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ORIGINAL ARTICLE

Classifying AML patients with inv(16) into high-risk and low-risk relapsed patients based on peritransplantation minimal residual disease determined by *CBFβ/MYH11* gene expression

Zhao Xiaosu[1,2,3] · Cao Leqing[1,2] · Qin Yazhen[1,2] · Wang Yu[1,2,3] · Zhang Xiaohui[1,2,3] · Xu Lanping[1,2] · Huang Xiaojun[1,2,3] · Chang Yingjun[1,2,3]

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Abstract

Ninety acute myeloid leukemia (AML) patients with inv(16) were monitored *CBFβ/MYH11* transcript around allogeneic hematopoietic stem cell transplantation (allo-HSCT). A total of 23 patients received HLA-matched sibling donor transplantation (MSDT) and 67 patients received unmanipulated haploidentical hematopoietic stem cell transplantation (haplo-HSCT) were analyzed in this study. Patients were divided into four groups based on *CBFβ/MYH11* expression prior to transplantation (pre-MRD): with negative (group 1)/positive (group 2) pre-MRD before MSDT; with negative (group 3)/positive (group 4) pre-MRD before haplo-HSCT. The results showed that patients in group 2 had the highest cumulative incidence of relapse (2-year CIR, 40.7%), the lowest leukemia-free survival (2-year LFS, 50.8%), and overall survival (2-year OS, 62.5%). The other three groups of patients had comparable outcomes. The patients were also classified into the other three groups according to *CBFβ/MYH11* value of + 1 month after transplantation: group 5: pre- and post-transplant MRD were both negative; group 6: the value of post-transplant MRD was lower than 0.2%; group 7: the value of post-transplant MRD was higher than 0.2%. Group 7 had the highest CIR and the lowest LFS. These results indicated that AML patients with inv(16) were able to be separated into high-risk and low-risk relapse groups based on peritransplant MRD determined by RQ-PCR-based *CBFβ/MYH11*. Haplo-HSCT might overcome the negative impact of pre-MRD on patient outcomes compared to MSDT.

Keywords Acute myeloid leukemia · Allogeneic hematopoietic stem cell transplantation · Minimal residual disease · *CBFβ/MYH11* gene

Introduction

A subtype of patients with core-binding factor acute myeloid leukemia (AML) is characterized by the presence of inv(16)-(p13q22) and rarely by a translocation t(16;16)(p13;q22). The final genetic product is the fusion of the CBFβ gene at 16q22 to the smooth muscle myosin heavy chain (MYH11) at 16p13, which is termed as *CBFβ/MYH11* fusion gene. Although this type of AML is considered a favorable cytogenetic subgroup both in NCCN guideline or ELN classification, in particular with the application of high-dose cytarabine-based consolidation chemotherapy regimens [1, 2], disease relapse remains one of the most important causes leading to death, occurring in around 35% of patients [3, 4]. Thus, for this cohort of patients, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is needed to as salvage treatment to improve the overall survival.

It has been reported that when patients achieved hematological remission, the MRD prior to transplantation (pre-MRD) has been demonstrated to be a useful parameter to predict leukemia relapse after allo-HSCT [5–8]. In addition to leukemia-associated immunophenotypes (LAIPs)

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✉ Chang Yingjun
pk200608@126.com

[1] Present address: Peking University People's Hospital, Peking University Institute of Hematology, No.11 Xizhimen South Street, Beijing 100044, China

[2] Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Peking University People's Hospital, Peking University Institute of Hematology, Beijing, China

[3] Collaborative Innovation Center of Hematology, Peking University, Beijing, China

measured by flow cytometry (FCM), leukemia-specific genes and other pan leukemia molecular marker, such as Wilms' tumor suppressor gene (*WT1*) have also been proved to be able to assist risk stratification before transplantation [5, 7, 9, 10]. Evaluating minimal residual disease (MRD) by real-time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) can quantify the expression level of fusion transcript at different time points along with the treatment. More recently, *CBFβ/MYH11* fusion gene has been confirmed to be a good MRD marker and further screening out the patients in high-risk of relapse either during the treatment or after transplantation [11–13]. However, due to the limit number of patients, there have been no studies about predictive value of *CBFβ/MYH11*expression after allo-HSCT.

