VOLUME 30 · NUMBER 25 · SEPTEMBER 1 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

RUNX1 Mutations Are Associated With Poor Outcome in Younger and Older Patients With Cytogenetically Normal Acute Myeloid Leukemia and With Distinct Gene and MicroRNA Expression Signatures

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Submitted November 22, 2011; accepted March 27, 2012; published online ahead of print at www.jco.org on July 2, 2012.

Supported in part by the National Cancer Institute (Grants No. CA101140, CA114725, CA140158, CA31946, CA33601, CA16058, CA77658, and CA129657), the Coleman Leukemia Research Foundation, the Deutsche Krebshilfe–Dr Mildred Scheel Cancer Foundation (H.B.), and the Conquer Cancer Foundation (J.H.M.).

Both G.M. and C.D.B. contributed equally to this work.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/12/3025-3109/$20.00

DOI: 10.1200/JCO.2011.40.6652

ABSTRACT

Purpose
To determine the association of *RUNX1* mutations with therapeutic outcome in younger and older patients with primary cytogenetically normal acute myeloid leukemia (CN-AML) and with gene/microRNA expression signatures.

Patients and Methods
Younger (< 60 years; n = 175) and older (≥ 60 years; n = 225) patients with CN-AML treated with intensive cytarabine/anthracycline-based first-line therapy on Cancer and Leukemia Group B protocols were centrally analyzed for *RUNX1* mutations by polymerase chain reaction and direct sequencing and for established prognostic gene mutations. Gene/microRNA expression profiles were derived using microarrays.

Results
RUNX1 mutations were found in 8% and 16% of younger and older patients, respectively ($P = .02$). They were associated with *ASXL1* mutations ($P < .001$) and inversely associated with *NPM1* ($P < .001$) and *CEBPA* ($P = .06$) mutations. *RUNX1*-mutated patients had lower complete remission rates ($P = .005$ in younger; $P = .006$ in older) and shorter disease-free survival ($P = .058$ in younger; $P < .001$ in older), overall survival ($P = .003$ in younger; $P < .001$ in older), and event-free survival ($P < .001$ for younger and older) than *RUNX1* wild-type patients. Because *RUNX1* mutations were more common in older patients and almost never coexisted with *NPM1* mutations, *RUNX1* mutation–associated expression signatures were derived in older, *NPM1* wild-type patients and featured upregulation of genes normally expressed in primitive hematopoietic cells and B-cell progenitors, including *DNTT*, *BAALC*, *BLNK*, *CD109*, *RBPMS*, and *FLT3*, and downregulation of promoters of myelopoiesis, including *CEBPA* and *miR*-223.

Conclusion
RUNX1 mutations are twice as common in older than younger patients with CN-AML and negatively impact outcome in both age groups. *RUNX1*-mutated blasts have molecular features of primitive hematopoietic and lymphoid progenitors, potentially leading to novel therapeutic approaches.

J Clin Oncol 30:3109-3118. © 2012 by American Society of Clinical Oncology

INTRODUCTION

Acute myeloid leukemia (AML) is a cytogenetically and molecularly heterogeneous disease characterized by maturation arrest and uncontrolled proliferation of abnormal myeloid precursors. Cytogenetically normal AML (CN-AML) is the largest cytogenetic group among both younger (< 60 years) and older (≥ 60 years) patients with AML and the one that is best characterized molecularly.[1-3] Several recurring mutations with prognostic significance in genes such as *FLT3*,[4,5] *NPM1*,[6,7] *CEBPA*,[8,9] *WT1*,[10,11] *MLL*,[12,13] *IDH1*,[14,15] *IDH2*,[14,15] and *TET2*[16] have been identified in CN-AML. These mutations are increasingly being used both as single markers and in combination to refine the outcome prediction of patients with CN-AML and to stratify them to risk-adapted treatments.[17] In addition to refining prognostication, mutations in some of these genes are associated with distinct gene

and microRNA expression signatures[9,18] that may provide clues regarding mutation-specific leukemogenic mechanisms.

The runt-related transcription factor 1 (*RUNX1*) gene encodes the α-subunit of core binding factor, a heterodimeric transcription factor required for definitive hematopoiesis.[19] Monoallelic germline mutations in this gene occur in rare cases of familial platelet disorder with predisposition to AML,[20,21] and acquired mutations have been identified in myelodysplastic syndromes[22,23] and AML.[24-34] Acquired *RUNX1* mutations have been associated with poor clinical outcome in younger patients with CN-AML[31,32]; however, these mutations were not found to impact outcome in older patients with CN-AML.[31] Moreover, previous studies analyzed patients heterogeneous with regard to cytogenetics,[31-33] AML type (primary or secondary),[32] and treatment received (including allogeneic stem-cell transplantation [alloSCT] in first complete remission [CR1]),[31-33] leaving the prognostic impact and molecular signatures associated with *RUNX1* mutations in patients with primary CN-AML as open questions that remain to be fully explored.

The aims of this study were to investigate the prognostic impact of *RUNX1* mutations in relatively large and well-characterized cohorts of younger and older patients with primary CN-AML treated similarly with intensive cytarabine/anthracycline-based first-line therapy and to assess whether *RUNX1* mutations are associated with specific gene/microRNA expression signatures in CN-AML.

PATIENTS AND METHODS

Patients, Treatment, and Sample Collection

Pretreatment bone marrow or blood samples were obtained from 175 younger patients (age 18 to 59 years) and 225 older patients (age 60 to 83 years) with primary CN-AML, who received intensive cytarabine/anthracycline-based first-line therapy on Cancer and Leukemia Group B trials. Per protocol, no patient received an alloSCT in CR1. For details regarding treatment protocols and sample collection, see the Data Supplement. 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.

