TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs)

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Oncogenic pathways underlying in the development of myelodysplastic syndromes (MDS) remain poorly characterized, but mutations of the *ten-eleven translocation 2* (*TET2*) gene are frequently observed. In the present work, we evaluated the prognostic impact of *TET2* mutations in MDS. Frameshift, nonsense, missense mutations, or defects in gene structure were identified in 22 (22.9%) of 96 patients (95% confidence interval [CI], 14.5-31.3 patients). Mutated and unmutated patients did not significantly differ in initial clinical or hematologic parameters. The 5-year OS was 76.9% (95% CI, 49.2%-91.3%) in mutated versus 18.3% (95% CI, 4.2%-41.1%) in unmutated patients ($P = .005$). The 3-year leukemia-free survival was 89.3% (95% CI, 63.1%-97.0%) in mutated versus 63.7% (95% CI, 48.2%-75.4%) in unmutated patients ($P = .035$). In univariate analysis (Cox proportional hazard model), the absence of *TET2* mutation was associated with a 4.1-fold (95% CI, 1.4-12.0-fold) increased risk of death ($P = .009$). In multivariate analysis adjusted for age, International Prognostic Scoring System, and transfusion requirement, the presence of *TET2* mutation remained an independent factor of favorable prognosis (hazard ratio, 5.2; 95% CI, 1.6-16.3; $P = .005$). These results indicate that *TET2* mutations observed in approximately 20% of patients, irrespective of the World Health Organization or French-American-British subtype, represent a molecular marker for good prognosis in MDS. (Blood. 2009;114:3285-3291)

Introduction

Myelodysplastic syndromes (MDSs) are hematopoietic stem cell disorders with impaired differentiation and risk of progression to acute myeloid leukemia (AML).[1] Initially, the French-American-British (FAB) and World Health Organization (WHO) classifications distinguished several subtypes.[2,3] The International Prognostic Scoring System (IPSS) considered the number of cytopenias, the karyotype, and the percentage of bone-marrow blasts.[4] Prognostic parameters for survival and progression to AML have been proposed, including circulating blasts, lactate dehydrogenase serum levels, and red blood-cell transfusion requirement.[5-7] However, prognostic factors are lacking for the early stages of the disease.

Little is known regarding the pathogenesis of MDS, although approximately 50% of MDS patients display karyotype aberrations in cytogenetic analyses.[8] Recurrent molecular anomalies have been identified in less than 10% of MDS mainly advanced stages (for a review, see Nimer[1]). High-resolution single nucleotide polymorphism (SNP) array–based karyotyping recently has contributed to the detection of small genomic imbalance and acquired segmental uniparental disomy in MDS.[9,10]

We previously described 4 MDS and AML patients with acquired interstitial deletions affecting the 4q24 chromosomal region.[11] Molecular and cytogenetic approaches allowed the identification of the *ten-eleven translocation 2 (TET2)* gene in a common 500-kb minimal deleted region.[12] The *TET2* gene comprises 11 exons spread over 150 kb.[12] The 2002 amino acids predicted TET2 protein exhibits 2 evolutionary conserved regions: one region located from amino acid 1134 until amino acid 1444 and a second region located near the carboxyterminal end from amino acid 1842 until amino acid 1921 that is related to the hydroxylase family depend on iron and 2 oxoglutarate.[13] We and others[12,14-19] recently have reported the recurrent alteration of the *TET2* gene in the stem cells of various myeloid disorders, including 20% of MDS and MDS/myeloproliferative neoplasm (MPN)–related AML and 8% to 15% of MPN. In the present study, we investigated *TET2*

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mutations in a series of MDS to describe the characteristics of *TET2*-mutated patients and to evaluate the prognostic value of *TET2* mutations.

