Multi-Institutional Study of Post-Transplantation Cyclophosphamide As Single-Agent Graft-Versus-Host Disease Prophylaxis After Allogeneic Bone Marrow Transplantation Using Myeloablative Busulfan and Fludarabine Conditioning

Christopher G. Kanakry, Paul V. O'Donnell, Terry Furlong, Marcos J. de Lima, Wei Wei, Marta Medeot, Marco Mielcarek, Richard E. Champlin, Richard J. Jones, Peter F. Thall, Borje S. Andersson, and Leo Luznik

Listen to the podcast by Dr van Besien at www.jco.org/podcasts

Christopher G. Kanakry, Marta Medeot, Richard J. Jones, Leo Luznik, Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD; Paul V. O'Donnell, Terry Furlong, Marco Mielcarek, Fred Hutchinson Cancer Research Center, Seattle, WA; Marcos J. de Lima, Wei Wei, Richard E. Champlin, Peter F. Thall, Borje S. Andersson, The University of Texas MD Anderson Cancer Center, Houston, TX.

Published online ahead of print at www.jco.org on September 29, 2014.

Supported in part by Otsuka Pharmaceutical and Grant No. P01-CA015396 from the National Institutes of Health.

Both C.G.K. and P.V.O. share first authorship of this article.

Presented at the 49th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, May 31-June 4, 2013.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00809276.

Corresponding author: Leo Luznik, MD, Cancer Research Building I, Room 2M88, 1650 Orleans St, Baltimore, MD 21287; e-mail: luznik@jhmi.edu.

© 2014 by American Society of Clinical Oncology

0732-183X/14/3231w-3497w/\$20.00

DOI: 10.1200/JCO.2013.54.0625

ABSTRACT

Purpose

The clinical safety and efficacy of intravenous busulfan and fludarabine (IV Bu/Flu) myeloablative conditioning as well as graft-versus-host disease (GVHD) prophylaxis with high-dose, post-transplantation cyclophosphamide (PTCy) have been demonstrated independently in several single-institutional studies. We hypothesized that combining these two promising approaches in a multi-institutional study of human leukocyte antigen (HLA)-matched bone marrow transplantation would provide low rates of severe acute and chronic GVHD, low toxicity, and effective disease control.

Patients and Methods

Ninety-two adult patients (median age, 49 years; range, 21 to 65 years) with high-risk hematologic malignancies were enrolled at three centers (clinical trial No. NCT00809276). Forty-five patients received related allografts, and 47 received unrelated allografts. GVHD prophylaxis was solely with PTCy at 50 mg/kg/day on post-transplantation days +3 and +4.

Results

The cumulative incidences of grades 2 to 4 acute, grades 3 to 4 acute, and chronic GVHD were 51%, 15%, and 14%, respectively. Nonrelapse mortality (NRM) at 100 days and 1 year were 9% and 16%, respectively. With a median follow-up period of 2.2 years, the 2-year disease-free survival (DFS) and overall survival (OS) rates were 62% and 67%, respectively. Donor relatedness did not affect NRM, DFS, or OS. Patients in complete remission (CR) without evidence of minimal residual disease (MRD) had markedly better DFS (80%) and OS (80%) than patients in CR with MRD or with active disease at the time of transplantation (DFS, $P = .0005$; OS, $P = .019$).

Conclusion

This multi-institutional study demonstrates that PTCy can be safely and effectively combined with IV Bu/Flu myeloablative conditioning and confirms PTCy's efficacy as single-agent, short-course GVHD prophylaxis for both acute and chronic GVHD after bone marrow transplantation from HLA-matched donors.

J Clin Oncol 32:3497-3505. © 2014 by American Society of Clinical Oncology

INTRODUCTION

Allogeneic blood or marrow transplantation (alloBMT) involves a multifaceted treatment platform, and each component (conditioning regimen, allograft source, and graft-versus-host disease [GVHD] prophylaxis) has significant implications for the overall success of the transplantation.¹⁻⁵ Busulfan (Bu)/fludarabine (Flu) reduced-toxicity myeloablative conditioning (MAC) has shown en-

couraging results in several prospective studies with low rates of nonrelapse mortality (NRM) and end-organ toxicity.⁶⁻⁹ Unlike ex vivo or in vivo T-cell depletion,¹⁰ most pharmacologic approaches for preventing acute GVHD (aGVHD) are less effective in preventing chronic GVHD (cGVHD). In contrast, high-dose, post-transplantation cyclophosphamide (PTCy) successfully prevents both aGVHD and cGVHD.¹¹ This clinical activity was demonstrated first in the reduced-intensity conditioning,

human leukocyte antigen (HLA)-haploidentical alloBMT setting^{12,13} and later as single-agent GVHD prophylaxis after busulfan/cyclophosphamide (Bu/Cy) MAC and HLA-matched-related or -unrelated allografting.¹⁴ In this latter study, the cumulative incidences of grades 3 to 4 aGVHD and cGVHD were each 10%.¹⁴

Therefore, we combined these two promising elements, intravenous (IV) Bu/Flu MAC and PTCy, in a multi-institutional study of patients receiving HLA-matched-related or -unrelated bone marrow allografting. We hypothesized that this transplantation platform would provide favorable patient outcomes by preventing grades 3 to 4 aGVHD and cGVHD, lowering regimen-related toxicity, and effectively controlling disease.

