DNMT3A mutations in acute myeloid leukemia: stability during disease evolution and clinical implications

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***DNMT3A* mutations are associated with poor prognosis in acute myeloid leukemia (AML), but the stability of this mutation during the clinical course remains unclear. In the present study of 500 patients with de novo AML, *DNMT3A* mutations were identified in 14% of total patients and in 22.9% of AML patients with normal karyotype. *DNMT3A* mutations were positively associated with older age, higher WBC and platelet counts, intermediate-risk and normal cytogenetics, *FLT3* internal tandem duplication, and *NPM1*, *PTPN11*, and *IDH2* mutations, but were negatively associated with *CEBPA* mutations. Multivariate analysis demonstrated that the *DNMT3A* mutation was an independent poor prognostic factor for overall survival and relapse-free survival in total patients and also in normokaryotype group. A scoring system incorporating the *DNMT3A* mutation and 8 other prognostic factors, including age, WBC count, cytogenetics, and gene mutations, into survival analysis was very useful in stratifying AML patients into different prognostic groups (*P* < .001). Sequential study of 138 patients during the clinical course showed that *DNMT3A* mutations were stable during AML evolution. In conclusion, *DNMT3A* mutations are associated with distinct clinical and biologic features and poor prognosis in de novo AML patients. Furthermore, the *DNMT3A* mutation may be a potential biomarker for monitoring of minimal residual disease. (*Blood*. 2012;119(2):559-568)**

Introduction

DNMT3A encodes the enzyme DNA methyltransferase (DNMT) 3A, which catalyzes the addition of methyl groups to the cytosine residue of CpG dinucleotides in DNA.[1,2] DNMT3A contains 3 main structure domains: an *ATRX*, *DNMT3*, and *DNMT3L*–type zinc finger domain, a proline-tryptophan-tryptophan-proline domain, and the methyltransferase (MTase) domain.[1] The proline-tryptophan-tryptophan-proline domain is responsible for targeting the enzyme to nucleic acid, whereas the zinc finger domain mediates protein-protein interactions with the transcription factors Myc and RP58, the heterochromatin protein HP1, histone deacetylases, and the histone methyltransferase Suv39h1.[2] Recently, mutations in *DNMT3A* were identified in patients with AML, myelodysplastic syndromes, and myeloproliferative neoplasms.[3-7] The incidences of this mutation in AML varied: 4.1% in a Japanese study,[8] 9% (among all AML, including M4/M5 and other subtypes) in a Chinese study,[9] and approximately 20% in 2 Western studies.[3,4] Whether there is a geographic difference in the incidence of *DNMT3A* mutations needs to be determined. Furthermore, sequential analyses to evaluate the stability of *DNMT3A* mutations during the clinical course were limited to a small number of patients. In the present study, we investigated the *DNMT3A* mutation in 506 patients with de novo AML and analyzed its interactions with 16 other gene alterations. Sequential analysis of the *DNMT3A* mutation during the clinical course was also performed on 138 patients to investigate the stability and pathogenic role of this mutation in AML. Further, to better stratify AML patients into different risk groups, a scoring system integrating *DNMT3A* mutations with 8 other prognostic factors, including age, WBC count, cytogenetics, *NPM1/FLT3* internal tandem duplication (*NPM1/FLT3*-ITD), *CEBPA*, *AML1/RUNX1*, *WT1*, and *IDH2* mutations, into survival analysis was proposed.

Methods

Subjects

This study was approved by the institutional review board of the National Taiwan University Hospital (NTUH), and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. From March 1995 to December 2008, a total of 506 adult patients who were newly diagnosed as having de novo AML at NTUH and had enough cryopreserved cells for analysis were enrolled consecutively.

The online version of this article contains a data supplement.

Patients with antecedent hematologic diseases or therapy-related AML were excluded. Diagnosis and classification of AML were made according to the French-American-British (FAB) Cooperative Group Criteria.

In total, 363 (71.7%) patients received standard induction chemotherapy (idarubicin 12 mg/m²/d on days 1-3 and cytarabine 100 mg/m²/d on days 1-7) and then consolidation chemotherapy with 2-4 courses of high-dose cytarabine (2000 mg/m² every 12 hours on days 1-4 for a total of 8 doses), with or without an anthracycline (idarubicin or Novantrone), after achieving complete remission (CR).[10,11] The patients with acute promyelocytic leukemia (M3 subtype) received concurrent all-*trans* retinoic acid and chemotherapy. The remaining 143 patients received palliative therapy with supportive care and/or low-dose chemotherapy because of underlying comorbidities or based on patient decision. Forty-five patients received allogeneic hematopoietic stem cell transplantation (HSCT) in first CR.

Cytogenetics

BM cells were harvested directly or after 1-3 days of unstimulated culture, as described previously.[12] Metaphase chromosomes were banded with the trypsin-Giemsa technique and karyotyped according to the International System for Human Cytogenetic Nomenclature.

Immunophenotype analysis

A panel of mAbs to myeloid-associated antigens, including CD13, CD33, CD11b, CD15, CD14, and CD41a, as well as lymphoid-associated antigens, including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage-nonspecific antigens HLA-DR, CD34, and CD56, were used to characterize the phenotypes of the leukemia cells, as described previously.[13]

Mutation analysis

Mutation analysis of *DNMT3*A exons 2-23 was performed by PCR and direct sequencing as described previously.[4] Abnormal sequencing results were confirmed by at least 2 repeated analyses. Sequential analysis of the *DNMT3*A mutation during the clinical course was performed in 316 samples from 138 patients. Mutation analyses of 16 other relevant molecular marker genes, including class I mutations, such as *FLT3*-ITD and *FLT3*-TKD,[13] *N-RAS*,[14] *K-RAS*,[14] *JAK2*,[14] *KIT*,[15] and *PTPN11*[16] mutations, and class II mutations, such as *MLL*-PTD,[17] *CEBPA*,[18] and *AML1/RUNX1*[11] mutations, as well as *NPM1*,[19] *WT1*,[10] *ASXL1*,[20] *IDH1*,[21] *IDH2*[22] (including R140 and R172 mutations), and *TET2*[23] mutations, were performed as described previously. To detect *DNMT3*A mutation at diagnosis, we used DNA amplified in vitro from BM cells with the Illustra GenomiPhi V2 DNA-amplification kit as described by the manufacturer (GE Healthcare). All mutations detected were verified in the original nonamplified samples. All nucleotide alterations causing premature truncation of the DNMT3A proteins (nonsense or frame-shift mutations) were regarded as true mutations. Missense mutations were regarded as true only if they were documented in other studies or could be verified by sequencing of normal somatic tissues or matched remission BM samples.

TA cloning analysis

For patients with double mutations, Taq polymerase-amplified (TA) cloning was performed to determine whether the 2 mutations were in the same or different alleles, as described previously.[15] Briefly, the cDNA was amplified to cover both mutations and the PCR products were then cloned into the TA-cloning vector pGEM-T Easy (Promega) and more than 10 clones were selected for sequencing.

