WT1 mutation in 470 adult patients with acute myeloid leukemia: stability during disease evolution and implication of its incorporation into a survival scoring system

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The impact of *WT1* mutations in acute myeloid leukemia (AML) is not completely settled. We aimed to determine the clinical implication of *WT1* mutation in 470 de novo non-M3 AML patients and its stability during the clinical course. *WT1* mutations were identified in 6.8% of total patients and 8.3% of younger patients with normal karyotype (CN-AML). The *WT1* mutation was closely associated with younger age ($P < .001$), French-American-British M6 subtype ($P = .006$), and t(7;11)(p15;p15) ($P = .003$). Multivariate analysis demonstrated that the *WT1* mutation was an independent poor prognostic factor for overall survival and relapse-free survival among total patients and the CN-AML group. A scoring system incorporating *WT1* mutation, *NPM1/FLT3-ITD*, *CEBPA* mutations, and age into survival analysis proved to be very useful to stratify CN-AML patients into different prognostic groups ($P < .001$). Sequential analyses were performed on 133 patients. *WT1* mutations disappeared at complete remission in all *WT1*-mutated patients studied. At relapse, 3 of the 16 *WT1*-mutated patients who had paired samples lost the mutation and 2 acquired additional mutations, whereas 3 of 110 *WT1*-wild patients acquired novel mutations. In conclusion, *WT1* mutations are correlated with poor prognosis in AML patients. The mutation status may be changed in some patients during AML progression. (*Blood*. 2010;115(25):5222-5231)

Introduction

The *Wilms' Tumor 1* (*WT1*) gene, encoding a zinc-finger transcription factor, is physiologically expressed in embryonic kidney cells[1] and hematopoietic stem cells.[2,3] *WT1* was first identified as a tumor suppressor gene in patients with the WAGR (Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation) tumor predisposition syndrome.[4] The *WT1* gene is demonstrated to be overexpressed in various leukemias, particularly acute myeloid leukemia (AML), as well as other cancers,[5,6] and thus is suggested to be an oncogene.[7,8] On the other hand, mutations in *WT1* gene are found in approximately 10% of AML patients with hotspots in the 4 Cys-His zinc finger domains on exons 7 and 9.[6,9] As a result, *WT1* as a tumor suppressor gene is suggested.[6,9] The precise role of *WT1* in leukemogenesis remains to be defined.[10]

Several reports addressed that *WT1* mutation was an independent poor risk factor for overall survival (OS) in cytogenetically normal AML (CN-AML) patients.[11-13] Accompanied with other mutations, such as *FLT3-ITD*, *WT1* mutations were associated with failure of standard induction chemotherapy.[14] However, different results were also reported by other groups.[15,16] Most studies on *WT1* mutations were restricted to CN-AML, and sequential analyses to evaluate the stability of this gene mutation during the clinical course were limited to a small number of patients. In this study, we delineated *WT1* mutation in 470 de novo non-M3 AML patients, both cytogenetically normal and abnormal, and investigated its association with other gene alterations. Sequential analysis of *WT1* mutation during the clinical course was also performed on 133 patients to investigate the stability and pathogenic role of this mutation in AML. Further, to better stratify CN-AML patients into different risk groups,[15,17] a simple scoring system integrating WT1 mutations with other gene alterations in survival analysis was proposed.

Methods

From November 1995 to March 2007, a total of 470 adult patients 15 years of age or older who were newly diagnosed as having de novo non-M3 AML at National Taiwan University Hospital (NTUH) and had cryopreserved bone marrow samples for genetic studies were enrolled. Patients with antecedent hematologic diseases, therapy-related AML, or acute promyelocytic leukemia (M3 subtype) were excluded because the pathogenesis of their leukemias and survival of these patients differ from other AML patients significantly. Among 470 patients, 330 (70.2%) patients received intensive induction chemotherapy (idarubicin 12 mg/m^2 per day on days 1-3 and cytarabine 100 mg/m^2 per day on days 1-7) and then consolidation chemotherapy with 2 to 4 courses of high-dose cytarabine (2000 mg/m^2 every 12 hours on days 1-4, total 8 doses), with or without an anthracycline if complete remission (CR) was achieved. The remaining 140 patients

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received low-dose chemotherapy and/or supportive care because of the poor performance status or the patients' will. Ninety-six patients received allogeneic hematopoietic stem cell transplantation (HSCT). This study was approved by the Institutional Review Board of the NTUH, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Cytogenetics

Bone marrow cells were harvested directly or after 1- to 3-day unstimulated culture as previously described.[18] Metaphase chromosomes were banded by the trypsin-Giemsa method and karyotyped according to International System for Human Cytogenetic Nomenclature.

Mutation analysis

Mutation analysis of *WT1* exons 7 and 9 was performed by genomic DNA polymerase chain reaction (PCR) and direct sequencing as previously reported.[14] The primers used were as follows: 7F 5′ GACCTACGTGAATG TTCACATG-3′ and 7R 5′-ACCAACACCTGGATCAGACCT-3′; 9F 5′-TGCA GACATTGCAGGCATGGCAGG-3′ and 9R 5′-GCACTATTCCT-TCTCTAACT GAG-3′. Abnormal sequencing results were confirmed by at least 2 repeated analyses. Sequential analysis of *WT1* mutation during the clinical course was performed in 309 samples from 133 patients. Mutation analyses of 11 other relevant molecular marker genes (*NPM1*,[19] *CEBPA*,[20] *FLT3/ITD* and *FLT3/TKD*,[19] *N-RAS*,[21] *K-RAS*,[21] *JAK2*,[21] *KIT*,[22] *MLL/PTD*,[23] *AML1/RUNX1*,[24] and *PTPN11*[25]) were performed as previously described.

GeneScan analysis

Percentage of *WT1* mutant was determined using PCR and fragment analysis as previously reported.[26] Two pairs of primers covering exon 7 and exon 9 were designed using PrimerQuest software (Integrated DNA Technologies). Each one of the primer pairs was labeled with appropriate fluorescence. The primers used for exon 7 were FAM-5′-ttactctctgcctgcag-gatgtgcgac-3′ and 5′-agcgggcacacttaccagt-3′, and those for exon 9 were VIC-5′-gccgaggctagaccttctct-3′ and 5′-tccaatccctctcatcacaa-3′, which would produce amplicons with sizes of 183 bp and 203 bp, respectively, for wild-type *WT1*. The amplified products were subsequently diluted in distilled water and mixed with deionized formamide and GeneScan 500 LIZ Size Standard (Applied Biosystems). These mixtures were then electrophoresed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). After electrophoresis, the fluorescence signals were analyzed using GeneScan, Volume 3.1 software (Applied Biosystems).

Taq polymerase–amplified cloning analysis

For patients with double *WT1* mutations, Taq polymerase–amplified cloning was performed to determine whether the 2 mutations were in the same or different alleles as previously described.[20] Briefly, the DNA was amplified to cover both mutations, the PCR products were then cloned into the Taq polymerase-amplified cloning vector pGEM-T Easy (Promega), and 10 clones were selected for sequencing.