Therefore, in this study, we attempted to investigate the impact of pre-MRD determined by RQ-PCR-based *CBFβ/MYH11* gene expression on transplant outcomes of AML patients with inv(16)(p13q22) or t(16;16)(p13;q22). Besides, our previous studies has demonstrated that in compared to HLA-identical transplantation, haploidentical allo-HSCT had superior graft-versus-leukemia (GVL) effect in AML patients with standard risk [5, 14–16]. Thus, here, we also wanted to know whether the level of *CBFβ/MYH11* pre-transplant could further divide this form of AML into high-risk and low-risk relapse patients before allo-HSCT.

Patients and methods

Study design

The study population consisted of 90 AML patients with *CBFβ/MYH11* fusion gene who were diagnosed and treated at Peking University People's Hospital between September 2006 and January 2018.These patients were diagnosed with inv(16) in bone marrow samples by karyotyping and/or detection of *CBFβ/MYH11* fusion gene. Patients were eligible for the study if they achieved complete remission (CR) before transplantation. Fifty-three of all 90 patients enrolled in this study were previously included in another investigation we previously reported (PMID: 27650511) and we updated these patients' follow-up. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Peking University. All of the included subjects provided written informed consent. Twenty-three patients who received HLA-matched sibling donor transplantation (MSDT) and sixty-seven patients who received unmanipulated haploidentical HSCT (haplo-HSCT) were enrolled in this study. Patient characteristics are summarized in Table 1.

Chemotherapy

Induction chemotherapy for these patients included daunorubicin (45 mg/m^2) or idarubicin (8–10 mg/m^2) for 3 days in combination with cytarabine (100 mg/m^2) for 7 days. The first two consolidation chemotherapies consisted of intermediate-dose cytarabine (IDAC; 1–2 g/m^2 every 12 h for 3 days) with or without an anthracycline (daunorubicin at 45 mg/m^2 or mitoxantrone at 8 mg/m^2 for 3 days). Then, patients received maintenance chemotherapy as described in our previous study [12].

Transplant protocol

All the patients in this study received myeloablative conditioning regimens. Unmanipulated haplo-SCT and HLA-identical sibling donor transplantation was performed according to protocols reported previously by our group [17, 18]. In brief, the conditioning therapy was busulfan (BU, 0.8 mg/kg i.v., q.i.d.) and cyclophosphamide (CTX, 1.8 g/m^2/d for 2 days) for patients who had a matched sibling. HLA-mismatched patients were conditioned with BU + CTX + human antithymocyte globulin (ATG, 2.5 mg/kg/d i.v. for 4 days) (Lyons, France). Prophylaxis against GVHD included treatment with CsA and short-term methotrexate (MTX) along with mycophenolatemofetil (MMF).

Donor lymphocyte infusion (DLI)

DLI was performed as previously described. The indications for DLI included hematological leukemia relapse, receiving chemotherapy followed by DLI, or positive MRD detection as previously described [19].

Detection of MRD

In this study, MRD was evaluated by the level of *CBFβ/MYH11* fusion gene. The pre-transplant *CBFβ/MYH11* measurement was performed using BM samples within a month before the transplant as a routine. The post-transplant scheduled time points were + 1, + 2, + 3, + 4.5, + 6, + 9, and + 12-month post-HSCT and every 6 months thereafter. More frequent MRD monitoring was performed in some patients depending on their individual intention.

RNA extraction and cDNA synthesis TRIzol reagent (Invitrogen, Carlsbad, CA) was used to extract total RNA. A High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) was used to synthesize cDNA.

TaqMan-based real-time quantitative polymerase chain reaction (RQ-PCR) technology was used to detect *CBFβ/MYH11* transcript levels, as described in our previous study [20]. The primers and probes for ABL and *CBFβ/MYH11* were taken from the report of the Europe Against Cancer

