Original Article

Feasibility of Allogeneic Stem Cell Transplantation after Azacitidine Bridge in Higher-Risk Myelodysplastic Syndromes and Low Blast Count Acute Myeloid Leukemia: Results of the BMT-AZA Prospective Study

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Running tytle: **Feasibility of HSCT in HR-MDS/AML after AZA bridge**

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ABSTRACT

Background Allogeneic stem cell transplantation (HSCT) is the only curative treatment in myelodysplastic syndromes (MDS). Azacitidine (AZA) is increasingly used prior to HSCT, however in Europe it is only approved for patients who are not eligible for HSCT.

Patients and Methods We conducted a phase II multicenter study to prospectively evaluate the feasibility of HSCT after treatment with AZA in 70 patients with a myelodysplastic syndrome (MDS), 19 with acute myeloid leukemia (AML), and 8 with chronic myelomonocytic leukemia (CMML). After a median of 4 cycles (range 1-11): 24% of patients achieved complete remission, 14% partial remission, 8% hematologic improvement, 32% had stable and 22% progressive disease. Ten patients discontinued treatment before the planned 4 cycles, due to an adverse event in 9 cases.

Results A HSC donor was identified in 73 patients, and HSCT was performed in 54 patients (74% of patients with a donor). Main reasons for turning down HSCT were lack of a donor, an adverse event, or progressive disease (9, 12 and 16 patients, respectively). At a median follow-up of 20.5 months from enrolment, response to AZA was the only independent prognostic factor for survival. Compared to baseline assessment, AZA treatment did not affect patients' comorbidities at HSCT: the HCT-CI remained stable in 62% patients, and worsened or improved in 23% and 15% of patients, respectively.

Conclusions Our study shows that HSCT is feasible in the majority of patients with HR-MDS/AML/CMML-2 after AZA treatment. As matched unrelated donor was the most frequent source of donor cells, the time between diagnosis and HSCT needed for donor search could be "bridged" using azacitidine. These data show that AZA prior to HSCT could be a better option than intensive chemotherapy in higher-risk MDS.

The trial has been registered with the EudraCT number 2010-019673-1.

Key words: azacitidine, hypomethylating treatment, high-risk MDS, allogeneic stem cell transplantation

KEY MESSAGE

In this study, we *prospectively* evaluated patient outcome from the time of HR-MDS/AML, diagnosis through azacitidine induction, followed by HSCT. We show that HSCT is feasible after azacitidine in 74% of HR-MDS/AML patients with a stem cell donor. In addition, AZA did not significantly impact patient comorbidities, but improved the disease status, which was independently associated to improved survival after HSCT.

INTRODUCTION

The prognosis of higher-risk MDS, including IPSS intermediate-2/high risks[1] or high and very-high risks according to the IPSS-R[2], closely resembles that of elderly AML, with a dismal predicted survival of one year or less. In this disease, HSCT remains the only curative option and is generally recommended for patients who are candidates to high-intensity treatment (NCCN Guidelines Version 1.2016).

After HSCT, other factors predicting survival in MDS are patient characteristics, as age and Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)[3], and disease-related factors, including prior duration of MDS, iron overload, karyotype, IPSS-R, and disease status at the time of HSCT conditioning.[4-5] The issue of donor selection is evolving and the increasing use of haplo-identical siblings has improved donor availability in recent years.[6] In parallel, the application of reduced-intensity conditioning (RIC) regimens has contributed to reduce transplant-related mortality, but at the cost of increasing rates of disease relapse.[7]

In this context, pre-transplant therapy represents a relevant issue in MDS, since conventional chemotherapy is associated with a high number of complications, besides low complete remission (CR) and high relapse rates.[8] In this regard, retrospective studies have shown similar survival outcomes in patients treated with intensive chemotherapy prior to HSCT compared to those transplanted upfront.[9-10] The EBMT group reported that, out of 341 evaluable patients including 244 MDS, only 16% finally underwent HSCT.[11] Similarly, a prospective study from the MD Anderson Cancer Research Center reported that less than 10% patients with HR-MDS or AML underwent HSCT after standard induction chemotherapy.[12]

Treatment outcomes in HR-MDS have significantly improved after the introduction of hypomethylating treatment (HMT), in particular azacitidine (AZA).[13] CR rates of 15-20%

after AZA treatment have been reported in prospective studies and confirmed in "real-life" patient cohorts, but the overall response rate, including PR and HI, reaches up to 50%.[13,14] In contrast with intensive chemotherapy, treatment complications are relatively low, with a vast majority of patients able to complete the 4-6 cycles necessary to obtain most of responses.

