



ORIGINAL ARTICLE

Low-dose decitabine plus venetoclax is safe and effective as post-transplant maintenance therapy for high-risk acute myeloid leukemia and myelodysplastic syndrome

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Abstract

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are usually associated with poor outcomes, especially in high-risk AML/MDS. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative option for patients suffering from high-risk AML/MDS. However, many patients relapse after allo-HSCT. Novel therapy to prevent relapse is urgently needed. Both the BCL-2 inhibitor venetoclax (VEN) and the hypomethylating agent decitabine (DEC) possess significant antitumor activity effects against AML/MDS. Administration of DEC has been shown to ameliorate graft-versus-host disease (GVHD) and boost the graft-versus-leukemia (GVL) effect post-transplantation. We therefore conducted a prospective study (ChiCTR1900025374) to examine the tolerability and efficacy of a maintenance therapy of low-dose decitabine (LDEC) plus VEN to prevent relapse after allo-HSCT for high-risk AML/MDS patients. Twenty patients with high-risk AML ($n = 17$) or high-risk MDS ($n = 3$) post-transplantation were recruited. Approximately day 100 post-transplantation, all patients received LDEC (15 mg/m^2 for 3 d) followed by VEN (200 mg) on d 1-21. The cycle interval was 2 mo, and there was 10 cycles. The primary end points of this study were rates of overall survival (OS) and event-free survival (EFS). The secondary endpoints included adverse events (AEs), cumulative incidence of relapse (CIR), nonrelapse mortality (NRM), incidences of acute GVHD (aGVHD) and chronic GVHD (cGVHD), and incidences of viral infection after allo-HSCT. Survival outcomes were assessed using Kaplan-Meier analysis. The median follow-up was 598 (149-1072) d. Two patients relapsed, 1 died, and 1 is still alive after the second transplant. The 2-y OS and EFS rates were 85.2% and 84.7%, respectively. The median 2-y EFS time was 525 (149-1072) d, and 17 patients still had EFS and were alive at the time of this writing. The most common AEs were neutropenia, anemia, thrombocytopenia, neutropenic fever, and fatigue. Grade 2 or 3 AEs were observed in 35% (7/20) and 20% (4/20) of the patients, respectively. No grade >3

Yunxiong Wei, Xia Xiong and Xin Li contributed equally to this work.

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AEs were observed. aGVHD (any grade) and cGVHD (limited or extensive) occurred in 55% and 20% of patients, respectively. We conclude that LDEC + VEN can be administered safely after allo-HSCT with no evidence of an increased incidence of GVHD, and this combination decreases the relapse rate in high-risk AML/MDS patients. This novel maintenance therapy may be a promising way to prevent relapse in high-risk AML/MDS patients.

KEYWORDS

allo-HSCT, decitabine, maintenance therapy, Venetoclax

1 | INTRODUCTION

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are 2 common hematological disorders and are usually associated with poor outcomes, especially for high-risk AML/MDS patients. The 5-y survival rates of AML and MDS are 28.7% and 8%, respectively. This dismal clinical outcome may be due to the advanced age of patients at diagnosis.^{1,2} As haploid donor hematopoietic stem cell transplantation and human leukocyte antigen (HLA)-matched hematopoietic stem cell transplantation achieved similar results, allogeneic hematopoietic stem cell transplantation (allo-HSCT) plays an increasingly important role in the management of AML and MDS.³⁻⁵ Allo-HSCT is the only curative option for patients with AML/MDS, while up to 70% of patients receiving allografts for high-risk AML are destined to relapse, and fewer than 10% survive long term.^{4,6} Therefore, relapse is now the primary cause of treatment failure in patients receiving allo-HSCT for AML/MDS.⁷ Moreover, most patients who relapse after allo-HSCT do not achieve long-term survival, and salvage regimens always have limited antileukemia effects. This result has led to an urgent exploration of new methods to prevent relapse.

Venetoclax (VEN) is an orally selective B-cell lymphoma-2 (BCL-2) inhibitor and BH3 mimetic, and its combination with hypomethylating agents (HMAs) has shown a promising antitumor effect for the treatment of patients with AML/MDS, including high-risk patients.⁸⁻¹⁰ Furthermore, several clinical trials have also shown that VEN plus decitabine (DEC) can be a safe and effective salvage treatment for patients with AML/MDS relapsing after allo-HSCT.¹¹⁻¹³ However, data on preventing relapse after allo-HSCT are limited, as its regimen-related toxicity may be an obstacle.

DEC has demonstrated promising activity in a variety of hematological disorders, including AML and MDS.^{14,15} Administration of DEC has been shown to enhance FOXP3 expression and can convert CD4⁺CD25⁻FOXP3⁻ T cells into CD4⁺CD25⁺FOXP3⁺ T regulatory cells (Tregs). A study on animals revealed that it can ameliorate graft-versus-host disease (GVHD) without sacrificing the graft-versus-leukemia (GVL) effect.¹⁶ Another animal study also showed that DEC can ameliorate GVHD and boost the GVL effect post-transplantation.¹⁷ Therefore, using DEC after transplantation may prolong the survival (OS) and disease-free survival (DFS) of patients.