Statistical analysis

The discrete variables of patients with and without gene mutation were compared using the χ^2 tests, but if the expected values of contingency tables were smaller than 5, the Fisher exact test was used. If the continuous data were not normally distributed, Mann-Whitney U tests were used to compare continuous variables and medians of distributions. To evaluate the impact of the *DNMT3* mutation on clinical outcome, only the patients who received conventional standard chemotherapy were included in the analysis.[10,11]

Overall survival (OS) was measured from the date of first diagnosis to the date of last follow-up or death from any cause, whereas relapse was defined as a reappearance of at least 5% leukemic blasts in a BM aspirate or new extramedullary leukemia in patients with a previously documented CR.[24] Relapse-free survival (RFS) was measured from the date of attaining the leukemia-free state until the date of AML relapse or death from any cause, whichever occurred first. Cox regression survival estimation was used to plot survival curves and to test the differences between groups. Multivariate Cox proportional hazard regression analysis was used to investigate independent prognostic factors for OS and RFS. The proportional hazards assumption (constant hazards assumption) was examined using time-dependent covariate Cox regression before conducting multivariate Cox proportional hazard regression. The variables including age, WBC counts, karyotype, *NPM1/FLT3*-ITD, *WT1*, *CEBPA*, *AML1/RUNX1*, *TET2*, *ASXL1*, *IDH2*, and *DNMT3A* mutations were used as covariates. Those patients who received HSCT were censored at the time of transplantation in survival analysis to ameliorate the influence of the treatment.[10,11] $P < .05$ was considered statistically significant. All statistical analyses were performed with SPSS Version 18 software and Statsdirect (2.7.8b, 2011).

Results

DNMT3A mutations in patients with de novo AML

Excluding the 8 single-nucleotide polymorphisms (P9P, S267S, G291G, A398A, P385P, L422L, V435V, and V563V) that were detected in 316 patients but did not alter the amino acid residues, and the 7 missense mutations (C586W, P896L, G543C, Y735C, A644T, G699D, and G707D) that were found in 6 patients but had uncertain biologic significance (because they were not reported previously and could not be verified because of lack of matched BM samples at CR), *DNMT3A* mutations at 30 different positions were identified in 70 patients (Table 1 and Figure 1). Twelve were missense mutations, 8 were nonsense mutations, 9 were frame-shift mutations, and 1 was an in-frame mutation. The most common mutation was R882H (n = 26), followed by R882C (n = 15), R882S (n = 3), R736H (n = 3), and R320X (n = 2). All other mutations were detected in only 1 patient each. Mutations at exon 23 occurred in 47 patients, including the 44 patients with R882 mutations. Four patients had double heterozygous mutations (patients 43, 64, 65, and 68); in 1 of them (patient 64), the 2 mutations were confirmed to be biallelic by DNA PCR and TA cloning, and for the other 3, the nature of the double mutations was not verified by this method because the 2 mutations were located in different exons too far apart to be amplified by a single-DNA PCR reaction. The remaining 66 patients showed only one mutation; all were heterozygous.

Correlation of *DNMT3A* mutations with clinical and laboratory features

In total, 500 de novo AML patients, including 70 (14%) *DNMT3A*-mutated and 430 *DNMT3A*-wild patients were enrolled into the study. The 6 patients with missense mutations of unknown significance were censored and were not included in the following analyses. A comparison of clinical characteristics of patients with and without distinct *DNMT3A* mutations is given in Table 2. *DNMT3A*-mutated patients were older (median, 61 vs 49 years, $P < .0001$) and had higher WBC, blast, and platelet counts than *DNMT3A*-wild patients ($P = .0018$, .0012, and .0001, respectively). Patients with the FAB M5 subtype of AML had the highest incidence (50%, $P < .0001$) of *DNMT3A* mutation, followed by those with the FAB M4 subtype (22.6%, $P = .0026$). *DNMT3A*

Table 1. Mutation patterns in 70 patients with *DNMT3A* mutations at diagnosis

UPN	Age, y/Sex	FAB	Karyotype	Location	DNMT3 mutation DNA change	DNMT3 mutation Protein change	Other accompanying gene mutations
1	79/M	M5	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, MLL/PTD, IDH2*
2	77/M	M1	46,XY	23	c.2646G > A	p.R882H	*AML1/RUNX1*
3	64/M	M5	NM	23	c.2646G > A	p.R882H	*PTPN11, NPM1*
4	73/M	M4	46,XY	23	c.2646G > A	p.R882H	*IDH2*
5	16/M	M4	46,XY	23	c.2645C > T	p.R882C	*FLT3/TKD, NPM1*
6	41/F	M4	46,XX	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
7	80/F	M4	46,XX	23	c.2646G > A	p.R882H	*PTPN11, NPM1*
8	61/F	M5	46,XX t(5;17)(q33;q21)	23	c.2645C > T	p.R882C	*FLT3/TKD, NPM1*
9	46/M	M4	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
10	35/F	M1	46,XX	18	c.2120delG	p.G707AfsX72	*NRAS, IDH1*
11	82/M	M0	ND	23	c.2645C > T	p.R882C	*FLT3/ITD, MLL/PTD, AML1/RUNX1*
12	79/F	M4	46,XX	23	c.2646G > A	p.R882H	*FLT3/ITD, FLT3/TKD, MLL/PTD, TET2*
13	51/M	M4	46,XY	23	c.2646G > A	p.R882H	*FLT3/TKD, NPM1*
14	55/M	M4	46,XY	16	c.1865_1866 insGT	p.Y623FfsX29	*NPM1*
15	54/M	M4	46,XY	8	c.890G > A	p.W297X	*PTPN11, ASXL1*
16	68/M	M2	46,XY	23	c.2645C > A	p.R882S	*FLT3/ITD, NPM1*
17	45/F	M5	46,XX	23	c.2645C > T	p.R882C	*FLT3/TKD, AML1/RUNX1, IDH2*
18	54/F	M2	46,XX	23	c.2646G > A	p.R882H	*NRAS, NPM1*
19	87/M	M4	46,XY	23	c.2606delG	p.G869VfsX12	*FLT3/TKD, NPM1*
20	51/F	M4	47,XX,+i(11)(q10)	20	c.2389A > T	p.N797Y‡	*ASXL1, IDH2*
21	78/M	M1	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
22	38/F	M5	46,XX	23	c.2645C > T	p.R882C	*NRAS, NPM1, IDH1*
23	72/F	M2	46,XX,del(20)(q11q13)	13	c.1477delA	p.I493SfsX158	*FLT3/ITD, NPM1*
24	65/F	M5	46,XX	23	c.2646G > A	p.R882H	*FLT3/ITD*
25	42/F	M4	46,XX	23	c.2646G > A	p.R882H	*FLT3/ITD, AML1/RUNX1, ASXL1*
26	78/M	M2	46,X,−Y,+4	19	c.2246_2249del	p.R749PfsX29	*FLT3/TKD, NPM1*
27	75/F	M1	46,XX	23	c.2645C > T	p.R882C	*FLT3/ITD, NPM1*
28	51/M	M4	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
29	60/F	M1	46,XX,t(9;22)(q34;q11)	18	c.2113A > T	p.I705F§	*IDH1*
30	73/F	M1	46,XX	23	c.2646G > A	p.R882H	*CEBPA, TET2*
31	22/F	M4	46,XX	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
32	38/M	M4	46,XY	23	c.2645C > T	p.R882C	*FLT3/ITD, NPM1*
33	31/F	M5	46,XX	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
34	46/M	M4	45X,−Y	23	c.2645C > A	p.R882S	*NRAS, FLT3/ITD, NPM1*
35	80/M	M4	46,XY	23	c.2645C > T	p.R882C	*FLT3/ITD, MLL/PTD*
36	52/M	M1	46,XY	23	c.2645C > T	p.R882C	*FLT3/ITD, IDH2*
37	44/M	M2	46,XY	23	c.2645C > T	p.R882C	*FLT3/ITD, NPM1*
38	33/F	M1	46,XX	8	c.958C > T	p.R320X	*FLT3/TKD, NPM1*
39	42/M	M4	45,X,−Y	15	c.1816C > T	p.Q606X	*NPM1*
40	78/F	M2	46,XX	19	c.2255_2257del	p.F752del	*FLT3/ITD, NPM1*
41	75/F	M2	47,XX,del(5)(q22q35),+8	8	c.958C > T	p.R320X	*IDH2*
42	49/M	M1	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
43	78/M	M4	46,XY	4	c.315C > A	p.S105R	*PTPN11*
44	64/M	M5	46,XY	23	c.2645C > A	p.R882S	*PTPN11, MLL/PTD*
45	40/M	M5	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1*
46	75/M	M4	46,XY	23	c.2646G > A	p.R882H	*NRAS, NPM1, TET2*
47	58/F	M2	46,XX	23	c.2645C > T	p.R882C	*NPM1*
48	48/F	M1	47,XX,+8	8	c.1001delG	p.G334AfsX11	*CEBPA, IDH2*
49	67/M	M5	47,XY,+8	23	c.2645C > T	p.R882C	*PTPN11, KRAS, AML1/RUNX1, IDH2*
50	51/M	M2	46XY	23	c.2646G > A	p.R882H	*IDH2*
51	85/M	M5	46,XY	7	c.767_770del	P256LfsX59	*FLT3/ITD, NPM1, WT1*
52	85/M	M1	45,XY,−7	4	c.327_328insG	Q110AfsX14	
53	67/M	M8	47,XY,+8	23	c.2646G > A	p.R882H	*NRAS, IDH2*
54	35/M	M4	46,XY	23	c.2645C > T	p.R882C	*FLT3/ITD, NPM1*
55	47/F	M2	46,XX	23	c.2646G > A	p.R882H	*ASXL1, IDH2*
56	50/F	M1	46,XX	23	c.2645C > T	p.R882C	*FLT3/ITD, MLL/PTD*
57	86/M	M5	46,XY	8	c.866delG	p.G289AfsX26	*FLT3/ITD*
58	69/M	M1	NM	23	c.2645C > T	p.R882C	*FLT3/ITD, NPM1, CEBPA*
59	75/M	M4	46,XY	23	c.2646G > A	p.R882H	*AML1/RUNX1*
60	79/F	M4	Cplx*	22	c.2510C > G	p.S837X	

