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

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Abstract

Background: MLL-PTD is a special MLL rearrangement gene that occurs in about 5-10% of acute myeloid leukemia (AML) with a normal karyotype and in 5-6% of myelodysplastic syndrome (MDS) patients. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is currently one of the curative therapies available for AML and MDS with excess blasts (MDS-EB). However, how the prognosis of patients with high levels of MLL-PTD after allo-HSCT, including AML and MDS, and whether MLL-PTD could be used as a reliable indicator for minimal residual disease (MRD) monitoring in transplant patients remains unknown. Our study purposed to analyze the dynamic changes of MLL-PTD peri-transplantation and the best threshold for predicting relapse after transplantation.

Methods: We retrospectively collected the clinical data of 48 patients with MLL-PTD AML or MDS-EB who underwent allo-HSCT in Peking University People's Hospital. The MLL-PTD was examined by real-time quantitative polymerase chain reaction (RQ-PCR) at the diagnosis, before transplantation and the fixed time points after transplantation. Detectable MLL-PTD/ABL>0.08% was defined as MLL-PTD positive in this study.

Results: The 48 patients included 33 AML patients and 15 MDS-EB patients. The median follow-up time was 26(0.7-56) months after HSCT. In AML patients, 7 patients (21.2%) died of treatment-related mortality (TRM), 6 patients (18.2%) underwent hematological relapse and died ultimately. Of the 15 patients with MDS-EB, 2 patients (13.3%) died of infection. The 3-year cumulative incidence of relapse (CIR), overall survival (OS), disease-free survival (DFS) and TRM were 13.7%±5.2%, 67.8%±6.9%, 68.1%±6.8% and 20.3%±6.1%, respectively. ROC curve showed that post-transplant MLL-PTD≥1.0% was the optimal cut-off value for predicting hematological relapse after allo-HSCT. There was statistical difference between post-transplant MLL-PTD≥1.0% and MLL-PTD<1.0% groups (3-year CIR: 75%±15.3% vs. 0%, P<0.001; 3-year OS: 25.0±15.3% vs. 80.7%±6.6%, P<0.001; 3-year DFS: 25.0±15.3% vs. 80.7%±6.6%, P<0.001; 3-year TRM: 0 vs. 19.3±6.6%, P=0.277). However, whether MLL-PTD≥1% or MLL-PTD<1% before transplantation has no significant difference on the prognosis.

Conclusions: Our study indicated that MLL-PTD had a certain stability and could effectively reflect the change of tumor burden. The expression level of MLL-PTD after transplantation can serve as an effective indicator for predicting relapse.

Background

Acute myeloid leukemia (AML) is a highly malignant hematopoietic system disease and myelodysplastic syndrome (MDS) is a type of heterogeneous myeloid malignancies and frequently progress to AML [1–4]. In previous studies, molecular genetic aberrations have become important approaches for minimal residual disease (MRD) detection for AML and MDS. Especially, the polymerase chain reaction (PCR)-based gene detection has been proven to be an effective MRD monitoring method for AML patients [5–7]. However, more than half of AML cases still lack effective specific MRD molecular markers [5].

The mixed-lineage leukemia (*MLL*) gene located on chromosome 11q23 is rearranged to generate partial tandem duplications (*MLL-PTD*) [8–10], which usually spans exons 2 to 6, 2 to 7, and 2 to 8, or exons 3–9, exons 3–10, exons 3–11, or exons 3–10 and exons 3–11 at the molecular level [11]. *MLL-PTD* has been detected in approximately 5–10% of AML and 5–6% of MDS patients [12–14]. Low level of *MLL-PTD* (< 0.08%) may also be present in the blood and bone marrow of healthy individuals [5]. Previous reports support that polymerase chain reaction (PCR)-based *MLL-PTD* is a reliable MRD marker and is associated with poor prognosis [5, 12–15]. For chemotherapy patients, a higher *MLL-PTD* level at initial diagnosis predicts a lower incidence of chemotherapy complete remission (CR) and a lower survival rate [13]. The dynamic changes of chemotherapy patients also show that *MLL-PTD* levels within the first six months after the start of therapy are useful for early risk assessment of AML patients, and that a reduction of *MLL-PTD* level ≥ 2 log is a good prognostic factor for overall survival [5]. Furthermore, compared with healthy donors, *MLL-PTD* level have no difference from that of non-transplanted patients in continuous CR, while was significantly higher than that of transplanted patients in continuous CR [15]. Taken together, these findings support that *MLL-PTD* is a specific clinical prognostic marker in the initial diagnosis and chemotherapy for AML patients. However, there are few reports on the dynamics of *MLL-PTD* peri-transplantation, especially after transplantation. Thus, whether *MLL-PTD* could be used as a stable and reliable MRD marker in the process of transplantation and whether there is an optimal value of *MLL-PTD* to predict relapse after transplantation will be explored for the first time in our study.

In this study, we investigated a consecutive cohort of 33 AML and 15 MDS patients with *MLL-PTD* who received allo-HSCT at our institute. Most *MLL-PTD* MDS cases are classified as MDS with excess blasts (MDS-EB) [16]. Our study purposed to analyze the dynamic changes of *MLL-PTD* peri-transplantation and the best threshold for predicting relapse after transplantation.

Methods

Patients

The consecutive patients diagnosed with *MLL-PTD* expression>0.08% AML or MDS undergoing allo-HSCT between January 2015 and March 2019 at the Peking University People's Hospital, Institute of Hematology were enrolled in this study. The patients' data were updated until September 31, 2020. The institutional review board at the hospital approved the protocol, and all patients or their guardians signed consent forms approved by the institutional review board.

Transplantation Protocol

All the patients in this study received myeloablative conditioning regimens. haploidentical HSCT (haplo-HSCT) and matched sibling donor transplantation (MSDT) were 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 anti-thymocyte 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 mycophenolate mofetil (MMF).