In the present multicenter study, we prospectively assessed the feasibility of HSCT in a large series of patients with HR-MDS, chronic myelomonocytic leukemia (CMML)-2, or AML with 20-30% blasts, following 4-6 standard courses of AZA, given with the purpose of reducing the disease burden, delaying disease progression and bridging the time to transplant.

PATIENTS AND METHODS

This prospective phase II non-randomized trial was conducted in 20 Hematology centers affiliated to the GITMO (Gruppo Italiano Trapianto di Midollo osseo e Terapie Cellulari) and/or GIMEMA (Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto) networks. The primary end-point of the study was the proportion of patients with HR-MDS, classified according to IPSS[1], able to perform HSCT after treatment with AZA. Further details regarding the study design are available in the Supplementary Materials, Online. The study was conducted in agreement with the Declaration of Helsinki, the ICH Harmonized Tripartite Guideline for Good Clinical Practice principles and procedures, and the Italian legislation requirements. The trial was approved by the ethic committees of all participating centers. All patients provided written informed consent before inclusion.

Treatment and Outcomes

Eligible patients received AZA 75mg/sqm/day subcutaneously for 7 days every 28 days for at least 4 cycles, followed by HSCT if a suitable sibling or unrelated donor was available. A minimum of 4 AZA cycles had to be given; however up to a total of 12 cycles were allowed. Pre-HSCT conditioning regimen was to be administered 4 to 8 weeks after the last AZA administration. Patients without a donor or unsuitable to or denying consent for HSCT, were allowed to continue AZA until a response persisted. Patients were followed and events recorded until progression to AML and/or death or to last available follow-up. Response was evaluated by BM aspirate and/or biopsy and cytogenetic analysis every 4 cycles of AZA, and before HSCT. The HCT-CI score was assessed at treatment start and before HSCT to identify three patient groups (low-risk: 0, intermediate: 1-2, high: $\geq$3).[3]

The primary endpoint was the proportion of patients who indeed underwent HSCT after bridge with AZA. Secondary endpoints were overall response rate (ORR) to AZA, safety of AZA, overall and disease-free survival (OS, DFS), transplant related mortality (TRM), and progression-free survival (PFS). Matched sibling or unrelated HLA 8/10 to 10/10 donor were allowed. Conditioning regimen before HSCT, and GVHD prophylaxis were according to the policy of the participating institutions.

This study was designed as a Simon optimal two-Stage Phase II clinical trial[15] to test the null hypothesis that $P \leq 0.180$ versus the alternative that $P \geq 0.300$ had an expected sample size of 50.78 and a probability of early termination of 0.681. Details on the statistical analysis are reported in Supplementary materials, online.

RESULTS

Feasibility of HSCT after AZA-bridge

From October 2010 to September 2014, 102 transplant-eligible patients were screened for enrolment into the study (Figure 1). Five patients did not enter the study due to rapid disease progression (*n*=2) or consent withdrawal (*n*=3). AZA was started at a median of 0.9 months (range 0-105 months) from diagnosis of higher-risk MDS, CMML or AML and at 0.4 months (range: 0-2.3) from registration in 97 patients (34 females, 63 males), with a median age of 59 years (range 21-66.5 years). Eleven patients had a prior diagnosis of lower-risk MDS. The main patient characteristics are shown in Supplementary Table 1. MDS 2016 WHO classification[16] and risk stratification, according to IPSS, WPSS and IPSS-R was re-assessed for all patients with available information.

Treatment was discontinued in 10 patients after a median of 2 cycles (range 1-3), mostly due to an adverse event (*n*=6, Figure 1). After 4 AZA cycles, CR was achieved in 21 patients (24%), partial remission (PR) in 12 patients (14%), hematologic improvement (HI) in 7 (8%), while the disease was considered stable in 28 (SD: 32%), and progressive in 19 patients (PD: 22%). Donor search was started at a median of 0.1 months from protocol inclusion (range -97 to +4.6 months), and was prematurely terminated due to progressive disease in 4 patients. A HSC donor was identified in 73 of 93 patients (78.5%) after a median of 3.4 months from AZA start (range 0.9-11.8 months).

