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RESEARCH ARTICLE

Azacitidine Maintenance Therapy Post-Allogeneic Stem Cell Transplantation in Poor-Risk Acute Myeloid Leukemia

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Abstract

Objective/Background: **Allogeneic hematopoietic stem cell transplant (HSCT) is the potential curative modality for poor-risk acute myeloid leukemia (AML), relapse remains the main reason for transplant failure. Early-phase studies showed azacitidine is safe for post-transplant maintenance therapy in AML.**

Methods: **We performed a single institutional prospective cohort study to evaluate the benefit of azacitidine maintenance therapy following allogeneic HSCT in poor-risk AML. The main objective of this study is to generate a hypothesis aiming to optimize post-transplantation outcomes in poor-risk AML. Forty-nine adults with poor-risk AML who underwent allogeneic HSCT were evaluated in a nonrandomized prospective cohort fashion. Thirty-one participants received post-transplant azacitidine (32 mg/m^2) on Days 1−5 for a 28-day treatment cycle beginning approximately 40 days after transplantation. The study was controlled using 18 matched individuals who were on a noninterventional surveillance protocol.**

Results: **The relapse rate was significantly higher in the control cohort (66.67%) versus (25.81%) in the azacitidine maintenance cohort ($p < .005$). Time to relapse was significantly prolonged by azacitidine maintenance, not reached versus 4.1 months in the control arm ($p < .0001$). In addition, median overall survival was lower in the control cohort at 7.6 versus 27.4 months in the interventional cohort ($p < .0001$). At a median follow-up of 24 months, incidence of graft-versus-host disease (GVHD) did not differ between study groups ($p = .325$). In both cohorts, minimal residual disease was correlated with higher hazard of relapse (95% confidence interval, 2.31−13.74; $p < .001$).**

Conclusion: **We conclude that low dose azacitidine maintenance following allogeneic HSCT in poor-risk AML, decreased relapse rate, and increased both the time to relapse and overall survival without increased risk of GVHD.**

Keywords: Clinical research, Poor-risk AML, Post-HSCT low dose azacitidine, Post-transplantation maintenance, Stem cell transplantation

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only potential curative modality for poor-risk acute myelogenous leukemia (AML) [1]. However, disease relapse after HSCT is responsible for 40% of transplant failures [2]. The overall prognosis for relapsed patients is poor, with 2-year survival rates below 20% [3−5].

One retrospective analysis of European Society for Blood and Marrow Transplantation (EBMT) registry data for 776 patients suffering de novo AML relapse following reduced-intensity HSCT showed 2-, 3-, and 5-year overall survival rates of 13.9%, 12.2%, and 9.8%, respectively [6]. Moreover, myeloablative conditioning failed to significantly improve overall survival owing to increased treatment-related mortality [7,8].

Detectable minimal residual disease (MRD) prior to transplant correlates with poor relapse-free survival and overall survival after transplant [9−11]. The median survival of relapsed AML is only

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3–4 months if no further definitive therapies were introduced [12]. Current salvage therapies are limited to either a second transplant or donor lymphocyte infusion (DLI). However, even with these treatments, the overall prognosis remains dismal [13].

Because of the availability of limited strategies for the management of relapsed AML after HSCT, and increasing incidence of disease relapse within the first 6 months after transplantation, consolidating the response to HSCT using a maintenance strategy to achieve a deeper response and decrease the probabilities of a relapse is crucial in poor-risk AML management algorithm.

An ideal drug for pharmacologic maintenance therapy should have activity against the disease, without excessive myelosuppression or eradication of donor cells, while also having a minimal risk of graft-versus-host disease (GVHD) exacerbation or other toxicity.

One such candidate would be azacitidine, a hypomethylating agent, which works epigenetically to modulate various genes, including tumor suppressor genes. The antineoplastic activity of azacitidine results from DNA hypomethylation and a cytotoxic effect on abnormal hematopoietic cells in bone marrow [14].

In vitro, azacitidine causes concentration-dependent apoptosis in myeloid cell lines and DNA damage in AML cell lines [15], induction of granulocytic differentiation in the leukemia cell line HL-60 [16], megakaryocytic differentiation in the primitive myeloid cell line 416B [17], and monocytic differentiation in the myeloid-B lymphoid leukemia cell line BW-90 [18].

