Favorable Prognostic Impact of *NPM1* Mutations in Older Patients With Cytogenetically Normal De Novo Acute Myeloid Leukemia and Associated Gene- and MicroRNA-Expression Signatures: A Cancer and Leukemia Group B Study

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ABSTRACT

Purpose

To analyze the prognostic significance of *NPM1* mutations, and the associated gene- and microRNA-expression signatures in older patients with de novo, cytogenetically normal acute myeloid leukemia (CN-AML) treated with intensive chemotherapy.

Patients and Methods

One hundred forty-eight adults age ≥ 60 years with de novo CN-AML, enrolled onto Cancer and Leukemia Group B protocols 9720 and 10201, were studied at diagnosis for *NPM1*, *FLT3*, *CEBPA*, and *WT1* mutations, and gene- and microRNA-expression profiles.

Results

Patients with *NPM1* mutations (56%) had higher complete remission (CR) rates (84% v 48%; $P < .001$) and longer disease-free survival (DFS; $P = .047$; 3-year rates, 23% v 10%) and overall survival (OS; $P < .001$; 3-year rates, 35% v 8%) than *NPM1* wild-type patients. In multivariable analyses, *NPM1* mutations remained independent predictors for higher CR rates ($P < .001$) and longer DFS ($P = .004$) and OS ($P < .001$), after adjustment for other prognostic clinical and molecular variables. Unexpectedly, the prognostic impact of *NPM1* mutations was mainly observed in patients ≥ 70 years. Gene- and microRNA-expression profiles associated with *NPM1* mutations were similar across older patient age groups and similar to those in younger (< 60 years) patients with CN-AML. These profiles were characterized by upregulation of *HOX* genes and their embedded microRNAs and downregulation of the prognostically adverse *MN1*, *BAALC*, and *ERG* genes.

Conclusion

NPM1 mutations have favorable prognostic impact in older patients with CN-AML, especially those age ≥ 70 years. The gene- and microRNA-expression profiles suggest that *NPM1* mutations constitute a marker defining a biologically homogeneous entity in CN-AML that might be treated with specific and/or targeted therapies across age groups.

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INTRODUCTION

The majority of patients with newly diagnosed acute myeloid leukemia (AML) in the United States are age ≥ 60 years.¹ Although the outcome of younger adults (< 60 years) has improved, the prognosis of older patients remains poor, with complete remission (CR) rates of 40% to 60% and long-term overall survival (OS) rates of 5% to 16%.²⁻⁶ The worse outcome of older patients has been attributed to such

factors as preceding hematologic disorders, overrepresentation of high-risk cytogenetics, or other adverse prognostic clinical characteristics.⁴⁻⁹ However, in approximately 65% to 75% of older patients, AML arises de novo,⁹⁻¹¹ and in 45% to 50% of these patients, leukemic blasts are cytogenetically normal (CN).^{3,6}

During the last decade, de novo CN-AML has been found to be heterogeneous at the submicroscopic level in younger adults,¹² with mutations in

the *NPM1* gene being the most frequent genetic alterations.¹³⁻²⁰ Indeed, AML with *NPM1* mutations has been added as a provisional entity in the 2008 World Health Organization classification of AML.²¹ The frameshift caused by an *NPM1* mutation results in aberrant cytoplasmic localization of the nucleophosmin protein, which likely interferes with its biologic functions in ribosomal assembly and the p53 and ARF pathways.²²

Most studies investigating the prognostic impact of *NPM1* mutations in CN-AML have been conducted in patients ≤ 60 years,^{13,14,19,20} or in cohorts comprising both younger and older patients.¹⁵⁻¹⁸ Although analyses of *NPM1* mutations alone yielded inconsistent results in CN-AML, when considered along with the *FLT3*-internal tandem duplication (*FLT3*-ITD) status, *NPM1* mutations conferred more favorable prognosis in patients lacking *FLT3*-ITD in comparison with the outcomes of patients with wild-type *NPM1* or *FLT3*-ITD, or both.¹³⁻²⁰

To our knowledge, no study has analyzed the predictive significance of *NPM1* mutations as a single marker in the context of other prognostic factors in older patients with CN-AML.^{6,23-25} Therefore, we evaluated *NPM1* mutations in a relatively large set of adults ≥ 60 years with CN-AML treated with intensive chemotherapy and characterized comprehensively with regard to clinical and molecular features. Furthermore, to gain biologic insights, we determined the gene- and microRNA-expression signatures associated with *NPM1* mutations in these older patients.

PATIENTS AND METHODS

Patients, Cytogenetic Analysis, and Treatment

We studied pretreatment bone marrow (BM) samples of 148 patients age ≥ 60 years with de novo CN-AML and material available for analysis. Cytogenetic analyses at diagnosis were performed by Cancer and Leukemia Group B (CALGB)-approved institutional cytogenetic laboratories as part of the cytogenetic companion study 8461 and confirmed by central karyotype review.^{26,27} To establish CN-AML, ≥ 20 metaphase cells from diagnostic BM had to be analyzed and the karyotype found to be normal.²⁷ Institutional review board-approved informed consent for participation in the studies was obtained from all patients. Patients with preceding hematologic disorders or treatment-related AML were excluded, as were those who later underwent transplantation in first CR. The patients were enrolled onto CALGB front-line treatment protocols 9720^{10,28} (n = 83) or 10201²⁹ (n = 65; for treatment details see Appendix, online only).

Analysis of NPM1 and Other Molecular Markers

Sample preparation is described in the Appendix. *NPM1* mutational analysis was performed as previously reported.¹⁴ Briefly, DNA fragments spanning the entire *NPM1* exon 12 were amplified by polymerase chain reaction and directly sequenced. Reference sequences for mutational analyses are under NCBI accession numbers NM_002520.5 and NP_002511.1. Other molecular markers, that is, *FLT3*-ITD,³⁰ *FLT3*-tyrosine kinase domain mutations (*FLT3*-TKD),³¹ *CEBPA* mutations,³² and *WT1* mutations³³ were assessed centrally as previously reported.