Nucleotide numberings are according to the National Center for Biotechnology Information reference sequence NM_024426.

UPN indicates unique patient number; NM, no mitosis; and ND, not done.

*Cplx: complex abnormalities, including del(2)(q31q35),der(2)del(2)(p12p22)del(2)(q31q35),−5,+6,del(7)(q11q36),+8,+11,del(12)(p11.1.p11.2),add(17)(p11).

†In addition to R882 mutations, missense mutations in patients 65, 66, 69, and 70 have been reported in previous studies.[4,7]

‡Missense mutations in patients 20, 29, 62, 64, and 68 were confirmed to be significant by the analysis of remission BM samples.

Table 1. Mutation patterns in 70 patients with *DNMT3A* mutations at diagnosis (continued)

UPN	Age, y/Sex	FAB	Karyotype	Location	DNMT3 mutation DNA change	DNMT3 mutation Protein change	Other accompanying gene mutations
61	61/M	M1	46,XY	23	c.2646G > A	p.R882H	*NPM1, WT1, TET2*
62	37/F	M2	46,XX	19	c.2312G > A	p.R771Q‡	*NPM1, TET2*
63	70/M	M2	46,XY	23	c.2646G > A	p.R882H	*FLT3/ITD, NPM1, IDH2*
64	46/M	M4	46,XY	19	c.2182G > C, c.2191T > C	p.G728R‡, p.F731L‡	*FLT3/ITD*
65	69/M	M4	47,XY,+X	8	c.941G > A	p.W314X	*NRAS, FLT3/TKD, AML1/RUNX1, IDH2*
				19	c.2207G > A	p.R736H†	
66	38/F	M2	46,XX	19	c.2207G > A	p.R736H†	*FLT3/ITD, NPM1, IDH1*
67	66	M1	47,XY,del(5)(q31q35), der(7)t(5;7)(q13;q11),+8	15	c.1792C > T	p.R598X	*IDH2*
68	81	M4	46,XY	17	c.2032C > T	p.Q678X	*NRAS, TET2*
				19	c.2210T > A	p.L737H‡	
69	50	M4	46,XX	15	c.1903C > T	p.R635W†	*PTPN11, NPM1, IDH2*
70	84	M0	ND	19	c.2207G > A	p.R736H†	*AML1/RUNX1, IDH2*

Nucleotide numberings are according to the National Center for Biotechnology Information reference sequence NM_024426.

UPN indicates unique patient number; NM, no mitosis; and ND, not done.

*Cplx: complex abnormalities, including del(2)(q31q35),der(2)del(2)(p12p22)del(2)(q31q35),−5,+6,del(7)(q11q36),+8,+11,del(12)(p11.1.p11.2),add(17)(p11).

†In addition to R882 mutations, missense mutations in patients 65, 66, 69, and 70 have been reported in previous studies.[4,7]

‡Missense mutations in patients 20, 29, 62, 64, and 68 were confirmed to be significant by the analysis of remission BM samples.

mutations were positively associated with the expression of CD13 ($P = .022$) and CD14 ($P = .0015$), but inversely associated with the expression of CD34 ($P = .0039$) on leukemic cells (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). There was no difference in the expression of other antigens between the patients with and without the *DNMT3A* mutation.

Association of *DNMT3A* mutations with cytogenetic abnormalities

Chromosome data were available for 482 patients at diagnosis, including 66 *DNMT3A*-mutated and 416 *DNMT3A*-wild patients (Table 3). *DNMT3A* mutations occurred more frequently in patients with intermediate-risk cytogenetics (19.5%) than in those with favorable karyotype or unfavorable cytogenetics (2.4%, $P = .0069$). There was a significant difference in the incidence of the *DNMT3A* mutation among patients with normal karyotype (22.9%), simple abnormalities with 1 or 2 changes (6.2%), and complex cytogenetics with 3 or more abnormalities (3.9%, $P < .0001$). None of the patients with t(8;21), t(15;17) inv(16), or 11q23 translocation had a *DNMT3A* mutation. There was no association of the *DNMT3A* mutation with other chromosomal abnormalities, including +8, +11, +13, +21, −5/del(5q), and −7/del(7q).

