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A Phase II Multicenter Study of the Addition of Azacitidine to Reduced-Intensity Conditioning Allogeneic Transplant for High-Risk Myelodysplasia (and Older Patients with Acute Myeloid Leukemia): Results of CALGB 100801 (Alliance)

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A B S T R A C T

Relapse remains the major cause of death in older patients transplanted for acute myeloid leukemia (AML) in first complete remission or for patients with advanced myelodysplastic syndrome (MDS) at any age. Conventional myeloablative conditioning followed by allogeneic blood or marrow transplantation is associated with significantly less relapse compared with reduced-intensity conditioning when performed in younger patients with AML or MDS, but the toxicity of this approach in older patients is prohibitive. We hypothesized that pharmacokinetic targeting to optimize busulfan (BU) exposure, combined with the administration of azacitidine (AZA) post-transplant would mitigate the risk of relapse while reducing nonrelapse mortality and ultimately improve progression-free survival (PFS). On this phase II multicenter study, 63 patients (40 unrelated donors and 23 matched related donors) received a uniform conditioning regimen consisting of fludarabine i.v. (days −7 to −3), BU targeted to a daily area under the curve (AUC) of 4000 μM/min (days −6 to −3) after the administration of a 25-mg/m^2 i.v. test dose on 1 day between days −14 to −9, and antithymocyte globulin (days −6, −5, and −4 (2 doses for matched related donors and 3 for matched unrelated donors only). Beginning on days +42 to +90, all patients were planned to receive up to 6 monthly cycles of AZA at 32 mg/m^2 subcutaneously for 5 days. The median age was 62 years (range, 44 to 74); 13 had AML and 50 had MDS; 87% of patients were within 20% of the target AUC based on a validation sample. Forty-one patients (65%) started AZA at a median of 61 days (range, 43 to 91) post-transplant, and 17 patients (41%) completed all 6 cycles of AZA. The cumulative incidence of nonrelapse mortality at 2 years was 33.4% (95% confidence interval [CI], 22%-45%). The cumulative incidence of relapse was 25% (95% CI, 15%-37%) at 2 years. With a median follow-up of 58.9 months, the estimated PFS probability at 2 years and 5 years after transplantation was 41.2% (80% CI, 33.9%-49.9%) and 26.9% (80% CI, 20.4%-35.5%), respectively, for the entire group with a median PFS of 15.8 months (95% CI, 6.7 to 28.3). The probability of overall survival at 2 and 5 years was 45.7% (95% CI, 34.9%-59.9%) and 31.2% (95% CI, 21.3% to 45.8%), respectively, for the entire group with a median overall survival of 19.2 months (95% CI, 8.7 to 37.5). In summary, we demonstrated the feasibility of a novel reduced-intensity conditioning regimen with test dose BU targeted to an AUC of 4000 μM/min. The feasibility of AZA in this setting appears to be limited if applied to an unselected population of older hematopoietic stem cell transplantation recipients. (ClinicalTrials.gov Identifier: NCT01168219.)

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for patients with myelodysplastic syndrome (MDS). Although reduced-intensity conditioning (RIC) is the preferred approach for most patients with high-risk MDS based on their age and performance status, the problem of relapse remains pressing. The Center for International Blood and Marrow Transplant Research registry data show 3-year probabilities of overall survival (OS) were 52% ± 2% and 49% ± 1% for recipients of matched related donor and unrelated donor transplants for early MDS, respectively. Among patients with advanced MDS, corresponding probabilities were 45% ± 1% and 41% ± 1% [1]. The prognosis for patients with acute myeloid leukemia (AML) ages 60 years or older at the time of initial diagnosis is also poor [2-7]. Despite first complete remission rates of up to 50% to 60%, prospects for long-term survival after chemotherapy are dismal because of the high risk of relapse [8].

Several investigators have reported on the feasibility of using a RIC approach in elderly patients with AML [9-14]. A prospective multicenter phase II trial study of allogeneic transplantation for older patients with AML in first complete remission using RIC (Cancer and Leukemia Group B 100103 [Alliance for Clinical Trials in Oncology]/Blood and Marrow Transplant Clinical Trial Network 0502) showed that disease-free survival and OS at 2 years after transplant were 42% and 48%, respectively, and the relapse rate was high at 44% at 2 years [15]. Strategies to improve outcomes of allogeneic transplants in patients with MDS and elderly patients with AML are needed and should focus on efforts to mitigate relapse.

Busulfan (BU) is commonly used in conditioning regimens for HSCT. Previous studies have suggested a therapeutic window for BU area under the curve (AUC) during allogeneic transplant, with decreased survival associated with both high and low levels [16]. High levels have been strongly associated with the risk of fatal liver toxicity from hepatic sinusoidal obstruction syndrome [17,18]. In the context of the fludarabine/BU regimen, an AUC $>$ 6000 μMol/min was found to be associated with increased toxicity and decreased survival [19]. Lower levels have been associated with both graft rejection and greater risk for relapse [20]. This has led to the exploration of personalized BU dose–based conditioning regimen [21]. We hypothesized that we could improve outcomes of RIC by improving disease control while controlling the risk of nonrelapse mortality (NRM) by using a higher BU dose, aiming for an AUC that is 75% of "standard" rather than the typical 50% used in RIC regimens. Also, DeLima et al. [22] treated 45 high-risk patients with MDS and AML (67% not in complete remission) on a phase I study with post-transplant azacitidine (AZA) for 4 cycles. They established the maximal tolerated dose as 32 mg/m^2 given for 5 days and reported a 1-year event-free survival and OS of 58% and 77%, respectively.

We sought to optimize the administration of BU and combine that with post-transplant AZA maintenance to reduce relapse without a substantial increase in toxicity to improve progression-free survival (PFS) after HSCT. We conducted a phase II multicenter study within the CALGB (now part of the Alliance) and report the final results here.

METHODS

Objectives

The primary objective of the study was to determine if this strategy could improve 2-year PFS in patients with high-risk MDS and in patients with AML age 60 and older responding to initial therapy. Secondary objectives were to determine the ability to use pharmacokinetic-directed BU to achieve an AUC of 4000 μM/min within 20% of target AUC in $>$80% of patients, the safety and feasibility of using post-transplant AZA, rates of grades II to IV and III to IV acute graft-versus-host disease (GVHD), incidence of extensive chronic GVHD, treatment-related mortality at 100 days, and 2- and 5-year OS.

