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RESEARCH

Impact of prior azacitidine on the outcome of allogeneic hematopoietic transplantation for myelodysplastic syndrome

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Abstract To clarify the clinical impact of prior use of azacitidine (AZA) on outcomes of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for myelodysplastic syndrome (MDS), we retrospectively reviewed the clinical outcomes of 15 MDS patients who were treated with AZA before allo-HSCT (AZA group). We compared the outcomes of these 15 patients with 52 MDS patients who were solely given the best supportive care (BSC) before allo-HSCT (BSC group). Although patients in the AZA group were older with higher International Prognostic Scoring System (IPSS) scores compared to patients in the BSC group, no significant differences were found between the two groups in overall survival (OS), disease-free survival (DFS), cumulative incidence of relapse (CIR) or non-relapse mortality. However, in patients with a higher IPSS score (Int-2/High), pre-transplant AZA may provide better OS and DFS and lower CIR. Acute graft-versus-host disease rates were similar between the two groups. These results should be reassuring to patients with high-risk MDS receiving AZA before allo-HSCT.

Keywords Myelodysplastic syndrome · Allogeneic hematopoietic stem cell transplantation · Azacitidine

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is currently the only curative approach for myelodysplastic syndrome (MDS); however, many patients are at substantial risk of relapse after transplantation. Pre-transplant induction chemotherapy could be used to reduce the incidence of post-transplant relapse, especially in cases of high disease burden, but this treatment is associated with considerable morbidity and mortality, which could prohibit proceeding to transplantation or interfere with the transplant outcome [1]. Azacitidine (AZA) has recently emerged as the first promising drug that significantly prolongs overall survival (OS), and is therefore considered the initial standard approach, particularly for patients with high-risk MDS [2, 3]. Considering its promising activity against MDS and its low toxicity profile, AZA appears to be an attractive alternative to induction chemotherapy as a pre-transplant treatment. Recent retrospective studies have demonstrated that AZA before allo-HSCT is not inferior to conventional induction chemotherapy [4–9]. However, a French group recently reported that pre-transplant AZA does not offer any advantages over best supportive care (BSC), regardless of the pre-transplant tumor burden or disease characteristics [10]. Thus, further clarification is needed regarding which patients to treat with AZA and whether AZA should be administered in a pre-transplant setting. The major objectives of this retrospective study were both to assess the clinical impact of AZA prior to transplantation on the subsequent survival, relapse, non-relapse mortality (NRM), and acute graft-versus-host disease (GVHD), and to compare MDS patients treated with AZA with patients who were treated solely with BSC before allo-HSCT in our institution.

Patients and Methods

Patients

Between January, 2005 and March, 2013, a total of 69 patients with MDS underwent allo-HSCT in our institution. Among these patients, two were excluded from analysis because data for either the initial French-American-British (FAB)/WHO category or the International Prognostic Scoring System (IPSS) score were unable to be retrieved. Of the remaining 67 patients, 15 had a history of AZA administration before allo-HSCT (AZA group), and 52 only received BSC, including blood transfusion, hormones, growth factors (erythropoietin, granulocyte colony-stimulating factor), vitamins (D, K), immunosuppressive treatment, hydroxycarbamide, and antibiotics, but no chemotherapy (BSC group). AZA was administered at doses of 75 mg/m^2/day on day 1 through 7 of a 28-day cycle. Responses to AZA were evaluated according to the International Working Group 2006 criteria [11].

This retrospective study was approved by the independent ethics committee at Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital.

Patient Characteristics

The characteristics of all patients at diagnosis are summarized in Table 1. Morphological classification according to FAB or WHO classifications was made at the initial diagnosis [12], and the IPSS score was also calculated as described [13]. Of the 67 MDS patients, 29 (43 %) were classified as having low-risk MDS and included refractory anemia, refractory anemia with ringed sideroblasts, refractory cytopenia with multilineage dysplasia and unclassified. Fifteen patients (22 %) had refractory anemia with excess blasts (RAEB-1), 18 (27 %) had RAEB-2, and five (8 %) had chronic myelomonocytic leukemia. Patients in the AZA group were older than those in the BSC group (median, 62 vs. 51 years, P=0.003). More patients in the AZA group had a higher IPSS score and a more advanced stage.

