\,\mu g\,kg^{-1}$ sc. daily from day $+7$ until achievement of an absolute neutrophil count (ANC) $>1.5\times10^9$ per liter for 3 days. Patients developing grade II or greater acute GVHD received methylprednisolone $2\,mg\,kg^{-1}$ per day as initial treatment.

Procurement of hematopoietic stem cells

Filgrastim mobilized peripheral blood progenitor cells were collected to achieve a cell dose of approximately 5×10^6 CD34+ cells per Kilogram. Stem cell products from unrelated donors were obtained through the National Marrow Donor Program.

Human leukocyte antigen typing

HLA typing for class I antigens (HLA-A, -B and -C) was performed using standard serologic and molecular techniques. Class II alleles (HLA-DRB1, -DQB1) were resolved with high resolution molecular typing. Class I alleles (HLA-A and -B) were resolved using high resolution molecular typing in all unrelated donor-recipient pairs.

Donor-recipient hematopoietic chimerism

Hematopoietic donor-recipient chimerism was evaluated by analysis of DNA microsatellite polymorphisms by polymerase chain reaction with D6S264, D3S1282, D18S62 and D3S1300 fluorescence-labeled primers. Mixed chimerism was defined as the presence of any detectable percentage of recipients DNA.

Definitions

Patients were scored as 'primary induction failure' after failing two or more courses of induction chemotherapy. Patients with persistent leukemia after high-dose Ara-C-containing therapy were labeled as 'refractory'. CR prior to HSCT was defined as less than 5% blasts in the bone marrow, with a platelet count $>100\times10^9$ per liter and an ANC $>1.5\times10^9$ per liter, and diploid cytogenetics. CR after transplant was defined as above, without the need for platelets $>100\times10^9$ per liter, with documentation of donor cell engraftment. Neutrophil and platelet engraftment were defined as the first of 3 consecutive days in which the ANC was $>0.5\times10^9$ per liter, and the first of 7 consecutive days in which a platelet count $>20\times10^9$ per liter was achieved without transfusions, respectively. Acute and chronic GVHD grading is described elsewhere.[9,10] Non-relapse mortality was death of any cause other than disease relapse or progression.

Statistical methods

Dose-finding: because the primary goal was to determine a dose of GO providing a desirable level of efficacy while still controlling toxicity, the Bayesian phase I/II dose finding method of Thall and Cook,[11,12] was employed. This method chooses doses based on both response and toxicity, and accounts for the trade-off between these two outcomes. For dose-finding, 'toxicity' was defined as grade III or IV renal, hepatic, intestinal, neurological, pulmonary or cardiac toxicity (NCI Common Toxicity Criteria version 1), graft failure or death during the first 30 days, and 'response' was defined as engraftment and CR on day 30. The scientific objective was to determine the best dose of GO having acceptable toxicity probability $\leqslant 0.24$ and acceptable response probability $\geqslant 0.69$, among the three doses 2, 4, $6\,mg\,m^{-2}$. These numerical acceptability limits were obtained from our historical data using the FM regimen.[13,14] Using this method, each cohort of patients is given the best acceptable dose, based on the dose-outcome data from the patients treated previously in the trial. Subject to the acceptability constraints, each cohort may receive a dose that is the same as, below, or above the dose given to the previous cohort. The 'best' dose is defined as that giving the largest response-toxicity trade-off. Figure 1 shows the trade-off contours used to conduct the GO trial, with all (Prob(response), Prob(toxicity)) pairs on each contour equally desirable.

