Maintenance Treatment With Low-Dose Decitabine After Allogeneic Hematopoietic Cell Transplantation in Patients With Adult Acute Lymphoblastic Leukemia

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Background: Post-transplant relapse remains a principal leading cause of failure after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with adult acute lymphoblastic leukemia (ALL). The aim of this study was to investigate the efficacy and safety of low-dose decitabine on the prevention of adult ALL relapse after allo-HSCT.

Methods: In this prospective study, we enrolled 34 patients with ALL who underwent allo-HSCT from August 2016 to April 2020 and received low-dose decitabine maintenance treatment after transplantation. The primary objectives were cumulative incidence of relapse rate (CIR), overall survival (OS), and disease-free survival (DFS). The secondary objectives were graft-versus-host disease (GVHD) and safety.

Results: Among the enrolled 34 patients, 6 patients relapsed and 6 patients died. The 2-year CIR, OS, and DFS were 20.2, 77.5, and 73.6%, respectively. Subgroup analysis revealed the 2-year CIR, OS, and DFS rates of 12 patients with T-ALL/lymphoblastic lymphoma (LBL) were 8.3, 90, and 81.5%, respectively. None of the seven patients with T-ALL relapsed. During maintenance treatment, only one patient (2.9%) developed grade IV acute GVHD and four (11.8%) patients had severe chronic GVHD. Thirty-two patients (94.1%) developed only grade I to II myelosuppression, and two patients (5.8%) developed grade III to IV granulocytopenia.

Conclusions: Maintenance treatment with low-dose decitabine after allo-HSCT may be used as a therapeutic option to reduce relapse in patients with adult ALL, especially in patients with T-ALL. Our findings require confirmation in larger-scale controlled trials.

Clinical Trial Registration: Chinese Clinical Trials Registry, identifier ChiCTR1800014888.

Keywords: allogeneic hematopoietic stem cell transplantation, decitabine, maintenance, prophylaxis, relapse, acute lymphoblastic leukemia
INTRODUCTION

Post-transplant relapse remains a leading cause of failure after allogeneic hematopoietic stem cell transplantation (allo-HSCT). In patients with adult acute lymphoblastic leukemia (ALL), the risk of relapse-related death is higher, up to 30–54% (1–3). At present, donor lymphocyte infusion (DLI) is the most widely used management approach to relapse after transplantation. However, the downregulation of human leukocyte antigen II (HLA-II) molecules leads to the inability of donor T cells to recognize leukemic cells, which limits the use of DLI in the treatment of relapse after transplantation in patients with acute myeloid leukemia (AML) (4, 5), and the 3-year overall survival (OS) rate of these patients is only 10–20% (6). In particular, DLI is not ideal for treatment of ALL relapse after transplantation. Given the difficulty in the treatment of post-transplant relapse, preventing relapse is more important than treatment. Therefore, it is urgent to explore novel approaches to prevent leukemia relapse after allo-HSCT in adult ALL.

Different from the relapse occurring after traditional chemotherapy, the elimination of leukemic cells after allo-HSCT mainly depends on the graft-versus-leukemia (GVL) effect (7). A mechanism of post-transplantation relapse involves the downregulation of HLA-class II molecules induced by epigenetic silencing to reduce the GVL effect, and the downregulation of HLA-II expression is caused by hypermethylation of the promoter of the class II major histocompatibility complex (MHC) transactivator (CIITA) (4, 5, 8). Most patients with T-ALL showed molecular loss of HLA-II (9), and only 5–17% of T-ALL expressed HLA-DR. A similar mechanism of loss of expression of HLA-II class molecules was also observed in B-cell lymphoma lines (10). In addition, many studies have shown that the degree of methylation of tumor suppressor genes is closely associated with the subtypes and prognosis of ALL (11–13). The above studies indicate the possibility of using hypomethylating agents (HMAs) as treatment in ALL after transplantation.

Both decitabine and azacitidine are HMAs and have been safely and effectively used for AML treatment and myelodysplastic syndrome (MDS) after transplantation (14–20). The main effect of post-transplantation hypomethylation treatment is to prevent primary disease relapse and reduce graft-versus-host disease (GVHD). The main mechanisms include increasing the number of regulatory T (Treg) cells and inducing cytotoxic CD8+ T cells (21, 22). Thus, considering the low hematological toxicity of maintenance treatment with low-dose decitabine after AML/MDS transplantation and the advantages of preventing relapse without affecting GVHD, we administered low-dose decitabine maintenance treatment to 34 patients with ALL after allo-HSCT. This was the first prospective study with the largest number of cases to date to describe the application of decitabine prophylaxis for relapse of transplanted ALL.

MATERIALS AND METHODS

Study Design

This was a single-center, prospective, single-arm study. Informed consent was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. This study is registered at www.chictr.org.cn (ChiCTR1800014888).

Patient Cohort

Eligible candidates met all of the following inclusion criteria: (1) age ≥14 years; (2) satisfied the diagnostic criteria of ALL or lymphoblastic lymphoma (LBL); (3) patients underwent allo-HSCT in the First Affiliated Hospital of Zhengzhou University; (4) Eastern Cooperative Oncology Group (ECOG) performance status score ≤2; (5) morphological complete remission (CR) before maintenance treatment; (6) estimated survival ≥3 months.

The exclusion criteria included (1) concomitant diagnosis of another cancer; (2) concomitant uncontrolled fungal, bacterial, or viral infection; (3) hypersensitivity to decitabine; (4) diagnosis of human immunodeficiency virus infection or in active stage of hepatitis B or C virus infection; (5) brain dysfunction or severe mental illness; (6) concomitant disease(s) that may seriously endanger the safety of patients or affect the completion of this study; and (7) participation in another drug clinical trial(s) 1 month before the trial.