Association of *DNMT3A* mutation with other molecular abnormalities

To investigate the interaction of gene mutations in the pathogenesis of adult AML, a complete mutational screening of 16 other genes was performed in all 500 patients (Table 4). Among the 70 patients with *DNMT3A* mutations, 68 (97.1%) showed additional molecular abnormalities at diagnosis (supplemental Table 2). Fifteen had 1 additional change, 37 had 2, 13 had 3, and 3 had 4. The most common associated molecular event was the *NPM1* mutation (n = 38), followed by *FLT3*-ITD (n = 30), *IDH2* (n = 16), and *FLT3*-TKD (n = 9) mutations. Patients with *DNMT3A* mutations had a significantly higher incidence of the *NPM1* mutation and *FLT3*-ITD, *IDH2*, and *PTPN11* mutations than those with *DNMT3A*-wild type (54.3% vs 15.3%, $P < .0001$; 42.9% vs 19.3%,
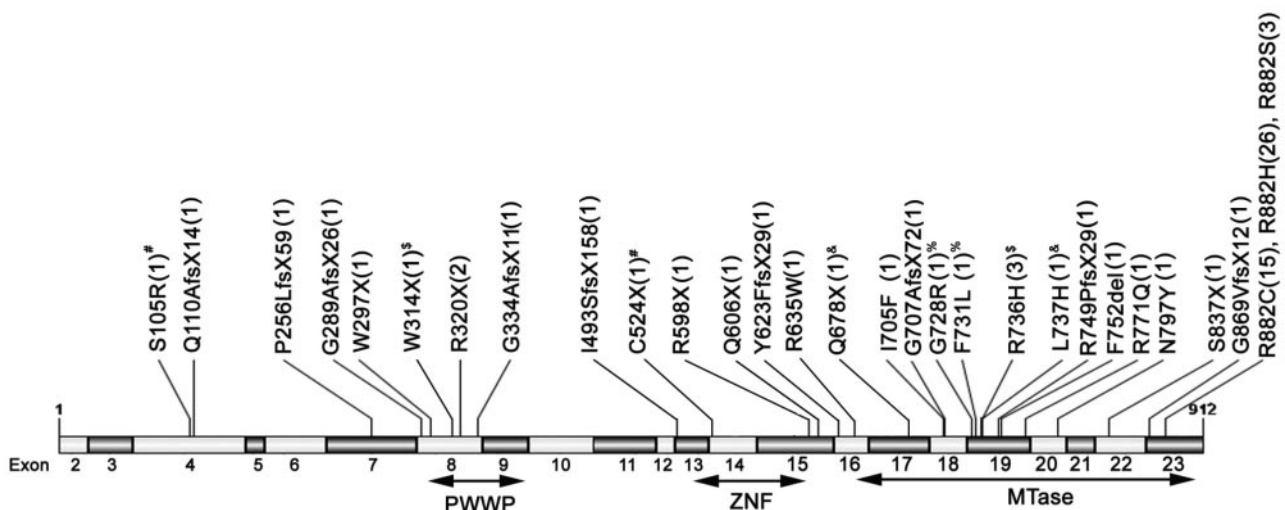

Figure 1. Patterns and locations of the 30 different positions of mutations. The positions and predicted translational consequences of *DNMT3A* mutations detected in 500 AML samples are shown. The number of patients with the mutation is indicated in the parentheses behind each mutation. #, %, &, and $ indicate that the patient has 2 mutations.

Table 2. Comparison of clinical and laboratory features between AML patients with and without the *DNMT3A* mutation

Variable	Total (n = 500)	*DNMT3A*-mutated (n = 70, 14%)	*DNMT3A*-wild (n = 430, 86%)	*P*
Sex, n				.7961
Male	285	41	244	
Female	215	29	186	
Median age, y (range)	51 (15-90)	61 (16-87)	49 (15-90)	< .0001
Laboratory data, median (range)				
WBCs, /μL	19 075 (120-627 800)	32 490 (650-340 400)	15 940 (120-627 800)	.0018
Hemoglobin, g/dL	8 (2.9-16.2)	8.7 (3.2-12)	7.9 (2.9-16.2)	.0675
Platelets, × 1000/μL	42 (2-802)	57 (10-436)	39 (2-802)	.0001
Blasts, /μL	7401 (0-456 725)	19 030 (111-283 212)	6263 (0-456 725)	.0012
Lactase dehydrogenase, U/L	889 (206-15 000)	1064 (250-8116)	832 (206-15 000)	.0883
FAB, n (%)				< .0001
M0	10	2 (20)	8 (80)	.6375
M1	112	14 (12.5)	98 (87.5)	.7572
M2	171	13 (7.6)	158 (92.4)	.0027
M3	38	0 (0)	38 (100)	.0056
M4	124	28 (22.6)	96 (77.4)	.0026
M5	24	12 (50)	12 (50)	< .0001
M6	12	0 (0)	12 (100)	.3889
Undetermined	9	1 (11.1)	8 (88.9)	> .9999

22.9% vs 9.1%, *P* = .0016; and 10% vs 3.5%; *P* = .007, respectively). Conversely, *CEBPA* mutation was rarely seen in patients with *DNMT3A* mutations (4.3% vs 14.7%, *P* = .0134). There was no difference in the incidence of other molecular mutations between patients with and without the *DNMT3A* mutation. Among the 68 patients with concurrent other genetic alterations, 51 (75%) had at least 1 concomitant class I mutation; 16 (23.5%) had class II mutations; and 38 (54.3%) had *NPM1* mutations, which behave more like class II mutations.[13] In total, 40 patients (58.8%) had concurrent class I and class II or *NPM1* mutations at diagnosis. Twenty-one patients had concomitant *FLT3*-ITD and *NPM1* mutations (supplemental Table 2).

Table 3. Association of *DNMT3A* mutation with chromosomal abnormalities

	Total	*DNMT3A*-mutated	*DNMT3A*-wild	*P*
Karyotype*				.0069
Favorable	99	0 (0)	99 (100)	< .0001
Intermediate	318	62 (19.5)	256 (80.5)	< .0001
Unfavorable	65	4 (6.2)	61 (93.8)	.0783
Unknown	18	4 (22.2)	14 (77.7)	
Normal	223	51 (22.9)	172 (77.1)	< .0001
Simple	208	13 (6.2)	195 (93.8)	< .0001
Complex	51	2 (3.9)	49 (96.1)	.0303
t(8;21)	42	0 (0)	42 (100)	.0034
t(15;17)	38	0 (0)	38 (100)	.0053
inv(16)	19	0 (0)	19 (100)	.0909
t(11q23)	16	0 (0)	16 (100)	.145
t(7;11)	10	0 (0)	10 (100)	.371
−5/5q−†	2	1 (50)	1 (50)	.2554
−7/7q−†	10	1 (10)	9 (90)	> .9999
+8‡	27	4 (14.8)	23 (85.2)	.7765
+11†	3	1 (33.3)	2 (66.7)	.3577
+13†	1	0 (0)	1 (100)	> .9999
+21†	9	0 (0)	9 (100)	.6178

Four hundred eighty-two patients, including 66 *DNMT3A*-mutated and 416 *DNMT3A*-wild patients, had chromosome data at diagnosis.

*Favorable, t(15;17), t(8;21), inv(16); unfavorable, −7, del(7q), −5, del(5q), 3q abnormality, complex abnormalities; and intermediate, normal karyotype and other abnormalities.

†Only including simple chromosomal abnormalities with ≤ 2 changes, but not those with complex abnormalities with ≥ 3 aberrations.

