RESEARCH ARTICLE

Allogeneic hematopoietic stem cell transplantation could improve survival of cytogenetically normal adult acute myeloid leukemia patients with DNMT3A mutations

Yang Xu,[1,2,3] Yanjun Sun,[1] Hongjie Shen,[1] Lin Ding,[4] Zhen Yang,[1] Huiying Qiu,[1,2] Aining Sun,[1,2,3] Suning Chen,[1,2]* and Depei Wu[1,2,3]*

DNMT3A mutations are frequent in cytogenetically normal acute myeloid leukemia (cn-AML) patients and associated with poor survival. The role of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in *DNMT3A*^{mut} cn-AML patients remains unclear. In this study, we retrospectively analyzed the prognostic impact of *DNMT3A* mutations and explored the role of allo-HSCT in 308 cn-AML patients who received consolidation of intensive chemotherapy or allo-HSCT in our center from March 2005 to May 2014. In the whole cohort, 63 patients (20.5%) were identified with *DNMT3A* exon 23 mutations and R882H was the most frequent variant. *DNMT3A*^{mut} patients had shorter overall survival (3-year OS: 31.9% vs. 52.0%, $P = 0.009$) and disease-free survival (3-year DFS: 21.8% vs. 40.1%, $P = 0.004$) compared with *DNMT3A*^{wt} patients. Based on *FLT3/NPM1/CEBPA* mutations, 308 cn-AML patients were divided into favorable/intermediate group ($n = 262$) and unfavorable group ($n = 46$). There were no significant differences in 3-year OS and 3-year DFS between *DNMT3A*^{mut} and *DNMT3A*^{wt} patients in both favorable/intermediate and unfavorable groups. Additionally, in multivariate analysis, *DNMT3A* mutation remained an independent adverse prognostic factor for the survival. In the *DNMT3A*^{mut} cohort, 23 complete remission (CR) patients received allo-HSCT consolidation and 32 CR patients received chemotherapy consolidation, dramatic differences were observed in 3-year OS (51.7% vs. 28.9%, $P = 0.048$) and 3-year DFS (41.6% vs. 14.9%, $P = 0.024$) between allo-HSCT group and chemotherapy group. Collectively, *DNMT3A* mutation is a poor prognostic factor for cn-AML patients and allo-HSCT could improve survival of cn-AML patients with *DNMT3A* mutations.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disorder with regard to morphology and chromosome aberrations detected in the leukemic cells. Clinical and genetic prognostic markers are now crucial in the evaluation of AML patients and in guiding rational management. Forty to fifty percent of patients do not have clonal chromosomal aberrations, and all such cases of cytogenetically normal AML (cn-AML) are currently categorized in the intermediate-risk group [1,2]. In recent years, extended molecular analyses have yielded novel molecular markers important for proper diagnostics and prognostics of AML, including fms-like tyrosine kinase-3 gene (*FLT3*), nucleophosmin gene (*NPM1*), and the CCAAT/enhancer binding protein gene (*CEBPA*), which provide risk classification for AML at diagnosis in current clinical practice [3–6].

With the development of whole genome sequencing technology, additional recurrently mutated genes in AML were identified. Many of the mutated genes were involved in epigenetic regulation of transcription, including *IDH1/2*, *TET-2*, and *DNMT3A* [7–11]. *DNMT3A*, located in chromosomal band 2p23 and, together with DNMT3B and *DNMT1*, is a member of a DNA methyltransferase (MTase) family, which catalyzes the addition of methyl groups to cytosine residues of CpG nucleotides [12]. According to published literatures, a range of 11–35% AML patients were

Additional Supporting Information may be found in the online version of this article.

[1]Jiangsu Institute of Hematology, Key Laboratory of Thrombosis and Hemostasis of Ministry of Health, The First Affiliated Hospital of Soochow University, Suzhou, People's Republic of China; [2]Collaborative Innovation Center of Hematology, Soochow University, Suzhou, People's Republic of China; [3]Suzhou Institute of Blood and Marrow Transplantation, Soochow University, Suzhou, People's Republic of China; [4]Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan

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Y.X., Y.S., and H.S. contributed equally to this work.
***Correspondence to:** Suning Chen; Jiangsu Institute of Hematology, Key Laboratory of Thrombosis and Hemostasis of Ministry of Health, The First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou 215006, Jiangsu province, P.R. China. E-mail: chensuning@sina.com (or) Depei Wu; Jiangsu Institute of Hematology, Key Laboratory of Thrombosis and Hemostasis of Ministry of Health, The First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou 215006, Jiangsu province, P.R. China. E-mail: wudepei@medmail.com.cn
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observed with *DNMT3A* mutations and the majority of *DNMT3A* mutations were missense mutations at residue 882. *DNMT3A* mutations were also associated with old age, higher white blood cells, normal cytogenetic, and the presence of *NPM1*, *FLT3-ITD*, and *IDH1* mutations [13–16]. The function and biological consequences of DNMT3A mutations have not yet to be fully understood, while a negative impact on outcomes in cn-AML patients was observed by a number of studies [17–21]. Currently, only three molecular markers (*NPM1*, *CEBPA*, and *FLT3-ITD*) have been included in the European Leukemia Net classification [6], whether *DNMT3A* will be included in the future is an interesting question. Moreover, allogeneic hematopoietic stem cell transplantation (allo-HSCT) were recommended to be the first-front therapy for the consolidation of AML patients with molecular unfavorable factors [6,22,23], however, the clinical benefit of allo-HSCT consolidation has not been clearly established in cn-AML patients with *DNMT3A* mutations.

As the *DNMT3A* often served as the poor risk factor and R882 mutations was the major mutation, we analyzed *DNMT3A* R882 mutations together with *FLT3*, *NPM1*, and *CEBPA* to provide risk classification, and retrospectively analyzed the clinical phenotype, prognostic impact of *DNMT3A* mutations and explored treatment value of allo-HSCT in 308 cn-AML patients who received intensive consolidation therapy or allo-HSCT.

Patients and Methods

Patients and treatments. Three hundred and eight adult patients diagnosed with de novo cn-AML were involved in this study with approval of the ethics committee of our hospital from March 2005 to May 2014. All cn-AML patients received induction chemotherapy consisted of standard first-line treatment of an IA (Idarubicin and cytarabine)-like regimen comprised of Idarubicin 8–12 mg/m^2 (Days 1–3) and cytarabine 100 mg/m^2 (Days 1–7), and after achieving first complete remission (CR) patients received consolidation of either at least four cycles of intermediate/high-dose cytarabine (1–2 g/m^2 over 3 hr for 3 days)-based combination chemotherapy ($n = 94$) or allo-HSCT treatment ($n = 214$).

HSCT patients received a conditioning regimen consisting of cytarabine (2 g/m^2/day for 2 days), busulfan (3.2 mg/kg/day for 3 days, intravenously), and cyclophosphamide (1.8 g/m^2/day for 2 days). Patients with high risk of central nervous system leukemia received a similar regimen, but with total body irradiation (8 Gy, lung shielding at 6.5 Gy, on Day -6) substituted for busulfan and cytarabine started on Day -8. The patients who are considered poor candidates for myeloablative conditioning due to advanced age or other concurrent medical conditions were conditioned with fludarabine 30 mg/m^2 (Days -6 to -2), cytarabine 2 g/day (on Days -5 and -2), rabbit antithymocyte globulin (ATG; Genzyme, Cambridge, MA) 1.5 mg/kg/day (on Days -5 to -2), and cyclophosphamide 30 mg/kg/day (on Days -3 to -2). Graft-versus-host disease (GVHD) prophylaxis consisted of continuous cyclosporine infusion at 3 mg/kg/day starting on Day -1 until patients could switch to oral intake (PO), with a target blood concentration ranging from 200 to 300 ng/ml, and short term methotrexate given on Days +1, +3, +6, and +11 at doses of 15, 10, 10, and 10 mg/m^2, respectively.

Cytogenetic and mutation analyses. The bone marrow (BM) samples of de novo AML patients were processed using standard 24-hr unstimulated cultures. A conventional R-banding assay was used for karyotypic analysis. When possible, at least 20 metaphases per sample were analyzed. Genomic DNA was extracted from BM-derived mononuclear cells using the PurelinkTM Genomic DNA mini kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A variety of acute leukemia-related gene mutations were evaluated, including *FLT3-ITD*, *FLT3-TKD*, *NPM1*, *CEBPA*, and *DNMT3A*. In this study, only *DNMT3A* exon 23 mutations were analyzed. The gene mutations were detected by PCR amplification of the entire or a portion of the coding region followed by direct bidirectional DNA sequencing in cn-AML patients.