Cytogenetic and Mutational Analyses

The diagnosis of CN-AML was based on the analysis of ≥ 20 metaphases in bone marrow specimens subjected to short-term cultures and confirmed by central karyotype review.[35] Exons 3 to 8 of *RUNX1* were amplified from genomic DNA by polymerase chain reaction and analyzed by direct sequencing. All mutations were validated by repeat polymerase chain reaction and sequencing. Patients were also characterized centrally for *FLT3* internal tandem duplication (ITD),[4] *FLT3* tyrosine kinase domain mutations,[36] *MLL* partial tandem duplication,[13,37] *NPM1*,[6,18] *WT1*,[10] *CEBPA*,[9] *IDH1*[14], *IDH2*,[14] *TET2*,[16] *ASXL1*,[38] and *DNMT3A*[39] mutations as previously reported. Methods regarding germline analysis and determination of phase in double-mutant patients are provided in the Data Supplement.

Microarray Experiments

Gene expression profiling was performed using Affymetrix (Santa Clara, CA) oligonucleotide microarrays, and microRNA expression profiling was performed using a custom microarray, as previously reported.[18] Differentially expressed probe sets or probes were identified by comparing *RUNX1*-mutated and *RUNX1* wild-type patients, using univariable significance levels of $P < .001$ for gene expression and $P < .005$ for microRNA expression profiles. For details regarding microarray and gene set analysis, see the Data Supplement.

Statistical Analyses

Baseline characteristics were compared between *RUNX1*-mutated and *RUNX1* wild-type patients using Fisher's exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Definitions of clinical end points (ie, complete remission [CR], disease-free survival [DFS], overall survival [OS], and event-free survival [EFS]) are provided in the Data Supplement. For time-to-event analyses, survival estimates were calculated using the Kaplan-Meier method, and groups 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 Alliance for Clinical Trials in Oncology Statistics and Data Center.

RESULTS

RUNX1 Sequence Variations and Description of Mutations

Of 400 patients, 60 (15%) had at least one *RUNX1* sequence variation. Patients with synonymous variations (n = 3) were considered equivalent to *RUNX1* wild type. Patients with intron sequence variations of more than 10 base pairs from an exon junction (n = 3) were considered unclassifiable between *RUNX1* mutated and *RUNX1* wild type and, therefore, were excluded from the analysis. Patients with the missense variation p.L56S (n = 5) were also excluded because of controversy over whether this is a polymorphism[32,34] or a true mutation.[40] The presence of p.L56S in the germline was confirmed in three of four patients with material available.

The remaining 392 patients were classified as either *RUNX1* wild type (n = 343) or *RUNX1* mutated (n = 49; Table 1; Data Supplement). Mutations were present in 8% of younger (< 60 years) patients (14 of 173 patients) and 16% of older ($\geq$ 60 years) patients (35 of 219 patients; $P = .02$). *RUNX1*-mutated patients had either a single mutation (n = 39) or two distinct mutations (n = 10). Among the latter, mutations were biallelic in all patients with tissue available (n = 6). Five *RUNX1*-mutated patients with single mutations had a homo-/hemizygous pattern, consistent with loss of heterozygosity for the wild-type *RUNX1* allele. Among *RUNX1*-mutated patients with germline material available (n = 35), mutations were rarely found in the germline (n = 3). Coding sequence mutations were missense (n = 24), nonsense (n = 4), frameshift (n = 22), and in-frame insertions/deletions (n = 2). A list of the specific mutations and germline status in each patient is provided in the Data Supplement.

Association of RUNX1 Mutations With Other Clinical and Molecular Characteristics

In the combined cohort of younger and older patients, *RUNX1* mutations were associated with a higher median age ($P <$ .001) and lower hemoglobin ($P = .01$), WBC count ($P = .04$), and blood blasts ($P = .006$; Table 1). *RUNX1*-mutated patients harbored *ASXL1* mutations more frequently ($P < .001$) and *NPM1* ($P < .001$) and *CEBPA* mutations ($P = .06$) less frequently than *RUNX1* wild-type patients.

Impact of RUNX1 Mutation Status on Treatment Outcome

Among all patients, the median follow-up for patients alive was 7.8 years (range, 2.3 to 13.1 years). *RUNX1*-mutated patients, compared with *RUNX1* wild-type patients, had an inferior CR rate (47% *v*

Table 1. Comparison of Demographics and Clinical and Molecular Characteristics by *RUNX1* Mutation Status in All Patients (N = 392) With Primary Cytogenetically Normal Acute Myeloid Leukemia