Overall, these studies provide a rationale for the administration of VEN and DEC after allo-HSCT for AML and MDS. We hypothesize that low-dose DEC plus VEN maintenance therapy may both provide direct antileukemic effects to eradicate minimal residual disease and decrease the incidence of GVHD. Moreover, there are no published reports on the use of VEN to prevent relapse after transplantation. Therefore, we designed a prospective study to examine the tolerability and efficacy of maintenance therapy with low-dose DEC (LDEC) plus VEN to prevent relapse after allo-HSCT for high-risk AML/MDS patients.

2 | PATIENTS AND METHODS

2.1 | Patients

We conducted a prospective study from April 2018 to April 2022. Twenty patients who underwent allo-HSCT with high-risk AML/MDS were enrolled in this study. More patients are still being recruited. We reported the clinical outcome of the past 3 y at this time. AML was classified as high risk if patients met the following criteria: (a) age 60 y or older; (b) history of MDS or myeloproliferative neoplasm (MPN); (c) treatment-related or secondary AML; (d) WBC $\geq 100 \times 10^9/L$; (e) combined central nervous system leukemia; (f) gene mutations with poor prognosis or complex karyotypes; or (g) 2 courses of standard induction chemotherapy that did not lead to complete remission. High-risk MDS was defined as World Health Organization Prognostic Scoring System (WPSS) points ≥ 3 or IPSS-R points > 4.5 .

All of the patients met the following criteria: (a) historically diagnosed with high-risk AML or MDS based on the 2016 World Health Organization guidelines (acute promyelocytic leukemia excepted); (b) underwent allo-HSCT within 2 mo; (c) aged ≥ 18 and ≤ 70 y with an ECOG score ≤ 2 , an absolute neutrophil count $\geq 500/\mu L$, and a platelet count $\geq 50\,000/\mu L$; and (4) no uncontrolled active infectious diseases. Patients with active acute GVHD (aGVHD) or chronic GVHD (cGVHD) or a history of grade 4 GVHD were excluded. Patients with impaired renal or hepatic function (defined as total bilirubin greater than or equal to 2.5 times the upper limit of normal [ULN], aspartate aminotransferase or alanine aminotransferase level ≥ 3.0 times

the ULN, and estimated glomerular filtration rate ≤ 40 mL/min) were excluded. Patients receiving clinical trials of other new drugs within 3 mo before the start of protocol treatment were also excluded.

All patients provided written informed consent before enrollment in accordance with the Declaration of Helsinki. The study was approved by the Tianjin First Central Hospital Medical Ethics Committee and registered in the Chinese Clinical Trial Registry (www.chictr.org) (ChiCTR1900025374).

2.2 | Treatment plan

The low-dose DEC plus VEN maintenance therapy regimen comprised 15 mg/m^2 DEC on day 1 to day 3 and 200 mg VEN daily on day 1 to day 21, beginning at approximately day 100 post-HSCT in patients with high-risk AML or MDS. The cycle interval was 2 mo, with 10 cycles. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03. The criteria for LDEC plus VEN discontinuation were the development of drug-related grade 3 or 4 organ toxicity or severe infection. Maintenance therapy was also discontinued if the platelet count dropped to $<10\,000/\mu\text{L}$, with a 50% dose reduction if the platelet count dropped to $<20\,000/\mu\text{L}$, or if the neutrophil count dropped to $<500/\mu\text{L}$. Patient enrollment flow chart and treatment plan are summarized in Figure 1.

2.3 | Clinical outcome assessment

Leukemia recurrence monitoring was performed to monitor the bone marrow every month in the first 6 mo after the transplant and every 2 mo or longer after half a year, depending on the patient's condition. We also used flow cytometry (FCM) and real-time quantitative polymerase chain reaction (RT-QPCR) to monitor minimal

residual disease (MRD). The MRD monitoring interval was the same as the bone marrow biopsy interval. MRD positivity was defined as $>0.01\%$ of cells with leukemia-associated aberrant immune phenotypes in the bone marrow or transcript level $\geq 0.001\%$ for leukemia-related genes, including AML1/ETO, FLT3-ITD, DNMT3A, MLLAF9, MLL/AF4, and so on. Patients were scored as MRD positive if they had 2 consecutive positive results using FCM or PCR or were both FCM and PCR positive in a single sample. Regimen-related hematological toxicity was monitored once a week in the outpatient clinic, including routine blood, liver, and kidney function, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) tests.

2.4 | Study end points, definitions, and statistical analysis

The study aimed to assess the safety and efficacy of LDEC plus VEN maintenance therapy after allo-HSCT for high-risk AML and MDS.

