

## ORIGINAL ARTICLE

# MicroRNA Expression in Cytogenetically Normal Acute Myeloid Leukemia

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## ABSTRACT

**BACKGROUND**

A role of microRNAs in cancer has recently been recognized. However, little is known about the role of microRNAs in acute myeloid leukemia (AML).

**METHODS**

Using microRNA expression profiling, we studied samples of leukemia cells from adults under the age of 60 years who had cytogenetically normal AML and high-risk molecular features — that is, an internal tandem duplication in the *fms*-related tyrosine kinase 3 gene (*FLT3*-ITD), a wild-type nucleophosmin (*NPM1*), or both. A microRNA signature that was associated with event-free survival was derived from a training group of 64 patients and tested in a validation group of 55 patients. For the latter, a microRNA compound covariate predictor (called a microRNA summary value) was computed on the basis of weighted levels of the microRNAs forming the outcome signature.

**RESULTS**

Of 305 microRNA probes, 12 (including 5 representing microRNA-181 family members) were associated with event-free survival in the training group ( $P < 0.005$ ). In the validation group, the microRNA summary value was inversely associated with event-free survival ( $P = 0.03$ ). In multivariable analysis, the microRNA summary value remained associated with event-free survival ( $P = 0.04$ ) after adjustment for the allelic ratio of *FLT3*-ITD to wild-type *FLT3* and for the white-cell count. Using results of gene-expression microarray analysis, we found that expression levels of the microRNA-181 family were inversely correlated with expression levels of predicted target genes encoding proteins involved in pathways of innate immunity mediated by toll-like receptors and interleukin-1 $\beta$ .

**CONCLUSIONS**

A microRNA signature in molecularly defined, high-risk, cytogenetically normal AML is associated with the clinical outcome and with target genes encoding proteins involved in specific innate-immunity pathways.

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**I**N ALMOST HALF OF PATIENTS WITH ACUTE myeloid leukemia (AML), no cytogenetic abnormality is detectable in the leukemic cells. Such patients are in an intermediate-risk prognostic category,<sup>1</sup> but among them are subgroups of patients who have molecular markers associated with either a favorable prognosis or an unfavorable prognosis.<sup>2</sup> Gene-expression profiling can also identify subgroups of patients who have cytogenetically normal AML with different outcomes.<sup>3-5</sup>

Patients with internal tandem duplication in the *fms*-related tyrosine kinase 3 gene (*FLT3*-ITD) and those without *FLT3*-ITD but with the wild-type nucleophosmin (*NPM1*) gene are in a high-risk group, whereas patients whose leukemia cells are negative for *FLT3*-ITD but have mutated *NPM1* constitute a low-risk group.<sup>6</sup> The group with a favorable risk profile can be further divided into subgroups on the basis of expression of the *vets* erythroblastosis virus E26 oncogene homologue (*ERG*) gene, with higher *ERG* expression associated with a worse outcome than is lower *ERG* expression.<sup>7</sup>

In this study, we examined microarray microRNA expression profiles in patients with cytogenetically normal AML. MicroRNAs are RNAs that contain 19 to 25 nucleotides and arise by cleavage from 70 to 100 nucleotide precursors. They hybridize to complementary messenger RNA (mRNA) targets and inhibit the translation of mRNA.<sup>8</sup> MicroRNAs have recently been shown to play a role in malignant transformation,<sup>9</sup> and microarray microRNA expression signatures have been associated with aggressive malignant phenotypes in chronic lymphocytic leukemia and solid tumors.<sup>10-12</sup> Little is known, however, regarding the role of microRNAs in the development of AML or its response to treatment.<sup>13</sup>

## METHODS

### PATIENTS AND STUDY DESIGN

Sixty-four adults with cytogenetically normal AML and unfavorable molecular characteristics (i.e., with *FLT3*-ITD, wild-type *NPM1*, or both) who were under the age of 60 years and were treated in the Cancer and Leukemia Group B (CALGB) 19808 study<sup>14</sup> constituted the training group. We analyzed leukemia cells from these patients to seek a microRNA expression signature associated with clinical outcome. Fifty-five similar patients who were enrolled in the CALGB 9621

study<sup>15</sup> constituted the validation group. (For details regarding the treatment regimens in these two studies, see the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).)