Low dose azacitidine (32 mg/m^2) given on Days 1–5 of 28-day treatment cycles for four total cycles commencing on Day + 40 after transplant is safe and tolerable.

The main objective of this study is to generate a hypothesis aiming to optimize post-transplantation outcomes in poor-risk AML. In this study, we set out to determine whether maintenance azacitidine administered to patients with poor-risk AML after allogeneic HSCT would improve either relapse-free survival or overall survival.

2. Patients and methods

2.1. Study design

This is a nonrandomized prospective cohort study performed on patients with poor-risk AML who had an allogeneic stem cell transplant between September 2013 and July 2018 in the Bone Marrow Transplant Program at the University of Oklahoma Health Sciences Center (OUHSC; Oklahoma City, OK, USA). The study was approved by the Institutional Review Board at the OUHSC.

Patients are included in the treatment cohort based on their preference to start maintenance therapy after providing informed consent. The control cohort was matched based on sex, age, AML risk status, disease status prior to transplant, and donor type.

Patients included in the treatment cohort received azacitidine 32 mg/m^2 on Days 1–5 of the 28-day treatment cycle for a total of four cycles. The intervention started at Day 40 after transplantation. A noninterventional cohort included matched controls of patients monitored on surveillance after transplantation.

The primary study objectives were to evaluate the benefit of using azacitidine maintenance therapy after HSCT and its impact on relapse-free and overall survival in poor-risk AML.

The secondary objectives were to evaluate the association between other clinical parameters that can affect transplant outcomes. The variables included in this study include: (a) disease status prior to transplant (MRD status), (b) donor status (related vs. unrelated), (c) source of stem cells (bone marrow vs. peripheral blood), (d) preparative regimen (reduced-intensity conditioning vs. myeloablative conditioning), and (e) stem cell dose. We also planned to evaluate its impact on the incidence of GVHD and infection rates.

2.2. Inclusion and exclusion criteria

Inclusion criteria called for individuals older than 18 years with poor-risk AML, based on cytogenetic and/or molecular profile at diagnosis, underwent matched or mismatched, related or unrelated, bone marrow or peripheral blood, cord blood, or haploidentical allogeneic HSCT in complete remission (CR) at time of transplant, and with no evidence of morphological disease by peripheral blood and bone marrow biopsy after transplantation.

Patients with poor-risk AML were defined by using cytogenetics and molecular profiles based on the European LeukemiaNet risk stratification by genetics [19]. We defined MRD status as persistent positivity for molecular markers and/or chromosomal abnormalities [20]. CR was defined, using the International Working Group response evaluation, as the presence of < 5% blasts in the bone marrow with the absence of circulating blasts and blasts with

Auer rods as well as the absence of extramedullary disease with adequate peripheral blood counts recovery [21].

2.3. Statistical analysis

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Time to relapse and relapse-free survival was calculated (in months) and defined as the period between transplant date and the date of relapse. Overall survival was calculated (in months) and defined as the period between transplant date and the date of death or last follow-up.

The relapse rate is the percentage of patients who relapsed within the study follow-up.

Unadjusted probabilities of relapse-free survival/time to relapse (TTR) and overall survival (OS) were estimated using the Kaplan–Meier method.

A multivariable Cox proportional hazards model was derived to adjust for covariates that differ between the two treatment arms and was used to analyze time to relapse and overall survival in both arms; results were graphed using Kaplan–Meier plot.

We also performed univariate analysis to determine the effect of MRD status (positive vs. negative), donor status, preparative regimen, stem cell dose, and source on time to relapse and overall survival.

For covariates analysis, we used a Student *t* test to determine differences between the two treatment groups with continuous variables and the chi-square test to determine differences in categorical covariates. Covariates that differed significantly at an alpha level of 0.05 were included in a multivariable Cox proportional hazards model.

For overall survival analysis, we censored patients still alive at the time of data analysis, and for time to relapse analysis, we censored patients who did not have a relapse at the time of data analysis.

