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TET2 Mutations in Acute Myeloid Leukemia (AML): Results From a Comprehensive Genetic and Clinical Analysis of the AML Study Group

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ABSTRACT

Purpose
The tet oncogene family member 2 (*TET2*) gene was recently identified to be mutated in myeloid disorders including acute myeloid leukemia (AML). To date, there is increasing evidence for a functional role of *TET2* mutations (*TET2*^{mut}) in AML. Thus, we explored the frequency, gene-expression pattern, and clinical impact of *TET2*^{mut} in a large cohort of patients with AML in the context of other AML-associated aberrations.

Patients and Methods
Samples from 783 younger adult patients with AML were analyzed for the presence of *TET2*^{mut} (coding exons 3 to 11), and results were correlated with data from molecular genetic analyses, gene-expression profiling, and clinical outcome.

Results
In total, 66 *TET2*^{mut} were found in 60 patients (60 of 783 patients; 7.6%), including missense (n = 37), frameshift (n = 16), and nonsense (n = 13) mutations, which, with one exception, were all heterozygous. *TET2*^{mut} were not correlated with distinct clinical features or genetic alterations, except for isocitrate dehydrogenase mutations (*IDH*^{mut}) that were almost mutually exclusive with *TET2*^{mut} ($P < .001$). *TET2*^{mut} were characterized by only a weak gene-expression pattern, which, nevertheless, reflected *TET2*^{mut}-associated biology. *TET2*^{mut} did not impact the response to induction therapy and clinical outcome; the combination of patients who exhibited *TET2*^{mut} and/or *IDH*^{mut} revealed shorter overall survival ($P = .03$), although this association was not independent from known risk factors.

Conclusion
TET2^{mut} were identified in 7.6% of younger adult patients with AML and did not impact the response to therapy and survival. Mutations were mutually exclusive with *IDH*^{mut}, which supported recent data on a common mechanism of action that might obscure the impact of *TET2*^{mut} if compared against all other patients with AML.

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INTRODUCTION

Genome-wide studies in patients with myeloid malignancies have provided major insights into the pathogenesis of these diseases,[1,2] and especially in acute myeloid leukemia (AML), an increasing panel of genetic markers has been identified that constitute a base for risk stratification and therapeutic decision making.[1,3-6]

Recently, the tet oncogene family member 2 (*TET2*) gene was identified to be mutated in a variety of myeloid disorders.[7] Subsequent sequencing analysis revealed *TET2* mutations (*TET2*^{mut}) in 7% to 23% in de novo AML[8-11] and 14% to 55% in other myeloid malignancies.[7,8,12-15] In some of these instances, *TET2*^{mut} were associated with uniparental disomies that involve *TET2* on 4q24.[7,16,17] Thus far, a leukemogenic role of ten-eleven translocation (*TET*) family gene members (*TET1*, *TET2*, and *TET3*) was only known for *TET1*, which is involved as a translocation partner in *MLL*-rearranged AML and rarely in acute lymphoblastic leukemias.[18]

The *TET* family members have two highly conserved regions, an *N*-terminal cysteine-rich domain followed by a 2-oxoglutarate (2OG) -Fe(II) oxygenase characteristic double-stranded b-helix.[16,19] Recently, TET proteins were also found to be homologues of 2OG-Fe(II) oxygenases catalyzing the

conversion of thymine to β-D-glucosyl hydroxymethyl uracil in trypanosomes[20]; in humans, 2OG oxygenases have been shown to be involved in various biologic functions including histone demethylation and DNA repair.[21]

TET1 is an enzyme involved in the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in DNA, which is a process thought to play an important role in DNA demethylation and, thus, epigenetic regulation.[20] On the basis of sequence homology, the TET2 protein is expected to also play a role in chromatin remodeling,[16] and it was demonstrated that TET2 also converts 5mC into 5hmC.[22] Furthermore, it was shown that *TET2*^{mut} samples display uniformly low levels of 5hmC compared with normal controls, supporting a functional relevance of *TET2*^{mut} in leukemogenesis.

Recently, Figueroa et al[10] found that activating mutations of *IDH1/2* are mutually exclusive with mutations of the 2OG-dependent *TET2*. Because *IDH*^{mut} interfere with the production of 2OG (α-ketoglutarate) by the aberrant production of 2-hydroxyglutarate, it was hypothesized that the 2OG-dependent catalytic function of *TET2* might be hampered by *IDH*^{mut}, which would explain the mutual exclusiveness of these mutations. In line, *IDH*^{mut} were associated with similar epigenetic defects as *TET2*^{mut}, and *IDH*^{mut} impaired the catalytic function of TET2.[10] Thus, there is evidence that *TET2*^{mut} and *IDH*^{mut} may lead to a biologically redundant hypermethylation phenotype. In accordance with current multistep pathogenesis models of leukemogenesis, *TET2*^{mut} co-occur with other mutations (eg, *JAK2V617F* in myeloproliferative neoplasia or *NPM1* in AML), but until recently, it was still questionable whether *TET2*^{mut} represent an early pathogenic event.[16,19] Data from two independent conditional mouse models demonstrated that Tet2 haploinsufficiency leads to increased stem-cell self-renewal and myeloproliferation, which, thereby, suggested that monoallelic *TET2*^{mut} are an early event in leukemogenesis because they can contribute to myeloid transformation.[23,24]