Impact of *DNMT3A* mutation on response to therapy and clinical outcome

Of the 363 AML patients undergoing conventional intensive induction chemotherapy, 284 (78.5%) patients achieved a CR. The probability of achieving a CR was similar between patients with and without *DNMT3A* mutations (74.4% vs 79%, *P* = .5531). However, the patients with *DNMT3A* mutations had a trend of higher relapse rate than those without (65.6% vs 48.8%, *P* = .0911). With a median follow-up of 55 months (range, 1.0-160), patients with the *DNMT3A* mutation had significantly poorer OS and RFS than those without the *DNMT3A* mutation (median, 14.5 months vs 38 months, *P* = .013, and median, 7.5 months vs 15 months, *P* = .012, respectively, Figure 2A-C). The same was true among patients with non-M3 AML (*P* = .04 and *P* = .036, respectively). In the subgroup of 130 younger patients (< 60 years) with normal karyotype AML (CN-AML), the differences between patients with and without the *DNMT3A* mutation in OS (median, 15.5 months vs

Table 4. Association of the *DNMT3* mutation with other gene mutations

|	Patients with alteration, n (%)			
Variable	Whole cohort (n = 500)	*DNMT3A*-mutated patients (n = 70)	*DNMT3A*-wild patients (n = 430)	*P*
FLT3/ITD	113 (22.6)	30 (42.9)	83 (19.3)	< .0001
FLT3/TKD	39 (7.8)	9 (12.9)	29 (6.7)	.087
N-RAS	61 (12.2)	8 (11.4)	53 (12.3)	> .9999
K-RAS	16 (3.2)	1 (1.4)	15 (3.5)	.7112
PTPN11	18 (3.6)	7 (10)	11 (2.6)	.007
KIT	15 (3.0)	0 (0)	15 (3.5)	.2451
JAK2	3 (0.6)	0 (0)	3 (0.7)	> .9999
WT1	33 (6.6)	2 (2.9)	31 (7.2)	.2946
NPM1	104 (20.8)	38 (54.3)	66 (15.3)	< .0001
CEBPA	66 (13.2)	3 (4.3)	63 (14.7)	.0134
AML1/RUNX1	62 (12.4)	8 (11.4)	54 (12.6)	> .9999
MLL/PTD	27 (5.4)	6 (8.6)	21 (4.9)	.2475
ASXL1	51 (10.2)	4 (5.7)	46 (10.7)	.2812
IDH1	27 (5.4)	4 (5.7)	23 (5.3)	.7812
IDH2	55 (11)	16 (22.9)	39 (9.1)	.0016
TET2	65 (13.0)	6 (8.6)	59 (13.7)	.3365

High intensity text content
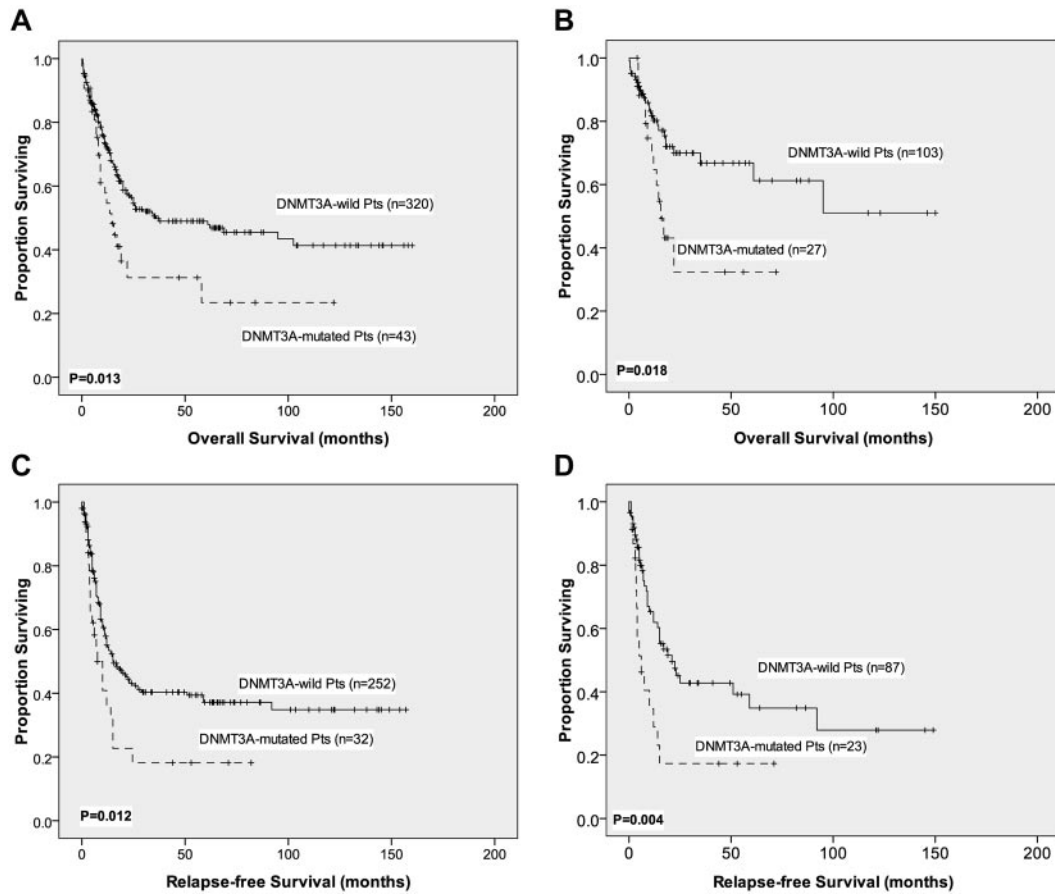
Figure 2. OS and RFS in total patients and in younger patients with CN-AML. Kaplan-Meier survival curves for OS and RFS in 363 AML patients (A and C) and 130 younger patients (< 60 years) with CN-AML (B and D) who received standard intensive chemotherapy.

not reached, $P = .018$, Figure 2B) and RFS (median, 6 months vs 21 months, $P = .004$, Figure 2D) were still significant. We also observed that the prognostic impact of the *DNMT3A* mutation could only be demonstrated in the patients with a poor prognostic genotype (*NPM1*-mutated (*NPM1*$^+$)/*FLT3*-ITD$^+$, *NPM1*-wild (*NPM1*$^-$)/*FLT3*-ITD$^+$ or *FLT3*/ITD$^-$), but not in those with favorable genotype (*NPM1*$^+$/*FLT3*-ITD$^-$) among total AML patients ($P < .001$ and $P = .823$, respectively) or in CN-AML

Table 5. Multivariate analysis (Cox regression) for relapse-free and overall survival

Variable	Relapse-free survival				Overall survival			
	HR	95% CI		P	HR	95% CI		P
		Lower	Upper			Lower	Upper	
Age*	1.150	0.803	1.648	.446	2.531	1.790	3.580	< .001†
WBC‡	1.649	1.120	2.428	.011†	1.970	1.358	2.857	< .001†
Karyotype§	2.577	1.433	4.633	.002†	3.078	1.849	5.123	< .001†
NPM1/FLT3-ITD¶	0.268	0.124	0.581	.001†	0.261	0.121	0.564	.001†
CEBPA#	0.629	0.362	1.093	.100	0.423	0.211	0.847	.015†
*IDH2***	0.775	0.420	1.430	.415	0.573	0.296	1.110	.099
WT1	2.823	1.680	4.743	<.001†	2.576	1.490	4.454	.001†
AML1/RUNX1	1.448	0.718	2.918	.301	1.963	1.129	3.414	.017†
ASXL1	0.739	0.293	1.863	.521	1.439	0.798	2.597	.227
TET2	1.125	0.625	2.026	.694	1.033	0.601	1.777	.906
DNMT3A	2.898	1.673	5.022	<.001†	2.218	1.333	3.692	.002†

HR indicates hazard ratio; and 95% CI, 95% confidence interval.
*Age > 50 relative to age ≤ 50 (the reference age).
†Statistically significant ($P < .05$).
‡WBCs > 50 000/μL versus < 50 000/μL.
§Unfavorable cytogenetics versus others.
¶*NPM1*^{mut}/*FLT3-ITD*^{neg} versus other subtypes.
#*CEBPA*$^{double-mutation}$ versus others.
***IDH2* mutations included R140 and R172 mutations.

patients ($P < .001$ and $P = .970$, respectively). There was no significant difference in survival between patients with mutations of R882 and those with other mutations ($P = .612$).

