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Original Article

Allogeneic hematopoietic stem cell transplantation overcomes the poor prognosis in MLL-rearranged solid tumor therapy related-acute myeloid leukemia

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Abstract: MLL rearrangement is very common in solid tumor therapy-related acute myeloid leukemia (t-AML). To investigated the prognosis of solid tumor MLL t-AML, 157 patients were divided into 3 groups: non-MLL t-AML (n=41), MLL t-AML (n=18) and MLL de novo AML (n=98). Of the 150 patients underwent anti-leukemia therapy, the complete remission (CR) was similar in MLL t-AML, non-MLL t-AML and MLL de novo AML (P=0.251). 3-years overall survival (OS) was 37.5%, 21.5% and 20.4% (P=0.046), and leukemia-free survival (LFS) was 28.0%, 32.2% and 22.7% (P=0.031), and the incidence of relapse was 30.0%, 50.4% and 53.5% (P=0.382), respectively, in the three groups. Multivariate analysis revealed that MLL t-AML was a risk factor while allo-HSCT was a protective factor for OS, LFS, and relapse (P<0.001, P<0.001 and P=0.005) (P=0.002, P<0.001 and P<0.001, respectively). The 3-years OS was 0%, 17.9% and 2.3% (P=0.038), and LFS was 0%, 23.1% and 3.3% (P=0.017), and relapse was 100%, 53.1% and 74.4% (P=0.001), respectively, among three groups in patients undergoing chemotherapy alone, while OS was 64.3%, 52.7% and 40.7% (P=0.713), LFS was 60.0%, 48.8% and 37.0% (P=0.934), and relapse was 25.0%, 47.4% and 47.5% (P=0.872), respectively, among these groups in patients undergoing allo-HSCT. Intriguingly, MLL t-AML was no longer risk factor for relapse and LFS (P=0.882 and P=0.484, respectively), and it became a favorable factor for OS (P=0.011) in patients undergoing allo-HSCT. In conclusion, MLL t-AML had poor prognosis compared with non-MLL t-AML and MLL de novo AML, but allo-HSCT might overcome the poor prognosis of MLL t-AML.

Keywords: MLL rearrangement, solid tumor therapy-related acute myeloid leukemia, prognosis, transplantation

Introduction

Therapy-related acute myeloid leukemia (t-AML) has frequently emerged as a long-term complication in the patients with malignancies who have underwent cytotoxic therapy (i.e., chemotherapy and radiotherapy) [1]. In general, t-AML carries poor cytogenetic and molecular genetic features, characterized by inferior response to conventional treatment and poor survival compared to de novo AML [2-6]. Traditionally, World Health Organization (WHO) classified t-AML into two types based on prior cytotoxic therapies. The first subtype of t-AML, occurring after exposure to radiation and/or alkylating agents, often has unbalanced cytogenetic abnormalities, such as the deletion of chromosomes 5 and/or 7 [7-9]. The second subtype of t-AML, occurring after exposed to agents targeting topoisomerase, usually accompanied by balanced chromosomal rearrangements, such as MLL rearrangment, PML-RARA and RUNX1 [9-14].

In de novo AML, the patients with MLL rearrangement usually had a worse prognosis compared with the patients without MLL rearrangement [15, 16]. The incidence of MLL rearrangement is more common in t-AML compared with that in de novo AML [17-19]. However, current studies have not drawn a distinction of prognosis between MLL t-AML and non-MLL t-AML. Additionally, these studies were lack of atten-

tion to the difference in prognosis between MLL t-AML and MLL de novo AML.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is recommended for MLL AML, and seemed to be the only potential curative option for MLL AML [20-22]. It is rarely reported whether allo-HSCT has a difference prognosis between MLL t-AML and MLL de novo AML. Here, we performed a retrospective analyses of solid tumor t-AML, and compared the prognosis of MLL t-AML with MLL de novo AML. Our results indicated that MLL t-AML had an inferior prognosis compared with no-MLL t-AML and MLL de novo AML, while allo-HSCT might overcome the poor prognosis of MLL t-AML.

Methods

Study design and patients

This retrospectively study was enrolled in all consecutive patients older than 14 years old, who diagnosed as solid tumor t-AML and MLL de novo AML between January 2008 and December 2018, and underwent anti-leukemia therapy including chemotherapy and/or allo-HSCT in Nanfang hospital. The diagnosis of t-AML was according to the WHO 2016 classification, which involves the primary disease history and laboratory findings. Bone marrow with >20% myeloblasts was diagnosed as AML [9]. Patients with a history of myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) and those without anti-leukemia therapy were excluded from this study. Medical records of all patients were reviewed for demographic data, primary diseases, treatment and transplant-related parameters, and so on. We collected and analyzed the follow-up data of 5 years post diagnosis or transplantation. This research was conducted in accordance with the Declaration of Helsinki and was approved by the Institution Review Board of our institution.

Cytogenetic and molecular genetic analysis

According to our previously described [23, 24], chromosome banding, fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) as well as next generation sequencing (NGS) were used for cytogenetic and molecular genetic analysis. MLL gene rearrangement is detected by FISH, NGS and chromosome banding method. MLL fusion gene was detected by PCR.

Definitions

The diagnosis of AML was according to WHO criteria [9]. Solid tumor t-AML is defined as AML secondary to solid tumors that had underwent chemotherapy and/or radiotherapy before T-AML. The patients of AML who with a history of solid tumor but did not undergoing cytotoxic therapy were classified to de novo AML. High white blood cells (WBC) defined as WBC $\geq 50\times10^9$/L. The outcomes evaluated included complete remission (CR), overall survival (OS), leukemia-free survival (LFS) and relapse incidence were followed recommended criteria [25]. According to the recommended criteria, cytogenetics were divided into favorable, intermediate and adverse risk groups [26].

Statistical analysis

Data processing was performed using SPSS (version 22.0) software for statistical analysis. Cumulative incidence of OS, LFS and relapse were calculated according to Kaplan-Meier (K-M) method with comparison by the log-rank (Mantel-Cox) test. Differences in proportions were evaluated using the chi-square test. Multivariate analysis was performed applying the Cox model. A p value of <0.05 was considered as statistically significant.

Results

Patients characteristics

A total of 157 consecutive patients with AML were enrolled in this study, including 18 cases with MLL t-AML, 41 no-MLL t-AML and 98 MLL de novo AML. There were 73 females and 84 males. The median age was 36 (range: 13-84) years old. The Patients demographics and baseline characteristics are detailed in **Table 1**. The study flow diagram is shown in **Figure 1**. The patients with MLL t-AML were younger than non-MLL t-AML cohort (P=0.036), while they were older compared with MLL de novo AML (P=0.019). And the ratio of female was higher in t-AML patients compared with de novo AML patients (P=0.020). The other characteristics were similar among three groups (all P values >0.05) (**Table 1**).