Patients and Donors

Patients were eligible if they met the following criteria for AML and MDS. For AML, criteria were age $\geq$ 60 years and $<$75 years, morphologic complete remission (leukemia-free state) defined as bone marrow blasts $<$ 5% (as determined by bone marrow within 4 weeks of beginning preparative regimen) but without requirement for normal peripheral blood counts, no extra medullary leukemia, and no blasts in peripheral blood. Patients with prior central nervous system involvement were eligible as long as disease was in remission at transplant. No more than 2 cycles of induction chemotherapy and no more than 2 cycles of consolidation therapy were permitted. Patients treated with hypomethylating agents (AZA or decitabine) who achieved a leukemia-free state could have received up to 4 cycles of therapy to reach this status. No more than 6 months could elapse from documentation of morphologic complete remission to transplant. Patients with AML after blast transformation of prior chronic myeloid leukemia or other myeloproliferative disease were excluded. For MDS, criteria were age $<$ 75 years and with high-risk features defined as 1 of the following: International Prognostic Scoring System risk $\geq$ intermediate-2, refractory anemia with excess blasts by French-American-British classification, high-risk cytogenetics (either complex karyotype or monosomy 7), and $<$10% bone marrow blasts determined by bone marrow biopsy within 4 weeks of beginning preparative regimen. Reduction in marrow blast percentage may have been achieved with chemotherapy or other therapy. Patients could have received treatment with AZA or decitabine before study enrollment. Patients who progressed from MDS to AML during treatment were not eligible for enrollment.

Donors were either an HLA-identical sibling (6/6) by serologic typing (A, B, DR) or low-resolution molecular HLA tests or an 8/8 locus matched unrelated donor using high-resolution DNA-based typing. Donors were required to be healthy and acceptable as per institutional standards for stem cell donation with no significant cardiopulmonary, renal, endocrine, or hepatic disease. There was no age restriction for related donors. Syngeneic donors were not eligible.

Conditioning Regimen

A BU test dose of 25 mg/m^{2}i.v. over 45 minutes was administered as a single i.v. infusion between days −14 and −9 (Figure 1). The test dose was infused over 45 minutes, and blood samples were drawn at the end of infusion and at 1, 2, 4, and 6 hours after test dose completion. (All samples for pharmacokinetics were sent to Emory Medical Laboratories or Seattle Cancer Care Alliance.) The pharmacokinetic- based targeted treatment dose of BU was calculated as follows: BU* (mg) = test dose (mg) × 4000/test AUC. Initially, the treatment dose was originally administered over 3 hours. The protocol was subsequently modified to infuse BU at the same infusion rate as the test dose. BU target level validation samples were obtained at the end of infusion and at 1, 2, 4, and 6 hours after the day −6 dose of BU. Fludarabine 30 mg/m^2/day was administered i.v. over 30 minutes for 5 days on days −7 through −3. Rabbit antithymocyte globulin (Thymoglobulin, sanofi-aventis U.S. LLC) was administered at 1.5 mg/kg/day i.v. over 6 hours for 2 doses on days −6 and −5 in case of related donors. In unrelated donors the dose was escalated to 1.5 mg/kg on day −6, 2.0 mg/kg on day −5, and 2.5 mg/kg on day −4.

Post-Transplant AZA

Post-transplant AZA was to be started as early as day +42 but not later than day +90 provided the following conditions were met: serum creatinine $<$ 2.0 mg/dL, serum bilirubin $<$ 2.0 mg/dL, aspartate aminotransferase $\leq$ 3 × upper limit of normal, platelets $\geq$ 30,000/μL without transfusion for the preceding 72 hours, absolute neutrophil count $\geq$ 500/μL (this may have been achieved with use of growth factors), no acute GVHD grades III or IV, and no life-threatening infections or bleeding. AZA was administered at a dose of 32 mg/m^2 s.c. daily for 5 days. Cycles were repeated for up to 6 courses every 4 weeks. If s.c. administration was not possible, i.v. administration was permitted. Before the start of cycles 2 to 6 the platelet count needed to be $>$20,000/μL attained with or without platelet transfusion and absolute neutrophil count $>$ 500/μL with or without the use of myeloid growth factors. If patients were unable to start a subsequent cycle of AZA because of toxicity or other reasons, the start of a subsequent cycle could be delayed up to 4 weeks. If the AZA could not be started after a 4-week delay, the patient could not receive any additional AZA. Patients who developed drug-related grade 3 or 4 renal, hepatic, cardiac, pulmonary, or neurologic toxicity had the AZA discontinued permanently. Patients with active acute GVHD grades III to IV were not eligible to receive AZA. If acute GVHD grades III to IV resolved, patients could receive the drug at 1 lower dose level in subsequent cycles dose level −1 at 16 mg/m^2 and dose level −2 (minimum dose) at 8 mg/m^2. Patients developing pneumonia or any infection deemed life-threatening by the attending physician had the AZA discontinued. If unexplained elevations of creatinine occurred to between 2 and 3 mg/dL, the next cycle was delayed

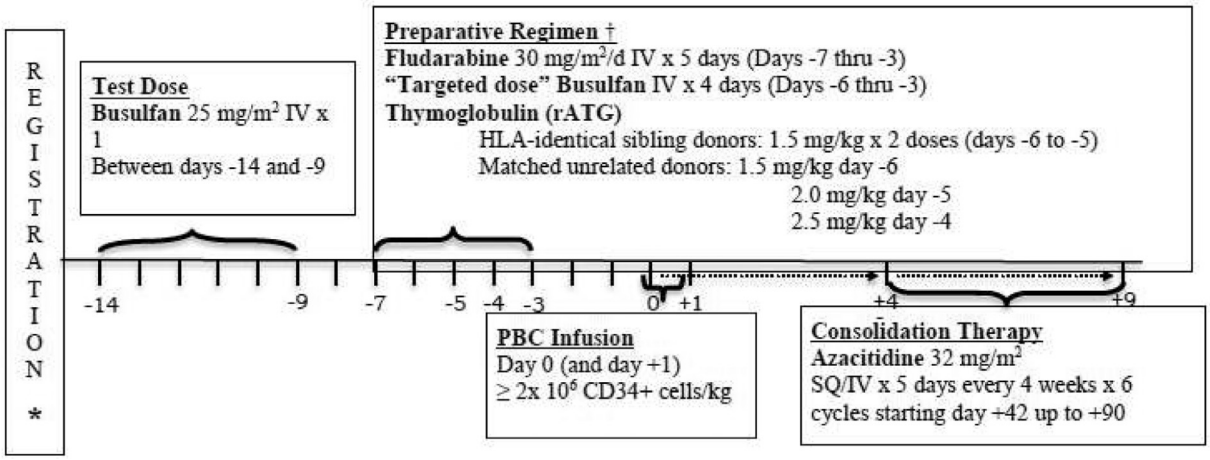

Figure 1. Treatment schema.

until values returned to normal or baseline, and the dose was reduced by 1 level for the next treatment course. AZA was discontinued for any unexplained increase in creatinine levels > 3 mg/dL.

