Biol Blood Marrow Transplant 20 (2014) 776–780

Pretransplantation 5-Azacitidine in High-Risk Myelodysplastic Syndrome

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Article history:
Received 27 December 2013
Accepted 11 February 2014

Key Words:
5-Azacitidine
MDS
Hypomethylating agent
Allogeneic hematopoietic cell transplantation

A B S T R A C T

We prospectively evaluated the allogeneic hematopoietic cell transplantation (HCT) outcomes in high-risk myelodysplastic syndrome (MDS) patients who received pretransplantation 5-azacitidine. Twenty-five patients evaluated for allogeneic HCT consult and considered medically eligible for a donor search were enrolled. Azacitidine was administered at 75 mg/m^2 for 5 to 7 days every 4 weeks until a suitable donor was found. A median of 3 (range, 0 to 6) cycles of 5-azacitidine were administered. Preallogeneic HCT responses to 5-azacitidine, based on the International Working Group criteria, were 48% partial response, 33% stable disease, and 19% progressive disease. Four patients did not proceed to allogeneic HCT. Twenty-one patients, a median age of 55 (range, 25 to 67) years, received allogeneic HCT after myeloablative pharmacokinetically targeted i.v. busulfan and fludarabine conditioning regimen. Donors were either HLA-matched related or unrelated, except for 1 mismatch unrelated donor. With a median follow-up of 30 months, 1-year relapse-free and overall survivals were 52% (95% confidence interval [CI], 30% to 71%) and 62% (95% CI, 38% to 79%), respectively. Preallogeneic HCT 5-azacitidine administration was well tolerated and provided reasonable disease control before allogeneic HCT. (Registered at ClinicalTrials.gov as NCT00660400).

INTRODUCTION

Myelodysplastic syndrome (MDS) is composed of groups of clonal bone marrow disorders characterized by peripheral blood cytopenias due to ineffective hematopoiesis and by a risk of progression to acute myelogenous leukemia (AML) [1]. The availability of hypomethylating agents, including 5-azacitidine and decitabine, has changed the landscape of MDS treatment. Azacitidine results in hematologic improvements in approximately 25% to 50% of cases and complete response (CR) in 10% to 20% with improved survival compared to supportive care alone in high-risk MDS [2,3]. Although hypomethylating agents can induce hematological and cytogenetic responses, these therapies do not appear to eradicate MDS clones. Allogeneic hematopoietic cell transplantation (HCT) is the only known curative therapy for MDS and is offered to selected patients who are considered to have higher risk of AML progression and who are fit for the procedure [4].

The use of hypomethylating agents as a bridge to more definitive therapy is increasing based on the premise that inhibition of DNA methyltransferases results in hypomethylation and, consequently, might result in reactivation of tumor suppressor genes, terminal differentiation, and apoptosis of neoplastic cells with reduction of tumor burden before allogeneic HCT [5]. Several studies have evaluated the role of hypomethylating agents given before allogeneic HCT [6-9], though very few were conducted prospectively [10].

We hypothesized that the administration of 5-azaciditine before the conditioning regimen may reduce the risk of MDS relapse after allogeneic HCT. In addition to its direct antitumor effect, 5-azaciditine may potentiate the effect of high-dose chemotherapy or promote the expression of targets critical to the effect of graft-versus-tumor response. Therefore, we designed a pilot prospective trial to evaluate the role of pretransplantation 5-azaciditine therapy in high-risk MDS patients on the outcomes after allogeneic HCT.

METHODS

Patient Population

Eligible patients were 18 to 68 years of age, had histologically confirmed high-risk MDS, defined by International Prognostic Scoring System [11] >1, AML deriving from background MDS, or therapy-related MDS. Patients must have had a serum bilirubin level ≤1.5 times the upper limit of normal (ULN) (higher levels were acceptable if attributed to active hemolysis or ineffective erythropoiesis), aspartate aminotransferase or alanine aminotransferase ≤2 times the ULN, serum creatinine ≤1.5 times the ULN, and Karnofsky performance status ≥70%. Patients were excluded if they had known or suspected hypersensitivity to 5-azacitidine or mannitol; were pregnant or lactating; or had active central nervous system malignancy, active infection, or a history of solid organ malignancy, with the exception of nonmelanoma skin cancers.