Methods

Patients

Ninety-six samples of MDS at diagnosis were collected at Cochin Hospital (Paris) and at Paoli-Calmettes Institute (Marseille). Clinical and hematological data were recorded after patients gave their informed consent in accordance with the Declaration of Helsinki. According to the FAB classification, patients were classified as refractory anemia (RA; n = 29), refractory anemia with ringed sideroblasts (RARS; n = 12), refractory anemia with excess blasts (RAEB; n = 47), and RAEB in transformation (RAEBt; n = 8). According to the WHO criteria, patients were classified as RA (n = 16), 5q-syndrome (n = 2), undefined MDS (MDS-U, n = 4), refractory cytopenia with multilineage dysplasia (RCMD, n = 7), RARS/RARS with thrombocytosis (RARS-T, n = 8), refractory cytopenia with multilineage dysplasia (RCMD) with ringed sideroblasts (n = 4), RA with an excess of blasts less than 10% (RAEB1, n = 25), RAEB more than 10% blasts (RAEB2, n = 23), and secondary AML with a bone-marrow blast count between 20% and 29% (n = 7). IPSS was Low in 31, Int-1 in 30, Int-2 in 24, and High in 11 patients. In 12 cases, samples were available before and after disease progression. Among these 96 patients, 88 patients had a follow-up for 15 days or longer and were studied for overall survival (OS), leukemia-free survival with AML transformation defined as 30% bone marrow blasts or greater according to the FAB classification, and event (death or AML transformation)-free survival (for flow chart, see supplemental Figure 1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). The follow-up information was updated by means of a telephone call to patients or their doctors or to town halls. Data concerning treatments received during the period of follow-up were recorded for all patients. This study has been approved by the Hopital Cochin (Paris) and Institute Paoli-Calmette (Marseille) ethics committees.

Nucleic acid methods

DNA (n = 96) was extracted from frozen samples by the use of commercial kits (QIAGEN). For *TET2* gene analysis, polymerase chain reaction (PCR) and direct sequencing were performed starting from 20 ng of genomic DNA, as previously described.[12] All reported *TET2* mutations were scored on both strands by use of ABI 3300 capillary sequencers. Sequence traces were analyzed with Mutation Surveyor (Softgenetics Inc) and reviewed manually. Missense mutations were considered only when located in conserved regions spanning from 1134 to 1444 and 1842 to 1921 amino acids of the TET2 protein. *TET2* anomalies were numbered according to European Molecular Biology Laboratory nucleotide sequence reference FM992369. Previously annotated single nucleotide polymorphisms in database (http//www.hapmap.org) were discarded. N- and K-*RAS* exons 2 and 3 DNA mutation screening were performed by high-resolution melting PCR with the use of LightCycler 480 in High Melting Resolution Master Mix 1X (Roche Applied Science) with 10 ng of genomic DNA, primers 0.1 μmol/L each, and 25 mmol/L $MgCl_2$. High-resolution melting-PCR cycling conditions were initial denaturation at 95°C for 10 minutes followed by 50 cycles at 95°C for 10 seconds, at 63°C for 15 seconds, and at 72°C for 25 seconds. Melting curve was measured from 72°C to 95°C with 25 acquisitions per degree centigrade. Primers used are listed as follows:
KRAS_X2_F4 GGCCTGCTGAAAATGACTGAA;
KRAS_X2_R4 AATTAGCTGTATCGTCAAGGCACTC;
KRAS_X3_F2 TCCCTTCTCAGGATTCCTACAGG;
KRAS_X3_R2 ACAGGGATATTACCTACCTCATAAACATT;
NRAS_X2_F2 CTGATTACTGGTTTCCAACAGGTTCT;
NRAS_X2_R2 TGGGTAAAGATGATCCGACAAGT;
NRAS_X3_F1 TTGTTGGACATACTGGATACAGCTG; and
NRAS_X3_R1 CATTTCCCCATAAAGATTCAGAACAC

SNP array

For SNP arrays, DNA from 23 cases was analyzed with the use of Affymetrix GeneChip Human Mapping SNP6.0 following the manufacturer's protocol (Affymetrix). In brief, 250 ng of DNA were digested with *Nsp*I and *Sty*I, and adapters were ligated to each restriction enzyme digestion, followed by PCR amplification. The 2 PCR products were combined, cleaned-up, and end-labeled to be hybridized to the SNP array containing 1.8 million features with half of them specifically designed to detect copy number variations. SNP array data were analyzed for signal intensity and SNP calls by use of the Gene Chip Genotyping Console 3.0.2 (GTC; Affymetrix). Areas of copy number change (CN) and loss-of-heterozygosity (LOH) were investigated by use of the bioinformatic tools GTC version 3.0.2 (Affymetrix), DChip (http://biosun1.harvard.edu/complab/dchip/), and the Partek Genomics Suite (www.partek.com/). All 23 chips passed Affymetrix QC standards (Contrast QC: minimum 2.4, average 3.1; QC call rate minimum 96%, average 98%, median of the absolute values of all pairwise difference: maximum 0.33, average 0.26). We paid special attention on the 106,287,392-106,420,407 region of chromosome 4, which contains the *TET2* gene (http://genome.ucsc.edu).