Table 1 Characteristics of patients

Characteristics	Total (n=67)		AZA (n=15)		BSC (n=52)		P
	No.	%	No.	%	No.	%	
Age, years							0.003
Median	55		62		51		
Range	16–69		25–70		16–69		
Sex							0.321
Male	49	73	13	87	36	69	
Female	18	27	2	13	16	31	
Diagnosis							0.026
RA/RCMD/RARS/U	29	43	4	27	25	48	
RAEB1	15	22	1	7	14	27	
RAEB2	18	27	8	53	10	19	
CMML	5	8	2	13	3	6	
Marrow blast, %							0.082
<5	32	48	4	27	28	54	
≥5	35	52	11	73	24	46	
IPSS score							0.042
Low/Int-1	39	58	5	33	34	65	
Int-2/High	28	42	10	67	18	35	
Donor							0.32
Related	17	25	2	13	15	29	
Unrelated	50	75	13	87	37	71	
Stem cell source							0.861
BM	54	80	13	87	41	79	
PB	8	12	1	7	7	13	
CB	5	8	1	7	4	8	
Conditioning							0.121
MAC	55	82	10	67	45	87	
RIC	12	18	5	33	7	13	

AZA-azacitidine, *BSC* best supportive care, *RA* refractory anemia, *RCMD* refractory cytopenia multilineage dysplasia, *RARS* refractory anemia with ringed sideroblasts, *U* unclassifiable, *RAEB1* refractory anemia with excess of blasts-1, *RAEB2* refractory anemia with excess of blasts-2, *CMML* chronic myelomonocytic leukemia, *IPSS* International prognostic scoring system, *Int-1* intermediat-1, *Int-2* intermediate-2, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood, *MAC* myeloablative conditioning, *RIC* reduce intensity conditioning

Transplant Modalities

Of the 67 patients, 55 (82 %) underwent myeloablative conditioning (MAC) and 12 (18 %) underwent reduced intensity conditioning (RIC). The following two typical conditioning regimens were included in the myeloablative procedure: one consisted of total body irradiation (TBI) (12 Gy) and cyclophosphamide (CY) (120 mg/kg), and the other consisted of busulfan (BU) (12.8 mg/kg intravenously) and CY (120 mg/kg). In contrast, several RIC regimens were administered; the most common consisting of fludarabine (125 mg/m^2), melphalan (80 mg/m^2), and either low-dose TBI (2 Gy×2) or fludarabine (180 mg/m^2) plus BU (6.4 mg/kg intravenously). Myeloablative and non-myeloablative regimens were differentiated according to a previous report. [14] All patients were given intravenous infusion of donor hematopoietic stem cells on day 0, and subsequently received acute GVHD prophylaxis with continuous intravenous infusion of cyclosporine A

or tacrolimus and additional short-term methotrexate. Transplantation modalities in the two groups are summarized in Table 1.

Statistical Analysis

The current analysis was performed on the reference date of September 30, 2013. OS was defined as the interval from allo-HSCT to death, regardless of the cause. Disease-free survival (DFS) was defined as survival with no evidence of relapse. Relapse was defined as the presence of more than 5 % marrow blasts and/or reappearance of major myelodysplastic features associated with cytopenia and evidence of autologous reconstitution when chimerism was available. NRM was defined as death resulting from the transplantation procedure without evidence of relapse. Categorical variables were described as frequencies and percentages. The two groups were compared using Fisher's exact test for categorical data. For continuous variables, medians and ranges were determined. The two groups were compared using the paired Student's *t* test. OS and DFS were estimated using the Kaplan-Meier method. The log-rank statistic was used to test the prognostic value of patient characteristics at transplantation for the occurrence of the event. Pre-transplantation treatment and variables with a significance level of $P<0.15$ from the univariate analyses were introduced into a multivariate Cox regression model. Adjusted hazard ratios and 95 % confidence intervals were computed, and $P<0.05$ was considered statistically significant. Relapse and NRM were summarized using cumulative incidence estimates, with NRM a competing risk for relapse and relapse a competing risk for NRM. The individual prognostic value of each variable was assessed with the Gray test. All

