Original Article

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

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BACKGROUND: Recurrence is a major cause of treatment failure after allogeneic transplantation for acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS), and treatment options are very limited. Azacitidine is a DNA methyltransferase inhibitor with activity in myeloid disease. The authors hypothesized that low-dose azacitidine administered after transplant would reduce recurrence rates, and conducted a study to determine a safe dose/schedule combination. **METHODS:** Forty-five high-risk patients were treated. Median age was 60 years; median number of comorbidities was 3; 67% were not in remission. By using a Bayesian adaptive method to determine the best dose/schedule combination based on time to toxicity, the authors investigated combinations of 5 daily azacitidine doses, 8, 16, 24, 32, and 40 mg/m^2, and 4 schedules: 1, 2, 3, or 4 cycles, each with 5 days of drug and 25 days of rest. Cycle 1 started on Day +40. **RESULTS:** Reversible thrombocytopenia was the dose-limiting toxicity. The optimal combination was 32 mg/m^2 given for 4 cycles. Median follow-up was 20.5 months. One-year event-free and overall survival were 58% and 77%, justifying further studies to estimate long-term clinical benefit. No dose significantly affected DNA global methylation. **CONCLUSIONS:** Azacitidine at 32 mg/m^2 given for 5 days is safe and can be administered after allogeneic transplant for at least 4 cycles to heavily pretreated AML/MDS patients. The trial also suggested that this treatment may prolong event-free and overall survival, and that more cycles may be associated with greater benefit. ***Cancer* 2010;116:5420–31.** © *2010 American Cancer Society*.

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Patients with acute myelogenous leukemia (AML) or advanced myelodysplastic syndrome (MDS) who fail to achieve a complete remission (CR) or are otherwise refractory to therapy have a poor prognosis. Allogeneic hematopoietic stem cell transplantation (HSCT) is frequently considered a salvage option for these patients, but disease recurrence and

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nonrecurrence mortality remain a major cause of treatment failure for patients transplanted without remission.[1,2] Preparative regimen dose escalation has failed to improve results significantly, in large part because of a direct relationship between dose intensity and treatment-related mortality. The CR rate with HSCT, however, is high, and most patients transplanted in recurrence will be in morphologic remission after HSCT, but these remissions are usually short-lived. Because most recurrences occur early, any preventative intervention must be implemented during the first 3 months after HSCT to be effective. In this scenario, new strategies for maintaining remission are needed.

Pharmacologic maintenance is difficult to achieve with traditional agents because of multiple drug interactions and myelosuppression risk. An ideal drug should have activity against the disease, without excessive myelosuppression. Azacitidine is effective in MDS in doses that are likely to induce severe pancytopenia after HSCT.[3] This hypomethylating agent appears to reverse DNA hypermethylation, leading to silencing of tumor-suppressing genes in malignant cells. Azacitidine and decitabine may also cause phenotypic modification of leukemic cells (including increased expression of major histocompatibility complex class I and human leukocyte antigen [HLA]-DR) and induction of expression of cancer antigens that could potentially enhance the graft-versus-leukemia effect.[4-9] We have shown that low-dose azacitidine is moderately active in reinducing remission and donor chimerism for patients with indolent AML/MDS recurrences after HSCT, using doses ranging from 16 to 40 mg/m^2 for 5 days in 28- to 30-day cycles.[10]

We therefore hypothesized that azacitidine might decrease the recurrence rate after HSCT. However, it might worsen graft-versus-host disease (GVHD), compromise graft function and immune recovery, or induce other adverse effects. A phase 1 study consequently was necessary. We were also interested in demonstrating that the drug can be administered repeatedly after transplant, assuming that patients treated early on, when grafts are vulnerable to myelosuppression, would be able to safely receive longer administration schedules in future studies. Herein we present the results of such study.

MATERIALS AND METHODS

Eligibility

Eligible were adult patients with AML or high-risk MDS (International Prognostic Scoring System[11] intermediate-2 or high-risk) aged 18 to 75 years, not in first CR (CR1), who were not candidates for myeloablative transplant regimens because of older age or comorbidities. After establishing the low toxicity profile of azacitidine, we amended the protocol to allow inclusion of high-risk CR1 patients. Donors could be related or unrelated, matched at HLA-A, B, C, DRB1, and DQB1 (1 mismatch allowed), typed as previously described.[12]

Other eligibility criteria included a left ventricular ejection fraction >40%, a forced expiratory volume at 1 second, forced vital capacity, and diffusing capacity of lungs for carbon monoxide >40%, creatinine <1.6 mg/dL, bilirubin <1.6 mg/dL, human immunodeficiency virus seronegativity, negative pregnancy test, absence of active infection, and ability to undergo the informed consent process. The protocol was approved by the institutional review board at The University of Texas M. D. Anderson Cancer Center.