The study was approved by the Institutional Review Board of the University of South Florida. All study participants provided voluntary written informed consent. This clinical trial was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonization for Good Clinical Practice. The trial was registered at www.clinicaltrials.gov as NCT00660400.

Financial disclosure: See Acknowledgments on page 780.

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1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.02.008

Study Design

The primary objective of the study was to evaluate relapse-free survival (RFS) 1 year after allogeneic HCT in MDS patients receiving at least 1 complete cycle of 5-azacitidine in the pretransplantation setting. Secondary objectives included assessment of response to 5-azacitidine, proportion of patients enrolled who subsequently proceeded to allogeneic HCT, and overall survival (OS) 1 year from the first dose of 5-azacitidine, time to progression of MDS, and time to progression to AML.

Patients with high-risk MDS evaluated in blood and marrow transplantation clinic who were potentially eligible for allogeneic HCT were considered for trial enrollment. After informed consent was documented and the donor search was initiated, 5-azacitidine was administered per standard practice. The recommended dose of 5-azacitidine was 75 mg/m^2/day intravenously or subcutaneously, daily for 5 to 7 days, every 4 weeks. After administration of the recommended dosage for the first cycle, dosage adjustments for the subsequent cycles were allowed based on the suggested dosing guidelines [12]. The number of cycles of 5-azacitidine treatment was not specified in the protocol as our prior retrospective review (data not shown) indicated potential benefit to MDS patients with any exposure to 5-azacitidine. If a suitable donor was found, patients proceeded to allogeneic HCT as soon as feasible, where the conditioning regimen was prescribed at the discretion of the treating physician per institutional guidelines. Additional cycles of 5-azacitidine therapy were allowed while waiting for allogeneic HCT. The conditioning regimen was prescribed no longer than 8 weeks and no earlier than 4 weeks from the last dose of 5-azacitidine. Post-transplantation disease staging studies were performed at 3, 6, and 12 months after allografting.

Patients without suitable donors continued 5-azacitidine per standard treatment. These patients were followed until progression of MDS to AML or death, for up to 1 year from time of first dose of 5-azacitidine.

Assessments

A bone marrow biopsy was performed for assessment of disease response either after the last cycle of 5-azacitidine before allogeneic HCT or after the fourth cycle of 5-azacitidine, whichever occurred earlier. Disease response was assessed per Cheson criteria [13] as modified by Silverman et al. [12]. Complete blood counts were performed as needed to monitor response and toxicity per standard practice. For monitoring, grade 3 or 4 toxicities (per Common Terminology Criteria for Adverse Events version 3.0) occurring up to 30 days after the last dose of 5-azacitidine or at the start of conditioning regimen considered related to 5-azacitidine were recorded.

Transplantation

Donors

Donors were related or unrelated and HLA-compatible for 7/8 or 8/8 at HLA-A, -B, -C, and -DRB1 loci. All patients received granulocyte colony–stimulating factor –mobilized unmanipulated peripheral blood hematopoietic cells.

Conditioning regimen

All patients who underwent transplantation received a modified de Lima regimen of busulfan plus fludarabine, which was adjusted to a specific busulfan area under the curve (AUC) target [14,15]. Fludarabine was administered at 40 mg/m^2 daily intravenously for 4 days, with each infusion followed immediately by intravenous busulfan. The dose of busulfan for days 1 and 2 was 130 mg/m^2. After the first dose of busulfan, serial blood samples were obtained for pharmacokinetic analysis. Accordingly, busulfan dose for days 3 and 4 were adjusted to achieve a steady state targeted busulfan AUC from 3500 to 7500 μM-min. One patient with a mismatched unrelated donor graft at HLA-A locus received rabbit antithymocyte globulin (ATG) at a total dose of 7.5 mg/kg over 3 days [16].