Maintenance Treatment Regimen

Maintenance treatment began more than 50 days after transplantation. This post-HSCT interval allows for adequate marrow recovery before starting decitabine. Decitabine 10 mg/d was planned for intravenous infusion 5 h on days 1 to 5, and every 4 weeks for eight cycles, based on comprehensive analysis of previously relevant studies (15, 23, 24). However, in the previous pretrial of low-dose decitabine maintenance treatment after AML/MDS transplantation at our center, patients who received decitabine for 5 days at 10 mg/d developed grade IV myelosuppression with granulocytic fever, requiring transfusion of approximately two units of platelets. Myelosuppression was alleviated, and blood products were not needed after adjusting to 10 mg/d for 3 days. Therefore, decitabine 10 mg/d (approximately 6 mg/m²/d) was ultimately administered as an intravenous infusion for 5 h on days 1, 3, and 5 every 4 weeks for eight cycles in this study. It should be noted that the number of cycles increased by four to six cycles based on the patients’ wishes, if they presented minimal residual disease (MRD) in the late period of maintenance treatment. The interval time of each cycle was also appropriately prolonged according to the recovery of the patient’s hemogram.

Routine blood parameters, bone marrow (BM) smear, and MRD were examined before each cycle. MRD detection methods included flow cytometry (FCM), quantitative detection of certain genes or WT1 via polymerase chain reaction (PCR), and donor chimerism. In addition, patients with T-LBL also underwent regular positron emission tomography-computed tomography (PET/CT) examinations. Routine blood parameters were examined intermittently during the period of drug administration. Granulocyte colony stimulating factor (G-CSF) or blood products were administered as required according to
the hemogram. Systemic anticancer drugs and other similar experimental treatments were banned during the trial. Withdrawal criteria included (1) patients who were unable to tolerate the treatment, (2) patients with relapse of primary disease, (3) patients developing severe GVHD or unacceptable infection, and (4) subjects who decided to withdraw from the trial.

Evaluation Parameters
Patients with ALL were divided into high-risk and standard-risk groups. High-risk ALL was defined based on at least one of the following criteria: (1) age ≥35 years; (2) white blood cell (WBC) counts >30×10⁹/L for B-cell precursor (BCP)-ALL or >100×10⁹/L for thymic T-ALL; (3) pro-B-ALL (CD10−), early T-ALL or mature T-ALL, hypodiploid ALL; (4) ALL with Philadelphia chromosome (Ph), with the t(4,11) translocation, or with complex karyotype; and (5) failure to achieve CR after the first induction therapy (25). The risk classification of LBL was based on the international prognostic index (IPI) score. CR from ALL was defined as BM blasts <5%, no primitive naive lymphocytes in the peripheral blood, and no extramedullary lesions. CR of LBL was based on the international prognostic classification, n (%)

RESULTS
Patient Characteristics
In total, 34 patients from our institution were enrolled between August 2016 and April 2020. The characteristics of patients are shown in Table 1. Our cohort comprised 34 patients with a median age of 20 years (range, 14–49 years), including 22 males and 12 females. Overall, 22 patients (64.7%) had B-ALL, 7 (20.6%) had T-ALL, and 5 (14.7%) had T-LBL. Nine patients

### Table 1 Patients’ characteristics (N = 34).

| Characteristic | Value |
|----------------|-------|
| Age at HSCT, year, range (median) | 15–49 (20) |
| Sex, n (%) | |
| Male | 22 (64.7) |
| Female | 12 (35.3) |
| Diagnosis, n (%) | |
| B-ALL | 22 (64.7) |
| T-ALL/T-LBL | 7/5 (35.3) |
| Risk classification, n (%) | |
| High risk | 25 (73.5) |
| Standard risk | 9 (26.5) |
| Subtype, n (%) | |
| Some-positive (Ph+) ALL | 7 (20.6) |
| Ph− ALL | 27 (79.4) |
| MRD after the 1st induction, n (%) | |
| Negative | 18 (64.3) |
| Positive | 10 (35.7) |
| MRD at allo-HSCT, n (%) | |
| Negative | 27 (79.4) |
| Positive | 7 (20.6) |
| Disease status at allo-HSCT, n (%) | |
| CR1 | 31 (91.2) |
| CR2 | 3 (8.8) |
| HCT-CI score, n (%) | |
| 0 | 21 (61.8) |
| 1 | 12 (35.3) |
| 2 | 1 (2.9) |
| EBMT risk score, n (%) | |
| 0 | 4 (11.8) |
| 1–2 | 25 (73.5) |
| 3–4 | 5 (2.9) |
| Conditioning regimen, n (%) | |
| mBu/Cy | 26 (76.5) |
| TBI/Cy | 8 (23.5) |
| Transplant resource, n (%) | |
| PBSC | 31 (91.2) |
| PBSC+BMT | 3 (8.8) |
| Donor/HLA match, n (%) | |
| Matched related | 21 (61.8) |
| Mismatched related | 11 (32.4) |
| Matched unrelated | 1 (2.9) |
| Mismatched unrelated | 1 (2.9) |
| CD34+ cells×10⁶/kg, range (median) | |
| 1.52–16.3 (5.7) |
| MNC cells×10⁶/kg, range (median) | |
| 1.2–11.5 (5.3) |
| Time of leukocyte engraftment, d (median) | |
| 8–21 (13) |
| Time of platelet engraftment, d (median) | |
| 10–22 (14) |

ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; Ph, Philadelphia chromosome; MRD, minimal residual disease; HCT-CI, hematopoietic cell transplantation comorbidity index; EBMT, European Society for Blood and Marrow Transplantation; Bu, busulfan; Cy, cyclophosphamide; TBI, total body irradiation; mBu/Cy, modified Bu/Cy; PBSC, peripheral blood stem cell; BM, bone marrow; MNC, mononuclear cell.
Decitabine Exposure and MRD

Outcomes of maintenance therapy with decitabine and the changes in MRD during this stage are shown in Table 2 and Figure 1. All four patients with MRD-positive disease before maintenance treatment turned negative after two or two cycles. Only three patients (8.8%) had positive MRD once during maintenance therapy. The median time from transplantation to the start of maintenance treatment was 96 days (range, 51–175 days), and the median number of decitabine cycles for all patients was seven (range, 1–14). Overall, 14 patients (41.1%) completed the study and entered the follow-up phase, including 12 patients with 8 cycles, 1 patient with 14 cycles, and 1 patient with 13 cycles of treatment. Patients No. 5 and No. 6 received more than eight cycles because they were MRD positive after the completion of eight cycles of maintenance treatment, and their MRD turned negative after an additional cycle of decitabine (Figure 1). At the data cut-off point, eight patients (23.5%) were in the maintenance phase, including six patients who entered the study later and two patients due to the delay caused by the coronavirus disease 2019 (COVID-19) epidemic. The reasons for discontinuation included relapse (n = 4, 11.7%), GVHD (n = 3, 8.8%), and withdraw consent (n = 5, 14.7%) (Table 2). Besides, as shown in Table 3, seven patients with Ph+ ALL were treated with TKI maintenance during pre-transplantation chemotherapy, conditioning regimen, and post-transplantation maintenance therapy. Notably, TKI was suspended temporarily to reduce the risk of infection in patients with neutropenia after chemotherapy or transplantation.

### Table 2 | Outcomes of transplantation and maintenance treatment (N = 34).

| Outcomes                                      | Data     |
|-----------------------------------------------|----------|
| MRD before maintenance treatment, n (%)      | Positive: 4 (11.7) Negative: 30 (88.3) |
| Start time of decitabine, d, median (range)   | 86 (51–175) |
| No. of cycles, median (range)                 | 7 (1–14) |
| Completed study, n (%)                        | 14 (41.1) |
| Maintenance period, n (%)                     | 8 (23.5) |
| Reason for discontinuation, n (%)             | Withdrew consent: 5 (14.7) Relapse: 4 (11.7) GVHD: 3 (8.8) |
| Hematological toxicity, n (%)                 | I–II: 32 (94.1) III–IV: 2 (5.8) |
| Relapse, n (%)                                | 1 (2.9) |
| Severe, n (%)                                 | 3 (8.8) |
| I–II                                           | 0 |
| III–IV                                        | 1 (2.9) |
| Chronic GVHD after maintenance treatment, n (%) | 7 (20.5) |
| Relapse, n (%)                                | 1,629 |
| GVHD, graft-versus-host disease.              | 154–1,629 |

At the data cut-off point (July 2020), the median follow-up time was 480.5 days (range, 154–1,629 days) (Table 2). A total of six patients relapsed (17.6%) with a median relapse time of 213 days (range, 156–551 days) after transplantation. Of these, five patients were at high risk (three patients with Ph+ B-ALL, two of which had T315I mutation at the time of relapse; one patient with pro-B-ALL; one patient with T-LBL in the leukemic phase had a WBC count >100×10^9/L at diagnosis), and one patient with B-ALL was at standard risk (Table 4). Among the 14 patients who completed the study, only patient No. 2 presented extramedullary recurrence at 551 days after transplantation. Patient No. 16 with Ph+ ALL only took imatinib but stopped decitabine on his own after five cycles. Relapse happened and T315I mutation was detected 2 months later, whereas this patient did not take ponatinib due to economic reasons. Patient No. 17 with Ph+ ALL relapsed for a second time, whereas this patient did not take ponatinib due to economic reasons. Patient No. 17 with Ph+ ALL relapsed for a second time, and the T315I mutation was detected after two cycles. Patient No. 18 with Ph+ ALL relapsed after five cycles and refused to test for mutations in the ABL kinase domain. Patients No. 3 with CR2 at 315I mutation was detected after two cycles. Patient No. 18 with Ph+ ALL relapsed after five cycles and refused to test for mutations in the ABL kinase domain. Patients No. 3 with CR2 at standard-risk, and 25 (73.5%) were at high-risk (including five patients with T-LBL). Seven patients (20.6%) were Philadelphia chromosome-positive (Ph+), and 27 patients (79.4%) were Philadelphia chromosome-negative (Ph−). Excluding six patients due to missing MRD data from other hospitals at initial treatment, 10 (35.7%) of 28 assessable patients were MRD positive (including two patients with non-CR after induction) and 18 patients (64.3%) who became MRD-negative after the first induction chemotherapy. Seven patients (20.6%) became MRD-positive, and 27 patients (79.4%) achieved MRD negativity at transplantation. All patients received myeloablative conditioning, including 26 patients (76.5%) receiving a modified busulfan (Bu)/cyclophosphamide (Cy) regimen and 8 (23.5%) patients receiving a total body irradiation (TBI)/Cy regimen (26). Prophylaxis against GVHD for all patients consisted of cyclosporine A and short-term methotrexate treatment with mycophenolate mofetil. In addition, patients without matched related donors were supplemented with anti-thymocyte globulin. The median number of infused CD34+ cells was 5.7 × 10^6/kg (range, 1.52–16.3 × 10^6/kg), and the median number of infused MNC cells was 5.3 × 10^9/kg (range, 1.2–11.5 × 10^9/kg). Neutrophils and platelets were implanted successfully in all patients. All patients achieved morphological CR and donor complete chimerism before maintenance treatment. Thirty patients (88.3%) achieved MRD negativity, and four patients (11.7%) were MRD positive before maintenance treatment (Table 2).
including one patient with early T-cell precursor ALL (ETP-ALL) and three patients at high risk. In the end, the 2-year CIR of all 34 patients was 20.0%, and the median CIR time was not reached (Figure 2A). Patients with T-ALL/LBL and B-ALL had a 2-year CIR of 8.3 and 25.8%, respectively (P = 0.34). Patients at high risk and standard risk had a 2-year CIR of 22.4 and 12.5%, respectively (P = 0.63). Patients with Ph+ ALL and Ph− ALL had a 2-year CIR of 42.8 and 14.5%, respectively (P = 0.08) (Figure 3A).

