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ORIGINAL REPORT

TET2 Mutations Improve the New European LeukemiaNet Risk Classification of Acute Myeloid Leukemia: A Cancer and Leukemia Group B Study

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ABSTRACT

Purpose
To determine the frequency of *TET2* mutations, their associations with clinical and molecular characteristics and outcome, and the associated gene- and microRNA-expression signatures in patients with primary cytogenetically normal acute myeloid leukemia (CN-AML).

Patients and Methods
Four-hundred twenty-seven patients with CN-AML were analyzed for *TET2* mutations by polymerase chain reaction and direct sequencing and for established prognostic gene mutations. Gene- and microRNA-expression profiles were derived using microarrays.

Results
TET2 mutations, found in 23% of patients, were associated with older age ($P < .001$) and higher pretreatment WBC ($P = .04$) compared with wild-type *TET2* (*TET2*-wt). In the European LeukemiaNet (ELN) favorable-risk group (patients with CN-AML who have mutated *CEBPA* and/or mutated *NPM1* without *FLT3* internal tandem duplication [*FLT3*-ITD]), *TET2*-mutated patients had shorter event-free survival (EFS; $P < .001$) because of a lower complete remission (CR) rate ($P = .007$), and shorter disease-free survival (DFS; $P = .003$), and also had shorter overall survival ($P = .001$) compared with *TET2*-wt patients. *TET2* mutations were not associated with outcomes in the ELN intermediate-I–risk group (CN-AML with wild-type *CEBPA* and wild-type *NPM1* and/or *FLT3*-ITD). In multivariable models, *TET2* mutations were associated with shorter EFS ($P = .004$), lower CR rate ($P = .03$), and shorter DFS ($P = .05$) only among favorable-risk CN-AML patients. We identified a *TET2* mutation-associated gene-expression signature in favorable-risk but not in intermediate-I–risk patients and found distinct mutation-associated microRNA signatures in both ELN groups.

Conclusion
TET2 mutations improve the ELN molecular-risk classification in primary CN-AML because of their adverse prognostic impact in an otherwise favorable-risk patient subset. Our data suggest that these patients may be candidates for alternative therapies.

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INTRODUCTION

The tet oncogene family member 2 (*TET2*) gene is located at chromosome band 4q24. Mutations in *TET2* were initially identified in myeloid neoplasms with a deletion or uniparental disomy of this chromosomal region,[1,2] and subsequently described in patients with myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), chronic myelomonocytic leukemia, and acute myeloid leukemia (AML).[3-7] *TET2* mutations are frequently acquired during progression of MPN or MDS to secondary AML[8,9] and have been associated with shorter overall survival (OS) in patients with chronic myelomonocytic leukemia or AML.[3,10] However, in other reports, *TET2* mutations were either not significantly correlated with survival in patients with AML[11] or were associated with a decreased risk of progression of MDS to AML and longer OS.[12] The prognostic relevance of *TET2* mutations in myeloid neoplasia thus remains controversial.

Only one recent study of *TET2* mutations[11] has focused specifically on primary (de novo) AML. Cytogenetically normal AML (CN-AML) represents

Table 1. Overview of *TET2* Sequence Variations Detected in 427 Patients With Primary Cytogenetically Normal Acute Myeloid Leukemia

Sequence Variations	No.	%
Patients with wild-type *TET2*	323	100
Patients with *TET2* sequence variation	104	32
Single heterozygous variation	62	19
Two sequence variations	35	10
Three sequence variations	1	0.3
Homozygous/hemizygous variations*	6	2
Total No. of sequence variations	141	100
Missense variation	37	26
Within *TET2* conserved domain†	28	20
Outside *TET2* conserved domain†‡	9	6
In-frame insertion/deletion	2	1
Nonsense variation	34	24
Insertion/deletion causing frame shift	59	42
Variation affecting splice site	9	6

NOTE. Single-nucleotide polymorphisms occurring in healthy individuals and synonymous variations were not included in the analysis.

*These six patients had sequence variations that appeared homozygous on the sequencing chromatograms, consistent with loss of heterozygosity (ie, either the remaining wild-type allele was lost or both *TET2* alleles were affected by the same mutation).

†The evolutionarily conserved domains within the *TET2* sequence, as defined by Langemeijer et al,[1] comprise codons 1104 to 1478 and 1845 to 2002.

‡For seven of the nine patients with missense variations outside the *TET2*-conserved domains, germline DNA was available for testing, and in all seven patients, the mutation was found in the germline sample. Therefore, all nine patients with such changes were excluded from analyses of clinical and molecular associations and outcomes.

the largest subgroup of adult primary AML,[13] and within this molecularly heterogeneous group, gene mutations are increasingly used to assess prognosis and guide risk-adapted treatment.[14,15] To integrate the prognostic information conveyed by cytogenetics and gene mutations and apply this information to the clinical management of patients with AML, an international expert panel on behalf of the European LeukemiaNet (ELN) recently recommended a classification scheme for adult AML based on karyotype, *FLT3*-internal tandem duplications (*FLT3*-ITD), and *NPM1* and *CEBPA* mutations.[16] According to the ELN classification, patients with CN-AML are assigned to either the favorable-risk category (*CEBPA*-mutated and/or mutated *NPM1* without *FLT3*-ITD) or to the intermediate-I–risk category (all remaining patients). Mutations in *TET2* and other genes were recognized as novel genetic abnormalities in the ELN report,[16] and such markers may potentially be useful to refine the existing ELN categories.