Statistical analyses. A student's *t*-test was used to compare the age, WBC (white blood cells) count, hemoglobin, platelet count, and BM blasts at diagnosis in the different groups. The χ^2 test was used to analyze the interrelation for different gene mutations. Overall survival (OS) was measured as the time from the date of disease diagnosis to the date of death or last follow-up. Disease-free survival (DFS) was defined as the time from disease diagnosis to CR, first relapse, or death. A Kaplan–Meier analysis was used to calculate the distribution of OS and DFS. All quoted *P* values were from two-tailed tests and confidence intervals refer to 95% boundaries. All calculations were performed using the SPSS software package (version 19.0; IBM).

Results

Clinical characteristics of the 308 cn-AML patients

Three hundred and eight adult de novo cn-AML patients were involved in this study from March 2005 to May 2014. The median age was 40 years (range: 16–68 years), and there are 144 males and 164 females. Based on French–American–British (FAB) criteria, there were 5 (1.6%) patients classified as M0, 55 (17.9%) as M1, 74 (24.0%) as M2, 53 (17.2%) as M4, 75 (24.4%) as M5, 16 (5.2%) as M6, 3 (1.0%) as M7, and 27 (8.8%) that were unclassified AML patients. The median blast in BM was 56.12%, and the median white cell counts was 25.22 (0.5–355.9)*10^9/L. Based on *FLT3-ITD/NPM1/CEBPA* risk classification, the total 308 cn-AML patients could be divided into unfavorable risk group ($n = 46$, harboring *FLT3-ITD* mutation) and favorable/intermediate risk group ($n = 262$, other mutations). Two hundred and twenty-six cn-AML patients achieved CR with one cycle of induction therapy and 271 cn-AML patients achieved CR with two cycles of induction therapy. In these CR patients, 188 patients received allo-HSCT and 83 patients received consolidation of intermediate/high-dose cytarabine chemotherapy. Additionally, 37 patients who were no remission with induction therapy received salvage therapy of allo-HSCT ($n = 26$) or Decitabine combined intermediate dose cytarabine chemotherapy ($n = 11$).

Frequencies of DNMT3A mutations and clinical features in cn-AML patients

As shown in Table I, 63 (20.4%) cn-AML patients were identified with *DNMT3A* exon 23 mutations in a total cohort of 308 cn-AML patients, and the distribution of mutations was as follows: R882H ($n = 36$), R882C ($n = 12$), R882P ($N = 6$), R882H&C ($n = 2$), and others ($n = 7$). The median age of *DNMT3A* mutated patients was elder than that of the control group ($P < 0.001$), while there were no significant differences in sex, white cell counts, and blast percentage in peripheral blood (PB) and BM between patients with and without *DNMT3A* mutations. However, regarding to FAB distributions, more M5 patients (38.1%) were observed in *DNMT3A* mutated group compared with the controls (20.8%) group ($P < 0.001$). *FLT3-ITD* and *NPM1* mutations were also more often observed in *DNMT3A* mutated group ($P < 0.001$).

Clinical outcome of cn-AML patients with DNMT3A mutations

The median follow-up time for cn-AML patients was 30 months (range: 6–98 months). Compared with *DNMT3A*^{wt} patients, *DNMT3A*^{mut} patients had a lower 3-year OS (31.9% vs. 52.0%, $P = 0.009$) and 3-year DFS (21.8% vs. 40.1%, $P = 0.004$) (Supporting Information), suggesting *DNMT3A* mutations might serve as a poor prognostic factor. The survival of all 308 cn-AML patients were further analyzed according to *FLT3-ITD/NPM1/CEBPA* risk classification, and the validity of *FLT3-ITD/NPM1/CEBPA* risk classification was verified by the median OS time (favorable: not reached; intermediate: 41 months; unfavorable: 12 months, $P < 0.001$) and DFS time (favorable: 36 months; intermediate: 15 months; unfavorable: 7 months, $P = 0.003$). Notably, there was no significant difference in OS (3-years OS: 39.5% vs. 53.5%, $P = 0.210$) and DFS (3-years DFS: 24.8% vs. 41.2%, $P = 0.091$) between *DNMT3A*^{mut} and *DNMT3A*^{wt} patients in favorable/intermediate group, and similar results were observed in OS and DFS between *DNMT3A*^{mut} and *DNMT3A*^{wt} patients in unfavorable group (3-year OS: 21.6% vs. 29.3%, $P = 0.582$; 3-year DFS: 17.4% vs. 23.9%, $P = 0.508$). (Fig. 1)