At 5 years, the 119 patients who had undergone genetic analysis and 19 patients who were not included in the analysis because of a lack of suitable samples had similar event-free survival (25% and 32%, respectively;  $P=0.95$ ), disease-free survival (31% and 38%,  $P=0.85$ ) and overall survival (31% and 35%,  $P=0.97$ ) (Table 1 of the Supplementary Appendix).

At a central location, we reviewed the results of pretreatment cytogenetic analyses and determined the allelic ratio between *FLT3*-ITD and wild-type *FLT3*, *NPM1* mutations, and expression of *ERG* and the brain and acute leukemia cytoplasmic (*BAALC*) gene, as described previously.<sup>6,7,16-19</sup> The protocols for treatment and cytogenetic and molecular studies were approved by the institutional review board at each participating CALGB institution, and written informed consent was obtained from all patients before enrollment.

Figure 1 illustrates the strategy we used to derive and validate a microRNA signature associated with the clinical outcome and to elucidate its biologic associations by means of gene-expression profiling. The clinical end point for this analysis was event-free survival, which was defined as the interval between study enrollment and removal from the study owing to a lack of complete remission, relapse, or death from any cause, with data censored for patients who did not have an event at the last follow-up visit.

### RNA EXTRACTION AND CHIP HYBRIDIZATION

For microRNA expression profiling, biotinylated first-strand complementary DNA was synthesized from total RNA extracted from pretreatment bone marrow and blood mononuclear cells and was hybridized to microRNA microarray chips.<sup>10</sup> Images of the microRNA microarrays were acquired,<sup>10</sup> and calculation, normalization, and filtering of signal intensity for each microarray spot and batch-effect adjustment were performed (see the Methods section of the Supplementary Appendix). A total of 305 microRNA probes met the filtering criteria for the training group and were included in subsequent analyses. For gene-expression profiling, RNA samples were analyzed with