Supportive care for transplantation

All patients received lorazepam for seizure prophylaxis. Regimens for graft-versus-host disease (GVHD) prophylaxis were tacrolimus and either methotrexate, mycophenophate mofetil, or sirolimus. Methotrexate was given at 15 mg/m^2 intravenously on day +1 and then 10 mg/m^2 intravenously on days +3, +6, and +11. The dose of methotrexate was adjusted for renal function. The dose of tacrolimus was adjusted to achieve levels of 10 to 15 ng/mL during the first month after allogeneic HCT and gradually decreased in the absence of GVHD [17]. Mycophenolate mofetil was administered at 30 mg/kg/day intravenously in 2 divided doses, beginning on day 0 at least 2 hours after the end of the infusion of donor cells. The tapering schedule began on day +240 in the absence of GHVD per institutional guidelines [17]. Sirolimus was administered at a loading dose of 12 mg on day -1, followed by maintenance to target sirolimus level of 8 to 14 ng/mL. For the tacrolimus/sirolimus combination, target tacrolimus level was 3 to 7 ng/mL [18].

Antimicrobial prophylaxis consisted of acyclovir, levofloxacin, and micafungin or voriconazole. Cytomegalovirus was monitored with weekly real-time polymerase chain reaction of blood samples for the first 100 days after allogeneic HCT and pre-emptive therapy with ganciclovir, valganciclovir, or foscarnet was initiated per the institutional guidelines. Prophylaxis for *Pneumocystis jiroveci* was with trimethoprim-sulfamethoxazole beginning at day +30. Granulocyte colony–stimulating factor was not routinely administered after allogeneic HCT.

Statistical Analysis

The study was design to detect an improvement in 1-year RFS rate in MDS patients who underwent transplantation from our institutional baseline of 50% (unpublished data) to 80%. A total of 19 evaluable patients (who were treated with 5-azacitidine and received allogeneic HCT) was estimated to be needed to achieve 90% power to detect a 30% increase (1-sided test) in 1-year RFS rate at a 10% significance level. If 13 or more of 19 evaluable patients were relapse free, then the null hypothesis was rejected and the study was deemed promising. The actual power and type I error are 93.2% and 8.4%, respectively.

For descriptive statistics, categorical and continuous variables are summarized as frequencies or percentages and as the median and range, respectively. OS and RFS were estimated using Kaplan-Meier method [19]. The cumulative incidences of relapse, nonrelapse mortality, and acute GVHD were estimated using the competing risk approach [20]. The 95% confidence interval (CI) for point estimates of cumulative incidences and survivals were derived based on the log-log transformation [21].

Neutrophil engraftment after allogeneic HCT was defined as the first day of absolute neutrophil count $>0.5 \times 10^9$/L on 3 consecutive days. Platelet engraftment was defined as the first day of platelet count $>20 \times 10^9$/L for 7 days without transfusion support. Chimerism analyses were performed at around day +30, +90, +180, and +365 using a polymerase chain reaction/short tandem repeat method on unsorted bone marrow samples. Peripheral blood CD3+ and CD33+ cell chimerism studies were also performed using the subsets isolated by fluorescence-activated cell sorting. Acute GVHD was graded based on consensus conference criteria [22]. Chronic GVHD was graded based on the National Institute of Health Consensus Development Project [23]. Relapse and nonrelapse mortality were considered competing risks of GVHD.

RESULTS

Patient, Disease, and Transplantation Characteristics

From May 2008 to August 2010, 25 patients with MDS were enrolled in the study. Four patients did not proceed to allogeneic HCT because of lack of insurance approval, suboptimal organ function, intracranial hemorrhage, and patient refusal of allogeneic HCT. Except for 1 patient who did not receive any 5-azacitidine, 3 non-HCT patients were followed as described previously. Patient, disease, and transplantation characteristics on 21 MDS patients who received allogeneic HCT are summarized in Table 1.