Donor lymphocyte infusion (DLI)

Prophylactic DLI was administered for patients in relapse or no remission (NR) state before transplantation. 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 Flow Cytometry (FCM) [20], the expression level of *WT1* and *MLL-PTD* determined by RQ-PCR. The pre-transplant FCM, *MLL-PTD* and *WT1* were performed using bone marrow (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 months post-HSCT and every 6 months thereafter.

The patients were analyzed for the presence of *MLL-PTD* and *WT1* as described previously [15,24]. Briefly, *MLL* primers and hybridization probes were placed in exons 8-10 and 3 of the *MLL* gene, allowing for detection of *MLL-PTD* with exon 8/exon 3 fusion, exon 9/exon 3 fusion, or exon 10/exon 3 fusion. The transcript level was calculated as target transcript copies/ABL copies in percentages. Detectable *MLL-PTD/ABL*>0.08% was defined as *MLL-PTD* positive [13]. A *WT1* transcript level less than 0.60% was defined as negative [24].

Definitions and Assessments

The day of neutrophil engraftment was defined as the first day of 3 consecutive post-transplantation days on which the absolute neutrophil count (ANC) exceeded 500/μL. Patients who survived at least 28 days were considered to have had successful engraftment. The criteria for grading acute graft versus host disease (aGVHD) have been previously published [21,22]. CR was defined as hematological CR that is, < 5% BM blasts, the absence of blasts in peripheral blood, the absence of extramedullary disease, an ANC> 1.0×10^9/L, and a platelet count >100×10^9/L with no red cell transfusions. Hematological relapse was defined by morphologic evidence of disease in the peripheral blood, marrow, or extramedullary sites.

Statistical Analysis

The primary study end point was the cumulative incidence of relapse (CIR). The secondary end points were the OS, disease-free survival (DFS) and treatment-related mortality (TRM). CIR, OS, DFS and TRM were defined as previously described [23]. Summary statistics, such as proportions, medians and ranges, were used to describe the patient characteristics and outcomes. The associations between *MLL-PTD* expression and post-transplantation outcomes were analyzed by the Kaplan-Meier method. Differences in CIR, DFS, OS and TRM between groups were calculated using the log-rank test. A two-sided P value of 0.05 was considered statistically significant. The independence of categorical parameters was calculated using the chi-square test or Fisher exact test, and the distribution of continuous variables was calculated

using the Mann-Whitney U-test. All statistical analyses were performed using SPSS 23.0 (Chicago, IL, USA).

Results

Patients Characteristics

A total of 33 AML patients included 13 males and 20 females, with a median age of 42 years (10–57 years) and 15 MDS-EB patients included 11 males and 4 females, with a median age of 51 years (4–60 years). The median follow-up time was 26 (0.7–56) months after HSCT. Patient characteristics are shown in Table 1. Of these 33 AML patients, 31 patients had gotten CR after chemotherapy, and 2 patients had gotten NR after 3 courses of chemotherapy. And 5 MDS-EB patients receiving chemotherapy including decitabine had gotten CR pre-transplantation. All patients had neutrophil engraftment, and 39 patients had platelet engraftment. Of the 33 patients with AML, 7 patients (21.2%) died of TRM and 6 patients (18.2%) underwent hematological relapse who died ultimately. The median hematological relapse time was 4.8 months (range 4–9 months) after HSCT in 6 relapsed patients. Of the 15 patients with MDS-EB, 2 patients (13.3%) died of infection. In addition, all enrolled patients had a 3-year CIR of 13.7%±5.2%, 3-year OS of 67.8%±6.9%, 3-year DFS of 68.1%±6.8% and 3-year TRM of 20.3%±6.1% (Fig. 1).

Table 1
Characteristics of acute myeloid leukemia and myelodysplastic syndrome patients.

Characteristic	AML N = 33	MDS-EB1/2 N = 15
Median age at allo-HCT, years (range)	42 (10–57)	51 (4–60)
Gender, n (%)		
Male	13 (39.4%)	11 (73.3%)
Female	20 (60.6%)	4 (26.7%)
Chromosome normal, n (%)	23 (69.7%)	9 (60.0%)
FLT3-ITD mutation, n (%)		
Yes	10 (30.3%)	0
No	23 (69.7%)	15 (100%)
NPM1 positive, n (%)	0	0
Median WT1 expression level at initial diagnosis	25.25 (0.23–83.20)	18.80 (1.40–53.50)
No remission before transplant, n (%)	2 (6.1%)	1 (6.7%)
Donor type, n (%)		
HLA-matched sibling	7(21.2%)	5(33.3%)
Haploidentical	26(78.8%)	10(66.7%)
ABO blood type match, n (%)		
Compatible	17 (51.5%)	7 (46.7%)
Incompatible	16 (48.5%)	8 (53.3%)
Conditioning regimen, n (%)		
Chemotherapy based	33 (100%)	15 (100%)
TBI based	0	0
Cell compositions in allografts		
Median MNC, $\times 10^8$/kg (range)	7.82 (6.04–10.86)	8.54 (6.10-10.86)
Median CD34 + count, $\times 10^6$/kg (range)	2.32 (0.27–6.67)	1.89 (0.84–5.34)

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; HLA, human leukocyte antigen; TBI, total body irradiation; MNC, mononuclear cell ;aGVHD, acute graft versus host disease; DLI, donor lymphocyte infusion.

Characteristic	AML N = 33	MDS-EB1/2 N = 15
Granulocyte engraftment time, day(range)	13 (8–25)	13 (11–19)
Platelet engraftment time, day (range)	14 (10–74)	13 (10–53)
II–IV°aGVHD	8 (24.2%)	1 (6.7%)
aGVHD	18 (54.5%)	4 (26.7%)
DLI after transplant, n (%)		
For relapse prevention	2 (6.1%)	0
For intervention	4 (12.1%)	2 (13.3%)
Prognosis, n (%)		
Relapse	6 (18.2%)	0
Treatment-related death	7 (21.2%)	2 (13.3%)
Relapse death	6 (18.2%)	0

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; HLA, human leukocyte antigen; TBI, total body irradiation; MNC, mononuclear cell ;aGVHD, acute graft versus host disease; DLI, donor lymphocyte infusion.