In multivariate analysis (Table 5), the independent poor risk factors for OS were older age (> 50 years), high WBC count ($> 50 000/\mu L$), unfavorable karyotype, *DNMT3A* mutation, *AML1/RUNX1* mutation, and *WT1* mutation. Conversely, *CEBPA*^double-mutation and *NPM1*^+/*FLT3*^-ITD^- were independent favorable prognostic factors. There was a trend of better OS in patients with the *IDH2* mutation (hazard ratio [HR], 0.573; 95% confidence interval [95% CI], 0.296-1.110, $P = .099$). The independent poor risk factors for RFS included high WBC count ($> 50 000/\mu L$), unfavorable karyotype, *DNMT3A* mutation, and *WT1* mutation. *NPM1*^+/*FLT3*-ITD^- was an independent favorable factor for RFS. In 130 CN-AML patients younger than 60 years, the *DNMT3A* mutation was still an independent poor prognosis for OS and RFS (HR, 2.303; 95% CI, 1.088-4.876, $P = .029$ and HR, 3.496; 95% CI, 1.773-6.896, $P < .001$, respectively, supplemental Table 3).

To better stratify the AML patients into different risk groups, a scoring system incorporating 9 prognostic markers—age, WBC count, cytogenetics at diagnosis, *NPM1/FLT3*-ITD, and mutations of *CEBPA*, *DNMT3A*, *AML1/RUNX1*, *IDH2*, and *WT1* mutation—into the survival analysis was formulated based on the results of our Cox proportional hazards model. A score of -1 was assigned for each parameter associated with a favorable outcome (ie, *CEBPA*^double-mutation, *IDH2* mutation, and *NPM1*^+/*FLT3*-ITD^-), whereas a score of $+1$ for each factor associated with an adverse outcome (ie, *DNMT3A*, *WT1*, and *AML1/RUNX1* mutations, older age, and higher WBC counts at diagnosis). The karyotypes were stratified into 3 groups ($+2$, unfavorable; $+1$, intermediate; and 0, favorable). The algebraic summation of these scores for each patient was the final score. This score system divided the AML patients into 5 groups with different clinical outcomes ($P < .001$ for both OS and RFS, Figure 3).

Sequential studies of *DNMT3A* mutations in AML patients

DNMT3A mutations were studied sequentially in 316 samples from 138 patients, including 35 patients with distinct *DNMT3A* mutations and 103 patients without mutations at diagnosis (Table 6). Among the 34 patients with *DNMT3A* mutations who had ever obtained a CR and had available samples for study, 29 lost the original mutation at remission status, but 5 (patients 5, 8, 28, 32, and 33) retained it (Table 6); all 5 patients relapsed within a median of 3.5 months and died of disease progression, suggesting the presence of leukemic cells. In the 13 patients who had available samples for serial study at relapse, 12 patients regained the original mutations, but 1 (patient 9) lost the mutation at relapse. Because direct sequencing might not be sensitive enough to detect low levels of *DNMT3A* mutation signal, we sequenced TA clones of the PCR product from patient 9 and 1 mutant clone of 17 was detected. Among the 103 patients who had no *DNMT3A* mutation at diagnosis, none acquired the *DNMT3A* mutation at relapse, whereas karyotypic evolution was noted at relapse in 39% of these patients (data not shown).

Discussion

In the present study, we found that the *DNMT3A* mutation was associated with distinct clinical and biologic features and was a poor prognostic factor in AML patients independent of age, WBC counts, karyotype, and other genetic markers.
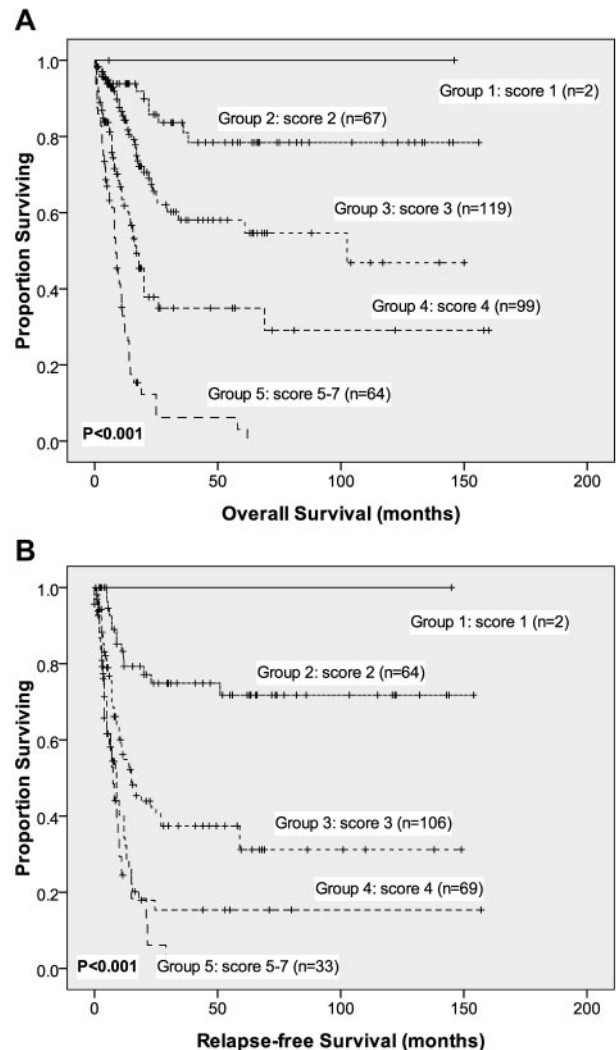

Figure 3. OS and RFS stratified by proposed scoring system. Kaplan-Meier survival curves for OS (A) and RFS (B) in AML patients based on our new scoring system ($P < .001$ for both OS and RFS). AML patients were grouped according to our scoring system based on the *DNMT3A* mutation and 8 other prognostic markers (ie, age, WBC count at diagnosis, and *CEBPA*^double-mutation, *NPM1/FLT3*-ITD, *IDH2*, *DNMT3A*, *WT1*, and *AML1/RUNX1* mutations). A score of -1 was assigned for each parameter associated with a favorable outcome (ie, *CEBPA*^double-mutation, *IDH2* mutation, and *NPM1*^+/*FLT3*-ITD^-); a score of $+1$ for each factor associated with an adverse outcome (ie, older age, higher WBC counts at diagnosis, and *DNMT3A*, *WT1*, and *AML1/RUNX1* mutations). The karyotypes were stratified into 3 groups ($+2$, unfavorable; $+1$, intermediate; and 0, favorable). The algebraic summation of these scores for each patient was the final score. The 12 patients without chromosome data were not included in the analysis.