Genome-Wide Expression Analyses

Gene- and microRNA-expression was profiled using the Affymetrix U133 plus 2.0 array (Affymetrix, Santa Clara, CA) and the Ohio State University custom microRNA array (OSU_CCC version 4.0), respectively, as reported previously,^{32,34,35} and described in the Appendix.

Statistical Analysis

Definitions of clinical end points (ie, CR, disease-free survival [DFS], and OS) are provided in the Appendix. Associations between patients with and without *NPM1* mutations for baseline demographic, clinical, and molecular

features were compared using the Fisher's exact and Wilcoxon rank sum tests for categorical and continuous variables, respectively. Estimated probabilities of OS and DFS were calculated using the Kaplan-Meier method, and the log-rank test was used to evaluate differences between survival distributions.

Multivariable analyses are detailed in the Appendix. Multivariable logistic regression models were constructed to analyze factors related to the probability of achieving CR using a limited backward selection procedure. Multivariable proportional hazards models were constructed for OS and DFS to evaluate the impact of *NPM1* mutations by adjusting for other variables using a limited backward selection procedure. For achievement of CR, estimated odds ratios, and for survival end points, hazard ratios with their corresponding 95% CIs, were obtained for each significant prognostic factor. All outcome end point models (CR, DFS, OS) were adjusted for treatment protocol (9720 v 10201).

For expression profiling, summary measures of gene and microRNA expression were computed, normalized, and filtered (Appendix). Expression signatures were derived by comparing gene and microRNA expression between *NPM1* mutated (*NPM1*mut) and *NPM1* wild-type (*NPM1*wt) patients. Univariable significance levels of .001 for gene- and .005 for microRNA-expression profiling were used to determine, respectively, the probe sets and microRNA probes that composed the signatures. Prediction of *NPM1* mutation status using gene- and microRNA-expression profiles is described in the Appendix. All analyses were performed by the CALGB Statistical Center.

RESULTS

Clinical Characteristics and Molecular Markers at Diagnosis

Overall, 83 (56%) patients harbored *NPM1* mutations. The most frequent was a type A mutation (n = 67 [81%]), that is, the duplication of TCTG at position c.860_863 (NCBI Accession NM_002520.5).¹³ Additional mutations included type B (n = 4 [5%]), type D (n = 3 [4%]), and others (n = 9 [11%]) (Appendix Table A1, online only).¹³ *NPM1*mut patients were younger ($P = .01$), had higher white blood counts (WBC; $P = .01$) and higher percentages of blood ($P = .01$) and BM ($P = .02$) blasts, experienced gum hypertrophy more often ($P = .02$), and were more frequently *FLT3*-ITD positive ($P = .01$) than *NPM1*wt patients (Table 1).

Prognostic Impact of NPM1 Mutations

*NPM1*mut patients had a significantly better CR rate than *NPM1*wt patients (84% v 48%; $P < .001$; Table 2). In multivariable analyses, mutated *NPM1* was independently associated with CR achievement ($P < .001$), after adjustment for WBC ($P = .001$). *NPM1*mut patients had more than nine-fold higher odds of achieving CR compared with *NPM1*wt patients (Table 3).

The median follow-up of living patients was 3.5 years (range, 2.3 to 9.7 years). Among patients who achieved CR, those with *NPM1* mutations had better DFS ($P = .047$; 3-year rates, 23% v 10%; Table 2; Fig 1A). In multivariable analyses, *NPM1* mutations were independently associated with longer DFS ($P = .004$), after adjustment for WBC ($P = .005$) and *FLT3*-ITD status ($P = .012$; Table 3).

*NPM1*mut patients also had a significantly longer OS compared with *NPM1*wt patients ($P < .001$; 3-year rates, 35% v 8%; Table 2; Fig 1B). In multivariable analyses, *NPM1* mutations were independently associated with longer OS ($P < .001$), after adjustment for WBC ($P = .008$) and *FLT3*-ITD status ($P = .014$; Table 3).

Prognostic Impact of NPM1 Mutations by Age Groups

The prognosis of adult patients with AML worsens with increasing age, with patients age ≥ 70 years having a particularly poor

Table 1. Clinical and Molecular Characteristics According to *NPM1* Mutation Status in Older Patients With Cytogenetically Normal De Novo Acute Myeloid Leukemia

Characteristic	<i>NPM1</i> Mutated (n = 83)		<i>NPM1</i> Wild-Type (n = 65)		P
	No.	%	No.	%	
Age, years					.01
Median	67		71		
Range	60-81		60-83		
Age group, years					.01
60-69	52	63	27	42	
≥ 70	31	37	38	58	
Sex					.40
Male	43	52	39	60	
Female	40	48	26	40	
Race					.36
White	77	94	56	89	
Nonwhite	5	6	7	11	
Performance status*					.84
Fully active (0)	20	24	18	29	0/1 v 2/3/4
Ambulatory (1)	46	56	31	50	
In bed < 50% of time (2)	14	17	13	21	
In bed > 50% of time (3)	1	1	0	0	
Completely bedridden (4)	1	1	0	0	
Hemoglobin, g/dL					.12
Median	9.4		9.3		
Range	6.0-15.0		6.5-13.6		
Platelet count, × 10 ⁹ /L					.32
Median	59.5		69.5		
Range	17-356		11-510		
WBC, × 10 ⁹ /L					.01
Median	26.2		7.0		
Range	1.0-249.3		1.0-434.1		
Percentage of PB blasts					.01
Median	44.5		30		
Range	0-97		0-96		
Percentage of BM blasts					.02
Median	66		47		
Range	11-93		7-96		
FAB†					—
M0	1	2	3	9	
M1	15	31	5	15	
M2	9	19	13	39	
M4	11	23	6	18	
M5	10	21	5	15	
M6	2	4	1	3	
Extramedullary involvement	20	25	15	23	.85
CNS	0	0	0	0	—
Hepatomegaly	3	4	3	5	1.00
Splenomegaly	5	6	8	12	.25
Lymphadenopathy	3	4	4	6	.70
Skin infiltrates	3	4	4	6	.70
Gum hypertrophy	12	15	2	3	.02
Mediastinal mass	0	0	0	0	—
<i>FLT3</i> -ITD					.01
Present	33	40	13	20	
Absent	50	60	52	80	
<i>FLT3</i> -TKD					.43
Present	10	12	5	8	
Absent	73	88	60	92	