Multivariate analyses for outcomes in cn-AML patients

In the total 308 DNMT3A mutated patients, 214 patients received allo-HSCT consolidation while 94 patients received at least four cycles

TABLE I. Clinical Characteristics of the 308 cn-AML Patients

	Total ($n = 308$)	DNMT3A^{mut} ($n = 63$)		DNMT3A^{wt} ($n = 245$)		P
Age (years)						
Median (range)	40 (16–68)	44 (16–68)		39 (18–65)		<0.001
Gender						0.420
Male	144	21	33.3%	123	50.2%	
Female	164	42	66.7%	122	49.8%	
ECOG						0.536
0	53	11	17.5%	42	17.1%	
1	164	30	47.6%	134	54.7%	
2	91	22	34.9%	69	28.2%	
FAB						<0.001
M0	5	2	3.2%	3	1.2%	
M1	55	0	0.0%	55	22.5%	
M2	74	15	23.8%	59	24.1%	
M4	53	14	22.2%	39	15.9%	
M5	75	24	38.1%	51	20.8%	
M6	16	0	0.0%	16	6.5%	
M7	3	0	0.0%	3	1.2%	
Unknown	27	8	12.7%	19	7.8%	
Blast in PB (%)						
Median	53.70	45.19		55.49		0.200
Blast in BM (%)						
Median	56.12	57.47		55.78		0.768
White cells (10^9/L)						
Median (range)	25.22 (0.5–355.9)	29.49 (0.67–204)		24.15 (0.5–355.9)		0.323
Hb (g/L)						
Median (range)	85 (29–157)	76.5 (36–157)		86 (29–157)		0.307
Platelets (10^9/L)						
Median (range)	45 (6–985)	56.5 (8–443)		38 (6–985)		0.132
FLT3-ITD						
mut	46	24	38.1%	22	9.0%	<0.001
wt	262	39	61.9%	223	91.0%	
NPM1						
mut	69	30	47.6%	39	15.9%	<0.001
wt	239	33	52.4%	206	84.1%	
CEBPA						
mut	24	4	6.3%	20	8.2%	0.795
wt	284	59	93.7%	225	91.8%	
FLT3-ITD/NPM1	27	19	70.4%	8	29.6%	<0.001
FLT3-ITD/CEBPA	4	3	75.0%	1	25.0%	0.028
NPM1/CEBPA/FLT3-ITD						
Favorable/intermediate	262	39	61.9%	223	91.0%	<0.001
Unfavorable	46	24	38.1%	22	9.0%	
CR	271	55	87.3%	216	88.2%	0.851

Abbreviation: PB: Peripheral blood; BM: Bone Marrow; Hb: Hemoglobin; CR: Complete Remission.

of intermediate dose cytarabine-based combination chemotherapy. We then performed multivariate analyses including age (<45 years vs. >45 years), treatment (allo-HSCT vs. chemotherapy), white blood cell counts, hemoglobin counts, platelet counts, ECOG score, *FLT3-ITD/NPM1/CEBPA* risk classification, and *DNMT3A* mutations on the end point OS and DFS in the total 308 cn-AML patients. The results revealed that age, treatment, *FLT3-ITD/NPM1/CEBPA* risk classification, and *DNMT3A* mutations were significantly and independently associated with a worse OS and DFS, while high white blood cell counts were associated with a worse OS and DFS and low ECOG score was associated with a better DFS in univariate analysis (Table II).

