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ORIGINAL REPORT

Clinical Role of microRNAs in Cytogenetically Normal Acute Myeloid Leukemia: *miR-155* Upregulation Independently Identifies High-Risk Patients

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See accompanying editorial on page 2065 and article on page 2219; listen to the podcast by Dr Estey at www.jco.org/podcasts

ABSTRACT

Purpose

To evaluate the impact of *miR-155* on the outcome of adults with cytogenetically normal (CN) acute myeloid leukemia (AML) in the context of other clinical and molecular prognosticators and to gain insight into the leukemogenic role of this microRNA.

Patients and Methods

We evaluated 363 patients with primary CN-AML. *miR-155* levels were measured in pretreatment marrow and blood by NanoString nCounter assays that quantified the expression of the encoding gene *MIR155HG*. All molecular prognosticators were assessed centrally. *miR-155*–associated gene and microRNA expression profiles were derived using microarrays.

Results

Considering all patients, high *miR-155* expression was associated with a lower complete remission (CR) rate ($P < .001$) and shorter disease-free survival ($P = .001$) and overall survival (OS; $P < .001$) after adjusting for age. In multivariable analyses, high *miR-155* expression remained an independent predictor for a lower CR rate ($P = .007$) and shorter OS ($P < .001$). High *miR-155* expressers had approximately 50% reduction in the odds of achieving CR and 60% increase in the risk of death compared with low *miR-155* expressers. Although high *miR-155* expression was not associated with a distinct microRNA expression profile, it was associated with a gene expression profile enriched for genes involved in cellular mechanisms deregulated in AML (eg, apoptosis, nuclear factor-κB activation, and inflammation), thereby supporting a pivotal and unique role of this microRNA in myeloid leukemogenesis.

Conclusion

miR-155 expression levels are associated with clinical outcome independently of other strong clinical and molecular predictors. The availability of emerging compounds with antagonistic activity to microRNAs in the clinic provides the opportunity for future therapeutic targeting of *miR-155* in AML.

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INTRODUCTION

MicroRNAs are short noncoding RNAs that regulate the expression of proteins involved in pivotal cellular mechanisms. Initially encoded as premicroRNAs, they undergo several steps of maturation before being incorporated into RNA-induced silencing complexes and hybridizing to target mRNAs.[1,2] This process results in either degradation or translation inhibition of the target mRNAs and, in turn, protein downregulation.

Aberrant expression levels of microRNAs and their target mRNAs occur in human cancer and likely contribute to malignant transformation. In acute myeloid leukemia (AML), microRNAs are involved in disruption of hematopoietic mechanisms of cell differentiation, proliferation, and survival; contribute to the molecular heterogeneity of the disease; and impact on treatment response and outcome.[2,3]

The *MIR155HG* gene located at chromosome band 21q21.3 encodes *miR-155*. In the normal host,

this microRNA is induced in hematopoietic stem cells and myeloid progenitor cells during inflammatory responses under the transcriptional control of nuclear factor-κB (NF-κB) and activator protein 1 (AP-1).[4] By preferentially targeting inhibitors of inflammation, *miR-155* sustains normal mechanisms of the innate immune response.[4-7] The potential oncogenic role of *miR-155* was identified early by showing its enhanced expression levels in lymphoma, aggressive molecular subtypes of chronic lymphocytic leukemia, and solid tumors.[4] Subsequently, high levels of *miR-155* were demonstrated to cause aggressive pre–B-cell leukemia and myeloproliferative disorders in murine models.[8,9]

In AML, we and others have reported that higher expression of *miR-155* is often associated with *FLT3* internal tandem duplication (*FLT3*-ITD), a genetic marker predicting poor outcome.[10-13] However, whether *miR-155* upregulation impacts on clinical outcome in patients with AML independently from *FLT3*-ITD and other established prognosticators is unknown. This is likely to be relevant not only for patients' risk stratification, but also for establishing specific clinical approaches. Indeed, compounds with antagonistic activity to microRNAs are emerging for clinical use, and *miR-155* could represent a novel therapeutic target in AML.[14] Thus, we assessed the clinical impact of this microRNA in a cohort of adult patients with primary (de novo) cytogenetically normal AML (CN-AML) who were well characterized molecularly at diagnosis. Furthermore, to gain biologic insights, we performed genome-wide gene and microRNA expression analyses.