Thirty-three patients did not undergo HSCT, due to lack of a suitable SC donor (*n*=9), PD (*n*=16), adverse event (*n*=3), consent withdrawal (*n*=4), or re-staging as low-risk MDS (*n*=1). Twenty patients continued AZA for a median of 7 cycles (range 5-12).

Fifty-four patients (56%) received an allogeneic HSCT, after a median of 5 cycles of AZA (range 1-11 cycles), and 6.4 months (range 4.2- 14.3 months) from study inclusion. One

patient underwent HSCT in SD after only one AZA cycle due to a medical decision. Feasibility of HSCT reached 74% when the analysis was restricted to the 73 patients with a suitable donor.

At the time of HSCT, 24 of the 54 patients were in CR (44.4%), 8 in PR (14.8%), 5 had HI (9.3%), 17 SD (31.5%). Compared to baseline, the HCT-CI re-evaluated prior to HSCT in 52 patients with available data indicated that 5 of 24 patients with low-HCT progressed to intermediate or high HCT-CI (21%), while 5 of 6 patients with high HCT-CI at baseline remained stable (Supplementary Table 2). In 22 patients classified as intermediate HCT-CI at baseline, 7 patients improved to low and 7 progressed to high HCT-CI (p=0.3). This translated into worsening of comorbidities in 22% and improvement in 15% of patients after AZA “bridge”.

Myeloablative and reduced intensity conditioning regimen was used in 28 and 26 patients, respectively. Donors were HLA-identical siblings in 16 patients (29.6%), MUD in 36 (66.7%), and haplo-identical siblings in 2 patients (3.7%). Although haplo-identical sibling transplantation was not foreseen by the protocol, we included these two patients in the analysis, according to the primary objective of the study, which was feasibility of HSCT. Stem cell source was mostly peripheral blood (*n*=40, 74.1%) and the remaining bone marrow (*n*=14, 25.9%). Median time to engraftment was 18 days (range 10-43 days) for neutrophils, and 17 days (9-186 days) for platelets.

Survival analysis

Median follow-up for surviving patients was 20.5 months (range 1.6-40.6). Median OS was 15.2 months on an intent-to-treat (ITT) basis (*n*=97 patients, Supplementary Figure 1A). HSCT considered as a time-dependent covariate was associated to significantly longer survival in patients who received HSCT (median OS 20.9 months; range: 6.8-40.6) compared with those who did not receiving HSCT (median OS 9.4 months; range: 0.23-21.3) (*p*=0.01, HR 0·41, 95% C.I. 0.22-0.78).

At univariate analysis, significant prognostic factors for OS were very-high WPSS risk, high HCT-CI, and treatment response (Table 1 and Figure 2A and 2B). Multivariate analysis, including HSCT as time-dependent covariate, confirmed AZA treatment response as the only independent prognostic factor for OS. Treatment response and low HCT-CI were prognostic factors for PFS (Table 1, Supplementary figure 1B, Figure 2C).

In the 54 patients who underwent HSCT, OS was not associated to status at HSCT (CR/PR/HI, versus SD, *p*=0.28), nor to IPSS-R at diagnosis (IPSS-R low vs Intermediate, vs high, vs very high, *n*=33 pts, *p*=0.492, Figure 3A-B). Low HCT-CI[3] at the time of transplant remained a statistically significant prognostic factor for OS (n=52 patients, High vs Low: *p*=0.09, HR: 4.33, 95%CI: 1.63-11.51; Intermediate vs Low: *p*=0.007, HR: 3.81, 95%CI: 1.45-10.00, Figure 3C).

Adverse events and causes of death

Sixty-four grade III-IV serious adverse events (SAEs) were reported in 58 patients. Adverse events were the cause of AZA discontinuation in 6 patients, and consisted of infections (4 pts), or hemorragic disorders (2 pts). SAE impeded HSCT in 3 patients, and consisted on an infection in 2 cases and an intra-abdominal hemorrage in 1 patient. Acute grade III-IV graft versus host disease (GVHD) was diagnosed in 3 patients (6%), while

chronic GVHD occurred in 14 patients (29%). At a median follow-up of 20.5 months from treatment start, 52 patients died. Causes of death in the non-HSCT group were disease progression or relapse (16 of 26 patients, 61.5%), followed by infectious (7 patients) and hemorrhagic complications (3 patients).