Demographic or Characteristic	*RUNX1*-Mutated Patients (n = 49) No.	%	*RUNX1* Wild-Type Patients (n = 343) No.	%	*P*
Age, years					< .001
Median	68		61		
Range	30-81		18-83		
Age group, years					.02
< 60	14	29	159	46	
≥ 60	35	71	184	54	
Male sex	28	57	169	49	.36
Race					.29
White	46	96	308	90	
Nonwhite	2	4	33	10	
Hemoglobin, g/dL					.01
Median	8.8		9.5		
Range	4.6-11.6		4.8-15.0		
Platelet count, × 10^9/L					.46
Median	77		60		
Range	8-309		4-850		
WBC count, × 10^9/L					.04
Median	21		28.6		
Range	0.9-434.1		0.9-450.0		
Blood blasts, %					.006
Median	37		58		
Range	0-96		0-99		
Bone marrow blasts, %					.54
Median	65		68		
Range	7-97		4-97		
Extramedullary involvement	11	23	90	27	.73
NPM1					< .001
Mutated	3	6	238	69	
Wild type	46	94	105	31	
FLT3-ITD					.87
Present	17	35	125	36	
Absent	32	65	218	64	
CEBPA					.06
Mutated	3	6	60	17	
Single mutated	2		26		
Double mutated	1		34		
Wild type	46	94	283	83	
ELN Genetic Group*					< .001
Favorable	4	8	186	54	
Intermediate-I	45	92	157	46	
FLT3-TKD					.15
Present	1	2	29	9	
Absent	47	98	303	91	
WT1					.61
Mutated	5	12	34	10	
Wild type	43	88	309	90	
TET2					1.00
Mutated	11	23	79	23	
Wild type	37	77	260	77	
ASXL1					< .001
Mutated	17	35	21	6	
Wild type	31	65	321	94	
DNMT3A					.15
Mutated	12	24	121	36	
R882	8	16	78	23	
Non-R882	4	8	43	13	
Wild type	37	76	214	64	
MLL-PTD					1.00
Present	3	7	19	6	
Absent	42	93	286	94	
IDH1					.24
R132	3	6	42	12	
Wild type	46	94	299	88	
IDH2					.84
IDH2	8	16	62	18	
R140	6		51		
R172	2		11		
Wild type	41	84	279	82	

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

*Favorable is defined as *CEBPA* mutated or *FLT3*-ITD negative and *NPM1* mutated. Intermediate-I is defined as *CEBPA* wild type and *FLT3*-ITD positive and *NPM1* mutated, *FLT3*-ITD negative and *NPM1* wild type, or *FLT3*-ITD positive and *NPM1* wild type.[17]

77%, respectively; $P < .001$) and shorter DFS ($P < .001$; 5-year DFS, 0% *v* 28%, respectively), OS ($P < .001$; 5-year OS, 2% *v* 30%, respectively), and EFS ($P < .001$; 5-year EFS, 0% *v* 22%, respectively; Table 2). Because consolidation treatment differed in intensity for younger and older patients, their outcomes were also analyzed separately. In younger patients, *RUNX1* mutations, compared with *RUNX1* wild-type status, were associated with an inferior CR rate (50% *v* 84%, respectively; $P = .005$) and worse DFS ($P = .06$; 5-year DFS, 0% *v* 42%, respectively), OS ($P = .003$; 5-year OS, 7% *v* 47%, respectively), and EFS ($P < .001$; 5-year EFS, 0% *v* 36%, respectively; Fig 1; Table 2). In older patients, *RUNX1* mutations, compared with *RUNX1* wild-type status, were also associated with an inferior CR rate (46% *v* 71%, respectively; $P = .006$) and worse DFS ($P < .001$; 3-year DFS, 0% *v* 18%, respectively), OS ($P < .001$; 3-year OS, 0% *v* 22%, respectively), and EFS ($P < .001$; 3-year EFS, 0% *v* 13%, respectively; Fig 2; Table 2).

Because *RUNX1* and *NPM1* mutations are nearly mutually exclusive and prognostically favorable *NPM1* mutations are prevalent in CN-AML (61% in our cohort), the prognostic impact of *RUNX1* mutations was assessed in *NPM1* wild-type patients separately. In younger *NPM1* wild-type patients, *RUNX1* mutations, compared with *RUNX1* wild-type status, were associated with a lower CR rate (50% *v* 84%, respectively; $P = .02$), borderline worse DFS ($P = .10$; 5-year DFS, 0% *v* 38%, respectively) and worse OS ($P = .04$; 5-year OS, 8% *v* 40%, respectively) and EFS ($P = .004$; 5-year EFS, 0% *v* 34%, respectively; Data Supplement). In older *NPM1* wild-type patients, *RUNX1* mutations, compared with *RUNX1* wild-type status, were associated with a shorter DFS ($P = .005$; 3-year DFS, 0% *v* 14%,

Table 2. Outcomes According to *RUNX1* Mutation Status in Primary Cytogenetically Normal Acute Myeloid Leukemia

End Point	*RUNX1* Mutated	*RUNX1* Wild Type	*P**	OR†	95% CI	HR‡	95% CI
All patients (N = 392)							
No. of patients	49	343					
CR			< .001	0.27	0.14 to 0.49		
No. of Patients	23	264					
%	47	77					
Disease-free survival			< .001			2.43	1.57 to 3.76
Median, years	0.6	1.1					
Disease free at 5 years, %	0	28					
95% CI	—	23 to 33					
Overall survival			< .001			2.32	1.69 to 3.18
Median, years	0.9	1.4					
Alive at 5 years, %	2	30					
95% CI	0 to 9	25 to 35					
Event-free survival			< .001			2.41	1.76 to 3.28
Median, years	0.2	0.8					
Event free at 5 years, %	0	22					
95% CI	—	18 to 26					
Younger patients (n = 173)							
No. of patients	14	159					
CR			.005	0.19	0.06 to 0.58		
No. of Patients	7	134					
%	50	84					
Disease-free survival			.06			2.09	0.96 to 4.54
Median, years	1.2	1.9					
Disease free at 5 years, %	0	42					
95% CI	—	33 to 50					
Overall survival			.003			2.34	1.30 to 4.20
Median, years	1.1	2.8					
Alive at 5 years, %	7	47					
95% CI	0 to 28	39 to 54					
Event-free survival			< .001			2.56	1.46 to 4.49
Median, years	0.3	1.1					
Event free at 5 years, %	0	36					
95% CI	—	28 to 43					
Older patients (n = 219)							
No. of patients	35	184					
CR			.006	0.35	0.17 to 0.73		
No. of Patients	16	130					
%	46	71					
Disease-free survival			< .001			2.61	1.52 to 4.50
Median, years	0.5	0.9					
Disease free at 3 years, %	0	18					
95% CI	—	12 to 25					
Overall survival			< .001			2.25	1.53 to 3.30
Median, years	0.8	1.3					
Alive at 3 years, %	0	22					
95% CI	—	16 to 28					
Event-free survival			< .001			2.17	1.49 to 3.18
Median, years	0.2	0.7					
Event free at 3 years, %	0	13					
95% CI	—	8 to 18					