Immunophenotype analysis

A panel of monoclonal antibodies to myeloid-associated antigens, including CD13, CD33, CD11b, CD15, CD14, and CD41a, lymphoid-associated antigens, including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage-nonspecific antigens HLA-DR, CD34, and CD56 was used to characterize the phenotypes of the leukemia cells, as previously described.[19]

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, Fisher exact test was used. Mann-Whitney tests were used to compare continuous variables and medians of distributions if the continuous data were not normally distributed. OS was measured from the date of first diagnosis to death from any cause, and relapse-free survival (RFS) was calculated from the time of CR until relapse, death, or end of study. Kaplan-Meier estimation was used to plot survival curves, and log-rank tests were used to test the difference 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, white blood cell (WBC), karyotype, and mutations of *CEBPA*, *WT1*, and *NPM1/FLT3-ITD*, were used as covariates. Those patients who received HSCT were censored at the time of HSCT in survival analysis to ameliorate the influence of this treatment. A P value less than .05 was considered statistically significant. All statistical analyses were performed with the SPSS, Version 15 software (SPSS Inc) and Statsdirect.

Results

WT1 mutations in 470 adult patients with de novo non-M3 AML

Mutation screening of the coding and intron-exon boundaries of *WT1* exons 7 and 9 was performed using a direct sequencing method on DNA samples from 470 de novo AML patients. Overall, 29 different kinds of *WT1* mutations were detected in 32 patients (6.8%, Table 1), including 26 on exon 7 and 3 on exon 9. Of them, 4 mutations (V382fsx385, N381fsx450, Q380fsx384, and S381X) occurred in more than one patient (nos. 1 and 7, 4 and 5, 6 and 16, and 9, 28, and 31, respectively).

The mutations in 2 patients (nos. 2 and 20) were double heterozygous with 2 exon 7 mutations in different alleles. The remaining 30 patients showed only one mutation; all are heterozygous. Of the 29 kinds of mutations, 3 were base substitutions, 19 with 1- to 13-bp insertions, and 7 with 1- to 11-bp deletions. Of them, 2 of the 3 base substitutions caused amino acid changes, the remaining one base substitution and 2 insertion mutations (patient nos. 11 and 30) created a stop codon, and the others resulted in reading frame shift, which was predicted to generate nonfunctional truncated proteins with loss of their DNA-binding sites (aa391-aa506), RNA recognition sites (zinc finger 1), and nuclear localization signal (aa359-aa418). Among 26 mutations on exon 7, 23 (88.5%) were frame-shift mutations, compared with one of the 3 exon 9 mutations ($P = .068$).

Correlation of *WT1* mutations with clinical features and immunophenotypes of leukemic cells

Among the 470 AML patients recruited, 266 were males and 204 were females with a median age of 52 years (range, 15-90 years). The comparison of clinical characteristics and laboratory data between patients with and without *WT1* mutation is summarized in Table 2. *WT1*-mutated patients were younger than *WT1*-wild patients (median, 38.5 years vs 53 years, $P < .001$). Patients with French-American-British (FAB) M6 subtype of AML had the highest incidence (33.3%) of *WT1* mutation, whereas those with M0 subtype had the lowest incidence ($P = .006$). There was no difference in other clinical parameters, including sex, WBC count, hemoglobin, platelet count, and lactate dehydrogenase value between patients with and without the mutation. The *WT1* mutation did not correlate with the expression of the antigens studied (data not shown).

Association of *WT1* mutations with cytogenetic abnormalities

Chromosome data were available in 452 patients at diagnosis, including 30 *WT1*-mutated and 422 *WT1*-wild patients (Table 3). *WT1* mutations were highly associated with t(7;11)(p15;p15)

Table 1. Mutation patterns in 32 patients with *WT1* mutations at diagnosis

UPN	Age, y/sex	FAB	Karyotype	*WT1* mutation			Other gene mutations detected
				Location	DNA change	Protein change	
1	30/M	2	46XY	Exon 7	nt1340/1341 (+TCGG),	V382fsX385	*FLT3/ITD*
2	64/M	1	46XY	Exon 7	nt1338/1339 (+A), nt1337/1338 (+ACGGT)	G382fsX384, Y381fsX450	*CEBPA*
3	59/F	2	47,XX, t(7;11)(p15;p15),+8	Exon 7	nt1333 (−A)	G380fsX448	*KRAS*
4	61/M	1	46,XY	Exon 7	nt1336/1337 (+AACGG)	N381fsX450	*NPM1*
5	69/M	2	NM	Exon 7	nt1336/1337 (+AACGG)	N381fsX450	*CEBPA*
6	35/F	2	46,XY,t(8;21)(q22;q22)	Exon 7	nt1334/1335 (+A)	Q380fsX384	—
7	25/M	2	46,XY,t(7;11)(p15;p15)	Exon 7	nt1340/1341 (+TCGG)	V382fsX385	—
8	31/M	2	46,XY,t(8;21)(q21;q22)	Exon 7	nt1420/1421 (+GAGCAGCCAA)	E409fsX421	—
9	15/M	2	46,XY,t(2;22)(p21;q11)	Exon 7	nt1338 (C>A)	S381X	*FLT3/ITD*
10	38/F	2	45,XX,t(4;12;11)(q21;q13;p15),-7	Exon 9	nt1590-1600 (−ACCTGAAGACC)	P465fsX472	—
11	20/F	6	46,XX	Exon 9	nt1574/1575 (+AAGT)	F460X	*CEBPA*
12	26/M	6	46,XY	Exon 7	nt1326/1327 (+AAGAGAC)	R378fsX386	—
13	25/F	2	46,XX,t(8;21)(q22;q22)	Exon 9	nt1536 (G>T)	G447V	—
14	43/M	6	46,XY	Exon 7	nt1331/1332 (+ACTCTTG)	D379fsX386	—
15	28/F	1	46,XX	Exon 7	nt1392/1393 (+A)	K399fsX400	*CEBPA*
16	85/M	5	46,XY	Exon 7	nt1334/1335(+A)	Q380fsX384	*FLT3/ITD, NPM1*
17	80/M	6	46,XY	Exon 7	nt1271-1275 (−CGAGG, +GCTCAGTGGGGGGAC)	A359fsX387	*RUNX1*
18	23/F	2	46,XX,t(7;11)(p15;p15)	Exon 7	nt1332-1339 (−TACGGTCG)	G379fsX381	*KRAS*
19	27/F	2	46,XX,t(7;11)(p15;p15)	Exon 7	nt1336/1337 (+G)	V381fsX384	*FLT3/ITD*
20	43/M	2	46,XY,del(20)(q11q13)[15]/ 46,idem,del(11)(p13p15)[3]	Exon 7 Exon 7	nt1302-1306 (−GACGT, +AAGA) nt1398 (G>A)	Q369fsX374 R401K	—
21	31/F	2	46,XX	Exon 7	nt1332-1333(−TA)	A379fsX383	*FLT3/ITD,NPM1, NRAS*
22	53/F	2	46,X,-X,+21	Exon 7	nt1323/1324 (+C)	D377fsX384	*CEBPA*
23	39/F	4	46,XX	Exon 7	nt1338/1339 (+TCTTGTACGGTCG)	S382fsX388	*FLT3/TKD, NPM1*
24	31/F	1	46,XX	Exon 7	nt1334 (−C, +GG)	G380fsX384	*FLT3/ITD*
25	16/M	4	46,XY	Exon 7	nt1328-1331 (−CTTG)	Y378fsX447	*FLT3/TKD, RUNX1*
26	48/M	4	NM	Exon 7	nt1326/1327 (+AAGAC)	R378fsX450	*FLT3/ITD, NRAS*
27	43/F	8	Cplx*	Exon 7	nt1303-1304 (−AC, +GCCTCCTATA)	P370fsX377	—
28	70/F	4	46,XX, t(11;12)(q23;q13), del(9)(q12q33)	Exon 7	nt1338 (C>A)	S381X	*FLT3/ITD, NPM1*
29	40/F	2	46,XX	Exon 7	nt1302-1303 (−GA, +CGG)	P369fsX384	*MLL/PTD*
30	45/F	1	48,XX,+4,+10[6]/ 47,XX,del(9)(q13q22),+10[4]	Exon 7	nt1400/1401 (+GAGAT)	Y402X	*CEBPA*
31	26/F	2	46,XX	Exon 7	nt1338 (C>A)	S381X	*FLT3/ITD, MLL/PTD*
32	40/M	1	46,XY	Exon 7	nt1393 (−T)	K399fsX448	*CEBPA*