PATIENTS AND METHODS

Patients, Treatment, and Cytogenetic Analysis

Three hundred sixty-three adults, including 153 younger (age < 60 years) and 210 older (age ≥ 60 years) patients, with untreated, primary CN-AML who received intensive first-line therapy on Cancer and Leukemia Group B (CALGB) trials and had diagnostic bone marrow (BM) or blood specimens available for molecular analyses were included. The diagnosis of normal cytogenetics was based on the analysis of ≥ 20 metaphases in BM specimens subjected to short-term culture and confirmed centrally.[15] All patients received cytarabine-daunorubicin–based induction chemotherapy. Younger patients were treated on CALGB trials 9621 or 19808.[16-18] Older patients were treated with less intense regimens on CALGB protocols 8525, 8923, 9420, 9720, and 10201.[19-24] Per protocol, no patient received allogeneic stem-cell transplantation during first complete remission (CR), defined according to published criteria.[25] See Data Supplement for treatment details.

When patients with CN-AML in the current study were compared with patients enrolled onto the same CALGB protocols who were not studied because they had no material available for analysis (n = 499), there were no statistically significant differences in any of the outcome end points analyzed (for other clinical characteristics, see Data Supplement). All patients provided written informed consent, and study protocols were in accordance with the Declaration of Helsinki and approved by Institutional Review Boards at each center.

Molecular Analyses

We measured the expression of *MIR155HG* transcript (hereafter referred to as *miR-155*) using an enzyme-independent probe-based quantification system that allows digital counting of individual mRNA molecules (nCounter; NanoString Technologies, Seattle, WA), as previously reported.[26] The method allows for direct measurement of actual levels of RNAs with no need for target amplification as used by other polymerase chain reaction (PCR) –based assays. The sensitivity, dynamic range, linearity, and reproducibility of the nCounter system have been validated previously,[26] and mRNA expression levels measured with this approach show excellent correlation with data obtained by oligonucleotide microarrays or quantitative PCR. *miR-155* levels were normalized using *ABL* as an internal control. Target/internal control ratios were $\log_2$ transformed for downstream analyses.

The presence or absence of additional molecular markers, that is, *FLT3*-ITD, *FLT3* tyrosine kinase domain mutations (*FLT3*-TKD), and *MLL* partial tandem duplication; mutations in *NPM1*, *CEBPA*, *WT1*, *TET2*, *ASXL1*, *DNMT3A*, *RUNX1*, *IDH1*, and *IDH2*; and expression levels of *BAALC*, *ERG*, and *miR-181a* were assessed centrally, as previously reported.[12,27-40]

Microarray Profiling

Gene and microRNA expression profiling was performed using HG-U133 plus 2.0 oligonucleotide microarrays (Affymetrix, Santa Clara, CA) and Ohio State University custom microRNA microarrays, respectively, as previously reported.[39-41] Expression signatures were identified by correlating the expression levels of *MIR155HG* with those of protein-coding genes and microRNAs using Spearman rank correlation.[42] The false discovery rate (FDR) was used to assess the multiple testing errors. A permutation test was computed based on 1,000 random permutations. The CI of FDR assessment was 80%, and the maximum allowed proportion of false-positive genes was 1%. Gene Ontology analysis to assess enrichment of genes in the *miR-155*–associated signature in distinct biologic processes was conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID).[43]

Definition of Clinical End Points and Statistical Analyses

For definitions of outcome end points, see the Data Supplement. The main objective of our study was to evaluate the association of *miR-155* expression levels with clinical and molecular characteristics and the impact of aberrant *miR-155* expression on outcome. We first divided patients into quartile groups based on the expression levels of *miR-155* and assessed the outcome associations by the trend test for disease-free survival (DFS; $P < .001$) and overall survival (OS; $P < .001$). This justified the use of the median cut to dichotomize patients into high and low expressers of *miR-155*.

The associations of *miR-155* expression status (low/high) with baseline clinical, demographic, and molecular features were compared using the Wilcoxon rank sum test and the Fisher's exact test for continuous and categorical variables, respectively.[42,44] Univariable logistic regression models were constructed to evaluate *miR-155* expression for achievement of CR, and univariable Cox proportional hazards models were used to evaluate the associations of *miR-155* expression with DFS and OS.[45,46] Multivariable logistic regression models were generated for attainment of CR, and multivariable proportional hazards models were constructed for DFS and OS, using a limited backward elimination procedure. Clinical variables that were considered for univariable analyses, in addition to *miR-155* expression, were age, sex, race, hemoglobin, platelet count, WBC count, and the centrally assessed molecular variables. Variables significant at $\alpha = .20$ from the univariable analyses were considered for multivariable analyses. For the time-to-event end points, the proportional hazards assumption was checked for each variable individually. All models considering both age groups were adjusted for an age group effect (≥ 60 *v* < 60 years).