In 54 patients undergoing HSCT, median survival after transplantation was 13.6 months. Mortality was transplant-related in 16 patients (30%, GVHD: 4 patients, infectious complication: 6 patients, multi-organ failure: 4 patients, other causes: 2 patients), disease relapse in 9 patients (17%), and a second malignant disease in 1 patient.

DISCUSSION

In this prospective study, we show that HSCT is feasible after AZA "bridge" in 74% of patients with HR-MDS or low-blast count AML with a suitable HSC donor. The trial included a population of patients with a median age of 59 years, with >10% bone-marrow blasts at protocol inclusion in 73% of cases, and adverse karyotype in 55%, which are negative prognostic factors for HSCT outcome in MDS.[4,5,17] In the context of a close collaboration between MDS teams and HSCT units, AZA induced responses in 49% of our patients, considering together CR, PR or HI.

Duration of response after AZA treatment is relatively short, of about 13-17 months, while HSCT is the only curative option in MDS.[13,14,18] This has been recently confirmed by a prospective observational study conducted in France, where patients with an HLA-matched donor had a significantly better 4-year OS, compared to those without a donor (37% vs 15%).[19] Most of the patients in that study (76%) had received hypomethylating treatment prior to HSCT.

It has been shown that best outcomes of HSCT in MDS rely on a shorter interval between diagnosis and transplantation.[20] In our study, we identified a donor in 78% of patients, at a median of 3 months from protocol inclusion. The rapid identification of a HSC donor today may favor the applicability of upfront HSCT without any prior treatment in HR-MDS. In our study, AZA responders had a significantly longer survival than non-responders, reaching a median survival of over 2 years in patients who achieved CR or PR. Different from a recent report from Yahng et al.[21], prolonged survival in our patients was not limited to patients achieving remission or HI, but was also observed in patients with SD, accounting for 29% of cases in our series. SD may also reflect a biologically less aggressive disease, independent from treatment, which would need to be characterized at initial diagnosis, most probably by identification of somatic mutations predictive not only of response, but also of SD after HMT.[22-24]

In our series, AZA did not significantly affect patients' comorbidities at HSCT. These data compare favorably to results of conventional chemotherapy schedules in HR-MDS. After HSCT, grade III-IV acute GVHD was rarely reported (6%), while grade III-IV chronic GVHD occurred in 29% of patients, similar to recent reports on HSCT preceded by HMT.[19] In this line, AZA administered as maintenance after HSCT has been shown to increase the number of T regulatory cells and of cytotoxic T-cells, as mechanisms likely to increase the graft vs leukemia effect, without a concomitant increase in GVHD.[25] Probably, changes related to AZA pretransplant do not play a significant role in post-transplant immunological changes.

Our study, in the setting of patients with MDS, "highly eligible for transplant", previously untreated, of a maximum age of 66, shows that HSCT is feasible in 74% of patients following AZA "bridge", at a significantly higher rate than conventional chemotherapy in this setting. The major limitation of the study is that it does not answer the question

whether the patients who did not proceed to HSCT due to an adverse event (13%) or progressed during AZA (20%) could have benefited from upfront HSCT. This issue could be addressed by a prospective randomized study where upfront HSCT would be tested against HSCT after AZA. A major challenge for this type of study is heterogeneity of MDS, whereby karyotype and blast proportion, together with patient-related factors, as age and comorbidities, should be considered for adequate patient stratification.

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DISCLOSURE OF CONFLICTS OF INTERESTS

MTV received honoraria from Celgene, GL and SS received research funds from Celgene, LF received honoraria from Celgene.