Statistical analysis

Continuous variables are reported as medians and interquartile range and were compared by use of the Wilcoxon test. Categorical variables are reported as count and percentage and were compared by the use of the χ^2 or Fisher exact tests. The prevalence of *TET2* mutation was estimated with the 95% confidence interval (95% CI). OS was measured as the time in months from diagnosis to death or censored at last patient's follow-up if no death occurred. The time to AML transformation was defined as the time between diagnosis and documented transformation (≥ 30% bone-marrow blasts). OS and time to AML transformation were studied with Kaplan-Meier estimates, and Log-rank test was used to compare the survival curves between mutated and unmutated patients. Multivariate Cox proportional hazards model was used to study the independent factors of death adjusted for *TET2* mutation status, age (by 10-year increments), IPSS, and treatment by red blood-cell transfusions, these variables being statistically significant in univariate analyses. Unadjusted and adjusted hazards ratio and their 95% CI are provided. All statistical analyses were 2-sided, and *P* values less than .05 were considered to have statistical significance. Statistical analyses were performed with the use of the SAS package version 9.01 (SAS Institute).

Results

Status of *TET2* locus in MDS

We analyzed the *TET2* coding sequence (exons 3-11) in a cohort of 96 MDS patients. According to the FAB classification, patients were classified as RA (n = 29), RARS (n = 12), RAEB (n = 47), and RAEBt (n = 8). According to the WHO criteria, patients were classified as RA (n = 16), 5q-syndrome (n = 2), undefined MDS (MDS-U, n = 4), refractory cytopenia with multilineage dysplasia (RCMD, n = 7), RARS/RARS with thrombocytosis (RARS-T, n = 8), RCMD with ringed sideroblasts (n = 4), RA with an excess of blasts less than 10% (RAEB1, n = 25), RAEB more than 10% blasts (RAEB2, n = 23), and secondary AML with a bone-marrow blast count between 20% and 29% (n = 7). The majority of patients had a rather good prognosis according to IPSS, including 63.5% of Low/Int-1 and 36.5% Int-2/High risk.

We observed 27 different anomalies of the *TET2* coding sequence in 22 patients, including 13 (48.1%) frameshifts, 8 (29.6%) nonsense mutations, and 4 (14.8%) missense mutations, 1 case with a potential structural abnormality of the gene (UPN 51), and 1 mutation affecting a splice site (UPN 137; Table 1). Mutations were spread all over the gene, mostly affecting exon 3 (10 events) and exon 11 (11 events; supplemen-