RESULTS

Associations of miR-155 *Expression With Clinical and Molecular Characteristics in Patients With CN-AML*

At diagnosis, high *miR-155* expressers had higher WBC counts ($P < .001$) and higher percentages of blood ($P = .004$) and BM blasts ($P < .001$) than low expressers (Table 1). High *miR-155* expressers were also more frequently *FLT3*-ITD positive ($P < .001$), *RUNX1* mutated ($P < .001$), *WT1* mutated ($P = .03$), and high *ERG* ($P = .02$) and *BAALC* ($P = .002$) expressers, and less frequently *CEBPA* mutated ($P = .003$), *IDH2* mutated ($P = .004$), and *FLT3*-TKD positive ($P = .08$; Table 1). However, when younger and older patients were considered separately, the frequencies of these molecular variables in

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Table 1. Comparison of Clinical and Molecular Characteristics With *miR-155* Expression in Patients With Primary Cytogenetically Normal Acute Myeloid Leukemia

Characteristic	Low *miR-155** (n = 182) No. of Patients	Low *miR-155** (n = 182) %	High *miR-155** (n = 181) No. of Patients	High *miR-155** (n = 181) %	*P*
Age, years					.60
Median	63		62		
Range	19-83		18-81		
Male sex	94	52	90	50	.75
Race					.73
White	160	89	164	91	
Nonwhite	19	11	17	9	
WBC, $\times 10^9$/L					< .001
Median	20.7		37.9		
Range	0.8-295.0		1.0-450.0		
Blood blasts, %					.004
Median	50		64		
Range	0-97		0-99		
Bone marrow blasts, %					< .001
Median	63		74		
Range	4-97		6-97		
Hemoglobin, g/dL					.75
Median	9.3		9.4		
Range	4.9-14.5		4.6-15.0		
Platelet count, $\times 10^9$/L					.22
Median	70		61		
Range	4-481		8-850		
Extramedullary involvement	43	24	51	29	.34
NPM1					.32
Mutated	109	61	117	66	
Wild type	70	39	60	34	
FLT3-ITD					< .001
Present	37	21	97	55	
Absent	143	79	80	45	
CEBPA					.003
Mutated	38	21	17	10	
Single mutated	14		12		
Double mutated	24		5		
Wild type	141	79	160	90	
ELN genetic group†					< .001
Favorable	119	66	55	31	
Intermediate-I	60	34	121	69	
TET2					.62
Mutated	42	24	46	26	
Wild type	134	76	129	74	
ASXL1					.86
Mutated	16	9	17	10	
Wild type	160	91	157	90	
DNMT3A					.36
Mutated	57	33	63	38	
R882	36		44		
Non-R882	21		19		
Wild type	118	67	104	62	
RUNX1					< .001
Mutated	9	5	33	20	
Wild type	157	95	128	80	
IDH1					.17
Mutated	15	8	23	13	
Wild type	163	92	151	87	
IDH2					.004
IDH2 mutated	44	25	22	13	
Codon R140 mutation	36		20		
Codon R172 mutation	8		2		
Wild type	134	75	152	87	
FLT3-TKD					.08
Present	24	13	13	7	
Absent	155	87	164	93	
WT1					.03
Mutated	11	6	23	13	
Wild type	168	94	154	87	
MLL-PTD					.53
Present	10	6	14	8	
Absent	170	94	167	92	
ERG expression group*					.02
High	79	43	102	56	
Low	103	57	79	44	
BAALC expression group*					.002
High	76	42	106	59	
Low	106	58	75	41	

(continued on following page)

Table 1. Comparison of Clinical and Molecular Characteristics With *miR-155* Expression in Patients With Primary Cytogenetically Normal Acute Myeloid Leukemia (continued)

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.
*The median expression value was used as a cut point. Gene expression was measured using NanoString.
†Within patients with cytogenetically normal acute myeloid leukemia (CN-AML), the ELN favorable genetic group comprises patients with mutated *CEBPA* and/or mutated *NPM1* without *FLT3*-ITD. All remaining patients with CN-AML (ie, those with wild-type *CEBPA* and *NPM1* mutation with *FLT3*-ITD or with wild-type *NPM1* with or without *FLT3*-ITD) belong to the ELN intermediate-I genetic group.

high *miR-155* expressers versus low expressers varied slightly according to the age group considered (Data Supplement).

Prognostic Value of miR-155 *Expression*

When all patients were considered and analyses were adjusted for age, high *miR-155* expressers had lower odds of achieving CR than low expressers ($P < .001$; odds ratio, 0.41; 95% CI, 0.25 to 0.68). With a median follow-up time for patients alive of 7.9 years (range, 2.3 to 12.9 years), high *miR-155* expressers had shorter DFS ($P < .001$; hazard ratio, 1.59; 95% CI, 1.20 to 2.09) and OS ($P < .001$; hazard ratio, 2.00; 95% CI, 1.58 to 2.53) than low expressers (Fig 1).