A reduced-intensity regimen of gemtuzumab ozogamicin 2 mg/m^2 (given to 40 CD33-positive patients on Day −12), fludarabine at a dose of 30 mg/m^2 (on Days −5, −4, −3, and −2), and melphalan at a dose of 140 mg/m^2 (Day −2) was used.[13] Patients with unrelated or mismatched-related donors received rabbit antithymocyte globulin (ATG) 0.5 mg/kg (Day −3) and 1.25 mg/kg (Days −2, −1). GVHD prophylaxis was tacrolimus and minimethotrexate (5 mg/m^2 on Days 1, 3, 6, and 11), or sirolimus, mycophenolate mofetil, and ATG (n = 5). Supportive care was as previously described.[13]

Eligibility to Receive Azacitidine

Patients in CR by HSCT Day +30 were eligible to receive azacitidine, whereas patients with persistent disease or without donor engraftment were removed from the study. Other eligibility criteria to start azacitidine were as follows: creatinine <1.6 mg/dL, bilirubin <1.6 mg/dL, alanine aminotransferase ≤3 × upper limit of normal, platelet count >15,000/mm^3, and absolute neutrophil count (ANC) >1000/mm^3. Patients could not have bleeding, uncontrolled infection, or grade III/IV acute GVHD. If not eligible for treatment during the first 3 months post-transplant, patients went off protocol.

Azacitidine was given for 1 to 4 30-day cycles. In each cycle, the drug was administered subcutaneously for 5 days, starting on the sixth week after HSCT at 1 of 5 dose levels (8, 16, 24, 32, or 40 mg/m^2).

Development of drug-related grade 3 or 4 organ toxicity or severe infection led to azacitidine discontinuation. Azacitidine was also discontinued if platelet count

Table 1. Primers and Conditions Used for PCR of Pyrosequencing

Primer	Sequences	Gene Bank Accession No.	Temperature, °C (Cycles)
LINE	F: 5-TTTTGAGTTAGGTGTGGGATATA-3 R: 5-AAAATCAAAAAATTCCCTTTC-3[a] Sequencing: 5-AGTTAGGTGTGGGATATAGT-3	X58075	56 (45)

PCR indicates polymerase chain reaction; LINE, long interspersed nuclear elements.
[a] Biotin-labeled.

dropped to $<10{,}000/mm^3$, with 50% dose reduction if platelet count dropped to $<20{,}000/mm^3$, or if ANC dropped to $<500/mm^3$, not responsive to growth factor. Granulocyte colony-stimulating factor administration was allowed.

Evaluation of Response and Definitions

Patients had a bone marrow aspiration on transplant Days +30 and +100 to +120, and at 9 and 12 months after transplantation. CR was defined as <6% bone marrow blasts and evidence of donor chimerism (>80%) by DNA microsatellite polymorphism analysis.

Bone marrow or peripheral blood donor-recipient chimerism was evaluated using DNA microsatellite polymorphism analysis by polymerase chain reaction (PCR). We also used conventional cytogenetic analysis with G-banding or fluorescent in situ hybridization studies for the Y chromosome in sex-mismatched transplants. Mixed chimerism was defined as the presence of any detectable percentage of unsorted recipient cells or DNA.

Analysis of DNA Methylation

We studied long interspersed nuclear elements methylation, a marker of global DNA methylation using bisulfite pyrosequencing.[14] Methods for bisulfite modification of DNA and subsequent PCR techniques are described in http://www3.mdanderson.org/leukemia/methylation and in Table 1. The degree of methylation was calculated using PSQHS 96A 1.2 software (Biotage AB, Uppsala, Sweden). Blood samples were obtained on Days 1, 5, and 21 of treatment with azacitidine (n = 38 patients).

Total Design and Statistical Analysis

The primary goal was to find the best combination of per-administration dose and schedule of azacitidine. Each patient was assigned 1 per-administration dose/schedule combination, with schedule = 1, 2, 3, or 4 cycles. The first cycle started approximately on Day 40 post-transplant. Under schedule 1, the assigned per-administration dose was given on transplant Days 40, 41, 42, 43, and 44; under schedule 2 on Days 40, 41, 42, 43, 44, 68, 69, 70, 71, and 72, and similarly for schedules 3 and 4. The outcome was the time to toxicity, where toxicity was defined as any of the following adverse events occurring within 116 days from the start of the first cycle: 1) National Cancer Institute grade 3 or higher renal, hepatic, cardiac, pulmonary, or neurologic toxicity; 2) grade III-IV acute GVHD; 3) serious infection; 4) severe hematologic toxicity/graft failure; or 5) >2 dose reductions for any reason. The Bayesian method of Braun et al[15] was used to adaptively choose each new patient's per-administration dose/schedule combination, based on the probability of toxicity within 116 days from the start of therapy, with the goal of choosing the per-administration dose/schedule combination having posterior mean probability of toxicity closest to .30, a criterion similar to that used by the Continual Reassessment Method.[16] Additional safety rules were 1) a per-administration dose/schedule pair was acceptably safe if the posterior probability of having likelihood of toxicity >.30 was not >.80, with no unacceptable pairs administered; and 2) when escalating to a per-administration dose/schedule pair that had not yet been tried, it was allowed to increase either the per-administration dose or schedule, a "do not skip" rule.