Table 2 Univariate analysis of transplantation outcome

Characteristics	No. of patients	1-year OS		1-year DFS		1-year relapse		1-year NRM	
		%	*P*	%	*P*	%	*P*	%	*P*
Age			0.201		0.044		0.224		0.192
<55	32	84.1		78.1		12.5		9.4	
≥55	35	72.7		60.6		18.8		20.6	
Sex			0.919		0.645		0.099		0.303
Male	49	81.1		68.5		19.3		12.3	
Female	18	72.2		72.2		5.6		22.2	
Marrow blast, %			0.009		0.004		0.0133		0.223
<5	32	90.6		84.4		6.2		9.4	
≥5	35	66.7		54.8		24.4		20.8	
IPSS			0.012		0.0004		0.0005		0.386
Low, Int-1	39	87.2		82.1		5.1		12.8	
Int-2, High	28	64.9		50.7		31.3		18	
Prior treatment			0.655		0.871		0.784		0.597
BSC	52	78.6		71.2		15.4		13.5	
AZA	15	79		59.2		19.7		21	
Conditioning			0.27		0.173		0.35		0.458
MAC	55	79.3		72.4		12.9		14.7	
RIC	12	74.1		56.2		27.1		16.7	
Donor			0.82		0.981		0.509		0.519
Related	16	87.5		68.8		18.8		12.5	
Unrelated	51	75.3		69.6		14.4		16	
Source			0.522		0.118		0.172		0.904
BM	54	79.3		71.7		13.4		15	
PB	8	87.5		75		12.5		12.5	
CB	5	40		26.7		53.3		20	

OS overall survival, *DFS* desease-free survival, *NRM* non-relapse mortality, *IPSS* International prognostic scoring system, *AZA* azaccitidine, *BSC* best supportive care, *MAC* myeloablative conditioning, *RIC* reduce intensity conditioning, *Int-1* intermediat-1, *Int-2* intermediate-2, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood

statistical analyses were performed using the EZR software package (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html) [15].

Results

The median follow-up period was 726 days after transplantation (range, 2–2915 days). For all patients, 1-year OS and DFS after allo-HSCT were 78.4 and 69.5 %, respectively. The cumulative incidence of relapse (CIR) and NRM over 1 year were 15.4 and 15.1 %, respectively. Concerning the AZA group, a median of four cycles of AZA (range, 1–13 cycles) was administered, and complete remission (CR) was obtained in only two patients (13.3 %). Marrow CR (mCR) was observed in two patients (13.3 %), and stable disease (SD) with hematological improvement (HI) was seen in two patients (13.3 %). The overall response rate including CR, mCR, and SD with HI was 40.0 %, which was essentially in line with previous reports [2, 3]. Patients' characteristics, responses to AZA, transplantation modalities and outcomes of transplantation are summarized in Table 2.

The unadjusted survival estimates for patients treated with AZA and BSC before transplantation were 79.0 versus 78.6 % at 1 year (P=0.655), respectively. No significant difference was observed between the two groups regarding OS, DFS, CIR, and NRM (Fig. 1). With univariate analysis, high marrow blast and a high IPSS score adversely influenced 1-year OS, DFS, and CIR, as expected. In addition, older patients (>55 years) tended to show worse DFS (Table 2). With multivariate analysis, only a higher IPSS score was found to adversely influence 1-year DFS (Table 3). AZA administration