In multivariable analyses (Table 2), high *miR-155* expressers were approximately 50% less likely to achieve CR ($P = .007$), after adjusting for *NPM1* mutation status ($P = .005$), *BAALC* expresser status ($P = .002$), WBC ($P < .001$), and age group ($P = .003$). High *miR-155* expressers also had a 60% increased risk of death ($P < .001$), after adjusting for *BAALC* expression status ($P < .001$), age group ($P < .001$), *FLT3*-ITD ($P < .001$), and race ($P = .03$). However, *miR-155* expresser status did not remain in the multivariable model for DFS.

Among younger patients, high *miR-155* expressers, compared with low expressers, had a lower CR rate ($P = .03$; 76% *v* 90%, respectively) and shorter DFS ($P < .001$; 5-year DFS, 27% *v* 55%,

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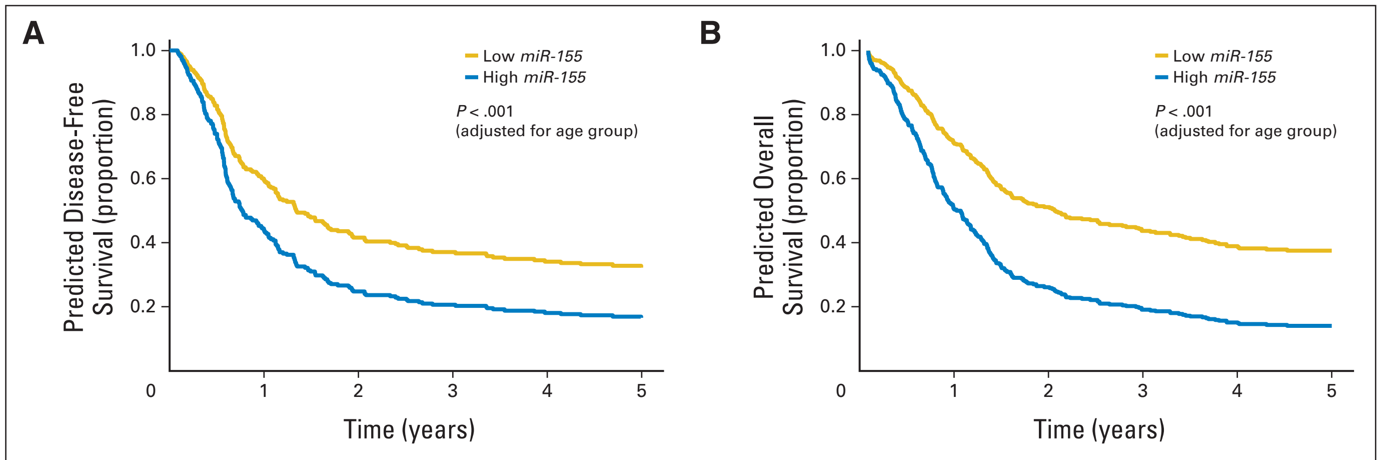

Fig 1. Age group–adjusted clinical outcome for patients with high and low *miR-155* expression levels: (A) disease-free survival and (B) overall survival.

respectively) and OS ($P < .001$; 5-year OS, 28% *v* 57%, respectively; Figs 2A and 2B; Data Supplement). In multivariable analyses (Table 2), high *miR-155* expresser status was associated with a trend for a worse CR rate ($P = .06$) and remained significantly associated with shorter DFS ($P = .003$), after adjustment for *FLT3*-ITD ($P < .001$), *FLT3*-TKD ($P < .001$), *BAALC* expression status ($P < .001$), and race ($P = .02$); high *miR-155* expresser status was also associated with shorter OS ($P = .01$), after adjustment for WBC ($P < .001$), *FLT3*-ITD

Table 2. Multivariable Analyses in Patients With Primary Cytogenetically Normal Acute Myeloid Leukemia