The trial was conducted as follows, where "escalation"("de-escalation") means increasing (decreasing) per-administration dose, schedule, or both:

- Treat the first patient at the lowest dose/schedule pair (8 mg/m^2, 1 cycle).
- For each patient after the first, based on the current data under the Bayesian model, determine the set of acceptably safe per-administration dose/schedule combinations.
- If none of the per-administration dose/schedule combinations are acceptably safe, then stop the trial and conclude that none of per-administration dose/schedule combinations are acceptable.
- If 1 or more per-administration dose/schedule combinations are acceptably safe, then assign the next patient to

the combination for which, based on the current data, the posterior mean of probability of toxicity is closest to the targeted value .30, subject to the escalation constraint of the "do not skip" rule.

- If the safe dose with probability of toxicity closest to the targeted value .30 is below the current per-administration dose/schedule combination, then there was no constraint on de-escalation.

It was planned initially to study the 3 per-administration doses of 8, 16, and 24 mg/m^2. When only 1 toxicity was observed in the first 27 patients, the design was extended to include 4 higher per-administration doses, 32, 40, 48, or 56 mg/m^2, of which 48 and 56 mg/m^2 were not studied.

Unadjusted probabilities of overall survival (OS) and event-free survival (EFS) were estimated using the Kaplan-Meier method.[17] The log-rank test[18] was used to compare unadjusted OS or PFS between subgroups. A Bayesian log-normal regression model was used to assess the effects of covariates and treatment on OS and PFS. Covariates included log(bone marrow blast), number of previous chemotherapy regimens ($\geq$2 vs $\leq$1), cytogenetics, number of comorbidities, dose, and number of cycles of azacitidine. The lognormal regression model was selected using the Bayes Information Criterion and the Bayesian chi-square method.[19] Each covariate parameter in the lognormal model linear term was assumed to follow a normal prior with mean 0 and variance 10,000, denoted N(0,10000), and the dispersion parameter followed a noninformative inverse-gamma prior with mean 1 and variance 10,000. A Bayesian logistic regression model was fit for the binary indicator of chronic GVHD, with each parameter in the linear term of covariates following a noninformative N(0,10000) prior. The Bayesian model fits were carried out in WinBugs1.4[20]; all other analyses were carried out in Splus6.1.[21] The Bayesian parametric model underlying the method[15] was fit to the final data to assess the joint effects of per-administration dose and schedule on the risk of toxicity.

RESULTS

Patients

Median age was 60.6 years (range, 24.3-73.8 years). Diagnoses were AML (n = 37) or MDS (n = 8); 67% of the patients were not in CR at HSCT. The median number of prior chemotherapy regimens was 2, 39 patients previously received high-dose Ara-C–based chemotherapy, and 18% of the patients had failed a previous allogeneic HSCT. The median number of clinical comorbidities was 3 (Table 2), and median performance status was 1 (range, 0-2).

Donors, Grafts, and Engraftment

Donors were unrelated (42%) and related (58%). All but 3 donor-recipient pairs were fully matched. Median number of infused CD34-positive and total nucleated cells was 4.3×10^6 (range, 1.04-13.3) and 8.0×10^8 (range, 0.4-26.9). Median time to neutrophil and platelet engraftment was 12 days (n = 45; range, 10-23) and 17 days (n = 43; range, 10-66). As expected with this preparative regimen, most patients (96%) exhibited 100% donor chimerism on Day 30 to 40. Azacitidine did not affect engraftment (median of 100% donor chimerism for evaluable patients at 6 and 12 months after HSCT).

Preparative Regimen and Azacitidine

Ninety patients were enrolled. Four never started the conditioning because of death or serious infections, 10 patients died early (up to Day +60), and 2 did not respond to transplant. Of the 74 patients potentially eligible to receive azacitidine, 45 (60%) actually received it and comprise the group described here. Reasons for never receiving azacitidine were refusal (n = 3), GVHD (n = 5), pancytopenia (n = 6), elevated creatinine (n = 4), and infections (n = 11). Patients received a total of 105 cycles of azacitidine.

EFS and OS

Median follow-up was 20.5 months (range, 7.7-39.6 months). Nineteen (42%) patients had died at a median of 30.8 months (95% confidence interval [CI], 14.3 months-upper limit not estimable) (Fig. 1). Causes of death included GVHD (n = 3), pneumonia and pulmonary hemorrhage (n = 1), and disease recurrence (n = 15). Nonrecurrence mortality rate was 9% (n = 4).

Twenty-four (53%) patients had developed disease recurrence. Seven recurrences occurred while on azacitidine: at 16 mg/m^2 for 2 cycles (n = 1, AML in CR3, second HSCT), 24 mg/m^2 for 1 cycle (n = 3, in first recurrence), 32 mg/m^2 for 1 cycle (n = 2, primary induction failure and first recurrence), and 40 mg/m^2 for 2 cycles (n = 1, primary induction failure).