DNMT3A mutations at 30 different positions, most commonly in the MTase domain, were demonstrated (Figure 1). All of the nonsense, frame-shift, and in-frame mutations generated truncated peptide with complete or partial deletion of the MTase domain and were thought to abolish the catalytic activity of this enzyme. The missense R882 mutations, the most common *DNMT3A* mutations, resulted in impaired enzyme activity,[4,9] but the influence of other missense mutations on the enzyme remains unclear. These missense mutations all involved amino acid residues well conserved through evolution. We censored 6 patients with missense variants of unknown significance and did not include them in the analyses because there were no available remission BM samples or normal tissues to verify that their *DNMT3A* variants were true somatic mutations. In contrast to the report of Thol et al,[3] who only found mutations between exons 15 and 23, 10 mutations in our patients

Table 6. Sequential studies in AML patients with *DNMT3A* mutations

UPN	Date	Status	Karyotype	*DNMT3A* mutation	Other mutations
4	10/31/2006	Initial	46, XY	p.R882H	*IDH2*
	11/29/2006			—	
5	7/27/2000	Initial	46,XY	p.R882C	*NPM1, FLT3/TKD*
	8/24/2000	CR1		p.R882C	—
	7/17/2001	Relapse 1	46,XY	p.R882C	*NPM1, FLT3/TKD, WT1*
	10/23/2001	CR2		p.R882C	—
	5/7/2002	Relapse 2	46,XY,del(6)(p21)	p.R882C	*NPM1, FLT3/TKD, WT1*
6	8/31/2004	Initial	46,XX	p.R882H	*NPM1, FLT3/ITD*
	9/14/2006	CR		—	—
8	9/16/2005	Initial	46,XX,t(5;17)(q33;q21)	p.R882C	*NPM1, FLT3/TKD*
	11/4/2005	CR	46,XX	p.R882C	—
9	5/27/1997	Initial	46,XY	p.R882H	*FLT3/ITD, NPM1*
	6/23/1997	CR		—	—
	7/30/1997	Relapse	46,XY	—*	*FLT3/ITD*
10	5/16/2000	Initial	46,XX	p.G707AfsX72	*NRAS, IDH1*
	6/7/2000	CR		—	—
13	7/26/2002	Initial	46,XY	p.R882H	*FLT3/TKD, NPM1*
	9/2/2002	CR		—	—
14	12/22/2003	Initial	46,XY	p.Y623FfsX29	*NPM1*
	3/5/2004	CR		—	—
15	11/21/2006	Initial	46,XY	p.W297X	*PTPN11, ASXL1*
	5/3/2007	CR		—	—
17	4/24/2007	Initial	46,XX	p.R882C	*FLT3/TKD, AML1/RUNX1, IDH2*
	6/28/2007	CR		—	—
18	10/15/1999	Initial	46,XX	p.R882H	*NRAS, NPM1*
	11/30/1999	CR		—	—
	1/18/2001	Relapse	46,XX	p.R882H	*NPM1*
20	12/28/2007	Initial	47,XX,+i(11)(q10)	p.N797Y	*ASXL1, IDH2*
	6/20/2008	CR	46,XX	—	—
	10/21/2008	Relapse	46,XX	p.N797Y	*ASXL1, IDH2*
22	9/16/2004	Initial	46,XX	p.R882C	*NRAS, NPM1, IDH1*
	10/28/2004	CR		—	—
28	8/7/2006	Initial	46,XY	p.R882H	*FLT3/ITD, NPM1*
	9/26/2006	CR		p.R882H	—
	1/18/2007	Relapse	ND	p.R882H	*FLT3/ITD, NPM1*
29	1/27/2004	Initial	46,XX,t(9;22)(q34;q11)	p.I705F	*IDH1*
	3/1/2004	CR	46,XX	—	—
	6/9/2005	Relapse	46,XX,t(9;22)(q34;q11)	p.I705F	*IDH1*
31	4/2/2001	Initial	46,XX	p.R882H	*FLT3/ITD, NPM1*
	5/11/2001	CR		—	—
	8/20/2001	Relapse	44-46,XX,del(20)(q11q13)[cp6]/46,XX[7]	p.R882H	*FLT3/ITD, NPM1*
32	4/12/2000	Initial	46,XY	p.R882C	*FLT3/ITD, NPM1*
	7/13/2000	CR		p.R882C	—
	10/5/2000	Relapse	46,XY	p.R882C	*FLT3/ITD, NPM1*
33	10/29/2007	Initial	46,XX	p.R882H	*FLT3/ITD, NPM1*
	3/18/2008	CR		p.R882H	—
	5/8/2008	Relapse	ND	p.R882H	*FLT3/ITD, NPM1*
34	6/25/1998	Initial	45,X,-Y	p.R882S	*NRAS, FLT3/ITD, NPM1*
	7/7/2000	Relapse	45,X,-Y	p.R882S	*FLT3/ITD, NPM1*
	8/11/2000	CR2	46,XY	—	—
37	2/3/2006	Initial	46,XY	p.R882C	*FLT3/ITD, NPM1*
	4/19/2006	CR		—	—
	5/3/2006	Relapse	ND	p.R882C	*FLT3/ITD*
38	8/15/2002	Initial	46,XX	p.R320X	*FLT3/TKD, NPM1*
	1/28/2003	CR		—	—
39	2/15/2002	Initial	45,X,-Y	p.Q606X	*NPM1*
	4/8/2002	CR	46,XY	—	—
45	2/1/2005	Initial	46,XY	p.R882H	*FLT3/ITD, NPM1*
	3/1/2005	CR		—	—
	11/24/2005	Relapse	46,XY	p.R882H	*FLT3/ITD, NPM1*
47	6/14/2000	Initial	46,XX	p.R882C	*NPM1*
	10/19/2000	CR		—	—

The results of serial studies in 103 patients without *DNMT3A* mutation at diagnosis are not shown in this table. None of these 103 patients acquired *DNMT3A* mutation at relapse.

UPN indicates unique patient number; CR, complete remission; −, negative; ND not done; and NM, no mitosis.

*Using the more sensitive TA cloning technique, 1 of 17 clones showed *DNMT3A* mutation.

Table 6. Sequential studies in AML patients with *DNMT3A* mutations (continued)

UPN	Date	Status	Karyotype	*DNMT3A* mutation	Other mutations
48	12/13/2006	Initial	47,XX,+8	p.G334AfsX11	*CEBPA, IDH2*
	2/9/2007	CR		—	
50	9/25/2003	Initial	46,XY	R882H	*IDH2*
	6/10/2005	CR		—	
51	5/29/2003	Initial	46,XY	p.P256LfsX59	*FLT3/ITD, NPM1, WT1*
	7/17/2003	CR		—	—
	12/26/2003	Relapse	ND	p.P256LfsX59	*FLT3/ITD, NPM1, WT1*
54	9/5/2002	Initial	46,XY	p.R882C	*FLT3/ITD, NPM1*
	5/28/2003	CR		—	—
55	2/21/2006	Initial	46,XX	p.R882H	*ASXL1, IDH2*
	9/14/2006	CR		—	—
56	3/24/2003	Initial	46,XX	p.R882C	*FLT3/ITD, MLL/PTD*
	5/21/2003	CR		—	—
	10/1/2003	Relapse	46,XX	p.R882C	*FLT3/ITD, MLL/PTD*
61	10/30/1995	Initial	46, XY	p.R882H	*NPM1, WT1, TET2*
	1/15/1996	CR		—	—
	10/22/1996	Relapse	ND	p.R882H	*NPM1, TET2*
62	9/8/1995	Initial	46,XX	p.R771Q	*NPM1, TET2*
	12/19/1995	CR		—	—
	9/23/1996	Relapse	46,XX	p.R771Q	NPM1, TET2
64	11/2/1999	Initial	46,XY	p.G728R, p.F731L	*FLT3/ITD*
	3/16/2000	CR1		—	—
	6/12/2000	Relapse 1	ND	p.G728R, p.F731L	*FLT3/ITD*
	7/14/2000	CR2	ND	—	—
	1/11/2001	Relapse 2	ND	p.G728R, p.F731L	*FLT3/ITD*
	3/13/2001	CR3		—	—
66	3/25/2003	Initial	46,XX	p.R736H	*FLT3/ITD, NPM1, IDH1*
	12/30/2003	CR		—	—
69	4/2/2001	Initial	46,XX	p.R635W	*PTPN11, NPM1, IDH2*
	5/17/2001	CR		—	—