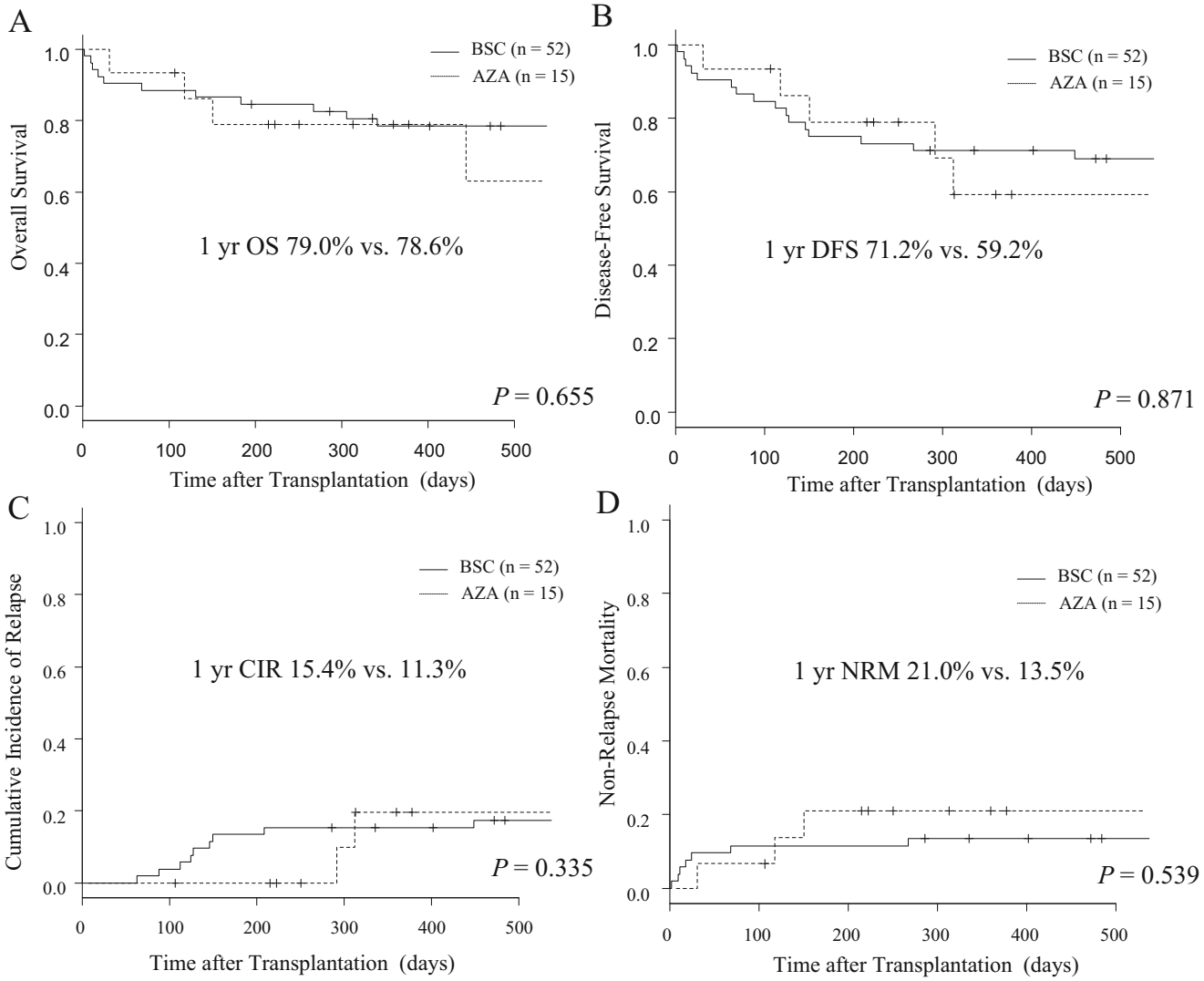

Fig. 1 Kaplan-Meier estimates of **a** 1-year overall survival, **b** 1-year disease-free survival, **c** cumulative incidence of 1-year relapse and **d** non-relapse mortality in all patients, according to the treatment received prior to transplantation

Table 3 Multivariate analysis of transplantation outcomes

Characteristics	1-year OS			1-year DFS			1-year relapse			1-year NRM		
	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*	HR	95 % CI	*P*
Age												
<55	–			1			–			–		
≥55				1.489	0.568–3.909	0.418						
Sex												
Male	–			–			1			–		
Female							0.72	0.037–13.88	0.83			
Marrow blast, %												
<5	1			1			1			–		
≥5	2.86	0.995–8.234	0.051	2.646	0.986–7.105	0.053	3.139	0.666–14.81	0.15			
IPSS												
Low, Int-1	1			1			1			–		
Int-2, High	2.334	0.910–5.985	0.078	2.889	1.098–7.602	0.032	6.042	0.658–55.44	0.11			
Source												
BM	–			1			–			–		
PB				0.524	0.118–2.327	0.396						
CB				2.59	0.724–9.270	0.144						