(continued in next column)

Table 1. Clinical and Molecular Characteristics According to *NPM1* Mutation Status in Older Patients With Cytogenetically Normal De Novo Acute Myeloid Leukemia (continued)

Characteristic	<i>NPM1</i> Mutated (n = 83)		<i>NPM1</i> Wild-Type (n = 65)		P
	No.	%	No.	%	
<i>WT1</i>					.73
Mutated	4	5	4	6	
Wild-type	79	95	61	94	
<i>CEBPA</i>					.07
Mutated	6	7	11	17	
Wild-type	77	93	54	83	
Protocol					.50
9720	49	59	34	52	
10201	34	41	31	48	
Induction treatment and protocol					—
ADE (9720)	44	53	32	49	
ADEP (9720)	5	6	2	3	
AD (10201)	21	25	17	26	
ADG (10201)	13	16	14	22	

Abbreviations: PB, peripheral blood; BM, bone marrow; FAB, French-American-British classification; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; *FLT3*-TKD, tyrosine kinase domain mutations of the *FLT3* gene; in induction treatment: A, cytarabine; D, daunorubicin; E, etoposide; P, valspodar (PSC 833); G, oblimersen sodium (Genasense, G3139; Genta, Berkeley Heights, NJ).

*Eastern Cooperative Oncology Group/Zubrod/WHO performance status score.

†FAB classifications are centrally reviewed.

outcome.^{4,7-9,36} Although age on its own was not significantly associated with outcome in our patient set, we observed an interaction between age groups (60 to 69 v ≥ 70 years) and *NPM1* mutational status for OS ($P = .057$). *NPM1* mutational status was significant for OS in patients age ≥ 70 years, but not in those age 60 to 69 years. Therefore, we dichotomized the study population into patients age 60 to 69 years ($n = 79$; median age, 64 years; Appendix Table A2, online only) and those age ≥ 70 years ($n = 69$; median age, 74 years; Appendix Table A3, online only), and assessed whether the prognostic value of *NPM1* mutations differed between the age groups.

In the 60 to 69 years age group, *NPM1* mutations were associated with a higher CR rate (83% v 59%; $P = .031$; Table 2) and remained independent predictors for achievement of CR ($P = .017$), after accounting for WBC ($P = .021$; Table 3). However, *NPM1* mutations did not significantly affect DFS or OS duration (Tables 2, 3).

In the ≥ 70 years age group, *NPM1*mut patients also had significantly better CR rates than *NPM1*wt patients (87% v 39%; $P < .001$; Table 2), and mutated *NPM1* was independently associated with achievement of CR ($P < .001$), after adjustment for WBC ($P = .017$; Table 3). In addition, in contrast to patients age 60 to 69 years, those age ≥ 70 years with *NPM1* mutations had longer DFS ($P = .018$; 3-year rates, 30% v 7%; Table 2; Fig 1C) and OS ($P < .001$; 3-year rates, 45% v 5%; Table 2; Fig 1D) than those with wild-type *NPM1*. In multivariable analyses, mutated *NPM1* was independently associated with longer DFS ($P = .003$) and OS ($P < .001$), whereas no other variables remained in the final models (Table 3). *NPM1* mutations conferred 69% and 73% reduction in the risk of relapse or death, respectively, compared with wild-type *NPM1* (Table 3).

Table 2. Outcomes According to *NPM1* Mutation Status in Older Patients With Cytogenetically Normal De Novo Acute Myeloid Leukemia, Separately in Those Age 60-69 Years and ≥ 70 Years

Outcome	<i>NPM1</i> Mutated	<i>NPM1</i> Wild-Type	<i>P</i>
All patients	n = 83	n = 65	
Complete remission			< .001
No.	70	31	
%	84	48	
Disease-free survival			.047
Median, years	1.1	0.7	
% disease free at 3 years	23	10	
95% CI	14-33	2-23	
Overall survival			< .001
Median, years	1.6	1.1	
% alive at 3 years	35	8	
95% CI	25-45	3-16	
Patients age 60-69 years	n = 52	n = 27	
Complete remission			.031
No.	43	16	
%	83	59	
Disease-free survival			.48
Median, years	0.9	0.7	
% disease free at 3 years	19	13	
95% CI	9-31	2-33	
Overall survival			.22
Median, years	1.3	1.2	
% alive at 3 years	29	11	
95% CI	17-41	3-26	
Patients age ≥ 70 years	n = 31	n = 38	
Complete remission			< .001
No.	27	15	
%	87	39	
Disease-free survival			.018
Median, years	1.5	0.7	
% disease free at 3 years	30	7	
95% CI	14-47	0-26	
Overall survival			< .001
Median, years	2.5	0.9	
% alive at 3 years	45	5	
95% CI	27-61	1-16	

Outcomes according to *NPM1* mutation status are indicated for the whole study population of 148 patients ≥ 60 years (range, 60-83 years) with cytogenetically normal de novo acute myeloid leukemia, and separately for the age groups comprising 79 patients age 60-69 years and 69 patients age ≥ 70 years.

Genome-Wide Gene-Expression Analysis

A gene-expression signature associated with *NPM1* mutations was derived from the whole study population, comprising 3,577 probe sets differentially expressed between *NPM1*mut (n = 74) and *NPM1*wt (n = 58) patients. A total of 1,719 probe sets corresponding to 912 named genes were upregulated, and 1,858 probe sets corresponding to 969 named genes were downregulated, in *NPM1*mut patients (Figs 2A, 2B; Data Supplement 1).