Table 1. Characteristics of patients

	MLL t-AML (n=18)	Non-MLL t-AML (n=41)	MLL de novo AML (n=98)	*P* value
Age (years, range)	38.5 (13-57)	47 (16-84)	29 (14-64)	<0.001
Gender				0.020
Female/Male, rate	1.25	1.93	0.58	
Peripheral blood				
WBC, 10^9/L (median, range)	13.2 (1.4-137.0)	7.2 (0.9-135.7)	19.9 (0.5-449.0)	0.674
Hemoglobin, g/L (median, range)	92 (53-115)	87 (45-132)	99 (37-144)	0.561
Platelet, 10^9/L (median, range)	42 (9-323)	55 (12-459)	51 (9-556)	0.139
Primary disease			-	
Breast cancer	7 (38.9%)	11 (26.8%)		0.373
Lymphoma	6 (33.3%)	3 (7.3%)		0.118
Hepatocellular cancer	2 (11.1%)	4 (9.8%)		0.999
Nasopharyngeal cancer	0	4 (9.8%)		0.302
Ovarian cancer	1 (5.6%)	3 (7.3%)		0.999
Cervical cancer	1 (5.6%)	3 (7.3%)		0.999
Colon cancer	0	4 (9.8%)		0.302
Thyroid cancer	0	3 (7.3%)		0.546
Gastric cancer	0	3 (7.3%)		0.546
Chorionic cancer	1 (5.6%)	0		0.305
Lung cancer	0	2 (3.7%)		0.999
Esophageal cancer	0	1 (4.9%)		0.999
Latency, months (median, range)	48.4 (11.4-60)	32.7 (1-133.5)	-	0.543

Abbreviations: WBC, white blood cells.

Cytogenetic and molecular genetic characteristics

The cytogenetic and molecular genetic characteristics at diagnoses are detailed in **Table 2**. Of the 153 evaluable cases for cytogenetics, the rate of favorable cytogenetic was 0.0%, 10.9% and 0% (P=0.026), the rate of intermediate cytogenetic was 38.9%, 45.9% and 38.8% (P=0.743) and the rate of adverse cytogenetic was 61.1%, 43.2% and 61.2% (P=0.158), respectively, in MLL t-AML, non-MLL t-AML and MLL de novo AML. In detail, the patients with MLL t-AML had more 5 chromosome deletion compared to those with non-MLL t-AML (16.7% vs. 10.8%, P=0.041) and MLL de novo AML (16.7% vs. 8.2%, P=0.036), and were more frequently with deletion of chromosome 7 compared to MLL de novo AML (16.7% vs. 10.2%, P=0.020, respectively).

The molecular genetic data were evaluated in 155 patients. The most common fusion gene in MLL t-AML was MLL-AF6 (11.1%), followed by MLL-AF9 (5.5%). MLL t-AML cohort showed more frequently with TP53 mutation compared to non-MLL t-AML and MLL de novo AML (11.1%, 0.0% and 0.0%, P<0.001), and less frequently with PML/RARa and NPM1 compared to non-MLL t-AML (P=0.002 and P=0.049). The other molecular genetic factors were similar among three groups (all *P* values >0.05).

Induction chemotherapy

Of the 157 patients enrolled in this primary study, 7 patients abandoned anti-leukemia treatments and 141 received standard-dose induction chemotherapy, including 17 in MLL t-AML, 30 in non-MLL AML and 94 in MLL de novo AML, and other 9 patients received reduced-dose induction chemotherapy, including 1 in MLL t-AML, 4 in non-MLL t-AML and 4 in MLL de novo AML (**Figure 1**). The outcomes of induction chemotherapy are summarized in **Table 3**. In the standard-dose cohort, the CR rate in 1 induction or ≥2 induction were similar among three groups (all *P* values >0.05). While

Figure 1. The study flow diagram.