We investigated the prevalence of *TET2* mutations in patients with primary CN-AML, their associations with clinical and molecular characteristics, and their impact on the prognosis of the CN-AML subgroups defined by the ELN. Furthermore, to gain insights into the role of *TET2* mutations in the pathobiology of AML, we derived genome-wide *TET2* mutation-associated gene- and microRNA-expression signatures.

PATIENTS AND METHODS

Patients, Treatment, and Cytogenetic Studies

Pretreatment bone marrow (BM) or blood samples were obtained from 427 patients with primary CN-AML, age 18 to 83 years, who received intensive cytarabine/daunorubicin-based first-line therapy on Cancer and Leukemia Group B (CALGB) trials. For details regarding treatment protocols and sample collection, see the Data Supplement. The diagnosis of normal cytogenetics was based on the analysis of ≥ 20 metaphases in BM specimens and confirmed by central karyotype review.[17] All patients provided written informed consent, and all study protocols were in accordance with the Declaration of Helsinki and approved by institutional review boards at each center.

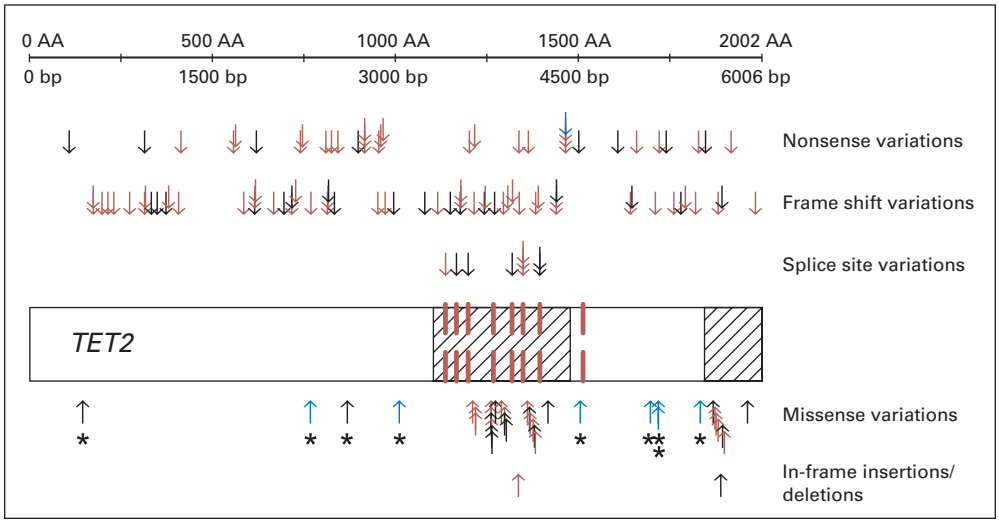

Fig 1. Localization of sequence variations in relation to the *TET2* coding sequence. The cross-hatched areas mark the two evolutionarily conserved domains of *TET2* (amino acids 1104 to 1478 and 1845 to 2002), and exon boundaries are shown as dashed red lines.[1] Each arrow represents one of the 141 nonsynonymous sequence variations in *TET2* found among 427 patients (except for known single-nucleotide polymorphisms, which were not considered). Nonsense and frame shift variations as well as variations affecting splice sites are shown in the upper part of the figure. Missense variations and in-frame insertions/deletions, which alter only one or two amino acids, are shown in the lower part. Variations that were absent in matched germline DNA and thus were proven to be somatically acquired mutations are shown by red arrows. Blue arrows represent changes that were also found in the corresponding germline sample, and black arrows represent sequence changes where corresponding germline DNA was not available. Nine of the missense changes (highlighted by asterisks) were located outside the conserved domains of *TET2*, and seven of them were present in the germline (two could not be tested). The nine patients harboring these changes were excluded from further analyses.

Table 2. Clinical and Molecular Characteristics According to *TET2* Mutation Status in 418 Patients With Primary Cytogenetically Normal Acute Myeloid Leukemia

