TET2 mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics

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The studies concerning clinical implications of *TET2* mutation in patients with primary acute myeloid leukemia (AML) are scarce. We analyzed *TET2* mutation in 486 adult patients with primary AML. *TET2* mutation occurred in 13.2% of our patients and was closely associated with older age, higher white blood cell and blast counts, lower platelet numbers, normal karyotype, intermediate-risk cytogenetics, isolated trisomy 8, *NPM1* mutation, and *ASXL1* mutation but mutually exclusive with *IDH* mutation. *TET2* mutation is an unfavorable prognostic factor in patients with intermediate-risk cytogenetics, and its negative impact was further enhanced when the mutation was combined with *FLT3*-ITD, *NPM1*-wild, or unfavorable genotypes (other than *NPM1*$^+$/*FLT3*-ITD$^-$ or *CEBPA*$^+$). A scoring system integrating *TET2* mutation with *FLT3*-ITD, *NPM1*, and *CEBPA* mutations could well separate AML patients with intermediate-risk cytogenetics into 4 groups with different prognoses ($P < .0001$). Sequential analysis revealed that *TET2* mutation detected at diagnosis was frequently lost at relapse; rarely, the mutation was acquired at relapse in those without *TET2* mutation at diagnosis. In conclusion, *TET2* mutation is associated with poor prognosis in AML patients with intermediate-risk cytogenetics, especially when it is combined with other adverse molecular markers. *TET2* mutation appeared to be unstable during disease evolution. (*Blood*. 2011;118(14): 3803-3810)

Introduction

Mutations in *Ten-Elevan-Translocation-2* (*TET2*) were first discovered in myeloid malignancies by high-resolution single nucleotide polymorphism (SNP) and comparative genomic hybridization arrays.[1] Subsequent studies using the same methods, direct sequencing, or next-generation sequencing confirmed that mutations in this gene were prevalent in myelodysplastic syndrome, myelodysplastic syndrome/myeloproliferative neoplasms, myeloproliferative neoplasms, and secondary acute myeloid leukemia (AML), with frequencies ~ 10%-26%, 22%-58%, 7%-13%, and 24%-32%, respectively.[2-14] The studies concerning clinical implications of *TET2* mutation in patients with primary AML are scarce. There are several unresolved issues relating to *TET2* mutations in primary AML. First, the association of *TET2* mutations with other genetic alterations has not been fully addressed. Whereas one study showed positive association of *TET2* mutation with *NPM1* mutation in AML patients achieving complete remission (CR),[15] other reports did not find such correlation.[16,17] Second, the prognostic significance of *TET2* mutation in AML is still controversial. Nibourel et al[15] did not find any prognostic impact of *TET2* mutation in primary AML achieving CR, whereas another study suggested an unfavorable effect of this mutation in primary cytogenetically normal AML patients bearing favorable genotypes (mutated *NPM1* without *FLT3*-ITD or *CEBPA* mutation, *NPM1*$^+$/*FLT3*-ITD$^-$ or *CEBPA*$^+$).[17] Third, the stability of *TET2* mutations during disease evolution in AML remains unknown.

To clarify these points, we analyzed *TET2* mutation in a cohort of 486 adult patients with de novo AML and correlated the result with clinical and biologic features and the status of 15 other important genetic mutations. Sequential studies were performed on 122 patients to investigate the serial changes of this mutation as the disease goes into CR and relapse. We found that the *TET2* mutation was associated with several distinct clinical features not reported so far. In AML patients bearing intermediate-risk cytogenetics according to Southwest Oncology Group (SWOG) criteria,[18] this mutation appears to be a poor prognostic factor for overall survival (OS). Importantly, the negative impact of *TET2* mutation in this group of AML patients was significantly enhanced by combination with other unfavorable factors, including the presence of *FLT3*-ITD, absence of *NPM1* mutation, or presence of molecular markers other than *NPM1*$^+$/*FLT3*-ITD$^-$ or *CEBPA*$^+$. A scoring system incorporating *TET2* mutation with *FLT3*-ITD, *NPM1*, and *CEBPA* double mutations (*CEBPA*^{double}) into survival analysis proved very useful to stratify AML patients with intermediate-risk cytogenetics into different prognostic groups ($P < .0001$). *TET2* mutation

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detected at diagnosis frequently disappeared at relapse. Infrequently, this mutation was acquired at disease relapse in patients without mutation in this gene at diagnosis.

Methods

Patients

A total of 486 consecutive adult patients (≥ 15 years of age) with newly diagnosed de novo AML from 1995 to 2007 at the National Taiwan University Hospital who had adequate cryopreserved bone marrow cells for complete mutation analyses were recruited. Written informed consent in accordance with the Declaration of Helsinki was obtained from all participants, and the study was approved by the Institutional Review Board of the National Taiwan University Hospital.

Among these 486 primary AML patients, 343 (70.6%) received standard intensive chemotherapy as described previously.[19] The remaining 143 patients (139 with non-M3 AML and 4 with acute promyelocytic leukemia) received palliative care or low-dose chemotherapy because of poor performance status or per patients' wish.