The nucleotide numberings are according to NCBI Reference Sequence: NM_024426.
FAB indicates French-American-British subtype; NM, no mitosis; and —, negative (no other mutation found).
*Cplx: 46,XX,add(5)(p15),del(5)(q11q35),add(6)(p23),add(8)(p21),add(10)(q25),del(12)(p11p13),der(14)t(7;14)(q11;p12),add(19)(q13).

($P = .003$), a chromosomal translocation resulting in fusion between *NUP98* on 11p15 and *HOXA9* on 7P15.[27,28] Four (40%) of the 10 patients with this cytogenetic abnormality showed concurrent *WT1* mutation. There was no association of *WT1* mutation with other chromosomal abnormalities, including +8, +11, +13, +21, −5/del(5q), and −7/del(7q). The incidences of *WT1* mutation were not significantly different between patients with normal karyotype (16 of 230, 7.0%) and those with abnormal cytogenetics (14 of 222, 6.3%; $P = .851$), and among patients with favorable (5.1%), intermediate (7.0%), and unfavorable karyotypes (6.1%). In younger patients (< 60 years of age) with normal karyotype (n = 121), the incidence of *WT1* mutation was 8.3%.

Association of *WT1* mutation with other molecular abnormalities

To investigate the interaction of gene mutations in the pathogenesis of adult AML, a complete mutational screening of 11 other genes was performed in all 470 patients. Among the 32 patients with *WT1* mutations, 23 (72%) showed additional molecular abnormalities at diagnosis (Table 4); 16 had one additional change, 6 had 2, and 1 had 3. Sixteen (69.6%) of them had at least one concurrent class 2 mutation that would impair differentiation of hematopoietic cells,[29] and 13 (56.5%) had a class 1 mutation that would confer proliferation advantage (Table 4). The most frequently associated molecular event was *FLT3/ITD* (9 cases), followed by *CEBPA* (7 cases), and *NPM1* (5 cases). However, there was no difference in the incidence of any mutation between patients with and without *WT1* mutation (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Sequential studies of *WT1* mutations in AML patients

A total of 309 samples from 133 patients were serially studied for *WT1* mutations using both direct sequencing and GeneScan analysis, including 23 patients with *WT1* mutations and 110 without mutation at diagnosis. Among the 23 patients with *WT1* mutations at diagnosis, all lost their mutations at remission status (Table 5). Among the 16 patients who had paired samples for serial study both at diagnosis and at relapse, 13 patients retained the original mutation at first relapse, but one of them (no. 26) lost the

Table 2. Comparison of clinical manifestations and laboratory features between AML patients with and without *WT1* mutation

Variable	Total (n = 470)	*WT1*-mutated (n = 32, 6.8%)	*WT1*-wild (n = 438, 93.2%)	*P*
Sex, no. (%) of patients				.250
Male	266	15 (46.9)	251 (57.3)	—
Female	204	17 (53.1)	187 (42.7)	—
Median age, y (range)	52.0 (15-90)	38.5 (15-85)	53 (15-90)	< .001
Laboratory data, median (range)				
WBCs, per μL	21 850 (120-627 800)	20 290 (1200-227 700)	21 850 (120-627 800)	.785
Hemoglobin, g/dL	8.0 (2.9-16.2)	7.65 (3-13.6)	8 (2.9-16.2)	.468
Platelets, × 1000/μL	45.0 (3-802)	46.5 (7-802)	44 (3-802)	.653
Blasts, per μL	9863 (0-456 725)	9927 (120-154 836)	9863 (0-456 725)	.928
LDH, U/L	890 (206-15 000)	1228 (301-7734)	856 (206-15 000)	.107
FAB, no. (%) of patients				.017
M0	10	0 (0.0)	10 (100.0)	—
M1	114	6 (5.3)	108 (94.7)	—
M2	174	16 (9.2)	158 (90.8)	—
M4	126	4 (3.2)	122 (96.8)	—
M5	25	1 (4.0)	24 (96.0)	—
M6	12	4 (33.3)	8 (66.7)	—
Undetermined	9	1 (11.1)	8 (88.9)	—
Induction response*	330	28	302	.516
CR	248 (75.2)	21 (75.0)	227 (75.2)	—
Refractory	59 (17.8)	6 (21.4)	53 (17.5)	—
Induction death	23 (7.0)	1 (3.6)	22 (7.3)	—
Relapse	134 (54.0)	18 (85.7)	116 (51.1)	.002

— indicates not applicable.

*Only the 330 patients (including 28 with the *WT1* mutation and 302 without) who received conventional intensive induction chemotherapy and then consolidation chemotherapy if CR was achieved, were included in the analysis.

mutation at second relapse (Table 5). Two patients (nos. 21 and 23) each acquired an additional *WT1* mutation on a different allele. In these 13 patients, the percentage of mutant at relapse, compared with that at diagnosis, was increased in 4 patients (nos. 12, 16, 22, and 29), decreased in 6 patients (nos. 7, 18, 23, 25, 26, and 32), had no much change in 2 patients (nos. 3 and 21), and cannot be evaluated in one patient (no. 13) because the mutation is single base substitution (Table 5). The remaining 3 patients (nos. 4, 11, and 19) lost *WT1* mutations at relapse. Among the 110 patients who had no *WT1* mutation at diagnosis, 3 acquired *WT1* mutation at relapse (Table 5). Patient 33 acquired *WT1* mutation at second relapse, 66 months after the initial study at diagnosis and 5 months after second human leukocyte antigen-matched sibling allogeneic HSCT. He also gained concurrently novel mutations of *NPM1* and *NRAS* at that time (Table 5; Figure 1). No bone marrow sample was available for mutation analysis at first relapse. Patient 34 acquired *WT1* mutation at first relapse, which disappeared at second CR but reappeared at second relapse; the same *NPM1* mutation detected at diagnosis was retained at first and second relapse. Patient 35 acquired novel *WT1* mutation and karyotypic evolution at first relapse, whereas the original *CEBPA* mutant remained the same (Table 5).