With regard to the prognostic impact of *TET2*^{mut}, initial studies in small cohorts with AML revealed inconclusive results.[8,9] A recent study of a large cohort with AML reported an adverse prognostic impact in the molecular favorable-risk cytogenetically normal (CN)-AML group,[11] whereas there was no impact of *TET2*^{mut} in the intermediate-risk-I group as defined by the current European LeukemiaNet (ELN) criteria.[3]

In this study, we aimed to further explore the frequency and clinical impact of *TET2*^{mut} in a large cohort of genetically and clinically well-characterized younger adult patients with AML. In addition, by using gene-expression profiling (GEP) in 333 patients, we sought to determine whether *TET2*^{mut} might be associated with a strong gene-expression signature that would further delineate *TET2*^{mut} AML as a biologic subset of AML.

PATIENTS AND METHODS

Patient Samples

Diagnostic bone marrow (BM) and/or peripheral blood (PB) samples were analyzed from 783 of 870 patients with AML (age range, 18 to 60 years) who were enrolled on the prospective German-Austrian AML Study Group multicenter treatment trial AML HD98A (NCT00146120) which was open from January 1998 until December 2004.[25] In brief, patients were treated by using double-induction therapy (idarubicin, cytarabine, and etoposide) and a first consolidation cycle of high-dose cytarabine; for the second cycle, patients were randomly assigned between high-dose cytarabine, autologous, or allogeneic hematopoetic stem-cell transplantation (family donor) treatment.

All patients gave informed consent for treatment and genetic analysis according to the Declaration of Helsinki. Patients were molecularly studied for the presence of the recurring gene fusions *RUNX1-RUNX1T1*, *CBFB-MYH11*, *MLL-MLLT3*, and *PML-RARA* and for mutations in *FLT3* (internal tandem duplications [ITD] and tyrosine kinase domain [TKD] mutations at codon D835 and I836), *NPM1*, *IDH1/IDH2*, *CEBPA*, *WT1*, and *RUNX1* genes (analyses of the latter two in CN-AML (CN-AML only]).[6,26-28]

Analysis of TET2^{mut}

The only criterion to include patients in this study was the availability of a diagnostic BM and/or PB sample for *TET2*^{mut} analysis. The entire coding region of *TET2* (exons 3 to 11) was amplified from DNA by using polymerase chain reaction (PCR) with exon flanking primer pairs and followed by direct sequencing of purified PCR products according to standard protocols (Data Supplement). PCR reactions and sequencing analyses were repeated in all instances that showed sequence variations. All *TET2* sequence variations were aligned to different single nucleotide polymorphism databases (dbSNP [http://www.ncbi.nlm.nih.gov/sites/snp], Genome Browser Gateway [http://genome.ucsc.edu/cgi-bin/hgGateway]; and Ensembl Genome Browser [http://www.ensembl.org/index.html]) to detect known polymorphisms. In 13 patients with *TET2*^{mut} AML in which germline material was available (DNA obtained from buccal swabs or from BM/PB in complete remission [CR]), we studied the presence of *TET2* germline mutations.

GEP

For a subset of AML HD98A patients with available high-quality RNA (n = 333), GEP data were available for additional analysis. GEP had been performed as described previously by using a cDNA microarray platform.[29,30] The complete GEP data set is accessible at Gene Expression Omnibus (accession GSE32246).

Statistical Analyses

Statistical analyses for GEP and for clinical outcome analyses were performed according to previous reports (Data Supplement).[6,26,27,29,31]

RESULTS

Frequency and Types of TET2^{mut}

We found 60 *TET2*^{mut} samples in 783 patients (7.6%), with six patients who exhibited two mutations (Data Supplement). Mutations were distributed all over the gene and most commonly affected exon 3 (n = 22), exon 11 (n = 26, which encodes the 2OG-binding domain), and exon 10 (n = 6) but also occurred in other exons (ie, exons 4 [n = 3], 6 [n = 3], 7 [n = 1], 8 [n = 1], and 9 [n = 4], but not exon 5; Appendix Fig A1, online only). All mutations but one were heterozygous. Frameshift mutations, which resulted from insertions or deletions (n = 16; 24%), and nonsense mutations (n = 13; 20%), which were predicted to result in protein truncation, accounted for approximately one-half of mutations; the remaining mutations were single nucleotide substitutions that led to missense mutations (n = 37; 56%). Analysis of germline materials in 13 patients (derived from buccal swaps or remission BM and/or PB) showed that all missense changes were acquired and not present in germline DNA.