Donor Mobilization and Target Allograft Composition

Donors received granulocyte colony-stimulating factor 10 μg/kg s.c. on days −5 through −2 (and, if necessary, day −1). On day −1 (and 0) donors underwent leukapheresis for 1 to 2 days to achieve a $CD34^+$ cell dose ≥ 2×10^6/kg (actual weight of recipient). If the yield of $CD34^+$ cells was $<2 \times 10^6$ /kg on day −1, an additional apheresis was permitted to be performed on day 0. If after 2 apheresis procedures the total $CD34^+$ cell dose was at least 2×10^6 /kg, no further apheresis was required. Target $CD34^+$ cell doses were based on institutional standards for sibling donors, as long as a minimum of 2×10^6 /kg was achieved. There was no maximum $CD34^+$ cell dose specified, and doses were not capped.

Supportive Care and Patient Assessments

Tacrolimus was targeted to a serum level of 5 to 10 ng/mL (not to exceed 15 ng/mL). The suggested starting dose was .03 mg/kg p.p. b.i.d. beginning on day −2 tapering between days +90 to +120 with a goal of stopping by days +150 to +180. Methotrexate was administered at 5 mg/m²/day i.v. on days +1, +3, and +6 (and day +11 in case of unrelated donor). Recipients received 5 μg/kg granulocyte colony-stimulating factor s.c. daily beginning on day +12 and continuing until absolute neutrophil count > 1500/μL for 2 consecutive days or > 5000/μL for 1 day. Acute GVHD and chronic GVHD were graded according to established criteria [16,17]. Patients were considered assessable for GVHD if they engrafted. Organ toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

Definitions

Neutrophil engraftment was defined as an increase in the absolute neutrophil count to 500/μL or greater after a conditioning regimen–induced nadir. Platelet engraftment was defined as the first day of 3 consecutive platelet count measurements greater than 20,000/μL without the aid of transfusion. NRM was defined as death in complete remission. Primary graft failure was defined as failure of neutrophil engraftment by day 30. Cytogenetic risk category was assigned based on the CALGB criteria [8].

Statistical Considerations

The primary endpoint was the probability of PFS at 2 years as estimated by the Kaplan-Meier estimator (if there is no censoring before 2 years, this is equivalent to a simple proportion of patients alive and progression free at 2 years). Disease progression and death due to any cause was considered an event. The time to PFS was the time interval between transplant and progression, death, or last follow-up, whichever occurred first. Patients without progression who were lost to follow-up before 2 years were censored at the time of their last follow-up. This study was designed as a single-arm single-stage trial. Based on previous studies a 2-year PFS of 25% or lower was considered clinically uninteresting. A 2-year PFS of 40% or higher would be considered clinically promising. Assuming a 2-year PFS of 40%, a sample size of 64 assessable patients provides 90% power at the 1-sided Type I error of .10 to reject the null hypothesis that the 2-year PFS was 25%. Based on this design, if at least 21 of 64 patients are alive and progression free for at least 2 years, it would be concluded that the 2-year PFS probability is greater than 25%.

Patient demographics and disease and treatment characteristics were summarized with median and range for continuous variables and frequency and percentage for categorical variables. PFS and OS were summarized using the Kaplan-Meier estimator. The cumulative incidence of relapse, NRM, and acute and chronic GVHD were summarized using the cumulative incidence function treating death as the competing risks. All patients who were lost to follow-up without experiencing the event of interest were censored at the time of their last follow-up. Data quality was ensured by review of data by the Alliance Statistics and Data Center and by the study chairperson following Alliance policies. Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center. All analyses were conducted using the SAS software version 9.4 (SAS Institute, Cary, NC) on the study database frozen on July 23, 2018.

RESULTS

Patient and Donor Characteristics

Patient and donor characteristics are provided in Table 1. In all, 68 patients were registered and 65 received transplantations at 10 centers between September 2010 and October 2013 (Figure 2). Each participant signed an institutional review board–approved, protocol-specific informed consent document in accordance with federal and institutional guidelines. Five patients who were registered (2 received transplants) were excluded from the primary analysis for the following reasons: 2 patients were taken off study before starting treatment, 1 patient withdrew consent before transplant, and 2 patients who underwent transplant had chronic myelomonocytic leukemia and were deemed ineligible for the study. Thus, the data analysis was limited to the 63 eligible recipients. The median patient age was 62 years (range, 44 to 74). Fifty patients (79.4%) had high-risk MDS and 13 (20.6%) had AML. Twenty-three patients (36.5%) received grafts from matched related donors and 40 (63.5%) from unrelated donor grafts. The median time from diagnosis to transplantation was 5.9 months (range, 1.8 to 109.7).

BU Dosing

Data on BU test dose and therapeutic dosing are shown in Table 2. The median AUC on the validation sample was 4143 μmol/min (range, 2400 to 6642). Thus, 87.1% of patients were within 20% of target AUC based on the validation sample.

Engraftment and Chimerism

The median number of $CD34^+$ cells infused was 5.0×10^6 /kg (range, 2.5 to 19.3). The median time to neutrophil engraftment was 13 days (range, 1 to 166) and to platelet engraftment was 11 days (range, 0 to 110) post-HSCT. Primary graft failure

Table 1
Patient Characteristics (N = 63)

Characteristic	Value
Age, years (median, range)	62 (44-74)
Gender	
Male	51 (81.0)
Female	12 (19.0)
ECOG performance status	
0	26 (41.3)
1	34 (54.0)
2	3 (4.8)
Disease	
AML	13 (20.6)
MDS	50 (79.4)
International Prognostic Scoring System (MDS only)	
Low	3 (7.3)
Intermediate-1	9 (22.0)
Intermediate-2	26 (63.4)
High	3 (7.3)
Missing	9
N/A (AML patients)	13
Cytogenetics risk	
Normal	29 (47.5)
Complex	18 (29.5)
−7, del(7)q	8 (13.1)
Other	6 (9.8)
Missing	2
Induction regimen	
7 + 3	12 (20.3)
DNA hypomethylating agent	41 (69.5)
Other	2 (3.4)
Unknown	4 (6.8)
No prior chemotherapy	4
Donor type	
Matched related	23 (36.5)
Matched unrelated	40 (63.5)
Months from diagnosis to transplant, median (range)	5.9 (1.8-109.7)
ABO Compatibility	
Match	29 (46)
Major mismatch	12 (19)
Minor mismatch	19 (30.2)
Bidirectional	3 (4.8)
Patient–donor cytomegalovirus serology	
Negative–negative	27 (42.9)
Negative–positive	7 (11.1)
Negative–unknown	1 (1.6)
Positive–negative	8 (12.7)
Positive–positive	19 (30.2)
Positive–unknown	1 (1.6)
Patient–donor gender	
Female–female	1 (1.6)
Female–male	11 (17.5)
Male–female	16 (25.4)
Male–male	35 (55.6)
No. of Cells Infused, $CD34^+ \times 10^6$/kg, median (range)	5.0 (2.5-19.3)