Dynamic changes of MLL-PTD **before and after transplantation**

Observing the changes in the expression level of *MLL-PTD* at different time points peri-transplantation is helpful to analyze the stability of *MLL-PTD* as an MRD indicator in the transplantation system. Our results showed that the *MLL-PTD* level before transplantation was significantly lower than that at the initial diagnosis, but there were still 37 cases were *MLL-PTD* positive before transplantation, and 33 of 37 cases became negative within post-transplant 1 month. However, during our follow-up period, 25 cases eventually occurred post-transplant *MLL-PTD* positive. The median *MLL-PTD* level in all enrolled patients was decreased by around 35 folds after transplantation compared with that of pre-transplant CR status and was similar to the healthy controls (Table 2). Furthermore, among the 6 relapsed patients after transplantation, 3 of them maintained *MLL-PTD* at the healthy level (< 0.08%) within a month after transplantation. But before relapse, the *MLL-PTD* level of these 3 patients gradually increased (> 0.08%) and reached the highest level at the time of relapse. The *MLL-PTD* level of the other 3 relapsed patients continuously remained > 0.08% after transplantation, and the *MLL-PTD* levels of these 3 patients suddenly increased by hundreds of times before relapse.

Table 2
Comparison of MLL-PTD and WT1 at the initial diagnosed and peri-transplant patients.

	MLL-PTD > 0.08% (n/total tests, positive rate)	Median level of MLL-PTD > 0.08% (range, %)	Median level of MLL-PTD (range, %)	WT1 > 0.6% (n/total tests)	Median level of WT1 > 0.6% (range, %)	P value (MLL-PTD + vs. WT1+)
The initial diagnosis	48/48(100%)	30.30 (1.20–631.00)	30.30 (1.20–631.00)	44/47(93.6%)	26.20 (0.82–83.20)	0.233
Pre-transplantation	37/48(68.8%)	6.10 (0.10-414.10)	1.70 (0.017–414.10)	28/47(59.6%)	6.20 (0.88–53.50)	0.351
Post-transplantation						
+ 1 month	8/43(18.6%)	0.115 (0.083–0.73)	0.046 (0.01–0.73)	1/46(2.2%)	0.82	0.027
+ 2 month	12/44(27.3%)	0.21 (0.09–0.82)	0.047 (0-0.82)	3/45(6.7%)	0.86 (0.74–2.4)	0.009
+ 3 month *	13/45(28.9%)	0.28 (0.086–104.70)	0.05 (0-104.70)	6/46(13.0%)	1.50 (0.75–32.70)	0.063
+ 4.5 month *	8/38(21.1%)	1.30 (0.082–55.30)	0.0515 (0-55.30)	9/39(23.1%)	3.90 (0.81–44.10)	0.524
+ 6 month*	11/39(28.2%)	1.40 (0.096–101.30)	0.053 (0.015–101.30)	12/39(30.8%)	1.30 (0.60–80.90)	0.500
+ 9 month*	5/27(18.5%)	0.09 (0.08–0.11)	0.0445 (0–1.00)	3/34(8.8%)	0.71 (0.63–0.74)	0.231
+ 12 month	1/30(3.3%)	0.45	0.049 (0-0.45)	5/32(15.6%)	0.88 (0.72-1.00)	0.113

*Patients underwent hematological relapse at that time point.

The effect of MLL-PTD level before and after transplantation on prognosis

Having analyzed the dynamic changes above which peri-transplant *MLL-PTD* can stably reflect the disease state we next studied the optimal threshold of post-transplant *MLL-PTD* for relapse. Our previous study shows that patients with *MLL-PTD/ABL* ≥ 1% based on initial diagnosis have a poor clinical

prognosis [13]. In order to explore whether *MLL-PTD* could be used as a MRD marker after transplantation, we performed a receiver operating characteristic (ROC) with the highest expression level of post-transplant *MLL-PTD* before hematological relapse in all patients to determine the optimal cut-off value to predict relapse. The area under the ROC curve value was 0.977 ($P < 0.001$, Fig. 2A). The optimal cut-off value was *MLL-PTD/ABL* = 1.0%. And as shown in Fig. 2B, most post-transplant patients with *MLL-PTD* maintained a low level of expression, only 8 patients had *MLL-PTD* ≥ 1%, and 6 of the 8 patients eventually relapsed, which also implied the importance of *MLL-PTD* ≥ 1% in predicting relapse after transplantation. Based on the optimal cut-off value, we divided the post-transplant patients into two groups of *MLL-PTD/ABL* < 1% and *MLL-PTD/ABL* ≥ 1% to analyzed the prognostic difference. Our study found that the group of *MLL-PTD/ABL* ≥ 1.0% had higher 3-year CIR (75%±15.3% vs. 0%, $P < 0.001$, Fig. 3A), and lower 3-year OS (25.0 ± 15.3% vs. 80.7%±6.6%, $P < 0.001$, Fig. 3B) and 3-year DFS (25.0 ± 15.3% vs. 80.7%±6.6%, $P < 0.001$, Fig. 3C) compared with that of group of *MLL-PTD/ABL* < 1%. However, there was no statistical difference between the two groups in TRM ($P > 0.05$, Fig. 3D).