Mutation analyses

Mutation analyses for *TET2* (NM_001127208) were performed by PCR largely as previously described but with mild modification.[1] The primer sequences and estimated product lengths were listed in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Mutations on *CEBPA*,[20] *WT1*,[21] *MLL*-PTD,[22] *PTPN11*,[23,24] *RUNX1*,[25] c-*KIT*,[26] *RAS*,[27] *FLT3*-TKD,[28] *IDH*,[29,30] *IDH2*,[31] *ASXL1*,[19] *NPM1*,[32] and *FLT3*-ITD[33,34] were performed as reported. To detect *TET2* mutation at diagnosis, we used DNA amplified in vitro from patients' bone marrow cells by Illustra GenomiPhi V2 DNA amplification kit as described by the manufacturer (GE Healthcare). All the mutations detected in such samples were verified in the original nonamplified samples. All the nucleotide alterations causing premature truncation of the TET2 proteins (non-sense or frame shifting) were regarded as true mutations. Missense mutations were regarded as true only if they were documented in other papers or could be verified in somatic tissue or remission marrow samples in our patients. The patients bearing other missense mutations not known to be somatic were censored from this study.

Cytogenetic analyses and immunophenotyping

Cytogenetic analyses and immunophenotyping were performed as previously described.[35,36]

Statistics

The discrete variables of patients with and without *TET2* mutation were compared using the χ^2 tests or Fisher exact test. Mann-Whitney tests were used to compare continuous variables and medians of distributions. OS was measured from the date of first diagnosis to death from any cause, and relapse-free survival was calculated from the time of CR until relapse, death, or end of study. Kaplan-Meier survival curves and log-rank test were used for estimation of survival and difference between groups. Multivariate Cox proportional hazard regression analysis was used to evaluate independent prognostic factors for survival. All statistical analyses were performed using XLSTAT statistical analysis software (edition 2010 Version 5.02, Addinsoft). Whole patient population (N = 486) was included for analyses of the correlation between *TET2* mutation and clinical characteristics, but only those 343 patients who received standard chemotherapy were included in analyses of survivals as previously described.[19,21,25] In patients who had received allogeneic stem cell transplantation, OS was censored on the date of stem cell infusion. Bootstrapping for Kaplan-Meier estimation was conducted using SAS Version 9.0.

Results

Correlation of *TET2* mutations with clinical features and laboratory data

A total of 486 patients, consisting of 212 females and 274 males with a median age of 51.5 years (range, 15-90 years) were recruited for *TET2* mutation analysis. Seventy-six different *TET2* mutations were detected in 64 patients (13.2%). Double mutations of *TET2* were detected in 27 patients (27 of 64, 42.2%), and 6 of them had homozygous *TET2* mutation. There were 27 non-sense, 33 frameshift, and 15 missense mutations, distributing across the whole coding sequence without obvious hot spots, some occurring in more than one patient (Figure 1). Some nucleotide variations were not regarded as true missense mutations: P29R, I1762V, and V218M were documented as SNPs in dbSNP (http://www.ncbi.nlm.nih.gov/snp/); P363L, L1721W, and H1778R were reported previously as SNPs[15]; and R814C, F868L, S1039L, E1513G, L1248T (resulted from in-frame deletion c.3742_3750del), and R1543P (resulted from in-frame deletion c.4627_4644del) were obviously retained in remission samples. The significance of some missense mutations, including C1374Y, H1219N, P1889H, H1868D, L1322Q, and L1326S, remained unknown, and these were thus censored from the analyses of this study. The detailed information of the 64 patients with *TET2* mutations is listed in supplemental Table 2. Patients with *TET2* mutation were significantly older than those without this mutation (68 years vs 48 years, $P < .001$); in patients older than 60 years, approximately one-fourth (24.5%, 42 of 172) had *TET2* mutation, in contrast to only 7% in younger patients (60 years of age or younger; Table 1). Patients with *TET2* mutations had significantly higher WBC count, higher peripheral blast count, higher serum lactate dehydrogenase level, and lower platelet count at diagnosis than those without *TET2* mutations, but the levels of hemoglobin were not different (Table 1). *TET2* mutations were not seen in patients with acute promyelocytic leukemia (Table 1). Leukemia cells with *TET2* mutations had higher frequency of CD14 and CD56 expression (supplemental Table 3). There were no differences in various clinical and laboratory characteristics and survival between patients with a single and those with double mutations (data not shown).

Correlation of *TET2* mutation with karyotype and other genetic alterations

Chromosome data were available in 467 patients (Table 1). *TET2* mutation occurred more frequently in patients with intermediate-risk cytogenetics according to SWOG criteria[18] (18.5% vs 7.1%, $P < .001$), normal karyotype (18.0% vs 9.2%, $P = .0064$), or isolated trisomy 8 (35.3% vs 12.6%, $P = .017$; Table 1). *TET2* mutation was mutually exclusive with *IDH* mutations ($P < .001$) but had positive association with *ASXL1* mutation ($P = .002$) and *NPM1* mutation ($P = .047$; Table 2).