Values are n (%) unless otherwise defined. ECOG indicates Eastern Cooperative Oncology Group.

was observed in 4 patients (6.5%). Beginning with the first planned sample on day +30, the median proportion of donor cells in samples of peripheral blood analyzed for myeloid chimerism was consistently higher than 64% (range, 64% to 100%) at all time points analyzed. Median $CD3^+$ cell chimerism values gradually increased over time in the surviving patients without relapse and were 93% (range, 0% to 100%) at day +30, 95.5% (range, 68% to 100%) at day +90, 100% (range, 0% to 100%) at day +180, and 100% (range, 0% to 100%) at day +365 (Table 3).

Post-Transplant AZA

Forty-one patients (65%) started AZA at a median of 61 days (range, 43 to 91) post-transplant. Twenty-two patients never started AZA. Seventeen patients (41% of 41) completed all 6 cycles of AZA, representing 27% of the study group originally intended to receive 6 cycles of AZA. Details on reasons for not starting AZA and for ending protocol treatment before completing AZA are shown in Table 4.

Acute and Chronic GVHD

The cumulative incidences of grades II to IV and III to IV acute GVHD at 100 days were 36.5% (95% confidence interval [CI], 24.7% to 48.3%) and 12.7% (95% CI, 5.9% to 22.2%), respectively (Figure 3A). For all patients experiencing grades II to IV acute GVHD, the median time to onset was 31 days (range, 3 to 339). The cumulative incidence of any chronic GVHD at 2 years was 30.2% (95% CI, 19.2% to 41.8%) (Figure 3B) and of extensive chronic GVHD at 2 years was 14% (95% CI, 7% to 24.1%). For all patients experiencing limited or extensive chronic GVHD, the median time to onset of chronic GVHD was 216 days (range, 50 to 391) post-transplant.

Nonhematologic Adverse Events and Opportunistic Infections

The post-transplant rates of at least grade 3 adverse events are shown in Table 5. The only grade 3 to 5 organ toxicity seen in >10% of patients was mucositis (31%) and rash (13%). No cases of hepatic sinusoidal obstruction syndrome were observed. Reactivation of cytomegalovirus (viremia) occurred in 14 of 34 donor–recipient pairs (41%) at risk. Three patients developed cytomegalovirus disease (gastrointestinal, 2; central nervous system, 1). No patients died of cytomegalovirus disease.

Results for PFS, OS NRM, and Relapse

The median follow-up of survivors was 58.9 months (95% CI, 53.1 to 62.6). At 2 years post-transplant, 25 patients (more than the decision threshold of 21 patients) were alive and progression free. Therefore, per study design, there is sufficient evidence to conclude that PFS probability at 2 years is higher than 25% with a 1-sided Type I error rate of 10%. Equivalently, the PFS probability at 2 and 5 years after transplantation was 41.2% (80% CI, 33.9% to 49.9%) and 26.9% (80% CI, 20.4% to 35.5%), respectively, for the entire group, with a median PFS of 15.8 months (95% CI, 6.7 to 28.3) (Figure 4). The PFS probability at 2 and 5 years after transplantation for patients with AML was 53.8% (95% CI, 32.6% to 89.1%) and 30.1% (95% CI, 13.6% to 69.5%), respectively, with a median PFS of 28.3 months (95% CI, 5.7 to not reached) (Figure 5). The PFS probability at 2 and 5 years after transplantation for patients with MDS was 37.8% (95% CI, 26.5% to 54%) and 25.9% (95% CI, 15.7% to 42.5%), respectively, with a median PFS of 10.9 months (95% CI, 5.7 to 27.1).

The primary reasons patients came off treatment are shown in Table 6. The 100-day mortality was 16% (n = 10). Twenty-four patients (57%) died from causes other than relapse at a median of 222 days (range, 5 to 1678) after transplantation. The cumulative incidence of NRM at 2 years was 33.4% (95% CI, 22% to 45%) (Figure 6). Twenty patients relapsed at a median of 140 days (range, 33 to 1352) post-HSCT. The cumulative incidence of relapse was 25% (95% CI, 15% to 37%) at 2 years.

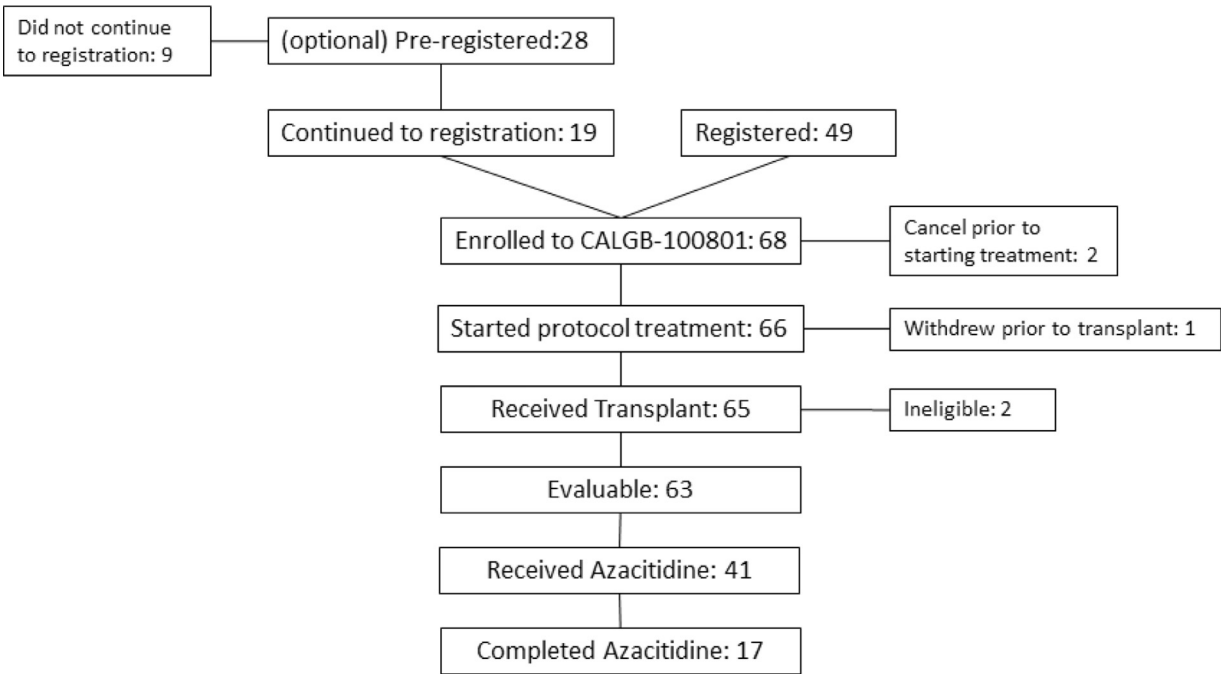

Figure 2. Consort diagram.