Both at the initial diagnosis and post-transplantation, it was analyzed that *MLL-PTD* = 1% was the optimal cut-off value, which implied that *MLL-PTD/ABL* = 1% was of important value in predicting prognosis. Therefore, we further analyzed whether *MLL-PTD/ABL* ≥ 1% before transplantation also indicated a poor prognosis after transplantation. However, our results showed that there was no statistical difference in prognosis between the *MLL-PTD/ABL* ≥ 1% and *MLL-PTD/ABL* < 1% group based on the level of *MLL-PTD* before transplantation (All $P > 0.05$, Fig. 4A-4D), but the group of *MLL-PTD/ABL* ≥ 1% tended to have lower OS ($P = 0.202$, Fig. 4B), DFS ($P = 0.202$, Fig. 4C), and have a higher TRM($P = 0.105$, Fig. 4D) compared with that of *MLL-PTD/ABL* < 1% group .

Factors affecting the prognosis of transplant patients with MLL-PTD

Factors affecting the prognosis were analyzed, including transplantation age, gender, disease type, donor type, blood type compatibility (Table 3). There was no statistical difference in TRM ($P = 0.675$), CIR ($P = 0.115$), DFS ($P = 0.151$) and OS ($P = 0.157$) between AML and MDS-EB. Among the 12 patients who received MSDT, 2 (16.7%) patients underwent hematological relapse both at 5 months after HSCT, and 1 patient died of pneumonia at 5.5 months. Among 36 patients who received haplo-HSCT, 4 patients (11.1%) underwent hematological relapse at a median of 4.5 months (range, 4–9 months) after HSCT, and 8 patients (22.2%) died due to TRM at a median of 5.3 months (range, 0.7–17.5 months). Based on the results of the analysis, it seemed that patients who received haplo-HSCT could achieve comparable outcomes compared to those who underwent MSDT (TRM: $P = 0.271$; CIR: $P = 0.653$; DFS: $P = 0.544$; OS: $P = 0.560$). The factor analysis of *MLL-PTD* level before and after transplantation showed that there was no statistical difference in pre-transplant *MLL-PTD* level. And post-transplant group of *MLL-PTD/ABL* ≥ 1% had a higher CIR, a lower OS and a lower DFS than that of group of *MLL-PTD/ABL* < 1% (all $P < 0.001$). In addition, other factors such as age, pre-transplant FCM, *WT1* status and prophylactic DLI have no significant impact on prognosis. The ABO blood type and *FLT3-ITD* mutation at first diagnosis were important risk factors of CIR and OS after transplantation, respectively. Incompatible ABO blood type

indicated a higher CIR than that of compatible ABO blood type, and patients with *FLT3-ITD* mutation had a low OS than that of without *FLT3-ITD* (Table 3).

Table 3. Univariate analysis of the variables affecting hematological TRM, CIR, DFS and OS in patients with MLL-PTD after allo-HSCT

Variables	Number (n, %)	P value			
		TRM	CIR	DFS	OS
Age of recipient		0.965	0.291	0.410	0.442
50 years	31(64.6%)				
≥50 years	17(35.4%)				
Underlying disease		0.675	0.115	0.151	0.157
AML	33(68.8%)				
MDS-EB1/2	15(31.2%)				
ABO compatibility		0.264	0.009	0.38	0.484
Compatible	24(50.0%)				
Incompatible	24(50.0%)				
Donor type		0.271	0.653	0.544	0.560
HLA-matched sibling	12(25.0%)				
Haploidentical	36(75.0%)				
Prophylactic DLI	2(4.2%)	0.325	0.591	0.735	0.702
FLT3-ITD positive	10(20.8%)	0.067	0.868	0.068	0.041
Pre-transplantation FCM		0.056	0.504	0.291	0.232
Negative	23(47.9%)				
Positive	25(52.1%)				
Pre-transplantation WT1		0.339	0.166	0.843	0.854
WT1<0.6%	19(40.4%)				
WT1≥0.6%	28(59.6%)				
Pre-transplantation MLL-PTD		0.105	0.967	0.202	0.202
MLL-PTD/ABL≥1.0%	25(52.1%)				
MLL-PTD/ABL1.0%	23(47.9%)				
Post-transplantation MLL-PTD		0.277	<0.001	<0.001	<0.001
MLL-PTD/ABL≥1.0%	8(16.7%)				
MLL-PTD/ABL1.0%	38(79.2%)				

TRM, treatment-associated mortality; CIR, cumulative incidence of relapse; DFS, disease-free survival; OS, overall survival; HLA, human leukocyte antigen; allo-HSCT, allogeneic hematopoietic stem cell transplantation; DLI, donor lymphocyte infusion; MLL-PTD, mixed lineage leukemia-partial tandem duplication; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome

Comparison of MLL-PTD **and other MRD parameters**

After transplantation, 8 patients were detected *MLL-PTD/ABL* ≥ 1.0% at a median of 3 months. Of the 8 patients, 7 patients were simultaneously (5 patients) or subsequently (2 patients) MRD positive detected by FCM at a median of 4.25 months (range,3–12 months), and 6 patients ultimately progressed to hematological relapse at a median of 2 months (range, 0.25–6 months) from the first time *MLL-PTD/ABL* ≥ 1.0%, half of whom receiving chemotherapy plus DLI. Finally, 2 patients receiving chemotherapy plus DLI became MRD negative gradually.

WT1 has been confirmed in previous studies to be an effective indicator of MRD monitoring and implementing interventions [24]. In order to analyze the specificity and sensitivity of *MLL-PTD* compared with *WT1*, we showed in Table 2 the dynamic changes of expression of *MLL-PTD* and *WT1* at the initial diagnosis and different time points before and after transplantation. All 6 relapsed patients were detected *MLL-PTD* positive prior to relapse, while only 4 patients were detected positive for *WT1*. As shown in Table 2, the expression levels of *MLL-PTD* and *WT1* both changed with the tumor burden. However, within post-transplant 3 months, *MLL-PTD* seemed be more sensitive than *WT1* for MRD monitoring ($P_{+1\ month}$=0.027; $P_{+2\ month}$=0.009; $P_{+3\ month}$=0.063).