	Complete Remission			Disease-Free Survival			Overall Survival		
Group	OR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
All patients									
miR-155 expression, high *v* low	0.46	0.27 to 0.81	.007	1.20	0.89 to 1.61	.23	1.62	1.25 to 2.09	< .001*
NPM1, mutated *v* wild type	2.42	1.31 to 4.48	.005						
BAALC expression, high *v* low	0.37	0.20 to 0.69	.002	1.73	1.28 to 2.34	< .001	2.16	1.68 to 2.76	< .001
WBC, each 50 units	0.65	0.51 to 0.82	< .001	1.17	1.02 to 1.34	.02			
Age group, older *v* younger	0.43	0.24 to 0.75	.003	2.48	1.86 to 3.33	< .001	2.38	1.84 to 3.08	< .001
FLT3-ITD, positive *v* negative				1.96	1.45 to 2.65	< .001	1.78	1.37 to 2.30	< .001
ERG expression, high *v* low				1.38	1.01 to 1.86	.04			
Race, white *v* nonwhite							1.62	1.04 to 2.52	.03
Patients, age < 60 years									
miR-155 expression, high *v* low	0.39	0.15 to 1.05	.06	2.13	1.29 to 3.51	.003	1.84	1.14 to 2.97	.01
RUNX1, mutated *v* wild type	0.21	0.06 to 0.72	.01						
Age, each 10-year increase	0.45	0.26 to 0.77	.004						
WBC, each 50 units							1.49	1.22 to 1.81	< .001
FLT3-ITD, positive *v* negative				2.82	1.70 to 4.66	< .001	1.82	1.15 to 2.87	.01
FLT3-TKD, present *v* absent				3.27	1.65 to 6.49	< .001			
BAALC expression, high *v* low				2.66	1.62 to 4.35	< .001	2.33	1.46 to 3.72	< .001
Race, white *v* nonwhite				2.81	1.19 to 6.66	.02			
CEBPA, mutated *v* wild type							0.47	0.26 to 0.86	.02
WT1, mutated *v* wild type							2.25	1.28 to 3.95	.005
Patients, age ≥ 60 years									
miR-155 expression, high *v* low	0.46	0.22 to 0.94	.03	0.93	0.64 to 1.35	.71	1.36	1.00 to 1.86	.05*
NPM1, mutated *v* wild type	2.45	1.10 to 5.47	.03						
BAALC expression, high *v* low	0.32	0.14 to 0.70	.004	2.07	1.42 to 3.01	.002	2.18	1.60 to 2.95	< .001
WBC, each 50 units	0.65	0.48 to 0.88	.005						
Age, each 10-year increase	0.48	0.26 to 0.90	.02						
FLT3-ITD, positive *v* negative				1.83	1.25 to 2.69	< .001	1.56	1.14 to 2.14	.006

NOTE. ORs greater than or less than 1.0 mean higher or lower complete remission rates, respectively, for the higher values of the continuous variables and the first category listed for the categorical variables. HRs greater than or less than 1.0 indicate higher or lower risk, respectively, for relapse or death (DFS) or death (OS) for the higher values of the continuous variables and the first category listed for the categorical variables.

Abbreviations: DFS, disease-free survival; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-TKD, tyrosine kinase domain mutation in the *FLT3* gene; HR, hazard ratio; OR, odds ratio; OS, overall survival.

**miR-155* expression did not meet the proportional hazards assumption. The model using a time-dependent covariate yielded similar results to the one using only the main effect and that is reported here.