Abbreviations: CR, complete remission; HR, hazard ratio; OR, odds ratio.
*P value is from Fisher's exact test for CR rate and from the log-rank test for time-to-event end points.
†An OR of < 1 or > 1 means a lower or higher CR rate, respectively, for *RUNX1*-mutated patients compared with *RUNX1* wild-type patients.
‡An HR of > 1 or < 1 corresponds to a higher or lower risk of an event, respectively, for *RUNX1*-mutated patients compared with *RUNX1* wild-type patients.

respectively), OS ($P = .002$; 3-year OS, 0% *v* 13%, respectively), and EFS ($P = .04$; 3-year EFS, 0% *v* 7%, respectively; Data Supplement). *RUNX1* mutations did not impact CR rate in older *NPM1* wild-type patients.

We next examined the prognostic impact of *RUNX1* mutations within the context of other molecular markers increasingly being used in the clinic. Recently, an international expert panel on behalf of the European LeukemiaNet (ELN) recommended a classification scheme

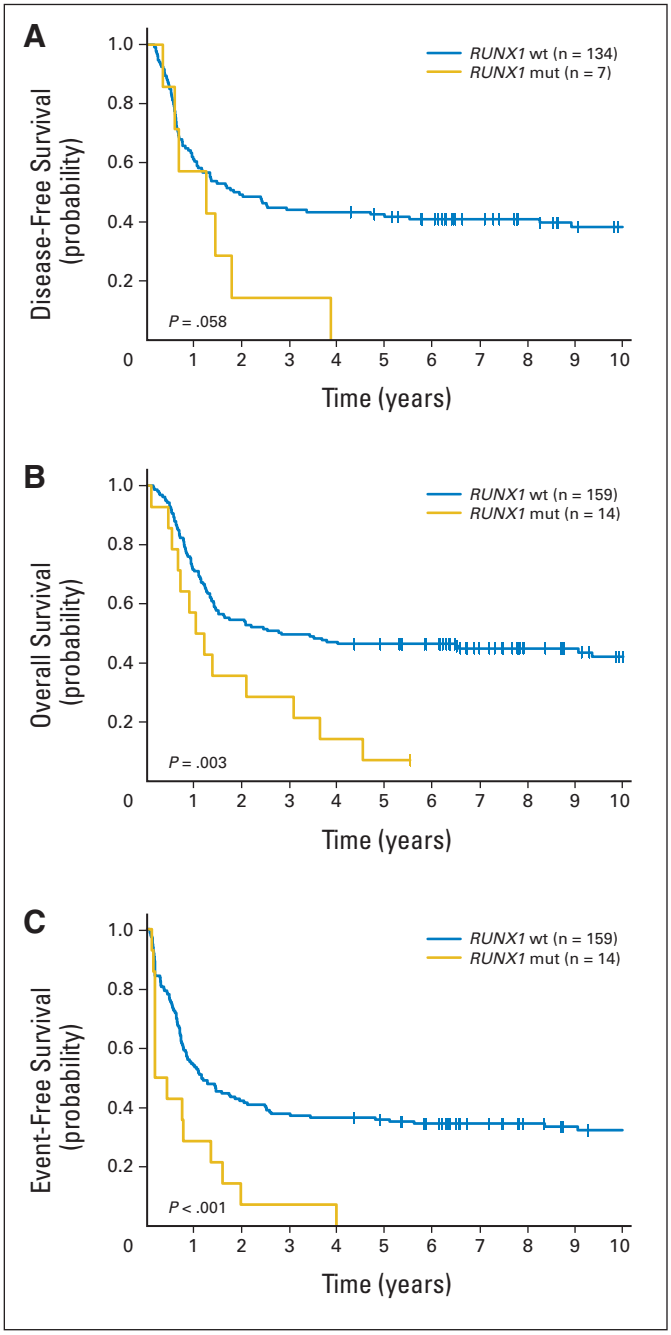

Fig 1. (A) Disease-free survival, (B) overall survival, and (C) event-free survival of patients younger than age 60 years with cytogenetically normal acute myeloid leukemia according to *RUNX1* mutation status. *RUNX1* mut, *RUNX1* mutated; *RUNX1* wt, *RUNX1* wild type.