Discussion

MLL-PTD is a special MLL rearrangement gene. No report had focused on the predictive significance of peri-transplant *MLL-PTD* expression on leukemia relapse after transplantation. In our retrospective study, results showed dynamic changes of *MLL-PTD* peri-transplantation, and the post-transplant *MLL-PTD* level is related to the prognosis of patients.

Previous reports have established the best threshold of *MLL-PTD* at the initial diagnosis for predicting the CR or relapse in AML patients [13, 15]. However, the AML patients with *MLL-PTD* analyzed in above reports included both non-transplanted patients and transplanted patients. Since different treatments (chemotherapy and transplantation) have a great impact on the prognosis of AML patients, they also have a certain impact on the accuracy of the *MLL-PTD* threshold for predicting relapse. Allo-HSCT is one of the curative therapies currently available for AML and *MDS-EB*, so it is very necessary to establish an optimal threshold of post-transplant *MLL-PTD* for relapse in transplanted AML patients. In the analysis of the post-transplant best cut-off value, we found that *MLL-PTD/ABL* = 1% can be used as the threshold for predicting relapse. Based on this result, physicians could need to pay more attention to the occurrence of relapse for post-transplant patients with *MLL-PTD/ABL* ≥ 1%. Under this condition, it is also necessary to shorten the MRD monitoring interval, or give appropriate relapse preventive interventions in combination with the clinical condition.

A stable and reliable MRD marker whose expression level needs to vary with the tumor burden. Our data showed that *MLL-PTD* levels in relapsed patients were significantly increased before relapse. Importantly, there was no occurrence of *MLL-PTD* turning negative or losing before relapse, which indicated that *MLL-PTD* had a certain stability and could effectively reflect the change of tumor burden. As expected, *MLL-PTD* was available prior to hematological relapse, but the relapse after *MLL-PTD* positive occurred at different rates. One of the explanations may be due to the patient's combination of additional mutations such as *FLT3-ITD*. Previous report confirms that *MLL-PTD* positive relapses harboring an additional *FLT3-ITD* mutation to relapse faster than other patients with MLL-PTD alone [15]. In our study, the initial diagnosis of 2 relapsed patients was accompanied by *FLT3-ITD* mutation. They respectively relapsed at 12 days and 35 days after post-transplant *MLL-PTD/ABL* ≥ 1%, and the relapse was significantly faster than that of other relapsed patients. These data suggested *MLL-PTD* patients with other mutations such as *FLT3-ITD* may need to be shortened intervals of MRD monitoring after transplantation. Of course, a larger sample size or data is needed in the future to further support the above result.

The timely monitoring of MRD in the early stage after transplantation was beneficial to guide early clinical intervention to improve the prognosis of patients. Some studies have confirmed that the *WT1* expression level is an independent prognostic indicator that can predict clinical outcome and combined use of *WT1* and flow cytometry monitoring can promote sensitivity of predicting relapse after allo-HSCT [24, 25]. For AML and MDS lacking specific markers, we usually need to combine FCM and *WT1* to evaluate MRD status. In the study, *MLL-PTD* became positive before relapse and prior to flow cytometry results. Thus, in contrast to FCM, PCR-based *MLL-PTD* detection have higher sensitivity. Our data showed that *MLL-PTD* seemed to be more sensitive than *WT1* in early MRD monitoring after transplantation. Furthermore, in contrast to *WT1*, *MLL-PTD* is more specific for the type of MLL-PTD positive AML and MDS. However, for post-transplant patients with *MLL-PTD*, in order to monitor MRD more effectively and accurately, there may not be a better way than monitoring FCM, *WT1* and *MLL-PTD* at the same time.

AML with *MLL-PTD* is considered to be a type of leukemia with a relatively poor prognosis compared with the standard-risk AML [13, 14]. In standard-risk AML, the post-transplant overall CIR and OS are around 15%-20% and 60%-70% at our institute, respectively [26, 27]. Our present results showed that the overall prognosis of post-transplant *MLL-PTD* patients (3-year OS: 67.8%; 3-year CIR: 13.7%) was similar to that of standard-risk patients. In addition, the other *MLL* rearrangement study about the transplant-related prognosis found that allo-HSCT would have a lower relapse risk and a higher survival probability compared to the results obtained from patients with chemotherapy alone [28]. The outcomes of patients with *MLL-PTD* are similar to the above results. The post-transplant OS in our study was significantly better than that of receiving chemotherapy alone (3-year OS < 40%) in previous study [5]. These data supported that allo-HSCT could achieve good therapeutic effect in patients with *MLL-PTD* at our institute.

Previous report shows that the OS of *MLL*-rearranged AL patients receiving haplo-HSCT was similar to that of receiving MSDT [29]. In order to explore whether our institution’s haplo-HSCT protocol has an impact on the prognosis of *MLL-PTD* patients, we further compared the overall prognosis between patients receiving haplo-HSCT and MSDT, and found that there was no statistical difference. Above

seemed that haplo-HSCT could achieve the similar therapeutic effect to the MSDT in patients with *MLL-PTD*. Therefore, our institution's transplant and relapse prevention system may be effective for *MLL-PTD* patients.

Conclusions

In conclusion, *MLL-PTD* expression is a sensitive and specific MRD marker for the *MLL-PTD* patients received allo-HSCT. *MLL-PTD* expression level higher than 1.0% suggested a high risk of hematological relapse and tended to have a worse prognosis. Furthermore, allo-HSCT could achieve good therapeutic effect in patients with *MLL-PTD* AML and MDS-EB. Of course, due to the limited number of patients with *MLL-PTD* patients, we still need to continue research to accumulate more cases to further confirm the significance of *MLL-PTD* for MRD monitoring around transplantation.