Characteristic	*TET2* Mutated (n = 95)		*TET2* Wild Type (n = 323)		*P*
	No.	%	No.	%	
Age, years					< .001
Median	66		60		
Range	20-80		18-83		
Age group, years					< .001
< 60	27	28	156	48	
≥ 60	68	72	167	52	
Female sex	56	59	154	48	.06
Race/ethnicity					1.0
White	85	91	290	91	
Nonwhite	8	9	30	9	
Hemoglobin, g/dL					.59
Median	9.3		9.5		
Range	6.5-15.0		4.8-13.6		
Platelet count, $\times 10^9$/L					.56
Median	70		64		
Range	8-510		4-850		
WBC count, $\times 10^9$/L					.04
Median	33.5		24.5		
Range	1.6-450.0		0.9-434.1		
Percentage of blood blasts					.96
Median	52		54		
Range	0-97		0-99		
Percentage of bone marrow blasts					.86
Median	67		67		
Range	7-97		7-99		
FAB category					—
M0	0	0	8	4	
M1	14	21	59	26	
M2	22	32	64	29	
M4	20	29	54	24	
M5	12	18	32	14	
M6	0	0	6	3	
Extramedullary involvement	26	27	81	26	.79
IDH1					< .001
Mutated	2	2	48	15	
Wild type	91	98	274	85	
IDH2					< .001
Mutated	2	2	74	23	
Codon R140 mutation	2		60		
Codon R172 mutation	0		14		
Wild type	91	98	248	77	
CEBPA					.07
Mutated	20	21	42	13	
Wild type	74	79	278	87	
NPM1					.34
Mutated	62	65	191	59	
Wild type	33	35	132	41	
FLT3-ITD					.54
Present	36	38	110	34	
Absent	59	62	213	66	
ELN risk group*					.08
Favorable-risk	53	56	146	45	
Intermediate-I-risk	42	44	177	55	
FLT3-TKD					1.0
Present	8	8	30	9	
Absent	87	92	290	91	

(continued on following page)

Table 2. Clinical and Molecular Characteristics According to *TET2* Mutation Status in 418 Patients with Primary Cytogenetically Normal Acute Myeloid Leukemia (continued)

Characteristic	*TET2* Mutated (n = 95) No.	%	*TET2* Wild Type (n = 323) No.	%	*P*
WT1					.16
Mutated	5	5	34	11	
Wild type	90	95	289	89	
MLL-PTD					.59
Present	6	8	17	6	
Absent	69	92	273	94	

Abbreviations: FAB, French-American-British classification; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; ELN, European LeukemiaNet; *FLT3*-TKD, tyrosine kinase domain mutation in the *FLT3* gene; *MLL*-PTD, partial tandem duplication of the *MLL* gene.

*The ELN favorable-risk group includes patients with cytogenetically normal acute myeloid leukemia with mutated *CEBPA* and/or mutated *NPM1* without *FLT3*-ITD. All remaining patients with cytogenetically normal acute myeloid leukemia (ie, those with wild-type *CEBPA* and *FLT3*-ITD and/or wild-type *NPM1*) belong to the ELN intermediate-I–risk category.

Mutational Analyses

All coding exons of the longest known *TET2* transcript were amplified from genomic DNA by polymerase chain reaction and were analyzed by direct sequencing. Synonymous sequence changes and known single nucleotide polymorphisms were not considered (Data Supplement).[1,18] If available, matched buccal swab specimens or BM aspirates obtained during complete remission (CR) were analyzed to assess whether observed sequence alterations represented somatic mutations or germline variants. Patients were also characterized for *FLT3*-ITD[19] and *FLT3* tyrosine kinase domain mutations,[20] *MLL* partial tandem duplications,[21,22] and mutations in *NPM1*,[23,24] *WT1*,[25] *CEBPA*,[26] and *IDH1*/*IDH2*,[27] as previously reported.

Microarray Experiments

Gene-expression profiling was performed using Affymetrix oligonucleotide microarrays (Affymetrix, Santa Clara, CA), and microRNA-expression profiling was performed by using a custom microarray, as previously reported.[24] Differentially expressed probe sets or probes were identified by comparing *TET2*-mutated and *TET2* wild-type (*TET2*-wt) patients, using univariable significance levels of $< .001$ for gene-expression and $< .005$ for microRNA-expression profiles (Data Supplement).

Statistical Analyses

Baseline characteristics were compared between *TET2*-mutated and *TET2*-wt patients by using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Clinical end points were defined according to published recommendations (Data Supplement).[28] For time-to-event analyses, survival estimates were calculated by using the Kaplan-Meier method, and groups in which at least 10 events had occurred were compared with the log-rank test. We constructed multivariable logistic regression models to analyze factors for the achievement of CR and multivariable Cox proportional hazards models for factors associated with survival end points. For details regarding statistical analyses, see the Data Supplement. All analyses were performed by the CALGB statistical center.

RESULTS

Prevalence and Spectrum of TET2 Mutations

We identified variations in the *TET2* coding sequence in 104 of 427 patients (Table 1). Mutations predicted to result in a truncated protein (nonsense and frame shift) occurred most frequently and were distributed throughout all coding exons. In contrast, missense changes mainly clustered in two evolutionarily conserved domains of the gene (Fig 1).[1] Analyses of buccal swab or remission BM samples showed that all evaluable missense changes outside these conserved domains (n = 7) were present in the germline (Data Supplement). Therefore, as done in previous studies,[1,10,12] we excluded all nine patients with missense changes outside the two conserved domains, leaving 418 patients for subsequent analyses. Thus, the prevalence of *TET2* mutations in our cohort was 23% (95 of 418).