DFS and OS
At the data cut-off point, 28 (82.3%) of the 34 patients were alive (82.3%), and 26 patients (76.5%) were alive without relapse/progression. Causes of death included relapse (n = 4), severe infection (n = 1), and GVHD (n = 1) (Table 2). The 2-year NRM was 6.3% (Figure 2A). The 2-year OS was 77.5%, and the 2-year DFS rate was 73.6% for the 34 patients (Figure 2B). The 2-year OS of patients with T-ALL/LBL and B-ALL were 90 and 72.5%, respectively (P = 0.37). The 2-year OS of patients at high risk and standard risk were 73.6 and 87.5%, respectively (P = 0.57). The 2-year OS of patients with Ph+ ALL and Ph− ALL were 68.6 and 79.1%, respectively (P = 0.53) (Figure 3B). For patients with T-ALL/LBL and B-ALL, the 2-year DFS was 81.5 and 69.6%, respectively (P = 0.52). For patients at high risk and standard risk, the 2-year DFS were 68.8 and 87.5%, respectively (P = 0.36). For patients with Ph+ ALL and Ph− ALL, the 2-year DFS were 57.1 and 77.3%, respectively (P = 0.23) (Figure 3C).

GVHD
One patient (2.9%) developed grade IV aGVHD, and seven (20.5%) patients developed cGVHD (three with mild cGVHD and four severe cGVHD) during maintenance treatment phase (Table 2). Among the eight patients with GVHD after

![FIGURE 1](https://www.frontiersin.org) | Changes in MDR and decitabine exposure in patients.
### TABLE 3 | Use of TKI in 7 Patients with Ph⁺ ALL.

| Patient No. | TKI Before HSCT | TKI in Conditioning | TKI after HSCT | Time of TKI Withdrawal (days) | Relapse |
|-------------|-----------------|---------------------|----------------|-----------------------------|---------|
| 16          | Dasatinib (100 mg/d) + chemotherapy | Dasatinib (100 mg/d) | Imatinib (400 mg/d)* | 276 | Yes |
| 17          | Imatinib (400 mg/d) + chemotherapy | Imatinib (400 mg/d) | Imatinib (400 mg/d) | 170 | Yes |
| 18          | Dasatinib (100 mg/d) + chemotherapy | Dasatinib (100 mg/d) | Dasatinib (100 mg/d) | 223 | Yes |
| 19          | Imatinib (400 mg/d) + chemotherapy | Imatinib (400 mg/d) | Imatinib (400 mg/d) | 365 | No |
| 20          | Imatinib (400 mg/d) + chemotherapy | Imatinib (400 mg/d) | Imatinib (400 mg/d) | 379 | No |
| 21          | Dasatinib (100 mg/d) + chemotherapy | Dasatinib (100 mg/d) | Dasatinib (100 mg/d) | 156 | No |
| 22          | Imatinib (300 mg/d) + chemotherapy | Imatinib (300 mg/d) | Imatinib (400 mg/d)* | 365 | No |

TKI, tyrosine kinase inhibitor; ALL, acute lymphoblastic leukemia; HSCT, hematopoietic stem cell transplantation.

*As the patient suffered from diabetes mellitus complicated with fundus disease, dasatinib was replaced with imatinib after transplantation. *Dasatinib was replaced with imatinib at 157 days after transplantation because of repeated pleural effusion after taking dasatinib. §The patient was intolerant to imatinib (400 mg/d), accompanied by severe nausea and vomiting, so imatinib was reduced to 300 mg/d.