Unadjusted overall and EFS were estimated using the method of Kaplan and Meier.[15] Unadjusted between-group comparisons of survival and EFS were made using the log-rank test.[16] The Cox proportional hazards regression model[17] was used to assess the ability of patient characteristics or treatments to predict survival and EFS, with goodness-of-fit assessed by the Grambsch–Therneau test[18] and martingale residual plots. CD33 percentage expression was fit as a continuous predictor in the Cox models. Fisher's exact test was used to assess possible associations between categorical variables. All computations were carried out in Splus.[19]

Results

We treated 52 patients with GO FM in this study. Their median age was 53 (range, 13–72) years (Table 1). The trial was

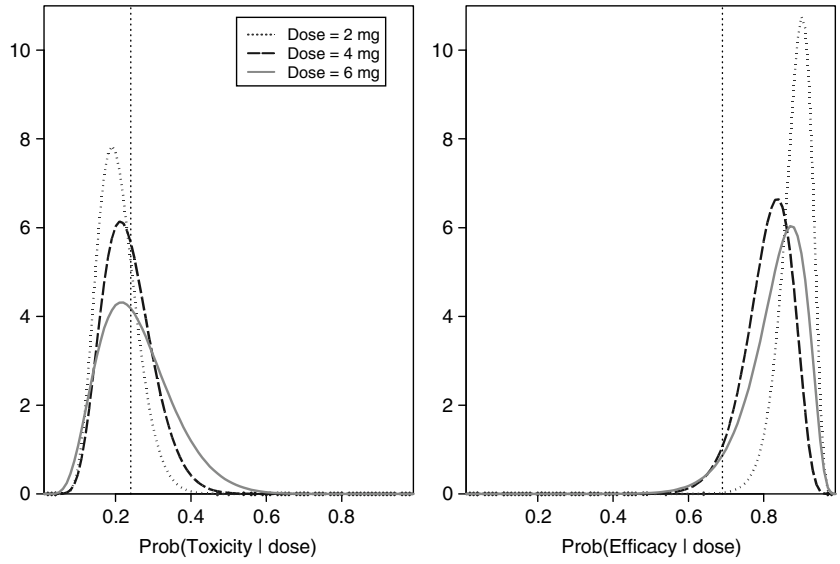

Figure 1 Posterior distributions of the probability of toxicity and the probability of response for each dose of GO. The posterior distributions at dose 6 mg m^{-2} are a consequence of the assumed model, since no patients were treated at that dose, and hence these are predictions. To interpret Figure 1, it must be kept in mind that, under the Bayesian model used for dose-finding, π_T (2 mg), π_T (4 mg), π_T (6 mg), represented by the curves in the left-hand figure and π_E (2 mg), π_E (4 mg), π_E (6 mg) in the right-hand figure are each random quantities, and thus each is represented by a curve for its posterior probability distribution based on the final data from the trial. For example, the posterior distribution of π_T (6 mg), given by the solid curve in the left-hand figure, has mode at about 0.20 and has the largest area to the right of the upper limit 0.25 for $\pi_{Toxicity}$ used in the trial, so it has the highest risk of toxicity. An interesting feature of these posteriors is that the lowest dose, 2 mg m^{-2}, has both the lowest toxicity and the highest efficacy, on average. The right-hand plots are, in fact, contrary to the conventional belief that response rate should increase with dose, which was not the case in this trial.

conducted from March 2002 to June 2006, and this report is based on data collected as of November 2006. Median follow-up was 37 months (range, 2–69). One patient has been lost to follow-up. AML was the most common diagnosis (90%), and 49 (94%) were not in remission at transplant. FLT3 mutations were present in 11% of 38 tested patients, and high-risk cytogenetics in 39% of 52 subjects. The median time from diagnosis to transplant was 14.4 months (range, 2.6–80.8). Most participants failed high-dose Ara-C treatment (94%), and the median number of chemotherapy cycles prior to transplant was five. A total of 11 patients received a previous allogeneic transplant (19%).

Seven patients (14%) had previously received GO-based salvage therapy prior to enrollment in this study. The median number of GO courses was 2 (range, 1–5) and the median time from last GO dose and transplant was 8.5 months (range, 1.5–35). Our patients were selected primarily due to their inability to undergo myeloablative regimens, and accordingly, the median Charlson's comorbidity score[20] was high, at three.