Table 3. Association of *WT1* mutation with chromosomal abnormalities*

Karyotype	Total	*WT1*-mutated	*WT1*-wild	*P*
Favorable	59	3 (5.1)	56 (94.9)	.953
Intermediate	327	23 (7.0)	304 (93.0)	—
Unfavorable	66	4 (6.1)	62 (93.9)	—
Unknown	18	2 (11.1)	16 (88.9)	—
Normal	230	16 (7.0)	214 (93.0)	> .999
Simple	169	11 (6.5)	158 (93.5)	—
Complex	53	3 (5.7)	50 (94.3)	—
t(8;21)	40	3 (7.5)	37 (92.5)	.740
inv(16)	19	0 (0.0)	19 (100.0)	.628
t(11q23)	16	1 (6.3)	15 (93.7)	> .999
t(7;11)	10	4 (40.0)	6 (60.0)	.003
−5/5q−†	1	0 (0.0)	1 (100.0)	> .999
−7/7q−†	10	1 (10.0)	9 (90.0)	.500
+8†	22	1 (4.5)	21 (95.5)	> .999
+11†	3	0 (0.0)	3 (100.0)	> .999
+13†	1	0 (0.0)	1 (100.0)	> .999
+21†	11	1 (9.1)	10 (90.9)	> .999

— indicates not applicable.

*A total of 452 patients, including 30 *WT1*-mutated and 422 *WT1*-wild patients, had chromosome data at diagnosis.

†Only including simple chromosomal abnormalities with 2 or fewer changes, but not those with complex abnormalities with 3 or more aberrations.

Impact of *WT1* mutation on response to therapy and clinical outcome

Of the 330 AML patients undergoing conventional intensive induction chemotherapy, 248 (75.2%) patients achieved CR. The probability of achieving a first CR was similar between patients with and without *WT1* mutations (75% vs 75.2%, $P = .985$). However, the patients with *WT1* mutations had a higher incidence of relapse than those without (85.7% vs 51.1%, $P = .002$; Table 2).

With a median follow-up time of 53 months (range, 1.0-160 months), patients with *WT1* mutation had a significantly poorer OS and RFS than those without *WT1* mutation (median, 14.0 months vs 29.5 months, $P = .021$; and 6 months vs 14 months, $P < .001$, respectively; supplemental Table 2; Figure 2). There was no significant difference in OS and RFS between patients with frame-shift mutations and other mutations ($P < .987$ and $P < .99$, respectively).

In multivariate analysis (Table 6), the independent poor risk factors for OS were age more than 50 years, WBC count more than 50 000/μL, unfavorable karyotype, and *WT1* mutation. $CEBPA^{double\text{-}mutation}$ and $NPM1^{mut}/FLT3\text{-}ITD^{-}$ were independent favorable prognostic factors. The independent poor risk factors for RFS included unfavorable karyotype and *WT1* mutation.

Table 4. Cooperative gene mutations in the 32 AML patients with WT1 mutations*

	Class 1 mutations				Class 2 mutations			
UPNs	*FLT3/ITD*	*FLT3/TKD*	*NRAS*	*KRAS*	*RUNX1*	*CEBPA*	*MLL/PTD*	*NPM1* mutation
1, 9, 19, 24	+	—	—	—	—	—	—	—
16, 28	+	—	—	—	—	—	—	+
21	+	—	+	—	—	—	—	+
26	+	—	+	—	—	—	—	—
31	+	—	—	—	—	—	+	—
25	—	+	—	—	+	—	—	—
23	—	+	—	—	—	—	—	+
18	—	—	—	+	—	—	—	—
3	—	—	—	+	—	—	—	—
2, 5, 11, 15, 22, 30, 32	—	—	—	—	—	+	—	—
17	—	—	—	—	+	—	—	—
29	—	—	—	—	—	—	+	—
4	—	—	—	—	—	—	—	+

A total of 23 of the 32 *WT1*-mutated patients concurrently had other gene mutations, including 13 (56.5%) with class 1 mutations and 16 (69.6%) with class 2 mutations or *NPM1* mutations. None of these patients had *KIT*, *PTPN11,* or *JAK2* mutation.

UPN indicates unique patient number; and —, negative (not mutated).

$CEBPA^{double\text{-}mutation}$ and $NPM1^{mut}/FLT3\text{-}ITD^{-}$ were independent favorable factors for RFS. Double *CEBPA* mutations ($CEBPA^{double\text{-}mutation}$) instead of all *CEBPA* mutations were used as a variable for survival analysis in this study because recent studies showed that only $CEBPA^{double\text{-}mutation}$, but not single mutation, were associated with favorable prognosis.[30,31]

Further, we made survival analysis in a relatively homogeneous population of younger patients (< 60 years) with normal karyotype (CN-AML). *WT1* mutation was still an independent poor prognosis for OS and RFS (relative risk = 3.752, 95% confidence interval 1.195-11.783, $P = .024$; and relative risk = 3.806, 95% confidence interval 1.588-9.112, $P = .003$, respectively, Table 6).

To better stratify the CN-AML patients into different risk groups according to gene mutations, a scoring system was formulated based on the results of our Cox proportional hazards model. Positivity of *WT1* mutations or older age was scored +1 individually, whereas $NPM1^{mut}/FLT3\text{-}ITD^{-}$ or $CEBPA^{double\text{-}mutation}$ was scored −1. The algebraic summation of these scores of each patient was the final score. This score system divided these CN-AML patients into 3 groups with different survival ($P < .001$ for both OS and RFS; Figure 3).

Discussion

In this study, we showed the incidence of *WT1* mutations was 6.8% for total de novo non-M3 AML patients, and 7.0% and 8.3%, respectively, for patients with CN-AML of all age groups and those younger than 60 years. Further, *WT1* mutations were distinctly associated with poor prognosis in AML patients.

Most studies concerning *WT1* mutations in AML were focused on patients with normal karyotype. In this study, we recruited both patients with CN-AML and those with chromosomal aberrations. We found that *WT1* mutations occurred with similar frequencies in patients with normal karyotype and those with abnormal cytogenetics. The mutation could be detected in patients with t(8;21), t(15;17) (not recruited in this study), and t(7;11). But none of the patients with inv(16) showed this gene mutation. In the limited reports in literature regarding *WT1* mutation in AML patients afflicted with abnormal cytogenetics, the mutation was also found in patients with t(15;17), t(8;21), t(6;9), and 11q23 abnormalities.[6,32-34]

To the best of our knowledge, this study recruited the largest number of *WT1*-mutated patients with abnormal cytogenetics to date. We demonstrated that t(7;11)(p15;15), a translocation resulting in *NUP98/HOXA9* fusion, was closely associated with the *WT1* mutation. This chromosomal translocation occurs more frequently in the East than in the West[35] and is associated with poor prognosis.[36] It is interesting that *NUP98* (on 11p15) and *WT1* (on p13) involved in these 2 gene alterations are located in proximity on chromosome 11p. Whether the occurrence of t(7;11) would cause any specific conformational change closed to *WT1* conferring its vulnerability to mutation warrants further study.