Twenty-eight patients (62%) died or developed disease recurrence. Median EFS was 18.2 months (95% CI, 11.9 months-upper limit not estimable) (Fig. 2). Cytogenetics or donor type did not affect EFS. There was, however, a significant EFS difference favoring patients in

Table 2. Patient Characteristics

Variable	No. (%)	Median (Range)
Age, y	45	60.6 (24.3-73.8)
Bone marrow blast at transplantation (%) (all patients)	45	6 (0-80)
Median bone marrow blasts at transplantation (in patients with active disease)	30	10 (6%-80%)
Sex		
Women	21 (46.7)	
Men	24 (53.3)	
Diagnosis		
AML	37 (82.2)	
MDS	8 (17.8)	
Cytogenetics[11,35]		
Bad[a]	18 (40.0)	
Intermediate	26 (57.8)	
Good	1 (2.2)	
No. of chemotherapy regimens received before transplantation		
0	2 (4.4)	
1	18 (40.0)	
2	17 (37.8)	
3	5 (11.1)	
4	3 (6.7)	
Complete remission at transplantation		
No	30 (66.7)	
Primary induction failure	16	
First and second recurrence	11 and 1	
Untreated high-risk MDS	2	
Yes	15 (33.3)[b]	
No. of comorbidities[c]		
0	5 (11.1)	
1	7 (15.6)	
2	5 (11.1)	
3	9 (20.0)	
4	10 (22.2)	
5	2 (4.4)	
6	4 (8.9)	
7	1 (2.2)	
8	2 (4.4)	
Median performance status at transplantation		1 (0-2)
Donor type		
Unrelated	19 (42.2)	
Related	26 (57.8)	
Stem cell source		
Bone marrow	11 (24)	
Peripheral blood	34 (76)	
Azacitidine dose, mg/m^2		
8	7 (15.6)	
16	4 (8.9)	
24	17 (37.8)	
32	15 (33.3)	
40	2 (4.4)	
No. of azacitidine cycles		
1	13 (28.9)	
2	13 (28.9)	
3	10 (22.2)	
4	9 (20.0)	

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome.

[a] Included 7 patients with chromosome 7 deletions.

[b] First complete remission (CR): 5 patients (2 without cytogenetic CR, 1 with minimal residual disease by flow cytometry and poor prognosis cytogenetics, and 2 requiring $\geq$2 cycles of chemotherapy to enter CR); second CR, 7 patients; third CR, 3 patients.

[c] Comorbidities were scored as in Sorror et al.[36]