Abbreviations

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; Allo-HSCT: Allogeneic hematopoietic stem cell transplantation; MRD: minimal residual disease; RQ-PCR: real-time quantitative polymerase chain reaction; TRM: treatment-related mortality; CIR: cumulative incidence of relapse; OS: overall survival; DFS: disease-free survival; MLL: mixed-lineage leukemia; PTD: partial tandem duplications; CR: complete remission; MSDT: matched sibling donor transplantation; NR: no remission; DLI: Donor lymphocyte infusion; BM: bone marrow; FCM: Flow Cytometry; ANC: absolute neutrophil count; aGVHD: acute graft versus host disease

Declarations

Acknowledgments

Not applicable.

Authors' Contribution

X.-S.Z. designed the study and was responsible for whole project administration. J.K., M.-G.G. and X.-S.Z. analyzed data and wrote the manuscript, J.K. and M.-G.G. contributed equally to this work.; Y.-Z.Q., Y.W., C.-H.Y., Y.-Q.S., Y.-J.C., L.-P.X., X.-H.Z., K.-Y.L. and X.-J.H. contributed to collect samples and validate results. All authors have read and approved the manuscript

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations Ethics approval and consent to participate

The study followed the principles of the Helsinki Declaration and was approved by the Ethics Committee of Peking University People's Hospital. All subjects obtained informed consent and all patients or their guardians signed consent forms approved by the institutional review board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Figures

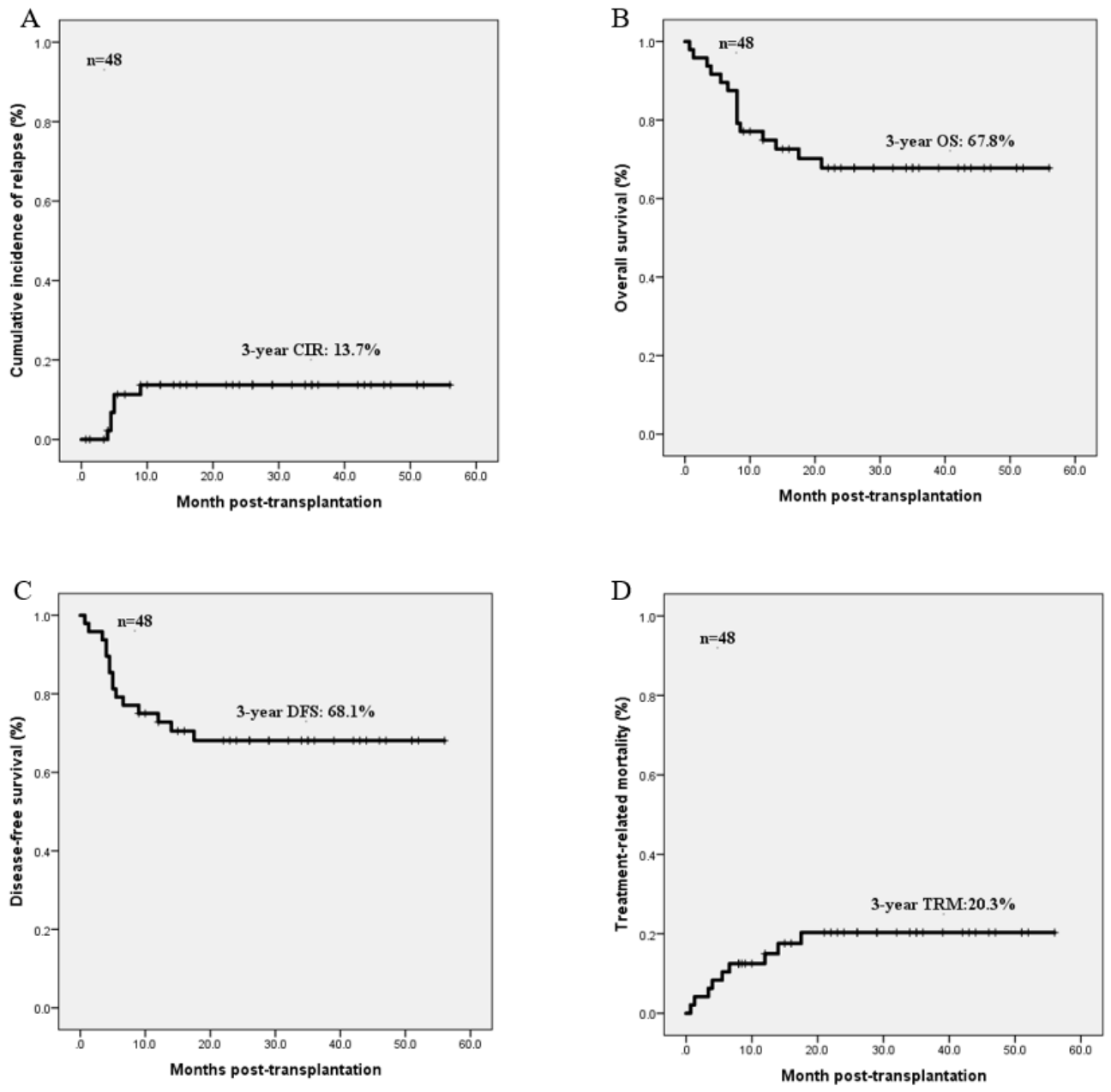

Figure 1

Cumulative incidence of relapse, overall survival, disease-free survival and treatment-related mortality of 48 MLL-PTD patients after allo-HSCT.