the use of Affymetrix U133 plus 2.0 GeneChips (Affymetrix).<sup>5,7</sup>

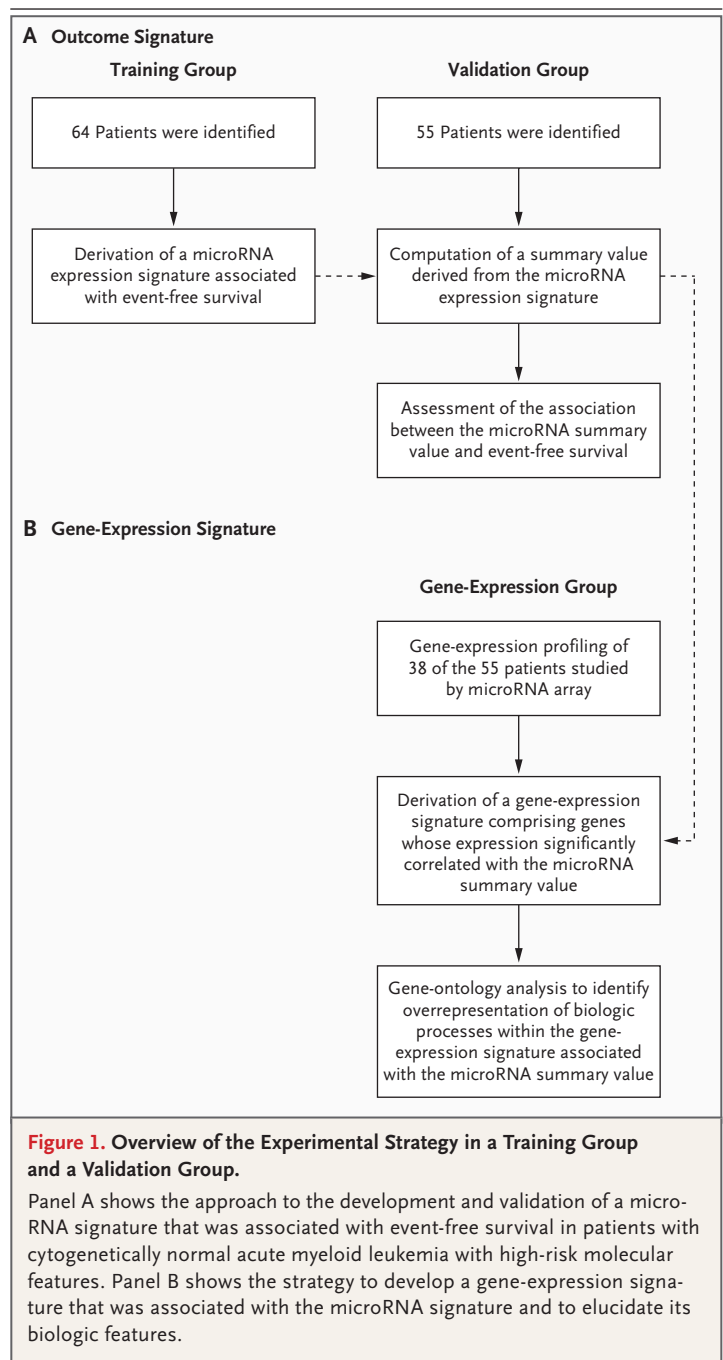
#### STATISTICAL ANALYSIS

The microRNA signature was developed by performing univariable Cox regression analyses that evaluated the association between the batch-adjusted expression values of each microRNA probe and event-free survival in the training group. The set of probes that was significantly associated with event-free survival ( $P < 0.005$ ) constituted a signature that was applied in the validation group. In this group, a compound covariate predictor, which was a linear combination of the expression values for the microRNAs that defined the signature,<sup>20</sup> was computed for each patient sample, and this predictor (called a microRNA summary value) was assessed for its association with event-free survival (for details, see the Supplementary Appendix).<sup>20</sup>

One-way analysis of variance was used to determine whether there was a linear relationship between the microRNA summary value, which was considered as a continuous variable, and other pretreatment variables of interest. Univariable Cox regression analysis was used to evaluate the association between the summary value and event-free survival in the validation group. A multivariable Cox regression model was constructed with the use of a limited backward-selection procedure for event-free survival. Variables that were considered in the model were those that were significant at an alpha level of 0.20 in the univariable models. Variables remaining in the final model were significant at an alpha level of 0.05. The proportional-hazards assumption was checked individually for each variable entered in the multivariable analysis. The Akaike information criterion was used to test whether the final model was the most appropriate fit for the data. Estimates for hazard ratios and corresponding 95% confidence intervals were obtained for each significant outcome factor.

Kaplan–Meier curves were generated for event-free survival in the validation group, with data stratified according to the median microRNA summary value. The median value was used to dichotomize the data for graphic display only; all statistical analyses were performed with the use of continuous microRNA summary values.

Microarray gene-expression profiles from an earlier study<sup>5</sup> were available for 38 patients with



microRNA-expression data in the validation group. Using these expression profiles, we derived a gene-expression signature that correlated with the microRNA summary value. The gene-expression signature was derived as follows: the Pearson correlation coefficient was computed for the correlation between expression of each probe set and the continuous microRNA summary value;

probe sets that correlated significantly with the microRNA summary value ( $P < 0.001$ ) constituted the gene signature.

We used GenMAPP version 2.1 and MAPPFinder version 2.1<sup>21</sup> to assess whether certain terms (as designated by the Gene Ontology project at [www.geneontology.org](http://www.geneontology.org)) were overrepresented among the genes that constituted the signature. An overrepresented term is one that has more associated genes (also referred to as members) in the gene-expression signature than is expected by chance. In our analysis, we considered only terms that were represented by at least five members among the genes that could be analyzed in our microarray-expression database. MAPPFinder uses a permutation procedure to determine overrepresent-

ed terms. An alpha level of 0.005 was used for identifying such terms. All analyses were performed by the CALGB Statistical Center.

## RESULTS

## MicroRNA SIGNATURE AND CLINICAL OUTCOME

In patients with cytogenetically normal AML, those whose leukemia cells had *FLT3*-ITD, wild-type *NPM1*, or both (approximately 65% of the patients) constituted a high-risk group. These patients had a worse outcome than did patients without *FLT3*-ITD and with *NPM1* mutations in leukemia cells ( $P < 0.001$ ). At 5 years, rates of event-free survival were 26% for the high-risk group and 53% for the low-risk group (Fig. 1 of the