### TABLE 4 | Characteristics and outcomes of six patients with relapse.

| Patient No. | Diagnosis | High-Risk Factor at Diagnosis | Disease Status at HSCT | Starting Time of Decitabine (days) | Cycles of Decitabine | Reason for Discontinuation of Decitabine | Bone Marrow Results at Relapse | Time From HSCT to Relapse (days) | Treatment After Relapse | Overall Survival (days) |
|-------------|-----------|-------------------------------|------------------------|------------------------------------|---------------------|------------------------------------------|-------------------------------|-----------------------------|--------------------------|-------------------------|
| 16          | B-ALL     | Ph⁺ WBC >100×10⁹/L            | CR1                    | 63                                 | 5                   | Withdrew consent                         | Marrow blast 97.6% T315I mutation | 276                         | DLI + chemotherapy; TBI | 433                     |
| 2           | B-ALL     | CD10⁻                         | CR1                    | 97                                 | 8                   | Completed study                           | Extramedullary                | 551                         | Chemotherapy; TBI       | 725                     |
| 17          | B-ALL     | Ph⁺ Age >35                   | CR2                    | 110                                | 2                   | Relapse                                   | Marrow blast 42% T315I mutation | 168                         | DLI + chemotherapy      | 213                     |
| 3           | B-ALL     | No                            | CR2                    | 84                                 | 3                   | Relapse                                   | Marrow blast 49.6% T315I mutation | 205                         | Automatic discharge     | 244                     |
| 30          | T-LBL     | Leukemic phase; WBC >100×10⁹/L| CR1                    | 93                                 | 3                   | Relapse                                   | Extramedullary                | 156                         | Automatic discharge     | >205                    |
| 18          | B-ALL     | Ph⁺ WBC >100×10⁹/L            | CR1                    | 60                                 | 5                   | Relapse                                   | Marrow blast 16.4%            | 221                         | Chemotherapy            | >427                    |

WBC, white blood cell; CR, complete remission; DLI, donor lymphocyte infusion; HSCT, hematopoietic stem cell transplantation.
maintenance treatment, three patients had reduced the dose of immunosuppressive drugs before developing GVHD (one withdrew immunosuppressants and developed grade IV aGVHD during the second cycle; then, the patient stopped using decitabine and received intensive immunosuppressive treatment, but the response was poor and the patient eventually died). Two patients presented cGVHD before maintenance treatment. Of the remaining three patients, two developed cGVHD after one cycle and one developed cGVHD after three cycles of maintenance treatment. Among the eight patients with GVHD, organ involvement included the skin in eight patients, the intestinal tract in two patients, the liver in three patients, the oral cavity in three patients, and the eye in one patient. No significant worsening or relief was observed in patients with GVHD due to the use of decitabine.

Adverse Events
The main adverse event caused by low-dose decitabine was hematological toxicity. Among the 34 patients, 32 (94.1%) developed grade I to II myelosuppression after maintenance treatment with low-dose decitabine (Table 4), and no infection occurred after timely administration of G-CSF. Only two patients (5.8%) developed grade III to IV myelosuppression. Patient No. 9 developed degree IV granulocytopenia and mild pulmonary fungal infection after one cycle, which improved after administration of G-CSF and oral voriconazole. Patient No. 22 developed grade III granulocytopenia and mild pulmonary bacterial infection after one cycle, which improved after administration of G-CSF and oral azithromycin. Their granulocytes returned to normal after 14 days and 12 days of treatment, respectively. None of the patients required blood transfusion during the period of myelosuppression, and none of the patients interrupted treatment because of infection.

DISCUSSION
Disease relapse is a major therapeutic challenge in patients with adult ALL that have undergone allo-HSCT, and treatment
options are limited. The risk of relapse-related death in this population was as high as about 30–54% (1–3, 28), leaving preventing post-transplantation relapse necessary. At present, the downregulation of HLA-II molecules on leukemic cells caused by epigenetic silencing (such as the hypermethylation of CIITA) leads to immune escape of leukemic cells, which is a main mechanism of relapse post-transplantation (4, 8). Moreover, abnormalities in DNA methylation are common in ALL (11–13, 29, 30). Therefore, combined with the clear benefits of HAMs in maintenance therapy after AML/MDS transplantation (14, 15), we first evaluated the use of low-dose decitabine as maintenance therapy after allo-HSCT for adult ALL to reduce relapse and improve survival of this population. In this study, we achieved a 2-year CIR (20%) that was lower than that reported in previous studies, and the 2-year OS (77.5%) was satisfactory. Even in the high-risk group, the 2-year OS was 73.6%. To some extent, our data also indicated that maintenance therapy with decitabine may be used as a treatment option to prevent relapse after ALL transplantation.

The prognosis of adult T-ALL is unsatisfactory, with a 5-year OS of only 30–50% (31–33). Furthermore, the prognosis of patients who relapse is poorer, with a reported 5-year OS of 5% (34). Although allo-HSCT has improved the prognosis of this population, there is still a CIR of 12.4% in the low-risk group and 41.2% in the high-risk group (35). However, the 2-year CIR of patients with T-ALL/LBL in our study was only 8.3%, while OS and DFS were as high as 90 and 81.5%, respectively. Surprisingly, none of the patients with T-ALL experienced relapse, which is encouraging. Katagiri et al. (36) also reported that successful maintenance treatment was achieved with azacitidine in a patient diagnosed with myeloid/lymphoid neoplasm with FGFR1 (located on chromosome 8p11.2) rearrangement after allo-HSCT. In addition, ETP-ALL has a higher rate of remission failure and subsequent relapse than typical T-ALL (37). Meng et al. (38) reported that six patients with relapsed/refractory ETP-ALL were treated with decitabine combined with the CAG regimen (aclarubicin, cytarabine, and G-CSF), and five patients achieved CR. In this study, one patient with ETP-ALL initiated maintenance treatment with decitabine and has completed four cycles and is currently well at the date of last follow-up. The above evidence supports the feasibility of low-dose decitabine maintenance therapy in T-ALL.