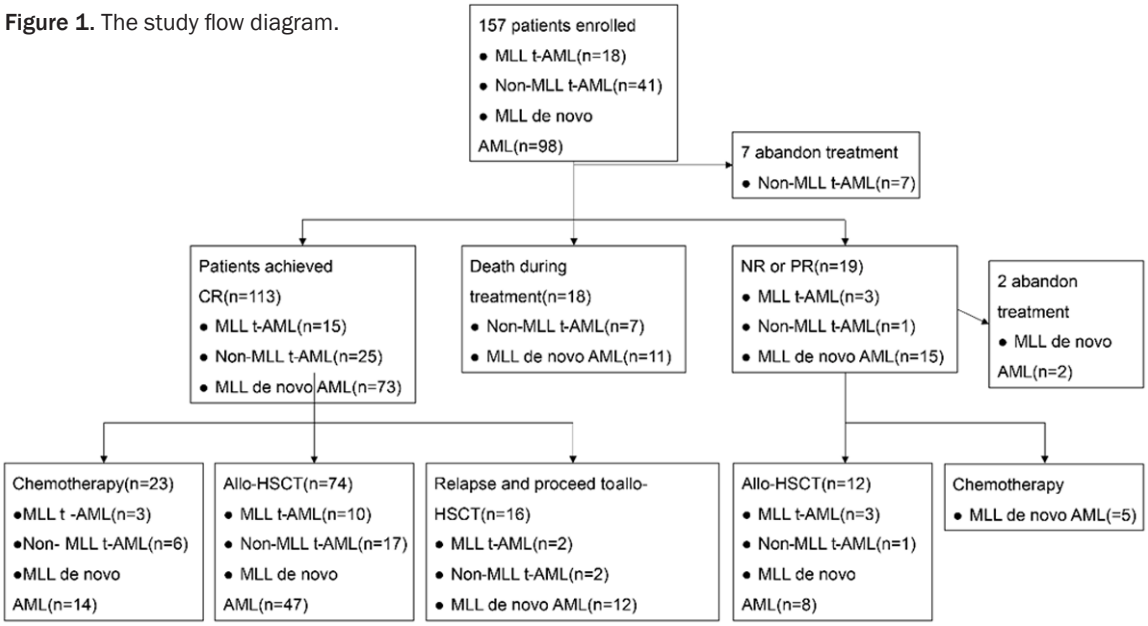

Table 2. Cytogenetic and molecular genetic characteristics

	MLL t-AML (n=18)	Non-MLL t-AML (n=41)	MLL de novo AML (n=98)	*P* value
Cytogenetic risk category				
Good	0 (0.0%)	4 (10.9%)	0 (0.0%)	0.026
Intermediate	7 (38.9%)	17 (45.9%)	38 (38.8%)	0.743
Adverse	11 (61.1%)	16 (43.2%)	60 (61.2%)	0.158
Missing	0	4	0	
Cytogenetic abnormalities				
-5 or 5q-	3 (16.7%)	4 (10.8%)	8 (8.2%)	0.022
-7 or 7q-	3 (16.7%)	5 (13.5%)	10 (10.2%)	0.039
t(15,17)	0 (0.0%)	3 (8.1%)	0 (0.0%)	0.002
t(8,21)	0 (0.0%)	1 (2.7%)	0 (0.0%)	0.104
Complex karyotype	1 (5.6%)	3 (7.3%)	5 (5.1%)	0.224
Others	4 (22.2%)	4 (10.8%)	37 (37.8%)	
Molecular genetic abnormalities				
MLL-AF4	0 (0.0%)	0 (0.0%)	6 (6.1%)	0.163
MLL-AF6	2 (11.1%)	0 (0.0%)	6 (6.1%)	0.165
MLL-AF9	1 (5.5%)	0 (0.0%)	1 (1.0%)	0.539
TP53	2 (11.1%)	0 (0.0%)	0 (0.0%)	<0.001
EP300	0 (0.0%)	0 (0.0%)	3 (3.1%)	0.372
NPM1	0 (0.0%)	2 (5.1%)	0 (0.0%)	0.049
PML/RARa	0 (0.0%)	3 (7.6%)	0 (0.0%)	0.002
AML1/ETO	0 (0.0%)	1 (2.6%)	0 (0.0%)	0.773
Others	0 (0.0%)	8 (20.5%)	3 (3.1%)	
Missing	0	2	0	

in the reduced-dose cohort, the CR rate in 1 induction of MLL t-AML was inferior compared to that in de novo AML (P=0.048). In total, the CR rates were 83.3%, 85.5% and 86.2%,

Table 3. The outcomes of chemotherapy

	MLL t-AML (n=18)	Non-MLL t-AML (n=34)	MLL de novo AML (n=98)	*P* value
Total CR	15 (83.3%)	23 (85.5%)	75 (86.2%)	0.251
Standard-dose chemotherapy				
CR				
CR with 1 induction	12 (70.6%)	17 (63.3%)	58 (61.7%)	0.783
CR with ≥2 induction	2 (11.8%)	5 (20.0%)	13 (13.8%)	0.696
PR	2 (11.8%)	0 (0.0%)	3 (3.2%)	0.149
NR	1 (5.9%)	1 (3.3%)	9 (9.6%)	0.516
Invalid	0	4	11	
Reduced-dose chemotherapy				
CR				
CR with 1 induction	0 (0.0%)	1 (100%)	4 (100%)	0.048
CR with ≥2 induction	1 (100%)	0 (0.0%)	0 (0.0%)	0.048
Invalid	0	3	0	

Abbreviations: CR, complete remission; PR, partial remission; NR, non-remission.

Table 4. Patients characteristics at transplantation

	MLL t-AML (n=15)	Non-MLL t-AML (n=20)	MLL de novo AML (n=67)	*P* value
Patient age (years, range)	36 (13-53)	38 (16-55)	29 (15-51)	0.433
Patient gender				0.006
Male	6 (40.0%)	4(20.0%)	40 (59.7%)	
Female	9 (60.0%)	16(80.0%)	27 (40.3%)	
Disease status at transplants				0.579
CR	10 (66.7%)	17 (85.0%)	47 (70.1%)	
PR	3 (20.0%)	1 (5.0%)	8 (12.0%)	
NR	2 (13.3%)	2 (10.0%)	12 (17.9%)	
Donor type				0.958
MSD	7 (46.7%)	10 (50.0%)	37 (55.2%)	
MUD	1 (6.6%)	2 (10.0%)	5 (7.5%)	
HID	7 (46.7%)	8 (40.0%)	25 (37.3%)	
Conditioning regimen[a]				0.347
Myeloablative	7 (46.6%)	14 (70.0%)	37 (55.2%)	
Intensified	8 (53.4%)	6 (30.0%)	30 (44.8%)	
GVHD prophylaxis				0.832
CsA + MTX	4 (26.7%)	5 (25.0%)	19 (28.4%)	
CsA + MTX + MMF	5 (33.3%)	10 (50.0%)	30 (44.8%)	
CsA + MTX + MMF + ATG	6 (40.0%)	5 (25.0%)	18 (26.8%)	

Abbreviations: MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; HID, haplo-identical donor; GVHD, graft-versus-host disease; ATG, antithymocyte globulin; CsA, ciclosporin A; MMF, mycophenolate mofetil. MTX, methotrexate. [a]Myeloablative conditioning regimens include TBI (total body irradiation) + Cy (cyclophosphamide), Bu (busulfan) + Cy, and Bu + Flu (fludarabine). Intensified conditioning regiments include TBI + Cy + etoposide, and Flu + cytarabine + TBI+ Cy.

respectively, in the MLL t-AML, non-MLL t-AML and MLL de novo AML groups (P=0.251).

Treatments post-remission

Of the 113 patients achieved CR after induction chemotherapy, 23 patients received chemotherapy as treatment post-remission, including 3 in MLL t-AML, 6 in no-MLL t-AML and 14 in MLL de novo AML, and 74 underwent allo-HSCT, including 10 in MLL t-AML, 17 in no-MLL t-AML and 47 in MLL de novo AML. Among those patients received chemotherapy, 16 patients undergoing relapse also proceed to