Association of TET2 Mutations With Pretreatment Demographic, Clinical, and Molecular Characteristics

TET2-mutated CN-AML patients were significantly older than *TET2*-wt patients ($P < .001$; Table 2). The prevalence of *TET2* mutations gradually increased with age, from 7% in adults younger than 30 years of age to 32% in patients age 70 years or older (P for trend $< .001$; Data Supplement). *TET2*-mutated patients presented with a higher WBC ($P = .04$) and were more likely to be female ($P = .06$) than *TET2*-wt patients.

Regarding other molecular markers, *IDH1* and *IDH2* mutations were less frequent in *TET2*-mutated than in *TET2*-wt patients ($P < .001$), with mutations at *IDH2* codon R172 being mutually exclusive with *TET2* mutations. We also observed a trend toward a higher prevalence of *CEBPA* mutations among *TET2*-mutated patients ($P = .07$; Table 2).

Impact of TET2 Mutation Status on Treatment Outcomes in All Patients With CN-AML

The median follow-up for patients still alive was 6.8 years (range, 2.3 to 11.7 years). Overall, *TET2*-mutated patients compared with *TET2*-wt patients showed a trend toward shorter event-free survival (EFS; $P = .07$; Table 3) but no significant differences in CR rate ($P = .28$), disease-free survival (DFS; $P = .16$; Fig 2A), or OS ($P = .14$; Fig 2B). No differences in outcomes were observed between patients with single or double *TET2* mutations nor between different types of mutations (data not shown).

TET2 Mutations Are Associated With Inferior Outcomes in the ELN Favorable-Risk Group but Not in the Intermediate-I–Risk Group

The ELN guidelines introduced a standardized reporting system for genetic abnormalities, classifying patients with CN-AML into favorable-risk or intermediate-I–risk categories on the basis of specific genetic characteristics.[16] Following the ELN recommendations, we

Table 3. Treatment Outcomes According to *TET2* Mutation Status in 418 Patients With Primary Cytogenetically Normal Acute Myeloid Leukemia

	TET2 Mutated			*TET2* Wild Type			
End Point	No.	%	95% CI	No.	%	95% CI	*P*
All patients (N = 418)	95			323			
Event-free survival							.07
Median, years		0.6			0.8		
Event-free at 3 years		19	12% to 27%		24	20% to 29%	
Complete remission	67	71		246	76		.28
Disease-free survival							.16
Median, years		0.8			1.2		
Disease-free at 3 years		25	16% to 36%		32	26% to 38%	
Overall survival							.14
Median, years		1.3			1.4		
Alive at 3 years		27	19% to 36%		35	30% to 40%	
ELN favorable-risk group* (n = 199)	53			146			
Event-free survival							< .001
Median, years		0.7			1.8		
Event-free at 3 years		21	11% to 32%		42	34% to 50%	
Complete remission	39	74		131	90		.007
Disease-free survival							.003
Median, years		1.1			2.5		
Disease-free at 3 years		26	13% to 40%		47	39% to 56%	
Overall survival							.001
Median, years		1.5			3.8		
Alive at 3 years		32	20% to 44%		54	46% to 62%	
ELN intermediate-I–risk group* (n = 219)	42			177			
Event-free survival							.45
Median, years		0.5			0.6		
Event-free at 3 years		17	7% to 29%		9	5% to 14%	
Complete remission	28	67		115	65		1.0
Disease-free survival							.36
Median, years		0.6			0.7		
Disease-free at 3 years		25	11% to 42%		14	8% to 21%	
Overall survival							.72
Median, years		0.9			1.1		
Alive at 3 years		21	11% to 35%		19	14% to 25%	

Abbreviation: ELN, European LeukemiaNet.
*The ELN favorable-risk group is defined as patients with mutated *CEBPA* and/or mutated *NPM1* without internal tandem duplication of the *FLT3* gene (*FLT3*-ITD). All remaining patients with cytogenetically normal acute myeloid leukemia (ie, those with wild-type *CEBPA* and *FLT3*-ITD and/or wild-type *NPM1*) belong to the ELN intermediate-I–risk category.

investigated the prognostic impact of *TET2* mutations within these categories. Among 418 patients, 199 (48%) were in the favorable-risk category (Data Supplement), and 219 (52%) were in the intermediate-I–risk category. Favorable-risk CN-AML patients had superior EFS ($P < .001$), a higher CR rate ($P < .001$), and longer DFS ($P < .001$) and OS ($P < .001$) than intermediate-I–risk patients (Data Supplement). *TET2* mutations tended to be more frequent in favorable-risk than in intermediate-I–risk patients (27% *v* 19%; $P = .08$). However, types and locations of *TET2* mutations were similar in both groups (data not shown).

Among favorable-risk CN-AML patients, those with *TET2* mutations had shorter EFS than those with *TET2*-wt ($P < .001$; Table 3). This difference was due to a lower CR rate ($P = .007$) and shorter DFS ($P = .003$; Fig 2C) of *TET2*-mutated patients who also had shorter OS ($P = .001$; Fig 2D).