Survival analysis

TET2-mutated patients showed only a trend of shorter OS in all 343 patients who received standard intensive chemotherapy (median, 61.1 vs 22.2 months, $P = .107$; Figure 2A). Subgroup analysis showed that, among 310 non-M3 AML patients, *TET2* mutation did not have a significant impact on OS or relapse-free survival (Figure 2B; and data not shown). However, *TET2* mutations were associated with shorter OS in the patients with intermediate-risk cytogenetics (N = 171)

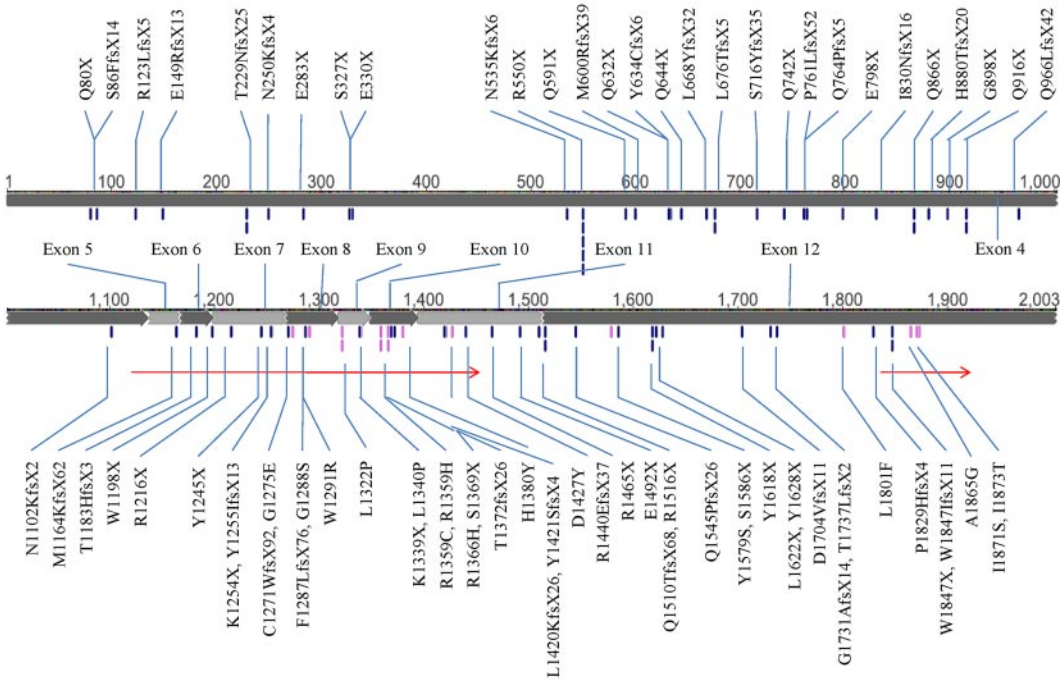

Figure 1. The mutations of *TET2*. The black ticks and gray ticks represent nonsense/frameshift and missense mutations, respectively. The 2 arrows indicate conserved regions (amino acids 1134-1444 and 1842-1921). Some mutations occurred in > 1 patient.

and a trend of shorter OS in those with a normal karyotype (N = 158, median not reached [NR] vs 14.7 months, $P = .021$ and NR vs 22.1 months, $P = .076$, respectively; Figure 2C-D). There was no difference in induction-related mortality, CR rate, or relapse-free survival between *TET2*-mutated patients and *TET2*-wild patients in any subgroup (data not shown).

Univariate analyses in patients with intermediate-risk cytogenetics (N = 171) showed that *TET2* mutation, as well as older age, higher WBC count, and mutations of *RUNX1* and *ASXL1*, was an unfavorable prognostic factor for OS, whereas *CEBPA*^{double} was a good prognostic factor and mutation of *NPM1* without *FLT3*-ITD (*NPM1*$^+$/*FLT3*-ITD$^-$) had a trend to be a favorable factor for OS (Table 3). In multivariate analysis, only 3 factors remained to be independent prognostic factors for OS: WBC count, *NPM1*$^+$/*FLT3*-ITD$^-$, and *CEBPA*^{double}; old age and *TET2* mutation had a trend toward poor OS (Table 4). *TET* mutation was not an independent prognostic factor for OS in patients with a normal karyotype, either, probably because of smaller patient number. We also analyzed the effects of 3 common SNPs in *TET2,* including P29R (N = 168), I1762V (N = 164), and V218M (N = 66) on OS but could not find any significant prognostic impact of these SNP variants (data not shown).