The median age was 55 (range, 26 to 67) years and the majority of allografted subjects were females (67%). Eleven (58%) received hypomethylating agent(s) before enrollment, with a median of 2 cycles (range, 1 to 5). Busulfan AUC target levels were 3500 μM-min in 4 (19%), 5300 μM-min in 14 (67%), 6000 μM-min in 1 (5%), and 7500 μM-min in 2 (9%). The majority received HLA-matched related or unrelated donor grafts, except for 1 with a mismatched unrelated donor (7/8). GVHD prophylaxis was predominantly tacrolimus plus methotrexate. ATG was given to 1 patient (Table 1).

Response and Safety of 5-Azacitidine

Twenty-one patients received a median of 3 (range, 0 to 6) cycles of 5-azacitidine on the study before allogeneic HCT. The median total number of cycles (combining cycles before and after enrollment) of 5-azacitidine before HCT was 4 (range, 1 to 6). Responses to 5-azacitidine included partial response in 10 (48%), stable disease in 7 (33%), and progressive disease in 4 (19%). Three patients received AML-type induction therapy before allogeneic HCT because of disease progression during 5-azacitidine therapy. Grade 3 and 4

Table 1
Patient, Disease, and Transplantation Characteristics (N = 21)

Characteristic	Value
Age, median (range), yr	55 (26-27)
Gender	
Male	7 (33%)
Female	14 (67%)
Disease at diagnosis	
RCMD	4 (19%)
RAEB-1	4 (19%)
RAEB-2	11 (52%)
CMML-1	1 (5%)
AML	1 (5%)
IPSS at diagnosis (n = 18)*	
Intermediate-1	3 (17%)
Intermediate-2	10 (56%)
High	5 (27%)
IPSS-R at diagnosis (n = 17)	
Intermediate	3 (18%)
High	6 (35%)
Very high	8 (47%)
5-Azacitidine cycles, median (range)	3 (0-6)
Disease at allogeneic HCT	
RCMD	9 (43%)
RAEB-1	6 (29%)
RAEB-2	3 (14%)
CMML	2 (10%)
AML	1 (5%)
Myeloblasts at allogeneic HCT	
<5%	10 (48%)
5%-10%	7 (33%)
11%-20%	4 (19%)
>20%	0 (0%)
Donor	
HLA-matched sibling	8 (38%)
HLA-matched unrelated (8/8)	12 (57%)
HLA-mismatched unrelated (7/8)	1 (5%)
GVHD prophylaxis	
Tac/MTX	19 (90%)†
Tac/MMF	1 (5%)
Tac/Rapa	1 (5%)

RCMD indicates refractory cytopenias with multilineage dysplasia; RAEB, refractory anemia with excess blasts; CMML, chronic myelomonocytic leukemia; AML indicates acute myeloid leukemia; IPSS, International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; Tac, tacrolimus; MTX, methotrexate; MMF, mycophenolate mofetil; Rapa, rapamycin.
Data presented are n (%) unless otherwise indicated.
* IPSS information not available in 1 patient with RCMD.
† One patient received 50% dose reduction of MTX on day +11.

toxicities of 5-azacitidine include febrile neutropenia (5%), *Clostridium difficile* colitis (5%), nodular pneumonia (presumed fungal, 5%), perirectal abscess (5%), deep venous thrombosis (5%), and cerebrovascular accident (5%).

Transplantation Outcomes

Neutrophil engraftment was achieved at a median of 15 (range, 12 to 22) days and platelet engraftment was achieved at a median of 16 (range, 12 to 26) days. Two patients did not engraft platelets. Early toxicities (within 30 days) after allogeneic HCT include hemorrhagic esophagitis (n = 1), lower extremity fasciitis after knee amputation (unrelated to HCT) (n = 1), posterior reversible encephalopathy syndrome (n = 1), and human herpes virus 6 encephalitis (n = 1). The median donor chimerisms on unsorted bone marrow and peripheral blood CD3, and CD33 were as follows: at day +30, 95% (range, 18% to 100%), 84% (range, 30% to 100%), and 100% (range, 97% to 100%); at day +100, 95% (range, 25% to 100%), 85% (range, 23% to 100%), and 100% (range, 97% to 100%), respectively.

