RESEARCH ARTICLE

Blood Science

OPEN

Pre-transplantation levels of lysine (K)-specific methyltransferase 2A (*KMT2A*) partial tandem duplications can predict relapse of acute myeloid leukemia patients following haploidentical donor hematopoietic stem cell transplantation

Dao-Xing Deng[a], Xiao-Hang Ma[a], Ze-Hua Wu[a,b], Xiao-Hui Zhang[a], Lan-Ping Xu[a], Yu Wang[a], Chen-Hua Yan[a], Huan Chen[a], Yu-Hong Chen[a], Wei Han[a], Feng-Rong Wang[a], Jing-Zhi Wang[a], Xiao-Jun Huang[a,c], Xiao-Su Zhao[a,d,*], Xiao-Dong Mo[a,d,*]

[a]Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China; [b]Department of Hematology, Peking University Shenzhen Hospital, Shenzhen, China; [c]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China; [d]Research Unit of Key Technique for Diagnosis and Treatments of Hematologic Malignancies, Chinese Academy of Medical Sciences, 2019RU029, Beijing, China.

Abstract

We aimed to identify dynamic changes of lysine (K)-specific methyltransferase 2A partial tandem duplications (*KMT2A*-PTD) before and after haploidentical donor hematopoietic stem cell transplantation (HID HSCT) and explore the prognostic value of pre-transplantation levels of *KMT2A*-PTD in acute myeloid leukemia (AML) receiving HID HSCT. Consecutive 64 AML patients with *KMT2A*-PTD positivity at diagnosis receiving HID HSCT were included in this study. Patients with *KMT2A*-PTD ≥1% before HSCT had a slower decrease of *KMT2A*-PTD after HID HSCT. Patients with *KMT2A*-PTD ≥1% before HID HSCT had a higher cumulative incidence of relapse (36.4%, 95% confidence interval [CI]: 6.3%–66.5%) at 2 years after HSCT than those with *KMT2A*-PTD <1% (7.5%, 95% CI: 0.3%–14.7%, $P = .010$). In multivariable analysis, *KMT2A*-PTD ≥1% before HID HSCT was the only independent risk factor for relapse (hazard ratio [HR]: 4.90; 95% CI: 1.22–19.59; $P = .025$). Thus, pre-transplantation levels of *KMT2A*-PTD could predict relapse in AML patients following HID HSCT.

Key Words: Acute myeloid leukemia; Allogeneic hematopoietic stem cell transplantation; Haploidentical; *KMT2A*-PTD; Relapse

**Address correspondence: Prof. Xiao-Su Zhao and Xiao-Dong Mo, Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, No. 11 Xizhimen South Street, Xicheng District, Beijing 100044, China. E-mail address: zhao.xiaosu@outlook.com (X.-S. Zhao) and mxd453@163.com (X.-D. Mo).*

Conflict of interest: The authors declare that they have no conflict of interest.

D.-X.D., X.-H.M., and Z.-H.W. contributed equally to this work.

The work was supported by the National Key Research and Development Program of China (2021YFC2500300, 2022YFC2502606), the Grants from the Beijing Municipal Science and Technology Commission (Z221100007422008), Major Program of the National Natural Science Foundation of China (82293630, 82293633), CAMS Innovation Fund for Medical Sciences (2022-I2M-C&T-B-121, 2023-I2M-C&T-B-123), Peking University People's Hospital Research and Development Funds (RD2023-08), Tongzhou District Distinguished Young Scholars (JCQN2023009), and the Fundamental Research Funds for the Central Universities.

Blood Science (2024) 6, 1–8:e00207.

Received July 11, 2024; Accepted August 29, 2024.

http://dx.doi.org/10.1097/BS9.0000000000000207

1. INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most important curative therapy for acute myeloid leukemia (AML).[1–5] Because both the human leukocyte antigen (HLA) matched sibling donors (MSD) and unrelated donors (URD) are usually unavailable in China, haploidentical-related donors are the most important alternative donors for Chinese AML patients.[1–3,6,7] Haploidentical donor (HID) HSCT could achieve superior clinical outcomes compared with consolidation chemotherapy for intermediate- and high-risk AML.[8,9] Furthermore, HID HSCT can achieve clinical outcomes similar to[10,11] or even superior to[12,13] those of MSD HSCT for AML patients.

Lysine (K)-specific methyltransferase 2A (*KMT2A*), which used to be named as mixed-lineage leukemia (*MLL*) gene, is located on chromosome 11q23.[14] There are more than 100 types of *KMT2A* rearrangements, and *KMT2A*-partial tandem duplications (PTD) is formed when the *KMT2A* gene is rearranged to generate PTD.[15–17] *KMT2A*-PTD is reported in approximately 5% to 10% of cases of AML.[18–20]

AML patients with a high level of *KMT2A*-PTD were unlikely to achieve complete remission (CR) and long-time survival.[19,21–23] Kong et al[23] reported that in AML patients, a high initial *KMT2A*-PTD level (ie, *KMT2A*-PTD ≥1%) was the only independent risk factor for CR after induction chemotherapy, and patients with a higher initial level of *KMT2A*-PTD had a shorter survival period compared to those with a lower level of *KMT2A*-PTD. The level of *KMT2A*-PTD after chemotherapy

could also predict relapse of AML patients. Weisser et al[24] reported that *KMT2A*-PTD level decreasing at least 2 logs after chemotherapy was associated with a superior overall survival (OS) in AML patients. Lastly, Kong et al[25] reported that *KMT2A*-PTD level after allo-HSCT could predict relapse; and myelodysplastic syndrome (MDS)/AML patients with post-transplant *KMT2A*-PTD levels ≥1% had a higher cumulative incidence of relapse, a lower probability of OS, and a lower probability of disease-free survival (DFS) compared to those with *KMT2A*-PTD levels <1%.

However, most of the studies included other hematologic malignancies apart from AML (eg, MDS), and no study had identified the influence of *KMT2A*-PTD on the post-transplant outcomes in the specific population with AML. In addition, no studies had identified the impact of pre-HSCT level of *KMT2A*-PTD on the clinical outcomes after HID HSCT, and whether *KMT2A*-PTD levels before HID HSCT could predict post-transplant relapse of AML patients was unclear.

Thus, we aimed to identify the dynamic changes of *KMT2A*-PTD before and after HID HSCT, particularly, we aimed to explore the prognostic value of pre-HSCT *KMT2A*-PTD level in AML patients receiving HID HSCT.