3. Results

3.1. Patient characteristics

A total of 49 patients undergoing allogeneic HSCT for poor-risk AML between September 2013 and July 2018 were evaluated. The treatment cohort included 31 patients who were started on the post-transplant maintenance azacitidine protocol between 2013 and 2018, and the control cohort included 18 patient who were started on post-transplant surveillance protocol between 2013 and 2015.

The median age was 47 years in the azacitidine arm and 54 years in the control arm (range, 19–68 years); 26.5% of patients had detectable MRD at the time of transplant (22.58% in the azacitidine arm and 33.3% in the control arm).

The majority of patients in the treatment cohort were able to finish the recommended four cycles of azacitidine maintenance therapy (23/31 patients, 76.6%). Whereas 22.5% of participants received only two to three cycles owing to either early relapse or intolerability caused by neutropenia and infections, three participants received more than four treatment cycles (Table 1).

3.2. Overall survival and time to relapse

At a median follow-up of 27 months after transplant for study cohorts, a significantly higher proportion of patients in the control cohort died (88.89%) than those in the azacitidine group (25.81%) ($p < .001$); most deaths in the control group were attributable to relapse (12/16 deaths, all within 5 months after transplant).

The relapse rate was also significantly higher in the control cohort (66.67%) than in the azacitidine group (25.81%) ($p < .005$), and the difference in the time to relapse between the two groups (Fig. 1) was also significant; median time to relapse was 4.1 months in the control arm and not reached in the azacitidine group by the end of the median follow-up time (log-rank $p < .0001$)

Unadjusted probabilities of overall survival were different between the two arms (log-rank $p < .0001$) with median overall survival being 7.6 months in the control arm versus 25th survival percentile was 27.4 months in the azacitidine arm (Fig. 2).

Twenty-nine participants from both arms were relapse-free (23 were in the treatment arm and 6 in the control arm). Overall survival analysis on this subgroup (OS in relapse-free patients) showed significant differences between the two arms (log-rank $p = .003$). Median survival time among those in the control group was 9.8 months, whereas in the azacitidine arm only two events occurred and the remaining 21 participants were alive (censored) (Fig. 3).

3.3. Donors and grafts

Most participants received matched unrelated donor stem cells (67.3% overall; 23/31 in the azacitidine arm and 10/18 in the control arm), whereas 32.7% received related donor cells and only two from each group received haploidentical donor

Table 1. Baseline Descriptive Statistics.

Covariate	Statistics	Level	Azacitidine arm ($N = 31$)	Control arm ($N = 18$)	Parametric p[a]
Sex	*N* (Col %)	F	15 (48.39)	9 (50)	0.913
	N (Col %)	M	16 (51.61)	9 (50)	
Age	Mean		44.48	53.39	0.044
	Median		47	54	
CR	*N* (Col %)	MRD +	7 (22.58)	6 (33.33)	0.411
	N (Col %)	MRD −	24 (77.42)	12 (66.67)	
# cycles	*N* (Col %)	2 or 3	7 (22.6)		
	N (Col %)	4	20 (71.43)		
	N (Col %)	6	1 (3.57)		
	N (Col %)	12	2 (7.14)		
GVHD	*N* (Col %)	No	12 (38.71)	12 (66.67)	0.059
	N (Col %)	Yes	19 (61.29)	6 (33.33)	
Donor type	*N* (Col %)	Unrelated	23 (74.19)	10 (55.56)	0.180
Prep regimen	*N* (Col %)	Related	8 (25.81)	8 (44.44)	0.019
	N (Col %)	MAB	24 (77.42)	8 (44.44)	
	N (Col %)	RIC	7 (22.58)	10 (55.56)	
Stem cells source	*N* (Col %)	BM	19 (63.33)	6 (35.29)	0.064
	N (Col %)	PB	11 (36.67)	11 (64.71)	
Cell dose	*N*		30	13	0.353
	Mean		3.69	4.55	
	Median		3.64	3.55	

Note. BM = Bone marrow; CR = complete remission; MAB = ; GVHD = graft-versus-host disease; MRD = Minimal residual disease; PB = Peripheral blood ; RIC = Reduced intensity conditioning.