Table 1 Patient and donor characteristics

Characteristics	All patients	MDST ($n = 23$)		p value	HBMT ($n = 67$)		p value
		Pre-MRDneg	Pre-MRDpos		Pre-MRDneg	Pre-MRDpos	
Number of patients	90	14 (60.9%)	9 (39.1%)		30 (44.8%)	37 (55.2%)	
Median age (range),years	31 (5–56)	37 (6–56)	45 (19–56)	0.183	27 (5–51)	30 (15–54)	0.169
Male sex, *n* (%)	61 (67.7%)	5 (35.7%)	9 (55.6%)	0.417	19 (63.3%)	28 (75.7%)	0.272
Disease status, *n* (%)				1.000			0.867
CR1	70 (77.7%)	11 (78.6%)	7 (77.8%)		23 (76.7%)	29 (78.4%)	
CR > 1	20 (22.2%)	3 (21.4%)	2 (22.2%)		7 (23.3%)	8 (21.6%)	
c-KIT mutation	22 (24.4%)	1 (7.1%)	1 (11.1%)	1.000	7 (23.3%)	13 (35.1%)	0.233
HLA-A, B, DR mismatched grafts, *n* (%)				NA			0.249
0	23 (25.6%)	14 (60.9%)	9 (39.1%)		0	0	
1	2 (2.2%)	0			2 (6.7%)	0	
2	10 (11.1%)	0			3 (10.0%)	7 (18.9%)	
3	55 (61.1%)	0			25 (83.3%)	30 (81.1%)	
Donor-recipient relationship, *n* (%)				0.688			0.213
Parent-child	31 (32.5%)	1 (7.1%)	1 (11.1%)		16 (53.3%)	13 (35.1%)	
Sibling-sibling	40 (44.4%)	12 (85.7%)	8 (88.9%)		9 (30.0%)	11 (29.7%)	
Child-parent	16 (17.8%)	1 (7.1%)	0		5 (16.7%)	10 (27.0%)	
Other	3 (3.3%)	0	0		0	3 (8.1%)	
ABO matched grafts, *n* (%)				0.610			0.145
Matched	44 (48.9%)	8 (57.1%)	5 (55.6%)		12 (40.0%)	19 (51.4%)	
Major mismatch	15 (16.7%)	4 (28.6%)	1 (11.1%)		6 (20.0%)	4 (10.8%)	
Minor mismatch	26 (28.9%)	1 (7.1%)	2 (22.2%)		9 (30.0%)	14 (37.8%)	
Bi-directional mismatch	5 (5.6%)	1 (7.1)	1 (11.1%)		3 (10.0%)	0	
Cell compositions in allografts							
Infused nuclear cells, (range) 10^8/kg	7.73 (1.73–11.55)	7.82 (4.06–9.15)	7.33 (4.37–9.97)	0.988	8.05 (6.03–11.55)	7.67 (1.73–11.48)	0.118
Infused $CD34^+$ cells, (range) 10^6/kg	2.61 (0.45–7.2)	2.67 (1.07–6.41)	2.78 (1.06–6.43)	0.491	2.49 (1.16–6.7)	2.63 (0.45–7.2)	0.441
II–IV°aGVHD	52 (21.1%)	1 (7.1%)	1 (11.1%)	1.000	9 (30.0%)	8 (21.6%)	0.064
cGVHD	47 (52.2%)	7 (50.0%)	4 (44.4%)	0.712	12 (40.0%)	24 (64.7%)	0.101
DLI after transplant, *n* (%)				NA			NA
For intervention	9 (10.8%)	0	1 (11.1%)		1 (3.3%)	1 (2.7%)	
For relapse treatment	5 (6.0%)	0	0		1 (3.3%)	2 (5.4%)	

Abbreviations: *HLA*, human leukocyte antigen; *MSDT*, HLA-matched sibling donor transplantation; *haplo-HSCT*, haploidentical blood and marrow transplantation; *MRD*, minimal residual disease; *Pre-MRDpos*, positive MRD status before transplantation; *Pre-MRDneg*, negative MRD status before transplantation; *CR*, complete remission; *DLI*, donor lymphocyte infusions

Program [21, 22]. The transcript level was calculated as the percentage of *CBFβ/MYH11* transcript copies/ABL copies. As we previously reported, cDNA was used to perform PCR to detect c-KIT mutations in exons 17 and 8 [23].

Definitions

The primary study end point was the cumulative incidence of leukemia relapse (CIR). The secondary end points were the cumulative incidences of treatment-related mortality (TRM) and the probabilities of leukemia-free survival (LFS) and overall survival (OS). Engraftment, infection, TRM, relapse, LFS, OS, acute graft-versus-host disease (aGVHD), and chronic GVHD (cGVHD) were defined as previously described [24]. The time to GVHD was defined as the time from transplantation to the onset of GVHD of any grade.