The primary end points of this study were the rates of OS and event-free survival (EFS). The secondary endpoints included adverse events (AEs), cumulative incidence of relapse (CIR), nonrelapse mortality (NRM), incidences of aGVHD and cGVHD, and incidences of viral infection after allo-HSCT. OS was measured from the time of transplantation to death from any cause. EFS was defined as the time from transplantation to recurrence, progression, or death. CIR was defined as the time from transplantation to disease recurrence or progression. NRM was measured from the time of transplantation to death from any cause other than disease relapse or disease progression. OS and EFS were estimated using the Kaplan-Meier method. The results were generated by IBM SPSS Statistics 25.

3 | RESULTS

3.1 | Patient characteristics

Twenty patients met our eligibility criteria and were enrolled in this stage of the prospective feasibility study. The baseline characteristics of the patients are summarized in Table 1. The median age of the patients at the time of transplantation was 35.5 y (range, 21-64 y). Seven patients had complex karyotypes, and the remaining 13 patients had normal karyotypes. The 20 patients included 3 patients with high-risk myelodysplastic syndrome, and the remaining patients were diagnosed with high-risk AML (2 with prior diagnosis of MDS). AML patients were at high risk because of refractory disease at the time of allo-HSCT ($n = 7$), relapsed AML ($n = 5$), and molecular characteristics with poor prognosis ($n = 13$, including 6 primary refractory patients). Furthermore, 3 MDS patients were classified as high risk according to the International Prognostic Scoring System-Revised (IPSS-R) and the WPSS criteria (Table 1).

At the time of allo-HSCT, 7 patients were in first hematologic complete remission (CR), 6 were in second hematologic CR, and 4 patients were in third hematologic CR. Twelve patients with hematologic

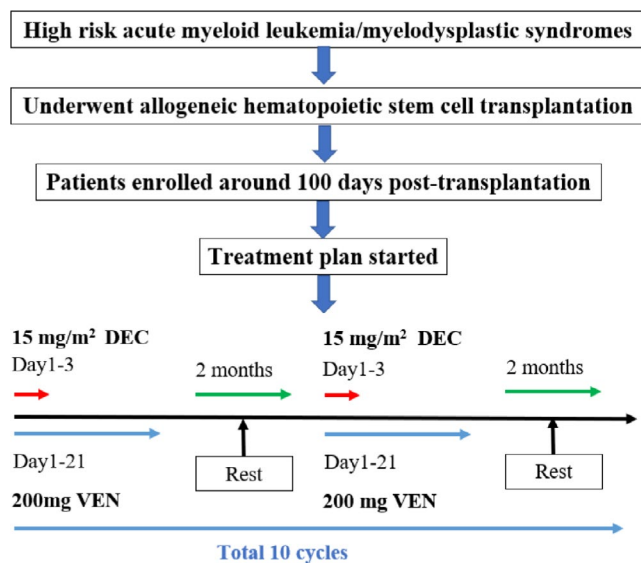


FIGURE 1 Patient enrollment flow chart and treatment plan

TABLE 1 Patient characteristics

Patient characteristics		
Total patients	20	
Median age at transplantation, y (range)	35.5 (21-64)	%
Gender		
Male	8	40
Female	12	60
Disease type		
AML	15	75
MDS	3	15
MDS transformed to AML	2	10
CR1	7	35
CR2	6	30
Others	7	35
MRD detection method		
Flow cytometry	20	100
PCR	15	75
Disease status at transplant		
CR	17	85
MRD-	5	25
MRD+	12	60
PR	3	15
High-risk factor		
Primary refractory/ relapsed	12	60
>60 y old	3	15
Complex cytogenetic aberrations	7	35
Molecular characteristics with poor prognosis ^a	13	65
WBC > 100 × 10 ⁹ at diagnosis	2	10
WPSS points ≥3 or IPSS-R points >4.5	3	15
Conditioning regimens	Bu/Flu/Cy/Ara-C/ATG	
Median CD34 ⁺ cells at transplant, 10 ⁶ (range)	4.79 (3-8.25)	
Donor type		
Haplo HLA-matched donor	17	85
Matched sibling donor	3	15
Median days of neutrophils ≥0.5 × 10 ⁹ /L after transplant	12 (10-16)	
Median days of platelets ≥20 × 10 ⁹ /L after transplant	17 (10-29)	
Median follow-up time (range)	598 (149-1072)	

^aMolecular characteristics with poor prognosis are defined as DNMT3A, TP53, FLT3-ITD, MLL, C-KIT, ASXL1, and WT-1 overexpression.