2. MATERIAL AND METHODS

2.1. Patients

Consecutive 64 AML patients with *KMT2A*-PTD positivity at diagnosis receiving HID HSCT at Peking University Institute of Hematology (PUIH) from April 2016 to December 2020 were enrolled in this study (refer to Supplementary Figure 1, http://links.lww.com/BS/A105, for detailed inclusion criteria). The last follow-up was on January 13, 2023. The study was approved by the institutional review board of Peking University People's Hospital and was conducted in accordance with the *Declaration of Helsinki*.

2.2. Transplant regimen

The preconditioning regimen consisted of cytarabine, busulfex, cyclophosphamide, simustine, and rabbit anti-thymocyte globulin (ATG).[26] Cyclosporine A (CSA), mycophenolate mofetil (MMF), and methotrexate (MTX) were used to prevent GVHD.[27]

2.3. *KMT2A*-PTD and multiparameter flow cytometry (MFC) monitoring

KMT2A-PTD expression levels were determined by real-time quantitative polymerase chain reaction (RQ-PCR) technology.[25] The transcript level was calculated as *KMT2A*-PTD transcript copies/ABL copies in percentage. According to our experiment method, *KMT2A*-PTD/ABL ≥0.1% was considered as *KMT2A*-PTD positivity. Measurable residual disease (MRD) detected by MFC was based on leukemia-associated aberrant immune phenotypes (LAIPs). The different-from-normal (DfN) approach was used when LAIPs at diagnosis were not available. MFC MRD positivity was defined as ≥0.1% of cells with LAIPs or DfN abnormalities in bone marrow (BM) samples in accordance with the European LeukemiaNet (ELN)-2022 criteria.[28] Patients with post-transplant *KMT2A*-PTD or MFC MRD positivity were routinely treated with preemptive interventions including donor lymphocyte infusion (DLI) and interferon-α (IFN-α).[25,29]

2.4. Definitions of clinical outcomes

Relapse was defined as recurrence of at least 5% blasts in BM, reappearance of blasts in peripheral blood, or development of extramedullary disease. Non-relapse mortality (NRM) was defined as death of any cause beyond relapse. Leukemia-free survival (LFS) was defined as survival period without relapse or death of any cause. OS was defined as survival period without death from any cause. The primary endpoint was relapse, and the secondary endpoints included NRM, LFS, and OS.

2.5. Statistical analysis

Categorical variables were compared using χ^2 or Fisher exact tests, and continuous variables were compared using the Mann–Whitney *U* test. Survival analysis was calculated by the Kaplan–Meier method. Relapse and NRM were estimated by competing risk analysis. Relapse was the competing event for NRM and vice versa. Multivariable analysis was performed by Cox's proportional hazards model. Estimate the hazard ratios of clinical factors using univariable and multivariable Cox regression analysis. The following factors for multivariable analysis were included: age, genetic risk classification, disease status before allo-HSCT, hematopoietic cell transplantation–specific comorbidity index (HCT-CI) score, donor–recipient sex matched and *KMT2A*-PTD before allo-HSCT. The *P* values were 2-sided, and $P < .05$ was considered statistically significant. Statistical analysis was performed by the SPSS 27 (SPSS Inc./IBM, Armonk, NY) and the R software package (version 4.2.1; http://www.r-project.org).

3. RESULTS

3.1. Patient characteristics

The characteristics of 64 patients with initial *KMT2A*-PTD positivity are shown in Table 1, and 39, 14, and 11 patients showed *KMT2A*-PTD < 0.1% (group 1), ≥0.1% but <1% (group 2), and ≥1% (group 3) before HID HSCT, respectively. All of the clinical characteristics including age, genetic risk classification, and disease status before allo-HSCT were comparable among these 3 groups.

3.2. Dynamic changes of *KMT2A*-PTD after HID HSCT

A total of 36 (92.3%), 13 (92.9%), and 8 (72.7%) patients achieved MRD negativity at 1 month after HID HSCT, respectively, in group 1, group 2, and group 3 ($P = .175$). A total of 37 (94.9%), 14 (100.0%), and 8 (72.7%) patients achieved MRD negativity at 2 months after HID HSCT, respectively, in group 1, group 2, and group 3 ($P = .042$). A total of 35 (89.7%), 13 (92.9%), and 6 (54.5%) patients achieved MRD negativity at 3 months after HID HSCT, respectively, in group 1, group 2, and group 3 ($P = .018$). A total of 37 (94.9%), 14 (100.0%), and 8 (72.7%) patients achieved MRD negativity ultimately after HID HSCT, respectively, in group 1, group 2, and group 3 ($P = .042$) (**Fig. 1**). The dynamic variation of *KMT2A*-PTD before and after HID HSCT is shown in Figure 2, and patients with *KMT2A*-PTD ≥1% before HSCT had a slower decrease of *KMT2A*-PTD after HID HSCT. Meanwhile, in 57 patients achieving MRD negativity after HID HSCT, patients with *KMT2A*-PTD ≥1% before HSCT were more likely to convert to *KMT2A*-PTD positivity again (87.5% vs 24.5%, $P < .001$) and experience relapse compared to those with *KMT2A*-PTD <1% before HSCT (50.0% vs 8.2%, $P = .002$).

3.3. The association between *KMT2A*-PTD and MFC MRD

KMT2A-PTD ≥1% before HSCT was significantly associated with MFC MRD positive (Spearman's correlation coefficient: 0.260, $P = .038$), although there was no association between *KMT2A*-PTD ≥0.1% before HSCT and MFC MRD positivity (Spearman's correlation coefficient: 0.196, $P = .120$).

Table 1

Patients' clinical characteristics.