**Table 1. Clinical and Molecular Characteristics of the Patients.**

Characteristic	Training Group (N=64)	Validation Group (N=55)
Age — yr		
Median	45	46
Range	19–59	21–59
Male sex — no. (%)	30 (47)	26 (47)
Race — no. (%)*		
White	56 (88)	48 (89)
Nonwhite	8 (12)	6 (11)
Hemoglobin — g/dl		
Median	9.7	9.1
Range	6.4–13.6	4.6–12.9
Platelet count — $\times 10^9$ /liter		
Median	55	54
Range	8–395	7–208
White-cell count — $\times 10^9$ /liter		
Median	21.7	37.3
Range	1.1–210.0	0.9–295.0
Blood blasts — %		
Median	58	68
Range	0–95	0–97
Bone marrow blasts — %		
Median	69	63
Range	21–99	30–90
Extramedullary involvement — no. (%)	12 (19)	15 (27)
Ratio of <i>FLT3</i> -ITD to wild-type <i>FLT3</i> — no. (%)†		
Low	43 (67)	45 (82)
High	21 (33)	10 (18)

Table 1. (Continued.)		
Characteristic	Training Group (N=64)	Validation Group (N=55)
<i>NPM1</i> — no. (%)		
Wild-type	36 (56)	32 (58)
Mutated	28 (44)	23 (42)
<i>BAALC</i> expression — no. (%)‡		
Low	30 (47)	16 (37)
High	34 (53)	27 (63)
<i>ERG</i> expression — no. (%)§		
Low	19 (43)	30 (73)
High	25 (57)	11 (27)
Complete remission — no. (%)		
Relapse — no. (%)	32 (63)	30 (70)
Event-free survival		
Median — yr	1.0	0.8
At 5 yr — % (95% CI)	30 (19–41)	24 (13–35)

\* Race was self-reported. In the validation group, race was reported for 54 patients.

† The ratios of *FLT3* with internal tandem duplication (*FLT3*-ITD) to wild-type *FLT3* alleles were dichotomized at the median (0.78) to define the high-value group and the low-value group.<sup>17</sup>

‡ Values for *BAALC* expression were dichotomized at the median to define the high-value group and the low-value group.<sup>18</sup>

§ Values for *ERG* expression were dichotomized at the median in the training group<sup>7</sup> and at the 75th percentile in the validation group<sup>19</sup> to define patients with high *ERG* expression (which was associated with an increased risk of an event) and low *ERG* expression (which was associated with a decreased risk of an event).

Supplementary Appendix). This result was consistent with the data that have been reported previously.<sup>6</sup> No microRNA probes were found to be associated with outcome in the low-risk group, which was therefore not considered for further analysis (data not shown).

Among the 75 patients with *FLT3*-ITD, wild-type *NPM1*, or both who were enrolled in CALGB 19808, samples for microRNA-expression analyses were available for 64 patients, with a median follow-up of 2.9 years for patients who were still alive with no event. These 64 patients constituted the training group (Table 1). We derived a microRNA signature in which each probe was significantly associated with event-free survival ( $P < 0.005$ ) from this group of patients. The signature contains 12 probes (Table 2). Expression levels of five probes corresponding to microRNAs 181a and 181b were inversely associated with the risk of an event (i.e., lack of complete remission, relapse, or death); expression levels of the remaining seven probes were positively associated with the risk of an event.

#### VALIDATION GROUP

Fifty-five of the 63 patients with *FLT3*-ITD, wild-type *NPM1*, or both who were enrolled in the CALGB 9621 study and had samples available for microRNA analysis constituted the validation group. For this group, the median follow-up of patients who had no event was 7.0 years. The training and validation groups differed significantly with respect to the white-cell count ( $P = 0.01$ ), the percentage of bone marrow and circulating blasts ( $P = 0.05$  for both comparisons), and the proportion of patients with high levels of *ERG* expression by leukemia cells ( $P = 0.008$ ). (High levels of *ERG* expression are associated with decreased event-free survival.<sup>7</sup>) The training group was similar to the validation group with respect to other pretreatment characteristics and clinical outcomes (Table 1).