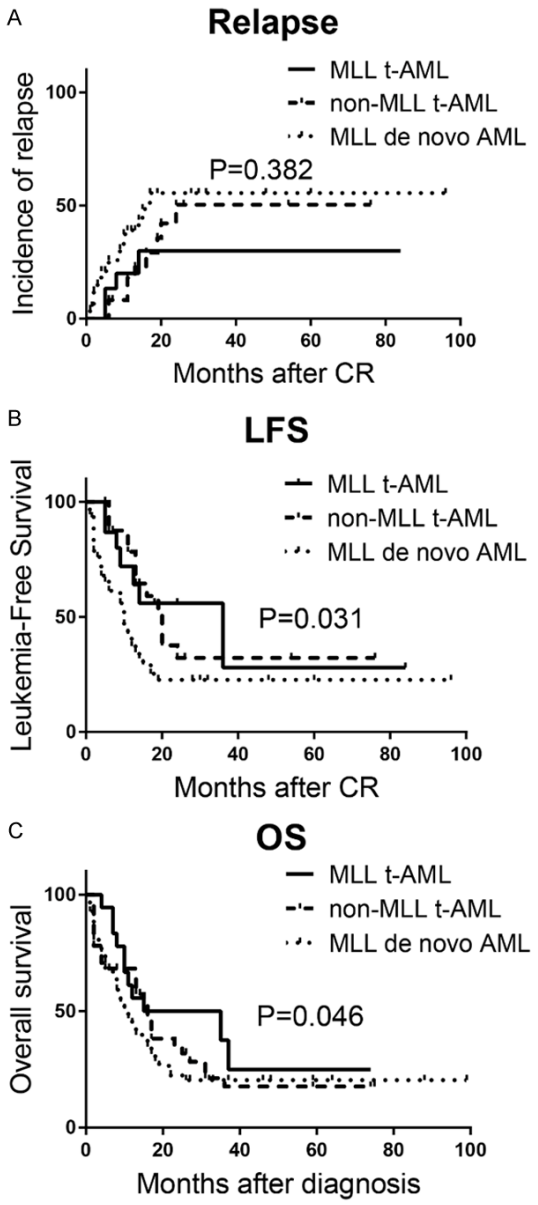

Figure 2. Kaplan-Meier outcome curves for all patients received anti-leukemia therapy. A. The 3-years relapse incidence were not difference between MLL t-AML, non-MLL t-AML and MLL de novo AML. B. The 3-years LFS in MLL t-AML were not difference compared to non-MLL t-AML and MLL de novo AML (P=0.991 and P=0.055, respectively). C. The 3-years OS in MLL t-AML was higher than MLL de novo AML group (P=0.029), while it did not differ significantly compared to non-MLL t-AML (P=0.141).

allo-HSCT, including 2 in no-MLL t-AML, 2 in MLL t-AML and 12 in MLL de novo AML (**Figure 1**).

Ultimately, 102 patients underwent allo-HSCT, including 15 in MLL t-AML, 20 in no-MLL t-AML, and 67 in MLL de novo AML. There were 52 females and 50 males, with a median age of 49 (range: 17-65) years. Fifty-four patients were translated with HLA matched sibling donor (MSD), 8 with HLA matched unrelated donor (MUD) and 40 with HLA haplo-identical donor (HID). The donor sources were not different among 3 groups (P=0.958). All patients received myeloablative conditioning regimens according to our previously described [23]. The patient characteristics at transplantation were summarized in **Table 4**.

Relapse and survival

Of the 150 patients received anti-leukemia therapy, 69 patients were alive at a median follow up of 13 months (range, 1 to 60 months). Causes of death included leukemia relapse (n=27), infections (n=26), leukemia progression (n=12), GVHD (n=5), and therapy-related mortality (TRM, n=7). Overall, the 3-years OS was 37.5%, 21.5% and 20.4% (P=0.046), respectively, in MLL t-AML, non-MLL t-AML and MLL AML groups. OS in MLL t-AML was higher than MLL de novo AML group (P=0.029), while it did not differ significantly compared to non-MLL t-AML (P=0.141). LFS was 28.0%, 32.2% and 22.7% (P=0.031) respectively in three groups. LFS in MLL t-AML did not differ significantly with non-MLL t-AML and MLL de novo AML (P=0.991 and P=0.055, respectively), while it was higher in non-MLL t-AML compared to MLL de novo AML (P=0.024). the 3-year cumulative incidence of leukemia relapse was 30.0%, 50.4% and 53.5% (P=0.382) (**Figure 2**). Multivariate analysis indicated that MLL t-AML was the risk factor (P=0.005, P<0.001 and P<0.001, respectively), while allo-HSCT was the protective factor for relapse, LFS, and OS (P<0.001, P<0.001 and P=0.002, respectively). In addition, WBC $\geq 50\times10^9$/L was a risk factor for relapse (P=0.026) while CR was a favorable factor for OS (P<0.001) (**Table 5**).

To further clarify the efficacy of allo-HSCT in MLL t-AML patients, we divided patients into two subgroups: patients with chemotherapy alone and patients underwent transplantation. In 48 patients undergoing chemotherapy alone, 23 experienced relapse with a median time of 5 (range, 1.0 to 24.0) months after CR. The 3-years OS was 0%, 17.9% and 2.3% (P=0.038), respectively, in MLL t-AML, non-MLL t-AML and

Table 5. Univariate/Multivariate analysis of outcomes in all patients

	Relapse		LFS		OS	
	Univariate	Multivariate (HR, 95% CI)	Univariate	Multivariate (HR, 95% CI)	Univariate	Multivariate (HR, 95% CI)
Gender, female vs. male	NS	NS	NS	NS	NS	NS
Median age, <40 vs. ≥40 (median)	NS	NS	NS	NS	NS	NS
WBC, <50.0 vs. ≥50.0 (10^9/L)	0.015	0.026 (1.474, 1.245-1.916)	NS	NS	NS	NS
Cytogenetic, unfavorable vs. good/intermediate	NS	NS	NS	NS	NS	NS
Disease status after chemotherapy, CR vs. non-CR	NS	NS	NS	NS	<0.001	<0.001 (0.354, 0.224-0.561)
MLL t-AML vs. non-MLL t-AML vs. MLL de novo AML	0.053	0.005 (1.196, 1.079-1.488)	0.07	<0.001 (1.307, 1.174-1.543)	0.06	<0.001 (2.137, 1.519-2.466)
Allo-HSCT, yes vs. no	<0.001	<0.001 (0.146, 0.069-0.306)	<0.0001	<0.001 (0.137, 0.079-0.236)	0.0001	0.002 (0.092, 0.055-0.156)