Characteristics	*KMT2A*-PTD <0.1% (n = 39)	*KMT2A*-PTD 0.1%–1% (n = 14)	*KMT2A*-PTD ≥1% (n = 11)	*P*
Median age at allo-HSCT, y (range)	42 (14–62)	43 (14–57)	33 (16–56)	.278
Sex, male/female, n	23/16	6/8	4/7	.359
Normal karyotype, n (%)	24 (61.5)	11 (78.6)	8 (72.7)	.637
FLT3-ITD mutation, n (%)				.896
Yes	6 (15.4)	3 (21.4)	2 (18.2)	
No	33 (84.6)	11 (78.6)	9 (81.8)	
Genetic risk classification				.940
Favorable	4 (10.3)	1 (7.1)	0 (0.0)	
Intermediate	24 (61.5)	8 (57.1)	7 (63.6)	
Adverse	11 (28.2)	5 (35.8)	4 (36.4)	
HCT-CI score, n (%)				.922
Low risk	30 (76.9)	10 (71.4)	8 (72.7)	
Intermediate risk	9 (23.1)	4 (28.6)	3 (27.3)	
High risk	0 (0.0)	0 (0.0)	0 (0.0)	
Disease status before allo-HSCT				1.000
CR1	35 (89.7)	13 (92.9)	10 (90.9)	
CR2	4 (10.3)	1 (7.1)	1 (9.1)	
Donor–recipient sex matched, n (%)				.254
Female to male	9 (23.1)	2 (14.3)	0 (0.0)	
Others	30 (76.9)	12 (85.7)	11 (100.0)	
Donor–recipient relationship, n (%)				.619
Mother–child	3 (7.7)	0 (0.0)	1 (9.1)	
Others	36 (92.3)	14 (100.0)	10 (90.9)	
ABO compatibility, n (%)				.241
Compatible	24 (61.5)	6 (42.9)	4 (36.4)	
Incompatible	15 (38.5)	8 (57.1)	7 (63.6)	
Median mononuclear cell counts, $\times 10^8$/kg (range)	8.4 (2.7–15.7)	8.7 (5.2–12.4)	9.1 (6.1–12.5)	.898
Median $CD34^+$ cell counts, $\times 10^6$/kg (range)	2.7 (0.5–6.7)	2.8 (0.3–5.6)	1.8 (0.7–6.0)	.141
Neutrophil engraftment, n (%)	39 (100.0)	14 (100.0)	11 (100.0)	1.000
Median time from HSCT to neutrophil engraftment, d (range)	12 (8–21)	13 (10–23)	13 (11–20)	.849
Platelet engraftment, n (%)	37 (94.9)	12 (85.7)	11 (100.0)	.331
Median time from HSCT to platelet engraftment, d (range)	15 (9–83)	15.5 (11–56)	20 (10–102)	.422

Allo-HSCT = allogeneic hematopoietic stem cell transplantation, CR = complete remission, *FLT3-ITD* = FMS-like tyrosine kinase 3 (*FLT3*) internal tandem duplication, HCT-CI = hematopoietic cell transplantation–specific comorbidity index, *KMT2A*-PTD = lysine (K)-specific methyltransferase 2A partial tandem duplication.

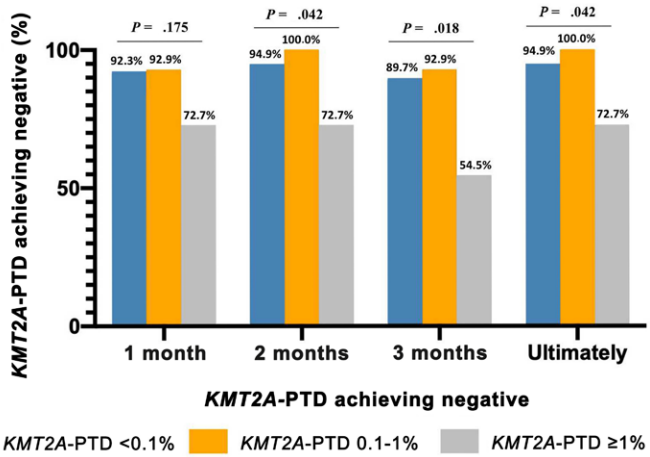

Figure 1. *KMT2A*-PTD achieving negative at 1, 2, 3 mo and ultimately after HID HSCT. HID HSCT = haploidentical donor hematopoietic stem cell transplantation, *KMT2A*-PTD = lysine (K)-specific methyltransferase 2A partial tandem duplication.

Five patients showed *KMT2A*-PTD and MFC MRD positivity simultaneously after HSCT, and 1 and 1 of them showed *KMT2A*-PTD positivity 2 and 4 months prior to MFC MRD positivity, respectively. None showed MFC MRD positivity before *KMT2A*-PTD positivity. *KMT2A*-PTD positivity after HSCT was significantly associated with MFC MRD positivity (Spearman's correlation coefficient: 0.448, $P < .001$) within 1 year after HID HSCT, and *KMT2A*-PTD ≥1% after HID HSCT was more significantly associated with MFC MRD positivity within 1 year after HSCT (Spearman's correlation coefficient: 1.000, $P < .001$).

Five patients showed *KMT2A*-PTD ≥1% and MFC MRD positivity simultaneously after HSCT, and none showed *KMT2A*-PTD ≥1% prior to MFC MRD positivity and vice versa.