PATIENTS AND METHODS

Study Design

This phase II study (clinical trial No. NCT00809276) was designed to determine whether cyclophosphamide on days +3 and +4 after transplantation would provide sufficient postgrafting immunosuppression in patients with high-risk hematologic malignancies conditioned with fludarabine and once-daily IV busulfan. To assess this in a nonrandomized fashion, three possible GVHD prophylactic regimens were considered (Data Supplement; Table A1 [online-only]). The primary end point was the cumulative incidence of grades 3 to 4 aGVHD at 100 days after alloBMT. A sequentially adaptive Bayesian design,¹⁵ defined in terms of the 100-day grades 3 to 4 aGVHD rate compared with the historic rate of 11%,^{7,14} was followed by a data safety monitoring board (Data Supplement; Methods). Enrollment was begun using PTCy on days +3 and +4 as the sole GVHD prophylaxis. Because there were no excess grades 3 to 4 aGVHD, all 92 patients enrolled were treated using PTCy as the only GVHD prophylaxis. Predefined secondary end points included primary and secondary graft failure, NRM, cGVHD, disease-free survival (DFS), and overall survival (OS).

Patients

Eligibility criteria included the following: patient age 65 years or younger; bone marrow donor who was either a genotypically HLA-identical sibling, HLA-matched first-degree relative, or unrelated donor (10/10 HLA-matched at HLA-A, -B, -C, -DRB1, and -DQB1); and a high-risk hematologic malignancy as defined (Data Supplement; Table A2). Patients with Eastern Cooperative Oncology Group performance status¹⁶ of 3 or higher, active serious infections, significant end-organ dysfunction (Data Supplement; Table A2), positive serology for HIV or human T-cell lymphotropic virus (HTLV), or pregnancy were excluded. The institutional review boards of all participating institutions approved this study. All patients provided written informed consent before enrollment.

Regimen

The treatment schema is shown in Figure 1. The busulfan dose of 130 mg/m² IV daily for 4 days was adjusted based on measured pharmacokinetics.

Fig 1. Transplantation platform. The treatment schema is shown. No additional graft-versus-host disease prophylaxis was administered after post-transplantation cyclophosphamide on days +3 and +4. (*) Busulfan dosing was adjusted based on measured pharmacokinetics, which at Johns Hopkins Hospital and MD Anderson Cancer Center used a test dose and at Fred Hutchinson Cancer Research Center used busulfan targeting in real time. IV, intravenously.

We used a test dose of Bu to estimate a targeted daily systemic exposure of 4,600 μ Mol-min at Johns Hopkins Hospital (JHH; with an acceptable range of 3,600 to 5,600 μ Mol-min) and a targeted daily systemic exposure of 5,000 μ Mol-min at MD Anderson Cancer Center (with an acceptable range of 4,700 to 5,800 μ Mol-min). Bu levels were adjusted in real time to a targeted steady-state concentration goal of 900 ± 100 ng/mL at Fred Hutchinson Cancer Research Center (FHCRC). Patients received 40 mg/m²/day IV Flu immediately before Bu on all four days to achieve synergistic cytotoxicity.¹⁷⁻¹⁹ One or two days of rest were allowed after conditioning and before infusion of a T-cell-replete bone marrow allograft. Patients received 50 mg/kg/day IV cyclophosphamide on days +3 and +4, with the first dose starting 62 to 72 hours after the start of allograft infusion. Mesna was administered in four doses each on days +3 and +4 at a total daily dose of 80% of the cyclophosphamide dose. Supportive care measures were administered according to standard practices at each institution. Growth factors were not allowed, except in cases of delayed engraftment or secondary graft failure.

Engraftment

Neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count $\geq 500/\mu$ L. Platelet engraftment was defined as the first day of achieving a platelet count $\geq 2 \times 10^4/\mu$ L, without receiving a platelet transfusion in the preceding 7 days. Primary graft failure was defined as failure to achieve a sustained neutrophil count of $\geq 500/\mu$ L in the absence of disease relapse. Secondary graft failure was defined as a decline in the neutrophil count to less than 500/ μ L after initial engraftment, which was unrelated to infection, medications, or disease relapse and was unresponsive to growth factor stimulation.

Diagnosis and Treatment of GVHD

Acute GVHD was scored centrally by a third-party reviewer (Paul Martin at FHCRC), using Modified Keystone Criteria.^{20,21} Chronic GVHD diagnosis and grading were based on National Institutes of Health criteria²² and were scored by third-party investigators at each participating institution. A pathologic confirmation of clinically suspected GVHD was obtained when feasible. GVHD was treated according to the standard practice at each institution.

Pretransplantation Disease Status Criteria

Patients' disease status was defined based on standard criteria²³⁻²⁷ and was divided into three groups: complete remission (CR) without detectable minimal residual disease (MRD), CR with MRD, and active disease (Data Supplement; Table A3).

Statistical Methods

NRM was defined as patient death without disease persistence or progression. NRM and relapse were considered competing risks. Events for defining DFS were relapse or death from any cause. Competing risks for GVHD were graft failure, relapse, or death without GVHD. Patient data collection was locked for analysis on February 28, 2013.