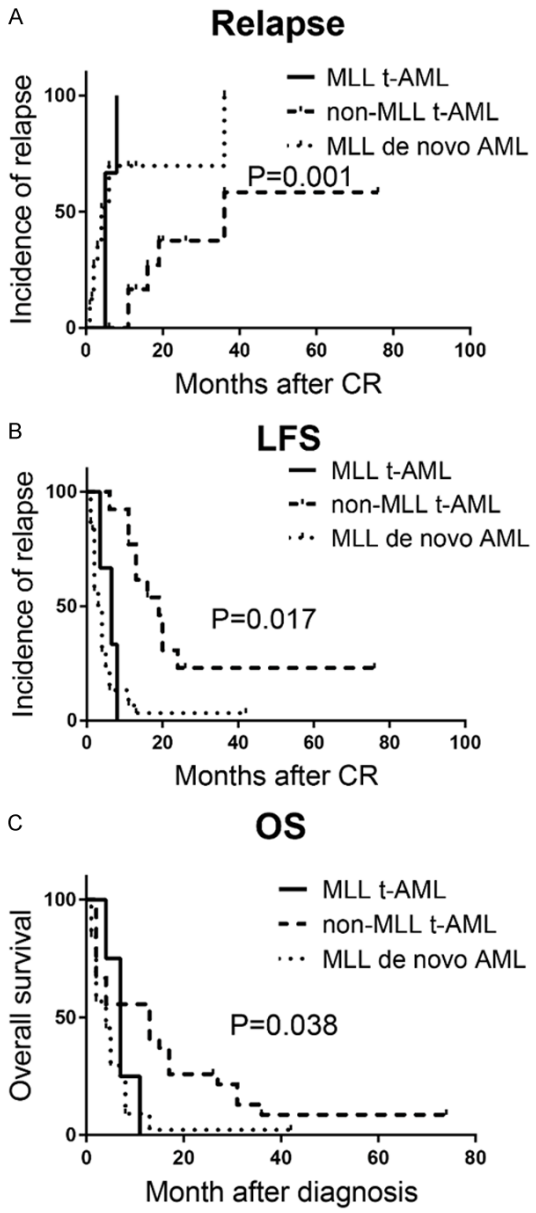

Figure 3. Kaplan-Meier outcome curves for patients with chemotherapy alone. A. The 3-years incidence of relapse in MLL t-AML was higher than non-MLL t-AML and MLL de novo AML (P=0.001 and P=0.034, respectively). B. The 3-years LFS in MLL t-AML was inferior to non-MLL t-AML (P=0.001), while it was not difference compared to MLL de novo AML (P=0.991). C. The 3-years OS in MLL t-AML was inferior than non-MLL t-AML (P=0.019), and it was not difference with MLL de novo AML (P=0.715).

MLL de novo AML groups. OS was longer in non-MLL t-AML than MLL t-AML and MLL de novo AML groups (0.019 and 0.030, respectively), while it was similar between MLL t-AML and MLL de novo AML (P=0.715). The 3-years LFS was 0%, 23.1% and 3.3% (P=0.017), in MLL t-AML, non-MLL t-AML and MLL de novo AML groups, respectively. LFS in MLL t-AML was inferior to non-MLL t-AML (P=0.001), while it was similar when comparing MLL t-AML and MLL de novo AML or comparing non-MLL t-AML and MLL de novo AML (P=0.991 and P=0.075, respectively). The incidence of relapse was 100%, 53.1% and 74.4% (P=0.001), respectively, in MLL t-AML, non-MLL t-AML and MLL de novo AML groups. The relapse in MLL t-AML was inferior than non-MLL t-AML and MLL de novo AML (P=0.001 and P=0.034, respectively), while it was similar between non-MLL t-AML and MLL de novo AML (P=0.318) (**Figure 3**). MLL t-AML was a risk factor for relapse, LFS and OS (P=0.002, 0.001 and 0.015, respectively), and the unfavorable cytogenic was also a risk factor for relapse (P=0.026), while CR was a favorable factor for OS (P<0.001) (**Table 6**).

To analyzed the outcomes in the 102 patients who underwent transplantation, the result showed that 35 relapsed at a median time of 6 (range, 2-15) months post transplantation, including 3 in MLL t-AML, 6 in non-MLL t-AML and 26 in MLL de novo AML. With a median follow up of 18 months (range, 5 to 60 months) post-transplantation, 64 patients were alive and 38 died. The causes of death were summarized in **Table 7**. The 3-years OS was 64.3%, 52.7% and 40.7% (P=0.713), LFS was 60.0%, 48.8% and 37.0% (P=0.934), and the 3-years incidence of relapse post-transplantation was 25.0%, 47.4% and 47.5% (P=0.872), respectively, in MLL t-AML, non-MLL t-AML and MLL de novo AML. There were no significantly difference in OS, LFS and incidence of relapse among three groups (**Figure 4**). Multivariate analysis revealed that the non-CR at transplantation was a risk factor for relapse (P=0.018), LFS (P=0.032) and OS (P=0.034), and the WBC $\geq 50\times10^9$/L at diagnosis was also a risk factors for relapse (P=0.023), LFS (P=0.009,) and OS (P=0.001). While the MSD donor type was a favorable factor for LFS and OS (P=0.001 and P=0.007, respectively), and the intensified conditioning was a protect factor for relapse, LFS and OS (P=0.002, P=0.004 and P=0.001, respectively). In addition, the unfavorable cytogenic was a risk factor for OS (P=0.020). Intriguingly, MLL t-AML was no longer the risk factor for relapse and LFS, while it became a

Table 6. Univariate/Multivariate analysis in chemotherapy

	Relapse		LFS		OS	
	Univariate	Multivariate (HR, 95% CI)	Univariate	Multivariate (HR, 95% CI)	Univariate	Multivariate (HR, 95% CI)
Gender, female vs. male	0.044	NS	0.061	NS	NS	NS
Median age, <40 vs. ≥40	0.078	NS	0.006	NS	0.004	NS
WBC, <50.0 vs. ≥50.0 (10^9/L)	NS	NS	0.042	NS	0.025	NS
Cytogenetic, unfavorable vs. good/intermediate	NS	0.026 (1.337, 1.105-1.522)	NS	NS	NS	NS
Disease status after chemotherapy, CR vs. non-CR	NS	NS	NS	NS	<0.001	<0.001 (0.176, 0.092-0.335)
MLL t-AML vs. non-MLL t-AML vs. MLL de novo AML	0.001	0.002 (1.182, 1.063-1.524)	0.001	0.001 (2.142, 1.639-2.465)	0.001	0.015 (1.583, 1.198-1.861)

Table 7. Cause of death post-transplantation (n=38)

Cause of death	Total	MLL t-AML	Non-MLL t-AML	MLL de novo AML	*P* value
Relapse	20	2 (5.3%)	4 (10.5%)	14 (36.8%)	0.884
GVHD	7	0 (0.0%)	2 (5.3%)	5 (13.2%)	0.578
Infection	7	0 (0.0%)	1 (2.7%)	5 (13.2%)	0.687
Others	4	1 (2.7%)	1 (2.7%)	2 (5.3%)	0.391