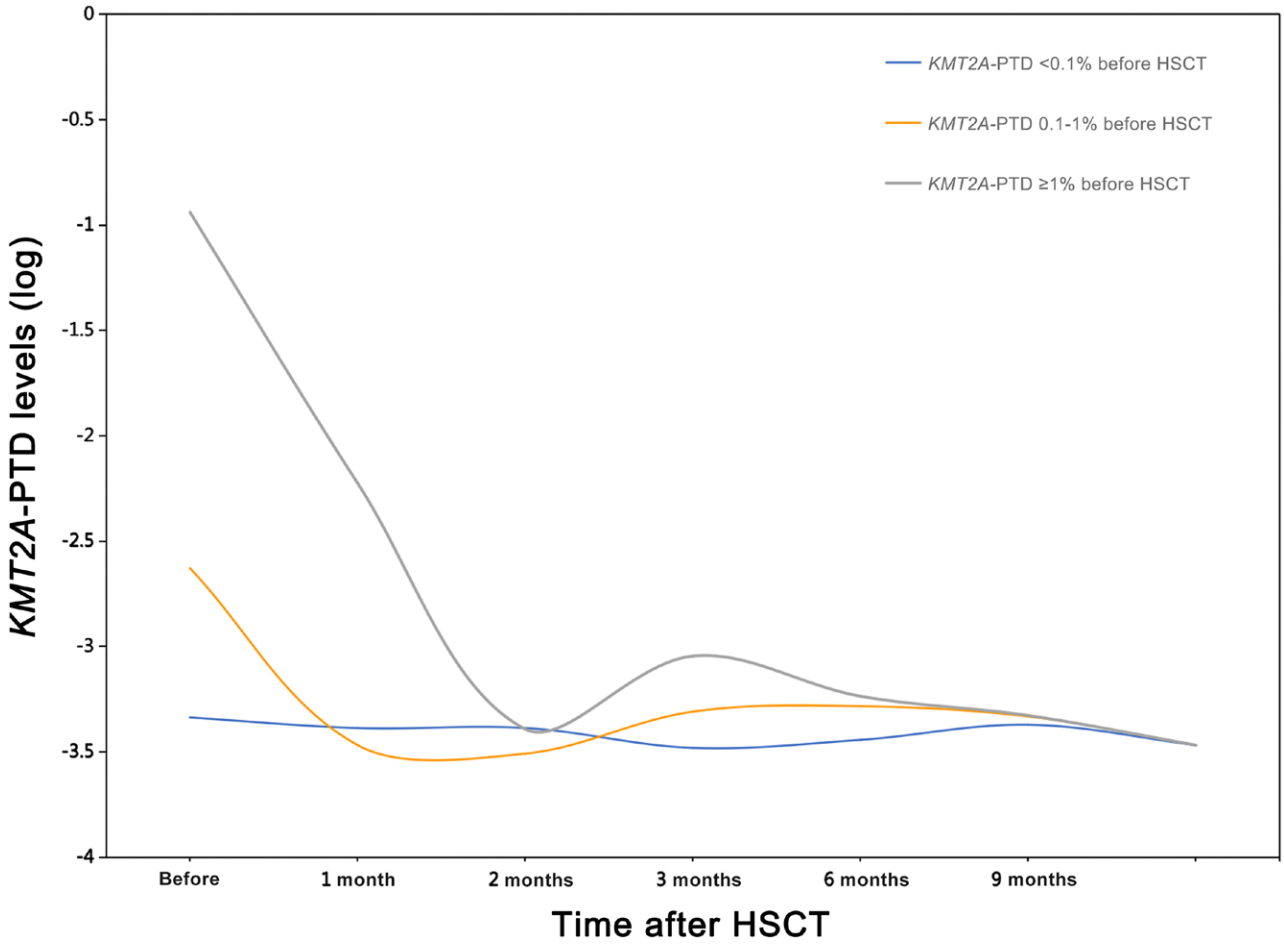

Figure 2. The dynamic variation of *KMT2A*-PTD before and after HID HSCT. HID HSCT = haploidentical donor hematopoietic stem cell transplantation, *KMT2A*-PTD = lysine (K)-specific methyltransferase 2A partial tandem duplication.

3.4. Comparison of clinical outcomes between patients with *KMT2A*-PTD <0.1% (group 1), ≥0.1% but <1% (group 2), and ≥1% (group 3) before HID HSCT

Patients in group 3 had a higher 2-year cumulative incidence of relapse after HID HSCT (36.4%, 95% confidence interval [CI]: 6.3%–66.5%) compared with those in group 1 (7.7%, 95% CI: 0.0%–16.2%) (P = .016), and showed a trend of higher 2-year cumulative incidence of relapse compared with those in group 2 (7.1%, 95% CI: 0.0%–21.1%) (P = .091) (**Fig. 3A**).

The 2-year probabilities of NRM after HID HSCT were 10.3% (95% CI: 0.6%–20.0%), 21.4% (95% CI: 0.0%–43.9%), and 9.1% (95% CI: 0.0%–27.4%) (P = .531), respectively, for group 1, group 2, and group 3 (**Fig. 3B**). The 2-year probabilities of LFS after HID HSCT were 82.1% (95% CI: 70.1%–94.1%), 71.4% (95% CI: 47.7%–95.1%), and 54.5% (95% CI: 25.1%–83.9%) (P = .222), respectively, for group 1, group 2, and group 3 (**Fig. 3C**). The 2-year probabilities of OS after HID HSCT were 84.6% (95% CI: 73.2%–96.0%), 71.4% (95% CI: 47.7%–95.1%), and 72.7% (95% CI: 46.4%–99.0%) (P = .516), respectively, for group 1, group 2, and group 3 (**Fig. 3D**).

Because all clinical outcomes were comparable between patients in group 1 and group 2, we combined these 2 groups as *KMT2A*-PTD <1% in the following analysis. Patients with *KMT2A*-PTD ≥1% before HID HSCT had a higher 2-year cumulative incidence of relapse (36.4%, 95% CI: 6.3%–66.5%) after HSCT than those with *KMT2A*-PTD <1% (7.5%, 95% CI: 0.3%–14.7%, P = .010) (**Fig. 4A**). The 2-year probabilities of NRM, LFS, and OS after HID HSCT were 13.2% (95% CI: 4.0%–22.4%) vs 9.1% (95% CI: 0.0%–27.4%) (P = .670), 79.2% (95% CI: 68.2%–90.2%) vs 54.5% (95% CI: 25.1%–83.9%) (P = .120), and 81.1% (95% CI: 70.5%–91.7%) vs 72.7% (95% CI: 46.4%–99.0%) (P = .596), respectively, for patients with *KMT2A*-PTD <1% and those with *KMT2A*-PTD ≥1% before HID HSCT (**Fig. 4B–D**).

In patients with *KMT2A*-PTD ≥1% before HID HSCT, a total of 4 patients suffered post-transplant relapse, and 2 of them died of relapse while the other 2 still survived due to receiving DLI. In addition, in those with *KMT2A*-PTD <1% before HID HSCT, a total of 4 patients suffered post-transplant relapse, and 3 of them died of relapse, while only 1 was still alive due to receiving DLI.

In multivariable analysis, *KMT2A*-PTD ≥1% before HID HSCT was the only independent risk factor for post-transplant relapse (hazard ratio [HR]: 4.90; 95% CI: 1.22–19.59; P = .025). No risk factors were associated with NRM, LFS, and OS in multivariable analysis (data not shown).

3.5. *KMT2A*-PTD levels before HID HSCT did not influence the efficacy of post-transplant preemptive interventions

Among 8 patients who developed MRD positivity post-HSCT (*KMT2A*-PTD positivity alone: 5; both *KMT2A*-PTD and MFC MRD positivity: 3), we preemptively intervened with DLI in 2 patients and IFN-α in 6 patients. Two patients subsequently relapsed. Pre-transplant *KMT2A*-PTD levels were <1% in 4 patients and ≥1% in the other four. Notably, 1 patient from