[a] The parametric p-value is calculated using analysis of variance (ANOVA) for numerical covariates and chi-square test for categorical covariates.

transplants. Neither donor type ($p = .18$) nor stem cell dose ($p = .353$) differed between groups.

Univariate analysis for the effect of donor types (related vs. unrelated donor) showed no difference between study groups in terms of either overall survival (hazard ratio [HR] = 1.18; 95% confidence interval [CI], 0.49–2.85; log-rank $p = .709$) or time to relapse (HR = 0.85; 95% CI, 0.34–2.13; log-rank $p = .724$).

In terms of stem cell source, although most patients in the azacitidine arm received bone marrow stem cells and most in the control received

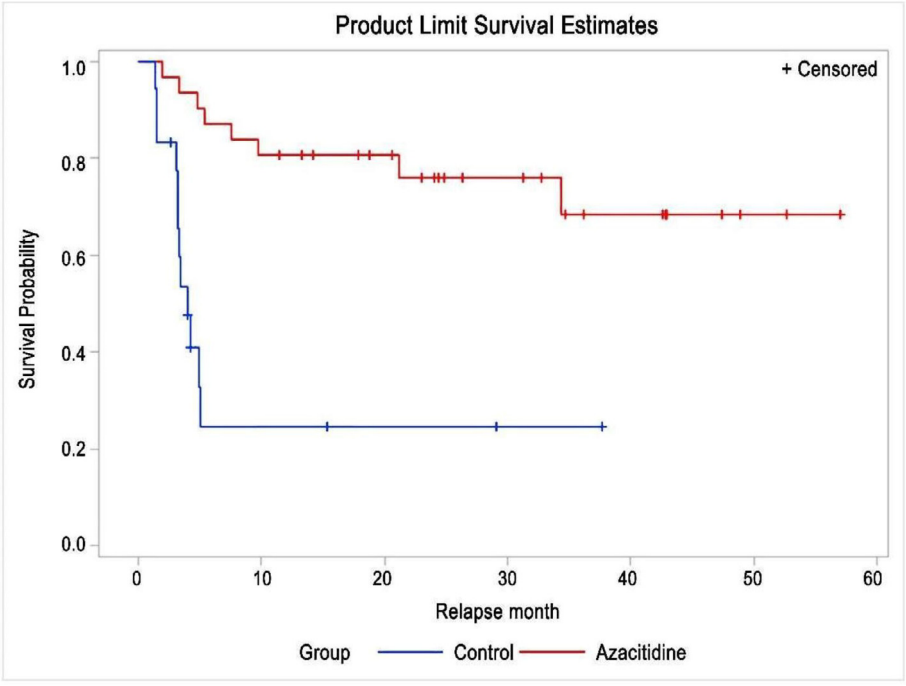

Fig. 1. Time to relapse Kaplan–Meier plot of azacitidine arm and control arm show median time to relapse of not reached versus 4.1 months.