Table 1. Details of *TET2* anomalies in 22 of 96 patients with MDS

UPN	Sex	Age	FAB	WHO	Karyotype	IPSS	Exon	Nucleotide change	Consequence	Type of mutation	*TET2* locus status*
10	F	56	RAEB	RAEB2	46, XX,del(5)(q12q34)(2)/ 46, id,add(1)(p3?3),r(13) (8)/ 45, idem,del(9)(p13),−17 (2) / 46,XX (10)	Int-2	3	delA 3166	p.Gln769 FS	Frameshift	1
12	F	56	RA	RA	47, XX, +min1, +mar	Int-1	3 and 7	c.4755A>G + c.2490C>T	p.Lys1299Glu+ p.Arg544X	Missense + Nonsense	2
30	M	73	RAEB	RAEB1	46, XY (20)	Int-1	11	insA 5540	p.Tyr1560 FS	Frameshift	1
38	M	61	RAEB	RAEB1	46, XY (20)	Int-1	3	c.2913 C>T	p.Gln685X	Nonsense	2 (UPD)†
51	F	49	RA	RA	46, XX (20)	Low	11		No amplification of 5′ Exon 11		1
59	M	68	RAEB	RAEB1	46, XY (20)	Int-1	11	c.6475T>C	p.Leu1872Pro	Missense	1
72	M	80	RARS	RARS-T	46, XY (20)	Low	3	del 2834_2835	p.His658 FS	Frameshift	1
79	F	65	RAEB	RAEB2	46, XX (20)	Int-2	3	delT 2685 + c.6316T>G	p.Ser609 FS + p.Leu1819X	Frameshift + Nonsense	2
84	M	74	RAEB	RAEB1	46, XY (20)	Int-1	3	ins2540_2544	del/p.Leu560 FS	Deletion + Frameshift	2‡
91	M	75	RAEB	RAEB2	46, XY (20)	Int-2	3	delT 2944	p.Leu699X	Nonsense	1
110	M	92	RA	RA	46, XY (20)	Low	11	p.6360C>T	p.Gln1834X	Nonsense	1
111	M	75	RARS	RCMD-RS	46, XY (20)	Low	3	delG 2994	p.Glu711 FS	Frameshift	1
115	M	71	RAEB	RAEB2	46, XY (20)	Int-2	3 and 11	p.3688C>T + delA 6507	p.Gln943X + p.Thr1883 FS	Nonsense + Frameshift	2
116	M	83	RA	RCMD	45,XY,−20 (2) / 46, XY (18)	Int-1	10	p.5253C>T	p.Arg1465X	Nonsense	1
129	M	60	RAEB	RAEB1	46,XY,del(11)(q13) (20)	Int-1	4 and 11	delG 4271 + c.6478T>C	p.Glu1137 FS + p.Ile1873Thr	Frameshift + Missense	2
137	M	70	RAEB	RAEB1	46, XY (20)	Int-1	5	g.4366-1G>T	mutation of splice acceptor site	Mut splice	2 (UPD)†
143	M	79	MDS-U	RA	46, XX (20)	Low	11	insC 6507	p.Thr1883 FS	Frameshift	1
146	M	80	RAEBt	AML	43,XY,-4,-5,-6,-7, +?der(5;7)(p10;q10), add(11)(p15),-17,-18, +r(?),+mar,+var(17) /46,XY (4)	High	3	insCT 3581	pGly908 FS	Frameshift	2‡
150	F	61	RA	RA	46, XY (20)	Low	9 and 11	delG 4932 + del5521_5524	p.Glu1357 FS + pThr1554 FS	Frameshift × 2	2
187	F	74	RAEB	RAEB1	46, XX, −7, +mar (3) / 46, XX (17)	High	11	del 5583_5608	p.Pro1575 FS	Frameshift	1
210	M	65	RAEB	RAEB1	45,Y,t(X;4)(p21;q24)	Int-1	11	c.5730C>T	del + p.Gln1624X	Deletion + Nonsense	2†‡
211	F	56	RAEB	RAEB2	46, XX, t(3;4)(q26;q24)	High	11	del + c.6478T>C	del + p.Ile1873Thr	Deletion + Missense	2†‡

AML indicates acute myeloid leukemia; F, female; FAB, French-British-American; IPSS, International Prognostic Scoring System; M, male; MDS-U, undefined myelodysplastic syndromes; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB1, RA with an excess of blasts less than 10%; RAEB2, RAEB more than 10% blasts; RAEBt, RAEB in transformation; RARS, refractory anemia with ringed sideroblasts; RARS-T, RARS with thrombocytosis; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia with ringed sideroblasts; *TET*, *ten-eleven translocation*; and WHO, World Health Organization.

**TET2* locus status corresponds to the number of anomalies detected by PCR and sequencing and to the anomalies of the 4q24 region.

†Anomalies detected by the combination of sequencing and SNP arrays.

‡Anomalies detected by the combination of sequencing and conventional cytogenetics.

Table 2. Clinical and biologic characteristics of the population of 96 MDS patients