In contrast, in the intermediate-I–risk group, no significant difference in EFS ($P = .45$), CR rates ($P = 1.0$), DFS ($P = .36$), or OS ($P = .72$) was found between *TET2*-mutated and *TET2*-wt patients (Table 3). Therefore, since the adverse impact of *TET2* mutations appeared to be limited to the favorable-risk subgroup, we formally tested for an interaction between *TET2* mutation status and ELN risk category with regard to clinical end points. Indeed, we found statistically significant interactions between *TET2* mutation status and ELN risk category for all four outcome end points (EFS, $P = .001$; CR rate, $P = .03$; DFS, $P = .007$; OS, $P = .01$), confirming a differential effect of *TET2* mutations on outcome in the two ELN risk groups for CN-AML.

Multivariable Analyses

To assess whether *TET2* mutations provide additional prognostic value in the context of the ELN classification and other clinical and molecular prognosticators, we constructed multivariable models including all 418 patients with CN-AML and used an interaction term to account for the differential effect of *TET2* mutations in the favorable-risk and intermediate-I–risk subgroups (Table 4). In the model for EFS, *TET2* mutations conferred a 71% increased risk of an event

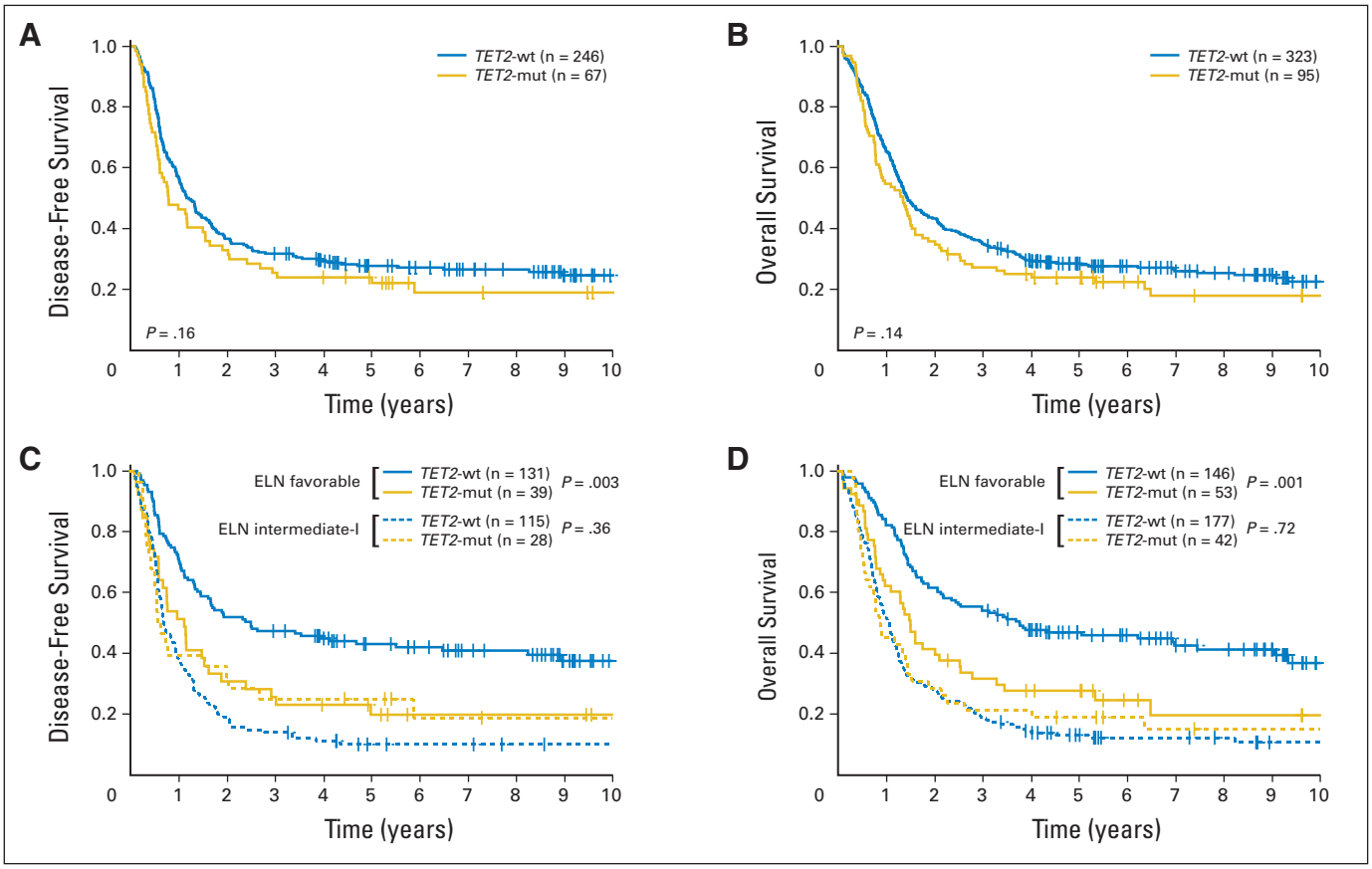

Fig 2. (A) Disease-free survival and (B) overall survival of all patients with cytogenetically normal acute myeloid leukemia according to *TET2* mutation status. (C) Disease-free survival and (D) overall survival of patients in the European LeukemiaNet (ELN) favorable-risk and intermediate-I–risk groups, according to *TET2* mutation status. *TET2*-mut, mutated *TET2*; *TET2*-wt, wild-type *TET2*.