An association of *WT1* mutation with FAB M6 subtype and younger age was also found in this study (Table 2). The *WT1* mutation has been demonstrated in AML-M6 patients,[11,37] but no correlation of this gene mutation with FAB subtype was demonstrated in previous reports.[11,34] Four of 12 AML-M6 patients in this study had *WT1* mutations. Clinical profiles of these patients were reviewed, and the diagnosis was confirmed by the overwhelming percentage of erythroblasts (> 50% of total nucleated cells) in bone marrow aspirate smears and/or the expression of glycophorin-A on blast cells. The cause of the close association between M6 and *WT1* mutations is not clear. Kirschner et al[38] revealed the permissive role of *WT1* gene on the expression of erythropoietin receptor in human erythroid progenitors. This indirectly offered a connection between *WT1* gene and erythroblasts. But additional elaborate studies are needed to clarify this point. In this study, patients with *WT1* mutations were much younger than those without the mutation (38.5 years vs 53 years), a result similar to the reports of Renneville et al[11] and Gaidzik et al,[16] but not others.[12,13] In another study focused on pediatric AML patients, the patients older than or equal to 3 years and less than 10 years of age had higher frequency of *WT1* mutations than those younger than 3 years or those 10 years or older.[34]

Most patients (23 of 32, 72%) with *WT1* mutations showed concurrent mutations of other genes, more frequently class 2 (16 of 23), but also class 1 mutations (13 of 23, Table 4). Although *FLT3/ITD* and *CEBPA* mutations were the most frequent gene alterations accompanied with *WT1* mutation, there was no difference in the incidence of these 2 gene mutations between patients with and without *WT1* mutation (supplemental Table 1). Significantly higher incidences of *FLT3/ITD*[11,16] and *CEBPA* mutation in patients with *WT1* mutations[16] were reported by some researchers, but not others.[12,13]

Table 5. Sequential studies of *WT1* mutations in AML patients

UPN*	Date	Status	Chromosomal abnormality	*WT1* mutation		Other genetic mutations
				Amino acid change	Mutant by gene scan, percentage	
1	7/29/1994	Initial	—	V382fsx385	54.02	*FLT3/ITD*
	2/13/1995	CR1	—	—		—
3	4/9/1999	Initial	t(7;11)(p15;p15),+8	G380fsX448	39.53	*KRAS*
	7/9/1999	CR1	—	—		—
	5/3/2000	Relapse1	t(7;11)(p15;p15),+8	G380fsX448	35.10	*KRAS*
4	10/30/1995	Initial	—	N381fsX450	41.55	*NPM1*
	1/15/1996	CR1	—	—		—
	10/22/1996	Relapse1	ND	—	0	*NPM1*
	12/21/1999	CR2	—			
	8/1/2000	Relapse2	—7,t(12;18)(p11;q11)	—	0	—
6	12/12/1995	Initial	t(8;21)(q22;q22)	Q380fsX384	8.71	—
	11/26/2002	CR1	—	—		—
7	10/8/1996	Initial	t(7;11)(p15;p15)	V382fsX385	59.24	—
	11/8/1996	CR1	—	—		—
	8/22/1997	Relapse1	t(7;11)(p15;p15)	V382fsX385	40.45	—
8	4/6/1999	Initial	t(8;21)(q21;q22)	E409fsX421	44.27	—
	7/23/2002	CR1	—	—		—
10	6/30/2000	Initial	t(4;12;11)(q21;q13;p15),—7	P465fsX472	46.43	—
	9/22/2000	CR1	—	—		—
11	9/1/2000	Initial	—	F460X	21.07	*CEBPA*
	9/22/2000	CR1	—	—		—
	9/24/2001	Relapse1	NM	—	0	*CEBPA*
	1/25/2005	CR2	—	—		—
12	3/15/2001	Initial	46,XY	R378fsX386	47.27	—
	11/16/2001	CR1	—	—		—
	10/8/2002	Relapse1	add(19)(q13)	R378fsX386	88.36	*FLT3/ITD*
13	6/26/2001	Initial	t(8;21)(q22;q22)	G447V	NA	—
	7/31/2001	CR1	—	—		—
	5/10/2002	Relapse1	t(8;21)(q22;q22),t(3;12)(p21;q24)	G447V	NA	—
14	4/3/2002	Initial	—	D379fsX386	21.93	—
	5/14/2002	CR1	—	—		—
16	5/29/2003	Initial	—	Q380fsX384	11.9	*FLT3/ITD*, *NPM1*
	7/17/2003	CR1	—	—		—
	12/26/2003	Relapse1	ND	Q380fsX384	37.76	*FLT3/ITD*, *NPM1*
18	8/7/2003	Initial	t(7;11)(p15;p15)	G379fsX381	34.16	*KRAS*
	11/20/2003	CR1	—	—		—
	1/4/2005	Relapse1	t(7;11)(p15;p15)	G379fsX381	8.65	—
19	8/17/2007	Initial	t(7;11)(p15;p15)	V381fsX384	47.43	*FLT3/ITD*
	9/28/2007	CR1	—	—		—
	2/14/2008	Relapse1	t(7;11)(p15;p15)	—	0	—
21	3/8/2005	Initial	—	A379fsX383	35.63	*FLT3/ITD*, *NRAS*, *NPM1*
	5/10/2005	CR1	—	—		—
				A379fsX383	40.46	
	12/2/2005	Relapse1	—	S378fsX387	39.56	*FLT3/ITD*, *NPM1*
22	2005/5/20	Initial	−X,+21	D377fsX384	11.71	*CEBPA*
	6/22/2005	CR1	—	—		—
	5/16/2006	Relapse1	−X,+21	D377fsX384	61.55	*CEBPA*
23	6/15/2005	Initial	—	S382fsX388	42.20	*NPM1*, *FLT3/TKD*
	9/13/2005	CR1	—	—		—
				S382fsX388	33.32	
	12/9/2005	Relapse1	—	V382fsX385	27.44	*NPM1*, *FLT3/TKD*
25	7/21/2006	Initial	—	Y378fsX447	49.63	*FLT3/TKD*, *RUNX1*
	9/21/2006	CR1	—	—		—
	7/15/2008	Relapse1	—	Y378fsX447	6.32	*FLT3/TKD*
26	10/19/2006	Initial	NM	R378fsX450	53.90	*FLT3/ITD*, *NRAS*
	1/30/2007	CR1	—	—		—
	6/5/2007	Relapse1	ND	R378fsX450	36.44	*FLT3/ITD*, *NRAS*
	11/2/2007	CR2	ND	—		—
	12/3/2007	Relapse 2	t(8;11)(q24;p15),del(12)(p13),add(19)(p13), t(2;17)(q21;q25)	—	0	*FLT3/ITD*

UPN indicates unique patient number; CR, complete remission; ND, not done; NM, no mitosis; —, negative; and NA, not applicable because the mutations are point mutations that cannot be determined by GeneScan.

*Patients 1 through 31 had *WT1* mutations at diagnosis; patients 32 through 35 had no *WT1* mutations at diagnosis but acquired the mutation at relapse. The data of serial studies in another 107 patients, who did not have *WT1* mutation either at diagnosis or at relapse, are not shown in this table.