Lockhart et al. (39) described a child with Ph+ ALL having mixed donor chimerism and persistent BCR-ABL transcripts after allo-HSCT. There was no response to TKI treatment, but her clonal cytogenetic abnormalities were resolved after decitabine treatment. Cui et al. (40) also described 12 patients with relapse ALL after transplantation who were treated with decitabine alone or in combination with chemotherapy and DLI, and found that patients with Ph+ ALL achieved higher survival than patients with Ph− ALL. However, the effects of decitabine maintenance treatment on patients with Ph+ ALL was not significant, and the 2-year CIR was much higher than that of patients with Ph− ALL in this study. Although all seven patients with Ph+ ALL received oral TKI after transplantation, three patients still relapsed. However, this may be related to the presence of the T315I mutation, as in two of the three relapsed patients the T315I mutation was detected at relapse, and one patient was not tested voluntarily. For patients with Ph+ ALL after transplantation, exploring treatment with next-generation TKI may be more meaningful than low-dose decitabine.

HAMs can upregulate the expression of FOXP3 in CD4+CD25−T cells, thus increasing the number of Treg cells and mitigating GVHD (41, 42). In this study, only one patient developed grade IV aGVHD, while four patients presented severe cGVHD. Most cases occurred when immunosuppressants were reduced or prior to maintenance treatment. Only three patients stopped maintenance treatment because of GVHD. It is a pity that no aggravation or relief was observed in patients with GVHD due to the use of decitabine.

In this study, 94.1% patients developed grade I–II myelosuppression after receiving low-dose decitabine. Only two patients (5.8%) developed grade III–IV granulocytopenia and mild pulmonary infection. None of the patients required blood transfusion, and no one stopped this trial because of hematological toxicity. Pusic et al. (15) divided 24 patients with AML/MDS into four groups after transplantation, and each group was given different doses of decitabine for maintenance therapy. The authors found that the 10 mg/m²/d group presented fewer hematological adverse reactions and that decitabine was well tolerated, which was similar to our results. Further, our study showed that maintenance treatment with low-dose decitabine after transplantation had low hematological toxicity and is well tolerated.

Obviously, this study also has some limitations. First, our patients exhibited selection bias. Risk of disease relapse after allo-HSCT is a composite of multiple factors, including age, risk stratification at diagnosis, remission status at the time of transplantation, and duration of remission after transplantation. This study enrolled patients who were relatively young, and the sample population included 26.9% low-risk patients and several patients who were still in CR about 6 months after transplantation, which would lead to a better overall prognosis. Conversely, patients were enrolled without severe complications such as severe GVHD and were selected after day +50 of HSCT, which necessarily excluded those who relapsed early. Therefore, some transplanted patients were excluded because of early relapse or non-relapse mortality within the first 2 months. Secondly, this study did not detect changes in DNA methylation level before and after treatment, which would further support the use of HAMs. Finally, this study is limited by the small number of patients and lack of controls.

In conclusion, although the current data do not provide definitive evidence supporting the effects of low-dose decitabine maintenance treatment on the prevention of relapse after ALL transplantation, the overall results are encouraging and still indicate a positive trend. Low-dose decitabine maintenance treatment may be used as an option to prevent relapse after transplantation in patients with adult ALL, especially in patients...
with T-ALL. Our findings require confirmation in larger-scale controlled trials.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin.

**AUTHOR CONTRIBUTIONS**

RG, JL, and Z-XJ conceived, designed, and planned the study. All authors acquired the data. JL analyzed the data. JL and RG interpreted results. JL, FH, and RG drafted the report. RG, R-QL, and WL were involved in the critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

**REFERENCES**

1. Yonal-Hindilerden I, Kalayoglu-Besisik S, Gurses-Koc N, Hindilerden F, Sargin D. Allogeneic Hematopoietic Stem Cell Transplantation for Adult Acute Lymphoblastic Leukemia: Results From a Single Center, 1993-2011. *Int J Hematol Oncol Stem Cell Res* (2017) 11(1):58–62.

2. Lussana F, Intermesoli T, Gianni F, Boschini C, Masciulli A, Spinelli O, et al. Achieving Molecular Remission Before Allogeneic Stem Cell Transplantation in Adult Patients With Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia: Impact on Relapse and Long-Term Outcome. *Biol Blood Marrow Transplant* (2016) 22(11):1983–7. doi: 10.1016/j.bbmt.2016.07.021.

3. Wang L, Wang Y, Tang W, Dou H, Shan J, Hu J. The Superiority of Allogeneic Hematopoietic Stem Cell Transplantation From Unrelated Donor Over Chemotherapy for Adult Patients With High-Risk Acute Lymphoblastic Leukemia in First Remission. *Int J Hematol* (2013) 98(5):569–77. doi: 10.1007/s12185-013-1442-5.

4. Toffalori C, Zito L, Gambacorta V, Riba M, Oliveira G, Bucci G, et al. Immune Signature Drives Leukemia Escape and Relapse After Hematopoietic Cell Transplantation. *Nat Med* (2019) 25(4):603–11. doi: 10.1038/s41591-019-0400-z.

5. Christopher MJ, Petti AA, Rettig MP, Miller CA, Chandamari E, Duncavage EJ, et al. Immune Escape of Relapsed Aml Cells After Allogeneic Transplantation. *N Engl J Med* (2018) 379(24):2330–41. doi: 10.1056/NEJMoa1808777.

6. Schmid C, Labopin M, Nagler A, Bornhäuser M, Finke J, Fassas A, et al. Donor Lymphocyte Infusion in the Treatment of First Hematological Relapse After Allogeneic Stem-Cell Transplantation in Adults With Acute Myeloid Leukemia: A Retrospective Risk Factors Analysis and Comparison With Other Strategies by the EBMT Acute Leukemia Working Party. *J Clin Oncol* (2007) 25(31):4938–45. doi: 10.1200/JCO.2007.11.6053.