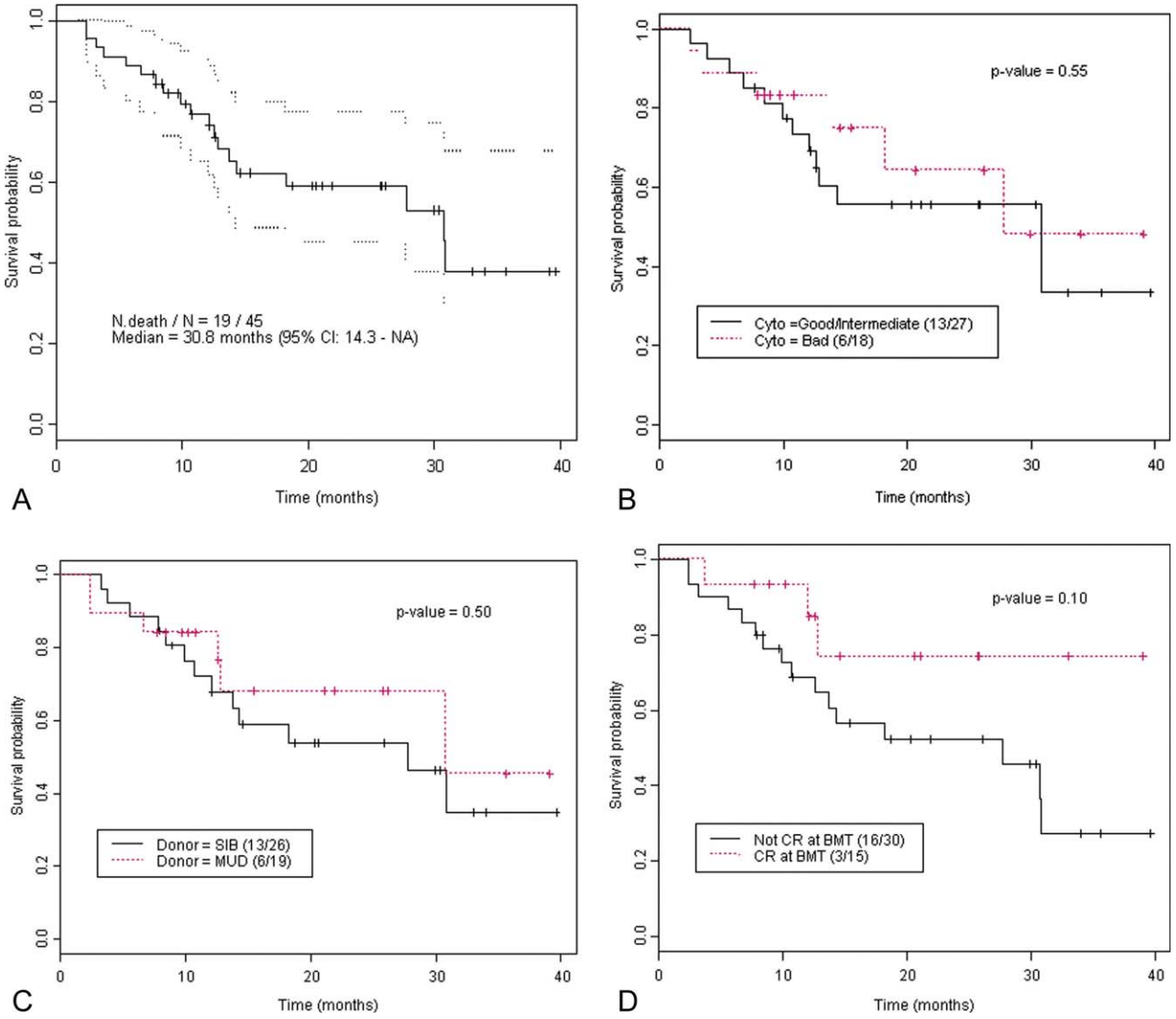

Figure 1. Kaplan-Meier estimates of overall survival (n = 45) are shown for (A) all patients, (B) patients by cytogenetics risk group, (C) patients by donor type, and (D) patients by remission status at the time of transplantation. There was no significant difference noted among the subgroups for any of the 3 variables (log-rank *P* values of .55, .50, and .10, respectively). CI indicates confidence interval; NA, not available; Cyto, cytogenetics; SIB, sibling; MUD, matched unrelated donor; CR, complete remission; BMT, bone marrow transplantation.

CR versus those with active disease (median of 27.2 vs 12 months; $P = .05$, log-rank test).

The fitted Bayesian model indicates that longer OS was significantly associated with having fewer bone marrow blasts, a smaller number of previous chemotherapies, fewer comorbidities, and more cycles of azacitidine (posterior probability .95 of a beneficial effect) (Table 3). There was no significant association between azacitidine dose and OS. Similar results were noted in the EFS model (Table 4).

Acute and Chronic GVHD

Grade 2-3 and grade 3 acute GVHD rates were 27% and 9%, respectively. Because most GVHD started before azacitidine initiation, and patients who developed severe GVHD earlier were excluded, these results should be interpreted with caution.

Eighteen (37%) of 43 patients at risk developed chronic GVHD. The probability of developing chronic GVHD decreased significantly with the number of azacitidine cycles, but was unaffected by dose (Table 5).