The prognostic values of *TET2* mutation in context with other mutations for patients with intermediate-risk cytogenetics

When the patients with intermediate-risk cytogenetics were stratified by the status of *TET2* mutation and *FLT3*-ITD, those with *TET2* wild-type and *FLT3*-ITD wild-type (*TET2*$^-$/*FLT3*-ITD$^-$) had the longest OS, those with *TET2* mutation and *FLT3*-ITD (*TET2*$^+$/*FLT3*-ITD$^+$) had the shortest OS, and others (*TET2*$^-$/*FLT3*-ITD$^+$ and *TET2*$^+$/*FLT3*-ITD$^-$) had OS in between (NR vs 5.0 vs 16.9 months, $P < .0001$; Figure 3A). In addition, the negative impact of *TET2*$^+$/*FLT3*-ITD$^+$ genotype on OS appeared to be independent of age, WBC count, and *CEBPA*^{double} and *NPM1* mutation (hazard ratio = 3.84; 95% CI, 1.46-10.69; $P = .007$). *TET2* mutation combined with absence of *NPM1* mutation (*NPM1*$^-$/*TET2*$^+$) also showed a strong trend of shorter OS compared with other genotypes (median 12.3 vs 61 months, $P = .055$; Figure 3B), and the negative effect was also independent of age, WBC count, and *CEBPA*^{double} (hazard ratio = 2.25; 95% CI, 1.05-4.83; $P = .037$). Although among patients with favorable molecular markers (*NPM1*$^+$/*FLT3*-ITD$^-$ or *CEBPA*^{double}), *TET2* mutation did not have significant impact on OS (Figure 3C), those without these favorable genotypes fared much worse if they also harbored *TET2* mutation (median 7 vs 20 months, $P = .004$; Figure 3D).

To further explore the prognostic impact of *TET2* mutation in primary AML patients with intermediate-risk cytogenetics, a scoring system was generated based on the status of *TET2* mutation and 3 other genetic alterations (*NPM1* mutation, *CEBPA*^{double} mutation, and *FLT3*-ITD), which were all well-documented prognostic factors in AML. The good prognostic mutations (*NPM1* mutation and *CEBPA*^{double}) were scored +1 point each, and poor prognostic mutations (*FLT3*-ITD and *TET2* mutation) −1 point each. All the scores were summated as the final score for each patient. This scoring system could well separate AML patients with intermediate cytogenetics into 4 groups of distinct prognosis; the median survival was NR (N = 46), 58 months (N = 92), 11 months (N = 31), and 4.3 months (N = 2) in patients with a score of +1, 0, −1, and −2 points, respectively ($P < .0001$, Figure 4). We validated this algorithm by carrying out 100 times bootstrapping for this Kaplan-Meier estimation with SAS Version 9.0. The results confirmed that this scoring system was reliable to predict the prognosis of primary AML patients with intermediate-risk cytogenetics (supplemental Table 4). These results suggested a close interaction among *TET2* mutation and other mutations in affecting patients' prognosis.

Sequential mutation analyses of *TET2*

Sequential analysis of *TET2* mutation was performed in 122 patients: 23 with *TET2* mutation at diagnosis and 99 without. Among the 23 *TET2*-mutated patients who had ever obtained a CR and had

Table 1. Correlation of *TET2* mutations with clinical data, FAB subtypes, and chromosomal abnormalities in AML patients (n = 486)

Variant	Total (N = 486)	*TET2* mutation (N = 64, 13.2%)	*TET2* wild-type (N = 422, 86.8%)	*P*
Median age, y (range)	51.5 (15-90)	68 (21-90)	48 (15-89)	< .001
Age in groups, y				< .001
15-20	23	0	23 (100)	
21-30	57	3 (5.3)	54 (94.7)	
31-40	78	7 (9.0)	71 (91.0)	
41-50	78	4 (5.1)	74 (94.9)	
51-60	78	8 (10.3)	70 (89.7)	
61-70	78	15 (19.2)	63 (80.8)	
71-80	69	17 (24.6)	52 (75.4)	
> 80	25	10 (40.0)	15 (60.0)	
Sex				.136
Female	212	22 (10.4)	190 (89.6)	
Male	274	42 (15.3)	232 (84.7)	
Median laboratory data (range)				
WBC, $\times 10^3/\mu L$	17.91 (0.12-627.8)	43.16 (1.68-277.25)	15.44 (0.12-627.8)	< .001
Blasts, $\times 10^3/\mu L$	7.33 (0-456.73)	26.53 (0-260.62)	5.80 (0-456.73)	< .001
Hemoglobin, g/dL	8.0 (2.9-16.2)	8.1 (3.5-16.2)	8.0 (2.9-13.9)	.196
Platelets, $\times 10^3/\mu L$	43 (3-802)	33 (6-157)	45 (3-802)	.017
LDH, U/L	855.5 (206-13 130)	1248 (365-7930)	823 (206-13 130)	.004
FAB				
M0	10	0	10 (100)	.371
M1	108	13 (12.0)	95 (88.0)	.749
M2	168	28 (16.7)	140 (83.3)	.169
M3	38	0	38 (100)	.005
M4	121	20 (16.5)	101 (83.5)	.290
M5	21	3 (14.3)	18 (85.7)	.748
M6	11	0	11 (100)	.375
Undetermined	9	0	9 (100)	
Karyotype* SWOG				
Favorable	96	5 (5.2)	91 (94.8)	.007
Intermediate	244	45 (18.5)	198 (81.5)	< .001
Unfavorable	128	11 (8.6)	117 (91.4)	.091
Normal karyotype	218	39 (18.0)	178 (82.0)	.006
Isolated trisomy 8	17	6 (35.3)	11 (64.7)	.017