Unadjusted event time distributions were estimated using the Kaplan-Meier method.²⁸ Cumulative incidences were estimated using the Gray method.²⁹ Unadjusted comparisons of DFS and OS between patient groups were performed using the log-rank test.³⁰ Relationships between covariates and each of NRM, DFS, and OS were assessed using Bayesian survival time regression.³¹ Goodness-of-fit was assessed using Bayes Information Criterion,³² which showed that the log-normal distribution fit the data best for all time-to-event end points. In this model, $\log(\text{time-to-event} | \text{covariates})$ was assumed to follow a normal distribution with mean $\beta_0 + \beta_1(\text{female-into-male alloBMT}) + \beta_2(\text{patient cytomegalovirus [CMV] seropositive}) + \beta_3(\text{related donor}) + \beta_4(\text{CR without MRD at alloBMT}) + \beta_5(\text{MRD at alloBMT}) + \beta_6(\text{patient age}) + \beta_7(\text{total nucleated cell [TNC] dose})$. For each covariate that had coefficient β in this linear component, $\Pr(\text{beneficial effect})$ was the posterior probability $\Pr(\beta > 0 | \text{data})$, which quantifies the strength of association, with large (small) values associated with a longer (shorter) mean event time. Priors and posterior computations are described in the Data Supplement (Methods). All statistical computations were performed using SAS 9.2 for Windows (SAS Institute, Cary, NC) or R 2.15 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient, Donor, and Allograft Characteristics

Patient, donor, and allograft characteristics are summarized in Table 1. A total of 92 adult patients with high-risk hematologic malignancies were treated between July 2009 and September 2011 at three institutions: JHH (42 patients), FHCRC (38 patients), and MD An-

derson Cancer Center (12 patients). Median patient age was 49 years (range, 21 to 65 years). Sixty-seven patients (73%) were in morphologic CR at alloBMT, 25 patients (27%) had active disease, and 18 patients (20%) had evidence of minimal residual disease. The allograft source was a genotypically identical HLA-matched-sibling donor for 44 patients (48%), a 10/10 HLA-matched familial donor for one patient (1%), and a 10/10 HLA-matched-unrelated donor (MUD) for 47 patients (51%; Table 1).

Engraftment

The median times to neutrophil and platelet engraftment were 21 days (range, 15 to 42) and 24 days (range, 12 to 65), respectively, for patients with primary engraftment. Chimerism studies, performed in

Table 1. Patient and Donor Demographic and Allograft Characteristics

Characteristic	Related (n = 45)		Unrelated (n = 47)		Total (N = 92)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
Age, years						
Median	47		53		49	
Range	22-65		21-64		21-65	
Female sex	26	58	26	55	52	56
Disease						
AML/MDS/CMML	31	69	37	79	68	74
De novo AML	16	36	21	45	37	40
Secondary AML	8	18	8	17	16	17
MDS	7	16	6	13	13	14
CMML	0	0	2	4	2	2
ALL	9	20	6	13	15	16
Chronic myelogenous leukemia	3	7	2	4	5	5
Non-Hodgkin lymphoma	2	4	1	2	3	3
Multiple myeloma	0	0	1	2	1	1
Disease status at BMT						
Morphologic CR	32	71	35	74	67	73
Morphologic CR with MRD	8	18	10	21	18	20
Active disease	13	29	12	26	25	27
Donor/recipient sex						
Female into male	14	31	11	23	25	27
Donor age, years						
Median	51		32		41.5	
Range	17-70		20-55		17-70	
CMV serologies						
CMV D ⁻ R ⁻	13	29	12	26	25	27
CMV D ⁺ R ⁻	9	20	5	11	14	15
CMV D ⁺ R ⁺	11	24	13	28	24	26
CMV D ⁻ R ⁺	12	27	16	34	28	30
CMV D ^{unk} R ⁺	0	0	1	2	1	1
Infused cell dose*						
Total nucleated cells × 10 ⁶ /kg						
Median	3.80		3.78		3.79	
Range	0.32-6.93		0.52-8.97		0.32-8.97	
CD34 ⁺ cells × 10 ⁶ /kg						
Median	3.35		3.16		3.19	
Range	1.07-7.80		1.00-9.87		1.00-9.87	
CD3 ⁺ cells × 10 ⁷ /kg						
Median	3.20		3.20		3.20	
Range	0.84-7.00		1.02-7.20		0.84-7.20	

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BMT, bone marrow transplantation; CMML, chronic myelomonocytic leukemia; CMV, cytomegalovirus; CR, complete remission; D, donor; MDS, myelodysplastic syndrome; MRD, minimal residual disease; R, recipient; Unk, unknown.

*Though data regarding nucleated cell doses are available for all patients, CD34⁺ cell doses are available for only 78 patients (85%), and CD3⁺ cell doses are available for 71 patients (77%).

Table 2. Toxicities and Causes of Death

Toxicity or Cause of Death	Related (No. of patients)	Unrelated (No. of patients)	Total	
			No. of Patients	%
Grade 3-4 toxicities up to day 100*				
AST elevation	11	6	17	18
Grade 3	9	5	14	15
Grade 4	2	1	3	3
ALT elevation	15	13	28	30
Grade 3	15	13	28	30
Grade 4	0	0	0	0
Hyperbilirubinemia	6	2	8	9
Grade 3	4	1	5	5
Grade 4	2	1	3	3
Acute renal failure	1	2	3	3
Mucositis, grade 3	11	8	19	21
Hemorrhage†	3	1	4	4
Cardiovascular‡	1	1	2	2
Pulmonary§	0	1	1	1
Dermatologic	1	0	1	1
Causes of death				
Relapsed or progressive disease	9	6	15	16
Nonrelapse mortality	7	8	15	16
Bacterial infection¶	2	3	5	5
Respiratory viral infection	1	1	2	2
Diffuse alveolar hemorrhage	2	0	2	2
Acute respiratory distress syndrome#	0	1	1	1
Stroke	0	1	1	1
Multiorgan failure	0	1	1	1
GVHD	1	1	2	2
Veno-occlusive disease	1	0	1	1

Abbreviations: GVHD, graft-versus-host disease; PTCy, post-transplantation cyclophosphamide.