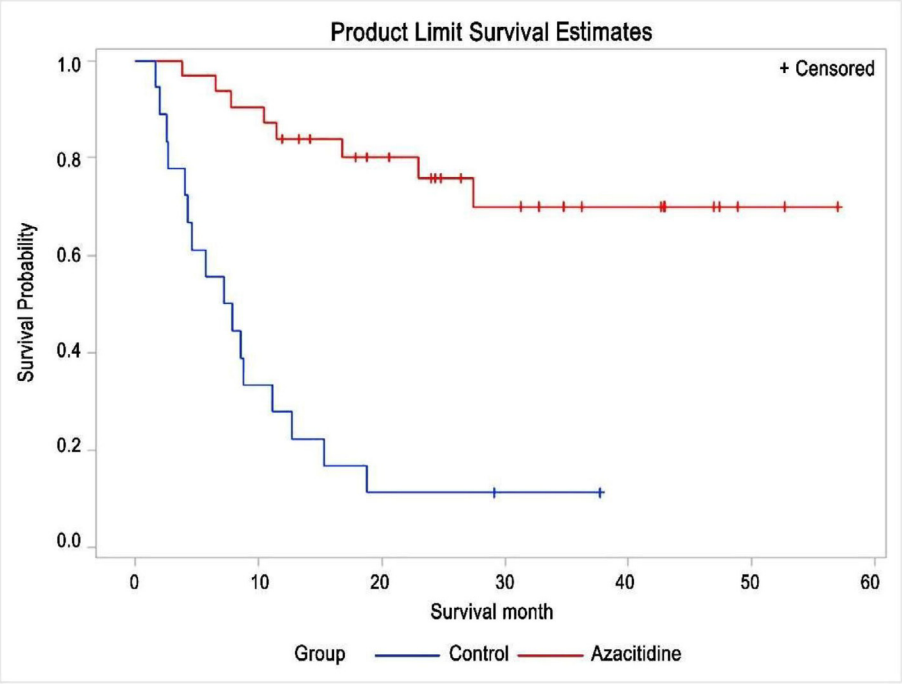

Fig. 2. Overall survival Kaplan–Meier plot of azacitidine arm and control arm shows 25th percentile survival of 27.6 months versus median survival of 7.6 months, respectively.

peripheral blood stem cells, there was no statistical difference between the groups ($p = .064$); univariate analysis also showed no difference in either overall survival (HR = 0.49; 95% CI, 0.21–1.15; log-rank $p = .095$) or time to relapse (HR = 0.61; 95% CI, 0.25–1.51; log-rank $p = .278$).

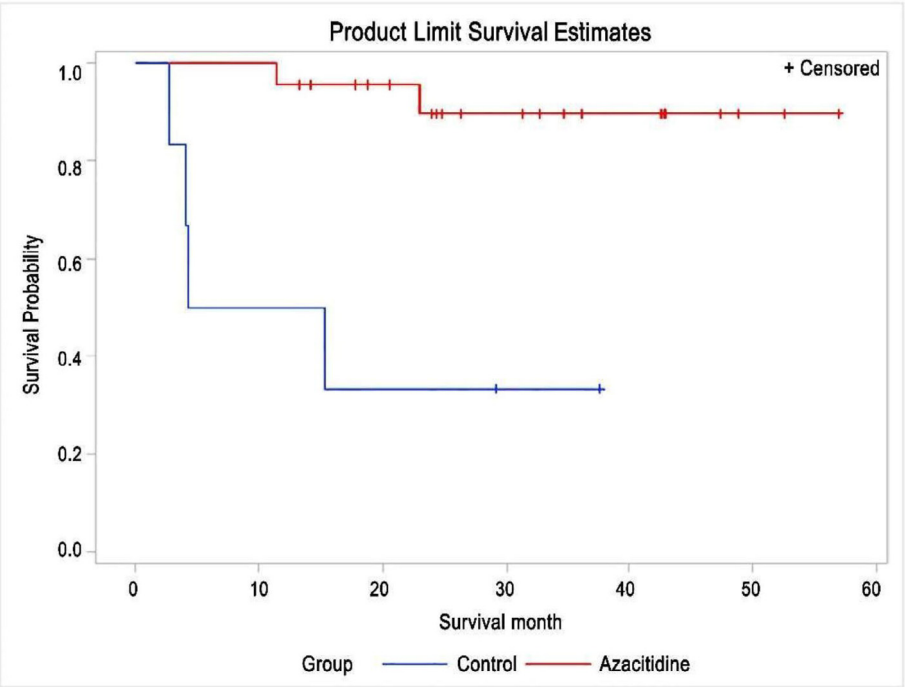

Fig. 3. Overall survival Kaplan–Meier plot among those relapse free in azacitidine arm and control arm shows two events occurred in 23 participants versus median survival 9.8 months, respectively.

3.4. Conditioning regimens

Myeloablative conditioning was used in 65.3% of participants (24/31 in the azacitidine arm and 8/18 in the control arm). The remaining patients (34.7%) received reduced-intensity conditioning (10/18 in the control arm and 10/31 in the azacitidine arm); there was a statistically significant difference in the proportion of patients receiving reduced-intensity conditioning between the study arms ($p = .019$).