Table 2
Busulfan Dosing

	No. of Cases	Result
Test dose AUC, μmol/min	60	903.5 (100-1294)
Body surface area	63	1.9 (1.5-2.3)
Busulfan total dose, mg	63	800 (194-1502)
AUC on validation sample, μmol/min	62	4143 (2400-6642)
Validated sample AUC/target dose	62	1.04 (.60-1.66)
Achieved AUC within 20% of target AUC, n (%)	62	54 (87.1)

Values are median (range) unless otherwise defined.

Table 3
Chimerism

	$CD3^+$	Total
Day 30	35; 93 (0-100)	40; 100 (64-100)
Day 60	22; 95 (68-100)	31; 100 (95-100)
Day 90	24; 95.5 (0-100)	31; 100 (92-100)
Day 180	20; 100 (0-100)	26; 100 (90-100)
Day 365	11; 100 (0-100)	15; 100 (95-100)

Values are n; median percent (range).

Forty-two patients have died. As shown in Table 7, treatment-related death (n = 18, 43%) and death from disease (n = 18, 43%) were equally likely to be the causes of death, representing 86% of all deaths. The OS probability at 2 and 5 years was 45.7% (95% CI, 34.9% to 59.9%) and 31.2% (95% CI, 21.3% to 45.8%), respectively, for the entire group, with a median OS of 19.2 months (95% CI, 8.7 to 37.5). The OS probability at 2 and 5 years for the patients with AML was 61.5% (95% CI, 40.0% to 94.6%) and 44% (95% CI, 23.3% to 83%), respectively, with a median OS of 30.4 months (95% CI, 7.1 to not reached). The OS probability at 2 and 5 years for the patients with MDS was 41.7% (95% CI, 30.0% to 58.0%) and 27.8% (95% CI, 17.4% to 44.5%), respectively, with a median OS of 15.7 months (95% CI, 6.9 to 37.5).

Table 4
AZA Dosing

	Result
No. of patients starting AZA	41
Reasons for ending protocol treatment before starting AZA	
Death	8 (36)
Progression	4 (18)
Adverse event	5 (23)
Refusal	2 (9)
Other*	3 (14)
Time from transplant to start of AZA, days, median (range)	61 (43-91)
No. of AZA doses received	
1	4 (9.8)
2	4 (9.8)
3	5 (12.2)
4	5 (12.2)
5	5 (12.2)
6	18 (43.9)†
Completed AZA	17 (41)
Reasons for ending protocol treatment before completing AZA	
Death	4 (17)
Progression	6 (25)
Adverse event	5 (21)
Refusal	8 (33)
Other‡	1 (4)

Values are n (%) unless otherwise defined.

* Patient scheduled to receive bone marrow stem cells not allowed per protocol; could not start AZA due to low absolute neutrophil count on day 90; patient started on Valcyte for cytomegalovirus, which caused low counts, never met criteria to start AZA.

† One patient stated cycle 6 but only received doses 1 and 2; doses 3-5 were missed due to sepsis. Therefore they did not complete treatment per protocol.

‡ Treatment delayed greater than 4 weeks due to adverse event not related to protocol.

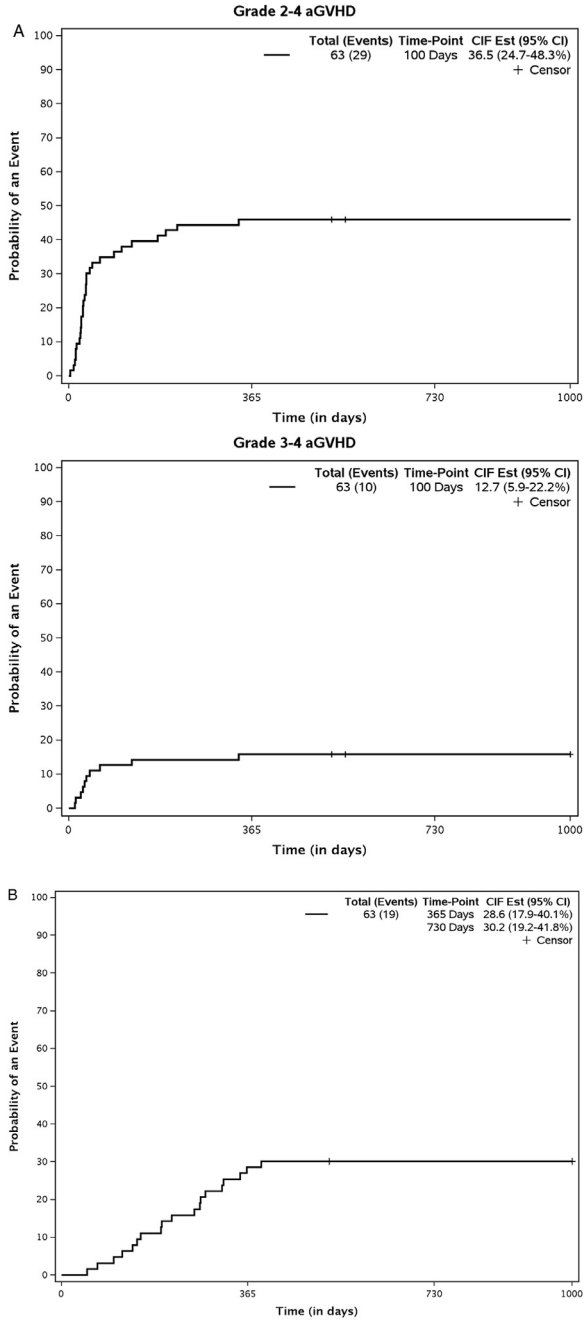

Figure 3. (A) Cumulative incidence of acute GVHD. (B) Cumulative Incidence of any chronic GVHD.