*Toxicities listed are nonhematologic and noninfectious that were either reported as severe adverse events or thought possibly related to PTCy. Toxicities were graded according to standard National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

†One patient with diffuse alveolar hemorrhage, one with subdural hematoma in the setting of thrombocytopenia from relapsed disease, and two patients with BK virus-related hemorrhagic cystitis.

‡One patient experiencing cardiac arrest and one experiencing left-ventricular systolic dysfunction.

§One patient with idiopathic pulmonary syndrome.

||Pruritic rash that was not GVHD.

¶No patients died as a result of bacterial infection before neutrophil engraftment. Three patients died in the setting of GVHD treatment, and one patient died after receiving a contaminated platelet product.

#Unclear stimulating etiology.

a subset of patients, showed that the vast majority of these patients were fully donor when assessed between days +60 to 100 (Data Supplement; Fig A1). Primary graft failure occurred in five patients (5.4%). All three patients who received MUD allografts and experienced primary graft failure had allograft transit times of longer than 24 hours, and two of these three allografts had low TNC doses of $\leq 1.3 \times 10^8/\text{kg}$, variables that have been suggested individually to be risk factors for graft failure in unrelated allografting.³³ Four patients with primary graft failure achieved full neutrophil and platelet engraftment after second alloBMTs using standard institutional protocols.^{34,35} The fifth patient was found to have disease relapse on day +83. Secondary graft failure occurred in two additional patients (2.2%), both of whom received MUD allografts. One fully engrafted after a reduced-intensity conditioning, HLA-haploidentical alloBMT as previously described,¹³ and the other had documented disease relapse 1 month after secondary graft failure occurred.

Toxicity and NRM

Grades 3 to 4 toxicities occurring during the first 100 days after alloBMT are listed in Table 2. The most common toxicities were grade

3 transaminase elevation and mucositis. Veno-occlusive disease occurred in two patients (2.2%) and was mild in one patient but fatal in the other.^{36,37}

The cumulative incidences of NRM were 9% and 16% at 100 days and 1 year, respectively, and did not differ by donor type (Figure 2A) nor by time to neutrophil engraftment (Data Supplement; Tables A4 and A5).³⁸ Of the 15 patients experiencing NRM (Table 2), seven patients died as a result of infections (five bacterial and two respiratory viral). Of the five patients who died as a result of bacterial infections, none died before neutrophil engraftment, three died during aGVHD treatment, and one died after receiving a contaminated platelet product. Multivariable Bayesian analysis showed that patients in CR with or without MRD at alloBMT had posterior probabilities 0.92 and > 0.99 , respectively, of having lower NRM compared with patients with active disease, suggesting that active disease at transplantation was associated with higher NRM (Table 3). No patients died as a result of invasive fungal infection or CMV disease. Detectable peripheral blood CMV reactivation occurred in 31 patients (34%) at a median of 42 days (range, 12 to 324) after transplantation. Consistent with a recent

Fig 2. Transplantation outcomes for nonrelapse mortality and graft-versus-host disease (GVHD) by donor type. Cumulative incidences are shown for (A) nonrelapse mortality; (B) grades 2 to 4 acute GVHD; (C) grades 3 to 4 acute GVHD; and (D) chronic GVHD.

Table 3. Fitted Bayesian Regression Models

Predictor	Posterior Quantities											
	Nonrelapse Mortality				Disease-Free Survival				Overall Survival			
	Mean	SD	95% CI	Pr(beneficial effect)	Mean	SD	95% CI	Pr(beneficial effect)	Mean	SD	95% CI	Pr(beneficial effect)
Intercept, β_0	6.90	1.04			5.25	0.64			6.72	0.81		
Female into male	-0.36	1.04	-2.40 to 1.73	0.36	0.91	0.64	-0.30 to 2.22	0.93	0.81	0.78	-0.65 to 2.42	0.86
CMV serology patient positive	0.87	0.95	-0.90 to 2.89	0.82	0.61	0.56	-0.47 to 1.74	0.87	0.27	0.67	-1.01 to 1.65	0.66
Related donor	0.48	0.94	-1.25 to 2.45	0.69	-0.25	0.52	-1.23 to 0.78	0.31	-0.32	0.62	-1.56 to 0.93	0.29
Disease status at alloBMT												
CR v AD	2.64	1.02	0.79 to 4.79	> 0.99	2.33	0.63	1.17 to 3.65	> 0.99	1.70	0.79	0.26 to 3.38	0.99
MRD v AD	1.70	1.23	-0.63 to 4.21	0.92	0.23	0.71	-1.13 to 1.64	0.63	-0.21	0.87	-1.91 to 1.53	0.40
Age, years	-0.56	0.55	-1.70 to 0.47	0.15	-0.06	0.30	-0.66 to 0.52	0.41	-0.12	0.35	-0.82 to 0.58	0.37
TNC dose, $\times 10^8/\text{kg}$	0.23	0.52	-0.79 to 1.26	0.68	0.34	0.29	-0.23 to 0.39	0.88	0.28	0.35	-0.41 to 0.98	0.80

NOTE. For each covariate x having coefficient β in the linear component of the lognormal model, $\text{Pr}(\text{beneficial effect})$ is the posterior probability $\text{Pr}(\beta > 0 \mid \text{data})$. Larger values of this probability (eg, above 0.95) imply that a larger value of x , or $x = 1$ for an indicator variable, was associated with a larger mean event time. Smaller values of this probability (eg, below 0.05) imply that a smaller value of x , or $x = 0$ for an indicator variable, was associated with a larger mean event time. $\text{Pr}(\beta > 0 \mid \text{data})$ near 0.50 corresponds to no effect of x on mean event time. For example, in the fitted models, being in CR without MRD is strongly beneficial for all three outcomes, whereas female-into-male alloBMT was moderately beneficial for disease-free survival.