FAB indicates French-American-British; and LDH, lactate dehydrogenase.
*Only 468 patients had available cytogenetic data at diagnosis (62 *TET2*-muted patients and 406 *TET2*-wild patients).

available samples for study, the initial *TET2* mutation disappeared at CR in 18 patients, but 5 (patients 2, 4, 28, 40, and 43) retained it (supplemental Table 5); all these 5 patients relapsed finally, suggesting the presence of residual leukemia cells. The mutations in 4 of these 5 patients were frameshift or non-sense mutations and the remaining one (patient 28) was missense mutation in the conserved region and the skin tissue from this patient was devoid of this mutation, so they were all significant mutations but not somatic changes. Ten of 23 patients remained in continuous CR at the time of this study, and none had detectable *TET2* mutation after achieving CR. Eight of them had concurrent other genetic changes at diagnosis, which also disappeared in CR. Interestingly, among the 13 patients who got relapse, 6 patients (patients 2, 4, 19, 20, 47, and 58) lost the original *TET2* mutations at relapse by sequencing. Patient 4 retained the original *TET2* mutation at the first relapse, but the mutation was no more detectable at the second relapse; patient 2 gained a novel mutation in *TET2* (c.2366_2367insG, p.N789KfsX13) but lost the original one (c.1799delT, p.M600RfsX39). Because direct sequencing might not be sensitive enough to detect the low level of *TET2* mutation signal, we therefore sequenced 20 TA clones of the PCR product from these 6 patients and searched for any mutant clone. All the relapsed samples from these patients were devoid of mutant clone, except for the one from patient 47, in which 1 mutant clone of 20 was detected. To further explore whether low amounts of *TET2* mutants, undetectable by direct sequencing or TA cloning, existed in these 5 patients, we set out allele-specific PCR, with sensitivities ranging from 1 of 625 to 1 of 125 (data not shown), to test the relapsed DNA from these 5 patients (supplemental Table 6). We found that 2 (patients 2 and 20) of these 5 patients still retained the *TET2* mutant by this sensitive method; however, the other 3 were

Table 2. Correlation of *TET2* mutation with other gene alterations

Mutation	Total (n = 486)	*TET2*-mutated (n = 64)	*TET2*-wild (n = 422)	*P*
NPM1	102 (21.0)	20 (31.3)	82 (19.4)	.047
FLT3-ITD	113 (23.3)	14 (21.9)	99 (23.5)	.847
NPM1$^+$/*FLT3*-ITD$^-$	50 (10.3)	10 (15.6)	40 (9.5)	.181
CEBPA^{double}	45 (9.3)	8 (12.5)	37 (8.8)	.353
WT1	32 (6.6)	2 (3.1)	30 (7.1)	.291
RUNX1	58 (11.9)	8 (12.5)	50 (11.8)	.837
IDH	80 (16.5)	1 (1.6)	79 (18.7)	< .001
FLT3-TKD	40 (8.2)	4 (6.3)	36 (8.5)	.806
MLL-PTD	25 (5.1)	2 (3.1)	23 (5.5)	.759
KIT	14 (2.9)	0	14 (3.3)	.233
NRAS	56 (11.5)	6 (9.4)	50 (11.8)	.678
KRAS	17 (3.5)	1 (1.6)	16 (3.8)	.712
ASXL1	53 (10.9)	15 (23.4)	38 (9)	.002

Values are no. (%) of patients.