DISCUSSION

This prospective study demonstrates the feasibility and challenges of implementing strategies designed to mitigate relapse in patients with high-risk hematologic malignancies undergoing HSCT in a multicenter setting. The study met the primary endpoint with the conclusion that PFS probability at 2 years post transplant was greater than 25% with a 1-sided Type I error of 10% as designed (equivalently, 80% CI was presented above). A test dose of BU to achieve targeted AUC of 4000 μM/min is both feasible and effective even in a predominantly older patient population, as most patients (87.1%) were within 20% of the targeted value without experiencing grade 4 toxicity. The planned administration of post-transplant maintenance AZA in older patients with AML and high-risk MDS is much more challenging, and one-third of our patients could not receive AZA as planned and only 27% of all recipients were able to receive the intended 6 cycles of maintenance therapy. Although we achieved our goal of at least 25% patients who were progression free at 2 or more years post-HSCT, we conclude that post-transplant maintenance strategies that are less toxic and more effective than s.c. AZA should be sought.

We have also demonstrated the clear value of long-term follow-up to achieve a more accurate assessment of the value of any transplant strategy. It is unusual for the first report of a prospective HSCT trial to contain data on a group of patients with a median follow-up of nearly 5 years. We demonstrated that many adverse relapse and nonrelapse events can occur between years 2 and 5 in a high-risk population and caution broad interpretation of studies with less than 2 years of median follow-up.

Prospective randomized trials and analysis of registry data comparing myeloablative conditioning with RIC in MDS and AML have yielded mixed results [23-27]. The Center for International Blood and Marrow Transplant Research registry data

Table 5
Summary of Adverse Events Regardless of Attribution (N = 63)

Adverse Event	No. of Patients	Percent of Patients
Total		
Grade 1 event	0	.0
Grade 2 event	0	.0
Grade 3 event	4	6.3
Grade 4 event	42	66.7
Grade 5 event	17	27.0
Hematologic adverse events		
Grade 1 event	0	.0
Grade 2 event	1	1.6
Grade 3 event	6	9.5
Grade 4 event	54	85.7
Grade 5 event	0	.0
Nonhematologic adverse events		
Grade 1 event	0	.0
Grade 2 event	2	3.2
Grade 3 event	37	58.7
Grade 4 event	7	11.1
Grade 5 event	17	27.0

Summaries are based on available patient data.

Table 6
Patient Disposition (N = 63)

Characteristic	Value
Months of follow-up for survivors, median (95% CI) Primary off-treatment reason	58.9 (53.1-62.6)
Treatment completed per protocol	17 (27.0)
Disease progression	10 (15.9)
Adverse event	10 (15.9)
Died during treatment	12 (19.0)
Patient refused further protocol treatment	10 (14.9)
Other*	4 (6.4)

Values are n (%) unless otherwise defined.
* Patient scheduled to receive bone marrow stem cells not allowed per protocol; could not start AZA due to low absolute neutrophil count on day 90; patient started on Valcyte for cytomegalovirus, which caused low counts, never met criteria to start AZA; treatment delayed greater than 4 weeks due to adverse event not related to protocol.

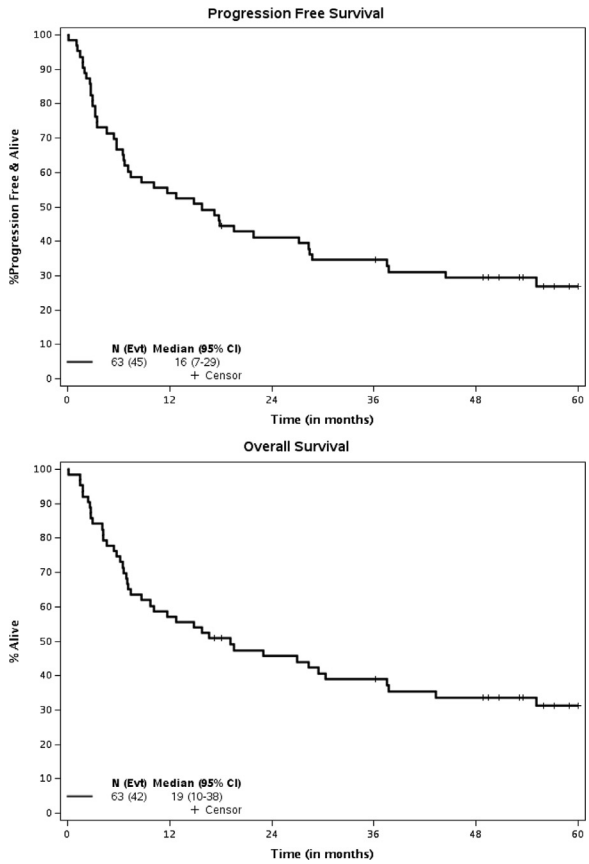

Figure 4. Kaplan-Meier survival curves (all patients).

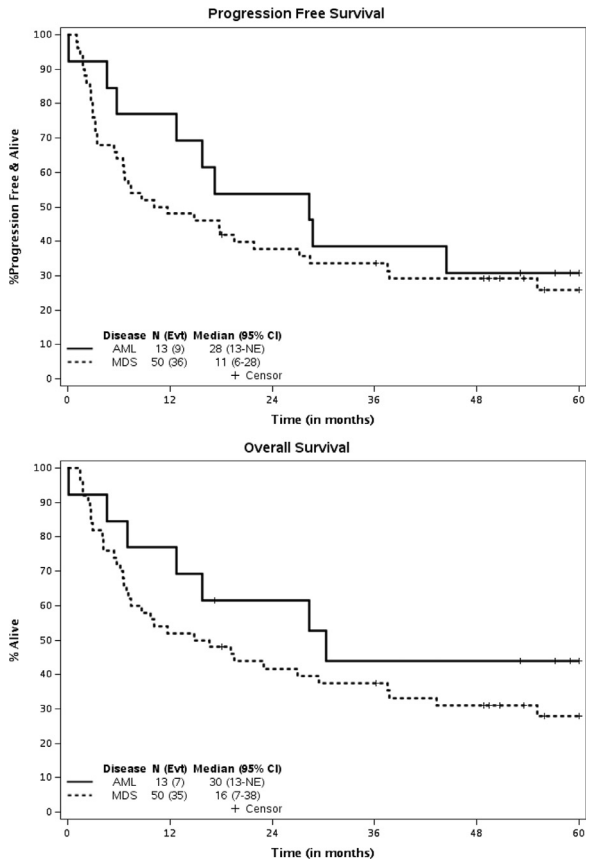

Figure 5. Kaplan-Meier survival curves (by disease).