Noteworthy features associated with *NPM1* mutations were upregulation of *HOXA* and *HOXB* genes, HOX cofactors *MEIS1* and *PBX3*, and other genes involved in embryonic development, for example, *FOXC1*, *NKX2-3*, *IRX3*, and *ZNF503*.³⁷⁻⁴¹ One of the most upregulated probe sets in *NPM1*mut patients recognizes six members of the *CT45* family.⁴² Other upregulated genes were *CTSG*, encoding the promyelocyte-specific serine protease cathepsin G⁴³; *CCL23*, en-

coding a protein capable of suppressing myeloid progenitor proliferation⁴⁴; *CCNA1*, the overexpression of which in transgenic mice leads to abnormal myelopoiesis⁴⁵; and several histone genes.⁴⁶

Downregulated in the *NPM1*mut group were genes whose low expression is associated with better prognosis in CN-AML, that is, *BAALC*,^{47,48} *MN1*,^{49,50} and *ERG*,^{51,52} and multidrug resistance genes *ABCB1*(*MDR1*)² and *ABCG2*(*BCRP*).⁵³ Other downregulated genes included *APP*, which is overexpressed in complex karyotype AML with 21q amplification⁵⁴; *HEMGN*, which is upregulated in CN-AML with high *ERG* expression⁵²; and *HGF* and *CD200*, genes reported to adversely affect AML outcome.^{55,56} Among the most downregulated genes were *CD34*, *PROM1*(*CD133*), and *CD48*. Also downregulated in the *NPM1*mut group were *DNTT*, which is regulated through histone modifications⁵⁷; *HOPX*, which mediates transcriptional repression by recruiting histone deacetylase activity⁵⁸; and *SPARC*, a gene with low expression in several neoplasms that can be upregulated in myelodysplastic syndromes responsive to lenalidomide.^{59,60}

The expression pattern of genes composing the signature was similar between the subsets of patients age 60 to 69 years and those age ≥ 70 years (Fig 2A). Among the probe sets forming the signature, no significant interaction effects between age group (60 to 69 and ≥ 70 years) and *NPM1* mutation status on gene-expression levels were observed.

We also tested whether *NPM1* mutation status could be predicted using gene-expression profiles. In leave-one-out cross-validated analysis, the mutation status of 89.4% of patients was correctly predicted (sensitivity = 91.9%, specificity = 86.2%; Table 4). Moreover, we applied the predictor derived from the gene-expression signature associated with *NPM1* mutations in older patients to a cohort of younger (< 60 years) CALGB patients with CN-AML.^{50,61} *NPM1* mutation status was accurately predicted for 86.3% of these patients (sensitivity = 89.4%, specificity = 80.6%; Table 4), similar to the performance of the predictor in the older cohort. This suggests that the expression patterns of the genes forming the signature associated with *NPM1* mutations are age independent (Appendix Fig A1).

Genome-Wide MicroRNA-Expression Analysis

We also derived a microRNA-expression signature associated with *NPM1* mutations in our cohort of older patients. Sixty-eight microRNA probes were differentially expressed between *NPM1*mut (n = 78) and *NPM1*wt (n = 56) patients. Thirty-four microRNA probes were upregulated and 34 downregulated in *NPM1*mut patients (Figs 2C, 2D; Data Supplement 2).

The prominent feature of the signature associated with *NPM1* mutations was upregulation of microRNAs embedded in the *HOX* cluster: *miR-10a*, *miR-10b*, *miR-196a* and *miR-196b*.⁶² Also overexpressed were members of the *let-7* family, which target oncogenes *RAS* and *MYC*⁶³; *miR-100*, upregulated in t(15;17)-positive AML^{64,65}; and *miR-9*, which targets *NFκB* and can be hypermethylated in cancer.^{66,67} Downregulated were *miR-126*, the expression of which we recently positively correlated with *MN1* expression in younger patients with CN-AML,⁵⁰ and *miR-130a* and *miR-451*, which are involved in megakaryocytic differentiation and erythropoiesis, respectively.^{68,69}

Similar to the gene-expression signature, the microRNA-expression pattern in the signature was comparable between patients

Table 3. Multivariable Analyses for Outcome According to the *NPM1* Mutation Status in All Older Patients With Cytogenetically Normal De Novo Acute Myeloid Leukemia, and Separately in Those Age 60-69 Years and ≥ 70 Years

Group	CR			DFS			OS		
	OR	95% CI	P	HR	95% CI	P	HR	95% CI	P
All patients									
<i>NPM1</i> , mutated v wild-type	9.35	3.73 to 23.26	< .001	0.48	0.29 to 0.78	.004	0.43	0.30 to 0.63	< .001
WBC, each 50 unit increase	0.43	0.26 to 0.71	.001	1.78	1.26 to 2.51	.005	1.19	1.05 to 1.36	.008
<i>FLT3</i> -ITD, present v absent				1.87	1.10 to 3.18	.012	1.66	1.11 to 2.48	.014
Patients age 60-69 years									
<i>NPM1</i> , mutated v wild-type	4.7	1.3 to 16.8	.017						
WBC, each 50 unit increase	0.4	0.2 to 0.9	.021						
Patients age ≥ 70 years									
<i>NPM1</i> , mutated v wild-type	19.23	3.98 to 90.90	< .001	0.31	0.14 to 0.68	.003	0.27	0.15 to 0.48	< .001
WBC, each 50 unit increase	0.43	0.22 to 0.86	.017						

NOTE. ORs > 1 (< 1) mean higher (lower) CR rate for the first category listed for dichotomous variables and for the higher (lower) values of continuous variables. HRs < 1 (> 1) indicate lower (higher) risk for an event for the first category listed for dichotomous variables and for the lower (higher) values of continuous variables. Variables considered in the model were those significant at $\alpha = .20$ from the univariable models. Variables considered in the models were as follows: for CR, *NPM1* (mutated v wild-type), platelets, WBC, and age; for DFS, *NPM1* (mutated v wild-type), *CEBPA* (mutated v wild-type), *FLT3*-ITD (present v absent), WBC, and race (white v nonwhite); for OS, *NPM1* (mutated v wild-type), *FLT3*-ITD (present v absent), *WT1* (mutated v wild-type), platelets, WBC, and race (white v nonwhite). Patients who had both *FLT3*-ITD and *FLT3*-TKD present were considered as *FLT3*-ITD present only for all outcome analyses.