In this cohort of patients, cumulative incidence of grade 2 to 4 acute GVHD was 100% with grade 3 to 4 acute GVHD of 14%. The organs involved for acute GVHD were the gastrointestinal tract in 90% (with 24% with upper gastrointestinal tract involvement), skin in 67%, and liver in 17%. The cumulative incidence of moderate to severe chronic GVHD was 38%. Day +30 disease response was 95% CR with 1 patient progressing to AML. Day +100 disease response was CR in 18 patients, 1 AML progression, 1 disease relapse, and 1 death. The figures demonstrate OS (Figure 1), RFS (Figure 2), nonrelapse mortality (Figure 3), and cumulative incidence of relapse (Figure 4), with a median follow-up of 30 months. One-year RFS and OS were 52% (95% CI, 30% to 71%) and 62% (95% CI, 38% to 79%), respectively. Two-year RFS and OS were 47% (95% CI, 25% to 66%) and 51% (95% CI, 28% to 70%), respectively. Cumulative incidence of relapse was 14% (95% CI, 3% to 33%) at 6 months and 24% (95% CI, 8% to 44%) at 1 year. Cumulative incidence of nonrelapse mortality was 5% (95% CI, .3% to 20%) at 100 days, 24% (95% CI, 8% to 44%) at 1 year, and 29% (95% CI, 11% to 50%) at 2 years. Causes of death include 4 disease relapses, 3 infectious complications, and 3 with GVHD and infections.

Outcomes of non-HCT Patients

The outcomes of the 3 non-HCT patients (except for 1 who did not receive further 5-azacitidine therapy) were the following. One patient received achieved CR after 7 cycles of 5-azacitidine; however, this patient later died because of progression to AML (no HCT was performed because of patient's refusal). Another patient continued with 20 cycles of 5-azacitidine and then died because of progressive MDS. The other patient, who received 1 cycle of 5-azacitidine, died because of complications from pneumonia.

DISCUSSION

In this pilot study, with 21 MDS patients with adverse risk factors who were prospectively treated with 5-azacitidine before allogeneic HCT, we found that pre-HCT hypomethylating agent therapy with 5-azacitidine is feasible and provides a reasonable disease control before HCT with busulfan/fludarabine regimen. Additionally, as our institutional reference, we evaluated 63 patients from July 2004 to December 2010 who did not receive 5-azacitidine before

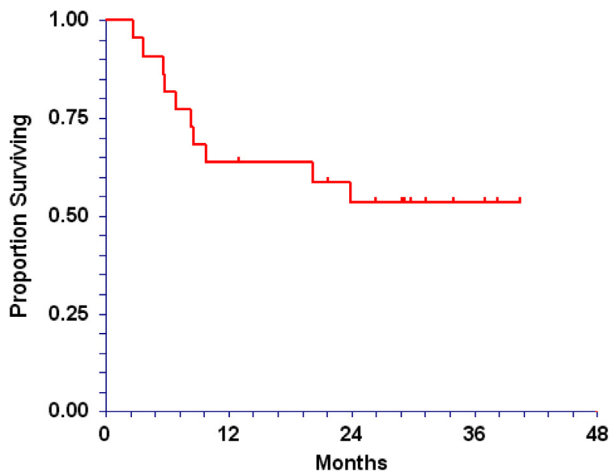

Figure 1. Overall survival from the time of allogeneic hematopoietic cell transplantation.

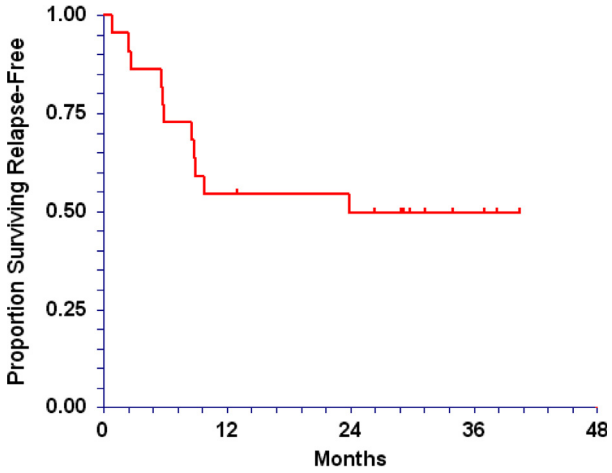

Figure 2. Relapse-free survival from the time of allogeneic hematopoietic cell transplantation.