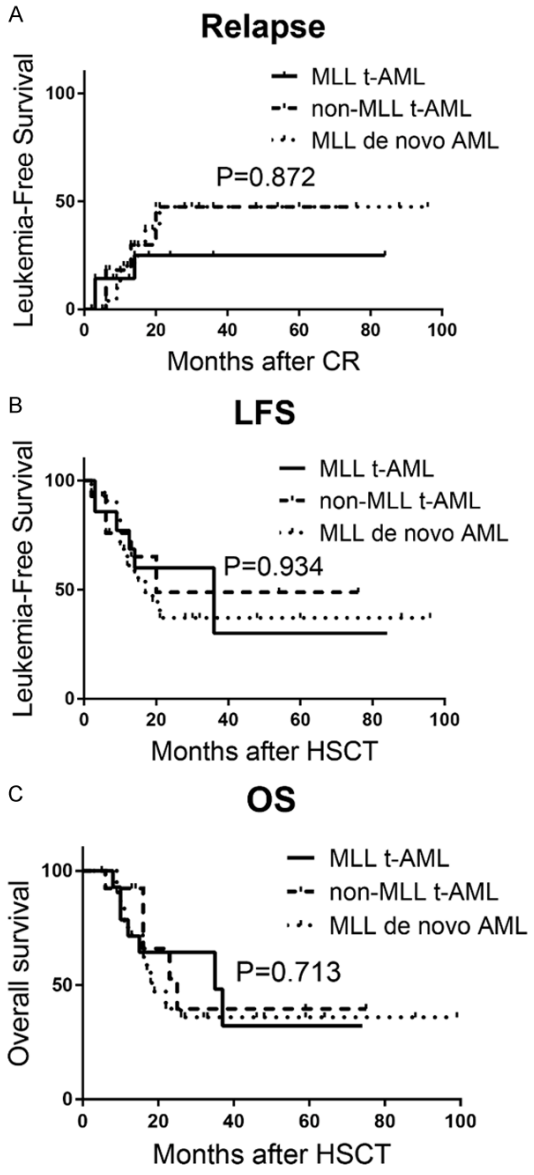

Figure 4. Kaplan-Meier outcome curves for patients with allo-HSCT. A. The 3-years incidence of relapse were not difference between MLL t-AML, non-MLL t-AML and MLL de novo AML. B. The 3-years LFS were not difference among MLL t-AML, non-MLL t-AML and MLL de novo AML. C. The 3-years OS were not difference among MLL t-AML, non-MLL t-AML and MLL de novo AML.

favorable factor for OS (P=0.011) compared with non-MLL t-AML and MLL de novo AML (**Table 8**).

To clarify the influence of primary disease and related therapies on the outcomes of MLL t-AML, we divided primary disease into breast cancer (n=7, 38.9%), lymphoma (n=6, 33.3%) and others (n=5, 27.8%); and we divided the related therapies into chemotherapy alone (n=13, 72.2%) and chemotherapy combined with radiotherapy (n=5, 27.8%). The multivariate analysis indicated that the primary disease and related therapies had no influence on the OS, LFS and relapse of the patients with MLL t-AML (all P>0.05, Supplementary Table 1).

Discussion

Therapy related-AML frequently exhibits abnormalities in genetics and molecular due to the cytotoxic therapy for primary malignancy. Therefore, understanding the cytogenetic and molecular genetic characteristics associated with the outcome of treatments in t-AML is very important for making therapy recommendations. Previous studies indicated that the MLL rearrangement was found in approximately 10% of t-AML [27-29]. But in recent years, due to the combinations of topoisomerase II inhibitors and alkylation were increasingly used in the therapies of primary malignancies, the incidence of MLL rearrangement in t-AML has greatly increased [30, 31]. In this study, we observed that MLL rearrangement is the most common genetic abnormality in t-AML, reached up to 30.5% in solid tumor t-AML, and alterations on chromosomes 5 and/or 7 reached up to 33.4% in this cohort, which is significantly higher than 7%-13% previously reported in t-AML and de novo MLL AML [6, 16]. A possible interpretation of our finding is that 94.4% of our patients had used topoisomerase II inhibitor as treatment for their primary malignancy, which is associated with MLL rearrangement [32], and 77.8% were combined with alkylating agents, which is contributed to the abnormal of chromosomes 5 and 7 [33, 34].

Generally, de novo AML with MLL rearrangement had a poor prognosis compared with those without MLL rearrangement, such as lower remission, higher relapse, and inferior

Table 8. Univariate/Multivariate analysis in transplantation

	Relapse		LFS		OS	
	Univariate	Multivariate, *P* value, (HR, 95% CI)	Univariate	Multivariate, *P* value, (HR, 95% CI)	Univariate	Multivariate, *P* value, (HR, 95% CI)
Gender, male vs. female	NS	NS	NS	NS	NS	NS
Median age, <49 vs. ≥49 (median)	NS	NS	NS	NS	NS	NS
WBC, <50.0 vs. ≥50.0 (10^9/L)	0.030	0.023 (2.644, 1.144-6.108)	0.004	0.009 (2.193, 1.203-3.999)	0.006	0.001 (2.862, 1.474-5.559)
Cytogenic, unfavorable vs. good/intermediate	NS	NS	NS	NS	0.030	0.020 (2.348, 1.144-4.820)
HLA type, MSD vs. MUD vs. HID	NS	NS	0.071	NS	0.050	0.029 (0.873, 0.736-0.938)
Disease status, non-CR vs. CR	0.0001	0.018 (1.751, 1.065-2.585)	0.0001	0.032 (1.394, 1.034-1.878)	0.001	0.034 (1.444, 1.028-2.028)
Conditioning regimen[a], Intensified vs. myeloablative	NS	0.002 (0.196, 0.071-0.541)	NS	0.004 (0.346, 0.166-0.719)	NS	0.001 (0.305, 0.155-0.600)
MLL t-AML vs. non-MLL t-AML vs. MLL de novo AML	NS	NS	NS	NS	0.081	0.011 (0.439, 0.162-1.191)

[a]Myeloablative conditioning regimens include TBI (total body irradiation) + Cy (cyclophosphamide), Bu (busulfan) + Cy, and Bu + Flu (fludarabine). Intensified conditioning regiments include TBI + Cy + etoposide, and Flu + cytarabine + TBI + Cy.

survivals [35-37]. But whether the outcomes of MLL t-AML is as inferior as that of MLL de novo AML was rarely reported. In this study, our results showed that the CR rates of chemotherapy were not difference among MLL t-AML, non-MLL t-AML, and MLL de novo AML, but MLL t-AML was a risk factor for relapse, LFS and OS in those underwent chemotherapy alone. In these patients, the 3-years OS was longer in non-MLL t-AML than MLL t-AML and MLL de novo AML groups, while it was similar between MLL t-AML and MLL de novo AML. The 3-years LFS in MLL t-AML was inferior to non-MLL t-AML, while it was similar when comparing MLL t-AML and MLL de novo AML or comparing non-MLL t-AML and MLL de novo AML. And the incidence of relapse in MLL t-AML was higher than non-MLL t-AML and MLL de novo AML, while it was similar between non-MLL t-AML and MLL de novo AML.