Characteristic	All	*TET2* mutated	*TET2* unmutated	*P*
Demographics				
n (%)	96 (100)	22 (22.9)	74 (77.1)	
Median age (IQR*)	71.5 (63.5-79)	70.5 (61-75)	72 (64-77)	.306
Sex ratio, M/F	1.5	2.3	1.3	.459
ATCD chemotherapy or radiotherapy, yes/no (%)	23/57 (28.7)	5/13 (27.8)	18/44 (29.0)	.671
FAB classification, n (%)				.803
RA	29 (30.2)	6 (27.3)	23 (31.1)	
RARS	12 (12.5)	2 (9.1)	10 (13.5)	
RAEB	47 (49.0)	13 (59.1)	34 (45.9)	
RAEBt	8 (8.3)	1 (4.5)	7 (9.5)	
WHO classification, n (%)				.909
RA	16 (16.7)	4 (18.2)	12 (16.2)	
5q− syndrome	2 (2.1)	0 (0.0)	2 (2.7)	
MDS-U	4 (4.2)	1 (4.5)	3 (4.1)	
RCMD	7 (7.3)	1 (4.5)	6 (8.1)	
RARS/RCMD-RS/RARS-T	12 (12.5)	2 (9.1)	10 (13.5)	
RAEB1	25 (26.0)	8 (36.5)	17 (23.0)	
RAEB2	23 (23.9)	5 (22.7)	18 (24.3)	
sAML	7 (7.3)	1 (4.5)	6 (8.1)	
Peripheral blood, median (IQR*)				
Hb, g/dL	9.4 (8.6-10.9)	9.4 (8.6-10.4)	9.4 (8.6-10.4)	.322
MCV, fL	99 (90-104)	99 (89-11.8)	98 (90-105)	.658
Leukocytes, g/L	3.9 (2.8-6.3)	5.0 (2.6-6.3)	3.8 (2.8-6.3)	.654
Neutrophils, g/L	1.8 (1.0-3.9)	2.3 (0.8-3.7)	1.8 (1.0-4.0)	.872
Platelets, g/L	164 (85-248)	178 (92-247)	156 (80-250)	.845
Reticulocytes, g/L	58 (35-76)	58 (40-63)	58 (27-88)	.431
Bone marrow				
Blasts, median % (IQR*)	6 (3-11)	6 (3-10)	5 (3-11)	.751
Multilineage dysplasia, yes/no (%)	58/27 (68.2)	13/7 (65.0)	45/20 (70.2)	.786
Karyotype, n (%)				> .999
Favorable	63 (65.6)	16 (72.7)	48 (64.9)	
Intermediate	20 (20.8)	4 (18.2)	16 (21.6)	
Unfavorable	13 (13.5)	3 (13.6)	10 (13.5)	
IPSS, n (%)				.761
Low	31 (32.3)	7 (31.8)	24 (32.4)	
Int-1	30 (31.2)	8 (36.4)	22 (29.7)	
Int-2	24 (25.0)	6 (27.3)	18 (24.3)	
High	11 (11.5)	1 (4.5)	10 (13.5)	
Treatments, n (%)				
None	40 (41.7)	9 (40.9)	31 (41.9)	.792
Red blood cell transfusions	26 (27.1)	6 (27.3)	20 (27.0)	> .999
Erythropoiesis-stimulating agent with or without G-CSF	33 (34.4)	7 (31.8)	26 (35.1)	.812
Lenalidomide/thalidomide	5 (5.2)	1 (4.5)	4 (5.4)	.901
Demethylating agents	5 (5.2)	1 (4.5)	4 (5.4)	.901
Low doses or intensive chemotherapy	7 (7.3)	2 (9.1)	5 (6.8)	.625

Comparison between categorical variables was performed by χ^2 or Fisher exact tests. Comparison between continuous variables was performed by use of the univariate Wilcoxon test.

ATCD indicates antecedent; G-CSF, granulocyte colony-stimulating factor; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndromes; MCV, mean corpuscular volume; and sAML, secondary acute myeloid leukemia. Other abbreviations as in Table 1.

*IQR indicates Interquartile Range (25%-75%).

tal Figure 2). Missense mutation leading to I1873T substitution was absent in nontumoral DNA in one case, suggesting that this substitution was not a polymorphism. The acquired nature of the K1299G or L1872P substitutions could not be checked in 2 other patients, but substitution at K1299 and L1872 has already been reported as acquired.[17-19] Likewise, L1872 and I1873 targeting has been described and lies within the catalytic domain of the TET2 homologue TET1.[13] It is therefore unlikely that these alterations would be of germinal origin in these specific patients. Interestingly, 2 different anomalies of the *TET2* coding sequence were detected in 5 patients, suggesting the alteration of the 2 copies of the *TET2* gene (Table 1). The type and localization of mutations were not related to WHO, FAB, or IPSS classes (Table 1 and supplemental Figure 2).

By conventional cytogenetics, deletion of the 4q24 region was detected in 4 cases. A first case (UPN 84) with a deletion of the 4q24 region and a second case (UPN 146) with a complete loss of one chromosome 4 both had a frameshift in the coding sequence of the remaining copy. In the 2 other cases (UPN 210 and 211), the deletion of 4q24 was associated to a missense or a nonsense mutation on the remaining copy (Table 1). High-resolution SNP-arrays were used to compare tumor to normal DNA in 23 tumor samples. We detected 2 cases with LOH without change in copy number (UPN 38, 137). These 2 patients carried mutation in the *TET2* coding sequence (Table 1), indicating that the LOH of chromosome 4q resulted in the loss of a wild-type *TET2* copy. Overall, genetic defects likely targeting the 2 *TET2* copies were detected in 11 (50%) of 22 mutated patients.