Abbreviations: CR, complete remission; DFS, disease-free survival; OS, overall survival; OR, odds ratio; HR, hazard ratio; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene.

age 60 to 69 years and those age ≥ 70 years (Fig 2C). Among the probes forming the signature, no significant interaction effects between age group and *NPM1* mutation status on microRNA-expression levels were observed. Furthermore, *NPM1* mutation status could be pre-

dicted with a high degree of accuracy on the basis of microRNA-expression profiles. In leave-one-out cross-validated analysis, the mutation status of 92.5% of patients in our cohort was correctly predicted (sensitivity = 96.2%, specificity = 87.5%).

Fig 1. (A) Disease-free survival and (B) overall survival of patients age ≥ 60 years with cytogenetically normal de novo acute myeloid leukemia according to *NPM1* mutation status. (C) Disease-free survival and (D) overall survival of patients age ≥ 70 years with cytogenetically normal de novo acute myeloid leukemia according to *NPM1* mutation status. *NPM1*mut, *NPM1* mutated; *NPM1*wt, *NPM1* wild-type.

Fig 2. Heat maps of the (A) gene- and (C) microRNA-expression signatures associated with *NPM1* mutations in older patients with cytogenetically normal de novo acute myeloid leukemia. Rows represent probe sets (A) or microRNA probes (C), and columns represent patients. Patients are grouped by *NPM1* mutational status and ordered by age within these groups. Expression values of the probe sets and microRNA probes are represented by color, with green indicating expression less than, and red indicating expression greater than, the median value for the given probe set or microRNA probe. (B) Fold-changes for selected genes of the signature. Genes are sorted by fold-change in expression between the *NPM1*mut and *NPM1*wt groups (MUT/WT). For genes with more than one corresponding probe set in the signature, the geometric mean of the fold-changes of these probe sets is displayed. (D) Fold-changes for selected microRNAs in the signature. MicroRNAs are sorted by fold-change in expression between the *NPM1*mut and *NPM1*wt groups (MUT/WT). Fold-change is displayed for those probes hybridizing with the respective mature microRNA. For microRNAs with more than one corresponding probe in the signature, the geometric mean of the fold-changes of these probes is given. Selection of genes and microRNAs is based on the fold-change and previous reports of these genes and microRNAs associated with *NPM1* mutations, leukemia, or other malignancies.

Table 4. Prediction Accuracy of *NPM1* Mutation Status of Patients Age $\geq$ 60 Years and $<$ 60 Years With Cytogenetically Normal De Novo Acute Myeloid Leukemia, Using the Gene-Expression Profile Derived in Patients Age $\geq$ 60 Years

Predictor	$\geq$ 60 years (n = 132)	$<$ 60 years (n = 102)
Overall accuracy	89.4%	86.3%
Sensitivity	91.9%	89.4%
Specificity	86.2%	80.6%

Prediction of *NPM1* mutation status by leave-one-out cross-validated analysis using the gene-expression profiles derived in patients age $\geq$ 60 years with cytogenetically normal de novo acute myeloid leukemia (AML). The same predictor was then applied to a cohort of younger ($<$ 60 years) patients with cytogenetically normal AML. Sensitivity is the probability that gene-expression analysis correctly predicts the presence of *NPM1* mutation; specificity is the probability that gene-expression analysis correctly predicts wild-type *NPM1*.

DISCUSSION

CN-AML in older patients is generally associated with poor prognosis, but the reasons why outcome of this age group is worse than that of younger patients, and to what extent prognosis of older patients depends on molecular markers, are largely unknown. In this study, we evaluated the prognostic impact of *NPM1* mutations in a relatively large series of patients age $\geq$ 60 years with de novo CN-AML, treated with intensive chemotherapy, in the context of molecular variables known to affect the outcome of younger patients with CN-AML.¹²

The incidence of *NPM1* mutations at diagnosis and their association with *FLT3*-ITD, higher WBC, and increased blast percentages in our older patients' cohort were similar to data reported in studies comprising younger patients.¹³⁻²⁰ However, in contrast to these studies, in which analyses of *NPM1* mutations as a single outcome predictor yielded inconsistent results,¹³⁻²⁰ we found that in older CN-AML patients, *NPM1* mutations had a favorable prognostic impact independent of other molecular and clinical prognosticators.

We showed that *NPM1* mutations were associated with relatively high odds of achieving CR even after adjustment for other predictive variables. These results are consistent with a recent report by Schneider et al,²⁴ although these authors tested the predictive value of *NPM1* mutations using only univariable analysis. Importantly, we showed that the CR rate of older CN-AML patients with *NPM1* mutations is similar to that observed in younger *NPM1*mut patients treated in CALGB front-line clinical trials.⁶¹ This suggests that *NPM1* mutations may represent a marker that can be used to stratify older patients with CN-AML to up-front intensive chemotherapy. More intensive treatment has often been avoided in older patients because of disappointing clinical results and low therapeutic index. However, the favorable outcome associated with *NPM1* mutations was even more pronounced in the oldest subgroup ($\geq$ 70 years) of our cohort, thereby emphasizing that older age alone should not exclude patients from more intensive chemotherapy.

We also demonstrated that *NPM1* mutations in older patients with CN-AML independently predicted better DFS and OS. The impact of *NPM1* mutations on survival of such patients was analyzed in two recent studies.^{6,25} In a smaller study, Schlenk et al²⁵ reported that relapse-free survival and OS were longer for *NPM1*mut patients than for those with wild-type *NPM1*, but only in the absence of *FLT3*-ITD and only in patients receiving all-*trans*-retinoic acid in addition to

chemotherapy. Similarly, in a study focused on age-related risk profiles, Büchner et al⁶ evaluated the prognostic value of *NPM1* mutations only together with *FLT3*-ITD mutational status, and showed that patients with *NPM1* mutations lacking *FLT3*-ITD appeared to have longer remission duration and OS than the remaining patients. In contrast to our study, neither of these reports established the prognostic impact of *NPM1* mutations as a single marker, evaluated separately patients age $\geq$ 70 years, or assessed the biologic significance of *NPM1* mutations in older patients with CN-AML using gene- and microRNA-expression profiling.