Association of TET2^{mut} With Clinical Characteristics and Genetic Alterations

Patients with *TET2*^{mut} were in trend older and had in trend higher WBC counts ($P = .08$ and $P = .11$, respectively; Table 1). There was no significant difference with respect to platelet counts, BM or PB

Table 1. Patient Demographics and Clinical and Genetic Characteristics of 783 Younger Adult Patients With AML According to *TET2*-Mutation Status

Characteristic	*TET2*^{wt} (n = 723)		*TET2*^{mut} (n = 60)		*P*
	No. of Patients	%	No. of Patients	%	
Age, years					.08
Median	47		51		
Range	16-64		19-60		
Sex					.42
M	378	52.3	28	46.7	
F	345	47.7	32	53.3	
WBC, G/l					.11
Median	13.2		20.9		
Range	0.2-427		0.8-192		
No. of missing values	12		0		
LDH, U/l					.39
Median	425		475		
Range	90-7627		98-2689		
No. of missing values	26		1		
BM blasts, %					.49
Median	75		80		
Range	0-100		10-95		
No. of missing values	76		4		
PB blasts, %					.25
Median	38		50		
Range	0-99		0-98		
No. of missing values	61		7		
Platelet counts, G/l					.11
Median	50		42.5		
Range	2-746		4-263		
No. of missing values	13		0		
Cytogenetic classification*					
Favorable risk	144	21.9	11	19.3	.54
Intermediate risk	398	60.7	39	68.4	
Adverse risk	114	17.4	7	12.3	
No. of missing/excluded values	67		3		
NPM1					
Mutated	183	25.5	18	31.0	.35
No. of missing values	6		2		
CEBPA					
Mutated	43	11.6	1	3.0	.24
No. of missing values	353		27		
FLT3-ITD					
Mutated	159	22.7	17	29.3	.26
No. of missing values	22		2		
FLT3-TKD					
Mutated	70	12.7	3	5.6	.35
No. of missing values	72		6		
IDH1/IDH2					
Mutated	125	17.4	1	1.6	<.001
No. of missing values	0		0		
RUNX1					.99
Mutated	37	6.3	2	4.5	
No. of missing values	139		6		
Type of AML					.99
De novo AML	669	92.7	56	93.3	
s/t AML	53	7.3	4	6.7	
No. of missing values	1		0		

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; ITD, internal tandem duplication; LDH, lactate dehydrogenase; PB, peripheral blood; s/t, secondary/therapy associated; *TET2*^{mut}, *TET2* mutated; *TET2*^{wt}, *TET2* wild type; TKD, tyrosine kinase domain.
*On the basis of European LeukemiaNet guidelines.[3]

Table 2. Patient Demographics and Clinical and Genetic Characteristics of 330 Younger Adult Patients With Cytogenetically Normal AML According to *TET2*-Mutation Status

Characteristic	*TET2*^{wt} (n = 301) No. of Patients	%	*TET2*^{mut} (n = 29) No. of Patients	%	*P*
Age, years					.03
Median	48		52		
Range	16-60		32-60		
Sex					.07
M	138	45.8	8	27.6	
F	163	54.2	21	72.4	
WBC, G/l					.03
Median	15.0		36.9		
Range	0.2-372		0.8-192		
No. of missing values	3		0		
LDH, U/l					.30
Median	443		505		
Range	108-6676		121-2420		
No. of missing values	8		0		
BM blasts, %					.56
Median	80		76		
Range	2-100		10-90		
No. of missing values	21		1		
PB blasts, %					.32
Median	37.5		54.5		
Range	0-98		0-94		
No. of missing values	17		1		
Platelet counts, G/l					.20
Median	65		54		
Range	5-746		4-263		
No. of missing values	3		0		
NPM1					.57
Mutated	147	49.2	16	55.2	
No. of missing values	2		0		
CEBPA					.34
Mutated	37	13.3	1	4.0	
No. of missing values	23		4		
FLT3-ITD					.53
Mutated	96	32.4	11	39.3	
No. of missing values	5		1		
FLT3-TKD					.71
Mutated	27	9.6	1	4.0	
No. of missing values	20		4		
IDH1/IDH2					.005
Mutated	75	24.9	1	3.4	
No. of missing values	0		0		
RUNX1					.39
Mutated	21	9.0	0	0	
No. of missing values	68		9		
Type of AML					.38
De novo AML	285	94.7	29	100.0	
s/t AML	16	5.3	0	0.0	
No. of missing values	0		0		

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; ITD, internal tandem duplication; LDH, lactate dehydrogenase; PB, peripheral blood; s/t, secondary/therapy associated; *TET2*^{mut}, *TET2* mutated; *TET2*^{wt}, *TET2* wild type; TKD, tyrosine kinase domain.

blast counts, and type of AML between *TET2*^{mut} and *TET2* wild type (*TET2*^{wt}). *TET2*^{mut} were found in all major cytogenetic subsets, and there was also no association with other AML-associated molecular markers (*NPM1*^{mut} [$P = .35$], *FLT3*-ITD [$P = .26$], and *FLT3*-TKD mutations [$P = .35$], *CEBPA*^{mut} [$P = .24$], and *RUNX1*^{mut} [$P = .99$]), with the exception of *IDH*^{mut} that were almost mutually exclusive with *TET2*^{mut} ($P < .001$; Table 1).

Within the subgroup of CN-AML, patients with *TET2*^{mut} were older, and they had higher WBC counts ($P = .03$ and $P = .03$, respectively), and mutations were again inversely correlated with *IDH*^{mut} ($P = .005$; Table 2).