allo-HSCT could improve survival of cn-AML with DNMT3A mutations

Fifty-five (55/63, 87.3%) *DNMT3A* mutated patients finally achieved CR with induction therapy. In these patients, 23 patients received allo-HSCT consolidation while 32 patients received at least four cycles of high-dose cytarabine-based combination chemotherapy. No significant differences were observed in age, sex, FAB distribution, BM blast, and other mutations in these two groups (Supporting Information). Interestingly, the 3-year OS (51.7% vs. 28.9%, $P = 0.048$) and 3-year DFS (41.6% vs. 14.9%, $P = 0.024$) were dramatically improved in allo-HSCT group. When we limited this comparison to the favorable/intermediate risk group only, significant differences were also observed in both 3-year OS (56.0% vs. 34.8%; $P = 0.036$) and 3-year DFS (41.9% vs. 16.7%; $P = 0.047$) between these two groups (Fig. 2). Meanwhile, based on *DNMT3A* mutations, the total HSCT cohort ($n = 214$) were further divided into *DNMT3A*^{wt} group ($n = 189$) and *DNMT3A*^{mut} group ($n = 25$) and the patients characteristic were listed in Supporting Information. No differences were observed in age, donor type, condition regimen, GVHD prophylaxis between two groups, and the 3-year OS (46.7% vs. 57.2%, $P = 0.731$) and 3-year DFS (35.2% vs. 38.5%, $P = 0.871$) were similar in these two groups. In addition, with respect to favorable/intermediate risk group, there were also no differences in 3-year OS (52.7% vs. 59.1%; $P = 0.599$) and 3-year DFS (39.4% vs. 40.7%; $P = 0.569$) between *DNMT3A*^{wt} and *DNMT3A*^{mut} groups (Supporting Information). These results might indicate that allo-HSCT could reverse the poor outcome of cn-AML with *DNMT3A* mutations.

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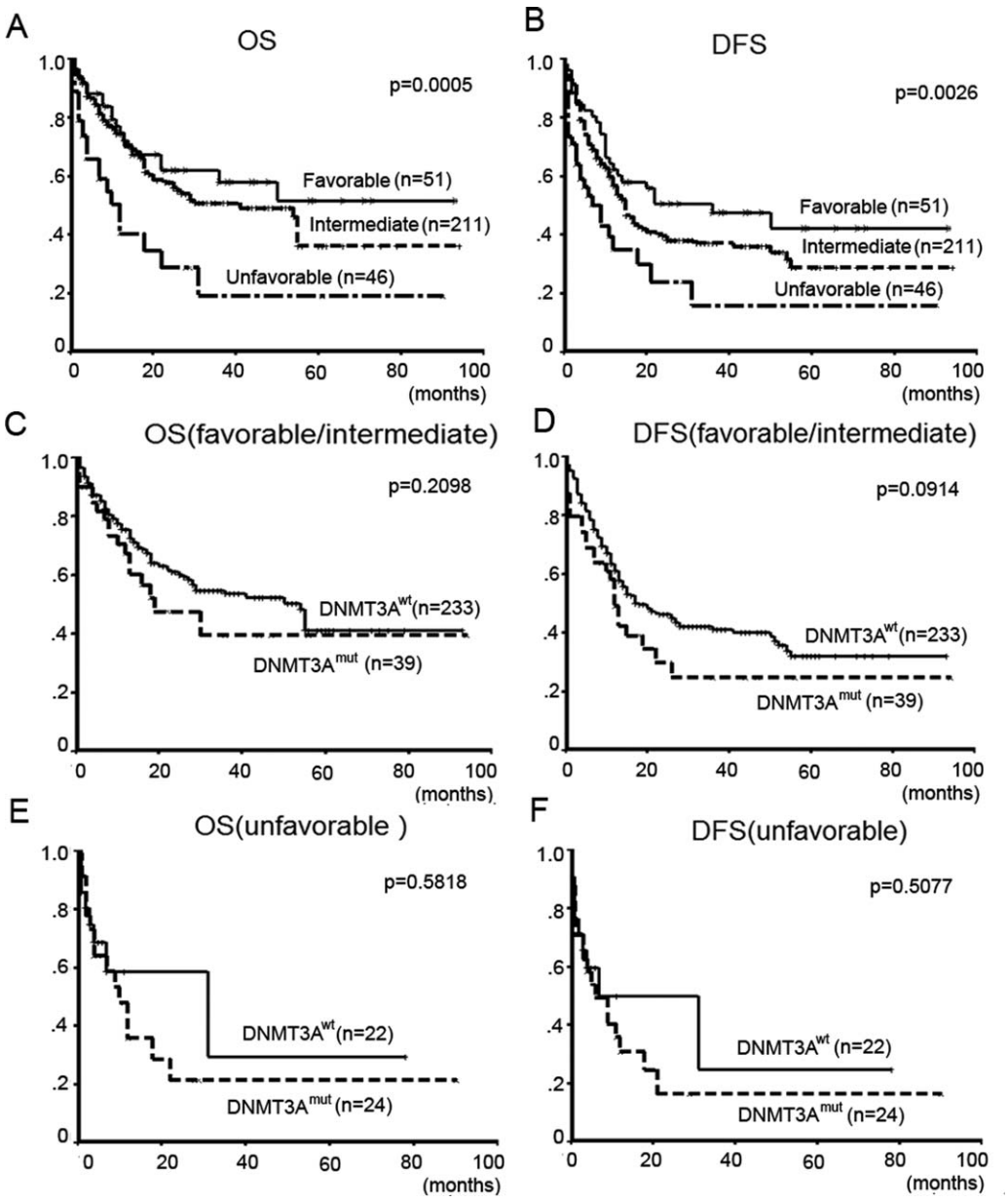