CR had detectable MRD before allo-HSCT. Three patients presented with partial remission (PR) at the time of allo-HSCT, and the remaining 5 patients were MRD negative. The median interval from CR to allo-HSCT was 40.5 d (range, 15-155 d). All of the patients received peripheral blood stem cells as the stem cell source (3 from a matched

sibling and 17 from a haploidentical donor). A myeloablative conditioning regimen was used in 17 patients, whereas a sequential regimen was used in the 3 remaining patients for their active disease before allo-HSCT. All patients received in vivo T-cell depletion with antithymocyte globulin before undergoing allo-HSCT. The median number of CD34-positive infused stem cells was 4.79 × 10⁶/kg (range from 3-8.25 × 10⁶/kg) for peripheral blood stem cell recipients.

All patients received the LDEC plus VEN maintenance therapy regimen approximately 100 d after allo-HSCT. Ten patients did not have exposure to the HMAs before this study started. No patients received VEN before transplantation. The median number of treatment cycles was 5 (range, 1-10 cycles); 13 patients received at least 5 cycles, and 2 patients received all 10 cycles.

3.2 | Regimen-related toxicity

Tumor lysis syndrome (TLS) is a common side effect in patients with high tumor burden who are treated with VEN. VEN poses a risk for TLS at initiation and during the ramp-up phase. Therefore, it was necessary to assess the TLS risk for patients. During our study, we found no TLS for our patients. Prior to maintenance treatment initiation, all patients achieved sustained, full donor chimerism by day +30 after allo-HSCT. The median time to neutrophil count ≥0.5 × 10⁹/L after transplantation was 12 (range, 10-16), and the median time to platelet count ≥20 × 10⁹/L after transplantation was 17 (range, 10-29) d. Grade ≥2 AEs (listed in Table 2) occurred in 11 (55%) patients during the maintenance treatment of LDEC and VEN. No grade > 3 AEs were observed. Thirty-three AEs were observed, and the most common AEs were neutropenia (*n* = 7), anemia (*n* = 6), thrombocytopenia (*n* = 4), diarrhea (*n* = 4), neutropenic fever (*n* = 1), and fatigue (*n* = 2). All toxicities were tolerable and reversible. Patient 8 reduced treatment dose for 1 cycle due to persistent severe neutropenia. No cardiac, pulmonary, renal, or neurologic toxicities, or treatment-related deaths were observed.

3.3 | Long-term clinical outcomes

Up to the last follow-up, the median follow-up was 598 (149-1072) d, the median 2-y EFS time was 525 (149-1072) d, and 17 patients still

TABLE 2 Grade 2 or greater possibly related AEs

Event	Grade 2	Grade 3	Grade 4	Grade 5
Neutropenia	6	3	0	0
Anemia	5	3	0	0
Thrombocytopenia	4	2	0	0
Neutropenic fever	1	1	0	0
Diarrhea	4	1	0	0
Fatigue	2	1	0	0

Abbreviation: AEs, adverse events.

had EFS and were alive at the time of this writing (Table 3). Patient 7 and patient 8 relapsed on day 274 and 516, respectively. Patient 8 died from relapse, and the other patient continued our treatment plan followed by second allo-HSCT. Patient 7 achieved CR with MRD positive for several months after second allo-HSCT, and her treatment plan is still ongoing. Impressively, patient 7 remained continuously MRD positive after first allo-HSCT, while the remaining 19 patients achieved MRD-negative CR after allo-HSCT. Her treatment plan was still ongoing at the time of her second transplantation, and we continued her maintenance treatment plan approximately 3 mo after the second transplantation. Unfortunately, patient 7 still relapsed after the second transplantation and survived with a low tumor burden. Moreover, patient 9 died of lung infection on day 309 after transplantation. Two patients completed our 10 total cycles, and they are still alive with no AEs. The clinical outcome of maintenance therapy after transplantation for 20 enrolled patients are summarized in Figure 2. Overall, the 2-y OS and EFS after LDEC + VEN were 85.2% and 84.7%, respectively (Figure 3). The 2-y cumulative incidence of relapse (CIR) after LDEC + VEN was 15.3%, and the 2-y nonrelapse mortality was 6.1% (Figure 4).

3.4 | GVHD

We added cyclosporin A, methotrexate, and mycophenolate mofetil for GVHD prophylaxis.¹⁸ As for the treatment of GVHD, we used cyclosporin A combined with oral glucocorticoids in most cases, and we adjusted the dosage of cyclosporin A and glucocorticoids according to the actual situation of the patients. In 3 patients, we replaced cyclosporin A with tacrolimus for the treatment of GVHD.

No existing studies have investigated the application of LDEC + VEN in GVHD, so we observed the incidence of GVHD before and after this maintenance therapy. The 100-day incidence of any grade aGVHD was 55%. The incidence of cGVHD was 20%. Before our treatment plan started, 9 patients suffered from aGVHD (grade I $n = 6$, grade II $n = 2$, grade III $n = 1$). The clinical features of cGVHD in 4 patients were mild and limited according to the National Institutes of Health classification (Table 3). There were no GVHD-related deaths. However, 1 patient suffered grade 3 diarrhea after our therapy, and whether the diarrhea was GVHD or regimen-related was unclear.