Our subset outcome analyses of patients age 60 to 69 years and those age $\geq$ 70 years revealed that in the 60 to 69 years group, *NPM1* mutations predicted higher CR rates but had no significant impact on survival. In contrast, in the $\geq$ 70 years group, *NPM1* mutations were a strong, independent predictor not only for higher CR rates, but also for longer DFS and OS. To our knowledge, this is the first example of a molecular marker whose favorable prognostic impact seems to become stronger with increased age in adults.

It should be underscored that despite the favorable outcome of older *NPM1*mut patients compared with that of *NPM1*wt patients, their DFS and OS remain disappointing and worse than those in younger *NPM1*mut patients with CN-AML.^{14-20,61} Whether this is caused by differences in the postremission therapy administered, disease biology, or other age-related factors remains to be established. To gain insight into potential biologic differences associated with age, we performed genome-wide gene- and microRNA-expression analyses. We found that expression of genes in the *NPM1* mutation-associated signature appeared similar in both groups of older patients (ie, 60 to 69 and $\geq$ 70 years). Moreover, the main features of our gene- and microRNA-expression signatures were similar to those reported by other groups in studies comprising mainly younger patients.^{65,70-72} Indeed, we showed that the gene-expression signature derived from older patients could predict accurately the *NPM1* mutation status in younger (18 to 59 years) CALGB patients with CN-AML. Similar gene-expression patterns across the age groups support the notion that *NPM1*mut CN-AML is a unique biologic entity and that differences in outcome may be related to differences in treatment or the influence of other clinical and molecular factors.

A noteworthy feature of our gene-expression signature associated with *NPM1* mutations in older patients was upregulation of homeobox genes, including *HOXA* and *HOXB*. This was accompanied by upregulation of *miR-10a*, *miR-10b*, *miR-196a*, and *miR-196b*, which are colocalized with and target various *HOX* genes.⁶² Moreover, consistent with the favorable prognostic impact of *NPM1* mutations, the multidrug resistance genes *ABCB1* (*MDR1*) and *ABCG2* (*BCRP*),^{2,53} and *BAALC*, *MN1*, and *ERG*, the high expression of which is associated with poor prognosis in CN-AML,⁴⁷⁻⁵² were downregulated in the *NPM1*mut group. As in our previous study of younger patients with CN-AML,⁵⁰ a positive correlation between *MN1* expression and *miR-126* was also observed in the older patients. *MIR-126* is reportedly hypermethylated in various neoplasms,^{64,67} and downregulation of *miR-126* increases apoptosis in leukemia cells.⁶⁴ Finally, in accordance with our recently described signature associated with *BAALC* expression in younger CN-AML patients,⁴⁸ *CT45* was highly upregulated in the *NPM1*mut group.

In conclusion, we show that *NPM1* mutations constitute a strong, independent prognostic factor for favorable treatment response and survival in older patients with CN-AML treated with

intensive chemotherapy. The gene- and microRNA-expression signatures of older *NPM1*mut CN-AML patients appear similar to those in younger patients, thereby suggesting that CN-AML with *NPM1* mutations may be a single entity in all age groups. Future studies should prospectively validate our findings and test whether older patients with CN-AML with *NPM1* mutations would benefit from up-front allocation to therapies similar to those received by the younger patients with *NPM1* mutations.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

- Ries LAG, Melbert D, Krapcho M, et al: SEER Cancer Statistics Review, 1975-2005. National Cancer Institute, Bethesda, MD. http://seer.cancer.gov/csr/1975_2005, 2008
- Leith CP, Kopecky KJ, Godwin J, et al: Acute myeloid leukemia in the elderly: Assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood* 89:3323-3329, 1997
- Farag SS, Archer KJ, Mrózek K, et al: Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: Results from Cancer and Leukemia Group B 8461. *Blood* 108:63-73, 2006
- Fröhling S, Schlenk RF, Kayser S, et al: Cytogenetics and age are major determinants of outcome in intensively treated acute myeloid leukemia patients older than 60 years: Results from AMLSG trial AML HD98-B. *Blood* 108:3280-3288, 2006
- Kantarjian H, O'Brien S, Cortes J, et al: Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: Predictive prognostic models for outcome. *Cancer* 106:1090-1098, 2006
- Büchner T, Berdel WE, Haeflrich C, et al: Age-related risk profile and chemotherapy dose response in acute myeloid leukemia: A study by the German Acute Myeloid Leukemia Cooperative Group. *J Clin Oncol* 27:61-69, 2009
- Appelbaum FR, Gundacker H, Head DR, et al: Age and acute myeloid leukemia. *Blood* 107:3481-3485, 2006
- Estey E: Acute myeloid leukemia and myelodysplastic syndromes in older patients. *J Clin Oncol* 25:1908-1915, 2007
- Juliusson G, Antunovic P, Derolf A, et al: Age and acute myeloid leukemia: Real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood* 113:4179-4187, 2009
- Baer MR, George SL, Dodge RK, et al: Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B study 9720. *Blood* 100:1224-1232, 2002
- Goldstone AH, Burnett AK, Wheatley K, et al: Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: The results of the United Kingdom Medical Research Council AML11 trial. *Blood* 98:1302-1311, 2001
- Mrózek K, Marcucci G, Paschka P, et al: Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: Are we ready for a prognostically prioritized molecular classification? *Blood* 109:431-448, 2007
- Falini B, Mecucci C, Tiacci E, et al: Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med* 352:254-266, 2005
- Döhner K, Schlenk RF, Habdank M, et al: Mutant nucleophosmin (*NPM1*) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: Interaction with other gene mutations. *Blood* 106:3740-3746, 2005
- Schnittger S, Schoch C, Kern W, et al: Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood* 106:3733-3739, 2005
- Boissel N, Renneville A, Biggio V, et al: Prevalence, clinical profile and prognosis of *NPM* mutations in AML with normal karyotype. *Blood* 106:3618-3620, 2005
- Suzuki T, Kiyoi H, Ozeki K, et al: Clinical characteristics and prognostic implications of *NPM1* mutations in acute myeloid leukemia. *Blood* 106:2854-2861, 2005
- Thiede C, Koch S, Creutzig E, et al: Prevalence and prognostic impact of *NPM1* mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood* 107:4011-4020, 2006
- Schlenk RF, Döhner K, Krauter J, et al: Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med* 358:1909-1918, 2008
- Gale RE, Green C, Allen C, et al: The impact of *FLT3* internal tandem duplication mutant level, number, size, and interaction with *NPM1* mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood* 111:2776-2784, 2008
- Arber DA, Vardman JW, Brunning RD, et al: Acute myeloid leukaemia with recurrent genetic abnormalities, in Swerdlow SH, Campo E, Harris NL, et al (eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France, IARC, 2008, pp 110-123
- Falini B, Bolli N, Liso A, et al: Altered nucleophosmin transport in acute myeloid leukaemia with mutated *NPM1*: Molecular basis and clinical implications. *Leukemia* 23:1731-1743, 2009
- Scholl S, Theuer C, Scheble V, et al: Clinical impact of nucleophosmin mutations and *FLT3* internal tandem duplications in patients older than 60 yr with acute myeloid leukaemia. *Eur J Haematol* 80:208-215, 2008
- Schneider F, Hoster E, Unterhalt M, et al: *NPM1* but not *FLT3-ITD* mutations predict early blast cell clearance and CR rate in patients with normal karyotype AML (NK-AML) or high-risk myelodysplastic syndrome (MDS). *Blood* 113:5250-5253, 2009
- Schlenk RF, Döhner K, Kneba M, et al: Gene mutations and response to treatment with all-trans retinoic acid in elderly patients with acute myeloid leukemia. Results from the AMLSG Trial AML HD98B. *Haematologica* 94:54-60, 2009
- Byrd JC, Mrózek K, Dodge RK, et al: Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and