For each patient in the validation group, we computed a summary value for expression levels of the microRNAs that formed the signature in the training group (Fig. 1A). All statistical analyses for the validation group were performed with

**Table 2. MicroRNA Probes Forming the Outcome Signature in the Training Group.**

Target MicroRNA*	MicroRNA Probe Sequence	Cox Regression Coefficient†	P Value‡
<b>Increased expression associated with decreased risk</b>			
181a	TGAGGTTGCTTCAGTGAACATTCAACGCTGTCGGTGAGTT	-0.61	1.4×10 <sup>-4</sup>
181a	TCAGAGGACTCCAAGGAACATTCAACGCTGTCGGTGAGTT	-0.52	0.002
181b	TTAAAAGGTCAACATCAACATTATTGCTGTCGGTGGGT	-0.57	3.0×10 <sup>-4</sup>
181b	CTGATGGCTGCACTCAACATTATTGCTGTCGGTGGGTTT	-0.41	0.001
181b	AACATTATTGCTGTCGGTGGGTTGAACTGTGTGGACAAG	-0.40	0.004
<b>Increased expression associated with increased risk</b>			
124	TTAAGGCACGCGGTGAATGCCAAGAGCGGAGCCTACGGCT	0.97	0.004
128-1	TTGGATTCGGGGCCGTAGCACTGTCTGAGAGGTTTACATT	1.73	0.002
194	TGTAACAGCAACTCCATGTGGACTGTGTACCAATTTCCAG	1.02	0.004
219-5p	ATTGTCCAAACGCAATTCTCGAGTCTATGGCTCCGGCCGA	1.75	7.1×10 <sup>-5</sup>
220a	TGTGGCATTGTAGGGCTCCACACCGTATCTGACACTTTGG	0.77	0.001
320	GAGTCGGGAAAAGCTGGGTTGAGAGGGCGAAAAGGATGA	1.40	0.003
320	GAAAAGCTGGGTTGAGAGGGCGAAAAGGATGAGGTGACT	1.25	0.003

\* Multiple probes representing the same microRNA were included on the microarray chips used in the analysis.

† Cox regression models were used to assess the association between individual microRNA probes and event-free survival.

The 12 probes with a significant association ( $P < 0.005$ ) constituted the microRNA outcome signature. A negative regression coefficient (which corresponds to a hazard ratio of  $< 1$ ) indicates that increased expression was associated with a decreased risk of an event (i.e., lack of complete remission, relapse, or death), and a positive coefficient (which corresponds to a hazard ratio of  $> 1$ ) indicates that increased expression was associated with an increased risk of an event.

‡ P values show the significance of the Cox regression coefficient and were calculated with the use of the Wald test.

the use of the microRNA summary value as a continuous variable. The microRNA summary value in the validation group was inversely associated with the percentage of circulating blasts ( $P = 0.004$ ) and was positively associated with *ERG* expression ( $P = 0.04$ ) (Table 2 of the Supplementary Appendix). The microRNA summary value was also inversely associated with event-free survival ( $P = 0.03$ ). To display the relation between the microRNA summary value and the clinical outcome, the validation group was dichotomized at the median microRNA summary value (Fig. 2). The estimated 5-year event-free survival rate was 36% for patients with microRNA summary values above the median and 11% for those with values below the median. In a multivariable model, the microRNA summary value as a continuous variable was associated with event-free survival ( $P = 0.04$ ), even after adjustment for the allelic ratio of *FLT3*-ITD to wild-type *FLT3* ( $P = 0.02$ ) and for the white-cell count ( $P = 0.04$ ) (Table 3).

With regard to other molecular markers, *BAALC* expression and *NPM1* mutations did not meet the statistical criteria for inclusion in the multivariable models, and the number of patients with available data regarding *ERG* expression was too small to draw conclusions about an interaction between microRNA summary values and *ERG* expression levels.

#### CORRELATION WITH GENE EXPRESSION

Of the 12 microRNA probes in the signature of the training group, 5 represented members of the microRNA-181 family. This family is expressed at relatively low levels in undifferentiated hematopoietic precursor cells,<sup>22</sup> and expression of microRNA 181a has been associated with AML.<sup>13</sup> Among other microRNAs in the signature, microRNAs 124, 128, and 219 have been associated with neuronal differentiation,<sup>23,24</sup> whereas definitive targets or functions for microRNAs 194, 220, and 320 are unknown.