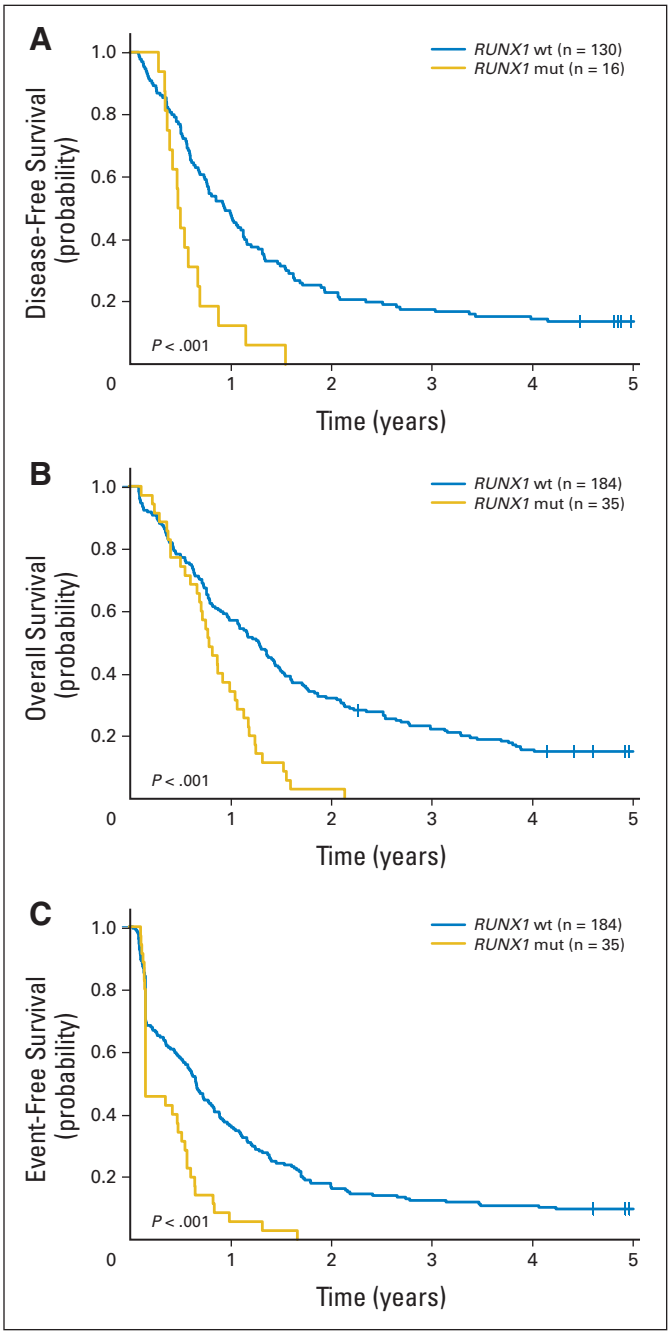

Fig 2. (A) Disease-free survival, (B) overall survival, and (C) event-free survival of patients age 60 years and older with cytogenetically normal acute myeloid leukemia according to *RUNX1* mutation status. *RUNX1* mut, *RUNX1* mutated; *RUNX1* wt, *RUNX1* wild type.

for CN-AML based on *NPM1*, *CEBPA*, and *FLT3*-ITD mutations.[17] According to this classification, patients with CN-AML are assigned to either the Favorable Genetic Group (*NPM1* mutated without *FLT3*-ITD and/or *CEBPA* mutated) or the Intermediate-I (all remaining patients) Genetic Group. Because *RUNX1* mutations rarely coexist with either *CEBPA* or *NPM1* mutations, *RUNX1*-mutated patients fall almost exclusively into the ELN Intermediate-I Group (Table 1). Even within this already high-risk molecular group, *RUNX1* mutations, compared with *RUNX1* wild-type status, were associated with an inferior CR rate in younger patients (50% *v* 77%, respectively; $P = .05$) and worse EFS in older patients ($P = .03$; 3-year EFS, 0% *v* 6%, respectively; Data Supplement).

Because there is an association between *RUNX1* and *ASXL1* mutations, and *ASXL1* mutations have a negative impact on outcome in older patients,[38] we assessed their relative prognostic impact in this age group. Among *ASXL1* wild-type patients, *RUNX1*-mutated patients, compared with *RUNX1* wild-type patients, had an inferior CR rate (40% *v* 74%, respectively; $P = .003$), DFS ($P = .007$), OS

($P < .001$), and EFS ($P < .001$; Data Supplement). Among *RUNX1* wild-type patients, *ASXL1*-mutated patients, compared with *ASXL1* wild-type patients, had an inferior CR rate (42% *v* 74%, respectively; $P = .01$), borderline inferior DFS ($P = .12$), and inferior OS ($P = .01$) and EFS ($P < .001$; Data Supplement). Older patients harboring both mutations had a similar prognosis to those harboring either mutation alone (Data Supplement).

Multivariable Analyses

To assess whether *RUNX1* mutations provide additional prognostic value in the context of other clinical and molecular prognosticators, we constructed multivariable models including all patients (N = 392; Table 3). *RUNX1*-mutated patients were four times less likely to achieve a CR ($P < .001$), after adjustment for *IDH2* mutation status ($P = .04$), WBC ($P = .004$), and age group ($P = .02$). For DFS, patients with *RUNX1* mutations were more than twice as likely to experience relapse or die ($P < .001$) after adjustment for *FLT3*-ITD status ($P < .001$), WBC ($P = .02$), and age group ($P < .001$). For OS, *RUNX1*-mutated patients had a 65% higher risk of death ($P < .001$) after adjustment for *FLT3*-ITD status ($P < .001$), *WT1* mutation status ($P < .001$), WBC ($P = .02$), and age group ($P < .001$). For EFS, *RUNX1*-mutated patients were more than twice as likely to experience an event ($P < .001$) after adjustment for *FLT3*-ITD status ($P < .001$), *WT1* mutation status ($P = .04$), WBC ($P = .006$), and age group ($P < .001$).

Gene and MicroRNA Expression Signatures Associated With RUNX1 Mutations in CN-AML

To gain molecular insight into *RUNX1*-mutated CN-AML, gene/microRNA expression signatures were derived. We focused only on older *NPM1* wild-type patients because *RUNX1* mutations are more common in this age group and almost never coexist with *NPM1* mutations. This enabled the derivation of gene/microRNA expression signatures without interference from *NPM1* mutations, which are themselves associated with strong gene/microRNA expression signatures.[18] This yielded a signature composed of 484 probe sets representing 245 named genes differentially expressed between *RUNX1*-mutated (n = 31) and *RUNX1* wild-type (n = 45) patients (Fig 3A; Data Supplement).