Response to Induction Therapy

In the entire cohort, no significant differences between patients with *TET2*^{wt} and *TET2*^{mut} with respect to rates of CR (71% [513 of 723 patients] and 73% [44 of 60 patients], respectively; $P = .77$), refractory disease (18% [129 of 723 patients] and 15% [nine of 60 patients]; $P = .72$), and early or hypoplastic death (11% [81 of 723 patients] and 12% [seven of 60 patients]; $P = .83$; Table 3) were found. In multivariable analysis, age, WBC, cytogenetic risk group, *FLT3*-ITD, and *NPM1*^{mut} were significantly associated with CR achievement; *TET2*^{mut} had no impact (Data Supplement). Within the subgroup of patients with CN-AML, there was a trend toward higher CR rates in patients with *TET2*^{mut} ($P = .12$; Table 3; Data Supplement).

We also evaluated the clinical impact of *TET2*^{mut} according to the ELN classification that groups CN-AML into a molecular favorable group (*CEBPA*^{mut} and/or *NPM1*^{mut} without *FLT3*-ITD) and unfavorable group (intermediate-I, all remaining patients with CN-AML).[3] There was no significant difference with regard to CR rates in the ELN favorable-risk group (77.8% [seven of nine patients] in *TET2*^{mut} *v* 84.7% [72 of 85 patients] in *TET2*^{wt}; $P = .63$), whereas in the intermediate-I risk group, we observed a significantly higher CR rate for patients with *TET2*^{mut} (90.0% [18 of 20 patients] in *TET2*^{mut} *v* 66.2% [143 of 216 patients] in *TET2*^{wt}; $P = .04$; Table 3). In multivariable analysis of the intermediate-I risk group, *TET2*^{mut} was a significant factor that predicted CR achievement ($P = .03$, odds ratio [OR], 5.59; 95% CI, 1.20 to 26.10; Data Supplement).

Table 3. Response to Induction Therapy and Outcome in 783 Younger Adult Patients With AML According to *TET2*-Mutation Status

Response to Double Induction	*TET2*^{wt} No. of Patients	%	*TET2*^{mut} No. of Patients	%	*P*
Entire cohort	723		60		
CR	513	71.0	44	73.3	.77
RD	129	17.8	9	15.0	.72
ED/HD	81	11.2	7	11.7	.83
CN-AML	301		29		
CR	215	71.4	25	86.2	.12
RD	54	17.9	2	6.9	.19
ED/HD	32	10.6	2	6.9	.75
CN-AML, ELN favorable	85		9		
CR	72	84.7	7	77.8	.63
RD	5	5.9	0	0.0	.99
ED/HD	8	9.4	2	22.2	.24
CN-AML, ELN intermediate-I	216				
CR	143	66.2	18	90.0	.04
RD	49	22.7	2	10.0	.26
ED/HD	24	11.1	0	0.0	.24

Abbreviations: AML, acute myeloid leukemia; CN, cytogenetically normal; CR, complete remission; ED, early death; ELN, European LeukemiaNet; HD, hypoplastic death; RD, refractory disease; *TET2*^{mut}, *TET2* mutated; *TET2*^{wt}, *TET2* wild type.