De novo AML with MLL rearrangement and t-AML were both belong to high-risk-AML, allo-HSCT is recommended for both subtype of AML [20-22]. But even with allo-HSCT, the outcomes of de novo AML with MLL rearrangement were inferior than those without MLL rearrangement de novo AML [38, 39]. In this study, we found that the relapse, LFS and OS were not significant difference among MLL t-AML, non-MLL t-AML and MLL de novo AML in these patients undergoing allo-HSCT. Allo-HSCT was protective factor for the relapse, LFS and OS, while MLL t-AML was no longer a risk factor for relapse and LFS. Inspiringly, MLL t-AML became a favorable factor for OS compared with non-MLL t-AML and MLL de novo AML. The mechanism of this result is worthy of further study.

In summary, MLL rearrangement is the most common genetic abnormality in solid tumor t-AML. MLL t-AML had poor prognosis compared with non-MLL t-AML and MLL de novo AML with chemotherapy alone. And allo-HSCT might overcome the poor prognosis of MLL t-AML. Our single-center retrospective study is limited by the sample size and retrospective data, a larger prospective cohort is needed to further confirm these results.

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Disclosure of conflict of interest

None.

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References

[1] Granfeldt Ostgard LS, Medeiros BC, Sengelov H, Norgaard M, Andersen MK, Dufva IH, Friis LS, Kjeldsen E, Marcher CW, Preiss B, Severinsen M and Norgaard JM. Epidemiology and clinical significance of secondary and therapy-related acute myeloid leukemia: a national population-based cohort study. J Clin Oncol 2015; 33: 3641-3649.

[2] Swaika A, Frank RD, Yang D, Finn LE, Jiang L, Advani P, Chanan-Khan AA, Ailawadhi S and Foran JM. Second primary acute lymphoblastic leukemia in adults: a SEER analysis of incidence and outcomes. Cancer Med 2018; 7: 499-507.

[3] Giri S, Chi M, Johnson B, McCormick D, Jamy O, Bhatt VR and Martin MG. Secondary acute lymphoblastic leukemia is an independent predictor of poor prognosis. Leuk Res 2015; 39: 1342-1346.

[4] Claerhout H, Lierman E, Michaux L, Verhoef G and Boeckx N. A monocentric retrospective study of 138 therapy-related myeloid neoplasms. Ann Hematol 2018; 97: 2319-2324.

[5] Li Z, Labopin M, Ciceri F, Blaise D, Tischer J, Ehninger G, Van Lint MT, Koc Y, Santarone S, Forcade E, Castagna L, Polge E, Mailhol A, Ruggeri A, Mohty M, Savani BN and Nagler A. Haploidentical transplantation outcomes for secondary acute myeloid leukemia: acute leukemia working party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT) study. Am J Hematol 2018; 93: 769-777.

[6] ESPíRITO Santo AE, Chacim S, Ferreira I, Leite L, Moreira C, Pereira D, Dantas Brito MD, Nunes M, Domingues N, Oliveira I, Moreira I, Martins A, Viterbo L, Mariz JM and Medeiros R. Effect of therapy-related acute myeloid leukemia on the outcome of patients with acute myeloid leukemia. Oncol Lett 2016; 12: 262-268.

[7] Yoshizato T, Nannya Y, Atsuta Y, Shiozawa Y, Iijima-Yamashita Y, Yoshida K, Shiraishi Y, Suzuki H, Nagata Y, Sato Y, Kakiuchi N, Matsuo K, Onizuka M, Kataoka K, Chiba K, Tanaka H, Ueno H, Nakagawa MM, Przychodzen B, Haferlach C, Kern W, Aoki K, Itonaga H, Kanda Y, Sekeres MA, Maciejewski JP, Haferlach T, Miyazaki Y, Horibe K, Sanada M, Miyano S, Makishima H and Ogawa S. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. Blood 2017; 129: 2347-2358.

[8] Breems DA, Van Putten WL, De Greef GE, Van Zelderen-Bhola SL, Gerssen-Schoorl KB, Mellink CH, Nieuwint A, Jotterand M, Hagemeijer A, Beverloo HB and Löwenberg B. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol 2008; 26: 4791-4797.

[9] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M and Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016; 127: 2391-2405.

[10] Ganser A and Heuser M. Therapy-related myeloid neoplasms. Curr Opin Hematol 2017; 24: 152-158.

[11] Godley LA and Larson RA. Therapy-related myeloid leukemia. Semin Oncol 2008; 35: 418-429.

[12] Rund D, Krichevsky S, Bar-Cohen S, Goldschmidt N, Kedmi M, Malik E, Gural A, Shafran-Tikva S, Ben-Neriah S and Ben-Yehuda D. Therapy-related leukemia: clinical characteristics and analysis of new molecular risk factors in 96 adult patients. Leukemia 2005; 19: 1919-1928.

[13] Larson RA. Cytogenetics, not just previous therapy, determines the course of therapy-related myeloid neoplasms. J Clin Oncol 2012; 30: 2300-2302.

[14] McNerney ME, Godley LA and Le Beau MM. Therapy-related myeloid neoplasms: when genetics and environment collide. Nat Rev Cancer 2017; 17: 513-527.

[15] Ruggeri A, Labopin M, Ciceri F, Mohty M and Nagler A. Definition of GvHD-free, relapse-free survival for registry-based studies: an ALWP-EBMT analysis on patients with AML in remission. Bone Marrow Transplant 2016; 51: 610.

[16] Schoch C. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood 2003; 102: 2395-2402.

[17] Aldoss I, Stiller T, Tsai NC, Song JY, Cao T, Bandara NA, Salhotra A, Khaled S, Aribi A, Al Malki MM, Mei M, Ali H, Spielberger R, O'Donnell M, Snyder D, Slavin T, Nakamura R, Stein AS, Forman SJ, Marcucci G and Pullarkat V. Therapy-related acute lymphoblastic leukemia has distinct clinical and cytogenetic features compared to de novo acute lymphoblastic leukemia, but outcomes are comparable in transplanted patients. Haematologica 2018; 103: 1662-1668.