Genes overexpressed in early hematopoietic stem/progenitor cells (HSPCs) relative to more mature progenitors, including *BAALC*, *CD109*, *P2RY14*, *CRHBP*, *NPTX2*, *GNAI1*, *HGF*, and *FHL1*, were upregulated in *RUNX1*-mutated patients.[39-44] Genes upregulated (*SETBP1*, *RBPMS*, and *SLC37A3*) and downregulated (*CCNA1* and *RNASE3*) in AML stem cells relative to AML progenitors were similarly deregulated in the *RUNX1*-mutated signature.[45,46] Several genes normally expressed in early lymphoid precursors,[47] including *DNTT*, *BLNK*, *IGHM*, *IRF8*, *FOXO1*, *FLT3*, and genes encoding multiple class II major histocompatibility complex molecules, were also upregulated in *RUNX1*-mutated patients, whereas *CEBPA*, a key promoter of granulopoiesis, and *AZU1*, *MPO*, and *CTSG*, components of neutrophil granules, were downregulated. Overexpression of genes known to negatively impact prognosis in CN-AML,[48-50] including *MN1* and the aforementioned *BAALC*, was also part of the *RUNX1*-mutated signature. Gene set analysis was performed to identify sets of genes representing canonical biologic pathways deregulated in patients with mutated *RUNX1*. Eighteen gene sets were significantly deregulated (Data Supplement), 16 of which were upregulated in *RUNX1*-mutated patients compared with *RUNX1* wild-type patients. *RUNX1*-mutated blasts were more likely to overexpress genes involved in platelet activation, vascular endothelial growth factor signaling, G protein–coupled receptor signaling, and intestinal immune function relative to *RUNX1* wild-type blasts.

Eight microRNAs were differentially expressed between *RUNX1*-mutated and *RUNX1* wild-type patients (Fig 3B; Data Supplement). Two members of the *let-7* tumor suppressor family, which represses self-renewal and promotes differentiation of stem cells,[51] were downregulated, as was *miR-223*, a positive regulator of granulopoiesis.[52] *MiR-99a* and *miR-100*, microRNAs upregulated in AML with inv(16)(p13q22),[53] were also downregulated, and *miR-211*, *miR-220*, and *miR-595*, with unknown functions in leukemogenesis, were upregulated in *RUNX1*-mutated blasts.

Table 3. Multivariable Analysis of Outcome According to the *RUNX1* Mutation Status in All Patients (N = 392) With Primary Cytogenetically Normal Acute Myeloid Leukemia

	Complete Remission			Disease-Free Survival			Overall Survival			Event-Free Survival		
Variable	OR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
RUNX1: mutated *v* wild type	0.25	0.13 to 0.47	< .001	2.34	1.49 to 3.66	< .001	1.65*	1.13 to 2.42	< .001	2.27	1.65 to 3.12	< .001
IDH2: mutated *v* wild type	0.55	0.30 to 0.98	.04	—	—	—	—	—	—	—	—	—
FLT3-ITD: present *v* absent	—	—	—	2.50*	1.83 to 3.42	< .001	1.56	1.22 to 1.99	< .001	1.57	1.27 to 1.95	< .001
WT1: mutated *v* wild type	—	—	—	—	—	—	1.99	1.39 to 2.84	< .001	1.44	1.02 to 2.01	.04
WBC: continuous, 50-unit increase	0.74	0.60 to 0.91	.004	1.17	1.03 to 1.32	.02	1.10	1.01 to 1.19	.02	1.13	1.04 to 1.23	.006
Age group: ≥ 60 *v* < 60 years	0.55	0.33 to 0.91	.02	2.19	1.67 to 2.88	< .001	2.46	1.93 to 3.15	< .001	1.80	1.46 to 2.22	< .001

NOTE. An OR of > 1 or < 1 corresponds to a higher or lower odds, respectively, of achieving a complete remission for higher values of continuous variables and the first level listed of a dichotomous variable. For time-to-event end points, an HR of > 1 or < 1 corresponds to a higher or lower risk, respectively, for higher values of continuous variables and the first level listed of a dichotomous variable. Variables were considered for inclusion in the multivariable models if they had a univariable $P < .20$. See Data Supplement for a full list of variables evaluated in univariable analysis. Variables with insufficient overlap with *RUNX1* mutations could not be evaluated by multivariable models investigating the impact of *RUNX1* mutations. These were *NPM1*, *CEBPA*, and *ASXL1* mutations, *MLL* partial tandem duplication, and the European LeukemiaNet Genetic Groups. On the basis of univariable analyses, variables considered in the model for complete remission were *RUNX1* mutations, *WT1* mutations, *IDH2* mutations, hemoglobin, platelet count, WBC, and age group (≥ 60 *v* < 60 years). Variables considered in the model for disease-free and overall survival were *RUNX1* mutations, presence of *FLT3*-ITD, *WT1* mutations, WBC, and age group (≥ 60 *v* < 60 years).

Abbreviations: HR, hazard ratio; ITD, internal tandem duplication; OR, odds ratio.

*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 HR estimate is provided at 6 months.