among favorable-risk patients ($P = .004$) after adjusting for *WT1* mutation status ($P = .006$), WBC ($P < .001$), platelet count ($P = .01$), and age group ($P < .001$). *TET2*-mutated patients in the favorable-risk group had 38% lower odds of achieving CR ($P = .03$) after adjusting for WBC ($P = .001$), platelet count ($P = .02$), and age group ($P = .009$). In the model for DFS, favorable-risk patients with *TET2* mutations had a 54% higher risk of relapse or death than *TET2*-wt patients ($P = .05$) after adjusting for *WT1* mutation status ($P = .03$), WBC ($P = .003$), and age group ($P < .001$). In these multivariable models, among intermediate-I–risk patients, *TET2* mutations were not significantly associated with EFS ($P = .14$), CR rate ($P = .18$), or DFS ($P = .13$).

Gene- and MicroRNA-Expression Signatures Associated With TET2 *Mutations*

To gain insights into the biology of *TET2*-mutated CN-AML, we studied mutation-associated gene-expression signatures. Within favorable-risk patients, 150 Affymetrix probe sets (representing 91 named genes) were upregulated and 63 probe sets (representing 45 named genes) were downregulated in *TET2*-mutated patients (n = 41) compared with *TET2*-wt patients (n = 93; Data Supplement). In contrast, a significant *TET2* mutation-associated gene-expression signature could not be identified in intermediate-I–risk patients. Among the genes upregulated in favorable-risk *TET2*-mutated patients were *DPPA4* (a marker of pluripotency highly expressed in hematopoietic stem and progenitor cells[29]), *MS4A3* (a cell cycle regulator in hematopoietic cells[30]), *NCAM1* (*CD56*; associated with poor outcomes in AML[31]), *LMO4* (involved in hematopoietic stem-cell development[32]), *APP* (overexpressed in complex karyotype AML with 21q amplification[33] and downregulated in *NPM1*-mutated CN-AML[24]), and the granulocytic transcription factor *CEBPA* and *IDH1* (both frequently targeted by mutations in CN-AML[15,26,27]). Downregulated in favorable-risk *TET2*-mutated patients were the cancer-testis antigen *CT45* gene family (highly expressed in CN-AML with *NPM1* mutations,[24] lymphoma, and epithelial cancers[34]), *HBG1/HBG2* (hemoglobin gamma), and *MLL* (implicated in stem-cell self-renewal,[35] frequently targeted by genomic rearrangements in AML[36]).

MicroRNA-expression profiles were available for a subset of older (age ≥ 60 years) patients in whom 72% of *TET2* mutations were found in our study. We identified distinct microRNA-expression signatures in favorable-risk and intermediate-I–risk patients. In favorable-risk *TET2*-mutated patients (n = 28) compared with favorable-risk *TET2*-wt patients (n = 50), five microRNAs (represented by six microarray probes) were upregulated and two microRNAs were downregulated (Data Supplement). Among the upregulated microRNAs were *miR-148a* (targeting DNA methyltransferases, highly expressed in refractory chronic lymphocytic leukemia[37]) and *miR-24* (stimulating myeloid cell proliferation and blocking granulocytic and erythroid differentiation[38,39]). One of the

Table 4. Multivariable Models for the Associations Between Patient Characteristics and Treatment Outcomes

Variable	Event-Free Survival			Complete Remission			Disease-Free Survival		
	HR	95% CI	*P*	OR	95% CI	*P*	HR	95% CI	*P*
Interaction of *TET2* status and ELN risk category*			.002			.05			.01
Within ELN favorable-risk group: *TET2* mutated *v* *TET2* wild type	1.71	1.19 to 2.47	.004	0.62	0.41 to 0.96	.03	1.54	1.00 to 2.35	.05
Within ELN intermediate-I–risk group: *TET2* mutated *v* *TET2* wild type			.14			.18			.13
WT1 (mutated *v* wild type)	1.64	1.15 to 2.34	.006			N/S	1.63	1.04 to 2.56	.03
WBC (continuous, 50-unit increase)	1.23	1.13 to 1.35†	< .001	0.71	0.58 to 0.87	.001	1.37	1.14 to 1.65†	.003
Platelet count (continuous, 50-unit increase)	1.09	1.02 to 1.17	.01	0.83	0.72 to 0.97	.02			N/S
Age group, years (≥ 60 *v* < 60)	2.00	1.59 to 2.51	< .001	0.51	0.31 to 0.84	.009	2.17	1.65 to 2.85	< .001