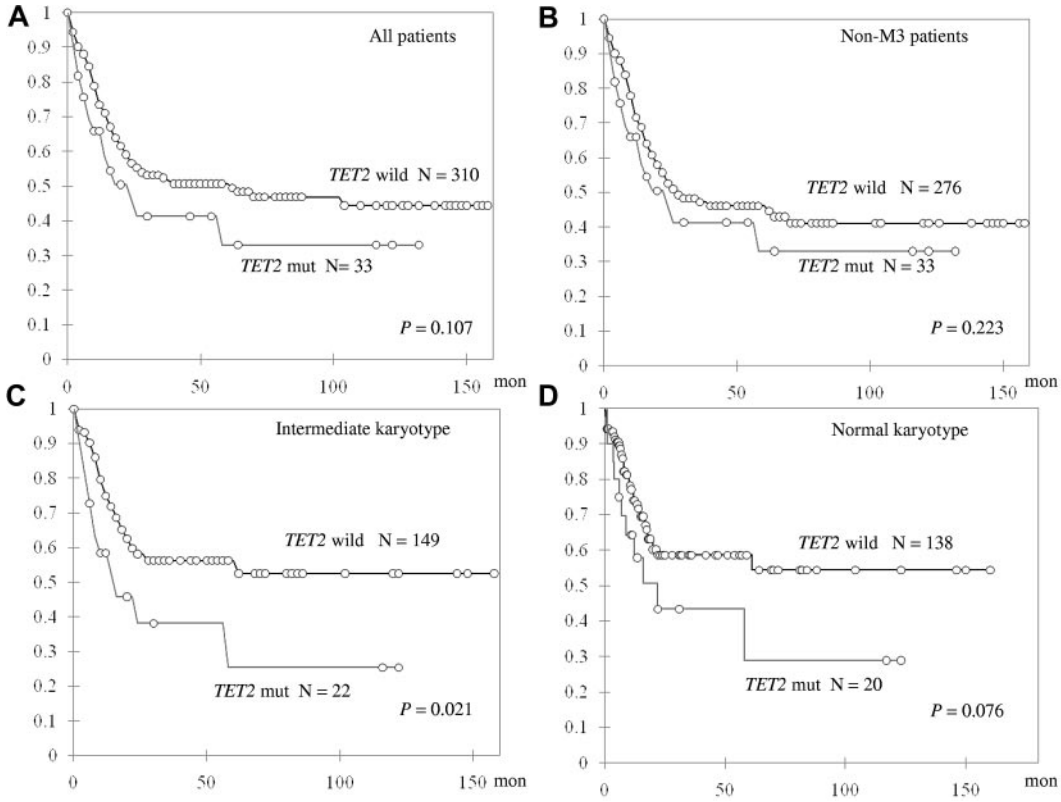

Figure 2. Kaplan-Meier curves for OS stratified by the status of *TET2* mutations in different subgroups of patients.

still devoid of the original *TET2* mutations, even by this PCR assay. We also analyzed *TET2* mutation in the relapsed samples from 99 patients without this mutation at diagnosis; only 1 of them gained *TET2* mutation at relapse (data not shown). These findings suggested that *TET2* mutation was not stable as the disease evolved.

Table 3. Univariate analysis of the impact of clinical parameters and molecular alterations on OS in AML patients with intermediate-risk karyotype (N = 171)

Variable	OS, mo, median ± SD	*P*
Age, y		< .001
≤ 60	NR	
> 60	12.3 ± 4.6	
WBC		.001
≤ 50 000/μL	NR	
> 50 000/μL	14.5 ± 3.6	
***NPM1*⁺/*FLT3*-ITD⁻**		.080
Yes	NR	
Others	61.0	
CEBPA		.010
Double mutation	NR	
Others	22.0 ± 16.7	
RUNX1		.015
Mutated	14.5 ± 3.8	
Wild	NR	
TET2		.021
Mutated	14.7 ± 7.8	
Wild	NR	
ASXL1		.014
Mutated	10.0 ± 3.2	
Wild	NR	

Only variables with $P < 0.1$ are shown. *KRAS, NRAS, WT1, MLL-PTD, FLT3-TKD, cKIT*, and *IDH1* mutations, and many other clinical parameters, such as hemoglobin, platelet counts, and sex, were not significantly associated with survival ($P > 0.1$) in this group of patients with intermediate-risk karyotype, and so were not listed in the table.

Discussion

This study recruits a large cohort of adults with de novo AML for *TET2* mutation for analysis. It reveals several distinct clinical and biologic features not reported before in patients with this gene mutation.

TET2 protein functions as an enzyme catalyzing conversion of methylcytosine to hydroxymethylcytosine, with ferrous iron and α-ketoglutarate as cofactors.[37,38] The mutation pattern in our patients indicated that *TET2* mutation was a loss-of-function mutation. Other reports that showed uniparental disomy or loss of corresponding chromosomal region of *TET2* gene also provide strong evidence for this hypothesis.[1-3,39] However, the findings that most *TET2*-mutated patients had single allele mutation and there was no difference in clinical characteristics between patients with single and those with double mutations of *TET2* in this study

Table 4. Multivariate analysis (Cox regression) for the OS in AML patients with intermediate-risk karyotype (N = 171)

Variable	Hazard ratio	95% CI	*P*
Age*	1.692	0.967-2.961	.065
WBC†	2.216	1.296-3.787	.004
NPM1⁺/*FLT*-ITD⁻	0.335	0.140-0.798	.014
CEBPA^{double}	0.285	0.119-0.685	.005
TET2	1.804	0.934-3.484	.079

*Age older than 60 years versus age 60 years or younger.
†WBC > 50 × 10^3/μL versus ≤ 50 × 10^3/μL.