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FIGURE LEGENDS

Figure 1

Consort diagram

Figure 2

Survival Outcomes

A) Overall Survival by response to AZA (*n*=87 patients) and B) by HCT-CI (*n*=97 patients, months from AZA treatment start)

C) Progression-free survival by response to AZA (*n*=87 evaluable patients, months from AZA treatment start)

HSCT considered as time-dependent covariate was associated with a significantly longer survival (*p*=0.01, HR 0.41, 95% CI: 0.22-0.788). Multivariate analysis showed that treatment response was the only independent prognostic factor for survival (*p*=0.0007)

Figure 3

Overall survival after HSCT

A) by AZA response at HSCT (*n*=54 pts)

B) by IPSS-R (*n*= 33 MDS pts)

C) by HCT-CI (*n*=52 pts with available HCT-CI at the time of HSCT)

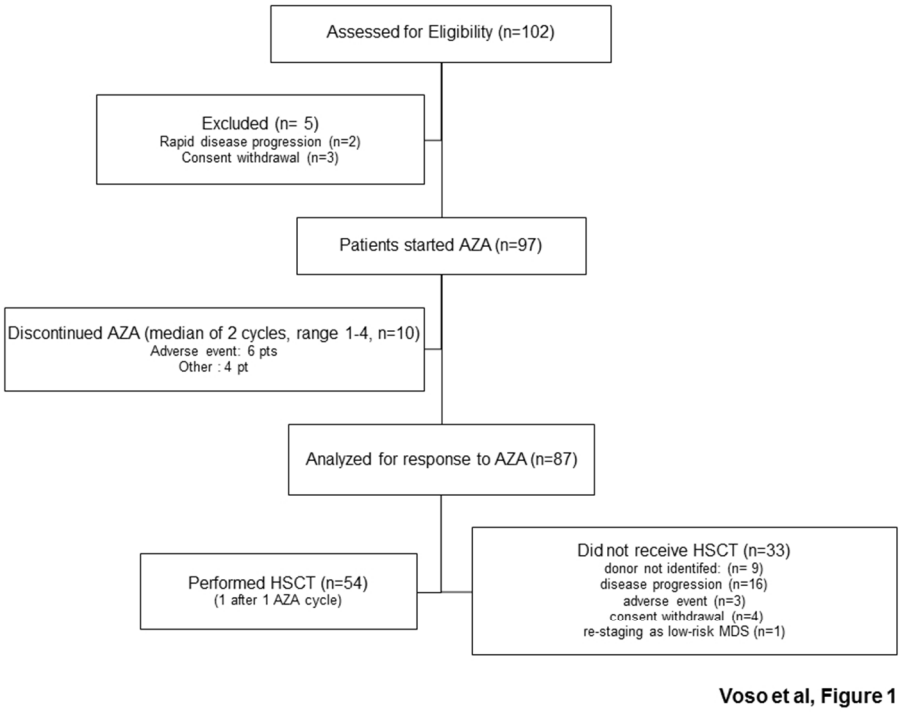

Figure 1

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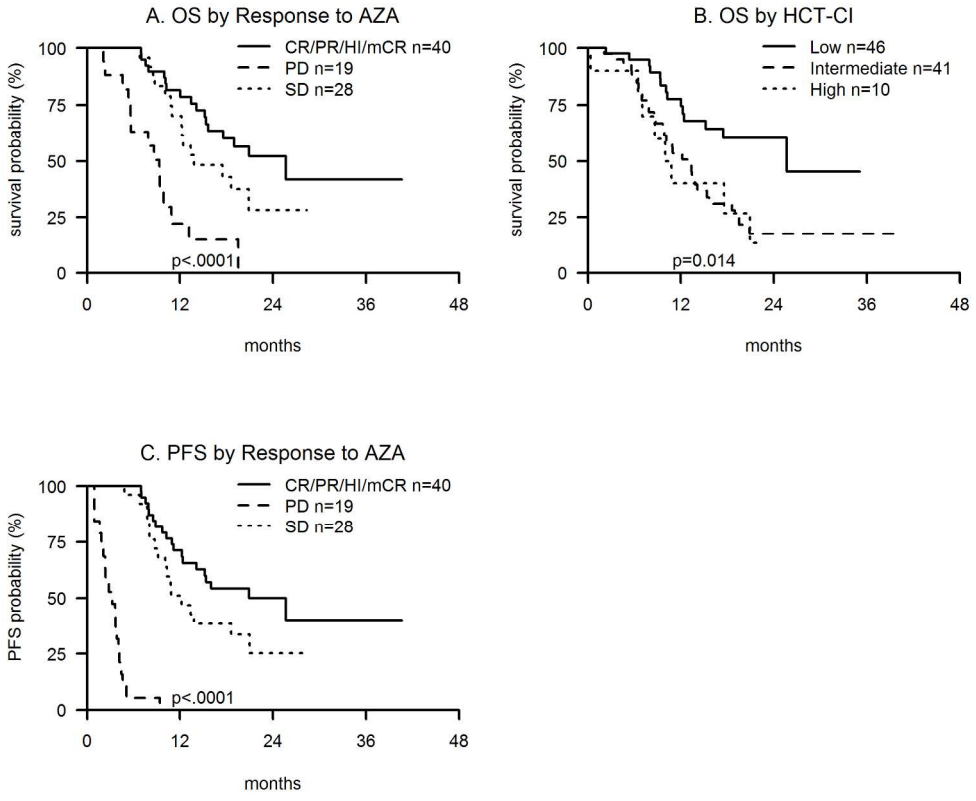