Furthermore, we also observed the relationship between regimen-related toxicity and side effects of the therapy for GVHD. We have observed that the most common side effects of the treatment of GVHD include increased blood pressure, increased blood glucose, and obesity. We found that our maintenance therapy did not increase the occurrence of these side effects, and these side effects had no effect on the implementation of our maintenance treatment.

We also compared the GVHD and overall survival (OS) rate of the 2 groups of patients who used HMA and did not use HMA before transplantation. We found that there was no difference in the occurrence of GVHD between these 2 groups. This is different from the

previously reported results.¹⁹ The reason for this result may be that the number of cases in our 2 groups is too small. There was also no significant difference in OS between these 2 groups.

3.5 | Infection complications after transplantation

The numbers of patients experiencing at least 1 bacterial and 1 invasive fungal infection (IFI) were 15% (3/20) and 10% (2/20), respectively. Bacterial infections included *Escherichia coli* ($n = 1$), *Enterococcus faecium* ($n = 2$), and *Pseudomonas aeruginosa* ($n = 2$). All patients responded to antibiotics. Viral infection was also recorded in all patients. The 2-y cumulative incidences of CMV viremia and EBV viremia were 25.0% and 15%, respectively.

4 | DISCUSSION

Leukemia recurrence after allogeneic hematopoietic stem cell transplant (allo-HSCT) is the major cause of treatment failure for patients with AML and MDS. Regardless of standard chemotherapy or transplantation, high-risk AML/MDS patients seem to be more likely to relapse than other patients. The salvage treatment effect is limited in relapsed patients, and very few of these patients can survive for a long period of time. Furthermore, most relapses occur in the first year after allo-HSCT. Therefore, it is urgent to consider preventive maintenance therapy post-transplant. In current stage, the most common treatments aimed at preventing relapse after allo-HSCT include tyrosine kinase inhibitors, HMAs, immune checkpoint inhibitors, donor lymphocyte infusions, and immunotherapy.¹⁹⁻²⁴

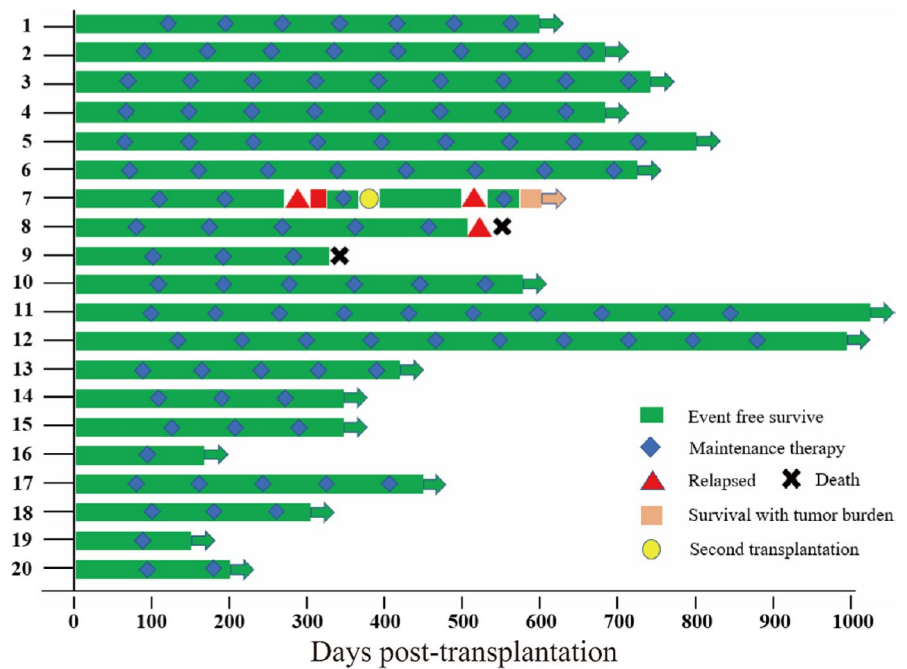
In 2020 ASH, a study revealed that venetoclax is safe and tolerable as post-transplant maintenance therapy for AML patients at high risk for relapse.²⁵ They recruited 23 post-transplantation patients (22 AML [6 with prior diagnosis of MDS] and 1 MDS), median age 65 (range 19-73). The 6-mo OS and relapse-free survival were both 87%. This demonstrated that venetoclax is tolerable in the post-transplantation maintenance setting without unexpected side effects.