Figure 1. Kaplan–Meier survival curves for cn-AML patients with different *FLT3-ITD/CEBPA/NPM1* risk classification. (A) OS of cn-AML patients in favorable, intermediate, and unfavorable groups. (B) DFS of cn-AML patients in favorable, intermediate, and unfavorable groups. (C) OS of cn-AML patients with and without *DNMT3A* mutations in favorable/intermediate group. (D) DFS of cn-AML patients with and without *DNMT3A* mutations in favorable/intermediate group. (E) OS of cn-AML patients with and without *DNMT3A* mutations in unfavorable group. (F). DFS of cn-AML patients with and without *DNMT3A* mutations in unfavorable group.

TABLE II. Results of Univariate and Multivariate Analysis for OS and DFS in cn-AML

	OS				DFS			
	Univariate		Multivariate		Univariate		Multivariate	
	P	HR	95%CI	*P*	*P*	HR	95%CI	*P*
Age (<45 years vs. >45 years)	0.004	2.07	1.19–3.61	0.011	0.002	1.75	1.17–2.63	0.007
White cells (<median vs. >median)	0.041	1.41	0.85–2.35	0.179	0.044	1.12	0.83–1.65	0.237
DNMT3A (*mut* vs. *wt*)	0.011	1.50	1.08–3.07	0.047	0.005	1.72	1.14–3.44	0.044
FLT3-ITD/NPM1/CEBPA (favorable/intermediate vs. unfavorable)	0.000	2.74	1.09–7.57	0.014	0.002	2.14	1.13–4.07	0.020
Treatment (allo-HSCT vs. chemotherapy)	0.002	1.56	1.08–3.23	0.046	0.029	1.98	1.11–3.93	0.048
ECOG score (<2 vs. >=2)	0.068	1.38	0.96–2.02	0.919	0.025	1.09	0.69–1.71	0.712
Hb (<median vs. >median)	0.523	0.92	0.55–1.53	0.744	0.801	0.92	0.62–1.36	0.870
Platelet (<median vs. >median)	0.809	1.06	0.63–1.78	0.829	0.566	0.99	0.66–1.47	0.952

Abbreviation: OS: Overall survival; DFS: Disease-free survival; HR: Hazard ratio; CI: Confidence interval; allo-HSCT: allogeneic hematopoietic stem cell transplantation; Hb: Hemoglobin.