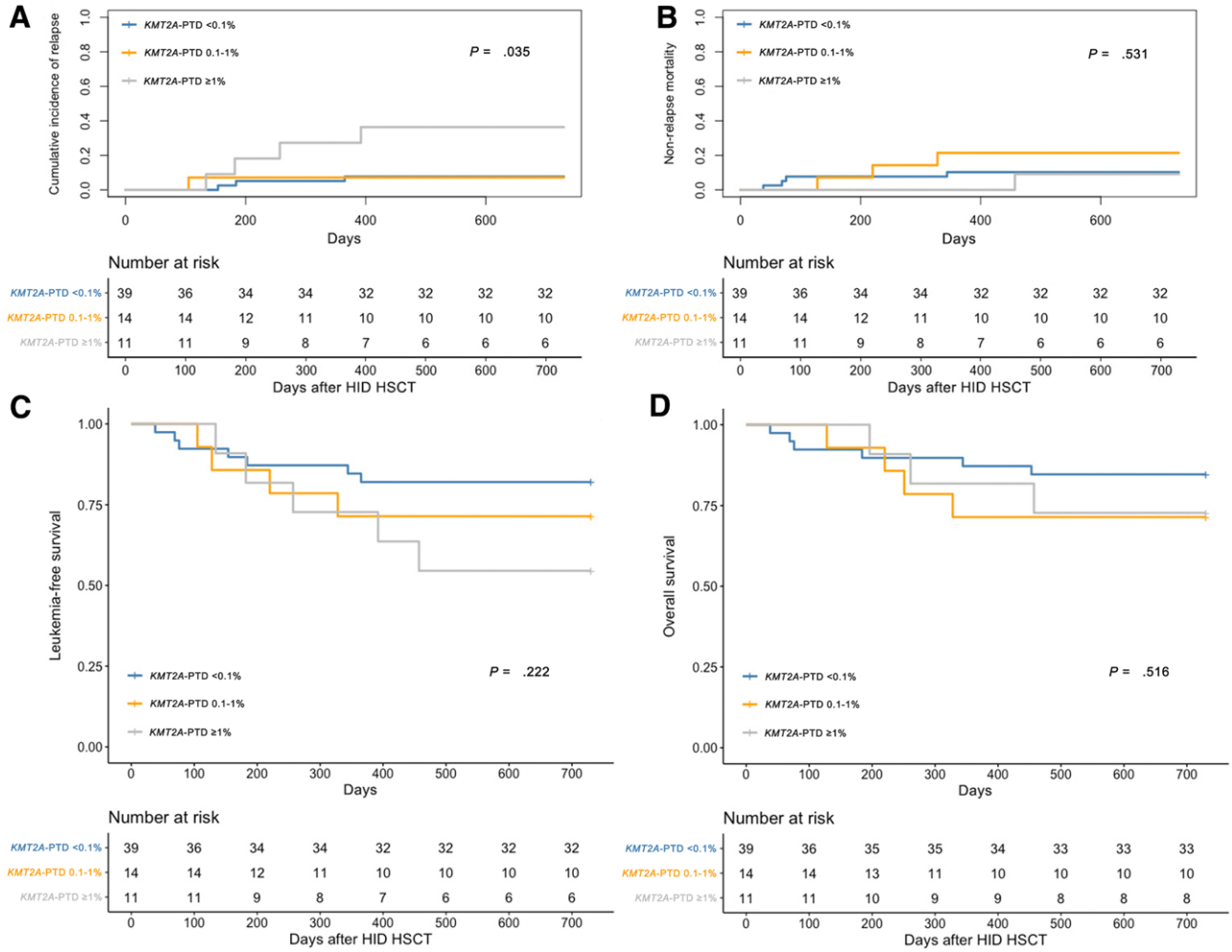

Figure 3. Comparison of clinical outcomes between patients with *KMT2A*-PTD <0.1%, ≥0.1% but <1%, and ≥1% before HID HSCT. (A) Cumulative incidence of relapse, (B) non-relapse mortality, (C) leukemia-free survival, (D) overall survival. HID HSCT = haploidentical donor hematopoietic stem cell transplantation, *KMT2A*-PTD = lysine (K)-specific methyltransferase 2A partial tandem duplication.

each group relapsed. The relapse rates were not significantly different between the 2 groups (25.0% vs 25.0%, $P = 1.000$).

4. DISCUSSION

In this study, *KMT2A*-PTD ≥1% before allo-HSCT had a significant impact on MRD achieving negativity after HID HSCT, and it could also be associated with a higher incidence of relapse after HID HSCT. To the best of our knowledge, this study first described the dynamic changes of *KMT2A*-PTD levels before and after HID HSCT in AML patients and first showed that pre-transplant *KMT2A*-PTD levels could predict relapse after HID HSCT.

This study showed that *KMT2A*-PTD positivity after HID HSCT, especially *KMT2A*-PTD ≥1%, was highly correlated with MFC MRD positivity. In addition, *KMT2A*-PTD ≥1% before HID HSCT was also associated with MFC MRD positivity. A large number of studies had proved that MFC MRD was a reliable and efficient MRD marker for AML patients.[28,30–33] Thus, based on the strong correlation between *KMT2A*-PTD positivity and MFC positivity, *KMT2A*-PTD had the potential to be an excellent MRD marker for AML patients receiving HID HSCT.

This study also found that AML patients with *KMT2A*-PTD ≥1% before HID HSCT had a significantly high incidence of relapse than those with *KMT2A*-PTD <1%. Previous studies had found that, compared to MSD HSCT, HID HSCT had a stronger graft-versus-leukemia (GVL) effect and could decrease relapse more significantly in high-risk AML patients.[12,13,31,34] However, in this study, HID HSCT could not overcome the adverse effect of a higher level of *KMT2A*-PTD on post-transplant relapse. Therefore, how to further reduce relapse of these patients was critical to improve the prognosis of AML patients with *KMT2A*-PTD.

Previous studies reported that the incidence of relapse after allo-HSCT could be decreased by some strategies. For example, intensive preconditioning regimen (eg, decitabine-based preconditioning regimen) could effectively reduce relapse in relapsed/refractory AML patients.[35–37] In addition, preemptive DLI after allo-HSCT could also effectively prevent post-transplant relapse of high-risk AML patients; and Yan et al reported a prospective study on preemptive DLI, which included 814 standard-risk AL patients who underwent allo-HSCT. The results demonstrated that preemptive DLI significantly reduced relapse rates ($P = .001$), improved both OS ($P = .022$) and DFS ($P = .002$) compared to these MRD$^+$ subjects who received only low-dose IL-2 in MRD positivity patients after HSCT.[38–42] Thirdly, maintenance therapy,[43–45] such as hypomethylating agents, could decrease relapse of high-risk AML patients. However, we do not recommend using Venetoclax (VEN) combined with azacitidine (AZA) for maintenance therapy post-HSCT due to its significant myelosuppressive effects. Our center conducted a study on the efficacy of VEN + AZA in 60 de novo unfit and relapsed/refractory AML patients. While this regimen achieved high