In our current study, we examined the efficacy and feasibility of low-dose DEC (LDEC) plus venetoclax (VEN) as a maintenance therapy after allo-HSCT for patients with high-risk AML/MDS. To our knowledge, this is the first report of the use of LDEC + VEN in this setting. Our study demonstrated that LDEC + VEN can be administered safely in the outpatient setting to this group of post-allo-HSCT patients. Approximately 70% ($n = 13$) of our patients received at least 5 cycles, and 2 patients received all 10 cycles. The major side effects of VEN + LDEC include TLS and severe neutropenia.²⁶ Therefore, we chose 2 mo, rather than the 4 wk that are usually used to treat AML and MDS, as the cycle interval to facilitate count recovery. In addition, most patients needed fungal infection prevention strategies after transplantation; voriconazole or fluconazole have been used, so we halved the amount of VEN from the normal 400 mg to 200 mg daily.²⁶ No TLS was observed in our study, which may be due to the

TABLE 3 Regimen-related toxicity and clinical outcomes

Median day of the first cycle post-transplant, d (range)	97.5 (90-110)	
Median cycles of maintenance therapy	5 (1-10)	%
Death within the first cycle	0	0
Exposure to HMA	10	50
Regimen-related adverse events		
≥Grade 2	11	55
aGVHD before maintenance therapy	9	45
aGVHD after maintenance therapy	2	10
cGVHD	4	20
Relapsed	2	
NRM	1	
OS	18	
EFS	17	

not be related to our maintenance therapy. Two patients have relapsed to date, and the 2-y CIR after LDEC + VEN was 15.3%. c-KIT mutations in t (8;21)-positive AML has a poor prognostic.²⁸ Patient 7 had such a mutation, and she was still refractory to relapse. Her disease status remained MRD+ until transplantation. After transplantation, her disease state only remained MRD- for a few months, and c-KIT mutation still existed. After our maintenance treatment, although MRD+ was temporarily converted to MRD-, she unfortunately relapsed at last. We hypothesized that her recurrence may be related to the c-KIT mutation. There is no relevant reports in this field, so more clinical trials are needed to confirm our hypothesis. Another patient who relapsed was complex karyotype with FLT3 and ASXL1 mutations. She had a relapse before transplantation, and then bridged the transplantation after chemotherapy. Her recurrence may indicate that the complex karyotype with FLT3 and ASXL1 mutations may not be effective for LDEC plus VEN maintenance therapy. The reason why our results are different from those

FIGURE 2 Clinical outcome of maintenance therapy after transplantation for 20 enrolled patients

following 2 reasons: (1) patients had no tumor burden 100 d after transplantation; or (2) no observation of TLS was reported with the combined use of VEN and DEC in patients with AML.⁸ Furthermore, as both VEN and DEC were dose reduced, there was no irreversible regimen-related toxicity caused by this maintenance therapy. No serious infection occurred as a result of the combined administration.

What is more, according to our clinical application of VEN in the treatment of AML, the plasma concentration of VEN >800 ng/mL had obvious antitumor effects with well tolerance. In our study, we found that while applying antifungal drugs, 200 mg of VEN could not only maintain the plasma concentration of 600-800 µg/mL, but it was also tolerable to patients after transplantation.

In our study, the 2-y nonrelapse mortality was 6.1%, which may also be 1 of the reasons for haploidentical transplantation²⁷ and may

reported in this study may be that our patients had relapsed before transplantation.²⁹

The 2-y OS and EFS after LDEC + VEN are 85.2% and 84.7%, respectively. It seems that the recurrence has no relationship with the state before the transplant in our study, whether it was CR, PR, or MRD negative or positive. This result was different from a previous report³⁰ that indicated that the rates of OS and DFS in MRD-negative patients were higher than those in MRD-positive patients. This interesting result may be related to our maintenance treatment plan. Furthermore, in our study, 12 of 20 patients were MRD positive before transplantation, and 3 of 20 achieved PR before transplantation. Only 1 of the 20 reduced the dose per cycle due to side effects, which may have caused her relapse. In addition, this maintenance therapy seemed to not have much impact on GVHD. In our