Abbreviations: AD, active disease; alloBMT, allogeneic bone marrow transplantation; CI, credible interval; CMV, cytomegalovirus; CR, complete remission without MRD; MRD, minimal residual disease; Pr, probability; SD, standard deviation; TNC, total nucleated cell.

report,³⁹ there were no incidents of post-transplantation lymphoproliferative disease.

GVHD

The cumulative incidence of grades 2 to 4 aGVHD at 100 days was 51% (95% CI, 41% to 61%) and was significantly higher in patients receiving HLA-matched-unrelated (60%; 95% CI, 45% to 74%) versus HLA-matched-related allografts (42%; 95% CI, 28% to 57%; $P = .027$; Fig 2B). The onset of grades 2 to 4 aGVHD occurred at a median of 34 days (range, 15 to 88) after alloBMT. The cumulative incidence of grades 3 to 4 aGVHD at 100 days was 15% (95% CI, 8% to 23%) for all patients and was similar between patients who received HLA-matched-related (11%; 95% CI, 2% to 20%) or HLA-matched-unrelated allografts (19%; 95% CI, 8% to 31%; $P = .44$; Fig 2C). Acute GVHD was the cause of death in two patients (2%).

Acute GVHD was treated with corticosteroids alone in 10 patients (21%), corticosteroids plus a calcineurin inhibitor (CNI) in 27 patients (57%), or a CNI alone in one patient (2%). Corticosteroids and a non-CNI agent were used in eight patients (17%), including seven treated with mycophenolate mofetil versus placebo on the Blood or Marrow Transplantation Clinical Trials Network study 0802 (randomization has not been unblinded). One patient (2%) with grade 2 aGVHD did not receive any systemic immunosuppression. Sixty-five percent of patients with grade 2 aGVHD who were alive at 1 year had stopped receiving all immunosuppressive therapy by that time and never required further immunosuppression. Overall, 35% of all patients and 39% of patients alive at last follow-up never required immunosuppression after PTCy on day +4 for any reason.

The cumulative incidence of cGVHD at 2 years was 14% (95% CI, 7% to 21%) for all patients and was lower in patients receiving HLA-matched-related (7%; 95% CI, 0% to 14%) versus HLA-matched-unrelated allografts (22%; 95% CI, 10% to 34%; $P = .041$; Fig 2D). All instances of cGVHD occurred within the first year. By National Institutes of Health criteria,²² cGVHD was moderate in 58%

of patients and severe in 42% of patients. Two patients had bronchiolitis obliterans syndrome as the only manifestation of cGVHD.

Relapse, DFS, and OS

The median follow-up period of surviving patients was 794 days (2.2 years; range, 0.61 to 3.52 years). At 2 years, the cumulative incidence of relapse by competing-risk analysis was 22% (95% CI, 13% to 31%), DFS probability was 62% (95% CI, 52% to 73%; Fig 3A), and OS probability was 67% (95% CI, 58% to 77%; Fig 3B). Relapse, DFS, and OS outcomes did not differ by donor type (Figs 3C and 3D); however, disease status did have a significant impact. The 2-year cumulative incidence of relapse for patients in CR without MRD was 10% (95% CI, 2% to 19%) compared with 33% for patients in CR with MRD (95% CI, 11% to 56%) and 38% for patients with active disease (95% CI, 18% to 58%; $P = .03$). Correspondingly, patients in CR without MRD had a 2-year DFS of 80% (95% CI, 69% to 92%) compared with 50% for patients in CR with MRD (95% CI, 32% to 79%) and 33% for patients with active disease (95% CI, 18% to 59%; $P = .0005$; Fig 3E). Likewise, patients in CR without MRD had an 80% 2-year OS (95% CI, 68% to 92%) compared with 50% for patients in CR with MRD (95% CI, 32% to 79%) and 54% for patients with active disease (95% CI, 38% to 78%; $P = .019$; Fig 3F). Multivariable Bayesian analysis showed that predictors for beneficial effects on DFS were female-into-male alloBMT and being in CR without MRD at the time of alloBMT with posterior probabilities 0.93 and > 0.99, respectively (Table 3). Multivariable Bayesian analysis for OS showed that being in CR without MRD was the only predictor of improved OS with posterior probability > 0.99 (Table 3).

DISCUSSION

In this multi-institutional study, the combination of IV Bu/Flu MAC, T-cell-replete bone marrow allografting, and PTCy as sole GVHD prophylaxis was a well-tolerated and effective platform for facilitating

Fig 3. Disease-free and overall survival. Disease-free and overall survival probabilities are shown for (A and B) all patients, (C and D) patients by donor type, and (E and F) patients by disease status at the time of transplantation. (A and B) Dashed lines represent 95% CIs. AD, active disease; CR, complete remission without MRD; MRD, minimal residual disease.