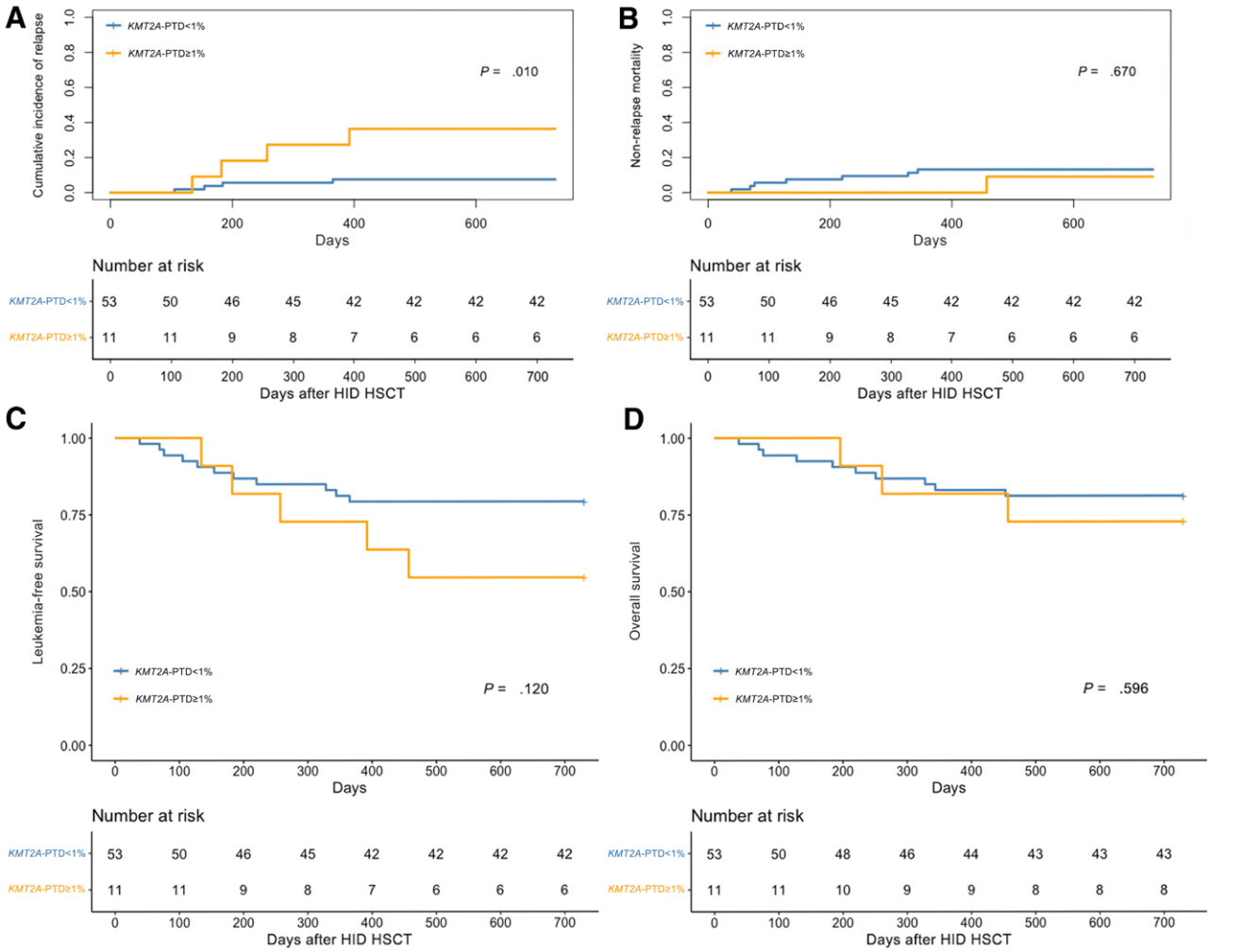

Figure 4. Comparison of clinical outcomes between patients with *KMT2A*-PTD <1%, and ≥1% before HID HSCT. (A) Cumulative incidence of relapse, (B) non-relapse mortality, (D) leukemia-free survival, (D) overall survival. HID HSCT = haploidentical donor hematopoietic stem cell transplantation, *KMT2A*-PTD = lysine (K)-specific methyltransferase 2A partial tandem duplication.

response rates, it also resulted in a 100% incidence of hematological adverse effects, including 58.3% leukopenia, 28.3% anemia, and 28.3% thrombocytopenia.[46] Furthermore, a prospective study has shown that T-cell reconstitution was delayed in HID HSCT compared to HLA-matched unrelated donor transplantation (MUDT).[47] Given that our center primarily performs haploidentical transplants, poor graft function remains a significant complication of allo-HSCT and presents a substantial clinical challenge. Consequently, we are particularly concerned about the risks associated with VEN + AZA. Moreover, our well-established preemptive intervention system, which has achieved long-term survival rates exceeding 70% through the use of IFN-α and DLI, enables precise intervention following MRD positivity and helps avoid overtreatment in patients who achieve deep remission.[29,42]

In this study, the relapse rate after post-transplant preemptive interventions was the same for AML patients with *KMT2A*-PTD <1% and ≥1% before HID HSCT, which suggested that preemptive interventions may help to reduce relapse in AML patients with a high level of *KMT2A*-PTD before HID HSCT. We believe this may be explained by preemptive interventions that enhance the GVL effect. A previous prospective study indicated that preemptive DLI increased the incidence of acute graft-versus-host disease (GVHD).[42] However, Morris et al confirmed that the use of G-CSF during blood-cell mobilization enhances natural killer and T-cell–dependent CD8+ cytotoxicity, which may help to separate GVHD from GVL effects.[42,48,49] IFN-α and DLI have similar effects. In GVHD mouse models, IFN signaling in recipient tissues has been shown to inhibit CD4-dependent GVHD while promoting CD8-mediated GVHD and enhancing the GVL effect.[50] In a prospective study conducted at our center, we observed that severe chronic GVHD can occur following IFN-α treatment. Nonetheless, the incidence of severe chronic GVHD after IFN-α treatment was only 6%, indicating that the severity of chronic GVHD induced by IFN-α was well-controlled.[51] However, the number of cases were not sufficient in the study, so the result needed to be confirmed by further research. Nevertheless, methods mentioned above may help to further decrease the risk of relapse in AML patients with high levels of *KMT2A*-PTD before HID HSCT.

In this study, AML patients with *KMT2A*-PTD ≥1% before HID HSCT were more likely to suffer post-transplant relapse than those with *KMT2A*-PTD <1%. And the NRM rates were comparable between these 2 groups. However, the OS rates were not significantly different. This is also consistent with the previous report. Kong et al[25] conducted a retrospective study on the impact of *KMT2A*-PTD levels before transplantation on the prognosis of 48 *KMT2A*-PTD-positive AML or MDS-EB patients. The results indicated that there was no significant difference in OS between patients with *KMT2A*-PTD levels ≥1% and those with levels <1% before HSCT.[25] One reason why AML patients with KMT2A-PTD ≥1% before HID HSCT

had comparable OS with those with KMT2A-PTD <1% in our study may be the small sample size of the study; secondly, some relapsed patients achieved long-term survival due to receiving DLI.