Prevalence of *TET2* mutations and characteristics of the mutated population

We found mutations of the *TET2* coding sequence in 22 of 96 MDS patients. Overall prevalence was 22.9% (95% CI, 14.5%-31.3%), including 20.7% in RA, 16.7% in RARS, 27.6% in RAEB, and 12.5% in RAEBt according to the FAB classification (Table 2). The prevalence was 22.7% in RA/5q-syndrome/MDS-U, 16.7% in RARS/RARS-T/RCMD-RS, 14.3% in RCMD, 32% in RAEB1, and 21.7% in RAEB2 according to the WHO classification. The prevalence was 22.6%, 26.7%, 25.0%, and 9.0% in Low, Int-1, Int-2, and High IPSS risk groups, respectively. The differences in the prevalence were not statistically significant within subgroups of WHO, FAB, or IPSS classifications (Table 2).

Among the 96 patients, 12 patients had samples available at diagnosis and after disease progression. In these 12 cases, the *TET2* mutation pattern remained unchanged. Mutations detected in 7 patients at diagnosis were always present, and no other change in the *TET2* coding sequence was observed after progression. None of the 5 patients lacking *TET2* mutations had gained mutations at the time of AML transformation. This finding indicates the stability of *TET2* mutations during the course of the disease.

The clinical and hematologic characteristics of mutated and unmutated patients are compared in Table 2. There was no significant difference in age, sex, previous exposure to chemotherapy or radiotherapy, peripheral blood parameters, circulating blast cells, multilineage dysplasia, and IPSS-based karyotype distribution between mutated and unmutated cases. When analyzing separately IPSS Low/Int-1 (n = 61) and Int-2/High (n = 35) risk groups, clinical and hematologic parameters were not different (supplemental Table 1). These parameters were also equivalent between the groups of patients with 1 versus 2 targeted copies of *TET2*.

Additionally, the mutational status of N- or K-*RAS* exons 2 and 3 was determined in 84 among the 96 samples. The frequency of N- or K-*RAS* mutation was 2.3% (2/84) in this group of patients. The 2 patients with N- or K-*RAS* mutation also had *TET2* mutation: one patient with RA of Low IPSS had a mutation of exon 2 in K-*RAS*, and one patient with RAEB1 of Int-1 IPSS had a mutation of exon 2 in K-*RAS*. None of the patients devoid of *TET2* mutation were mutated in the N- or K-*RAS* genes. In this cohort, no statistically significant link could be established between these 2 molecular events (χ^2 test, $P = .06$).

Impact of *TET2* mutations on survival

The prognostic impact of *TET2* mutations was evaluated in 88 MDS patients with a follow-up of 15 days or more (supplemental Figure 1). The prevalence of *TET2* mutation was 21 (23.8%) in 88 patients. At the time of last follow-up, 4 of 21 patients with *TET2* mutation had died compared with 27 of 67 in the unmutated group. Treatments received by *TET2* mutated and unmutated patients did not differ significantly (Table 2). Treatments had consisted in supportive care with red blood-cell transfusion in 27.1% of cases, erythropoiesis-stimulating agents with or without granulocyte colony-stimulating factor in 34.4%, thalidomide or lenalidomide in 5.2%, demethylating agents in 5.2%, and low doses or intensive chemotherapy in 7.3%. More than 40% of the patients did not receive any therapy. A Kaplan-Meier curve showed that OS was significantly increased in patients with *TET2* mutations (Figure 1A). The 5-year OS was 76.9% (95% CI, 49.2%-91.3%) in mutated patients versus 18.3% (95% CI, 4.2%-41.1%) in unmutated patients ($P = .005$). Kaplan-Meier analysis also showed that the freedom from AML progression within 3 years was 89.3% (95% CI, 63.1%-97.0%] in the mutated group versus 63.7% [95% CI, 48.2%-75.4%) in the unmutated group (Figure 1B). This difference was significant ($P = .035$). The event-free survival (death or transformation in AML) within 5 years was 76.9% (95% CI, 49.2%-90.7%) versus 18.1% (95% CI, 3.9%-40.7%) in the mutated and unmutated groups ($P = .002$; Figure 1C). This finding suggests that a lower rate of AML transformation in mutated patients might account for a longer survival in MDS with *TET2* mutation.