7. Fleischhauer K, Shaw BE. HLA-DR in Unrelated Hematopoietic Cell Transplantation Revisited: Challenges and Opportunities. *Blood* (2017) 130(9):1089–96. doi: 10.1182/blood.2017-03-742346.

8. Wright KL, Ting JP. Epigenetic Regulation of MHC-II and CIITA Genes. *Trends Immunol* (2006) 27(9):405–12. doi: 10.1016/j.ti.2006.07.007.

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9. von Dongen JI, Quertermous T, Bartram CR, Gold DP, Wolvers-Tettero IL, Comans-Bitter WM, et al. T Cell Receptor-CD3 Complex During Early T Cell Differentiation. Analysis of Immature T Cell Acute Lymphoblastic Leukemias (T-ALL) at DNA, RNA, and Cell Membrane Level. *J Immunol* (1987) 138:1260–9.

10. Holling TM, Schooten E, Langerak AW, van den Elsen PJ, Regulation of MHC Class II Expression in Human T-Cell Malignancies. *Blood* (2004) 103(4):1438–44. doi: 10.1182/blood-2003-05-1491.

11. Roman-Gomez J, Jimenez-Velasco A, Castillo JA, Agirre X, Barrios M, Navarro G, et al. Promoter Hypermethylation of Cancer-Related Genes: A Strong Independent Prognostic Factor in Acute Lymphoblastic Leukemia. *Blood* (2004) 104(8):2492–9. doi: 10.1182/blood-2004-03-0954.

12. Roman-Gomez J, Castillo JA, Jimenez A, Gonzalez M, Moreno F, Rodriguez, et al. 5’ CpG Island Hypermethylation Is Associated With Transcriptional Silencing of the P21(C1P1/WAF1/SDI1) Gene and Confers Poor Prognosis in Acute Lymphoblastic Leukemia. *Blood* (2002) 99(7):2291–6. doi: 10.1182/blood.v99.7.2291.

13. Garcia-Manero G, Daniel J, Smith TL, Kornblau SM, Lee M, Kantarjian HM, et al. DNA Methylation of Multiple Promoter-Associated CpG Islands in Adult Acute Lymphocytic Leukemia. *Clin Cancer Res* (2002) 8(7):2217–24.

14. Gao L, Zhang Y, Wang S, Kong P, Su Y, Hu J, et al. Effect of rG-CSF Combined With Decitabine Prophylaxis on Relapse of Patients With High-Risk Mrd-Negative AML After HSCT: An Open-Label, Multicenter, Randomized Controlled Trial. *J Clin Oncol* (2020) 38(36):4249–59. doi: 10.1200/JCO.19.03277.

15. Pusic I, Choi J, Fiala MA, Gao F, Holt M, Cashen AF, et al. Maintenance Therapy With Decitabine After Allogeneic Stem Cell Transplantation for Acute Myelogenous Leukemia and Myelodysplastic Syndrome. *Biol Blood Marrow Transplant* (2015) 21(10):1761–9. doi: 10.1016/j.bbmt.2015.05.026.

16. Ali N, Tomlinson B, Metheny L, Goldstein SC, Fu P, Cao S, et al. Conditioning Regimen Intensity and Low-Dose Azacitidine Maintenance After Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia. *Leuk Lymphoma* (2020) 61(12):2839–284. doi: 10.1080/10428194.2020.1789630.