show that between 2000 and 2015 the combination of fludarabine and BU (6.4 mg/kg total dose) was the most commonly used RIC regimen for AML and MDS, accounting for 32% of the transplants [1]. This regimen uses 50% of the standard BU dose without targeting. The toxicity seen with the BU-containing RIC regimen using 3.2 to 6.4 mg/kg has been minimal. We aimed to study a pharmacokinetic-based targeted BU preparative regimen with a target AUC of 75% of the full dose. We hypothesized that the enhanced antitumor effect of this higher BU exposure would also allow sufficient time for the alloimmune effect to emerge. The target AUC of 4000 μM/min was achieved in 87% of patients. Again, as anticipated the toxicity attributable to the conditioning regimen was generally mild to moderate and reversible. Optimizing BU pharmacokinetics through targeted dosing seems rational if the goal is to achieve maximal exposure while limiting toxicity. For instance, the Blood and Marrow Transplant Clinical Trials Network 0901 study demonstrated substantially lower rates of relapse but increased NRM in the recipients of fully myeloablative conditioning [23]. Combining myeloablative conditioning with ex vivo T cell depletion may also be another useful strategy to prevent relapse while minimizing transplant-related toxicity [28,29].

We also aimed to study the use of post-transplant AZA to reduce early post-transplant relapse, directly through the effect of AZA on MDS or alternatively by altering the post-transplant immune environment in a manner that facilitates the graft-versus-leukemia effect. Several authors have reported on the use of DNA hypomethylating agents after allogeneic transplant [22,30-33]. In contrast to our cohort, most patients in these studies had a diagnosis of AML. Patients with MDS accounted for nearly 80% of patients in our cohort, and we allowed patients with up to 10% blasts to be eligible. Forty-one patients (65%) started AZA and 17 (41%) completed all 6 cycles of AZA. This experience is similar to that reported by other authors. If the goal is to administer maintenance therapy for prolonged periods post-HSCT (up to 12 months or longer) in older patients, s.c. AZA may not be an optimal strategy based on our study results, given that less than half of the

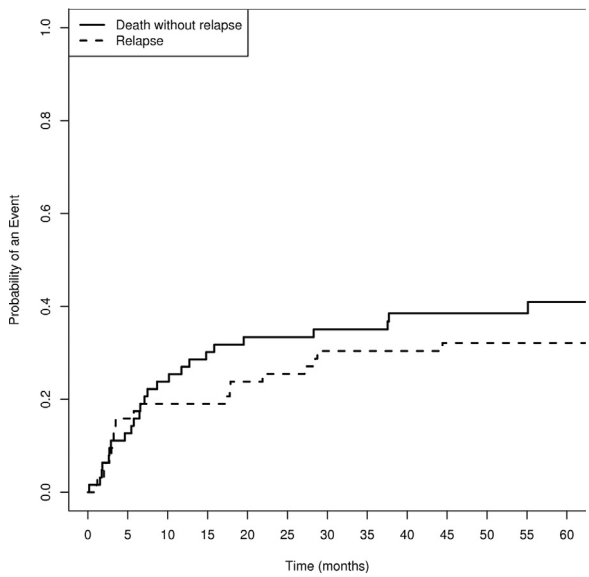

Figure 6. Cumulative incidence of NRM and relapse.

Table 7
Causes of Death (n = 42)

Cause of death	n (%)
Protocol treatment related	18 (42.9)
Protocol disease related	18 (42.9)
Not related to protocol treatment or protocol	5 (11.9)
Unknown	1 (2.4)

patients could complete the planned course. Oral maintenance agents or cell/antibody-based relapse mitigation strategies may be more attractive alternatives.

We incorporated rabbit antithymocyte globulin (Thymoglobulin) into the conditioning regimen to reduce the rates of both severe acute and chronic GVHD, as supported by both retrospective and prospective controlled data [34,35]. Whether this strategy led to a higher risk of relapse in the patients on this study is a matter of speculation and cannot be addressed with certainty. That said, the overall relapse rate of 25% is in line with reports from high-risk patients reported using non-antithymocyte globulin–containing regimens.

Overall, the conditioning regimen was reasonably well tolerated with acceptable and supportable rates of mucositis and cumulative incidences of grades II to IV and III to IV acute GVHD at 100 days of 36.5% and 12.7%, respectively. The cumulative incidence of chronic GVHD at 2 years of 30.2% with extensive chronic GVHD in 14% of patients is also in line with previous reports, particularly when peripheral blood progenitor cells are used as a graft source. The 100-day cumulative incidence of NRM was 16% and cumulative incidence of NRM at 2 years 33.4%, which, although appearing to be high, are also consistent with previous studies that included patients with high-risk MDS. The PFS probability at 2 years after transplant was 41.2% for the entire group (AML, 53.8%; MDS, 37.8%), and the OS probability at 5 years was 31.2% (AML, 44%; MDS, 27.8%), with death due to disease accounting for 43% of all deaths. The heterogeneity of the patient cohort and the single-arm design of the study make firm conclusions difficult. Also, this study was conducted at a time before incorporation of matched related donor assessment before transplant was routine, and collection of these data will be critical for future prospective trials. However, the results in this elderly group of patients are encouraging.

In a multicenter study of post-transplant AZA maintenance in older AML patients, quantification of circulating tumor-specific $CD8^+$ T cells was evaluated, and their presence was associated with freedom from relapse [32]. The potential induction of these tumor-specific cells by AZA provides good rationale for its use in this setting. Unfortunately, we did not analyze for the presence of these cells in our study, but this clearly should be incorporated into future studies planning to administer AZA or other hypomethylating agents after HSCT.

In conclusion, we have demonstrated the feasibility of a novel RIC regimen with test dose BU targeted to an AUC of 4000 μM/min and report on the largest series of patients to date with both AML and MDS given post-transplant AZA. The feasibility of AZA in this setting appears to be limited if applied to an unselected population of older HSCT recipients but may be beneficial if used in a more targeted group of patients most likely to benefit. However, the true value of this approach can only be evaluated in a randomized clinical trial. The results of a recently completed randomized study (NCT00887068) will be very helpful in determining the ultimate future of this strategy.