NOTE: All multivariable models contained an interaction term to account for the interaction between European LeukemiaNet (ELN) risk group assignment and *TET2* mutation status. A hazard ratio (HR) > 1 (< 1) corresponds to a higher (lower) risk for higher values of continuous variables and the first category listed of a dichotomous variable. Variables were considered for inclusion in the multivariable models if they had a univariable *P* value of < .2. See Data Supplement for a full list of variables evaluated in univariable analyses. Since internal tandem duplication of the *FLT3* gene (*FLT3*-ITD), *NPM1*, and *CEBPA* mutations are integrated in the ELN risk classification, they were not additionally considered as individual variables. Several variables were considered for model inclusion. In the model for event-free survival: *TET2* mutations, ELN risk group, and their interaction term; *WT1* mutations, WBC, platelet count, age group (< 60 *v* ≥ 60 years), and race (white *v* nonwhite). In the model for achievement of complete remission: *TET2* mutations, ELN risk group, and their interaction term; *WT1* mutations, WBC, platelet count, and age group (< 60 *v* ≥ 60 years). In the model for disease-free survival: *TET2* mutations, ELN risk group, and their interaction term; *WT1* mutations, WBC, age group (< 60 *v* ≥ 60 years), and race (white *v* nonwhite).

Abbreviations: OR, odds ratio; ELN, European LeukemiaNet; N/S, not significant.

*The ELN favorable-risk group is defined as patients with mutated *CEBPA* and/or mutated *NPM1* without *FLT3*-ITD. All remaining patients with cytogenetically normal acute myeloid leukemia (ie, those with wild-type *CEBPA* and *FLT3*-ITD and/or wild-type *NPM1*) belong to the ELN intermediate-I–risk category.

†This variable did not meet the proportional hazards assumption. The *P* value corresponds to the Wald statistic of a 2-*df* test evaluating whether the coefficients for the variable and an artificial time-dependent covariate were equal to 0 to account for nonproportionality. The estimate of the HR is provided at 3 months.

two downregulated microRNAs was *miR-135a*, which targets *JAK2* and, when downregulated, is associated with shorter DFS in patients with Hodgkin's lymphoma.[40] In the intermediate-I–risk group, six microRNAs were upregulated in *TET2*-mutated (n = 21) versus *TET2*-wt (n = 79) patients, and seven microRNAs (represented by nine probes) were downregulated (Data Supplement). Notably, there was no overlap between the microRNA-expression signatures identified in favorable-risk and intermediate-I–risk patients. Among the upregulated microRNAs in intermediate-I–risk *TET2*-mutated patients was *miR-204*, which targets *HOXA10* and *MEIS1* and shows low expression in *NPM1*-mutated AML.[41] In contrast, *miR-126*, targeting *HOXA9* and showing low expression in *NPM1*-mutated AML,[24] was downregulated in intermediate-I–risk *TET2*-mutated patients.

DISCUSSION

Since their initial discovery, *TET2* mutations have been extensively studied in MPN and MDS, and reports involving patients with AML[2-4,7,12,42] have often focused on secondary AML arising from these disorders. In contrast, little is known about the prevalence and clinical relevance of *TET2* mutations in primary AML. In two relatively small studies,[1,11] *TET2* mutations were found in 17% and 19% of patients with primary AML with various karyotypes and 22% of patients with CN-AML.[11] In our larger cohort, the prevalence of *TET2* mutations in primary CN-AML was 23%. Our findings that most *TET2* mutations are frame shift or nonsense changes (likely resulting in a truncated protein) and that missense mutations cluster in two evolutionarily conserved domains of *TET2* are in agreement with previous MDS and MPN series.[1,2,5,12] We analyzed matched germline DNA samples to ascertain the somatic nature of sequence changes. In our cohort, all evaluable missense changes outside the two conserved domains were germline variants and thus likely nonpathogenic. Therefore, patients carrying these variations were excluded from our analyses.

We focused on patients with CN-AML because the prognosis of this cytogenetic subset is affected by molecular markers that are increasingly used for prognostication and risk-adapted management decisions.[14-16,23-27] We demonstrated that *TET2* mutations are associated with older age (as reported for MPN[5]) and higher pretreatment WBC and that they rarely occur together with *IDH1* or *IDH2* mutations. Consistent with our previous report[27] that mutations of *IDH2* codon R172 are mutually exclusive with other gene mutations in CN-AML, no patient concurrently harbored a *TET2* mutation and an *IDH2* R172 mutation.

To the best of our knowledge, our study is the largest report on the prognostic implications of *TET2* mutations in primary CN-AML. Nibourel et al,[11] who focused on patients achieving CR, found no differences in DFS or OS between 12 *TET2*-mutated and 42 *TET2*-wt patients with CN-AML. In another study[3] of 93 patients with primary or secondary AML, *TET2* mutations were associated with inferior OS, but this analysis did not consider cytogenetics or other potentially confounding variables.