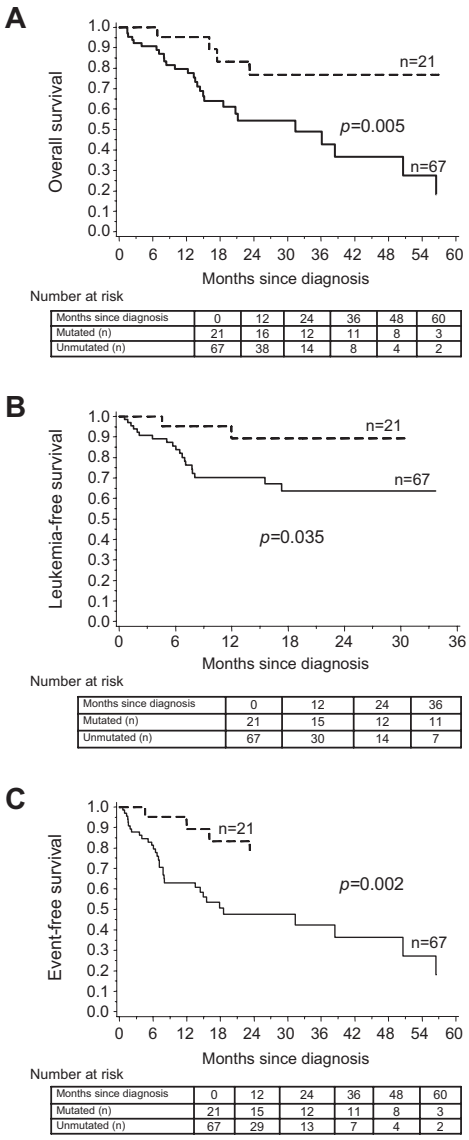

Figure 1. Kaplan-Meier curves for overall, leukemia-free, and event-free survival comparisons. (A) Overall survival in mutated (····) and unmutated (—) MDS patients (n = 88, log-rank test, $P = .005$), (B) freedom from AML progression in mutated (····) and unmutated (—) MDS patients (n = 88, log-rank test; $P = .035$), and (C) event of death or AML transformation-free survival in mutated (····) and unmutated (—) MDS patients (n = 88, log-rank test, $P = .032$).

Overall survival also was analyzed according to the IPSS in the group of 88 patients. In the group of Low and Int-1 MDS (n = 54), mutated patients (n = 14) had significant longer survival within 5 years than unmutated patients (n = 40; $P = .020$). However, the difference in OS at 3 years between mutated (n = 7) and unmutated (n = 27) patients within the Int-2/High risk group (n = 34) did not

Table 3. Univariate and multivariate Cox regression analyses of the risk of death of mutated (n = 21) and unmutated (n = 67) groups of patients

	Univariate		Multivariate	
Statistical analysis	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
***TET2* status**				
Mutated	1		1	
Unmutated	4.1 (1.4-12.0)	.010	5.2 (1.6-16.3)	.005
Age/10 y	1.8 (1.3-2.6)	.002	1.7 (1.1-2.6)	.020
Number of cytopenias				
1	1			
2 or 3	4.3 (2.0-9.3)	< .001		
Percentage bone marrow blasts	1.1 (1.0-1.1)	.002		
IPSS-based karyotype				
Favorable	1			
Intermediate	2.1 (0.9-4.8)	.078		
Unfavorable	2.7 (1.1-6.8)	.030		
IPSS risk groups				
Low/Int-1	1		1	
Int-2/High	5.0 (2.3-10.7)	< .001	9.2 (3.7-22.9)	< .001
Transfusions	2.2 (1.1-4.4)	.030	2.6 (1.2-5.8)	.021

Age is indicated as 10-year increments.
CI indicates confidence interval; and HR, hazard ratio.

reach the significance (p = 0.092) probably as a result of the small size of this subgroup (supplemental Figure 3).

In univariate analysis, age, IPSS, number of cytopenia, percentage of bone-marrow blasts, IPSS-based unfavorable karytoype, and transfusion requirement were identified as OS prognostic factors. The absence of *TET2* mutation was associated with a 4.1-fold (95% CI, 1.4-12.0-fold) increased risk of death ($P = .010$). In multivariate analysis, including mutational status, age, IPSS, and transfusion requirement, the absence of *TET2* mutation is associated with a 5.2-fold (95% CI, 1.6-16.3-fold) increased risk of death ($P = .005$), suggesting that *TET2* mutation was an independent favorable prognostic factor (Table 3).