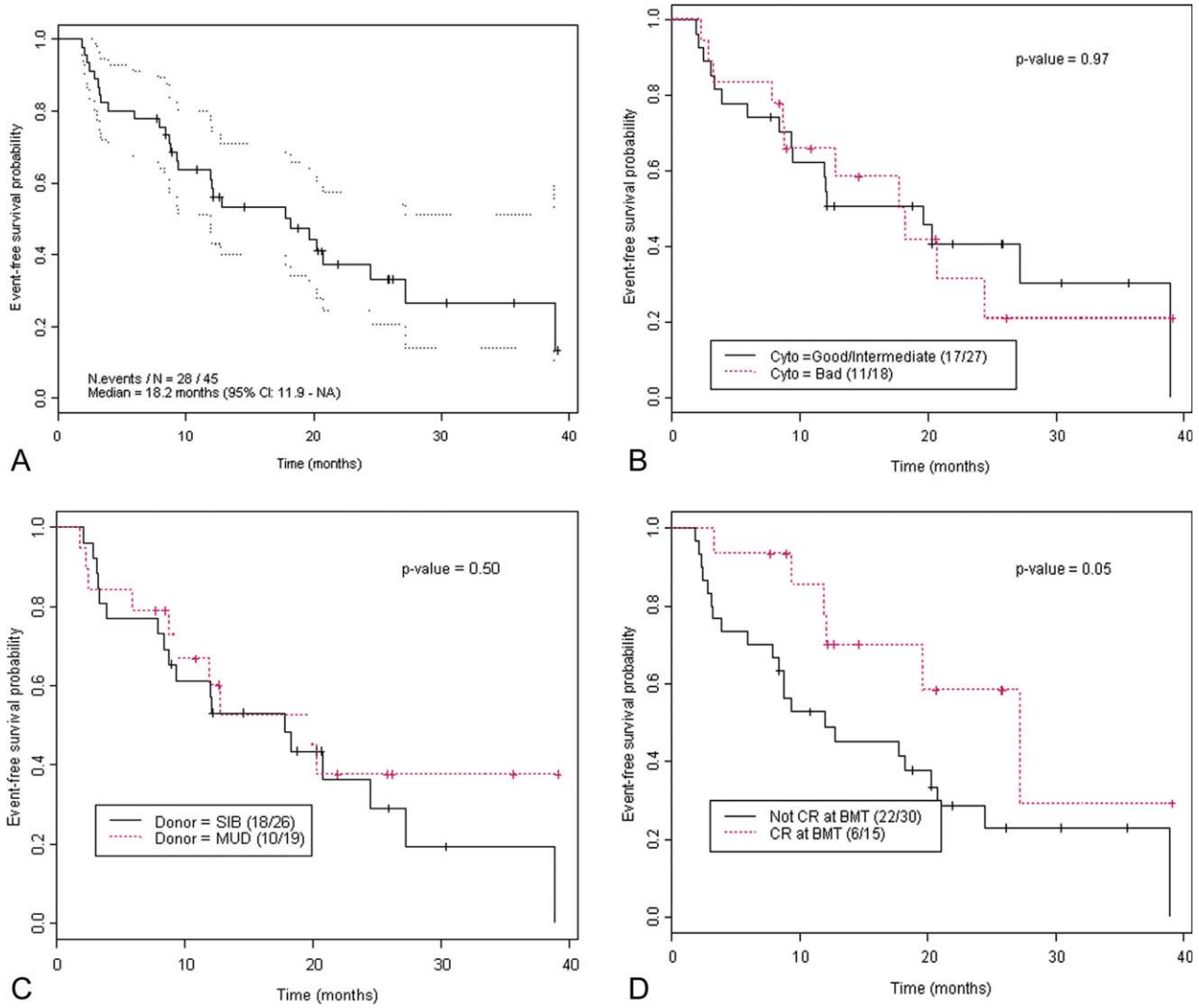

Figure 2. Kaplan-Meier estimates of event-free survival (EFS) (n = 45) are shown for (A) all patients, (B) patients by cytogenetics risk group, (C) patients by donor type, and (D) patients by remission status at the time of transplantation. There was no significant difference noted among the subgroups for cytogenetics or donor type ($P = .97$ and $P = .50$, respectively; log-rank test). However, there was a significant difference in EFS that favored patients in complete remission (CR) (6 events; median, 27.2 months with a lower 95% confidence interval [CI] of 12.1 months) versus those with active disease at hematopoietic stem cell transplantation (22 events; median, 12.0 months with 95% CI, 8.4-24.4 months [$P = .05$, log-rank test]). NA, not available; Cyto, cytogenetics; SIB, sibling; MUD, matched unrelated donor; BMT, bone marrow transplantation.

Toxicities and Infections

Median platelet count at the start of azacitidine was 113,000/mm^3 (range, 16,000-302,000; lower quartile, 69,500), median white blood cell count was 5600/mm^3 (range, 2800-18,000), and median ANC was 3000/mm^3 (range, 1220-15,800). There was no correlation in this relatively small series between white blood cell or platelet count at the start of maintenance and development of hematologic toxicities. Hematologic toxicities associated/possibly associated with azacitidine included reversible grade 1-2 or 3 thrombocytopenia (n = 7 and n = 2), which was documented more often with 32 mg/m^2, and in 1 of 2 patients receiving 40 mg/m^2. Grade 1-2 neutropenia was documented in 7 cases.

Other toxicities included grade 1 nausea (n = 9), grade 2 fatigue (n = 6), grade 1-2 transaminases elevation (n = 3), pruritus (n = 1), grade 1 confusion (n = 2), grade 2 creatinine elevation (n = 1), and oral ulcers (n = 2). There were 3 cases of possible ocular toxicity: conjunctival erythema, retina hemorrhage with platelet count drop to 50,000/mm^3 (possibly pre-existing), and papilledema. One patient developed cholecystitis. The most serious

Table 3. Fitted Bayesian Lognormal Survival Model for Overall Survival (N=45)

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect[a]
			2.5%	97.5%	
Intercept	1.474	0.337	0.787	2.174	—
log (bone marrow blast)	−0.108	0.062	−0.253	−0.003	.022
No. of chemotherapy regimens ≥2 (vs 0, 1)	−0.334	0.187	−0.707	−0.005	.023
No. of comorbidities	−0.086	0.041	−0.176	−0.013	.010
Azacitidine dose	0.006	0.009	−0.010	0.026	.716
No. of cycles	0.118	0.074	−0.029	0.263	.946
R	0.676	0.272	0.247	1.293	—

SD indicates standard deviation.
[a] Values in the last column close to either 1 or 0 correspond to a significant effect. Higher number of administered cycles of azacitidine, but not dose, was associated with improved survival.

Table 4. Fitted Bayesian Log-Normal Model for Event-Free Survival (N=45)

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect[a]
			2.50%	97.50%	
Intercept	1.573	0.327	0.937	2.213	—
log (bone marrow blast)	−0.140	0.058	−0.264	−0.030	.007
No. of chemotherapy regimens ≥2 (vs 0, 1)	−0.429	0.183	−0.798	−0.078	.006
No. of comorbidities	−0.085	0.040	−0.169	−0.015	.008
Azacitidine dose	−0.006	0.008	−0.020	0.011	.208
No. of cycles	0.137	0.076	−0.003	0.299	.971
R	0.816	0.260	0.390	1.402	—

SD indicates standard deviation.
[a] Values in the last column close to either 1 or 0 correspond to a significant effect. Higher number of administered cycles of azacitidine, but not dose, was associated with improved event-free survival.