On univariate analysis, the conditioning regimen had a significant impact on time to relapse with a hazard of relapse 2.63 (95% CI, 1.08–6.41) times greater in the control group than in the azacitidine group ($p = .027$). Similarly, the hazard of death was 2.6 (95% CI, 1.17–5.85) times greater in the control group than in the treatment group ($p = .015$). The preparative regimen meets the mathematical criteria for a confounding variable.

After adjusting for conditioning regimen on a multivariable Cox proportional hazard model to compute for HR, the hazard of relapse and hazard of death were respectively 5.26 (95% CI, 1.99–13.93) and 5.41 (95% CI, 2.02–14.45) times greater in the control group than in the azacitidine group ($p < .001$ for both overall survival and time to relapse).

3.5. MRD status

Prior to transplantation, 73.5% of all participants were MRD-negative (12/18 in the control arm and 24/31 in the treatment arm); there was no difference between the study groups ($p = .411$). Participants with detectable MRD had a 5.64 times greater hazard of relapse (95% CI, 2.31–13.74; $p < .001$) and 3.52 times greater hazard of death (95% CI, 1.56–7.93; $p < .001$) than MRD-negative patients.

3.6. Graft-versus-host disease

There was no difference in the numbers of patients diagnosed with GVHD in the treatment and control arms; 19 participants in the azacitidine arm and six in the control arm developed GVHD ($p = .059$). Inclusion of GVHD in the cause of death analysis revealed no statistical difference between study groups ($p = .325$)

3.7. Cause of death analysis

At a median follow-up of 24 months (range, 4–57 months), there were eight deaths in the azacitidine arm and 16 in the control arm. The cause of death did not differ between groups, and most deaths were attributed to relapsed disease (62.5% in both arms). The use of azacitidine did not increase the risk for either GVHD or infection rate.

4. Discussion

Disease relapse is a major cause of treatment failure after allogeneic HSCT in patients with poor-risk AML [22]. Detectable MRD is a predictor of relapse disease [9–11]. There are limited data regarding effective therapies to treat recurrence after allogeneic HSCT. Salvage using azacitidine followed by DLI has an overall response rate of 30% [23], although the response is temporary [24]. Participants in our control arm who relapsed failed salvage azacitidine followed by DLI.

There is no standard guideline on modality to reduce relapse disease after allogeneic HSCT in poor-risk AML. Oran et al. [25] examined the same hypothesis using low dose azacitidine maintenance after HSCT, included patients with high risk AML and MDS in a randomized approach, and showed no statistical significance improvement in relapse-free survival or overall survival with the use of azacitidine as maintenance compared with the control group. Those results are not aligning with our findings, probably because including patients with active disease in the phase 3 study confounded their results [25]. Moreover, the investigators reported that the power to detect a statistically significant difference was lost because of fewer-than-expected events observed in the control arm.

We showed that low dose azacitidine maintenance therapy in poor-risk AML decreases relapse rate, increases the time to relapse, and improves overall survival without increasing adverse events, risk of GVHD, or infection risk. Our study is unique compared with other published studies [26–29] in that we included only patients with poor-risk AML and had a homogenous population in the treatment and control cohorts in terms of disease status. Also, we examined the role of MRD both with and without post-transplant azacitidine maintenance.

We demonstrated that low dose azacitidine administered early after allogeneic HSCT to poor-risk AML is well tolerated. Previous retrospective reviews of post-transplant azacitidine maintenance using a similar dose and schedule to ours show that most patients tolerated three or more cycles without affecting the quality of life or increased risk of GVHD [26,28,30].

Although most of our participants received four cycles of azacitidine, it is possible to prolong therapy as there was no increase in GVHD or infection risk;

a longer treatment duration may be beneficial in disease control. In our cohort, three patients received more than four cycles; one received six cycles and the other two continued maintenance therapy for up to 1 year. All three of these patients had relapsed refractory disease before transplant with positive MRD but none had relapsed by the end of our analysis although two had developed GVHD.

The potential benefit of extended disease control with up to 1 year of maintenance using a hypomethylating agent is supported by a recent study using oral azacitidine (CC-486) that had similar positive findings [31].