Table 5. Sequential studies of *WT1* mutations in AML patients (continued)

UPN*	Date	Status	Chromosomal abnormality	*WT1* mutation		Other genetic mutations
				Amino acid change	Mutant by gene scan, percentage	
29	1/2/2007	Initial	—	P369fsX384	11.38	*MLL/PTD*
	7/13/2007	CR1	—	—		—
	1/7/2008	Relapse1	—	P369fsX384	29.56	*MLL/PTD*
30	7/9/2007	Initial	+4, del(9)(q13q22),+10	Y402X	47.04	*CEBPA*
	8/21/2007	CR1	—	—		—
31	9/11/2007	Initial	—	S381X	NA	*FLT3/ITD*, *MLL/PTD*
	11/19/2007	CR1	—	—		—
32	12/4/2007	Initial	—	K399fsX448	28.70	*CEBPA*
	12/27/2007	CR1	—	—		—
	4/1/2008	Relapse1	—	K399fsX448	7.20	*CEBPA*
33	12/8/1996	Initial	—	—	0	
	1/21/1997	CR1	—	—		—
	9/11/2001	CR2	—	—		—
	6/10/2002	Relapse 2	ND	L381fsX452	11.09	*NRAS*, *NPM1*
34	7/27/2000	Initial	—	—	0	*NPM1*
	11/10/2000	CR1	—	—		—
	7/17/2001	Relapse1	—	D379fsX386	20.70	*NPM1*
	10/23/2001	CR2		—		—
	5/7/2002	Relapse 2	del(6)(p21)	D379fsX386	28.06	*NPM1*
35	4/18/2002	Initial	—	—	NA	*CEBPA*
	5/13/2002	CR1	—	—		—
	11/19/2002	Relapse1	t(1;3)(p36;q21),del(3)(p13p21)	R471T	NA	*CEBPA*
	12/16/2002	CR2	—	—		—

UPN indicates unique patient number; CR, complete remission; ND, not done; NM, no mitosis; —, negative; and NA, not applicable because the mutations are point mutations that cannot be determined by GeneScan.

*Patients 1 through 31 had *WT1* mutations at diagnosis; patients 32 through 35 had no *WT1* mutations at diagnosis but acquired the mutation at relapse. The data of serial studies in another 107 patients, who did not have *WT1* mutation either at diagnosis or at relapse, are not shown in this table.

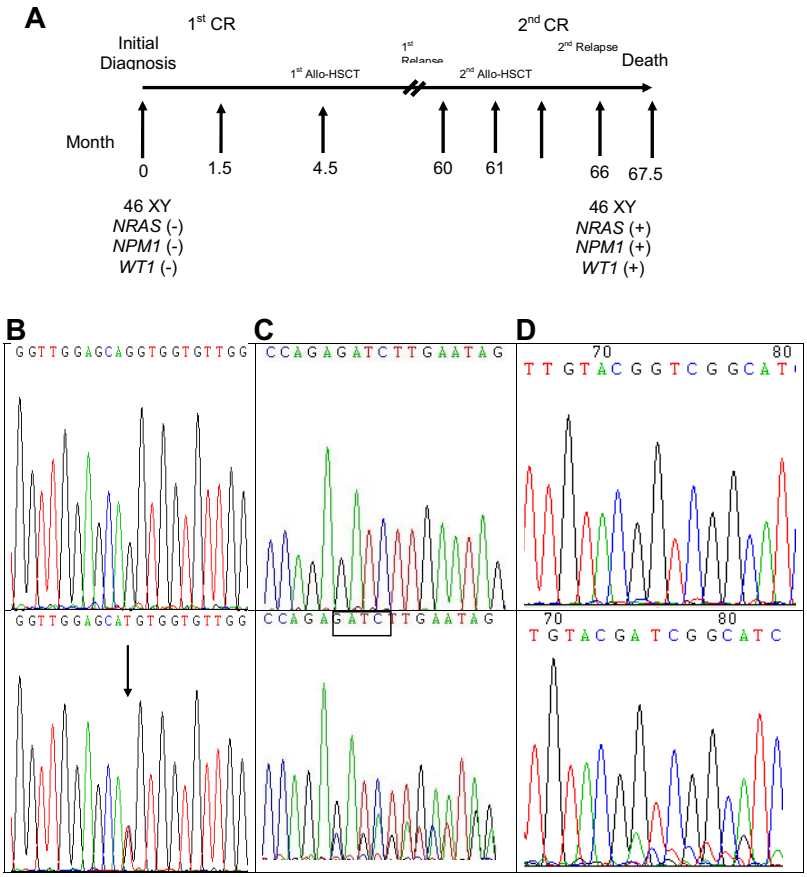

Figure 1. Sequential analyses of *NRAS*, *NPM1*, and *WT1* mutations in patient 33 showing acquisition of novel mutations in these 3 genes at relapse. (A) Sequential follow-up of mutation pattern at Patient 33. (B) Wild-type *NRAS* at diagnosis (top) and GGT>TGT at *NRAS* codon 12 at relapse (bottom). Arrows indicate the location of the mutation. (C) Wild-type *NPM1* at diagnosis (top); *NPM1* mutation with a TCTG insertion at relapse (bottom). The boxed tetranucleotides indicate the location of the mutation. (D) Wild-type *WT1* at diagnosis (top) and nt1335/1336 (+11 bp) on exon 7 at relapse (bottom).

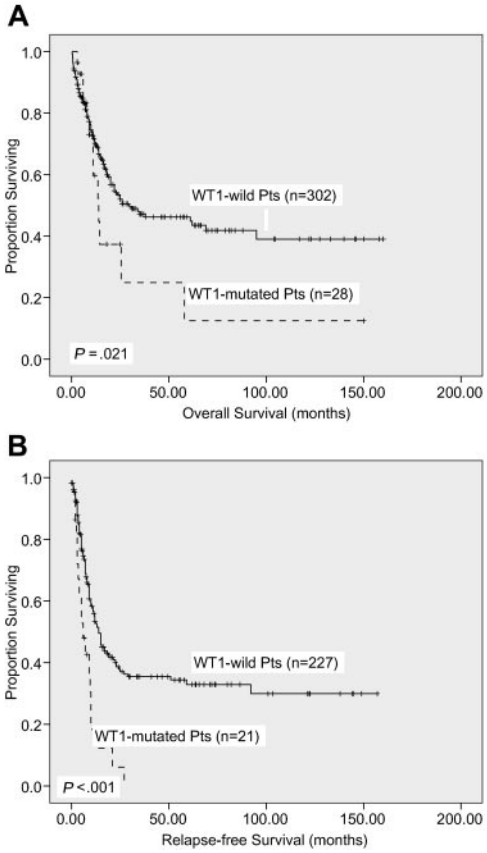

Figure 2. Kaplan-Meier survival curves. OS (A) and RFS (B) according to *WT1* mutation status in 330 AML patients receiving standard intensive chemotherapy.