Table 5. Fitted Bayesian Logistic Regression Model for Chronic GVHD (n=43)[a]

Variable	Mean	SD	Posterior 95% Credible Interval		Probability of a Beneficial Effect
			2.50%	97.50%	
Intercept	0.582	0.779	−0.887	2.111	—
Azacitidine dose	−0.0145	0.036	−0.083	0.057	.658
No. of cycles	−0.439	0.311	−1.073	0.159	.928

GVHD indicates graft-versus-host disease; SD, standard deviation.
[a] Two patients were not evaluable because of early deaths.

possibly drug-related adverse event was 1 case of pulmonary hemorrhage because of fungal pneumonia, which occurred in a patient receiving a second HSCT, who evolved with thrombocytopenia and multiorgan failure. Infections that occurred during the treatment period were considered to be within the expected profile seen in this population.

The fitted model for the risk of toxicity as a function of per-administration dose and number of cycles that was used as a basis for choosing per-administration dose/schedule pairs during the trial is summarized in Table 6. The risk of toxicity from 1 administration at a given dose is characterized by 3 parameters that determine a triangular hazard function. The 3 parameters are the hazard triangle's area, days to peak, and days from peak to the end. Additional details of the statistical model and method are given in Braun et al.[15] Table 7 gives the posterior mean of the probability of toxicity by Day 116 from the first

Table 6. Posterior values of Prob (ptox > .30)

No. of Cycles	Per Administration Dose of Azacitidine (mg/m^2)						
	8	16	24	32	40	48	56
4	.006/A	.034/A	.148/A	.269/A	.783/A	.920/T	.970/T
3	.001/A	.005/A	.030/A	.083/A	.558/A	.755/A	.883/T
2	.000/A	.000/A	.001/A	.004/A	.220/A	.371/A	.541/A
1	.000/A	.000/A	.000/A	.000/A	.025/A	.045/A	.076/A

Prob (ptox) indicates the probability of toxicity within 116 days. (a dose/schedule combination was considered to be excessively toxic if this probability exceeded .80); A, acceptable toxicity; T, unacceptable toxicity.

Table 7. Posterior Mean ptox

No. of Cycles	Per Administration Dose of Azacitidine (mg/m^2)						
	8	16	24	32	40	48	56
4	.105	.164	.225	.260	.407	.475	.531
3	.082	.129	.180	.211	.339	.394	.445
2	.056	.089	.125	.148	.246	.289	.331
1	.029	.046	.065	.077	.134	.160	.186

ptox indicates probability of toxicity by Day 116.
Although the pair 2 courses and dose of 48 mg/m^2 had a predicted mean ptox of .289, it was not selected as being the best because no patients were treated at this dose level.

Table 8. Posterior Mean and SD of the Per-Administration Hazard Parameters in the Bayesian Model for the Probability of Toxicity as a Function of PAD and Number of Cycles

PAD	Area [a], Mean (SD)	Days to Peak of Hazard [b], Mean (SD)	Days from Peak of Hazard to End [c], Mean (SD)	Duration [b+c], Mean d (SD)
8	0.0058 (0.0034)	14.5 (24.1)	8.7 (12.8)	23.2 (27.8)
16	0.0095 (0.0041)	14.9 (22.9)	14.4 (21.6)	29.4 (31.5)
24	0.0138 (0.0049)	11.7 (25.7)	20.3 (38.9)	32.0 (47.3)
32	0.0163 (0.0054)	15.9 (12.4)	31.3 (29.4)	47.3 (26.6)
40	0.0295 (0.0160)	14.0 (11.9)	32.0 (29.0)	46.0 (26.5)

SD indicates standard deviation; PAD indicates per-administration dose.

administration of azacitidine, as a function of per-administration dose and number of cycles (probability of toxicity). The design targeted a per-administration dose/schedule combination having posterior mean probability of toxicity closest to .30. Tables 6 and Table 8 show that, in terms of proximity to the targeted mean probability of toxicity of 0.30, 2 equally optimal safe combinations were 32 mg/m^2 for 4 cycles, which had posterior mean probability of toxicity = .26, and 40 mg/m^2 for 3 cycles), which had posterior mean probability of toxicity = .34. The dose 32 mg/m^2 for 4 cycles combination was chosen because of thrombocytopenia observed with 40 mg/m^2.

Induction of DNA Hypomethylation

As shown in Figure 3, we were unable to detect any significant induction of global DNA hypomethylation.

DISCUSSION

Disease recurrence is a major cause of treatment failure after transplant.[22-26] Results of most therapies given to treat recurrence are very poor. In view of this observation, we proposed to evaluate post-transplant azacitidine as a strategy for remission consolidation/maintenance.