Figure 2

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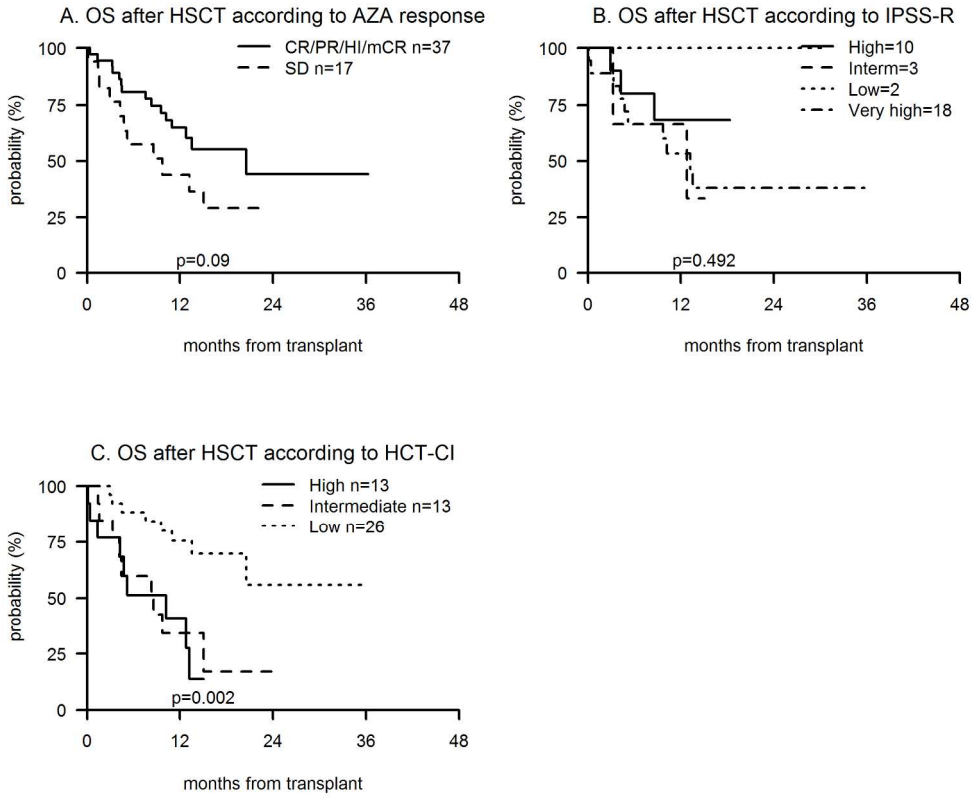

Figure 3

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Table 1 Prognostic factors for survival outcomes