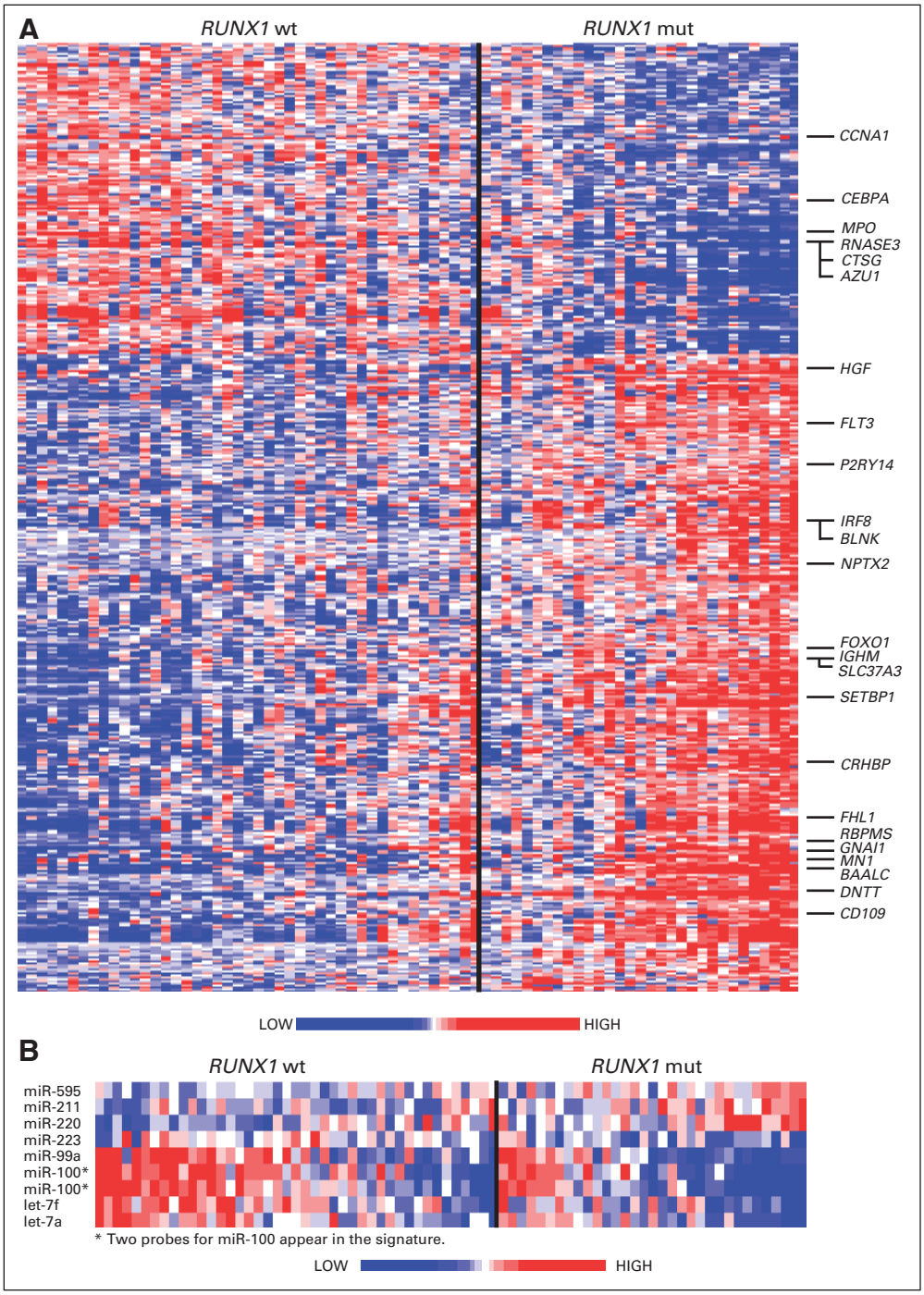

Fig 3. (A) Heat map of the gene expression signature associated with *RUNX1* mutations (mut) in older patients with *NPM1* wild-type (wt) status. Upregulated and downregulated genes mentioned in the text are indicated. (B) Heat map of the microRNA expression signature associated with *RUNX1* mutations in older patients with *NPM1* wild-type status.

DISCUSSION

The identification of novel, prognostically relevant molecular markers is of great importance in CN-AML, because this is the largest cytogenetic subset in both younger and older patients[2] and current molecular classification schemes do not fully capture the heterogeneity in outcome of these patients. Once a new marker is identified, its prognostic impact should be validated in both younger and older patients with CN-AML separately, because disease biology, treatment options, and outcomes differ between these age groups. Although prior studies have demonstrated a negative prognostic impact of *RUNX1* mutations on EFS in younger patients with CN-AML,[31,32] these mutations had no impact on outcome in older patients with CN-AML.[31] Consequently, our study was designed to more fully explore how *RUNX1* mutations impact on the prognosis of both younger and older patients with CN-AML and on global gene/microRNA expression.

We demonstrate that *RUNX1* mutations occur not infrequently in older patients with CN-AML. To our knowledge, our study is the first to report a prevalence of *RUNX1* mutations (16%) in a cohort of older patients with CN-AML. We confirm previous reports that

RUNX1 mutations occur less frequently in younger patients with CN-AML[32] and rarely coexist with either *NPM1* or *CEBPA* mutations[31-33] in either age group. This distinguishes *RUNX1*-mutated CN-AML primarily as a subset of *NPM1* wild-type/*CEBPA* wild-type disease; among patients with wild-type *NPM1* and *CEBPA*, *RUNX1* mutations are relatively frequent, occurring in 38% and 44% of younger and older patients, respectively.