Since our previous study confirmed that a *CBFβ/MYH11* expression > 0.2% after two courses of consolidation chemotherapy was an independent adverse prognostic factor for transplant outcomes and was regarded as an indication for allo-HSCT [12], positive pre-MRD (pre-MRDpos) was defined as a *CBFβ/MYH11* expression > 0.2% before transplant. Post-

transplant MRD (post-MRD) was mainly focused on the MRD status on + 1 month after HSCT. The cut-off value of *CBFβ/MYH11* expression on + 1 month after transplantation was also set at 0.2%. Positive post-MRD (post-MRDpos) was defined as a *CBFβ/MYH11* expression > 0.2% at + 1 month after transplantation.

Statistical analysis

The reference date of February 28, 2018, was used to define the end of follow-up. The median follow-up was 923 days (range: 55 to 4180 days). Patient characteristics of the MRDpos and MRDneg groups were compared with the χ^2 statistic for categorical variables and the Mann–Whitney test for continuous variables. To calculate TRM probabilities, cumulative incidence curves were used in a competing risk setting, with relapse being treated as a competing event, and with death from any cause being treated a competing risk for GVHD, engraftment, and relapse. The probabilities of LFS and OS were estimated with the Kaplan–Meier method. MRD status pre-transplantation, dynamic of MRD, and all variables in Table 1 were included in the univariate analysis. *p* values were based on two-sided hypothesis tests. Alpha was set at 0.05. Most analyses were performed with SPSS 22.0 (Mathsoft, Seattle, WA, USA).

Results

Patient characteristics

A total of 90 patients who received HLA-matched sibling donor transplantation (MSDT, $n = 23$) and unmanipulated haploidentical HSCT (haplo-HSCT, $n = 67$) were enrolled in this study. All patients had less than 5% bone marrow blasts and met the morphological criteria for a complete remission (including CR1/2/3). Table 1 included the characteristics of these patients, including their age, sex, disease status, c-KIT mutation status, HLA-mismatched grafts, donor-recipient relationship, ABO matched grafts, cell composition in allografts, grade 2–4 a & cGVHD, and DLI status based on transplant type. All patients achieved stable neutrophil engraftment at a median 13 days (range, 8–28 days). During the follow-up, 86 (95.6%) patients achieved platelet engraftment at a median 14 days (range, 8–108 days) after HSCT. Among the patients undergoing MSDT and haplo-HSCT, there was no significant difference between pre-transplant MRD (determined by *CBFβ/MYH11* gene expression) positive and negative subgroups in the above clinical characteristics. A total of 14 patients received DLI, which was given for relapse intervention ($n = 9$) and treatment ($n = 5$). The detailed numbers for patients who received DLI for various reasons are shown in Table 1.

Clinical outcomes based on the transplant type

Among the patients who received MSDT, the three patients (13.0%) underwent the hematological relapse at a median of 270 days (range, 85–300 days) after HSCT. Seven patients (10.4%) who received haplo-HSCT underwent hematological relapse at a median of 219 days (range, 101–523 days) after HSCT. Based on the results of the analysis, it seemed that patients who received haplo-HSCT were able to achieve comparable outcomes compared to those who underwent MSDT (2-year CIR: 15.2 ± 8.1% vs. 12.6 ± 4.5%, $p = 0.714$; 2-year TRM: 17.2 ± 9.2% vs. 11.0 ± 3.9%, $p = 0.872$; 2-year LFS: 75.6 ± 9.5% vs.77.6 ± 5.3%, $p = 0.803$; 2-year OS: 80.1 ± 8.9% vs. 80.2 ± 5.2%, $p = 0.857$).

Correlation between pre-MRD determined by *CBFβ/MYH11* expression and clinical outcomes after allo-HSCT

Among all 90 AML patients with *CBFβ/MYH11* expression, there were 46 (51.1%) patients with positive pre-MRD. The median expression level was 1.2% (0.22–51.5%). Patients undergoing MSDT and haplo-HSCT were classified into four groups based on the status of pre-MRD: group 1 with pre-MRD ≤ 0.2% before MSDT ($n = 14$), group 2 with pre-MRD > 0.2% before MSDT ($n = 9$), group 3 with pre-MRD ≤ 0.2% before haplo-HSCT ($n = 30$), and group 4 with pre-MRD > 0.2% before haplo-HSCT ($n = 37$). The incidences of grade 2–4 aGVHD and cGVHD between pre-MRDpos and pre-MRDneg patients in MDST group were comparable ($p = 1.000$ & $p = 0.845$, respectively, Table 2). Though the cumulative incidence of grade 2–4 aGVHD and cGVHD in pre-MRDpos patients was slightly higher than that of pre-MRDneg patients who received haplo-HSCT; there was no significant difference ($p = 0.228$ & $p = 0.320$, Table 2).