There were some limitations in the study. Firstly, it was a retrospective study. In addition, the sample size was relatively small, especially only 11 patients with *KMT2A*-PTD ≥1%, which was also a possible reason for no impacts of *KMT2A*-PTD on LFS and OS. Future studies with large samples may help to further identify the impact of *KMT2A*-PTD before HID HSCT on the prognosis of AML patients.

5. CONCLUSION

In conclusion, this study first described the dynamic changes of *KMT2A*-PTD before and after HID HSCT, and first observed that *KMT2A*-PTD levels before HID HSCT could effectively predict post-transplant relapse in AML patients. Future multicenter, large sample size studies may help to further validate our conclusions.

ACKNOWLEDGMENTS

The work was supported by the National Key Research and Development Program of China (2021YFC2500300, 2022YFC2502606), the Grants from the Beijing Municipal Science and Technology Commission (Z221100007422008), Major Program of the National Natural Science Foundation of China (82293630, 82293633), CAMS Innovation Fund for Medical Sciences (2022-I2M-C&T-B-121, 2023-I2M-C&T-B-123), Peking University People's Hospital Research and Development Funds (RD2023-08), Tongzhou District Distinguished Young Scholars (JCQN2023009), and the Fundamental Research Funds for the Central Universities.

The authors appreciate Dr. Feng-Ting Dao, Shuang Fan, Wen-Xuan Huo, and Meng-Zhu Shen for their help in data collection.

AUTHOR CONTRIBUTIONS

X.-D.M. and X.-S.Z. designed the study. D.-X.D., X.-H.M., Z.-H.W., X.-H.Z., L.-P.X., Y.W., C.-H.Y., H.C., Y.-H.C., W.H., F.-R.W., and J.-Z.W. conducted data collection. D.-X.D., X.-H.M., Z.-H.W., X.-D.M., and X.-S.Z. analyzed the data and drafted the manuscript. X.-D.M., X.-S.Z., and X.-J.H. reviewed the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

[1] Lv M, Shen M, Mo X. Development of allogeneic hematopoietic stem cell transplantation in 2022: regenerating "Groot" to heal the world. *Innovation (Camb)* 2023;4(1):100373.
[2] Lv M, Gorin NC, Huang XJ. A vision for the future of allogeneic hematopoietic stem cell transplantation in the next decade. *Sci Bull (Beijing)* 2022;67(19):1921–1924.
[3] Wang L, Zhang C, Fan S, Mo X, Hu X. Treatment options for adult intermediate-risk AML patients in CR1: allo-HSCT or chemotherapy? *Innovation (Camb)* 2023;4(4):100461.
[4] Cao Y, Zhang C, Cao L, Mo X, Hu X. Quizartinib is a good option for AML patients with FLT3-ITD mutations. *Innov Med* 2023;1(1):100007.
[5] Deng D, Shen M, Zhang X, et al. Basiliximab is the potential solution for severe liver chronic GVHD: a prospective pilot study. *Innov Med* 2023;1(1):100009. doi:10.59717/j.xinn-med.2023.100009.
[6] Xu LP, Lu DP, Wu DP, et al. Hematopoietic stem cell transplantation activity in China 2020-2021 during the SARS-CoV-2 pandemic: a report from the Chinese Blood and Marrow Transplantation Registry Group. *Transplant Cell Ther* 2023;29(2):136.e1–136.e7.
[7] Xu LP, Lu PH, Wu DP, et al. Hematopoietic stem cell transplantation activity in China 2019: a report from the Chinese Blood and Marrow Transplantation Registry Group. *Bone Marrow Transplant* 2021;56(12):2940–2947.
[8] Lv M, Wang Y, Chang YJ, et al. Myeloablative haploidentical transplantation is superior to chemotherapy for patients with intermediate-risk acute myelogenous leukemia in first complete remission. *Clin Cancer Res* 2019;25(6):1737–1748.
[9] Huang XJ, Zhu HH, Chang YJ, et al. The superiority of haploidentical related stem cell transplantation over chemotherapy alone as postremission treatment for patients with intermediate- or high-risk acute myeloid leukemia in first complete remission. *Blood* 2012;119(23):5584–5590.
[10] Wang Y, Liu QF, Xu LP, et al. Haploidentical vs identical-sibling transplant for AML in remission: a multicenter, prospective study. *Blood* 2015;125(25):3956–3962.
[11] Sanz J, Galimard JE, Labopin M, et al. Post-transplant cyclophosphamide after matched sibling, unrelated and haploidentical donor transplants in patients with acute myeloid leukemia: a comparative study of the ALWP EBMT. *J Hematol Oncol* 2020;13(1):46.
[12] Yu S, Huang F, Wang Y, et al. Haploidentical transplantation might have superior graft-versus-leukemia effect than HLA-matched sibling transplantation for high-risk acute myeloid leukemia in first complete remission: a prospective multicentre cohort study. *Leukemia* 2020;34(5):1433–1443.
[13] Zheng FM, Zhang X, Li CF, et al. Haploidentical- versus identical-sibling transplant for high-risk pediatric AML: a multi-center study. *Cancer Commun (Lond)* 2020;40(2-3):93–104.
[14] Poppe B, Vandesompele J, Schoch C, et al. Expression analyses identify MLL as a prominent target of 11q23 amplification and support an etiologic role for MLL gain of function in myeloid malignancies. *Blood* 2004;103(1):229–235.
[15] Meyer C, Burmeister T, Groger D, et al. The MLL recombinome of acute leukemias in 2017. *Leukemia* 2018;32(2):273–284.
[16] Schichman SA, Caligiuri MA, Gu Y, et al. ALL-1 partial duplication in acute leukemia. *Proc Natl Acad Sci U S A* 1994;91(13):6236–6239.
[17] Caligiuri MA, Schichman SA, Strout MP, et al. Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. *Cancer Res* 1994;54(2):370–373.
[18] Schnittger S, Kinkelin U, Schoch C, et al. Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. *Leukemia* 2000;14(5):796–804.
[19] Dohner K, Tobis K, Ulrich R, et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. *J Clin Oncol* 2002;20(15):3254–3261.
[20] Steudel C, Wermke M, Schaich M, et al. Comparative analysis of MLL partial tandem duplication and FLT3 internal tandem duplication mutations in 956 adult patients with acute myeloid leukemia. *Genes Chromosomes Cancer* 2003;37(3):237–251.
[21] Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* 2012;366(12):1079–1089.
[22] Caligiuri MA, Strout MP, Lawrence D, et al. Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics. *Cancer Res* 1998;58(1):55–59.
[23] Kong J, Zhao XS, Qin YZ, et al. The initial level of MLL-partial tandem duplication affects the clinical outcomes in patients with acute myeloid leukemia. *Leuk Lymphoma* 2018;59(4):967–972.
[24] Weisser M, Kern W, Schoch C, Hiddemann W, Haferlach T, Schnittger S. Risk assessment by monitoring expression levels of partial tandem duplications in the MLL gene in acute myeloid leukemia during therapy. *Haematologica* 2005;90(7):881–889.
[25] Kong J, Gao MG, Qin YZ, et al. Monitoring of post-transplant MLL-PTD as minimal residual disease can predict relapse after allogeneic HSCT in patients with acute myeloid leukemia and myelodysplastic syndrome. *BMC Cancer* 2022;22(1):11.
[26] Wang Y, Liu DH, Liu KY, et al. Long-term follow-up of haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for the treatment of leukemia: nine years of experience at a single center. *Cancer* 2013;119(5):978–985.
[27] Shen MZ, Hong SD, Lou R, et al. A comprehensive model to predict severe acute graft-versus-host disease in acute leukemia patients after haploidentical hematopoietic stem cell transplantation. *Exp Hematol Oncol* 2022;11(1):25.