Patients treated at the two GO dose levels 2 and 4 mg m^{-2} had similar characteristics. Median bone marrow blasts were 17 and 19% (P=not significant (NS)), respectively, for the lower and higher dose subgroups, while the 4 mg m^{-2} patients were younger (30 versus 56 years, $P=0.04$). In spite of no statistical difference, 4 mg m^{-2} patients had a lower proportion of recipients of MUD transplants (29 versus 38%), and a higher proportion of recipients of a previous allogeneic transplant (29 versus 19%).

Gemtuzumab ozogamicin dose

Of eight patients receiving GO 4 mg m^{-2}, two died early (due to pneumonia and renal failure) and two developed grade III gastro-intestinal and renal toxicity. In brief, 4 of 8 patients treated at 4 mg m^{-2} had toxicity, 4 had response. Of the four responders, two had no toxicity. This led the design to de-escalate to 2 mg m^{-2}, and the algorithm did not re-escalate thereafter. Of 44 patients then treated at 2 mg m^{-2}, 8 (18%) experienced toxicity and 36 (82%) responded, with the best outcome (response without toxicity) achieved in 31 (70%) patients at this dose. At the selected dose of 2 mg m^{-2}, the response rate of 86% (31/36) among the patients without toxicity was slightly higher than the response rate of 62% (5/8) among patients with toxicity. Although this negative association between response and toxicity at 2 mg m^{-2} was not statistically significant ($P=0.14$), it may explain in part why a higher overall response rate was seen at a lower dose of GO (82% at 2 mg m^{-2} versus 50% at 4 mg m^{-2}, $P=0.07$). Figure 1 gives the posterior distributions of the probabilities of toxicity and response for each dose of GO, showing that the lowest dose (2 mg m^{-2}) had a higher response rate and lower toxicity rate than 4 mg m^{-2}. Furthermore, the fitted model indicates, that the higher dose of 6 mg m^{-2} would be unlikely to improve the clinical results described here.

GO at 2 or 4 mg m^{-2} induced similar decreases in white blood cell and platelet counts (Figure 2). The median white cell count on day −12 (prior to GO administration) was 1.600 per mm^3 (range, 0.2–13.4) versus 1.100 per mm^3 (range, 0.1–24.6) on day −5, prior to initiation of fludarabine ($P=0.002$, Wilcoxon signed-rank test for paired data). The median platelet count on day −12 was 40 000 per mm^3 (range, 4–393), while it was 27 000 per mm^3 on day −5 (range, 5–499) ($P=0.0002$, Wilcoxon signed-rank test for paired data). Among patients with circulating blasts at initiation of GO ($n=17$), 50% had a decrease in the blast count prior to HSCT, while 6% had stable numbers and 44% had an increase (median number of blasts of 165 versus 220, P=NS). One patient did not receive the complete conditioning regimen and was not available. There

was a significant association between presence of bone marrow and peripheral blood blasts ($P=0.01$) and between bone marrow blasts and white cell count at study entry ($P=0.04$).

Table 1 Patient and disease characteristics

Variable	N = *52 patients*
Median age (years)	53 (range, 13–72)
Diagnosis	
AML	47 (90%)
MDS	4 (8%)
CML	1 (2%)
Disease status	
Complete remission	3 (6%)
Primary refractory	15 (29%)
Relapse	32 (63%)
First[a]	16
Second	16
Chemotherapy naïve MDS	1 (2%)
CML in second blast crisis	1 (2%)
Cytogenetics risk category[3,7]	
Intermediate	32 (61%)
Poor	20 (39%)
Previous allogeneic transplant	11 (21%)
Previous autologous transplant	3 (5%)
Therapy-related AML/MDS	8 (15%)
AML evolving from MDS	12 (23%)
Previous treatment with high-dose Ara-C	47 (94%)
Median number of chemotherapy cycles prior to HSCT	5 (range, 1–13)
Median Charlson's[b] comorbidity score[20] ($n=48$)	3 (range, 2–7)
	$\geqslant 2$ (92%)
	>2 (62%)
Median bone marrow blasts at study entry	17% (range, 0–95%)
Median bone marrow blast CD33 expression	89% (range, 22.5–99.6%)
Median platelet count at start of preparative regimen (per mm^3)	40 000 (4–393)
Median white cell count at start of preparative regimen (per mm^3)	1.600 (0.2–13.4)