Then, we compared the clinical outcomes of patients with or without pre-MRD who received either the MSDT or haplo-HSCT. The cumulative incidence of relapse (CIR) of group 1, group 3, and group 4 was comparable (2-year CIR: group 1 vs. group 3 vs. group 4, 0 vs. 8.4% vs. 16.0%, $p > 0.05$), and all of these values were significantly lower than those of group 2 (2-year CIR, 40.7%, 95%CI: 22.2–59.2%, for all $p < 0.05$) except group 4 ($p = 0.105$). However, the survival curves showed that the CIR of group 2 was still slightly higher than that of group 4 (Fig. 1a). The cumulative incidence of TRM was also comparable among these four groups (2-year LFS: group 1 vs. group 2 vs. group 3 vs. group 4, 8.3% vs. 14.3% vs. 6.8% vs. 13.7%, $p > 0.05$, Fig. 1b). The LFS probabilities of group 1, group 3, and group 4 were comparable (2-year LFS: group 1 vs. group 3 vs. group 4, 91.7% vs 85.4% vs. 72.2%, $p > 0.05$, Table 2 and Fig. 1c), and all of these values were obviously higher than those of group 2 (2-year LFS, 50.8%, 95%CI: 33.1–68.5%, $p < 0.05$ for all). Though the difference between group

2 and group 4 lacked statistical significance ($p = 0.140$), it seemed that the patients of group 2 had the lowest overall survival (2-year OS, 62.5%, 95%CI: 45.4–79.6%) among all four groups (group 1 vs. group 3 vs. group 4, 91.7% vs.85.2% vs. 76.3%, $p > 0.05$, Table 2 and Fig. 1d). Thus, the patients of group 2 would have a considerably worse prognosis than those of group 1 (CIR, $p = 0.017$; LFS, $p = 0.004$; OS, $p < 0.026$). However, there were no significant differences between patients with or without pre-MRD in the haplo-HSCT group. In addition, the clinical outcomes of patients receiving haplo-HSCT were comparable to those patients in group 2.

Correlation between post-MRD determined by *CBFβ/MYH11* expression and clinical outcomes after allo-HSCT

To further investigate the correlation between post-MRD and patient outcomes after allo-HSCT, the patients were also divided into the following three groups on the basis of *CBFβ/MYH11* value of + 1 month and pre-transplant MRD status: group 5: pre- and post-transplant MRD were both negative; group 6: the value of post-transplant MRD was lower than 0.2%; group 7: the value of post-transplant MRD was higher than 0.2%. The clinical outcomes of above three groups were shown in Fig. S1. It seemed that patients in group 7 had the highest 2-year CIR (50%) compared to groups 5 and 6 (12.5% & 10.0%, $p = 0.217$ & $p = 0.001$, respectively, Fig. S1A) and the lowest 2-year LFS (37.5% vs. 87.5% & 80.7%, $p = 0.072$ & $p = 0.002$, respectively, Fig. S1C), though the TRM and OS of these three groups were comparable ($p > 0.05$, Figs. S1B and S1D).

Discussion

In both NCCN clinical practice guidelines in oncology (NCCN guideline) and the European Leukemia Net (ELN) recommendations, AML with inv(16)(p13q22) or t(16;16)(p13;q22) is considered to have a favorable outcome [25]. Through examining the expression level of *CBFβ/MYH11* gene, the pending relapse of patients could be predicted well. Corbacioglu, A et al. has reported that *CBFβ/MYH11* monitoring during consolidation and the early follow-up period allowed for the identification of patients who are at high risk of relapse [11]. Similarly to their results, our study showed that for patients receiving chemotherapy/autologous HSCT, a *CBFβ/MYH11* level > 0.2% after course 2 consolidation indicated a higher CIR and a lower DFS & OS [12]. In addition, our recent study demonstrated that *CBFβ/MYH11*-based MRD status during the first 3 months after allo-HSCT, but not c-KIT mutations, can be used to identify patients with a high risk of relapse [13]. However, due to the small number of this type of patients, especially the much less patients who underwent an allo-HSCT, there has been no such study that investigates the impact of pre-transplant *CBFβ/MYH11* value on the outcomes. To our knowledge, it is the largest cohort of transplant patients with *CBFβ/MYH11*-positive AML.