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REFERENCES

1. D'Souza A, Fretham C. Current uses and outcomes of hematopoietic cell transplantation (HCT): CIBMTR summary slides, 2017. Vol 20192017, http://www.cibmtr.org. Accessed 9th Jan 2019.
2. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. 2006;107:3481–3485.
3. Cancer, Leukemia Group B, Farag SS, et al. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Blood*. 2006;108:63–73.
4. Lowenberg B, Ossenkoppele GJ, van Putten W, et al. High-dose daunorubicin in older patients with acute myeloid leukemia. *N Engl J Med*. 2009;361:1235–1248.
5. Oran B, Weisdorf DJ. Survival for older patients with acute myeloid leukemia: a population-based study. *Haematologica*. 2012;97:1916–1924.
6. Rowe JM, Tallman MS. How I treat acute myeloid leukemia. *Blood*. 2010;116:3147–3156.
7. Schoch C, Kern W, Schnittger S, Buchner T, Hiddemann W, Haferlach T. The influence of age on prognosis of de novo acute myeloid leukemia differs according to cytogenetic subgroups. *Haematologica*. 2004;89:1082–1090.
8. Vasu S, Kohlschmidt J, Mrozek K, et al. Ten-year outcome of patients with acute myeloid leukemia not treated with allogeneic transplantation in first complete remission. *Blood Adv*. 2018;2:1645–1650.
9. Farag SS, Maharry K, Zhang MJ, et al. Comparison of reduced-intensity hematopoietic cell transplantation with chemotherapy in patients age 60-70 years with acute myelogenous leukemia in first remission. *Biol Blood Marrow Transplant*. 2011;17:1796–1803.
10. McClune BL, Weisdorf DJ, Pedersen TL, et al. Effect of age on outcome of reduced-intensity hematopoietic cell transplantation for older patients with acute myeloid leukemia in first complete remission or with myelodysplastic syndrome. *J Clin Oncol*. 2010;28:1878–1887.
11. Aoudjhane M, Labopin M, Gorin NC, et al. Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European Group for Blood and Marrow Transplantation (EBMT). *Leukemia*. 2005;19:2304–2312.
12. Bertz H, Potthoff K, Finke J. Allogeneic stem-cell transplantation from related and unrelated donors in older patients with myeloid leukemia. *J Clin Oncol*. 2003;21:1480–1484.
13. Estey E, de Lima M, Tibes R, et al. Prospective feasibility analysis of reduced-intensity conditioning (RIC) regimens for hematopoietic stem cell transplantation (HSCT) in elderly patients with acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (MDS). *Blood*. 2007;109:1395–1400.

14. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood*. 2001;97:3390–3400.
15. Devine SM, Owzar K, Blum W, et al. Phase II study of allogeneic transplantation for older patients with acute myeloid leukemia in first complete remission using a reduced-intensity conditioning regimen: results from Cancer and Leukemia Group B 100103 (Alliance for Clinical Trials in Oncology)/Blood and Marrow Transplant Clinical Trial Network 0502. *J Clin Oncol*. 2015;33:4167–4175.
16. Andersson BS, Thall PF, Madden T, et al. Busulfan systemic exposure relative to regimen-related toxicity and acute graft-versus-host disease: defining a therapeutic window for i.v. BuCy2 in chronic myelogenous leukemia. *Biol Blood Marrow Transplant*. 2002;8:477–485.
17. Dix SP, Wingard JR, Mullins RE, et al. Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant*. 1996;17:225–230.
18. Grochow LB. Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimens. *Semin Oncol*. 1993;20:18–25. quiz 26.
19. Geddes M, Kangarloo SB, Naveed F, et al. High busulfan exposure is associated with worse outcomes in a daily i.v. busulfan and fludarabine allogeneic transplant regimen. *Biol Blood Marrow Transplant*. 2008;14:220–228.
20. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood*. 1997;89:3055–3060.
21. Palmer J, McCune JS, Perales MA, et al. Personalizing busulfan-based conditioning: considerations from the American Society for Blood and Marrow Transplantation Practice Guidelines Committee. *Biol Blood Marrow Transplant*. 2016;22:1915–1925.
22. de Lima M, Giralt S, Thall PF, et al. Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. *Cancer*. 2010;116:5420–5431.
23. Scott BL, Pasquini MC, Logan BR, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. *J Clin Oncol*. 2017;35:1154–1161.
24. Kroger N, Iacobelli S, Franke GN, et al. Dose-reduced versus standard conditioning followed by allogeneic stem-cell transplantation for patients with myelodysplastic syndrome: a prospective randomized phase III study of the EBMT (RICMAC trial). *J Clin Oncol*. 2017;35:2157–2164.
25. Bornhauser M, Kienast J, Trenschel R, et al. Reduced-intensity conditioning versus standard conditioning before allogeneic haemopoietic cell transplantation in patients with acute myeloid leukaemia in first complete remission: a prospective, open-label randomised phase 3 trial. *Lancet Oncol*. 2012;13:1035–1044.
26. Eapen M, Brazauskas R, Hemmer M, et al. Hematopoietic cell transplant for acute myeloid leukemia and myelodysplastic syndrome: conditioning regimen intensity. *Blood Adv*. 2018;2:2095–2103.
27. Sengsayadeth S, Gatwood KS, Boumendil A, et al. Conditioning intensity in secondary AML with prior myelodysplastic syndrome/myeloproliferative disorders: an EBMT ALWP study. *Blood Adv*. 2018;2:2127–2135.
28. Malard F, Labopin M, Cho C, et al. Ex vivo and in vivo T cell-depleted allogeneic stem cell transplantation in patients with acute myeloid leukemia in first complete remission resulted in similar overall survival: on behalf of the ALWP of the EBMT and the MSKCC. *J Hematol Oncol*. 2018;11:127.
29. Barba P, Martino R, Zhou Q, et al. CD34(+) cell selection versus reduced-intensity conditioning and unmodified grafts for allogeneic hematopoietic cell transplantation in patients age >50 years with acute myelogenous leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2018;24:964–972.
30. Maples KT, Sabo RT, McCarty JM, Toor AA, Hawks KG. Maintenance azacitidine after myeloablative allogeneic hematopoietic cell transplantation for myeloid malignancies. *Leuk Lymph*. 2018;59:2836–2841.
31. Pusic I, Choi J, Fiala MA, et al. Maintenance therapy with decitabine after allogeneic stem cell transplantation for acute myelogenous leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2015;21:1761–1769.
32. Craddock C, Jilani N, Siddique S, et al. Tolerability and clinical activity of post-transplantation azacitidine in patients allografted for acute myeloid leukemia treated on the RICAZA trial. *Biol Blood Marrow Transplant*. 2016;22:385–390.
33. Platzbecker U, Middeke JM, Sockel K, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. *Lancet Oncol*. 2018;19:1668–1679.
34. Soiffer RJ, Kim HT, McGuirk J, et al. Prospective, randomized, double-blind, phase III clinical trial of anti-T-lymphocyte globulin to assess impact on chronic graft-versus-host disease-free survival in patients undergoing HLA-matched unrelated myeloablative hematopoietic cell transplantation. *J Clin Oncol*. 2017;35:4003–4011.
35. Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood*. 2011;117:6963–6970.