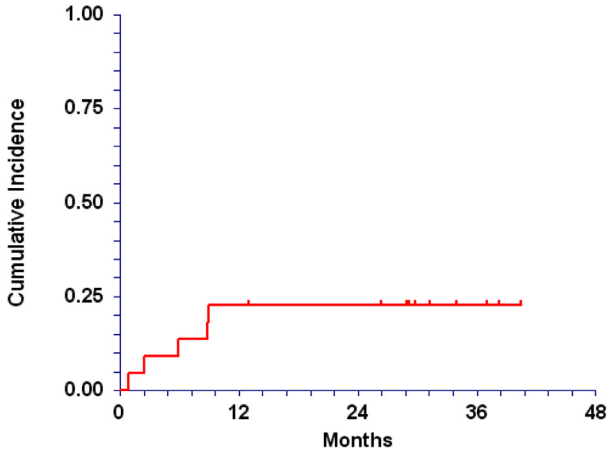

Figure 4. Cumulative incidence of relapse.

HCT, and their RFS after allogeneic HCT were 46% at 1 year and 36.4% at 2 years, respectively. Although no formal statistical analyses were performed, our study of pre-HCT hypomethylating agent resulted in favorable 1-year RFS and OS of 52% (95% CI, 30% to 71%) and 62% (95% CI, 38% to 79%), respectively. The median number of cycles of 5-azacitidine given was 3 (range, 0 to 6) and this strategy allowed ample time for a donor search before allogeneic HCT. Although there were 11 patients (58%) who received hypomethylating agents before enrollment, patients were enrolled to the study early in treatment and the authors do not believe that there was any significant impact on the study outcomes, even when accounting for the pre-enrollment treatment. Therapy with 5-azacitidine did not impose excess toxicity before allogeneic HCT. We did observe higher cumulative incidence of acute grade 2 to 4 GVHD, but this may be confounded by predominantly unrelated donor sources and a small sample size. Acute GVHD occurred predominantly in gastrointestinal tract with a quarter of patients having mostly upper gastrointestinal symptoms. Whether pre-HCT 5-azacitidine contributed to the development of acute GVHD will need to be evaluated in a larger cohort of patients. The limitations of our pilot study include single-arm study design and a small sample size, as the intent was to evaluate the feasibility of this pre-HCT approach.

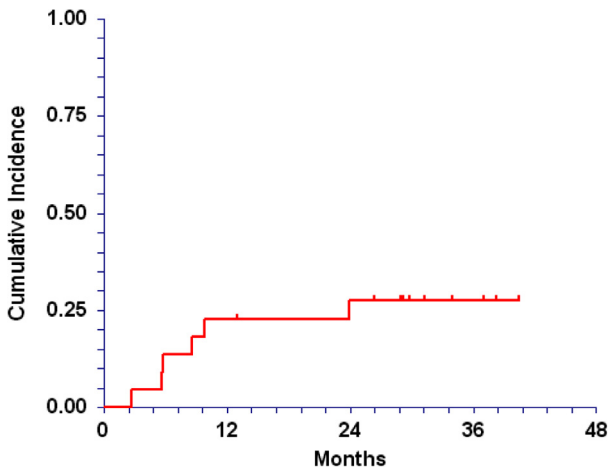

Figure 3. Nonrelapse mortality from the time of allogeneic hematopoietic cell transplantation.