For the AML patients with *CBFβ/MYH11* who met the indications for transplantation, there has been no precise and accepted parameter before transplant to predict the upcoming relapse. With regard to c-KIT mutation, emerging data including our results indicate that the presence of c-KIT mutations in patients with t(8;21), and to a lesser extent inv(16), confers a higher risk of relapse [23, 26–28]. Therefore, whether c-KIT mutation is a real marker to a poor prognosis is still unclear. Though multicolor flow cytometry has become one of the commonly used methods to monitoring MRD, the sensitivity and specificity are still not satisfactory in AML evaluation. Furthermore, the number of patients with MRD determined by FCM is limited. Thus, it is more interesting to explore the impact of pre-transplant *CBFβ/MYH11* value on the transplant outcomes. Our results proved that the expression level of this fusion gene before transplantation was able to provide important prognostic information for further risk stratification of

Table 2 Transplant outcomes for patients that underwent allogeneic stem cell transplantations

	MSDT		Haplo-HSCT	
	Pre-MRDneg	Pre-MRDpos	Pre-MRDneg	Pre-MRDpos
Grades 2–4 acute GVHD	7.1% (95%CI, 0.2 to 14.0%)	11.1% (95%CI, 0.6 to 21.6%)	51.2% (95% CI, 37.7 to 64.7%)	27.0% (95% CI, 18.5 to35.5%)
Chronic GVHD at 2 years	32.5% (95%CI, 20.4 to 44.6%)	66.7% (95%CI, 47.5 to 85.9%)	52.1% (95% CI, 41.5 to 62.7%)	70.2% (95% CI, 62.0 to 78.4%)
Cumulative incidence of relapse at 2 years	0	40.7% (95%CI, 22.2 to 59.2%)	8.4% (95% CI, 2.7 to 14.1%)	16.0% (95% CI, 9.4 to 22.6%)
Cumulative incidence of TRM at 2 years	8.3% (95%CI, 0.3 to 16.3%)	14.3% (95%CI, 1.1 to 27.5%)	6.8% (95% CI, 2.2 to 11.4%)	13.7% (95% CI, 8.0 to 19.4%)
Leukemia-free survival at 2 years	91.7% (95% CI, 83.7 to 99.7%)	50.8% (95% CI, 33.1 to 68.5%)	85.4% (95% CI, 78.6 to 92.2%)	72.2% (95% CI, 54.7 to 89.7%)
Overall survival at 2 years	91.7% (95%CI, 83.7 to 99.7%)	62.5% (95% CI, 45.4 to 79.6%)	85.2% (95% CI, 78.3 to 92.1%)	76.3% (95% CI, 68.8 to 83.8%)

Abbreviations: *MSDT*, human leukocyte antigen matched sibling donor transplantation; *haplo-HSCT*, haploidentical stem cell transplantation; *MRD*, minimal residual disease; *Pre-MRDpos*, positive MRD status before transplantation; *Pre-MRDneg*, negative MRD status before transplantation; *GVHD*, graft-versus-host disease; *TRM*, treatment-related mortality