	Overall Survival		Progression-Free Survival		Non-Relapse Mortality	
	HR (95%CI)	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Age ≤50 vs >51 years	0.53 (0.24-1.17)	0.118	0.59 (0.29-1.19)	0.140	0.58 (0.20-1.67)	0.310
BM-Blasts (below or over 10%)	0.54 (0.25-1.17)	0.118	0.55 (0.27-1.10)	0.090	0.54 (0.25-1.17)	0.118
IPSS (High vs Int-2)	1.62 (0.82-3.19)	0.162	1.28 (0.68-2.43)	0.440	1.69 (0.64-4.46)	0.287
WPSS (High vs V-High)	0.39 (0.19-0.84)	**0.015**	0.53 (0.27-1.05)	0.067	0.60 (0.21-1.72)	0.342
WPSS (Intermediate vs V-High)	0.34 (0.12-0.97)	**0.044**	0.49 (0.19-1.26)	0.137	0.35 (0.07-1.77)	0.206
IPSS-R (High vs V-High)	0.53 (0.21-1.33)	0.177	0.72 (0.33-1.58)	0.416	0.62 (0.19-1.96)	0.420
IPSS-R (Intermediate vs V-High)	0.96 (0.33-2.85)	0.949	1.20 (0.45-3.21)	0.718	0.46 (0.06-3.57)	0.458
HCT-CI (High vs Low)	2.91 (1.22-6.97)	**0.016**	2.10 (0.92-4.78)	0.076	1.88 (0.50-7.10)	0.352
HCT-CI High vs Intermediate	2.67 (1.41-5.04)	**0.002**	2.19 (1.25-3.82)	**0.006**	2.45 (1.05-5.74)	**0.040**
CR/PR/HI/mCR vs PD	0.22 (0.09-0.50)	**0.0001**	0.03 (0.01-0.08)	**<.0001**	0.13 (0.05-0.35)	**<.0001**
SD vs PD	0.38 (0.16-0.88)	**0.024**	0.06 (0.02-0.14)	**<.0001**	0.14 (0.04-0.48)	**0.0007**
Ferritin (as continuous variable)	1 (0.99-1.00)	0.526	1 (0.99-1.00)	0.648	1.00 (0.99-1.00)	0.322
HLA-id vs MUD (n=54)	0.50 (0.20-1.28)	0.149	0.45 (0.18-1.14)	0.093	0.57 (0.15-2.17)	0.412
Myeloablative vs RIC (n=54)	0.6 (0.27-1.32)	0.205	0.67 (0.32-1.42)	0.296	1.06 (0.32-3.48)	0.920

Supplementary Material

BMT-AZA Protocol eligibility criteria

Patients were eligible if they had a diagnosis of IPSS intermediate-2 or high-risk MDS, RAEB-t (at present classified as AML with 20-30% blasts, here defined as low-blast count AML), or CMML-2, with 10-29% bone marrow (BM) or peripheral blood (PB) blasts, and WBC < 20 x 10^9/L. Other inclusion criteria were age between 18 and 65 years, Eastern Cooperative Oncology Group (ECOG) performance status less than 3, and life expectancy of over 3 months.

A donor search activation was mandatory at the time of protocol enrolment. Patients with severe organ impairment or active viral infections were not included in the trial. Patients who had received prior HMT, chemotherapy or radiotherapy during the last 6 months, investigational drugs during the last 30 days or hematopoietic growth factors during the last 21 days were also excluded.

Statistical Analysis

This study was designed as a Simon optimal two-Stage Phase II clinical trial[19] to test the null hypothesis that $P \leq 0.180$ versus the alternative that $P \geq 0.300$ had an expected sample size of 50.78 and a probability of early termination of 0.681.

After testing the treatment on 31 patients in the first stage (interim analysis), the trial would have been terminated if 6 or fewer had achieved the primary end-point, i.e. HSCT. If more than 6 patients underwent HSCT, then the trial would proceed to the second stage, and a total of 93 patients would have to be enrolled. If the total number of patients undergoing HSCT were less than or equal to 22, the study would have failed to reach the primary end-point.

Baseline patient characteristics, disease history, and treatment-related variables were summarized using descriptive summary statistics and graphical approaches. Differences in the distribution of prognostic factors in patient subgroups were analyzed by the Chi-square

or Fisher's exact test and by the Wilcoxon test. PFS and OS were calculated using the Kaplan-Meier method. PFS was the time between the first dose of AZA and disease progression to AML in higher-risk MDS, and date of relapse in AML, or of death, whichever was first reported. Secondary endpoints are presented along with 95% confidence intervals for each stratum separately.

Differences in survival were calculated by the log rank test in univariate analysis and by the Cox regression model in multivariate analysis. The Cox proportional hazard regression model was used to examine the risk factors affecting time to event. All statistical analyses were performed using the statistical software SAS (release 9.4).