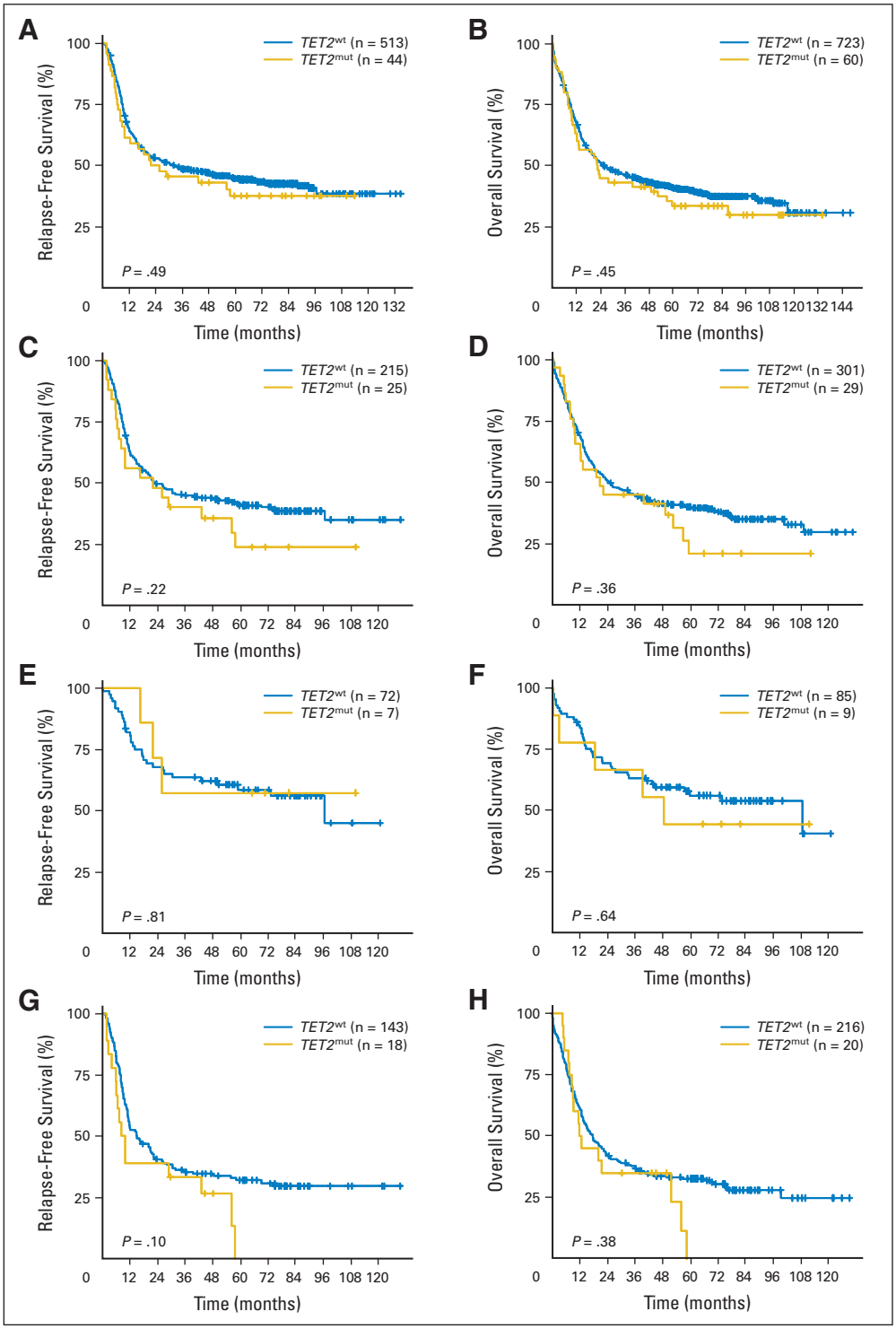

Fig 1. Kaplan-Meier survival estimates according to *TET2*-mutation status. Data (not censored for allogeneic hematopoietic stem-cell transplantation) are shown for (A) relapse-free survival (RFS) and (B) overall survival (OS) in the entire cohort, (C) RFS and (D) OS in patients with cytogenetically normal acute myeloid leukemia, (E) RFS and (F) OS in the favorable European LeukemiaNet (ELN) risk group, and (G) RFS and (H) OS in the intermediate-I ELN risk group. mut, mutated; wt, wild type.

Survival Analysis

The median follow-up time for survival was 6.5 years (95% CI, 6.3 to 6.72 years); the estimated 4-year relapse-free survival (RFS) and overall survival (OS) of the entire cohort were 43% (95% CI, 0.40% to 0.48%) and 43% (95% CI, 0.40% to 0.47%), respectively. Univariable survival analysis on the end points event-free survival (EFS), cumulative incidence of relapse, RFS, and OS showed no significant differences between patients with $TET2^{wt}$ and $TET2^{mut}$ ($P = .67$, $P = .32$, $P = .49$, and $P = .45$, respectively; Figs 1A and 1B).

Subset analyses in patients with CN-AML (n = 330) showed no significant impact of $TET2^{mut}$ on EFS, cumulative incidence of relapse, RFS, and OS, ($P = .94$, $P = .33$, $P = .22$, and $P = .36$, respectively; Figs 1C and 1D). Similarly, ELN subgroup analyses did not show an impact of $TET2^{mut}$ on EFS, RFS, or OS, neither in the

favorable-risk ($P = .65$, $P = .81$, and $P = .64$, respectively) nor in the intermediate-I group ($P = .81$, $P = .10$, and $P = .38$, respectively; Figs 1E and 1F).

In multivariable analysis, *TET2*^{mut} had no impact on RFS, EFS, and OS in both the entire cohort (RFS: OR 0.96; $P = .84$; EFS: OR, 0.93; $P = .65$; OS: OR, 0.94; $P = .72$) and subgroup of patients with CN-AML (RFS: OR, 1.06 [$P = .83$]; EFS: OR, 0.83 [$P = .41$]; OS: OR, 0.93 [$P = .77$]; Data Supplement). The only variables that consistently appeared in all models for survival end points were age, WBC, type of AML, cytogenetic risk, *NPM1*^{mut}, and *FLT3*-ITD (Data Supplement). Similarly, multivariable analyses within the ELN subgroups also revealed no significant impact of *TET2*^{mut} on outcome (Data Supplement).

Explorative Outcome Analyses According to TET2 *and* IDH *Mutational Status*

The observation that *TET2*^{mut} are mutually exclusive with *IDH*^{mut}[10,11] and recent data that suggested that these two aberrations might have a common mechanism of action[10] prompted us to perform clinical correlations for a cohort that comprised both the *TET2*^{mut} and *IDH*^{mut} groups. Compared with patients with *TET2*^{wt}/*IDH*^{wt}, patients with *TET2*^{mut} and/or *IDH*^{mut} were associated with older age ($P < .001$), higher BM blast counts ($P = .02$), and the cytogenetic intermediate-risk group ($P < .001$; Data Supplement). Univariable analysis revealed an inferior outcome as reflected by in trend shorter RFS and shorter OS for patients with *TET2*^{mut} and/or *IDH*^{mut} ($P = .12$ and $P = .03$, respectively; Figs 2A and 2B); in multivariable analysis, however, *TET2*^{mut}/*IDH*^{mut} was not independent of other known prognostic markers (Data Supplement).