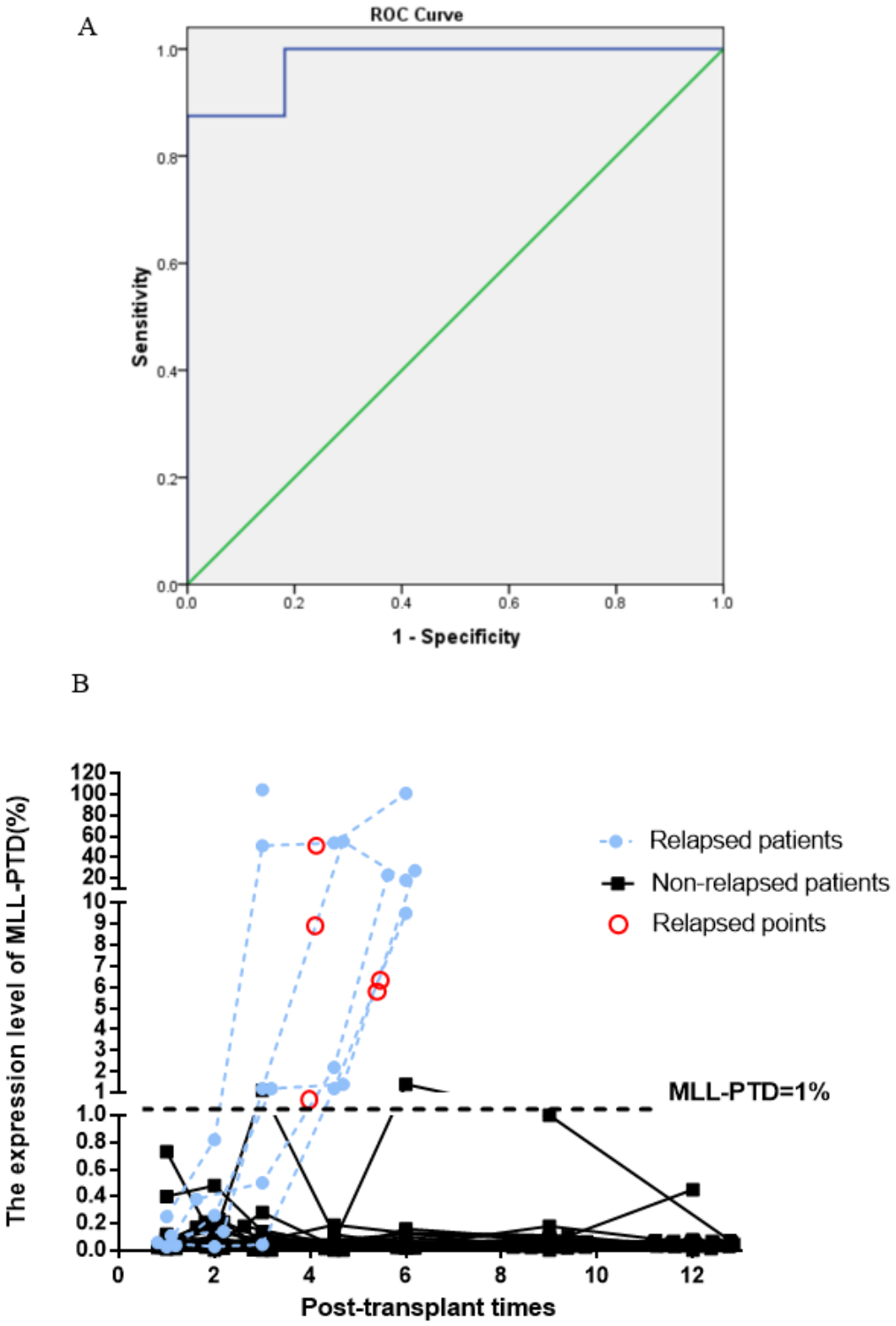

Figure 2

The curve of MLL-PTD expression levels post-transplantation. (A) Receiver operating characteristic (ROC) curve of MLL-PTD expression post-transplantation (AUC=0.977, P<0.001). (B) The level changes of post-transplant MLL-PTD.