[28] Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. *Blood* 2021;138(26):2753–2767.
[29] Shen MZ, Zhang XH, Xu LP, et al. Preemptive interferon-alpha therapy could protect against relapse and improve survival of acute myeloid leukemia patients after allogeneic hematopoietic stem cell transplantation: long-term results of two registry studies. *Front Immunol* 2022;13:757002.
[30] Cai Z, Fan S, Sun X, Mo X, Yang G. Novel microfluidic device for measurable residual disease detection in acute leukemia. *Innovation (Camb)* 2023;4(3):100408.
[31] Chang YJ, Wang Y, Liu YR, et al. Haploidentical allograft is superior to matched sibling donor allograft in eradicating pre-transplantation minimal residual disease of AML patients as determined by multiparameter flow cytometry: a retrospective and prospective analysis. *J Hematol Oncol* 2017;10(1):134.
[32] Terwijn M, van Putten WL, Kelder A, et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. *J Clin Oncol* 2013;31(31):3889–3897.
[33] Freeman SD, Virgo P, Couzens S, et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. *J Clin Oncol* 2013;31(32):4123–4131.
[34] Zhang XH, Chen J, Han MZ, et al. The consensus from The Chinese Society of Hematology on indications, conditioning regimens and donor selection for allogeneic hematopoietic stem cell transplantation: 2021 update. *J Hematol Oncol* 2021;14(1):145.
[35] Tong X, Li M, Jin J, et al. Cladribine- and decitabine-containing conditioning regimen has a low post-transplant relapse rate in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome. *Int J Cancer* 2023;152(10):2123–2133.
[36] Sun Y, Zhang J, Long B, et al. A novel intensive conditioning regimen for allogeneic hematopoietic stem cell transplantation in the treatment of relapsed/refractory acute myeloid leukemia. *Neoplasma* 2021;68(6):1351–1358.
[37] Li Y, Cheng L, Xu C, et al. A retrospective observation of treatment outcomes using decitabine-combined standard conditioning regimens before transplantation in patients with relapsed or refractory acute myeloid leukemia. *Front Oncol* 2021;11:702239.
[38] Fan S, Shen MZ, Zhang XH, et al. Preemptive immunotherapy for minimal residual disease in patients with t(8;21) acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation. *Front Oncol* 2021;11:773394.
[39] Biederstadt A, Rezvani K. How I treat high-risk acute myeloid leukemia using preemptive adoptive cellular immunotherapy. *Blood* 2023;141(1):22–38.
[40] Solomon SR, Sizemore CA, Zhang X, et al. Preemptive DLI without withdrawal of immunosuppression to promote complete donor T-cell chimerism results in favorable outcomes for high-risk older recipients of alemtuzumab-containing reduced-intensity unrelated donor allogeneic transplant: a prospective phase II trial. *Bone Marrow Transplant* 2014;49(5):616–621.
[41] Stadler M, Venturini L, Bunting I, et al. Navigating preemptive and therapeutic donor lymphocyte infusions in advanced myeloid malignancies by high-sensitivity chimerism analysis. *Front Oncol* 2022;12:867356.
[42] Yan C-H, Liu D-H, Liu K-Y, et al. Risk stratification–directed donor lymphocyte infusion could reduce relapse of standard-risk acute leukemia patients after allogeneic hematopoietic stem cell transplantation. *Blood* 2012;119(14):3256–3262.
[43] Molica M, Breccia M, Foa R, Jabbour E, Kadia TM. Maintenance therapy in AML: the past, the present and the future. *Am J Hematol* 2019;94(11):1254–1265.
[44] Nayak RK, Chen YB. Maintenance therapy for AML after allogeneic HCT. *Front Oncol* 2022;12:895771.
[45] Xuan L, Wang Y, Huang F, et al. Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. *Lancet Oncol* 2020;21(9):1201–1212.
[46] Yu WJ, Jia JS, Wang J, et al. [Short-term efficacy of venetoclax combined with azacitidine in acute myeloid leukemia: a single-institution experience]. *Zhonghua Xue Ye Xue Za Zhi* 2022;43(2):134–140.
[47] Cho BS, Min GJ, Park S, et al. Haploidentical vs matched unrelated donor transplantation for acute myeloid leukemia in remission: a prospective comparative study. *Am J Hematol* 2021;96(1):98–109.
[48] Mo X, Zhang W, Fu G, et al. Single-cell immune landscape of measurable residual disease in acute myeloid leukemia. *Sci China Life Sci* 2024. doi:10.1007/s11427-024-2666-8.
[49] Morris ES, MacDonald KP, Rowe V, et al. NKT cell-dependent leukemia eradication following stem cell mobilization with potent G-CSF analogs. *J Clin Invest* 2005;115(11):3093–3103.
[50] Robb RJ, Kreijveld E, Kuns RD, et al. Type I-IFNs control GVHD and GVL responses after transplantation. *Blood* 2011;118(12):3399–3409.
[51] Fan S, Pan TZ, Dou LP, et al. Preemptive interferon-α therapy could prevent relapse of acute myeloid leukemia following allogeneic hematopoietic stem cell transplantation: a real-world analysis. *Front Immunol* 2023;14:1091014.