Evaluation of the combined *TET2*^{mut}/*IDH*^{mut} group within the CN-AML subgroup also revealed a significant association with older age ($P = .02$) and inferior OS ($P = .03$; Fig 2), but again did not confer independent prognostic information (Data Supplement).

TET2^{mut}-Associated Gene-Expression Pattern

To evaluate the possible impact of *TET2*^{mut} on disease biology via epigenetic deregulation, we compared gene-expression profiles from 31 patients with *TET2*^{mut} with 302 patients with *TET2*^{wt}. Class-comparison analysis revealed a distinct *TET2*^{mut}-associated gene-expression profile that comprised 124 genes ($P < .005$), which was also shared by a group of *TET2*^{wt} samples (Fig A1; Data Supplement). Of note, a significant proportion of these *TET2*^{mut}-like instances comprised *IDH*^{mut} leukemia samples. Significantly deregulated candidate genes included *CD56* with higher expression levels and *JAK2* and *MEF2C* with lower expression levels in *TET2*^{mut} AML. Pathway-comparison analysis for Biocarta pathways revealed several pathways to be significantly enriched in this signature, such as the monocyte and its surface molecules pathway (including cell adhesion molecules [*PECAM1*] and integrins [*ITGAL*, *ITGB5*, *ITGA6*]) and candidates belonging to the nuclear factor of activated T cells pathway (including *MEF2C*, *EDN1*, *MAPK1*, and *RAF1*; Data Supplement).

DISCUSSION

TET2^{mut} have been found in various myeloid neoplasms, and their clinical impact has been investigated by several groups.[7-9,12-15] However, there are only a few studies that evaluated the frequency and clinical role of *TET2*^{mut} within large cohorts of patients with AML

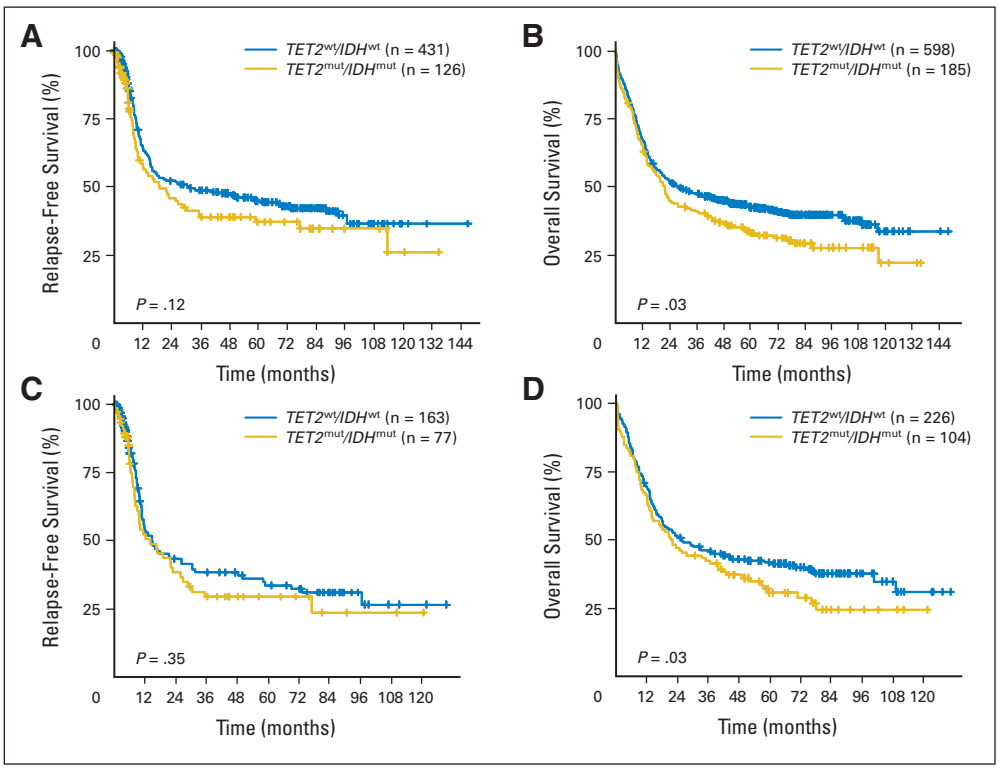

Fig 2. Kaplan-Meier survival estimates on the basis of the combined *TET2*^{mut}/*IDH*^{mut} status. Data are shown for (A) relapse-free survival (censored for allogeneic hematopoietic stem-cell transplantation in first complete remission) and (B) overall survival in the entire cohort as well as (C) relapse-free survival and (D) overall survival in cytogenetically normal acute myeloid leukemia. mut, mutated; wt, wild type.

derived from prospective clinical trials, and thus the clinical relevance still remains uncertain.