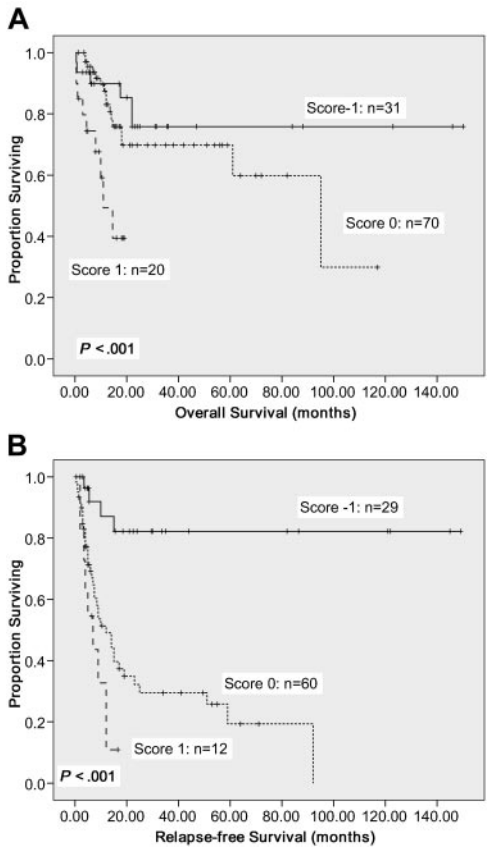

Figure 3. Kaplan-Meier survival curves. OS (A) and RFS (B) in 121 CN-AML patients based on scoring system ($P < .001$ for both OS and RFS). CN-AML patients were grouped according to scoring system based on 4 prognostic markers (*CEBPA*$^{double\text{-}mutation}$, *NPM1*/*FLT3-ITD*, *WT1* mutation, and age). A score of −1 was assigned for each parameter associated with a favorable outcome (*CEBPA*$^{double\text{-}mutation}$ and *NPM1*^{mut}/*FLT3-ITD*$^{-}$) and a score of 1 for each factor associated with an adverse outcome (*WT1* mutation and older age). The algebraic summation of these scores of each patient was the final score.

The percentage of *WT1* mutations in exons 7 and 9 in whole cohort in this study was 6.8%. Focused on 121 younger CN-AML, the percentage of *WT1* mutations was 8.3%, similar to larger cohort studies that analyzed exons 7 and 9 mutations in younger adults with CN-AML (8.5%–10.7%).[11-13] A slightly higher incidence (12.6%) of *WT1* mutations was reported by Gaidzik el al who analyzed all exons 1 to 10 but found the mutations were clustered in

Table 6. Multivariate analysis (Cox regression) on the RFS and OS

	RFS			OS		
Variable	**RR**	**95% CI**	***P***	**RR**	**95% CI**	***P***
Whole cohort (n = 330)						
Age†	1.334	0.929-1.915	.119	2.724	1.902-3.902	< .001*
WBC‡	1.377	0.941-2.014	.100	1.863	1.297-2.677	.001*
Karyotype§	1.851	1.017-3.368	.044*	2.684	1.626-4.432	< .001*
NPM1/FLT3-ITD‖	0.400	0.208-0.770	.006*	0.344	0.165-0.714	.004*
CEBPA¶	0.516	0.300-0.889	.017*	0.371	0.187-0.737	.005*
WT1	2.601	1.562-4.333	< .001*	2.141	1.188-3.859	.011*
Patients with normal karyotype and age < 60 y (n = 121)						
Age†	2.340	1.114-4.787	.020*	2.515	1.065-5.939	.035*
WBC‡	2.533	1.231-5.210	.012*	5.508	2.536-11.964	< .001*
NPM1/FLT3-ITD‖	0.151	0.059-0.387	< .001*	0.246	0.079-0.768	.016*
CEBPA¶	0.170	0.070-0.416	< .001*	0.146	0.045-0.475	.001*
WT1	3.806	1.588-9.122	.003*	3.752	1.195-11.783	.024*

RR indicates relative risk; and CI, confidence interval.
*Statistically significant ($P < .05$).
†Age more than 50 years relative to age less than or equal to 50 years (the reference).
‡WBC greater than 50 000/μL versus less than 50 000/μL.
§Unfavorable cytogenetics versus others.
‖*NPM1*^{mut}/*FLT3-ITD*$^{-}$ versus other subtypes.
¶*CEBPA*$^{double\text{-}mutation}$ versus others.

exons 7 and 9.[16] Hollink et al reported a high incidence (22%) of *WT1* mutations in pediatric CN-AML patients,[34] a finding compatible with the close association of *WT1* mutation with younger age[11,16] (and current study). Furthermore, they found that approximately half of *WT1*-mutated patients had double heterozygous mutations (15 of 35) or a homozygous mutation (2 of 35), in contrast to adults in whom most patients showed single heterozygous mutation[11-13,16] (and current study). Whether there is a true difference in *WT1* mutation pattern between childhood and adult AML and its significance needs to be clarified by further studies.

To the best of our knowledge, this study recruited the largest number of de novo adult AML patients for sequential analysis of *WT1* mutations during clinical follow-ups. All 23 *WT1*-mutated patients studied lost their *WT1* mutations at remission status, suggesting that these mutations were somatically acquired in the leukemogenesis. In contrast to *NPM1* mutations, which are quite stable during disease progression,[19] *WT1* mutation status may change at relapse. Three of the 16 patients with *WT1* mutations at diagnosis lost the mutations at relapse, whereas 2 others acquired additional mutations. On the other side, among the 110 patients who had no *WT1* mutation at diagnosis, 3 acquired a novel *WT1* mutation at relapse. These results were confirmed by reexamining the paired samples with GeneScan, a technique capable of detecting mutant DNA down to 5% of total DNA.[26] This phenomenon cast doubts on the appropriateness of mutated *WT1* as a surrogate marker in monitoring minimal residual disease. Only one report addressed the stability of *WT1* mutations in the literature[34]; they found that 4 of the 28 *WT1*-wild patients gained *WT1* mutations at relapse, but none of the 11 WT1-mutated patients lost the mutations. *WT1* mutations may play a role in the disease progression in some patients.

By multivariate analysis using the Cox regression model, we demonstrated that *WT1* mutation and older age were independent poor prognostic factors, whereas $CEBPA^{double\text{-}mutation}$ and $NPM1^{mut}/FLT3\text{-}ITD^{-}$ were good prognostic factors in both total cohort and CN-AML patients. Based on these results, a survival scoring system incorporated these 3 molecular markers and age into survival analysis was designed to better stratify CN-AML patients into different risk groups. Indeed, this scoring system was more powerful than single marker to separate patients into different prognostic groups; and unlike the sophisticated gene-expression profiling, it could be easily implemented in routine clinical laboratories.

In conclusion, this study demonstrated that *WT1* mutations occurred with similar frequencies in patients with normal karyotype and those with abnormal cytogenetics. The mutation was closely associated with younger age, FAB M6 subtype, and t(7;11)(p15;15), but inversely related to M0 subtype. Furthermore, the *WT1* 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 the instability of *WT1* mutations during disease progression. One should be cautious to use this mutation as a marker for minimal residual disease monitoring. Incorporation of the 3 gene mutations, including *NPM1/FLT3-ITD,* $CEBPA^{double\text{-}mutation}$, and *WT1*, and age at diagnosis, which are closely associated with prognosis, into survival analyses can better stratify patients into different risk groups.

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Authorship

Contribution: H.-A.H. was responsible for literature collection, data management and interpretation, and manuscript writing; T.-C.H. was responsible for data management and manuscript writing; L.-I.L. was responsible for mutation analysis and interpretation; C.-Y.L. was responsible for statistical analysis and interpretation of the statistical findings; 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. Chen contributed patient samples and clinical data; M.-H.T., C.-F.H., Y.-C. Chiang, F.-Y.L., and M.-C.L. performed the gene mutation and chromosomal studies; and H.-F.T. planned, designed, and wrote the manuscript and coordinated the study over the entire period.