Abbreviations: AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; HSCT, hematopoietic stem cell transplant; MDS, myelodysplastic syndrome.
[a]First relapse untreated ($n=6$) or refractory ($n=10$); median first remission duration 5.8 months (range, 1.5–25 months).
[b]Charlson's comorbidity score: data not available for four patients; score includes primary diagnosis.

Engraftment and chimerism

Donors were HLA-identical related in 59% ($n=31$) of the cases, unrelated in 37% ($n=19$) and mismatched related in 4% ($n=2$). Stem cell source was the bone marrow in 17% ($n=9$) and peripheral blood in 83% of the cases ($n=43$). Median infused CD34 and nucleated cell count was 4.54 (range, 1.73–15.41) and 5.75 (range, 0.59–13.88).

GO did not appear to affect engraftment. Neutrophil engraftment occurred in 96% of the patients, at a median of 13 days (range, 8–21), while 81% achieved platelet transfusion independence ($n=42$) at a median of 18 days (range, 9–52). A total of 29 patients achieved a platelet count $>100\,000$ per mm^3 (56%), at a median of 34 days (range, 16–295). Median donor cell chimerism was 100% (range, 0–100); 92% of the patients were 100% donor on the initial evaluation at approximately day 30 ($n=48$); one patient died early after engraftment, one patient had persistent disease with mixed chimerism and two died prior to neutrophil recovery.

Disease response

Among the 49 patients not in CR at HSCT, the remission rate was 90% ($n=44$), while three patients transplanted in remission remained in CR. Four patients (8%) were scored as 'early deaths' (deaths occurring during the first 30 days), and one patient did not respond (2%).

Toxicity, mortality and GVHD

Regimen-related toxicity was scored on day $+30$, and did not include GVHD-related or metabolic complications, such as electrolyte disturbances. Any death unrelated to leukemia relapse during the first 30 days was scored as 'Toxicity', as well as any grade III or IV renal, hepatic, intestinal, neurological, pulmonary or cardiac toxicity. Regimen-related toxicities are summarized in Table 2. Toxicity occurred in four (50%) of eight patients receiving GO 4 mg m^{-2} and in 8 (18%) of 44 patients treated at 2 mg m^{-2}. Grade III or greater toxicity rate was 10% for intermediate risk cytogenetics patients versus 35% for subjects with high-risk cytogenetics ($P=0.04$, Fisher's exact test). The number of previous chemotherapy cycles did not correlate with toxicity ($P=0.13$, Wilcoxon rank-sum test). One of the seven patients previously exposed to GO developed non-hepatic toxicity, and none had hepatic VOD.

Non-relapse mortality rate was 6, 13 and 29%, respectively, at 30 days, 100 days and 1 year after transplant. The corresponding numbers for those receiving GO 2 and 4 mg m^{-2} were 5 and 12.5% (30 days), 11 and 25% (100 days) and 27 and