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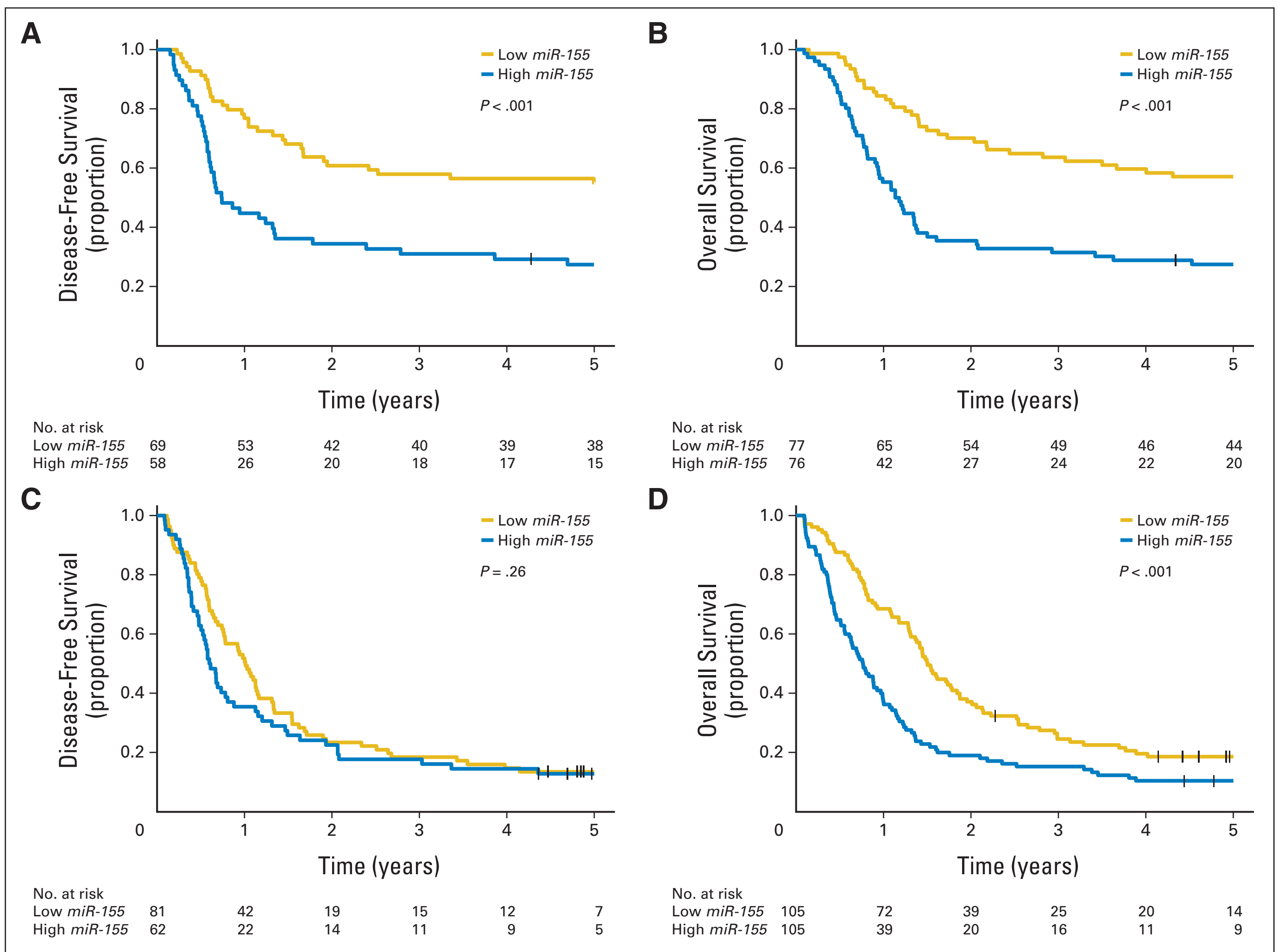

Fig 2. Survival curves according to age group and *miR-155* expression (high *v* low): (A) disease-free survival and (B) overall survival of younger patients and (C) disease-free survival and (D) overall survival of older patients.

($P = .01$), *BAALC* expression status ($P < .001$), and *CEBPA* ($P = .02$) and *WT1* ($P = .005$) mutation status. The risk of relapse or death of the younger high *miR-155* expressers was approximately twice that of the low expressers.

Among older patients (Data Supplement), high *miR-155* expressers, compared with low expressers, had a lower CR rate ($P = .008$; 59% *v* 77%, respectively) and shorter OS ($P < .001$; 3-year OS, 15% *v* 25%, respectively; Fig 2D). DFS was not significantly different between the two expression groups (Fig 2C). In multivariable analyses (Table 2), high *miR-155* expressers were 50% less likely to achieve CR ($P = .03$), after adjusting for *NPM1* mutation status ($P = .03$), *BAALC* expression status ($P = .004$), WBC ($P = .005$), and age ($P = .02$), and had a 40% increased risk of death ($P = .05$), once adjusted for *BAALC* expression status ($P < .001$) and *FLT3*-ITD ($P = .006$).

We previously reported that low expression levels of *miR-181a* had a negative prognostic impact on CN-AML.[40] Unfortunately *miR-181a* expression was available in only a subset of patients included in this study. However, in multivariable models limited to these patients (n = 298; Data Supplement), high levels of *miR-155* remained independently associated with shorter OS ($P < .001$) after adjusting for *miR-181a* ($P < .001$), *FLT3*-ITD ($P = .02$), *WT1* ($P = .004$), *IDH1* ($P = .03$), *ERG* ($P = .01$), *BAALC* ($P < .001$), WBC ($P = .02$), and age ($P < .001$).