OS overall survival, *DFS* desease-free survival, *NRM* non-relapse mortality, *HR* hazard ratio, *CI* confidence interval, *IPSS* International prognostic scoring system, *Int-1* intermediat-1, *Int-2* intermediate-2, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood

prior to allo-HSCT was not statistically associated with better survival; however, when considering the subgroup of patients with higher IPSS scores (Int-2/High), those who received AZA alone tended to have higher rates of 1-year OS and 1-year DFS and lower rates of CIR, although these differences did not reach statistical significance (Fig. 2). Moreover, in the AZA group, patients who reached CR, mCR, or SD with HI showed superior OS (100 versus 66.7 %, $P=0.066$) and DFS (75 versus 50 %, $P=0.305$) compared to those with SD without HI or treatment failure (data not shown). Cumulative rates of acute GVHD were similar in both groups; 27 % in the AZA group and 29 % in the BSC group showed grade II to IV (Table 4).

Discussion

Based on the National Comprehensive Cancer Network guidelines [16], the current recommendation does not always support routine use of AZA in the pre-transplant setting. However, pre-transplantation therapy with AZA can be considered in patients with higher-risk MDS or in patients with MDS who have a high burden of disease. Several reports have identified a positive role for AZA before allo-HSCT [4–9]. Damaj et al. recently showed similar survival data in their retrospective comparison of 48 MDS patients with AZA alone and 98 MDS patients with induction chemotherapy (ICT) [5]. Gerds et al. also reported that pre-transplant therapy with AZA is associated with lower toxicity than ICT and may lead to similar outcomes [6]. On the other hand, based on a more recent comparison between AZA and BSC in patients undergoing RIC, Damaj et al. concluded that the absence of cytoreduction before allo-HSCT with RIC does not alter the outcomes, regardless of the pre-transplant tumor burden and the main disease characteristics [10]. Thus, findings from these retrospective studies suggest that pre-transplant therapy with AZA is feasible and may not increase the risk of post-transplant toxicities compared with ICT or BSC. However, it remains controversial whether AZA therapy before allo-HSCT is necessary for all types of MDS.

Our study did not show any significant differences in outcomes after allo-HSCT for MDS between the AZA group and the BSC group among all patients. However, the AZA group included more patients with a higher IPSS score compared with the BSC group, and this may have adversely affected post-transplant outcomes in the AZA group, because a higher IPSS score was an independent adverse factor in post-transplant outcomes in our study. Therefore, we reanalyzed the data of patients with a higher IPSS score. A trend toward a decrease in an early relapse was seen in the AZA group, and this may contribute to better survival outcomes. Moreover,