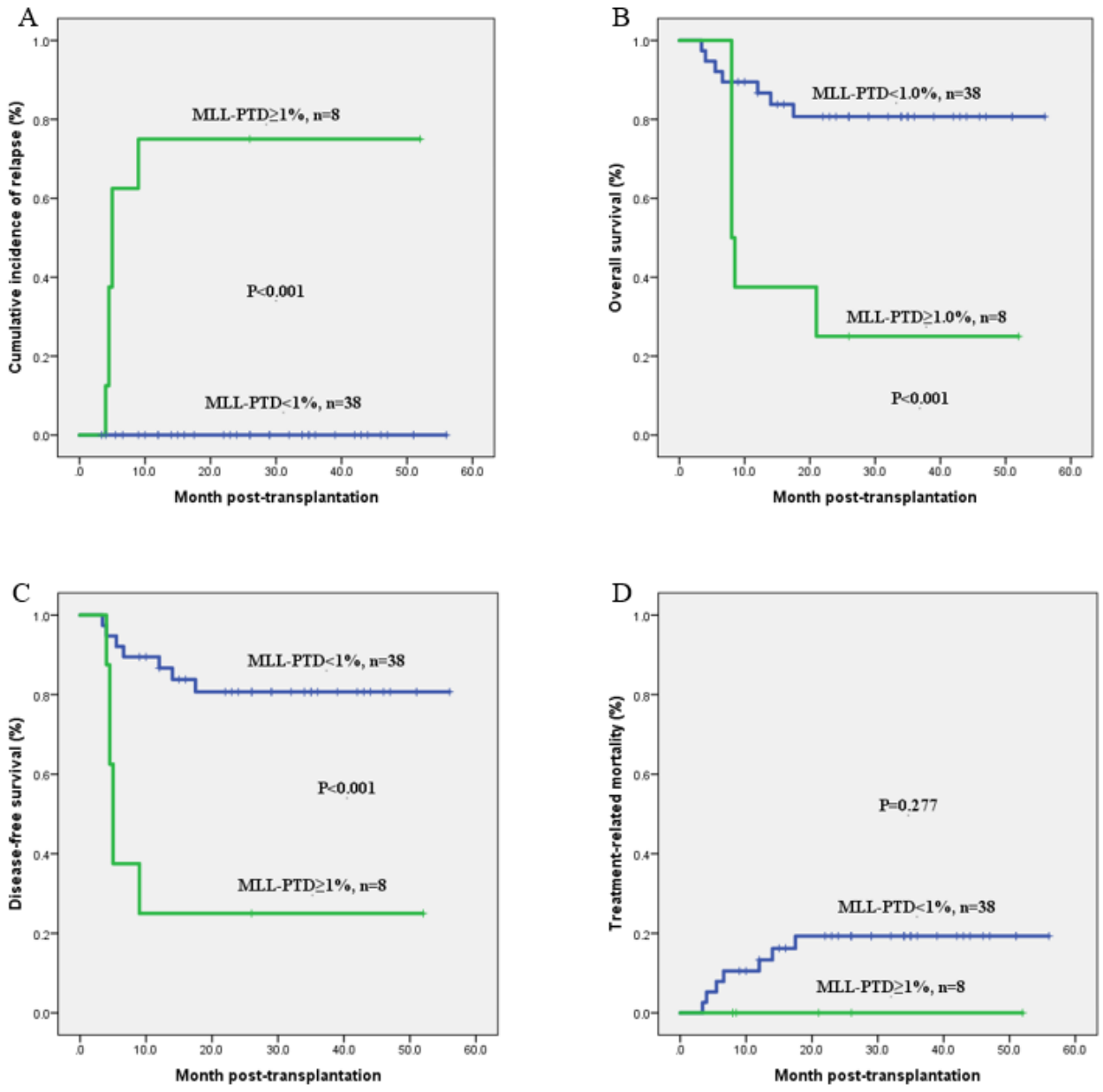

Figure 3

Kaplan-Meier survival curves analysis of patients between MLL-PTD<1% and MLL-PTD ≥1% after transplantation.

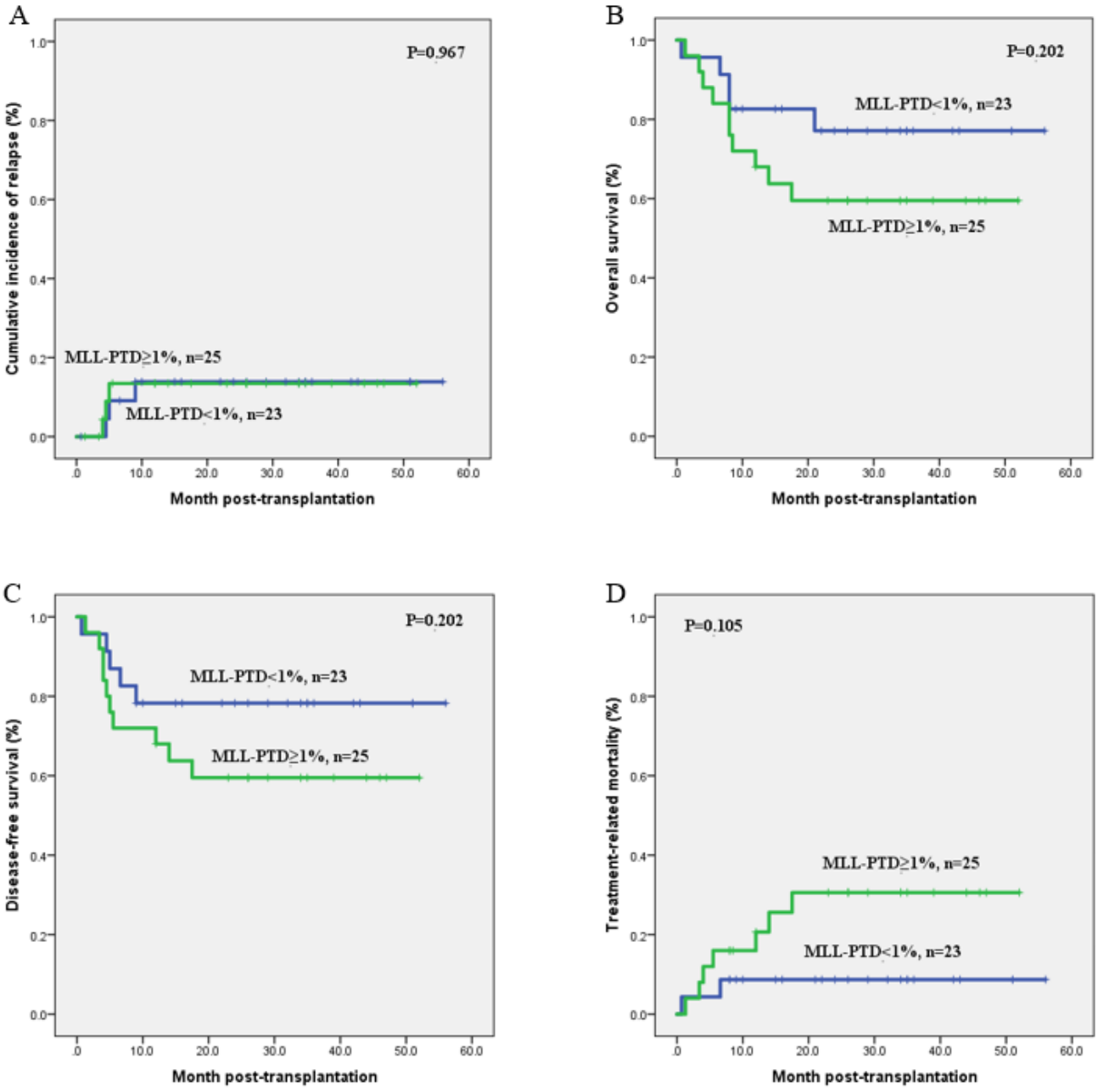

Figure 4

Kaplan-Meier survival curves analysis of patients between MLL-PTD<1% and MLL-PTD ≥1% before transplantation.