The results of serial studies in 103 patients without *DNMT3A* mutation at diagnosis are not shown in this table. None of these 103 patients acquired *DNMT3A* mutation at relapse.

UPN indicates unique patient number; CR, complete remission; –, negative; ND not done; and NM, no mitosis.

*Using the more sensitive TA cloning technique, 1 of 17 clones showed *DNMT3A* mutation.

were located outside of this region (Figure 1). Nine of these mutations were frame-shift or nonsense mutations and were expected to impair enzyme activity. Similar to our finding, Ley et al also detected mutations outside of exons 15 to 23.[4] In the study by Thol et al, all 23 exons of *DNMT3* were initially sequenced in 40 patients.[3] Because only mutations between exons 15 and 23 were found in these patients, they subsequently sequenced exons 15 to 23, but not other exons, in other patients. Because all but one mutation outside of exons 15 to 23 in our study were detected in only one patient each (an incidence of 1 in 500 for each mutation), the absence of mutation in this area in 40 patients might not mean that it would not happen in other patients.

In this study, *DNMT3A* mutations were found in 14%, 15.2%, 19.5%, and 22.9%, respectively, in whole cohort, non-M3 AML, intermediate-risk cytogenetics, and CN-AML groups, lower than the reports of Ley et al (22.1% for total patients, 33.7% for those with intermediate-risk cytogenetics, and 36.7% for CN-AML patients)[4] and Thol et al (17.8% in non-M3 and 27.2% in CN-AML patients).[3] In a study of Chinese AML patients by Yan et al, *DNMT3A* mutations were detected in 20.5% and 13.6%, respectively, of patients with the FAB M5 and M4 subtypes of AML, but none of the patients with FAB M1 or M2 subtypes had the mutation, leading to an overall incidence of 9% for the *DNMT3A* mutation in the entire group of AML patients.[9] Yamashita et al also reported a low incidence (4.1%) of *DNMT3A* mutations in Japanese AML patients.[8] The reason for the variability in the incidence of *DNMT3A* mutations in different studies is unknown, but may be because of the differences in ethnic background, patient popula-

tions recruited, and methods used. Whether *DNMT3A* mutations occur less frequently in Asian than in Western AML patients needs to be determined by further studies.

In our comprehensive analysis of the 17 gene mutations in 500 patients, we found that the *DNMT3A* mutation was the third commonest recurrent genetic alteration, followed by *FLT3*-ITD and *NPM1* mutations, in AML patients. In addition to its close association with *NPM1* mutations and *FLT3*-ITD, which has been shown previously,[3,4] we demonstrated herein that *DNMT3A* mutations were also positively associated with *PTPN11* and *IDH2* mutations and negatively associated with the *CEBPA* mutation. More intriguingly, we found that the *DNMT3A* mutation rarely occurred alone; all but 2 patients with *DNMT3A* mutations showed concurrent mutations of other genes, more frequently class I (51 of 68, 75%), but also class II mutations (16 of 68, 23.5%) and *NPM1* mutations (38 of 68, 54.3%, supplemental Table 2), which behave more like class II mutations.[13] In short, the development of AML may require concerted interaction among different genetic alterations.

The stability of *DNMT3A* mutations in the evolution of AML remains unclear. In a serial study of 5 patients with *DNMT3A* mutations at diagnosis, Thol et al found that the mutations disappeared at CR and reappeared at relapse in one patient tested.[3] To the best of our knowledge, the present study recruited the largest number of AML patients for sequential analysis of *DNMT3A* mutations during the clinical course. In contrast to the instability of *FLT3*-ITD during disease evolution, we found that the *DNMT3A* mutation seemed to be stable, analogous to *NPM1* and *IDH1/2*

mutations.[13,21,22] At relapse, all *DNMT3A*-mutated patients who had available samples for serial study regained the same mutations, including the one in whom the mutation could be detected by a sensitive gene-cloning technique, but not by direct sequencing. Conversely, all 103 patients without *DNMT3A* mutation at diagnosis remained *DNMT3A*-wild at relapse. These results suggested that although *DNMT3A* mutations are important for the development of AML, they may play little role in disease progression. Given the stability of the *DNMT3A* mutation during AML evolution, it may be a potential biomarker for monitoring minimal residual disease.

We found that AML patients with *DNMT3A* mutations had distinct clinical and laboratory characteristics and a poor prognosis. Recently, many gene mutations have been detected in AML and some found to be independent prognostic factors. In the present study, to better stratify AML patients into different risk groups, a survival scoring system incorporating the *DNMT3A* mutation and 8 other prognostic factors, including age, WBC count, cytogenetics, and *NPM1/FLT3*-ITD, *CEBPA*, *AML1/RUNX1*, *WT1*, and *IDH2* mutations, into the survival analysis was formulated. This scoring system was found to be more powerful than any single marker at separating patients into different prognostic groups. However, further study with an independent cohort will be needed to validate the proposed scoring system.

In summary, this study demonstrated that *DNMT3A* mutations could be detected in a substantial number of patients with de novo AML and were closely associated with older age and FAB M4/M5 subtypes. *DNMT3A* mutations occurred more frequently in patients with intermediate-risk cytogenetics and normal karyotype. They were mutually exclusive with *CEBPA* mutation, but were closely associated with *FLT3*/ITD, *NPM1*, *PTPN11*, and *IDH2* mutations. Furthermore, the *DNMT3A* mutation was an independent poor risk factor for OS and RFS among total cohort and CN-AML patients. Sequential study during the clinical course showed that the *DNMT3A* mutation was stable during AML evolution. We conclude that the incorporation of the *DNMT3A* mutation with 8 other prognostic factors into survival analyses can better stratify AML patients into different risk groups.

Authorship

Contribution: H.-A.H. collected the literature, managed and interpreted the data, performed the statistical analysis, and wrote the manuscript; Y.-Y.K. collected the literature, managed and interpreted the data, and wrote the manuscript; C.-Y.L. performed and interpreted the statistical analysis; L.-I.L. performed and interpreted the mutation analysis; C.-Y.C., W.-C.C., M.Y., S.-Y.H., J.-L.T., B.-S.K., S.-C.H., S.-J.W., W.T., and Y.-C.C. contributed patient samples and clinical data; M.C.L., M.-H.T., C.-F.H., Y.-C.C., C.-Y. L., F.-Y.L., and M.-C.L. performed the gene mutation and chromosomal studies; and H.-F.T. planned, designed, and coordinated the study and wrote the manuscript.

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