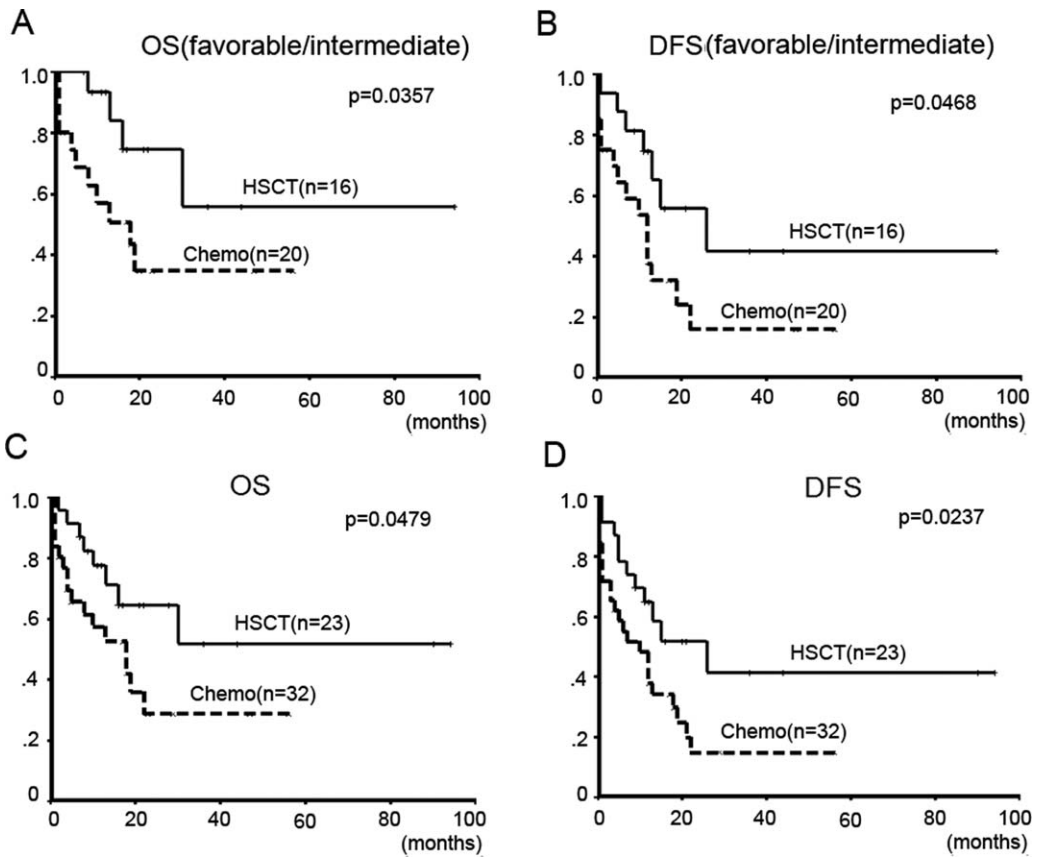

Figure 2. Kaplan–Meier survival curves for cn-AML patients with *DNMT3A* mutations followed allo-HSCT or chemotherapy. (A) OS of cn-AML patients with *DNMT3A* mutations in favorable/intermediate group. (B) DFS of cn-AML patients with *DNMT3A* mutations in favorable/intermediate group. (C) OS of cn-AML patients with *DNMT3A* mutations. (D) DFS of cn-AML patients with *DNMT3A* mutations.

Discussions

Somatic DNA methyl transferase 3A (*DNMT3A*) mutations are recurrent molecular aberrations in AML and associated with poor survival. In a cohort of 308 cn-AML patients, our data showed that *DNMT3A* mutations were among the most common mutations in cn-AML, occurring in 20.4% of patients, with more frequency in older patients, and were significantly associated with *NPM1* and *FLT3-ITD* mutations. Notably, Renneville et al. reported that in a 123 adult cn-AML cohort, 36 (29%) patients had *DNMT3A* mutations and 30 (83%) mutations affected the animo-acid residue R882 [16]. In this study, we found that there were no differences with respect to sex, BM blast percentage or WBC count between DNMT3A ^{wt} and DNMT3A^{mut} cases, which was inconsistent with other reports [15,16]. We speculated that the inconsistency might due to only DNMT3A exon23 mutated cases were included in this study. In addition, a series of studies have suggested that the presence of *DNMT3A* mutations in AML was associated with poor clinical outcomes. Thol et al. showed that *DNMT3A* mutations remained an independent negative prognostic marker for OS [15]. Marcucci et al. find adverse impact on OS in the subgroup analysis based on the age stratification [24]. In this report, we found that *DNMT3A* mutated cn-AML patients had shorter DFS and OS, and *DNMT3A* mutation remained an independent adverse prognostic factor for clinical outcome. Our study provided key insight into the prognostic value of *DNMT3A* mutations in cn-AML patients. However, it should be noted that *DNMT3A* non-R882 mutations were not included in this study. According to previous reports, non-R882 mutations accounted for minority of *DNMT3A* mutations (<40%), and the reason for the high prevalence of R882 was unclear [14]. It was reported that *DNMT3A* R882 and non-R882 mutations had different clinical implications in patients younger and older than 60 years of age [24]. Interestingly, Gaidzik et al. reported that in younger adults with AML *DNMT3A* R882 mutation was an unfavorable marker while non-R882 mutation was a favorable marker [25].