Supplementary Table 1

Patient Characteristics (n=97 patients)		
Age (median, range)		59 (21-66)
Disease duration (months, median, range)		0.9 (0-105)
BM-blasts % (median, range)		15 (0-30)
Blood counts (median, range)	Hb (g/dl) Neutrophils (10^9/L) Platelets (10^9/L)	9.3 (6.5-13.3) 0.7 (0.1-41) 74 (6-662)
ECOG (n)	0 1 2	70 (72%) 17 (17.5%) 10 (10%)
WHO 2016 Classification (n)	MDS-SLD/MLD MDS-EB-1 MDS-EB-2 AML (20-30% blasts) CMML-2	8 (8%) 11 (11%) 51 (53%) 19 (20%) 8 (8%)
Karyotype (n=87)	Normal Trisomy 8 -7 Del 5q Other monosomies Complex ($\geq$3 abn) Other	32 (37%) 4 (4.5%) 13 (15%) 3 (3.5%) 3 (3.5%) 23 (26.5%) 9 (10%)
IPSS (n=87)	Low/Int-1 Int-2 High	3 (3.5%) 43 (49.5%) 41 (47%)
WPSS (n=69)	Low/Interm. High Very high	9 (13%) 42 (61%) 18 (26%)
R-IPSS (n=69)	Very low/low Intermediate High Very high	4 (6%) 11 (16%) 21 (30%) 33 (48%)
HCT-CI (n=97)	Low (0) Intermediate (1-2) High ($\geq$3)	46 (47%) 41 (42%) 10 (10%)
Ferritin (ng/mL, median, range)	214 (1-909)	

Supplementary Table 2:

Changes in patients' HCT-CI from baseline to HSCT

(n=52 patients with available HCT-CI at HSCT)

HCT-CI at Baseline	HCT-CI at HSCT			
	Low (0)	Intermediate (1-2)	High (≥3)	Total
Low (0)	19	4	1	24
Intermediate (1-2)	7	8	7	22
High (≥3)	0	1	5	6
Total	26	13	13	52

*p=0.3

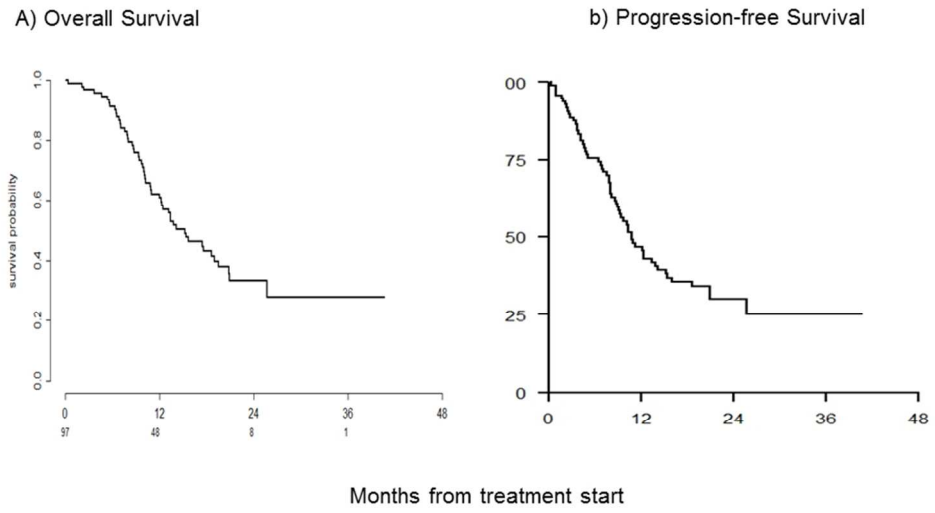
Supplementary Figure 1:
Changes in patients' HCT-CI from baseline to HSCT
(n=52 patients with available HCT-CI at HSCT)

HCT-CI at Baseline	HCT-CI at HSCT			
	Low (0)	Intermediate (1-2)	High (≥3)	Total
Low (0)	19	4	1	24
Intermediate (1-2)	7	8	7	22
High (≥3)	0	1	5	6
Total	26	13	13	52

*p=0.3

254x190mm (96 x 96 DPI)

Supplementary Figure 2: Survival
(n= 97 patients, intention to treat population)



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