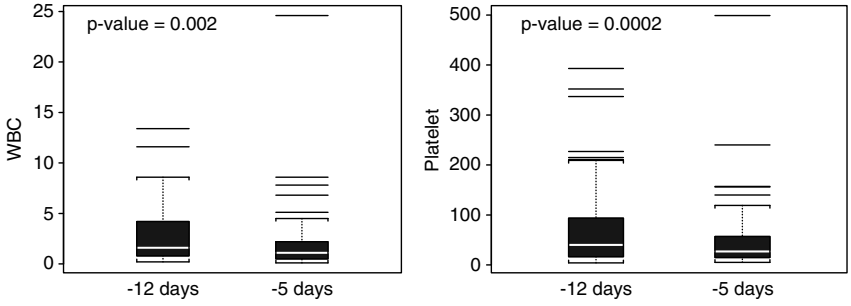

Figure 2 Effect of GO 2 or 4 mg m^{-2} on white blood cell and platelet counts. Box plots for white cell (left side) and platelet count (right side) on day -12 (prior to GO administration) and on day -5 (before fludarabine administration There was no statistically significant difference between the two GO dose levels in terms of the changes in white cell ($P=0.95$) or platelet ($P=0.35$) counts between day -12 and day -5. The data is therefore presented combining the two dose levels. '*P*' values determined using Wilcoxon signed-rank test for paired data.

Table 2 Regimen-related toxicity[a]; (defined as non-hematological toxicities documented during preparative regimen administration and the first 30 days after transplant)

Organ/system	*Grade I*	*Grade II*	*Grade III*	*Grade IV*
	N (%)	N (%)	N (%)	N (%)
Gastro-intestinal tract				
Mucositis	18 (35%)	22 (42%)	3 (6%)	0
Diarrhea	22 (42%)	14 (27%)	3 (6%)	0
Nausea/vomiting	14 (27%)	31 (70%)	0	0
Lower intestinal bleeding	1 (2%)	0	4 (8%)	0
Abdominal pain	8 (15%)	13 (25%)	0	0
Liver				
Ascites	2 (4%)	0	0	0
Veno-occlusive disease	Moderate = 1	(2%)	0	0
Transaminase elevation	9 (17%)	8 (15%)	1 (2%)	0
Bilirubin elevation	12 (23%)	5 (10%)	1 (2%)	0
Genito-urinary				
Creatinine elevation	10 (19%)	6 (12%)	1 (2%)	1 (2%)
Hemorrhagic cystitis	4 (8%)	4 (8%)	0	0
Metabolic[b]	27 (52%)	47 (90%)	5 (10%)	0
Neurological				
Headache	13 (25%)	10 (19%)	0	0
Confusion	3 (6%)	3 (6%)	1 (2%)	0
Extra-pyramidal	1 (2%)	2 (4%)	1 (2%)	0
Dizziness	5 (10%)	0	0	0
Mood changes	8 (15%)	5 (10%)	0	0
Visual changes	4 (8%)	7 (13%)	0	0
Constitutional symptoms[c]	35 (67%)	16 (31%)	2 (4%)	0
Fever	5 (10%)	12 (23%)	4 (8%)	0
Bone pain	6 (12%)	5 (10%)	1 (2%)	0
Skin rash	9 (17%)	14 (27%)	0	0
Cardiovascular[d]				
Hypotension	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Hypertension	3 (6%)	7 (13%)	1 (2%)	0
Cardiac arrhythmia	3 (6%)	3 (6%)	1 (2%)	0
Edema	5 (10%)	12 (23%)	0	0
Respiratory[e]				
Cough	7 (13%)	5 (10%)	0	0
Pleural effusion	4 (8%)	1 (2%)	1 (2%)	0
Pulmonary hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Dyspnea	6 (12%)	16 (31%)	4 (8%)	1 (2%)
Upper respiratory Hemorrhage/epistaxis	1 (2%)	2	0	1 (2%)

Abbreviations: FM, fludarabine/melphalan; GO, gemtuzumab ozogamicin; HSCT, hematopoietic stem cell transplantation.
Percentages refer to proportion of patients developing the toxicity. Single patients may have had multiple toxicities.
[a]No severe toxicities were documented during or after GO administration. Fever and chills were the most commonly documented events. The drug was given in an outpatient basis for most patients, and two patients were admitted to the hospital after GO due to fever and neutropenia. All patients received FM and HSCT in the inpatient service.
[b]Includes electrolyte and glicemia abnormalities.
[c]Includes asthenia, hiccups, skin dryness, hot flashes, lethargy.
[d]Not included: grade I ischemic abnormalities ($n=1$), pericarditis ($n=1$) and grade III thrombotic thrombocytopenic purpura.
[e]Not included: grade II pulmonary edema $N=1$), grade III pneumothorax ($n=1$), grade II ($n=2$) and grade III ($n=1$) pleuritic pain.