With a growing number of gene mutations being identified in CN-AML, it becomes increasingly important to consider individual markers in their genetic context. The prognostic impact of one mutation may vary depending on the presence or absence of other molecular markers (eg, *NPM1* mutations are associated with favorable outcomes particularly in the absence of *FLT3*-ITD).[14,23,43,44] For clinical decision making, risk stratification algorithms integrating prognostic information conveyed by a panel of molecular markers are needed. Recently, the ELN proposed a risk stratification scheme for

AML based on cytogenetics and three established molecular prognostic markers.[16] The relative prognostic importance of novel molecular markers should not only be evaluated in multivariable models but also needs to be investigated in the context of accepted classification systems. This approach allows judging the potential of new markers to be readily applicable in the clinic and to be incorporated in risk-adapted management decisions for patients with AML.

The ELN classification categorizes patients with CN-AML into a favorable-risk group (*CEBPA*-mutated and/or mutated *NPM1* without *FLT3*-ITD; ≈ 45% of patients with CN-AML[14]) and a less-favorable intermediate-I–risk group (all remaining patients with CN-AML). To examine whether *TET2* mutations can be used to improve this widely accepted classification, we studied their prognostic value within the ELN risk categories of CN-AML. Among ELN favorable-risk patients, those with *TET2* mutations had lower response rates and a higher risk of relapse or death than *TET2*-wt patients. The favorable-risk category comprises two molecularly defined subgroups of CN-AML: *CEBPA*-mutated patients and those with mutated *NPM1* without *FLT3*-ITD. Notably, when separately evaluating these two genotypes, we found that *TET2* mutations were associated with inferior outcomes in both subgroups (Data Supplement). In contrast, we observed no significant prognostic impact of *TET2* mutations in the ELN intermediate-I–risk category or any of its molecular subsets (Data Supplement), although some of these exploratory subgroup analyses were limited by small sample sizes.

Our study included patients across a broad range of ages, potentially introducing bias in our survival analyses. However, in multivariable analyses adjusting for age group and other variables, *TET2* mutations remained associated with inferior EFS, lower CR rates, and shorter DFS among favorable-risk patients. *FLT3*-ITD, *NPM1,* and *CEBPA* mutations do not appear in our multivariable models as individual variables, since they are already incorporated in the definition of the ELN risk categories. We did not separately consider *FLT3*-ITD:wt allelic ratio because the ELN favorable-risk group included only 19 patients with *FLT3*-ITD (all *CEBPA*-mutated) and because the adverse impact of a high allelic ratio has been found in younger, but not in older patients.[45]

Along with *TET2* mutations, *WT1* mutations were associated with shorter EFS and DFS after adjustment for ELN risk category and other covariables. In our model for CR rate, *TET*2 mutations were the only gene mutations providing additional prognostic information beyond ELN risk category. To the best of our knowledge, our study is the first to evaluate a novel molecular marker in CN-AML in the context of the current ELN classification and to suggest that *TET2* (and possibly *WT1*) mutations are candidate markers for a refined CN-AML classification scheme.

The function of the TET2 protein is not fully understood. Its paralogue TET1 was recently shown to catalyze conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA, suggesting a role of TET proteins in epigenetic regulation.[46,47] To gain insights into the biologic consequences of *TET2* mutations, we studied genome-wide gene- and microRNA-expression signatures. Among favorable-risk patients, we identified a *TET2* mutation-associated gene-expression signature comprising 136 differentially expressed named genes. *TET2* mutations were associated with deregulation of genes involved in stem-cell self-renewal, cell cycle control, and cytokine and growth factor signaling, which may help explain their adverse prognostic impact. In contrast, no significant gene-expression signature could be identified in the intermediate-I–risk cohort.

MicroRNA-expression profiling revealed distinct *TET2* mutation-associated signatures in both the favorable-risk and intermediate-I–risk groups, involving several microRNAs implicated in CN-AML and other hematologic malignancies. Interestingly, the microRNA-expression signatures in the two ELN risk groups did not overlap. Apparently, *TET2* mutations affect different sets of microRNAs and genes in favorable-risk and intermediate-I–risk patients with CN-AML. These differences show that the ELN classification identifies distinct biologic subsets of CN-AML and corresponds to our finding that the prognostic impact of *TET2* mutations also varies between the two ELN risk categories. Together, our results suggest that the biologic and clinical consequences of *TET2* mutations in CN-AML differ between the ELN risk groups, although the mechanisms underlying this differential impact remain unclear.

In conclusion, *TET2* mutations occur in > 20% of adult patients with primary CN-AML and may be useful for improving genetic risk classification schemes, such as the ELN classification, which are increasingly used in the clinic to guide personalized treatment decisions. The current ELN guidelines generally do not recommend allogeneic transplantation for favorable-risk patients in first CR,[16] and none of our patients received such treatment as postremission therapy. However, our results suggest that a subset of these patients, identified by mutated *TET2*, do not do well with conventional postremission treatment. If our results are corroborated, some of these patients might be considered candidates for alternative therapeutic approaches.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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