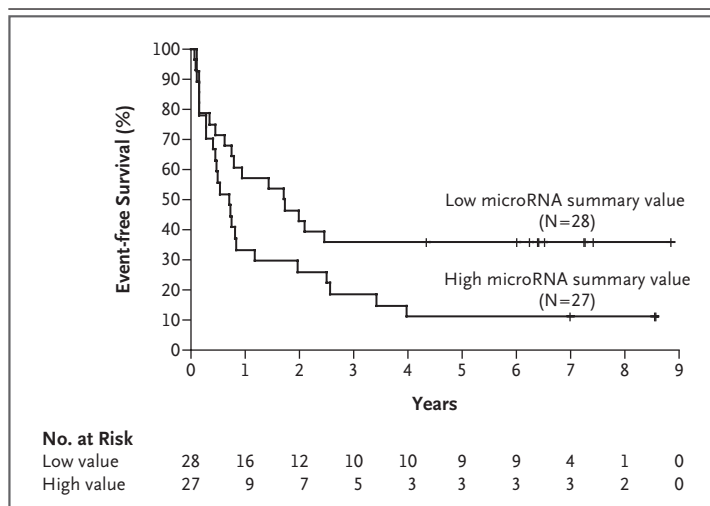
On the basis of the principle that microRNAs

regulate gene expression, we investigated whether the microRNA summary value correlated with expression of genes that were assessed in Affymetrix microarrays. Specifically, we sought a relation between expression of the microRNA members of the outcome signature and gene expression in AML (see the Supplementary Appendix). We included in this analysis 38 patients in the validation group for whom microarray gene-expression profiles were available in our database<sup>5</sup> (Fig. 1B). We found that expression levels of 452 genes correlated significantly with the microRNA summary value ( $P < 0.001$ ) (Table 3 of the Supplementary Appendix).

Increased microRNA summary values were associated with the increased expression of genes involved in mechanisms of innate immunity, including genes encoding toll-like receptors (*TLR2*, *TLR4*, and *TLR8*)<sup>25</sup> and those encoding interleukin-1 $\beta$  (*IL1B*) and upstream effectors that control the activation of this cytokine, including caspase recruitment domain (CARD) family member 8 (*CARD8*), *CARD12* (*NLRG4*), *CARD15* (*NOD2*), pyrin domain and CARD containing gene (*ASC* or *PYCARD*), and caspase 1 (*CASP1*)<sup>26</sup> (see Table 3 of the Supplementary Appendix).

To evaluate the relation between microRNA summary values and gene expression in another way, we used information from the Gene Ontology project to test which of the terms were overrepresented in the microarray gene-expression signature that correlated with the microRNA summary value. We defined an overrepresented term as one for which more members assigned to that term were found in the microarray gene signature than were expected by chance. We found 83 overrepresented terms. There was at least 50% representation in the microarray gene-expression signature for 16 of the 83 terms. Of these 16 terms, 15 included members that participate in mechanisms of innate immunity controlled by toll-like receptors and nucleotide-binding oligomerization domain (NOD)-like receptors. The latter receptors control activation of interleukin-1 $\beta$ , a cytokine that has been implicated in the promotion of autonomous growth of AML blasts, in addition to its proinflammatory role<sup>26-29</sup> (Table 4 of the Supplementary Appendix).

Because microRNAs suppress the expression of specific genes either directly, by down-modulating expression of the encoded protein, or indirectly, by controlling the expression of other tran-



**Figure 2. Event-free Survival in the Validation Group, According to the MicroRNA Summary Value.**

For the purpose of display, the microRNA summary value was dichotomized on the basis of the median value to separate patients into two groups, and Kaplan-Meier curves were generated to depict outcomes. The microRNA summary value reflects the expression levels of the microRNAs forming the outcome signature derived from the training group, as calculated for patients in the validation group.

scription factors or regulatory proteins, we also searched the Targetscan Release 4.1 database ([www.targetscan.org](http://www.targetscan.org)) to assess which of the 452 genes in the microarray gene-expression signature were predicted to be direct targets of the microRNAs forming the signature. Of these 452 genes, 32 — including *TLR4*, *CARD8*, *CASP1*, *IL1B*, solute carrier family 11 member 1 (*SLC11A1*), macrophage scavenger receptor 1 (*MSR1*), and Fc fragment of IgG high affinity Ia receptor (*CD64*) (*FCGR1A*) — were predicted targets of members of the microRNA-181 family, which is the most represented microRNA family in the outcome signature. The expression levels of these 32 genes were inversely correlated with the expression levels of microRNA-181 family members, with Pearson's correlation coefficient ranging from  $-0.84$  to  $-0.45$  for the probes (Fig. 2 of the Supplementary Appendix).