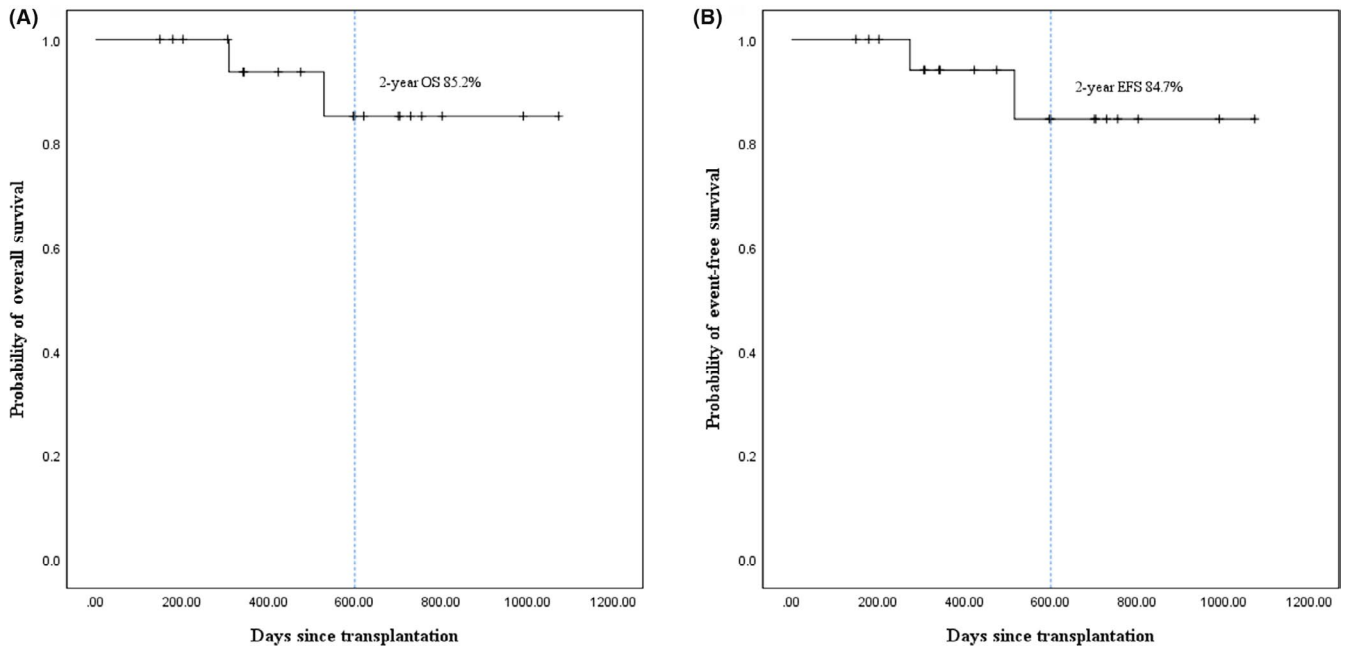


FIGURE 3 2-Year overall survival and event-free survival

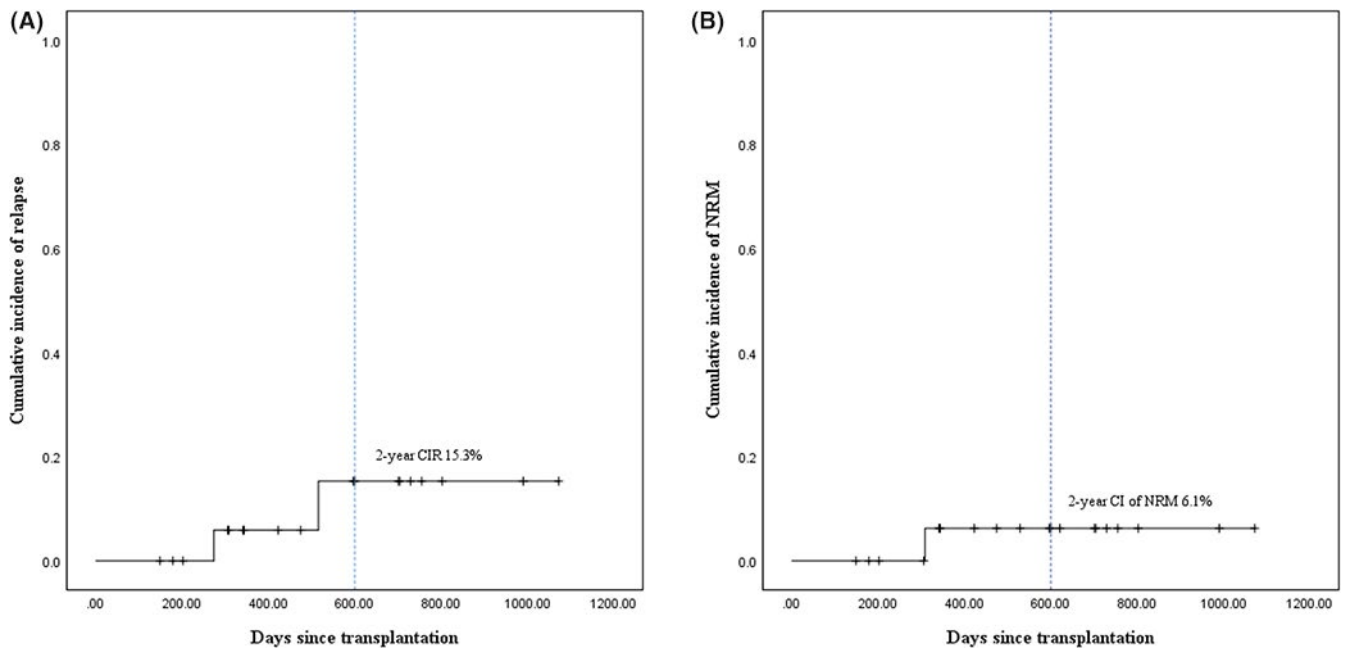


FIGURE 4 2-Year cumulative incidence of relapse and nonrelapse mortality

setting, 12 patients started this treatment plan within 100 d, and we did not observe aggravated acute GVHD (aGVHD). Our maintenance therapy also showed no influence on the incidence of chronic GVHD (cGVHD). Moreover, the GVL effect plays a significant role in preventing relapse after allo-HSCT. One study showed that azacitidine (AZA) and DEC may augment the GVL response through up-regulation and re-expression of epigenetically silenced genes related to major histocompatibility complex class I, HLA DR-1, and tumor-associated antigens.¹⁷ Another study also showed that venetoclax