In the study by Abdel-Wahab et al,[8] *TET2*^{mut} were found in 12% of patients with AML and were associated with decreased OS. In contrast, the French ALFA (Acute Leukemia French Association) cooperative group reported *TET2*^{mut} in 20% of 147 patients with AML and found no significant correlation with outcome in 111 patients who had achieved a CR (both in the entire cohort and in 54 patients with CN-AML).[9] In a recent study by the CALGB (Cancer and Leukemia Group B) of 427 adult patients with CN-AML, *TET2*^{mut} were found in 23% of patients. Mutations correlated with older age and higher WBC, and they predicted for inferior survival in the ELN molecular favorable-risk group of patients.[11] Although we also observed a trend for an association of *TET2*^{mut} with older age and higher WBC in our AML cohort, we did not find a prognostic impact of *TET2*^{mut} on clinical outcome in the entire patient cohort or subgroups of CN-AML or ELN molecular favorable-risk AML.

These discrepancies might have been due to differences in study populations because the CALGB study included only de novo AML patients, and older patients (> 60 years of age) were also enrolled. The higher *TET2*^{mut} incidence of 23% might reflect the age distribution that ranged from 18 to 83 years, with a median age of 66 years for patients with *TET2*^{mut}.[11] Similarly, the ALFA study reported a much higher *TET2*^{mut} incidence in 36 patients with AML who did not achieve a CR (27% compared with 12% in 111 patients with a CR), a cohort enriched for elderly patients.[9] In contrast, our study was restricted to younger patients with AML (age range, 18 to 60 years) with a median age of 47.5 years and included de novo and secondary/therapy-related AML. In agreement with the incidence of *TET2*^{mut} of 7.6% in our cohort, a recent ECOG study reported somatic *TET2*^{mut} in 7.3% of 385 patients with AML age 60 years or younger.[10]

Furthermore, on the basis of the age difference, the proportion of patients who received intensive therapies is likely to be different among the previously mentioned studies, which might also partly account for the differences in outcome. Although all patients in the CALGB study received intensive cytarabine/daunorubicin-based first-line therapy, only younger patients were assigned to a more intensive consolidation therapy. In our study, all patients with cytogenetic intermediate risk received intensive double-induction therapy and consolidation with repetitive cycles of high-dose cytarabine or autologous transplantation, and allogeneic transplantation was received by patients with a matched-related donor.[25]

In accordance with both the CALGB and ECOG studies that reported a significantly lower frequency of *IDH*^{mut} in *TET2*^{mut},[10,11] we also made the observation of almost mutual exclusiveness of *TET2*^{mut} and *IDH*^{mut}. This result points to a common pathomechanism, and this hypothesis is further strengthened by the fact that the altered enzymatic function of mutated *IDH* converts 2OG into 2-hydroxyglutarate,[33] and TET2 function is 2OG-dependent. Thus, *IDH*^{mut}-associated 2OG level changes might influence the catalytic TET2 function converting 5mC into 5hmC, which is also impaired in patients with *TET2*^{mut} who display uniformly low levels of 5hmC.[22] In agreement, epigenetic deregulation reflected by low 5hmC levels was also observed in a fraction of patients with *TET2*^{wt},[22] and patients with *IDH*^{mut} were shown to present with low 5hmC levels via the inhibition of TET2 function,[10] which explains a common hypermethylation phenotype of *IDH*^{mut} and *TET2*^{mut} AML. However, our combined analysis of patients with *TET2*^{mut} and *IDH*^{mut} did not reveal an impact on clinical outcome independent of other known markers.

These findings that *TET2*^{mut} and *IDH*^{mut} acted as epigenetic regulators, which may be biologically redundant, were further strengthened by our GEP data. The *TET2*^{mut}-associated expression pattern was not restricted to patients with *TET2*^{mut} but was also shared by a large number of patients with *TET2*^{wt}, including patients with *IDH*^{mut} and *TET2*^{mut}-like *IDH*^{wt}. Similarly, Metzeler et al[11] and Figueroa et al[10] found no strong gene-expression patterns for *TET2*^{mut} or *IDH*^{mut} AML, respectively,[10,11] but it was shown that *TET2*^{mut} AML displayed an hypermethylation signature overlapping with that of patients with *IDH*^{mut}.[10] In addition, other yet unknown factors might be involved in epigenetic deregulation such as recently discovered mutations in *EZH2*,[34] *ASXL1*,[35] and *DNMT3A*.[36]

Nevertheless, the gene-expression pattern seems to reflect in part the biologic impact of mutated *TET2*, as it is consistent with the recently observed impaired myeloid differentiation after shRNA-mediated *TET2* knockdown, which resulted in an expansion of monocyte/macrophage lineages.[22] In addition, the inverse correlation of *TET2*^{mut} and *JAK2* expression warrants further investigation as a result of a potential interplay of these genes in disease initiation and progression,[37] and the deregulation of the NFAT pathway members might play a pathogenic role in *TET2*^{mut} AML.[38]

With regard to the pathogenic relevance of *TET2*^{mut}, recent conditional mouse models proposed that *TET2*^{mut} occurs in a stem/progenitor cells, which creates a predisposition to the development of myeloid malignancy.[23,24] On the basis of these studies, *TET2*^{mut} might represent an early event that, in cooperation with secondary mutations, drives the phenotype of the disease. However, in AML, there are several findings that argue against this hypothesis: *TET2*^{mut} were spread over all cytogenetic subgroups and co-occurred with aberrations such as inv(16) or mutations of *NPM1* that are usually found in de novo AML but not as secondary events. Similarly, in our study, *TET2*^{mut} was not enriched in secondary or treatment-associated AML. Clonality studies as well as longitudinal studies that evaluated the potential involvement of *TET2*^{mut} in clonal evolution have to be performed to further elucidate the role of *TET2*^{mut} in the development of AML.