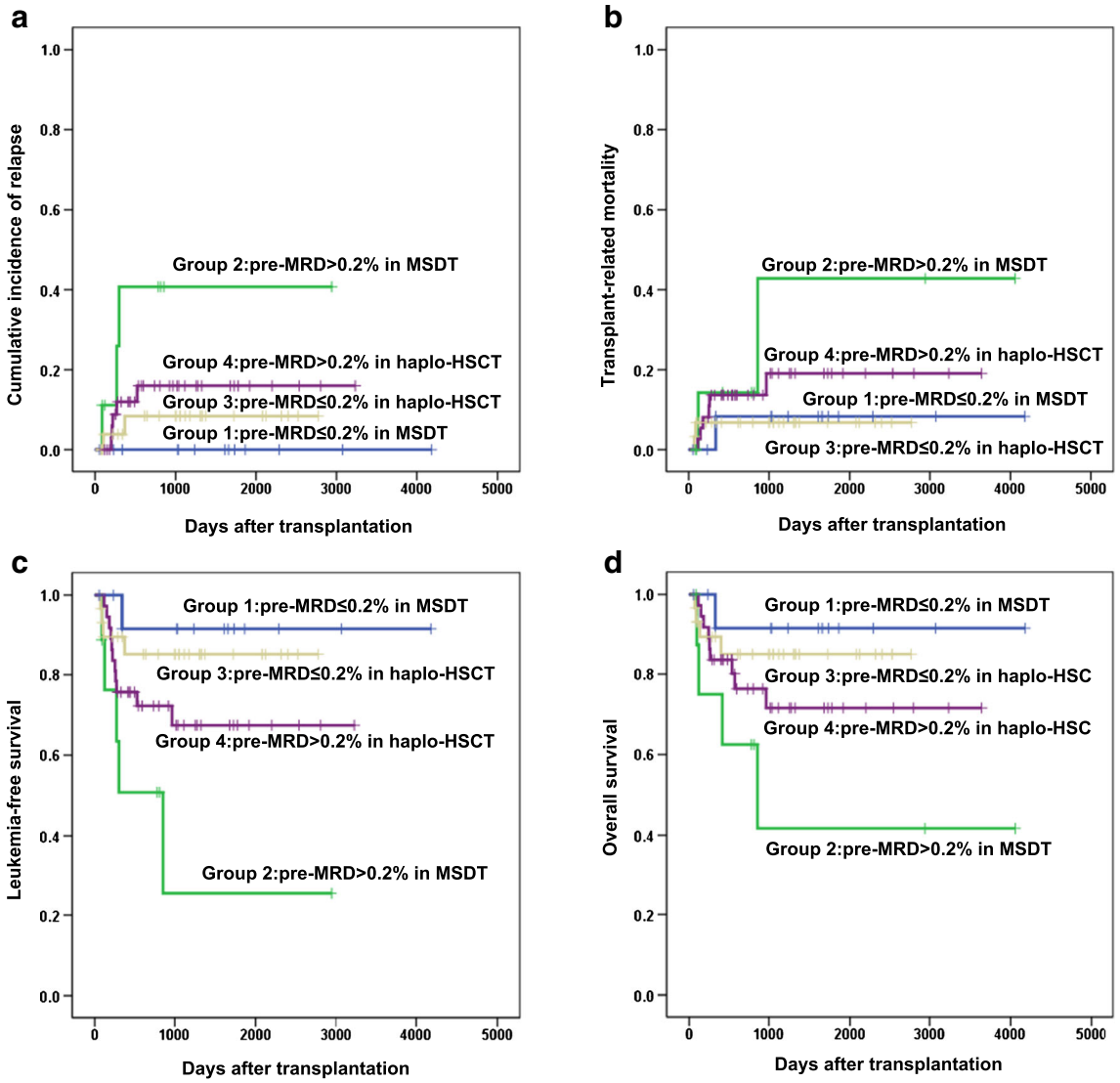

Fig. 1 The clinical outcomes of *CBFβ/MYH11*+ AML patients receiving MSDT and haplo-HSCT with or without positive MRD pre-transplant based on RQ-PCR ($n = 90$). Estimates of the **a** cumulative incidence of relapse (CIR), **b** cumulative incidence of treatment-related mortality (TRM), **c** leukemia-free survival (LFS), and **d** overall survival (OS). p values between the four groups: CIR: 1 vs. 2, $p = 0.017$; 1 vs. 3, $p = 0.323$; 1 vs. 4, $p = 0.152$; 2 vs. 3, $p = 0.026$; 2 vs. 4, $p = 0.105$; 3 vs. 4, $p = 0.431$. TRM: 1 vs. 2, $p = 0.170$; 1 vs. 3, $p = 0.973$; 1 vs. 4, $p = 0.396$; 2 vs. 3, $p = 0.154$; 2 vs. 4, $p = 0.416$; 3 vs. 4, $p = 0.305$; LFS: 1 vs. 2, $p = 0.004$; 1 vs. 3, $p = 0.549$; 1 vs. 4, $p = 0.108$; 2 vs. 3, $p = 0.007$; 2 vs. 4, $p = 0.071$; 3 vs. 4, $p = 0.192$. OS: 1 vs. 2, $p = 0.026$; 1 vs. 3, $p = 0.543$; 1 vs. 4, $p = 0.184$; 2 vs. 3, $p = 0.050$; 2 vs. 4, $p = 0.140$; 3 vs. 4, $p = 0.361$. Pre-MRD, MRD status before transplantation

AML patients with inv(16) and guide the appropriate and necessary strategy for relapse prevention.