Discussion

We recently identified *TET2* gene mutation in 19.8% of a series of MDS patients belonging to all WHO or FAB subtypes and in AML after MDS.[12] The present retrospective study confirms the high frequency (22.9%) of *TET2* mutations found in MDS. The prevalence was 24.6% in the Low/Int-1 MDS subgroup and 20% in the Int-2/High MDS subgroup, demonstrating that the frequency of mutations does not increase in advanced diseases. In 12 cases, the mutational status was stable before and after disease progression, suggesting that mutations of *TET2* gene are not linked to the onset of leukemia. N- or K-*RAS* mutations are detected in only 2.3% patients of the present MDS cohort. *TET2* mutations that appear as a frequent molecular event are an independent factor of good prognosis in MDS.

In half of the patients, a single alteration of the *TET2* coding sequence was observed. Therefore, a haploinsufficiency effect of *TET2* could not be excluded, as suggested for several genes of the 5q31 region such as *NPM1*or *RPS14*.[20-22] Although our results are compatible with *TET2* being haploinsufficient, we have not ruled out the inactivation of the wild-type copy of *TET2* gene by other mechanisms such as hypermethylation. With the use of SNP arrays, we detected LOH without change in copy number, including the 4q24 region in 2 of 23 cases, a frequency that might be underestimated but was in line with published results[9,10] and did not identify additional patients with *TET2* anomalies with respect to sequence analysis. Overall 2 anomalies of *TET2* were observed in the other half of the patients, which appeared preferentially to fall in the Low/Int-1 MDS. This situation contrasts with inactivation of both copies of other tumor suppressor genes, either bi-allelic mutation of *TP53*, hypermethylation of *p15INK4B* locus or hypermethylation of *CTNNA1* or *FZD9* gene together with deletion that is associated with disease progression or therapy-related MDS.[23-27]

MDS patients of this cohort had a rather good prognosis (63.5% Low/Int1 and 36.5% Int-2/High risk), and only few of them received MDS-related therapy. For the first time and despite the small size of the sample, we report that MDS patients with *TET2* mutations had a longer OS than unmutated patients. This result has to be confirmed with larger series of patients. The survival advantage resulted at least in part from less AML progression in the mutated patients. Multivariate analysis including age, IPSS, and transfusion requirement demonstrates that *TET2* mutation was an independent factor of favorable prognosis. Other mutations in *TP53*, *AML1/RUNX1*, and N- or K-*RAS* genes have been reported with low prevalence in MDS.[23,24,28-30] Some of the molecular abnormalities described in MDS have demonstrated unfavorable prognostic value, including *NRAS* and *TP53* mutations or hypermethylation of p15*INK4B*.[29-32] Those mutations were generally observed only in advanced stages or therapy-related diseases, and their prognostic value was generally not independent of other parameters, especially of IPSS. In sharp contrast, *TET2* mutation is a favorable prognostic factor in MDS that is independent of IPSS. *TET2* mutations are observed during the early steps of the disease[12,14,19] and might influence the phenotype and clinical behavior. In MPN, mutations in *JAK2* or *MPL* might cooperate with *TET2* mutations to induce the clinical phenotype.[12,15] The oncogenic events specifically cooperating with *TET2* abnormalities during MDS pathogenesis remain to be identified that will allow the evaluation of their respective contribution to phenotype and clinical behavior in these neoplasms.

Our results suggest that the determination of *TET2* mutational status might assist risk stratification. Prospective studies are required to determine whether response to treatments depends on *TET2* mutations.

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Authorship

Contribution: O.K., V.G.-B., and M.C. performed research and analyzed data; S.G. performed the statistical analyses; V.D.-V., F.P., F.V., and E.D. performed research; B.Q., E.S., O.B.-R., V.M.-D.M., M.H.-B., C.P., P.F., and F.D. provided patient samples; D.B. and M.F. planned research; E.S., W.V., O.A.B., D.B., P.G., and M.F. analyzed data and drafted the manuscript; and E.S., W.V., P.F., D.B., O.A.B., and M.F. finalized the manuscript. All authors reviewed and agreed with the final version of the manuscript

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A list of Groupe Francophone des Myelodysplasies participants can be found in the supplemental Appendix, available on the *Blood* website.

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