As corroborated by our previous retrospective study [9], several groups have retrospectively evaluated the role and feasibility of hypomethylating agent (either 5-azacitidine or decitabine) therapy before allogeneic HCT [6,7,9, 24-26]. Single-agent therapy with hypomethylating agent would be of value in stabilizing the disease or reverting it to an earlier stage, as was seen in our study, and allowing time for patients to reach the more curative allogeneic HCT. Some have suggested that achievement of CR with a hypomethylating agent might improve outcomes after allogeneic HCT [6]; however, although none of our study patients achieved CR, many proceeded with HCT, and the prospective validation of this observation is needed. In a large retrospectively study by the French Bone Marrow Transplant registry, Damaj et al. evaluated 48 patients treated with 5-azacitidine alone and 17 patients with 5-azacitidine followed by induction chemotherapy before allogeneic HCT compared with 163 MDS patients who received induction therapy alone [7]. Patients treated with 5-azacitidine alone had comparable OS and event-free survival compared with the induction therapy group. Gerds et al. evaluated the outcomes of 68 patients who underwent allogeneic HCT for MDS or AML transformed from MDS [26]. Thirty-five received 5-azacitidine before HCT and 33 received induction chemotherapy. One-year OS was 57% with azacitidine and 36% with induction therapy. After adjustment for cytogenetic risk, International Prognostic Scoring System, and the donor status, the rates of post-HCT relapse for 2 cohorts were similar [26].

Data on whether “debulking” before allogeneic HCT to suppress bone marrow blasts count to less than 10% or down-staging of MDS would improve post-HCT disease relapse are inconclusive [9,10]. Lubbert et al. argue that delaying HCT to achieve major cytoreduction may result in complications that prohibit HCT or development of drug resistance with concomitant disease progression [27]. Additionally, there is inherent physician bias to provide such therapy to high-risk MDS patients, and prospective studies to evaluate the role of debulking are needed before definitive conclusions can be made.

Disease that fails 5-azacitidine has a very poor prognosis. Prebet et al. investigated the outcomes in this group [28]. Out of 270 patients with available information of subsequent

treatments after failure of 5-azacitidine, 37 proceeded to allogeneic HCT with a median OS of 19.5 months. Twenty-eight patients received immediate allogeneic HCT and 9 went to HCT after 1 or more cycles of salvage therapy. Fourteen had progressive disease and underwent allogeneic HCT with a median OS of 14 months, whereas 14 with stable disease had not reached median survival after allogeneic HCT at the time of publication ($P = .08$), suggesting the benefit of allogeneic HCT, even without a favorable response to 5-azacitidine treatment [28].

Relapse after allogeneic HCT remains the major cause of treatment failure. A study by de Lima et al. evaluated the role of low-dose azacitidine (32 mg/m^2 given for 5 days at least 4 cycles) maintenance early after allogeneic HCT [29]. Approximately 60% of patients were able to receive at least 1 cycle of therapy and there was a suggestion that the maintenance therapy may prolong event-free and OS. The optimal allogeneic HCT strategy for high-risk MDS is not fully defined and current treatment options continue to evolve. Both pre-HCT disease-specific or disease-modifying therapy, incorporating a hypomethylating agent, and post-HCT maintenance or pre-emptive therapy to reduce disease relapse after allogeneic HCT could be incorporated in the ideal platform for allogeneic HCT in patients with MDS. Further research with prospective studies is needed to optimize allogeneic HCT strategy to produce long-term disease control.

CONCLUSIONS

Pretransplantation 5-azacitidine therapy was well tolerated and provided reasonable disease control. This regimen served adequately in our small patient cohort as a bridge to allogeneic HCT and did not impose any additional toxicity after allogeneic HCT with a promising 1-year RFS. Future clinical trials are needed to determine the optimal schedule and strategy to improve postallogeneic HCT relapse risk and survival with incorporation of pretransplantation 5-azacitidine therapy.

ACKNOWLEDGMENTS

We thank the patients, physicians, nurses and research staffs at Moffitt Cancer Center.

Financial disclosure: Research funding was provided by Celgene for this trial. Rami Komrokji was on the Speaker's Bureau and had research funding from Celgene. Alan List was a Consultant and received research funding from Celgene.

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