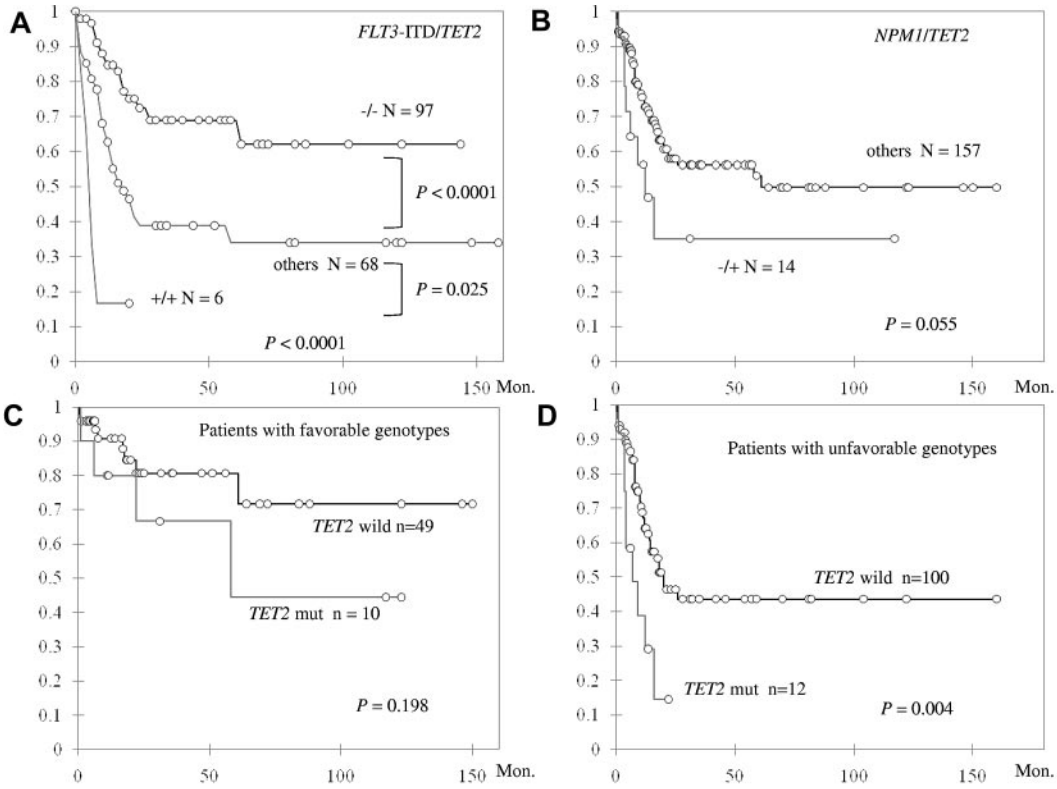

Figure 3. Kaplan-Meier curves for OS in 172 patients with intermediate-risk cytogenetics stratified by status. (A) *TET2* mutation and *FLT3*-ITD. (B) *TET2* and *NPM1* mutations. The OS was also stratified by the status of *TET2* mutation in patients with favorable molecular genotypes (*NPM1*$^{+}$/*FLT3*-ITD^{-} or *CEBPA*^{double}; C) and in patients without these favorable genotypes (D). Both the genotypes *TET2*$^{+}$/*FLT3*-ITD^{+} and *TET2*$^{+}$/*NPM1*$^{-}$ are independent unfavorable factors for OS.

suggested a role of haploinsufficiency of *TET2* in the development of leukemia.

The mutual exclusion between *TET2* mutation and *IDH* mutation, as shown in other reports[40-42] and in this study, suggests that mutations of the 2 genes may involve a common pathway in leukemogenesis. This is supported by a recent report showing that *TET2* loss-of-function mutations and *IDH* mutations are associated with similar abnormal global hypermethylation.[42] Another report also demonstrated that 2-hydroxyglutarate converted from α-ketoglutarate in *IDH*-mutated cells inhibited TET2-mediated hydroxymethylation of cytosine, indicating overlapping effects of these 2 mutations in cells.[40] However, some mysteries remain to be answered: although *IDH* mutations and *TET2* mutations show some common biologic effects in AML, many different clinical features exist between patients with *TET2* and *IDH* mutations. For example, *IDH1/2* mutations are closely associated with higher hemoglobin levels, higher platelet counts, and lower lactate dehydrogenase levels, but not higher WBC counts and older age, which are very different from the associations between these factors and *TET2* mutations. The impacts of *IDH* and *TET2* mutations on survival are also different.[31] *TET2* mutation and *IDH* mutation may have other different biologic