Impact on the European LeukemiaNet Genetic Groups

A modified classification of CN-AML has been recommended by an international expert panel on behalf of the European LeukemiaNet (ELN), in which the ELN favorable genetic group comprises patients with mutated *CEBPA* and/or mutated *NPM1* without *FLT3*-ITD, whereas the ELN intermediate-I genetic group consists of patients without *CEBPA* mutations who are either *NPM1* mutated with *FLT3*-ITD or have wild-type *NPM1* with or without *FLT3*-ITD.[47] When we analyzed the prognostic significance of *miR-155* in patients classified in the ELN favorable genetic group, younger high *miR-155* expressers, compared with low expressers, had a significantly lower CR rate ($P = .03$; 80% *v* 96%, respectively) and shorter DFS ($P = .04$; 5-year DFS, 45% *v* 66%, respectively) and OS ($P = .02$; 5-year OS, 48% *v* 71%, respectively; Data Supplement). In contrast, in the ELN intermediate-I genetic group, *miR-155* expression did not impact on the probability of CR attainment ($P = .99$), DFS ($P = .41$), or OS

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Table 3. Gene Ontology Terms Positively Correlated With *miR-155* Expression

Biologic Process	FDR	Fold Enrichment	No. of Genes
GO:0006915–apoptosis	7.18E-08	5.154073577	25
GO:0012501–programmed cell death	9.76E-08	5.078154327	25
GO:0042981–regulation of apoptosis	1.75E-07	4.322242001	28
GO:0043067–regulation of programmed cell death	2.18E-07	4.279658336	28
GO:0010941–regulation of cell death	2.37E-07	4.263904992	28
GO:0008219–cell death	2.66E-06	4.315371757	25
GO:0016265–death	3.05E-06	4.285569466	25
GO:0006916–antiapoptosis	1.52E-05	8.434666429	14
GO:0006954–inflammatory response	4.77E-04	5.72815808	15
GO:0043066–negative regulation of apoptosis	0.001336061	5.258902193	15
GO:0009611–response to wounding	0.001527465	4.21505972	18
GO:0043069–negative regulation of programmed cell death	0.001579	5.185658429	15
GO:0060548–negative regulation of cell death	0.001632093	5.171253823	15
GO:0006952–defense response	0.011381758	3.63249049	18
GO:0006955–immune response	0.013164608	3.417524265	19
GO:0043122–regulation of I-κB kinase/NF-κB cascade	0.037285223	9.279259196	8

Abbreviations: FDR, false discovery rate; GO, Gene Ontology; NF-κB, nuclear factor-κB.

($P = .20$) duration. Among older patients, higher *miR-155* expressers, compared with lower expressers, had a shorter OS both in the favorable ($P = .06$; 3-year OS, 23% *v* 34%, respectively) and intermediate-I genetic groups ($P = .05$; 3-year OS, 11% *v* 11%, respectively; median survival, 0.6 *v* 1.1 years, respectively).

Biologic Insights

To gain insight into the leukemogenic role of *miR-155* expression in CN-AML, we first derived an Affymetrix gene expression signature associated with *miR-155* expression in all patients. Using a conservative cutoff for significance (FDR < 0.001), we identified 196 mRNAs significantly correlated with *miR-155* expression. Of these mRNAs, 154 were correlated positively (Data Supplement), and 42 were correlated negatively (Data Supplement). Among the positively correlated mRNAs, the most significant was *MIR155HG*, the primary RNA for *miR-155*, thereby validating the results of the nCounter assay. The Gene Ontology analysis revealed that genes involved in biologic processes related to antiapoptotic, proliferative, and inflammatory activities were enriched in high *miR-155* expressers (FDR < 0.05; Table 3).

For microRNA expression profiling, younger and older patients were separately analyzed to avoid confounding batch effects. Testing for expressed microRNAs correlated with *miR-155* expression revealed no associated microRNAs other than *miR-155* itself. The mature *miR-155*, quantified by microRNA microarrays, was the only microRNA significantly correlated with *miR-155*, as measured by the nCounter assay ($P < .001$). This finding again validated the results obtained with the nCounter assay.

DISCUSSION

MicroRNAs have been shown to play a role in leukemogenesis and to impact on clinical outcome.[1-3] Furthermore, emerging proof-of-principle clinical studies support the potential therapeutic targeting of these small noncoding RNAs in human diseases including cancer.[14] In this study, we focused on the clinical significance of *miR-155*, which has a pivotal role in leukemogenesis.[6,9]

We show that *miR-155* expression levels constitute an independent prognostic factor in patients with primary CN-AML. Higher levels of *miR-155* expression were associated with lower odds of achieving CR and higher risk for disease relapse or death. Although higher *miR-155* expression impacted negatively on outcome of both younger and older patients, the association with outcome end points differed somewhat for the two age groups. For younger patients, higher *miR-155* expresser status was associated with a lower CR rate and shorter DFS and OS. For older patients, higher *miR-155* expresser status was associated only with a lower CR rate and shorter OS. This discrepancy between the age groups may be related not only to biologic differences but also to differences in the intensity of consolidation therapy administered to younger and older patients. Nevertheless, *miR-155* emerged from our study as a single noncoding RNA with a strong and independent prognostic impact in CN-AML, even when considered in the context of other validated molecular and clinical prognosticators. Thus, our results validate in the clinic previous data from preclinical models supporting a crucial role of *miR-155* in leukemia.[6,9]