did not impair human T-cell function in response to antigen stimuli and it also could increase the proportion of effector memory cells in the blood of human subjects.³¹ In view of the fact that both DEC and VEN have a regulatory effect on immune cells, but there has been no report on the study of immune cells after the 2 are combined. Therefore, in our study design, we hypothesized that the addition of DEC and VEN may influence the GVL effect. In summary, clarification of the influence on GVHD and the GVL effect requires more patients and a longer follow-up in the future.

Several studies have shown that leukemic stem cells (LSCs) rely on amino acid metabolism for oxidative phosphorylation and survival. VEN combined with AZA was able to induce LSC toxicity in vitro by decreasing amino acid uptake, as confirmed by decreased α -ketoglutarate and increased succinate levels, suggesting the inhibition of electron transport chain complex II. These metabolic perturbations suppress oxidative phosphorylation, which in turn efficiently and selectively targets LSCs.^{32,33} As both AZA and DEC are HMAs, we speculated that the scheme we designed can kill silent LSCs or eradicate MRD. One of the patients who relapsed had a second transplant after recurrence while continuing to receive our chemotherapy regimen. At the time of writing this article, patient 7 relapsed after the second transplantation and survived with a low tumor burden, which may be due to the antileukemia effect of our maintenance therapy on LSCs. DiNardo et al reported on 39 patients with relapsed/refractory AML who received VEN in combination with low-intensity therapies, and most patients (77%) had previous exposure to HMAs. The overall response in this cohort was dismal, with 12% of the patients attaining CR/CRi. This may be because the effect of combining HMAs with VEN after exposure to HMAs is worse than that without any previous HMA treatment. Therefore, another possible reason she achieved low tumor burden alive status after her second allo-HSCT may be that she was not exposed to an HMA before receiving LDEC + VEN, which facilitated the favorable antitumor effect of our maintenance therapy after relapse. The low recurrence rate was similar to that with the previous strategy of DEC alone to prevent relapse,³⁴ but the combination of LDEC plus VEN in our study not only prevented leukemia relapse but also exerted an antitumor effect as a salvage therapy when the patient relapsed.

Cytopenia was another severe complication when VEN was combined with HMA, which could make patients more susceptible to infection. Daniel et al previously reported a data set of 33 patients treated with AZA and VEN; 19 ultimately discontinued AZA for cytopenia (4 stopped all therapy). In addition, there is no clear evidence on the correlation between discontinuation and response duration.³² Moreover, infection was a primary or contributing cause of death in more than half of the patients who died in the follow-up period after allo-HSCT. One study revealed that 62% and 6% of patients experienced at least 1 bacterial infection and 1 IFI, respectively,³⁵ while in our trial, the rates were 15% and 10%. Furthermore, the rates of CMV viremia and EBV viremia in our trial did not appear to be increased compared with those in other studies.³⁶ Our results showed that, despite the long-term duration of pancytopenia, the novel maintenance treatment LDEC combined with VEN did not increase the risk of infection.

In addition, the impact of DEC and VEN on immune cells has been explored previously.^{16,31} We attributed our inspiring clinical outcome to 2 reasons: DEC increased the number of regulatory T cells; VEN increased the number of intratumoral effector T cells. What is the effect of the combination of these 2 drugs on immune cells? This is the question we will study in the next step.

In conclusion, the results of the current study suggested that maintenance treatment with LDEC combined with VEN introduced nearly 3 mo after allo-HSCT is efficacious, with an acceptable toxicity

profile and impressive long-term disease control. In addition, this treatment did not have much impact on GVHD, and its impact on GVL requires further clarification with more indicators. Whether combining DEC with VEN would be an effective, or even preferential, strategy for preventing relapse in AML/MDS after allo-HSCT deserves further investigation in larger cohorts with longer term follow-up.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethics Committee of New Technology/New Treatment Project of Tianjin First Center Hospital with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of New Technology/New Treatment Project of Tianjin First Center Hospital.

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DISCLOSURE

The authors declare no conflicts of interest related to this work.

AUTHOR CONTRIBUTIONS

MFZ designed the research. YXW, XX, XL, WYL, XYH, XJ, RS, HRL, XX, JXM, TY, and TTS performed the research. YXW, XX, XL, WYL, XYH, XJ, and RS analyzed the data. YXW, XL, and XX wrote the manuscript. YXW, XL, XX, and MFZ revised the manuscript. All authors approved the final version of the manuscript.

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