HLA-matched-related or -unrelated alloBMT. Although approximately half of the patients developed grades 2 to 4 aGVHD, only 15% and 14% developed grades 3 to 4 aGVHD and cGVHD, respectively. The low incidences of severe aGVHD and cGVHD observed in our study are consistent with previous trials using PTCy GVHD prophylaxis after bone marrow allografting, regardless of the specific transplantation platform used.^{13,14,40} Importantly, despite the low incidence of cGVHD, disease control remained excellent.

In a randomized study that compared cyclophosphamide with cyclosporine in the 1980s, cyclophosphamide was more effective at

preventing cGVHD.⁴¹ But cyclophosphamide-treated patients displayed slower hematopoietic reconstitution, higher aGVHD rates, and worse overall survival primarily because of toxicity; thus, that cyclophosphamide GVHD prophylactic approach was abandoned. However, cyclophosphamide was employed quite differently in that study, being administered serially (days +1, +3, +5, +7, +9, and weekly thereafter until day +100) at a low dose (7.5 mg/kg) rather than the high-dose (50 mg/kg) on days +3 and +4 as we used in our study. The effectiveness of high-dose PTCy in several studies including this current one likely reflects the timing and intensity of dosing and the absence of concurrent prophylactic methylprednisolone, which could ablate regulatory T-cell persistence after PTCy.⁴²

Even without prophylactic immunosuppression beyond day +4, the incidence of grades 2 to 4 aGVHD was similar in our patients to that observed using standard GVHD prophylaxis.^{43,44} Moreover, most of our patients had grade 2 disease, which had little impact on their ultimate clinical course. Only 15% of patients with grade 2 aGVHD went on to develop cGVHD, and approximately two thirds of patients had been taken off all immunosuppression permanently by 1 year after transplantation. Furthermore, patients with grade 2 aGVHD had encouraging 2-year DFS and OS rates of 64% (95% CI, 51% to 79%) and 70% (95% CI, 58% to 85%), respectively.

Outcomes for patients receiving MUD allografts were similar to those receiving HLA-matched-related allografts, including low rates of cGVHD despite no patients receiving prophylactic antithymocyte globulin. Furthermore, among all patients, only two patients died as a result of infections after day +100 and there were no incidents of post-transplantation lymphoproliferative disease, suggesting that PTCy may promote favorable immune reconstitution when compared with strategies utilizing either T-cell depletion or prolonged pharmacologic immunosuppression.⁴⁴⁻⁴⁶

NRM was higher in our study than was observed in initial reports of Bu/Flu conditioning with standard GVHD prophylaxis.^{6,7} These results suggest that the effectively higher intensity conditioning of adding PTCy to Bu/Flu may be associated with additional toxicity, but does provide cGVHD protection without requiring antithymocyte globulin. The low cGVHD rates observed in our study may be partly a result of the exclusive use of bone marrow allografts, but these cGVHD rates are still markedly lower than those reported when using bone marrow allografting without PTCy.² Given the higher but still favorable rates of cGVHD when using peripheral blood stem cells for HLA-haploidentical alloBMT with PTCy,⁴⁷ further study is needed to determine whether additional immunosuppression beyond PTCy is required when using peripheral blood stem-cell allografting for MAC, HLA-matched alloBMT.

Despite the promising outcomes seen for our patients, the optimal myeloablative conditioning approach for HLA-matched alloBMT in the context of PTCy GVHD prophylaxis is unknown. Using standard GVHD prophylaxis, retrospective data suggested a possible advantage for Bu/Flu over Bu/Cy MAC.^{8,48,49} However, recent prospective studies have failed to confirm this benefit with inferior results for Bu/Flu in one study⁵⁰ and similar outcomes in a second study.⁵¹ When comparing our results with those of our previous trial of Bu/Cy MAC alloBMT followed by PTCy,¹⁴ mucositis rates were higher, hepatotoxicity rates including veno-occlusive disease were lower, and GVHD rates were similar.¹⁴ Primary graft failure seemed to be slightly

higher using Bu/Flu than with Bu/Cy MAC and PTCy,¹⁴ but overall are in the range reported using bone marrow MUD allografting² and may have been affected by long transit times and low TNC counts for the individual cases of primary graft failure observed in this study. DFS and OS for patients in our current study were better than those observed for patients treated with Bu/Cy MAC followed by PTCy, but the included patient populations differed, with 54% of patients in the Bu/Cy MAC study not being in CR at the time of alloBMT.¹⁴ Indeed, outcomes for patients with active disease were similar between the two studies. Prospective, randomized studies are necessary to compare these two conditioning strategies using PTCy.

Our current study, as a collaborative effort by three academic centers, has confirmed the clinical efficacy of both PTCy and IV Bu/Flu reduced-toxicity MAC as previously seen in single-institutional studies. Moreover, the lack of a requirement for immunosuppression in a significant subset of patients can facilitate the early integration of adjunct therapies to prevent relapse.^{52,53} This advantage of PTCy single-agent GVHD prophylaxis is critical, given our finding, similar to a recent retrospective study,⁵⁴ that any amount of detectable disease at the time of alloBMT for patients in morphologic CR portends a worse prognosis. As PTCy is a promising strategy for GVHD prophylaxis in both HLA-matched and HLA-mismatched alloBMT,^{14,40,47} its efficacy relative to other approaches requires further study through randomized clinical trials.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory**

Role: Borje S. Andersson, Otsuka (C) **Stock Ownership:** None

Honoraria: None **Research Funding:** Paul V. O'Donnell, Otsuka;