## DISCUSSION

Altered expression of microRNAs has been observed in several cancers,<sup>10-12</sup> but little is known about microRNA expression in AML. In this study, we report a microRNA signature that is associated with clinical outcome in a subgroup of pa-

**Table 3. Multivariable Model of the Association between the MicroRNA Summary Value and Event-free Survival in the Validation Group.\***

Event-free Survival	Hazard Ratio for an Event (95% CI)	P Value
MicroRNA summary value (per increase of 4 units)	1.8 (1.0–3.0)	0.04
Ratio of <i>FLT3</i> -ITD to <i>FLT3</i> (high vs. low)	2.5 (1.2–5.4)	0.02
White-cell count (log)	1.4 (1.0–1.9)	0.04

\* The following variables were evaluated in univariable Cox regression models for outcome: age, sex, race, hemoglobin level, platelet count, white-cell count, percentage of blood blasts, percentage of bone marrow blasts, the presence or absence of extramedullary involvement, the microRNA summary value, the ratio between *FLT3* alleles with internal tandem duplication (*FLT3*-ITD) and wild-type *FLT3*, the presence or absence of a mutation in *NPM1*, and *BAALC* and *ERG* expression. Variables for which  $P < 0.20$  in univariable models were further evaluated in multivariable models.

tients with high-risk molecular features of AML (those who have *FLT3*-ITD, wild-type *NPM1*, or both). This subgroup constitutes approximately 65% of patients with cytogenetically normal AML and one third of all patients with AML who are under the age of 60 years. We also uncovered an association between the microRNA signature and expression of genes involved in innate immunity in AML.

The microRNA signature was obtained from a training group of patients and consisted of 12 probes that had a significant association with the clinical outcome. The signature was validated in a group of patients who received similar treatment on a different protocol from that used for the patients in the training group. By computing for each patient a summary value of the microRNA expression levels, we showed that the continuous microRNA summary value was associated with event-free survival. This approach eliminated the need for choosing a microRNA cutoff value that arbitrarily defined groups of patients for comparison. Furthermore, the microRNA signature appeared to be independent of the association between *FLT3*-ITD and outcome because its association with event-free survival in a multivariable model with adjustment for *FLT3*-ITD remained significant.

Several limitations of our study merit attention. First, our results are based on a retrospective analysis. Second, although the microRNA signature was derived in one group of patients and validated in another group, the numbers in both groups were small. Third, although the microRNA signature was independently associated with

event-free survival in a multivariable model, the P value for this association was only 0.04. We have not shown that the microRNA signature has greater clinical use than standard clinical or molecular markers. We also acknowledge that our results require confirmation in large prospective studies before the microRNA signature is ready for clinical application.

Our study points to an association in AML between microRNAs and genes that have a role in innate immunity. Of these genes, *TLR2*, *TLR4*, and *TLR8* encode proteins that are members of the family of toll-like receptors that recognize the so-called pathogen-associated molecular patterns of microbes.<sup>30</sup> Activation of toll-like receptors initiate signaling pathways that induce production of inflammatory cytokines through nuclear factor  $\kappa$ B. This transcription factor is constitutively activated in AML blasts but not in normal hematopoietic CD34-positive precursors.<sup>30</sup>

We also report the association between microRNA summary values and the expression of genes encoding the NOD-like receptor (NLR) family — *CARD8*, *CARD12* (*NLRC4*), and *CARD15* (*NOD2*) — that also recognize pathogen-associated molecular patterns. These proteins regulate inflammatory responses by controlling nuclear factor  $\kappa$ B through caspase 1 and its target, interleukin-1 $\beta$ .<sup>26</sup> In addition to its proinflammatory role, interleukin-1 $\beta$  promotes the survival and proliferation of AML blasts.<sup>27-29</sup>