We demonstrated that it is possible to administer azacitidine early after allogeneic HSCT to the majority of a group of high-risk AML/MDS patients. Approximately 60% of our cohort of heavily pretreated patients was able to receive at least 1 cycle of the drug. Our study was designed with only a maximum of 4 cycles because of the logistics of a phase 1 trial, but there are no reasons to believe we could not prolong the duration of treatment, considering that longer exposure may be important with hypomethylating agents. We used an innovative trial

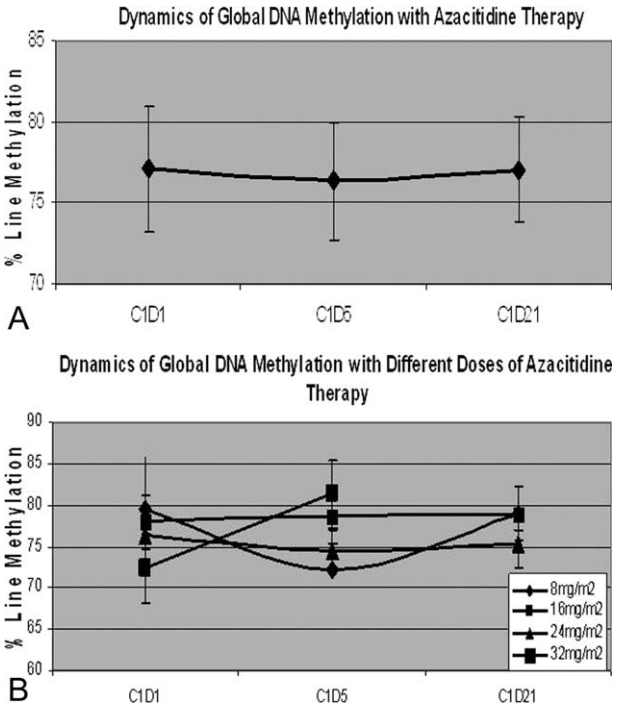

Figure 3. Mononuclear cell DNA global methylation is shown before and after the administration of azacitidine. C indicates cycle; D, day of the administration cycle. Global hypomethylation induction was analyzed using the long interspersed nuclear elements (LINE) bisulfite pyrosequencing assay. LINE methylation was measured on Day 1 before therapy and on Days 5 and 21 of therapy. (A) When all patients and dose levels/schedules were analyzed, no evidence of induction of LINE hypomethylation was observed. (B) The effect per dose level is shown. A nonsignificant hypomethylation trend was observed in patients treated at a dose of 8 mg/m^2.

design that allowed us to determine dose and schedule of administration, overcoming a major limitation of traditional phase 1 studies that do not address the issue of number of cycles that can be delivered with a given dose.

A maintenance of remission study does not provide direct evidence of drug activity. However, direct substantiation of the antileukemia effectiveness of low-dose azacitidine has been reported.[27] We have treated AML/MDS patients relapsing after allogeneic HSCT with doses ranging from 16 to 40 mg/m^2 for up to 2 years, and preliminary experience indicates a 20% long-term disease control rate for patients with indolent recurrences, even without the need for immunosuppression withdrawal.[10]

Given the timing of drug administration, we could not determine whether there was any effect on acute GVHD. However, the probability of developing chronic GVHD may have been decreased with longer schedules of azacitidine administration. This possible effect is intriguing and deserves further investigation.

There was no change in global DNA methylation with therapy, which is in contrast to studies in patients receiving standard-dose azacitidine.[14] Others have been unable to document a relationship between hypomethylation induction and disease response, however,[28] and it is possible that the potential therapeutic effects observed here are not directly related to hypomethylation.

It has been shown by other authors that epigenetic changes may lead to decreased expression of cancer testis antigens in malignant cells.[29-31] It seems reasonable to hypothesize that DNA hypomethylating agents could magnify the graft-versus-leukemia effect of allogeneic HSCT by increasing the immunogenicity of cancer cells through increased expression of tumor antigens. Azacitidine and decitabine may also induce increased FoxP3 expression and regulatory T lymphocyte generation, which could conceivably influence GVHD incidence.[32]

As expected, EFS was negatively influenced by disease burden, extent of prior treatment, and comorbidities. Longer schedules of azacitidine administration were associated with prolongation of EFS and OS, even with a median number of 2 cycles per patient. It is unlikely that patient selection per se explains the results. Although we excluded patients from receiving azacitidine for reasons described here, the final study cohort consisted of patients with a median age of 60 years, mostly with recurrent disease, and who had received a median of 2 chemotherapy regimens before transplant, a poor prognosis variable in the setting of refractory AML/MDS.[33,34]

Feasibility of maintenance therapy is likely higher than documented here. We arbitrarily limited eligibility for starting azacitidine to the first 2.5 months after HSCT, a decision made because of logistic reasons only. If patients are allowed to start the treatment in a more flexible schedule during the first 40-100 days, a larger fraction will be eligible to receive it. These findings provide the basis for an ongoing randomized trial comparing azacitidine given for 1 year after HSCT versus no maintenance.

In conclusion, azacitidine 32 mg/m^2 daily for 5 days in each of 4 30-day cycles is associated with acceptable toxicities when given after HSCT. Our trial also suggested that this treatment may prolong EFS and OS, and that more cycles may be associated with greater benefit.

CONFLICT OF INTEREST DISCLOSURES

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