38% (1 year), respectively. During the first 100 days, causes of death other than disease progression included pneumonia ($n=1$) and renal failure ($n=1$) for patients treated with GO 4 mg m^{-2}, and acute GVHD ($n=1$), sepsis with pulmonary bleeding ($n=3$) and pneumonia ($n=1$) for GO 2 mg m^{-2} patients. Overall, 34 (65%) patients have died. The median time to death was 11.2 months (95% CI: 7.3-NA months). Non-relapse mortality included GVHD ($n=8$), toxicity ($n=5$), infections ($n=2$) and other causes ($n=2$). Acute grades II–IV and III–IV GVHD rates were 42% ($n=21$) and 22% ($n=11$), respectively, while chronic GVHD rate was 52% ($n=23$).

Disease relapse, overall and event-free survival

A total of 39 patients progressed or died. The median time to event was 5.9 months (95% CI: 4.8–20.8 months). Figure 3 shows the Kaplan–Meier estimates of overall survival and EFS. The fitted model for EFS shows that log (WBC at entry), use of unrelated donors and higher leukemic blasts CD33 expression were each predictive of poorer EFS (Table 3a), and the same variables were predictive of overall survival (Table 3b). GO dose (2 versus 4 mg m^{-2}) did not affect either overall or EFS.

Discussion

In this study we demonstrate that GO can be safely added to the fludarabine and melphalan reduced intensity preparative regimen, and that the 2 mg m^{-2} dose was less toxic while appearing to have the same efficacy as the 4 mg m^{-2} dose. Our cohort had a median age of 53 years and was characterized by disease refractoriness and a high prevalence of comorbid conditions. Schmid *et al*.[21] recently reported worse survival in patients receiving more than two courses of pretransplant chemotherapy. The patients in this study had received a median of five chemotherapy cycles prior to study entry.

This is a challenging setting to determine toxicity of any agent, since the combination of chemotherapy drugs, immunodeficiency and GVHD can make assigning particular side effects to the drug a difficult task. Therefore, any serious organ toxicity or early death was ascribed to the treatment (that is, preparative regimen). The Thall–Cook dose-finding method allowed us to simultaneously take toxicity and efficacy into account, minimizing the number of patients treated at doses with either unacceptably high toxicity or unacceptably low efficacy. It succeeded in identifying the lowest dose as being both safer and more effective than 4 mg m^{-2}. If response had been ignored, as is done with conventional dose-finding methods, a traditional phase I algorithm likely would have identified the same dose but would have treated far fewer patients, so it would have been necessary to conduct a subsequent phase II trial. In contrast, the Bayesian phase I/II method moved seamlessly from phase I into the phase II portion.

We utilized a relatively low, but biologically active dose of GO. Our data are consistent with the study of Burnett *et al*.[22] outside of the HSCT setting, where they reported the safety and efficacy of GO 3 mg m^{-2}, which was added to standard induction chemotherapy, reducing the relapse risk among 1115 patients treated in the MRC AML15 trial.

The level of toxicity observed here was acceptable, considering the patients' disease status, comorbidities and age. In our historic experience using melphalan 140 mg m^{-2} with fludarabine, we observed grade III or greater toxicity rates of 38%, similar to that documented here with GO 2 mg m^{-2}. We had only one case of reversible hepatic VOD, and a somewhat increased frequency of gastro-intestinal side effects. Higher doses of GO (9 mg m^{-2}) have been associated to hepatic VOD. Phase II studies in AML indicate an incidence of 0.9% for patients that did not have a previous transplant, and of 19% for