Previous studies of salvage azacitidine plus DLI in patients with recurrent AML following transplantation reported acute and chronic GVHD incidence of 37% and 17%, respectively. This incidence was lower than expected as DLI can increase GVHD rates with reported rates of 40–60% [32–34]. Approximately 80% of patients receiving DLI had acute GVHD grade II–IV in one of the largest studies [35]. The lower incidence of GVHD in patients receiving DLI and azacitidine support a role for azacitidine in mitigating GVHD secondary to its ability to induce expansion of regulatory T cells [36,37].

Few published studies with azacitidine administered after allogeneic stem cell transplant have shown a decrease or no increase in the incidence of GVHD [26,28,29,38]. A small study of 10 patients receiving salvage azacitidine monotherapy for relapsed AML post-HSCT had no patients with GVHD flare [39]. However, in a relapsed setting, compromised alloreactivity of donor cells may explain the low incidence of GVHD [40–44].

Although these previous studies suggest that azacitidine may alleviate GVHD after transplant, our study failed to show any significant difference between groups, which might be related to a small number of patients.

The preparative regimen plays a significant role in disease control after transplantation; however, the benefit is negated by increased treatment-related mortality [8,45]. Our univariate analysis of the impact of the conditioning regimen on time to relapse and overall survival concluded that it is a confounding factor in our study; although this was corrected by adjusting our results based on the conditioning regimen through multivariable analysis, there was no difference between study arms.

MRD, defined as post-therapy persistence of leukemic cells at levels below morphologic detection, is a strong, independent prognostic marker of increased risk of relapse and shorter survival [10,11,46–48]. In the transplant setting, pre- and post-transplant MRD are important predictors of relapse [49,50]. Our study showed similar importance to MRD.

Currently, assessment of treatment response, including MRD, has been widely used, but there is no universal policy statement addressing how the detection of MRD before transplant should change practice. In our study, we used cytogenetics and molecular MRD to classify our patients. On univariate analysis, MRD positivity before transplant increased the hazard of relapse and had a statistically significant impact on overall survival.

There are several limitations to our study. The small sample size in the exposed cohort limits our conclusion; perhaps a more collaborative effort with additional institutions to increase our sample size would strengthen the validity of our results.

Sampling bias is encountered in our study; self-selection bias is obvious owing to the lack of randomization in our study. We tried to control for a self-selection bias by comparison to matched controls that were transplanted around the same time frame and have the same follow-up period.

Other confounders such as comorbidities and performance status were not assessed. Furthermore, restaging workup for disease status after transplant was not performed on all participants. Some studies have shown an improved outcome of administering azacitidine to those with positive MRD after transplant [49,50]. Both comorbidities [51] and disease status after transplant affect survival.

One of the difficulties of initiating azacitidine post-transplant is pancytopenia or viral reactivation. As the maintenance azacitidine dose was significantly lower than standard treatment dosing, whether to delay initiation of azacitidine post-transplant for pancytopenia or viral reactivation is unknown. This would be an important question to address in a larger randomized study.

Our limited single-institution study did show that post-transplant maintenance azacitidine is associated with statistically significant improvement in median overall survival and time to relapse in poor-risk AML.

In light of the potential side effects of azacitidine, a multi-institutional randomized placebo-controlled study on a larger scale is crucial to apply this protocol to strengthen its validity and aim to improve the standard of care treatment algorithm for poor-risk AML. Such a trial would evaluate the role of maintenance azacitidine after allogeneic HSCT for poor-risk AML in those with undetectable MRD and those with detectable MRD before and/or after transplant in enhancing the success of allogeneic HSCT.

A large trial would also allow assessment of potential adverse effects, effects on GVHD and infection, identify the population with the need for such therapy, or if it should only be initiated as a risk-adapted therapy.

5. Conclusions

Low dose azacitidine maintenance therapy following allogeneic HSCT in poor-risk AML decreased relapse rate and increased both the time to relapse and overall survival. This protocol needs to be applied on a large multi-institutional randomized placebo-controlled study to strengthen its validity and hopefully improve standard-of-care treatment algorithm for poor-risk AML.

Funding

Not applicable

- The data that support the findings of this study are available from the corresponding author upon reasonable request.
- The research protocol has been reviewed and approved by OUHSC institutional review board (IRB).
- No conflict of interest has been declared by the authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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