Richard E. Champlin, Otsuka; Borje S. Andersson, Otsuka,

sanofi-aventis; Leo Luznik, Otsuka **Expert Testimony:** None **Patents, Royalties, and Licenses:** Borje S. Andersson, Parenteral busulfan for the treatment of malignant disease, United States patents No. 5430057 and 5559148, Australia patent No. 694141 **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Paul V. O'Donnell, Terry Furlong, Marcos J. de Lima, Richard J. Jones, Peter F. Thall, Borje S. Andersson, Leo Luznik **Collection and assembly of data:** Christopher G. Kanakry, Paul V. O'Donnell, Terry Furlong, Marta Medeor, Borje S. Andersson, Leo Luznik

Data analysis and interpretation: Christopher G. Kanakry, Paul V. O'Donnell, Terry Furlong, Wei Wei, Marco Mielcarek, Richard E. Champlin, Peter F. Thall, Leo Luznik

Manuscript writing: All authors

Final approval of manuscript: All authors

REFERENCES

1. Horwitz ME: Reduced intensity versus myeloablative allogeneic stem cell transplantation for the treatment of acute myeloid leukemia, myelodysplastic syndrome and acute lymphoid leukemia. *Curr Opin Oncol* 23:197-202, 2011
2. Anasetti C, Logan BR, Lee SJ, et al: Peripheral blood stem cells versus bone marrow from unrelated donors. *N Engl J Med* 367:1487-1496, 2012
3. Couban S, Simpson DR, Barnett MJ, et al: A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood* 100:1525-1531, 2002
4. Mielcarek M, Storer B, Martin PJ, et al: Long-term outcomes after transplantation of HLA-identical related G-CSF-mobilized peripheral blood mononuclear cells versus bone marrow. *Blood* 119:2675-2678, 2012
5. Bacigalupo A, Van Lint MT, Occhini D, et al: Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. *Blood* 77:1423-1428, 1991
6. Russell JA, Tran HT, Quinlan D, et al: Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: Study of pharmacokinetics and early clinical outcomes. *Biol Blood Marrow Transplant* 8:468-476, 2002
7. de Lima M, Couriel D, Thall PF, et al: Once-daily intravenous busulfan and fludarabine: Clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood* 104:857-864, 2004
8. Andersson BS, de Lima M, Thall PF, et al: Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. *Biol Blood Marrow Transplant* 14:672-684, 2008
9. Alatrash G, de Lima M, Hamerschlak N, et al: Myeloablative reduced-toxicity i.v. busulfan-fludarabine and allogeneic hematopoietic stem cell transplant for patients with acute myeloid leukemia or myelodysplastic syndrome in the sixth through eighth decades of life. *Biol Blood Marrow Transplant* 17:1490-1496, 2011
10. Van Besien K: Allogeneic transplantation for AML and MDS: GVL versus GVHD and disease recurrence. *Am Soc Hematol Ed Book* 2013:56-62, 2013
11. Luznik L, O'Donnell PV, Fuchs EJ: Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. *Semin Oncol* 39:683-693, 2012
12. O'Donnell PV, Luznik L, Jones RJ, et al: Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant* 8:377-386, 2002
13. Luznik L, O'Donnell PV, Symons HJ, et al: HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant* 14:641-650, 2008
14. Luznik L, Bolaños-Meade J, Zahurak M, et al: High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. *Blood* 115:3224-3230, 2010
15. Thall PF, Sung HG: Some extensions and applications of a Bayesian strategy for monitoring multiple outcomes in clinical trials. *Stat Med* 17:1563-1580, 1998
16. Oken MM, Creech RH, Tormey DC, et al: Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 5:649-655, 1982
17. Gandhi V, Plunkett W: Cellular and clinical pharmacology of fludarabine. *Clin Pharmacokinet* 41:93-103, 2002
18. Valdez BC, Andersson BS: Interstrand cross-link inducing agents in pretransplant conditioning therapy for hematologic malignancies. *Environ Mol Mutagen* 51:659-668, 2010
19. Valdez BC, Li Y, Murray D, et al: The synergistic cytotoxicity of clofarabine, fludarabine and busulfan in AML cells involves ATM pathway activation and chromatin remodeling. *Biochem Pharmacol* 81:222-232, 2011
20. Przepiorka D, Weisdorf D, Martin P, et al: 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 15:825-828, 1995
21. Leisenring WM, Martin PJ, Petersdorf EW, et al: An acute graft-versus-host disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens. *Blood* 108:749-755, 2006
22. Filipovich AH, Weisdorf D, Pavletic S, et al: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I, Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 11:945-956, 2005
23. Cheson BD, Bennett JM, Kopecky KJ, et al: Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol* 21:4642-4649, 2003
24. Brüggemann M, Schrauder A, Raff T, et al: Standardized MRD quantification in European ALL trials: Proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. *Leukemia* 24:521-535, 2010
25. Cheson BD, Bennett JM, Kantarjian H, et al: Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood* 96:3671-3674, 2000
26. Baccarani M, Cortes J, Pane F, et al: Chronic myeloid leukemia: An update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol* 27:6041-6051, 2009
27. Cheson BD, Pfistner B, Juweid ME, et al: Revised response criteria for malignant lymphoma. *J Clin Oncol* 25:579-586, 2007
28. Kaplan EL, Meier P: Nonparametric-estimation from incomplete observations. *J Am Stat Assoc* 53:457-481, 1958
29. Gray RJ: A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 16:1141-1154, 1988
30. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 50:163-170, 1966
31. Gelman A: *Bayesian Data Analysis* (ed 2). Boca Raton, FL, Chapman & Hall/CRC, 2004
32. Schwarz G: Estimating dimension of a model. *Ann Stat* 6:461-464, 1978
33. Lazarus HM, Kan F, Tarima S, et al: Rapid transport and infusion of hematopoietic cells is associated with improved outcome after myeloablative therapy and unrelated donor transplant. *Biol Blood Marrow Transplant* 15:589-596, 2009
34. Bolaños-Meade J, Luznik L, Muth M, et al: Salvage transplantation for allograft failure using fludarabine and alemtuzumab as conditioning regimen. *Bone Marrow Transplant* 43:477-480, 2009
35. Gyurkocza B, Cao TM, Storb RF, et al: Salvage allogeneic hematopoietic cell transplantation with fludarabine and low-dose total body irradiation after rejection of first allografts. *Biol Blood Marrow Transplant* 15:1314-1322, 2009
36. Jones RJ, Lee KS, Beschoner WE, et al: Venooclusive disease of the liver following bone marrow transplantation. *Transplantation* 44:778-783, 1987
37. McDonald GB, Hinds MS, Fisher LD, et al: Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: A cohort study of 355 patients. *Ann Intern Med* 118:255-267, 1993
38. Offner F, Schoch G, Fisher LD, et al: Mortality hazard functions as related to neutropenia at different times after marrow transplantation. *Blood* 88:4058-4062, 1996
39. Kanakry JA, Kasamon YL, Bolaños-Meade J, et al: Absence of post-transplantation lymphoproliferative disorder after allogeneic blood or marrow transplantation using post-transplantation cyclophosphamide as graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant* 19:1514-1517, 2013
40. Raiola AM, Dominietto A, Ghiso A, et al: Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant* 19:117-122, 2013
41. Santos GW, Tutschka PJ, Brookmeyer R, et al: Cyclosporine plus methylprednisolone versus cyclophosphamide plus methylprednisolone as prophylaxis for graft-versus-host disease: A randomized double-blind study in patients undergoing allogeneic marrow transplantation. *Clin Transplant* 1:21-28, 1987
42. Kanakry CG, Ganguly S, Zahurak M, et al: Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. *Sci Transl Med* 5:211ra157, 2013
43. Yakoub-Agha I, Mesnil F, Kuentz M, et al: Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: A prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. *J Clin Oncol* 24:5695-5702, 2006
44. Finke J, Bethge WA, Schmoor C, et al: Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: A randomised, open-label, multicentre phase 3 trial. *Lancet Oncol* 10:855-864, 2009
45. Pasquini MC, Devine S, Mendizabal A, et al: Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. *J Clin Oncol* 30:3194-3201, 2012
46. Socié G, Schmoor C, Bethge WA, et al: Chronic graft-versus-host disease: Long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. *Blood* 117:6375-6382, 2011
47. Bashey A, Zhang X, Sizemore CA, et al: T-cell-replete HLA-haploidentical hematopoietic transplantation