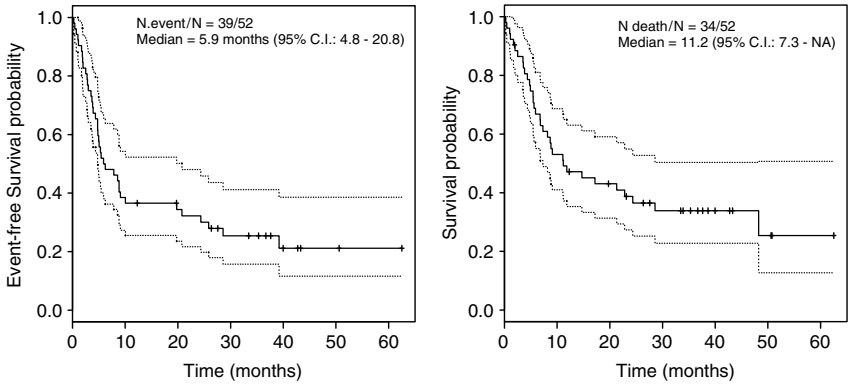

Figure 3 Event-free and overall survival of 52 patients treated with GO, fludarabine/melphalan and hematopoietic stem cell transplantation.

Table 3 Cox proportional hazards models for event-free and overall survival

Variable	*Relative Risk*	*95% Confidence Interval*	P-*value*
Event-free survival			
Log (WBC at study entry)	0.68	0.50–0.92	0.01
CD33 expression	1.02	1.00–1.04	0.05
Donor (related versus unrelated)	0.56	0.27–1.14	0.11
Overall survival			
Log (WBC at study entry)	0.70	0.50–0.97	0.03
CD33 expression	1.02	1.00–1.04	0.07
Donor (related versus unrelated)	0.37	0.17–0.80	0.01

Abbreviation: WBC, white blood cell count.

previously transplanted patients.[6] We did not observe an increase in hepatic or other toxicity among patients that received GO prior to treatment in this study, as observed by Wadleigh *et al*.[23] This may be due to the relatively long time from prior GO treatment to study entry.

In our analysis, blast CD33 expression was inversely correlated with event-free and overall survival. This effect was independent of the blast percentage in the bone marrow. We have no clear explanation for this. Others have found that higher CD33 levels correlate with better response to GO salvage monotherapy, although the statistical association was not present when adjusted for P-glycoprotein activity.[24] Since GO here was used in lower doses, in combination with other chemotherapy agents and donor cells, it is difficult to ascertain the impact of this observation, which should be confirmed in larger number of patients. Our multivariate analysis also showed that use of unrelated donors correlated with poorer outcomes. Accordingly, in our historic experience with the FM regimen, the subset of older patients receiving unrelated donor transplants for relapsed disease had the highest non-relapse mortality rates.[3] Presence of circulating blasts, higher white cell count and higher number of bone marrow blasts prior to transplantation have been shown to impact negatively survival and EFS of relapsed AML patients. These covariates were highly correlated, and did not retain their prognostic effect in this study. It is possible that the addition of GO is at least partially responsible for that lack of effect. Also, the relatively small sample size, comprised mostly of patients with several other poor prognostic characteristics, may have prevented adequate documentation of any possible interaction.

We postulated that the combination of monoclonal antibody and chemotherapy would increase the anti-leukemic cytoreduction achieved by the regimen. Among our patients with refractory AML or MDS treated with the fludarabine and melphalan regimen without GO, the median event-free and overall survival was 3 and 6 months, respectively. The results reported here would insinuate an improvement. However, this remains to be proven in a randomized controlled comparison.[2,14]

Our results suggest that this regimen of GO fludarabine and melphalan should be investigated in larger, controlled trials, ideally in less heavily pretreated patients. Furthermore, the EFS prolongation documented here may provide time for post-transplantation interventions to be conducted for relapse prevention. Currently, we are exploring the use of maintenance therapy with low-dose 5-azacitidine post-HSCT using the GO FM regimen.

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