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References

1. Buckler AJ, Pelletier J, Haber DA, Glaser T, Housman DE. Isolation, characterization, and expression of the murine Wilms' tumor gene (WT1) during kidney development. *Mol Cell Biol.* 1991;11(3):1707-1712.
2. Baird PN, Simmons PJ. Expression of the Wilms' tumor gene (WT1) in normal hemopoiesis. *Exp Hematol.* 1997;25(4):312-320.
3. Ellisen LW, Carlesso N, Cheng T, Scadden DT, Haber DA. The Wilms tumor suppressor WT1 directs stage-specific quiescence and differentiation of human hematopoietic progenitor cells. *EMBO J.* 2001;20(8):1897-1909.
4. Haber DA, Buckler AJ, Glaser T, et al. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. *Cell.* 1990;61(7):1257-1269.
5. Miwa H, Beran M, Saunders GF. Expression of the Wilms' tumor gene (WT1) in human leukemias. *Leukemia.* 1992;6(5):405-409.
6. King-Underwood L, Renshaw J, Pritchard-Jones K. Mutations in the Wilms' tumor gene WT1 in leukemias. *Blood.* 1996 15;87(6):2171-2179.
7. Yang L, Han Y, Suarez Saiz F, Minden MD. A tumor suppressor and oncogene: the WT1 story. *Leukemia.* 2007;21(5):868-876.
8. Ariyaratana S, Loeb DM. The role of the Wilms tumour gene (WT1) in normal and malignant haematopoiesis. *Expert Rev Mol Med.* 2007;9(14):1-17.
9. King-Underwood L, Pritchard-Jones K. Wilms' tumor (WT1) gene mutations occur mainly in acute myeloid leukemia and may confer drug resistance. *Blood.* 1998;91(8):2961-2968.
10. Renneville A, Roumier C, Biggio V, et al. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. *Leukemia.* 2008;22(5):915-931.
11. Renneville A, Boissel N, Zurawski V, et al. Wilms tumor 1 gene mutations are associated with a higher risk of recurrence in young adults with acute myeloid leukemia: a study from the Acute Leukemia French Association. *Cancer.* 2009;115(16):3719-3727.
12. Virappane P, Gale R, Hills R, et al. Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. *J Clin Oncol.* 2008;26(33):5429-5435.
13. Paschka P, Marcucci G, Ruppert AS, et al. Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol.* 2008;26(28):4595-4602.
14. Summers K, Stevens J, Kakkas I, et al. Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. *Leukemia.* 2007;21(3):550-551.
15. Santamaria CM, Chillon MC, Garcia-Sanz R, et al. Molecular stratification model for prognosis in cytogenetically normal acute myeloid leukemia. *Blood.* 2009;114(1):148-152.

16. Gaidzik VI, Schlenk RF, Moschny S, et al. Prognostic impact of WT1 mutations in cytogenetically normal acute myeloid leukemia: a study of the German-Austrian AML Study Group. *Blood.* 2009; 113(19):4505-4511.
17. Mrozek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? *Blood.* 2007;109(2):431-448.
18. Tien HF, Wang CH, Chuang SM, et al. Cytogenetic studies, ras mutation, and clinical characteristics in primary myelodysplastic syndrome: a study on 68 Chinese patients in Taiwan. *Cancer Genet Cytogenet.* 1994;74(1):40-49.
19. Chou WC, Tang JL, Lin LI, et al. Nucleophosmin mutations in de novo acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. *Cancer Res.* 2006;66(6): 3310-3316.
20. Lin LI, Chen CY, Lin DT, et al. Characterization of CEBPA mutations in acute myeloid leukemia: most patients with CEBPA mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. *Clin Cancer Res.* 2005;11(4):1372-1379.
21. Chen CY, Lin LI, Tang JL, et al. Acquisition of JAK2, PTPN11, and RAS mutations during disease progression in primary myelodysplastic syndrome. *Leukemia.* 2006;20(6):1155-1158.
22. Chen CY, Lin LI, Tang JL, et al. RUNX1 gene mutation in primary myelodysplastic syndrome: the mutation can be detected early at diagnosis or acquired during disease progression and is associated with poor outcome. *Br J Haematol.* 2007; 139(3):405-414.
23. Shiah HS, Kuo YY, Tang JL, et al. Clinical and biological implications of partial tandem duplication of the MLL gene in acute myeloid leukemia without chromosomal abnormalities at 11q23. *Leukemia.* 2002;16(2):196-202.
24. Tang JL, Hou HA, Chen CY, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. *Blood.* 2009;114(26):5352-5361.
25. Hou HA, Chou WC, Lin LI, et al. Characterization of acute myeloid leukemia with PTPN11 mutation: the mutation is closely associated with NPM1 mutation but inversely related to FLT3/ITD. *Leukemia.* 2008;22(5):1075-1078.
26. Lin LI, Lin TC, Chou WC, Tang JL, Lin DT, Tien HF. A novel fluorescence-based multiplex PCR assay for rapid simultaneous detection of CEBPA mutations and NPM mutations in patients with acute myeloid leukemias. *Leukemia.* 2006; 20(10):1899-1903.
27. Borrow J, Shearman AM, Stanton VP Jr, et al. The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. *Nat Genet.* 1996;12(2):159-167.
28. Nakamura T, Largaespada DA, Lee MP, et al. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15; p15) in human myeloid leukaemia. *Nat Genet.* 1996;12(2):154-158.
29. Kelly LM, Gilliland DG. Genetics of myeloid leukemias. *Annu Rev Genomics Hum Genet.* 2002;3: 179-198.
30. Hou HA, Lin LI, Chen CY, Tien HF. Reply to "Heterogeneity within AML with CEBPA mutations: only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis." *Br J Cancer.* 2009;101(4):738-740.
31. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations: only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. *Br J Cancer.* 2009;100(8):1343-1346.
32. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood.* 2008;112(12):4639-4645.
33. Miyagawa K, Hayashi Y, Fukuda T, Mitani K, Hirai H, Kamiya K. Mutations of the WT1 gene in childhood nonlymphoid hematological malignancies. *Genes Chromosomes Cancer.* 1999;25(2): 176-183.
34. Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, et al. Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. *Blood.* 2009;113(23):5951-5960.
35. Huang SY, Tang JL, Liang YJ, Wang CH, Chen YC, Tien HF. Clinical, haematological and molecular studies in patients with chromosome translocation t(7;11): a study of four Chinese patients in Taiwan. *Br J Haematol.* 1997;96(4):682-687.
36. Chou WC, Chen CY, Hou HA, et al. Acute myeloid leukemia bearing t(7;11)(p15;p15) is a distinct cytogenetic entity with poor outcome and a distinct mutation profile: comparative analysis of 493 adult patients. *Leukemia.* 2009;23(7):1303-1310.
37. Nyvold CG, Stentoft J, Braendstrup K, et al. Wilms' tumor 1 mutation accumulated during therapy in acute myeloid leukemia: biological and clinical implications. *Leukemia.* 2006;20(11): 2051-2054.
38. Kirschner KM, Hagen P, Hussels CS, Ballmaier M, Scholz H, Dame C. The Wilms' tumor suppressor Wt1 activates transcription of the erythropoietin receptor in hematopoietic progenitor cells. *FASEB J.* 2008;22(8):2690-2701.