overall survival in adult patients with de novo acute myeloid leukemia: Results from Cancer and Leukemia Group B (CALGB 8461). *Blood* 100:4325-4336, 2002

27. Mrózek K, Carroll AJ, Maharry K, et al: Central review of cytogenetics is necessary for cooperative group correlative and clinical studies of adult acute leukemia: The Cancer and Leukemia Group B experience. *Int J Oncol* 33:239-244, 2008

28. Baer MR, George SL, Caligiuri MA, et al: Low-dose interleukin-2 immunotherapy does not improve outcome of patients age 60 years and older with acute myeloid leukemia in first complete remission: Cancer and Leukemia Group B study 9720. *J Clin Oncol* 26:4934-4939, 2008

29. Marcucci G, Moser B, Blum W, et al: A phase III randomized trial of intensive induction and consolidation chemotherapy $\pm$ oblimersen, a pro-apoptotic Bcl-2 antisense oligonucleotide in untreated acute myeloid leukemia patients > 60 years old. *J Clin Oncol* 25:360s, 2007 (suppl; abstr 7012)

30. Thiede C, Steudel C, Mohr B, et al: Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. *Blood* 99:4326-4335, 2002

31. Whitman SP, Ruppert AS, Radmacher MD, et al: *FLT3* D835/I836 mutations are associated with poor disease-free survival and a distinct gene-expression signature among younger adults with de novo cytogenetically normal acute myeloid leukemia lacking *FLT3* internal tandem duplications. *Blood* 111:1552-1559, 2008

32. Marcucci G, Maharry K, Radmacher MD, et al: Prognostic significance of, and gene and microRNA expression signatures associated with, *CEPBA* mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: A Cancer and Leukemia Group B study. *J Clin Oncol* 26:5078-5087, 2008

33. Paschka P, Marcucci G, Ruppert AS, et al: Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: A Cancer and Leukemia Group B study. *J Clin Oncol* 26:4595-4602, 2008

34. Calin GA, Liu C-G, Sevignani C, et al: MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci U S A* 101:11755-11760, 2004

35. Radmacher MD, Marcucci G, Ruppert AS, et al: Independent confirmation of a prognostic gene-expression signature in adult acute myeloid leukemia with a normal karyotype: A Cancer and Leukemia Group B study. *Blood* 108:1677-1683, 2006

36. Peterson BA, Bloomfield CD: Treatment of acute nonlymphocytic leukemia in elderly patients: A prospective study of intensive chemotherapy. *Cancer* 40:647-652, 1977

37. Moens CB, Selli L: Hox cofactors in vertebrate development. *Dev Biol* 291:193-206, 2006

38. Pabst O, Zweigerdt R, Arnold HH: Targeted disruption of the homeobox transcription factor Nkx2-3 in mice results in postnatal lethality and abnormal development of small intestine and spleen. *Development* 126:2215-2225, 1999

39. Gómez-Skarmeta JL, Modolell J: Iroquois genes: Genomic organization and function in vertebrate neural development. *Curr Opin Genet Dev* 12:403-408, 2002

40. Lehmann OJ, Sowden JC, Carlsson P, et al: Fox's in development and disease. *Trends Genet* 19:339-344, 2003

41. McGlenn E, Richman JM, Metzis V, et al: Expression of the NET family member *Zfp503* is regulated by hedgehog and BMP signaling in the limb. *Dev Dyn* 237:1172-1182, 2008

42. Chen Y-T, Scanlan MJ, Venditti CA, et al: Identification of cancer/testis-antigen genes by massively parallel signature sequencing. *Proc Natl Acad Sci U S A* 102:7940-7945, 2005

43. Hanson RD, Connolly NL, Burnett D, et al: Developmental regulation of the human cathepsin G gene in myelomonocytic cells. *J Biol Chem* 265:1524-1530, 1990

44. Patel VP, Kreider BL, Li Y, et al: Molecular and functional characterization of two novel human C-C chemokines as inhibitors of two distinct classes of myeloid progenitors. *J Exp Med* 185:1163-1172, 1997