We also tested the prognostic significance of *miR-155* expression in the ELN genetic groups,[47] separately in younger and older patients.[48] In both age groups, *miR-155* expression had a prognostic impact in the ELN favorable genetic group. Most of these patients do not harbor *FLT3*-ITD (except for the relatively rare patients with *CEBPA* mutations and *FLT3*-ITD), suggesting that the clinical impact of *miR-155* is independent from the presence of *FLT3*-ITD. Indeed, high *miR-155* expressers had worse OS than low expressers among *FLT3*-ITD–negative younger ($P = .01$; 3-year OS, 35% *v* 61%, respectively) and older ($P = .02$; 2-year OS, 24% *v* 41%, respectively) patients (data not shown). In the ELN favorable genetic group, *miR-155* expression remained associated with CR rate ($P = .02$) and OS duration ($P = .003$) independently of *TET2* and *ASXL1* mutations, which we previously reported to impact negatively in this genetic group.[33,34] In contrast, the lack of the impact of *miR-155* overexpression on the ELN intermediate-I genetic group for younger patients could be related to the colinearity of high *miR-155* expression and *FLT3*-ITD.

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This made it difficult to establish whether the prognostic impact of these two variables was independent from each other. In contrast, the negative impact of *miR-155* overexpression on OS of older, ELN intermediate-I genetic group patients suggests a potential prioritized prognostic relevance of *miR-155* over *FLT3*-ITD in this age group.

We previously reported that *miR-181a* has prognostic impact only in molecular high-risk patients (ie, those with *FLT3*-ITD and/or wild-type *NPM1*), the majority of whom are classified in the ELN intermediate-I genetic group. Interestingly, in a multivariable model that included both *miR-155* and *miR-181a*, in addition to other clinical and molecular prognosticators, higher levels of *miR-155* and lower levels of *miR-181a* remained independently associated with shorter survival. Thus, our data indicate that expression levels of two distinct microRNAs, *miR-155* and *miR-181a*, provide additional and complementary prognostic information for molecular subsets of patients with CN-AML and may potentially be used to refine molecular risk classifications and provide treatment guidance.

To our knowledge, this is the first clinical study where *miR-155* was evaluated using nCounter, an amplification enzyme-independent quantification probe-based system that allows digital counting of RNA molecules. Using the nCounter assay as a primary quantification method has the potential to eliminate batch-related pitfalls intrinsic to quantitative PCR-based assays. This would make an accurate analysis of individual patients possible and allow the prospective use of *miR-155* expression in the clinic. The accuracy of *miR-155* measurements by the nCounter method was validated by their strong correlation with data obtained using two different microarray platforms (ie, Affymetrix oligonucleotide microarrays for the *MIR155HG* transcript and Ohio State University miR microarray for the mature *miR-155*).

To further understand how changes in *miR-155* expression affect the aggressiveness of the disease, response to treatment, and outcome of patients with CN-AML, we used the combination of genome-wide gene and microRNA expression and Gene Ontology analyses. We show that expression of genes involved in antiapoptotic, proinflammatory, and NF-κB activation processes positively correlated with *miR-155* expression. These results support the notion that *miR-155* expression contributes to different degrees of disease aggressiveness in leukemia and other types of cancer by increasing cell proliferation rate and survival.[7] Because *miR-155* is relatively easy to measure at diagnosis, it is possible to envision it as a marker for risk stratification and guidance for targeting treatments such as emerging compounds with antagonistic activity to microRNAs.[14] Furthermore, because *miR-155* is reported to be a target of NF-κB,[4,5] possible treatment approaches targeting this pathway may be a reasonable approach for patients with elevated *miR-155* expression.

In summary, we report that the expression of *miR-155* is independently associated with clinical outcome in CN-AML and may allow better characterization of molecular risk, especially in patients lacking *FLT3*-ITD, such as those classified in the ELN favorable genetic group. Although one of the limitations of the study was the retrospective nature of the analyses, it should be underscored that we were able to show independent prognostic significance of *miR-155* expression in two distinct age groups (younger and older) that were similarly treated with cytarabine-anthracycline–based induction chemotherapy but received consolidation treatments of different intensity. Nevertheless, the study of additional independent cohorts of patients is required before the *miR-155* quantification can be included in molecular panels adopted for risk stratification and treatment guidance for patients with CN-AML. Finally, the ongoing clinical development of compounds capable of downregulating microRNA expression in vivo offers hope for designing novel therapies for patients whose outcome is adversely impacted by high *miR-155* expression levels.[14]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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