Furthermore, among genes involved in innate immunity, we identified *TLR4*, *CARD8*, *CASP1*, and *IL1B* as putative targets of the microRNA-181 family and showed that the expression of these genes was inversely correlated with the expression of members of this microRNA family. We also showed that the expression of microRNA 181 correlated inversely with the expression of other putative targets, such as *SLC11A1* and *MSR1*, which encode proteins that enhance the activity of interleukin-1 $\beta$  and other cytokines during the inflammatory response,<sup>31,32</sup> and *FCGR1A*, which is coexpressed with *TLR4* in activated mast cells.<sup>33</sup>

Altogether, these data suggest that there is a functional relationship between microRNA expression and gene expression in a high-risk subgroup of patients with cytogenetically normal AML. It is likely that down-regulation of the microRNA-181 family contributes to an aggressive leukemia phenotype through mechanisms

## associated with the activation of pathways controlled by toll-like receptors and interleukin-1 $\beta$ .

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No potential conflict of interest relevant to this article was reported.

### APPENDIX

The following investigators participated in this study: *North Shore–Long Island Jewish Health System, Manhasset, NY*: D.R. Budman, P.R.K. Koduru; *Ohio State University Medical Center, Columbus*: C.D. Bloomfield, K.S. Theil, N.A. Heerema; *Wake Forest University School of Medicine, Winston-Salem, NC*: D.D. Hurd, W.L. Flejter, M.J. Pettenati; *University of Massachusetts Medical Center, Worcester*: P. Bhargava, V. Jaswaney, K. Richkind, M.J. Mitchell, P. Miron; *Vermont Cancer Center, Burlington*: H.B. Muss, E.F. Allenand, M. Tang; *Washington University School of Medicine, St. Louis*: N.L. Bartlett, M.S. Watson, J. Garcia-Heras; *Roswell Park Cancer Institute, Buffalo, NY*: E.G. Levine, A.W. Block; *Dartmouth Medical School, Lebanon, NH*: M.S. Ernstoff, T.K. Mohandas; *University of Chicago Medical Center, Chicago*: G. Fleming, D. Roulston, K.M. Carlson, Y. Zhang, M.M. Le Beau; *Duke University Medical Center, Durham, NC*: J. Crawford, M.B. Qumsiyeh; *Eastern Maine Medical Center, Bangor*: P.L. Brooks, L.J. Beauregard; *University of Iowa Hospitals, Iowa City*: G.H. Clamon, S.R. Patil; *Massachusetts General Hospital, Boston*: M.L. Grossbard, P. Dal Cin, C.C. Morton; *Mount Sinai School of Medicine, New York*: L.R. Silverman, V. Najfeld; *Weill Medical College of Cornell University, New York*: S. Wadler, P.R.K. Koduru, A.J. Carroll, S. Mathew; *University of Puerto Rico School of Medicine, San Juan*: E. Velez-Garcia, C.C. Morton, L.L. Atkins; *Christiana Care Health Services, Newark, DE*: S.S. Grubbs, D.S. Borgeonkar, J.M. Meck; *Western Pennsylvania Hospital, Pittsburgh*: R.K. Shaddock, G.R. Diggans; *Dana–Farber Cancer Institute, Boston*: G.P. Canellos, P. Dal Cin, C.C. Morton; *University of Missouri Ellis Fischel Cancer Center, Columbia*: M.C. Perry, T.H. Huang; *University of North Carolina, Chapel Hill*: T. Shea, K.W. Rao; *Ft. Wayne Medical College of Oncology/Hematology, Ft. Wayne, IN*: S. Nattam, P.I. Bader; *SUNY Upstate Medical University, Syracuse*: S.L. Graziano, C.K. Stein; *University of California at San Diego, San Diego*: J.E. Mortimer, M.L. Dell'Aquila; *Long Island Jewish Medical Center, Lake Success, NY*: K.R. Rai, P.R.K. Koduru; *Virginia Commonwealth University, Richmond*: J.D. Roberts, C. Jackson-Cook; *Medical University of South Carolina, Charleston*: M.R. Green, G.S. Pai; *Southern Nevada Cancer Research Foundation, Las Vegas*: J. Ellerton, M.L. Dell'Aquila; *Rhode Island Hospital, Providence*: W. Sikov, S.L. Kerman; *University of Nebraska Medical Center, Omaha*: M.A. Kessinger Wegner, W.G. Sanger; *University of California at San Francisco, San Francisco*: A.P. Venook, K.E. Richkind.

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