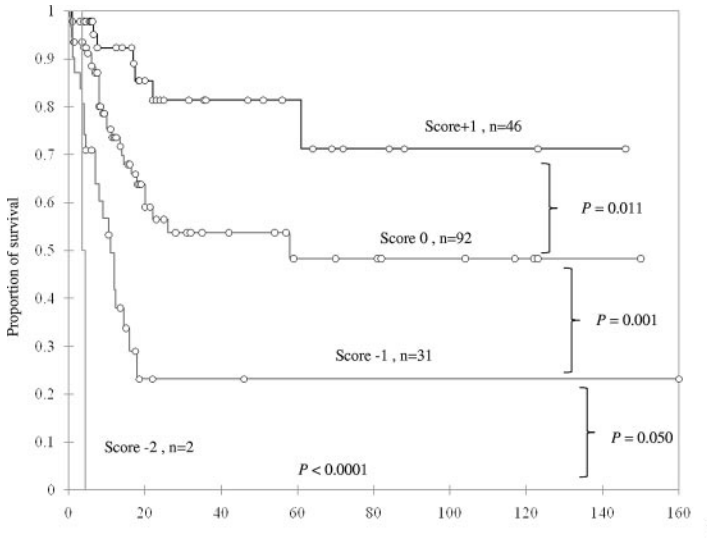

Figure 4. Kaplan-Meier survival curves according to a scoring system based on mutation status of *NPM1*, *CEBPA*^{double}, *TET2*, and *FLT3*-ITD. *CEBPA*^{double} and *NPM1* mutations, 2 favorable prognostic factors, were scored +1 each, whereas the other 2 unfavorable mutations were each scored −1.

effects, which await further studies. Other molecular alterations accompanied with *TET2* or *IDH* mutation may also influence the clinical characteristics in patients with either mutation.

We noted a close correlation of *TET2* mutation with *ASXL1* mutation. In human, the exact functions of ASXL1 remain to be defined, but it can bind steroid receptor coactivator 1 to activate retinoic acid pathway,[43] and is involved in regulation of histone methylation by cooperation with heterochromatin protein-1 to modulate the activity of LSD1,[44] a histone demethylase for H3K4 and H3K9.[45] It is interesting to see simultaneous mutations of *TET2* and *ASXL1* genes in the same patient, both related to epigenetic regulation but through different mechanisms. How these 2 mutations act together to influence epigenetic regulation of the whole genome remains to be defined.

In a report of the Cancer and Leukemia Group B, among cytogenetically normal AML patients with favorable genetic changes (mutated *CEBPA* or *NPM1*$^+$/*FLT3*-ITD$^-$), *TET2* mutations adversely affected CR rate and OS.[17] However, these findings could not be demonstrated in our current study as well as in an AML Study Group.[46] The reason that our results were different from those from Cancer and Leukemia Group B study was not clear but might be because of differences in patient characteristics between the 2 studies. The patients in Cancer and Leukemia Group B study were older than ours, especially in the *TET2*-wild group (median, 60 vs 48 years). This was also reflected in the higher prevalence of *TET2* mutations in their study than in ours (23% vs 18.2% in normal cytogenetic AML). The ethnic difference might be another factor. However, because our study was based on a retrospective analysis, further studies are necessary to confirm the results.

By combined analyses of *TET2* and other mutations, we found both *FLT3*-ITD$^+$/*TET2*$^+$ and *NPM1*$^-$/*TET2*$^+$ genotypes were independent poor prognostic factors, suggesting synergism of negative impact between *TET2* mutation and other adverse prognostic molecular markers. The impact of *TET2* mutation on prognosis in AML patients with intermediate cytogenetics was further highlighted by our scoring system.

We noted a frequent loss of *TET2* mutation in relapsed samples. Given the high blast percentages in the relapsed samples, it is highly probable that *TET2* mutation was really lost in the bulk of the tumor cells, although some residual cells may retain the mutation. The tendency of loss of *TET2* mutation in relapsed samples is analogous to *FLT3*-ITD and *RUNX1* mutation, but in contrast to *NPM1*, *IDH1*, and *IDH2* mutations, which usually persist in relapsed samples.[25,29-31,34,47,48] These findings suggest that *TET2* mutation may not be a good marker for monitoring minimal residual disease. *TET2* mutation may be a secondary event during initial leukemogenesis, and leukemic cells with this mutation can be selected away by chemotherapy, leaving *TET2*-wild residual leukemia cells to grow subsequently into major population at relapse. Alternatively, *TET2* mutation may be important for initiation of leukemogenesis, but not necessary for maintenance of the phenotype.

In conclusion, we showed distinct clinical and biologic characteristics of de novo AML with *TET2* mutation in a large cohort of adult patients. *TET2* mutation was a poor prognostic factor in patients with intermediate-risk cytogenetics, and a scoring system integrating *TET2* mutation with *FLT3*-ITD, *NPM1*, and *CEBPA*^{double} mutations could stratify these patients into 4 distinct prognostic groups.

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Authorship

Contribution: W.-C.C. and H.-F.T. designed the experiment; W.-C.C., S.-C.C., H.-F.T., and C.-Y.C. analyzed the data and wrote the paper; H.-A.H., Y.-C. Chang, F.-Y.L., M.-C. Liu, C.-W.L., Yuan-Yeh Kuo, M.-C. Lee, Yi-Yi Kuo, M.-H.T., and C.-F.H. performed the experiment; J.-L.T., M.Y., W.T., B.-S.K., S.-J.W., S.-Y.H., S.-C.H., K.-T.K., and Y.-C. Chen provided important materials; and C.-Y.L., S.-C.C., and W.-C.C. performed statistical analyses.

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