45. Liao C, Wang XY, Wei HQ, et al: Altered myelopoiesis and the development of acute myeloid leukemia in transgenic mice overexpressing cyclin A1. *Proc Natl Acad Sci U S A* 98:6853-6858, 2001

46. Marzluff WF, Wagner EJ, Duronio RJ: Metabolism and regulation of canonical histone mRNAs: Life without a poly(A) tail. *Nat Rev Genet* 9:843-854, 2008

47. Baldus CD, Tanner SM, Ruppert AS, et al: *BAALC* expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: A Cancer and Leukemia Group B study. *Blood* 102:1613-1618, 2003

48. Langer C, Radmacher MD, Ruppert AS, et al: High *BAALC* expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: A Cancer and Leukemia Group B (CALGB) study. *Blood* 111:5371-5379, 2008

49. Heuser M, Beutel G, Krauter J, et al: High meningioma 1 (*MN1*) expression as a predictor for poor outcome in acute myeloid leukemia with normal cytogenetics. *Blood* 108:3898-3905, 2006

50. Langer C, Marcucci G, Holland KB, et al: Prognostic importance of *MN1* transcript levels, and biologic insights from *MN1*-associated gene and microRNA expression signatures in cytogenetically normal acute myeloid leukemia: A Cancer and Leukemia Group B study. *J Clin Oncol* 27:3198-3204, 2009

51. Marcucci G, Baldus CD, Ruppert AS, et al: Overexpression of the ETS-related gene, *ERG*, predicts a worse outcome in acute myeloid leukemia with normal karyotype: A Cancer and Leukemia Group B study. *J Clin Oncol* 23:9234-9242, 2005

52. Marcucci G, Maharry K, Whitman SP, et al: High expression levels of the ETS-related gene, *ERG*, predict adverse outcome and improve molecular risk-based classification of cytogenetically normal acute myeloid leukemia: A Cancer and Leukemia Group B study. *J Clin Oncol* 25:3337-3343, 2007

53. Benderra Z, Faussat AM, Sayada L, et al: MRP3, BCRP, and P-glycoprotein activities are prognostic factors in adult acute myeloid leukemia. *Clin Cancer Res* 11:7764-7772, 2005

54. Baldus CD, Liyanarachchi S, Mrózek K, et al: Acute myeloid leukemia with complex karyotypes and abnormal chromosome 21: Amplification discloses overexpression of *APP*, *ETS2*, and *ERG* genes. *Proc Natl Acad Sci U S A* 101:3915-3920, 2004

55. Verstovsek S, Kantarjian H, Estey E, et al: Plasma hepatocyte growth factor is a prognostic factor in patients with acute myeloid leukemia but not in patients with myelodysplastic syndrome. *Leukemia* 15:1165-1170, 2001

56. Tonks A, Hills R, White P, et al: CD200 as a prognostic factor in acute myeloid leukaemia. *Leukemia* 21:566-568, 2007

57. Su R-C, Brown KE, Saaber S, et al: Dynamic assembly of silent chromatin during thymocyte maturation. *Nat Genet* 36:502-506, 2004

58. Kook H, Lepore JJ, Gitler AD, et al: Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeo-domain protein Hop. *J Clin Invest* 112:863-871, 2003

59. DiMartino JF, Lacayo NJ, Varadi M, et al: Low or absent *SPARC* expression in acute myeloid leukemia with *MLL* rearrangements is associated with sensitivity to growth inhibition by exogenous *SPARC* protein. *Leukemia* 20:426-432, 2006

60. Pellagatti A, Jädersten M, Forsblom A-M, et al: Lenalidomide inhibits the malignant clone and up-regulates the *SPARC* gene mapping to the commonly deleted region in 5q- syndrome patients. *Proc Natl Acad Sci U S A* 104:11406-11411, 2007

61. Paschka P, Marcucci G, Maharry K, et al: Prognostic impact of *NPM1* mutations in cytogenetically normal (CN) acute myeloid leukemia (AML) is adversely affected by high expression of *ERG* gene: A Cancer and Leukemia Group B (CALGB) study. *Haematologica* 92:145, 2007 (suppl 1; abstr 396)

62. Yekta S, Tabin CJ, Bartel DP: MicroRNAs in the Hox network: An apparent link to posterior prevalence. *Nat Rev Genet* 9:789-796, 2008

63. Büssing I, Slack FJ, Großhans H: Let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med* 14:400-409, 2008

64. Li Z, Lu J, Sun M, et al: Distinct microRNA expression profiles in acute myeloid leukemia with common translocations. *Proc Natl Acad Sci U S A* 105:15535-15540, 2008

65. Jongen-Lavrencic M, Sun SM, Dijkstra MK, et al: MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. *Blood* 111:5078-5085, 2008

66. Bazzoni F, Rossato M, Fabbri M, et al: Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. *Proc Natl Acad Sci U S A* 106:5282-5287, 2009

67. Lujambio A, Calin GA, Villanueva A, et al: A microRNA DNA methylation signature for human cancer metastasis. *Proc Natl Acad Sci U S A* 105:13556-13561, 2008

68. Garzon R, Pichiorri F, Palumbo T, et al: MicroRNA fingerprints during human megakaryocytopoiesis. *Proc Natl Acad Sci U S A* 103:5078-5083, 2006

69. Masaki S, Ohtsuka R, Abe Y, et al: Expression patterns of microRNAs 155 and 451 during normal human erythropoiesis. *Biochem Biophys Res Commun* 364:509-514, 2007

70. Verhaak RGW, Goudswaard CS, van Putten W, et al: Mutations in nucleophosmin (*NPM1*) in acute myeloid leukemia (AML): Association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. *Blood* 106:3747-3754, 2005

71. Alcalay M, Tiacci E, Bergamas R, et al: Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc⁺ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. *Blood* 106:899-902, 2005

72. Garzon R, Garofalo M, Martelli MP, et al: Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. *Proc Natl Acad Sci U S A* 105:3945-3950, 2008