An important question is which *RUNX1* sequence variations represent true, disease-associated *RUNX1* mutations. We suspect that the germline *RUNX1* mutations in our study are disease associated because they involve functional domains and have not been described previously as polymorphisms. In the case of p.L56S, there is evidence both in our study and in a study by Gaidzik et al[32] that its presence is not always consistent between leukemic blasts and germline cells; thus, this variation can be disease associated. However, given the relatively few p.L56S cases in our study, we feel that the issue of whether this variant is truly a somatic disease allele or a polymorphism is still unresolved. Although the patients with p.L56S were excluded from our formal outcome analyses, inclusion of these patients in the *RUNX1*-mutated group did not change the overall results or conclusions (data not shown).

Our study shows that *RUNX1* mutations portend a worse prognosis in both younger and older patients with CN-AML. To our knowledge, we demonstrate for the first time that in older patients with CN-AML, *RUNX1* mutations are associated with a lower CR rate and shorter DFS, OS, and EFS relative to *RUNX1* wild-type patients. Coupled with the high risk of treatment-related complications in older patients receiving intensive chemotherapy, these patients should be strongly considered for novel therapeutic approaches. The negative impact of *RUNX1* mutations on EFS in the younger patients of our study confirms findings of others,[32,31] who also found worse EFS associated with *RUNX1* mutations in this age group. Unique to our study is the negative prognostic impact of *RUNX1* mutations on other outcome end points in younger patients (CR rate, DFS, and OS). The shorter DFS and OS in our study may be related to the lack of alloSCT in CR1, because there is evidence that *RUNX1*-mutated patients achieving CR have a better outcome with postremission alloSCT than chemotherapy.[32,33]

As the number of molecular markers with prognostic impact increases in CN-AML, it becomes difficult to discern whether there is additional prognostic value of a new marker. Because the patients in this study have been extensively characterized for the presence of multiple prognostic markers, the relative impact of *RUNX1* mutations could be determined. *RUNX1* mutations remained prognostic in multivariable models; however, these models were limited by the exclusion of *NPM1* and *CEBPA* mutational status, two well-characterized favorable prognostic markers,[18,54] because of insufficient sample size and overlap with *RUNX1* mutations. To address this limitation, analyses were conducted in *NPM1* wild-type and ELN Intermediate-I Group patient subsets. The fact that *RUNX1* mutations continued to associate with worse outcomes in both of these subsets suggests that they may add prognostic information to molecular markers currently being used in the clinic.

To gain insight into molecular features of *RUNX1*-mutated CN-AML, *RUNX1* mutation–associated gene/microRNA expression signatures were derived in CN-AML for the first time. Several of the most strongly upregulated genes in the signature are also expressed in HSPCs and/or B-cell progenitors, whereas genes normally expressed in myeloid-committed cells are among the most downregulated. These findings are consistent with prior studies demonstrating that *RUNX1* mutations occur more frequently in minimally differentiated (M0) AML[24,55] and are associated with upregulation of B-cell lineage genes in this French-American-British subtype relative to *RUNX1* wild-type AML M0 patients.[56] In fact, a substantial number of the genes in the *RUNX1* mutation–associated signature of our study were also associated with *RUNX1* mutations in AML M0.[56] In contrast, only five genes (2% of our signature) were common between the *RUNX1* mutation–associated gene expression signature reported by Gaidzik et al[32] and our signature. This is possibly because of age-related differences or because of the diverse cytogenetics of the patients in their study, including several patients with t(8;21)(q22;q22), inv(16)(p13q22), or t(15;17)(q24;q21), among others. The *RUNX1* mutation–associated microRNA expression signature also lacks myeloid features, with downregulation of microRNAs normally expressed in either definitive myeloid progenitors (*miR-223*) or distinctly myeloid AML blasts (*miR-99a* and *miR-100*).[52,53]

In summary, our data demonstrate that *RUNX1* mutations occur in a substantial proportion of patients with primary CN-AML with wild-type *NPM1* and *CEBPA*. These mutations are associated with a poor outcome in both younger and older patients with CN-AML treated with intensive induction chemotherapy and not receiving alloSCT in CR1. Thus, patients harboring *RUNX1* mutations warrant strong consideration of up-front novel therapies and/or early alloSCT. *RUNX1* mutation–associated expression signatures are characteristic of HSPCs and lymphoid cells and provide candidate molecules to guide development of novel therapeutic approaches.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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GLOSSARY TERMS

Biallelic: The condition in which both alleles of a gene are mutated.

Cytogenetically normal acute myeloid leukemia (AML): AML with a normal karyotype at diagnosis based on the microscopic analysis of $\geq$ 20 metaphase cells in bone marrow specimens subjected to short-term cultures; approximately 45% of patients with AML are cytogenetically normal.

Gene-expression profiling: Identifying the expression of a set of genes in a biologic sample (eg, blood, tissue) using microarray technology.

Germline mutation: An inherited variation in the lineage of germ cells. Germline mutations can be passed on to offspring.

MicroRNAs: Endogenous noncoding RNAs approximately 22 nucleotides long that regulate gene silencing by post-transcriptional mechanisms such as cleavage or translational repression.

Missense: A change (mutation) in one nucleotide that results in the coding of a different amino acid.

Nonsense: A mutation that changes a codon that codes for an amino acid into a stop codon, therefore terminating translation.

Polymorphism: Genetic polymorphisms are natural variations in the genomic DNA sequence present in greater than 1% of the population, with SNP representing DNA variations in a single nucleotide. SNPs are being widely used to better understand disease processes, thereby paving the way for genetic-based diagnostics and therapeutics.

***RUNX1*:** This gene encodes a subunit of core binding factor, a heterodimeric transcription factor involved in normal hematopoiesis.