[18] Mosad E, Abdou M and Zaky AH. Rearrangement of the myeloid/lymphoid leukemia gene in therapy-related myelodysplastic syndrome in patients previously treated with agents targeting DNA topoisomerase II. Oncology 2012; 83: 128-134.

[19] Faller BA, Robu VG and Borghaei H. Therapy-related acute myelogenous leukemia with an 11q23/MLL translocation following adjuvant cisplatin and vinorelbine for non-small-cell lung cancer. Clin Lung Cancer 2009; 10: 438-440.

[20] Tallman MS, Wang ES, Altman JK, Appelbaum FR, Bhatt VR, Bixby D, Coutre SE, De Lima M, Fathi AT, Fiorella M, Foran JM, Hall AC, Jacoby M, Lancet J, LeBlanc TW, Mannis G, Marcucci G, Martin MG, Mims A, O'Donnell MR, Olin R, Peker D, Perl A, Pollyea DA, Pratz K, Prebet T, Ravandi F, Shami PJ, Stone RM, Strickland SA, Wieduwilt M, Gregory KM, Hammond L and Ogba N. Acute myeloid leukemia, version 3.2019, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2019; 17: 721-749.

[21] Leukemia & Lymphoma Group, Chinese Society of Hematology, Chinese Medical Association. [Chinese guidelines for diagnosis and treatment of adult acute myeloid leukemia (not APL) (2017)]. Zhonghua Xue Ye Xue Za Zhi 2017; 38: 177-182.

[22] Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B and Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424-447.

[23] Yu S, Huang F, Fan Z, Xuan L, Nie D, Xu Y, Yang T, Wang S, Jiang Z, Xu N, Lin R, Ye J, Lin D, Sun

J, Huang X, Wang Y and Liu Q. Haploidentical versus HLA-matched sibling transplantation for refractory acute leukemia undergoing sequential intensified conditioning followed by DLI: an analysis from two prospective data. J Hematol Oncol 2020; 13: 18.

[24] Yu S, Huang F, Wang Y, Xu Y, Yang T, Fan Z, Lin R, Xu N, Xuan L, Ye J, Yu W, Sun J, Huang X and Liu Q. 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: 1433-1443.

[25] Cheson BD, Bennett JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Löwenberg B, Sanz MA, Head DR, Ohno R, Bloomfield CD and LoCocco F. Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003; 21: 4642-4649.

[26] Mrózek K, Heerema NA and Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004; 18: 115-136.

[27] Chen CW and Armstrong SA. Targeting DOT1L and HOX gene expression in MLL-rearranged leukemia and beyond. Exp Hematol 2015; 43: 673-684.

[28] Huret JL, Dessen P and Bernheim A. An atlas of chromosomes in hematological malignancies. Example: 11q23 and MLL partners. Leukemia 2001; 15: 987-989.

[29] Krivtsov AV and Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer 2007; 7: 823-833.

[30] Hartmann L, Nadarajah N, Meggendorfer M, Höllein A, Vetro C, Kern W, Haferlach T, Haferlach C and Stengel A. Molecular characterization of a second myeloid neoplasm developing after treatment for acute myeloid leukemia. Leukemia 2020; 34: 811-820.

[31] Brzezinka K, Nevedomskaya E, Lesche R, Steckel M, Eheim AL, Haegebarth A and Stresemann C. Functional diversity of inhibitors tackling the differentiation blockage of MLL-rearranged leukemia. J Hematol Oncol 2019; 12: 66.

[32] Super HJ, McCabe NR, Thirman MJ, Larson RA, Le Beau MM, Pedersen-Bjergaard J, Philip P, Diaz MO and Rowley JD. Rearrangements of the MLL gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. Blood 1993; 82: 3705-3711.

[33] Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, Lamprecht TL, Shen D, Hundal J, Fulton RS, Heath S, Baty JD, Klco JM, Ding L, Mardis ER, Westervelt P, DiPersio JF, Walter MJ, Graubert TA, Ley TJ, Druley T, Link DC and Wilson RK. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature 2015; 518: 552-555.

[34] Christiansen DH, Andersen MK and Pedersen-Bjergaard J. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 2001; 19: 1405-1413.

[35] Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W and Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood 2003; 102: 2395-2402.

[36] Gatwood KS, Labopin M, Savani BN, Finke J, Socie G, Beelen D, Yakoub-Agha I, Chevallier P, Ganser A, Blaise D, Milpied N, Bruno L, Mailhol A, Mohty M and Nagler A. Transplant outcomes for patients with therapy-related acute myeloid leukemia with prior lymphoid malignancy: an ALWP of EBMT study. Bone Marrow Transplant 2020; 55: 224-232.

[37] Aldoss I, Douer D and Pullarkat V. Therapy-related acute lymphoblastic leukemia: where do we stand with regards to its definition and characterization? Blood Rev 2019; 37: 100584.

[38] Kotani S, Yoda A, Kon A, Kataoka K, Ochi Y, Shiozawa Y, Hirsch C, Takeda J, Ueno H, Yoshizato T, Yoshida K, Nakagawa MM, Nannya Y, Kakiuchi N, Yamauchi T, Aoki K, Shiraishi Y, Miyano S, Maeda T, Maciejewski JP, Takaori-Kondo A, Ogawa S and Makishima H. Molecular pathogenesis of disease progression in MLL-rearranged AML. Leukemia 2019; 33: 612-624.

[39] Stavropoulou V, Kaspar S, Brault L, Sanders MA, Juge S, Morettini S, Tzankov A, Iacovino M, Lau IJ, Milne TA, Royo H, Kyba M, Valk PJM, Peters A and Schwaller J. MLL-AF9 expression in hematopoietic stem cells drives a highly invasive aml expressing EMT-related genes linked to poor outcome. Cancer Cell 2016; 30: 43-58.

Supplementary Table 1. Univariate/Multivariate analysis of MLL t-AML patients

	Relapse		LFS		OS	
	Univariate	Multivariate, *P* value, (HR, 95% CI)	Univariate	Multivariate, *P* value, (HR, 95% CI)	Univariate	Multivariate, *P* value, (HR, 95% CI)
Primary disease, breast cancer vs. lymphoma vs. others	0.039	NS	NS	NS	NS	NS
Primary disease therapy, chemo alone vs. chemo/radiotherapy	NS	NS	NS	NS	NS	NS
Allo-HSCT, yes vs. no	0.07	NS	0.001	0.009 (0.099, 0.017-0.561)	<0.001	0.004 (0.066, 0.011-0.418)