In conclusion, in our study of younger adult patients with AML, *TET2*^{mut} were only found in approximately 8% of patients and were not associated with a clinical phenotype or outcome. The impact of *TET2*^{mut} will need to be revaluated in the light of additional gene mutations that influenced epigenetic regulation in AML.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Manuscript writing: All authors
Final approval of manuscript: All authors

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Appendix

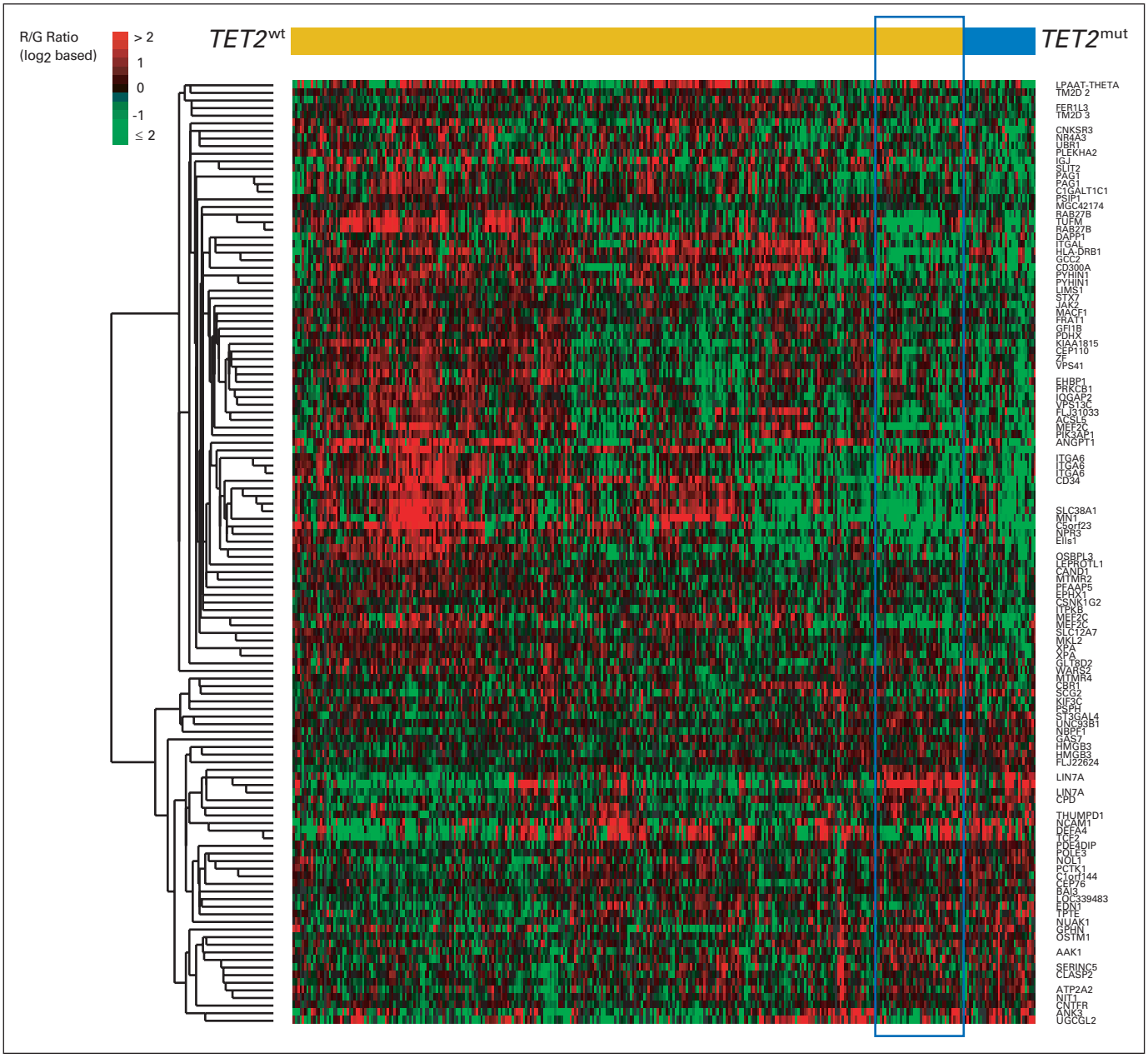

Fig A1. *TET2* mutation (*TET2*^{mut})–associated gene expression profile (top 124 genes). For data visualization, the ClassComparison-derived *TET2*^{mut}-associated genes (rows) were hierarchically clustered (average linkage clustering; similarity metric: correlation, uncentered; selected gene names are depicted). Although patients with *TET2*^{mut} (blue bar) exhibited a distinct gene-expression profile, this gene pattern was also shared by other *TET2*^{mut}-like patient cases (blue-box outline). R/G, red/green.