As *DNMT3A* mutations often confer a poor prognosis in cn-AML, we further investigated whether or not allo-HSCT could reverse the poor survival of cn-AML with *DNMT3A* mutation. In this study, we found that allo-HSCT significantly improved the OS of cn-AML with *DNMT3A* mutation, and *DNMT3A*^{mut} patients received allo-HSCT had the similar survival with the *DNMT3A*^{wt} cn-AML patients. Dose intensified chemotherapy was reported to improve the outcome of the *DNMT3A* mutated cases [26]. However, several studies also showed that *DNMT3A* mutations did not have any effect on RFS and OS of patients who have undergone allo-HSCT [17,26]. Our study might provide direct evidence for the benefit of allo-HSCT in *DNMT3A*^{mut} cn-AML patients. In a recent publication, Bejar et al. used massively parallel sequencing to investigate 40 recurrent mutations and explored these mutations predict outcome after allo-HSCT in 87 patients with MDS, 16 patients with *DNMT3A* mutations (18%) were identified and *DNMT3A* mutations were associated with decreased OS [27].

Integrated mutational profiling can improve initial risk stratification of AML patients, and the mutated genes with robust data supporting its independent prognostic value in AML patients could enter the integrated panel. To date, only *NPM1, CEBPA*, and *FLT3-ITD* mutations have entered clinical practice and affect risk stratification and treatment decision-making in cn-AML. Considering high occurrence and prognostic significance, it is likely that *DNMT3A* mutations belong to this category in cn-AML. In this study, we found that *DNMT3A* mutations were associated with *FLT3-ITD* and *NPM1*

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mutations in cn-AML patients. Based on *FLT-ITD/NPM1/CEBPA* gene mutations, the cn-AML patients were divided into two groups (favorable/intermediate and unfavorable). We compared the clinical outcomes in different groups and found that no significant differences were observed in OS and DFS between *DNMT3A*^{mut} and *DNMT3A*^{wt} patients in both unfavorable/intermediate and unfavorable groups. It was reported that in a multivariate analysis included *NPM1/FLT3-ITD/CEBPA* low- versus high-risk mutational status and *DNMT3A* as covariate in cn-AML patients, high WBC count at presentation, *NPM1/FLT3-ITD/CEBPA* high-risk genotypes and *DNMT3A* mutations were significantly and independently associated with a worse EFS and OS [16]. In addition, Ribeiro et al. also found that *DNMT3A* mutations were independent factor for worse OS in their multivariate analysis including *FLT3/NPM1* mutational status [17]. In this study, 211 patients (211/308, 68.5%) in this cohort were assigned to intermediate group and 46 patients (46/308, 15.0%) were assigned to unfavorable group. Most patients (214/308, 69.5%) patients of total cohort had received allo-HSCT treatment, which might improve survival of all cn-AML patients. Moreover, other gene mutations were likely to dictate both prognosis and potential therapeutic responsiveness in cn-AML. Pooling more clinical data in future and well-designed clinical trial will be helpful to learn more about prognostic value of *DNMT3A* in combination other recurrent gene mutations in cn-AML patients.

DNMT3A is a key gene for the epigenetic regulation of human cells as the encoded enzyme is known to be a major player in the de novo DNA methylation at CpG sites. Much remains to be learned on the precise leukemogenic effect of the epigenetic changes caused by *DNMT3A* mutations. Recently, de novo AML patients with concurrent *DNMT3A*, *FLT3*, and *NPM1* mutations had been suggested to represent a unique AML subset, and *DNMT3A* had a significant dominant effect on clinical outcomes of these subset patients [28]. It was reported that cells bearing mutations in *DNMT3A* but not *NPM1* were present at diagnosis in AML patients and persist at remission and relapse. The results indicated that *DNMT3A*^{mut} arises early in AML evolution, probably in HSCs, leading to a clonally expanded pool of preleukaemic HSCs from which AML evolves [29]. Interestingly, it was also reported that *DNMT3A* mutations presented in remission samples from 14 patients up to 8 years after initial AML diagnosis, and the presence of *DNMT3A* mutations in leukaemic blasts, but also at lower allele frequencies in T and B-cells from the same patients [30]. These results might provide a new insight into the role of *DNMT3A* in leukemogenic effect and management of AML therapy.

In conclusion, our results suggested that *DNMT3A* mutation was a poor prognostic factor for cn-AML patients and allo-HSCT could improve survival of cn-AML patients with *DNMT3A* mutations.

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