for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *J Clin Oncol* 31:1310-1316, 2013

48. Bredeson CN, Zhang MJ, Agovi MA, et al: Outcomes following HSCT using fludarabine, busulfan, and thymoglobulin: A matched comparison to allogeneic transplants conditioned with busulfan and cyclophosphamide. *Biol Blood Marrow Transplant* 14:993-1003, 2008

49. Chae YS, Sohn SK, Kim JG, et al: New myeloablative conditioning regimen with fludarabine and busulfan for allogeneic stem cell transplantation:

Comparison with BuCy2. *Bone Marrow Transplant* 40:541-547, 2007

50. Lee JH, Joo YD, Kim H, et al: Randomized trial of myeloablative conditioning regimens: Busulfan plus cyclophosphamide versus busulfan plus fludarabine. *J Clin Oncol* 31:701-709, 2013

51. Liu H, Zhai X, Song Z, et al: Busulfan plus fludarabine as a myeloablative conditioning regimen compared with busulfan plus cyclophosphamide for acute myeloid leukemia in first complete remission undergoing allogeneic hematopoietic stem cell transplantation: A prospective and multicenter study. *J Hematol Oncol* 6:15, 2013

52. Pavletic SZ, Kumar S, Mohty M, et al: NCI First International Workshop on the Biology, Prevention, and

Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: Report from the Committee on the Epidemiology and Natural History of Relapse following Allogeneic Cell Transplantation. *Biol Blood Marrow Transplant* 16:871-890, 2010

53. Bolaños-Meade J, Smith BD, Gore SD, et al: 5-azacytidine as salvage treatment in relapsed myeloid tumors after allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant* 17:754-758, 2011

54. Walter RB, Buckley SA, Pagel JM, et al: Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood* 122:1813-1821, 2013

Sign up for Alerts About Your Topic of Interest

Learn about new research in your field as it becomes available. Subscribe to a *JCO* e-mail alert to be notified immediately when new articles within your area of interest are posted.

Receive notification when:

- *JCO* releases a new issue's Table of Contents.
- A new issue of *JCO* is posted online.
- New articles are published online ahead of print publication.
- New content in your subspecialty is published.
- An article is published online from a specific author.

Go to jco.org/alerts to sign up.

Acknowledgment

We thank Paul Martin for scoring acute graft-versus-host disease (GVHD) and Javier Bolaños-Meade, Mary Flowers, and Patricia Cole for scoring chronic GVHD in our patients. We also thank Data Safety Monitoring Board members Lauri Burroughs, Ted Gooley, and Michael McDevitt. Finally, we thank our patients and research staff for making this study possible.