The results of our previous studies have indicated the more intensive GVL effect of haplo-HSCT than MSDT. Early data showed that the 2-year CIR of the patients with high-risk acute leukemia was significantly lower in haplo-HSCT (26%) than in MSDT (49%) [29]. A retrospective study of Zhao et al. also investigated a small cohort of AML patients in standard risk ($n = 86$) and found that the MRD status prior to haplo-HSCT might not affect leukemia relapse but this phenomena was not observed in MSDT. More recently, Chang et al. further confirmed this conclusion through a retrospective cohort ($n = 339$) and validated it by a prospective cohort of patients ($n = 340$) [16]. Their results indicated that, for pre-MRD-positive AML patients, haplo-HSCT was associated with lower incidence of relapse and better survival, suggesting a stronger GVL effect as well as in pediatric AML patients [15]. Moreover, for the patients with FLT3-ITD mutation, haplo-HSCT might also overcome the negative impact of pre-transplant MRD on patient outcomes compared to MSDT [14]. The objects of this study included the patients who belong to the group with favorable outcomes, but met the indications of allo-HSCT. In consistent with the above perspective, the patients with positive pre-MRD in MSDT group still

had the highest CIR in compared to those in the other three groups (groups 1, 3, & 4), especially in group 4. Our results further confirmed the more intensive GVL effect of haplo-HSCT.

Here, *CBFβ/MYH11* expression level pre-transplant was used as a marker to screening out the patients in high-risk. In our center, the median *CBFβ/MYH11* transcript level at diagnosis is around 200%, which is considered as the baseline. Our previous studies focusing on both after consolidation and after transplantation showed that achieving a 3-log reduction in *CBFβ/MYH11* transcript, which was defined as major molecular remission (MMR), indicated a better DFS [12, 13]. Thus, in this study, 0.2% was defined as the cut-off value to separate patients into a different risk group before allo-HSCT. The results showed that the CIR of group 2 (with pre-MRD > 0.2% before MSDT) was about 40% which was similar to that of refractory/relapsed AML patients. This data indicate that for this group of patients, more active intervention to prevent relapse after transplantation might be applied, for example, prophylactic DLI or interferon α injection. However, if the patients received haplo-HSCT, they might not need excessive intervention.

In this study, we also investigated the dynamic change of *CBFβ/MYH11* transcript around allo-HSCT. The results indicated that patients who achieved a MMR (< 0.2%) at the first month after transplantation would have a better CIR and LFS regardless of the expression level of *CBFβ/MYH11* transcript before transplantation. It suggested that for those patients in group 2, once they achieved MMR in the first month, above active treatment for preventing relapse might not be needed. Hence, we recommend that the relapse intervention should refer to the values of *CBFβ/MYH11* fusion gene both before and after transplantation.

Although two types of AML constitute core-binding factor AML (CBF-AML), and also share common clinical characteristics, they have different clinical features. The clinical outcome of AML with *CBFβ/MYH11* fusion gene was not similar to that of AML with t(8;21). It would have lower risk of relapse. Kuwatsuka Y et al. has demonstrated that the overall survival of patients with inv(16) was favorable beyond the first CR and patients with inv(16) in the second/third CR, or even non-CR patients, had comparable outcomes after allo-HSCT. But, it was not the case in AML with t(8;21) [30]. In consistent with their conclusion, we also found that the status of CR did not have any influence on CIR. Only the level of pre-MRD was associated to the relapse after transplantation in univariate analysis. Therefore, achieving MMR at the time of transplant was a very important prognostic factor regardless of the mutation status of c-KIT.

Although this study was a retrospective, single center study and the number of enrolled patients was limited, it included only AML with *CBFβ/MYH11* expression. Besides, the chemotherapy regimen was relative consistent. Thus, the conclusion of this study was reliable to some extent. In future, a multicenter, prospective, large-scale study is necessary.

In conclusion, as far as we know, this was the first study to show that AML patients with *CBFβ/MYH11* were able to be divided into high-risk and low-risk relapse groups based on pre-transplantation MRD determined by RQ-PCR. Moreover, haplo-HSCT might overcome the negative impact of pre-MRD on patient outcomes compared to MSDT.

Author contributions Y.-J.C. designed the study; X.-S. Z. and L.-Q.C. collected and analyzed the data; X.-S. Z. drafted the manuscript; all authors contributed to data interpretation, manuscript preparation, and approval of the final version.

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Compliance with ethical standards

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

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