17. Marini C, Brissot E, Bazarbachi A, Isnard F, Lapusan S, Adaeva R, et al. Donor Lymphocyte Infusion in the Treatment of First Hematological Relapse After Allogeneic Hematopoietic Stem Cell Transplantation Revisited: Challenges and Opportunities. *Blood* (2017) 130(9):1089–96. doi: 10.1182/blood.2017-03-742346.
28. Ribera J, Morgades M, Ciudad J, Montesinos P, Esteve J, Genescà E, et al.
27. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al.
25. Gökbuget N, Hoelzer D. Treatment of Adult Acute Lymphoblastic Leukemia.
24. Han S, Kim Y, Lee J, Jeon S, Hong T, Park G, et al. Model-Based Adaptive
30. Kimura S, Seki M, Kawai T, Goto H, Yoshida K, Isobe T, et al. DNA
29. Borsse
22. Goodyear O, Agathanggelou A, Novitzky-Basso I, Siddique S, McSkeane T,
31. Tavernier E, Le Q, de Botton S, Dhe
18. Oshrine BR, Shyr D, Hale G, Petrovic A. Low-Dose Azacitidine for Relapse
Prevention After Allogeneic Hematopoietic Cell Transplantation in Children
With Myeloid Malignancies. Pediatr Transplant (2019) 23(4):e13423.
doi: 10.1111/petr.13423
19. de Lima M, Oran B, Chapmlin RE, Papadopoulos EB, Giralt SA, Scott BL, et al.
CC-486 Maintenance After Stem Cell Transplantation in Patients With Acute
Myeloid Leukemia or Myelodysplastic Syndromes. Biol Blood Marrow
Transplant (2018) 24(10):2017–24. doi: 10.1016/j.bbmt.2018.06.016
20. Craddock C, Jilani N, Siddique S, Yap C, Khan J, Nagra S, et al. Tolerability and
Clinical Activity of Post-Transplantation Azacitidine in Patients
Allografted for Acute Myeloid Leukemia Treated on the RICAZA Trial. Biol
Blood Marrow Transplant (2016) 22(2):385–90. doi: 10.1016/j.
bbmt.2015.09.004
21. Goodyear OC, Dennis M, Jilani NY, Loke J, Siddique S, Ryan G, et al.
Azacitidine Augments Expansion of Regulatory T Cells After Allogeneic Stem
Cell Transplantation in Patients With Acute Myeloid Leukemia (AML). Blood
(2012) 119(14):3361–9. doi: 10.1182/blood-2011-09-377044
22. Goodyear O, Agathangelou A, Novitzky-Basso I, Siddique S, McSkeane T,
Ryan G, et al. Induction of a CD8+ T-Cell Response to the MAGE Cancer
Testis Antigen by Combined Treatment With Azacitidine and Sodium
Valproate in Patients With Acute Myeloid Leukemia and Myelodysplasia.
Blood (2010) 116(11):1908–18. doi: 10.1182/blood-2009-11-249474
23. Karachuca M, Momparler RL. Pharmacokinetic and Pharmacodynamic
Analysis of 5-Aza-2'-Deoxycytidine (Decitabine) in the Design of its Dose-
Schedule for Cancer Therapy. Clin Epigene (2013) 5(1):3. doi: 10.1186/1868-
7083-5-3
24. Han S, Kim Y, Lee J, Jeon S, Hong T, Park G, et al. Model-Based Adaptive
Phase I Trial Design of Post-Transplant Decitabine Maintenance in
Myelodysplastic Syndrome. J Hematol Oncol (2015) 8(1):118. doi: 10.1186/
s13045-015-0208-3
25. Goodyer O, McManus C, Palmqvist L, Karrman K, Abrahamsson J, Behrendtz M,
Heldrup Brien S, Cortes J, Giles F, Jeha S, et al. Long-Term Follow-Up Results of
Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, and
Dexamethasone (Hyper-CVAD), a Dose-Intensive Regimen, in Adult Acute
Lymphocytic Leukemia. Cancer (2004) 101(12):2788–801. doi: 10.1002/cncr.20668
33. Kantarjian H, Thomas D, O’Brien S, Cortes J, Giles F, Jeha S, et al. Outcome of
609 Adults After Relapse of Acute Lymphoblastic Leukemia (ALL); an MRC UKALL12/ECOG 2993 Study. Blood (2007) 109(3):944–50.
doi: 10.1182/blood-2006-05-18192
34. Fielding AK, Richards SM, Chopra R, Lazarus HM, Listow MB, Buck G, et al.
Outcome of 609 Adults After Relapse of Acute Lymphoblastic Leukemia (ALL); an MRC UKALL12/ECOG 2993 Study. Blood (2007) 109(3):944–50.
doi: 10.1182/blood-2006-05-18192
35. Xu M, Liu H, Liu Y, Ma X, Qiu H, Fu C, et al. Gene Mutations and
Pretransplant Minimal Residual Disease Predict Risk of Relapse in Adult
Patients After Allogeneic Hematopoietic Stem-Cell Transplantation for T Cell
Acute Lymphoblastic Leukemia. Leuk Lymphoma (2019) 60(11):2744–53.
doi: 10.1080/10428194.2019.1597270
36. Katagiri S, Umezzi T, Azuma K, Kobayashi C, Akahane D, Suguro T, et al.
Maintenance 5-Azacitidine Therapy by MRD Monitoring After Allogeneic
HSCT in Myeloid/Lymphoid Neoplasms With FGFR1 Rearrangement. Bone
Marrow Transplant (2019) 54(7):1148–50. doi: 10.1038/s41409-019-0436-1
37. Jain N, Lamb AV, O’Brien S, Ravandi F, Konopleva M, Jabbour E, et al. Early
T-Cell Precursor Acute Lymphoblastic Leukemia/Lymphoma (ETP-ALL/
LBL) in Adolescents and Adults: A High-Risk Subtype. Blood (2016) 127(15):
1863–9. doi: 10.1182/blood-2015-08
38. Meng T, Yao Y, Xu Y, Xue S, Han Y, Tang X, et al. Salvage Therapy With
Decitabine in Combination With Granulocyte Colony-Stimulating Factor,
Low-Dose Cytarabine, and Aclarubicin in Patients With Refractory or
Relapsed Early T-Cell Precursor Acute Lymphoblastic Leukemia. Hematol
 Oncol (2020) 38(5):834–7. doi: 10.1002/hon.2783
39. Lockhart S, McDonald L, Ryttig M, Chan KW. Clonal Cytogenetic
Abnormalities After Tyrosine Kinase Inhibitor Therapy in Ph+ All
Resolution After Decitabine Therapy. Pediatr Blood Cancer (2012) 59(3):
573–5. doi: 10.1002/pbc.23318
40. Cui J, Xiao Y, You Y, Shi W, Li Q, Luo Y, et al. Decitabine for Relapsed Acute
Lymphoblastic Leukemia: After Allogeneic Hematopoietic Stem Cell
Transplantation. Curr Med Sci (2017) 37(5):693–8. doi: 10.1007/s11596-
017-1790-0
41. Choi J, Ritchey J, Prior JL, Holt M, Shannon WD, Deych E, et al. In Vivo
Administration of Hypomethylating Agents Mitigate Graft-Versus-Host Disease
Without Sacrificing Graft-Versus-Leukemia. Blood (2010) 116(1):
129–39. doi: 10.1182/blood-2009-12-257253
42. Cooper ML, Choi J, Karpova D, Vij K, Ritchey J, Schroeder MA, et al.
Azacitidine Mitigates Graft-Versus-Host Disease via Differential Effects on
the Proliferation of Teffectors and Natural Regulatory T Cells In Vivo. J Immuno
(2017) 198(9):3746–54. doi: 10.4049/jimmunol.1502399

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