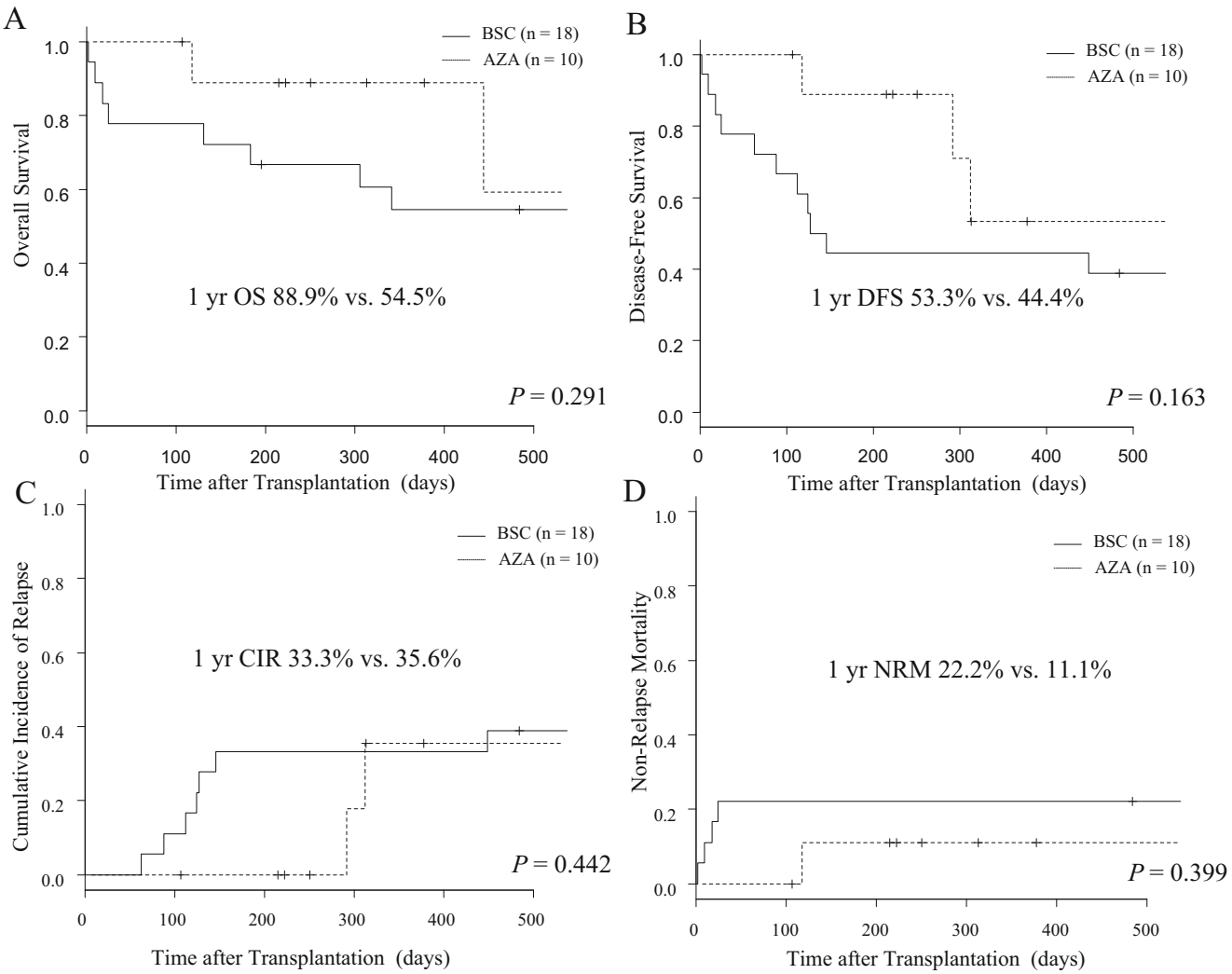

Fig. 2 Kaplan-Meier estimates of **a** 1-year overall survival, **b** 1-year disease-free survival, **c** cumulative incidence of 1-year relapse and **d** non-relapse mortality in the patients with a higher IPSS score (Int-2/High), according to the treatment received prior to transplantation

whether patients should be transplanted at their best response after AZA or after failing AZA is unclear; our results suggest that a good response to AZA appears to be a predictor of a better transplant outcome.

Several pre-clinical studies have shown the anti-GVHD effects of AZA [17, 18]. A retrospective analysis of post-transplant outcomes in patients treated with AZA or ICT prior to allo-HSCT showed a trend toward a lower rate of acute GVHD in patients pretreated with AZA [4]. These authors postulated that this result may be related to decreased tissue injury by AZA compared to ICT, or a direct anti-GVHD effect of AZA. However, in our study, we found no significant difference between the two groups in the incidence of severe acute GVHD.

In conclusion, for MDS patients with a higher IPSS score, AZA appears to be a valid therapeutic approach and may induce better OS and DFS with lower CIR and NRM. However, we emphasize that our results may have been influenced by the small number of patients in this study, and drawing a final conclusion will require studying a larger number of patients. Our experience with a small group of patients warrants a larger study with a series of patients to evaluate the efficacy of pre-transplant therapy with AZA.

Table 4 Incidence of acute GVHD

	Grade II–IV					Grade III–IV				
	No.	%	OR	95 % CI	*P*	No.	%	OR	95 % CI	*P*
					1					0.4
BSC	15	29	1			1	2	1		
AZA	4	27	0.9	0.180-3.690		1	7	3.55	0.043-290.7	

GVHD graft-versus-host desease, *OR* odds ratio, *CI* cinfidence interval, *AZA* azacitidine, *BSC* besty supportive care

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Conflicts of interest The authors declare no conflicts of interest.

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