Low expression of *MN1* associates with better treatment response in older patients with de novo cytogenetically normal acute myeloid leukemia

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Low *MN1* expression bestows favorable prognosis in younger adults with cytogenetically normal acute myeloid leukemia (CN-AML), but its prognostic significance in older patients is unknown. We analyzed pretherapy *MN1* expression in 140 older (≥ 60 years) de novo CN-AML patients treated on cytarabine/daunorubicin-based protocols. Low *MN1* expressers had higher complete remission (CR) rates (*P* = .001), and longer overall survival (*P* = .03) and event-free survival (EFS; *P* = .004). In multivariable models, low *MN1* expression was associated with better CR rates and EFS. The impact of *MN1* expression on overall survival and EFS was predominantly in patients 70 years of age or older, with low *MN1* expressers with mutated *NPM1* having the best outcome. The impact of *MN1* expression was also observed in the Intermediate-I, but not the Favorable group of the European LeukemiaNet classification, where low *MN1* expressers had CR rates and EFS similar to those of Favorable group patients. *MN1* expresser-status-associated gene- and microRNA-expression signatures revealed underexpression of drug resistance and adverse outcome predictors, and overexpression of *HOX* genes and *HOX*-gene–embedded microRNAs in low *MN1* expressers. We conclude that low *MN1* expression confers better prognosis in older CN-AML patients and may refine the European LeukemiaNet classification. Biologic features associated with *MN1* expression may help identify new treatment targets. (*Blood*. 2011;118(15): 4188-4198)

Introduction

Over the past 3 decades, there has been relatively steady improvement of outcomes of patients with acute myeloid leukemia (AML) younger than 60 years. However, this has not occurred in older AML patients. Despite advances in our understanding of disease mechanisms and investigation of new therapies targeting distinct clinical, cytogenetic, and molecular subsets, the outcome of AML patients older than 60 years remains poor, with long-term survival rates of ~ 7%-15%.[1-3] The shorter survival of older AML patients compared with younger patients is probably related to clinical and biologic differences between them, including the failure to achieve a complete remission (CR) as a result of an increased intrinsic resistance of leukemic blasts to chemotherapy and the presence of specific cytogenetic and/or molecular alterations associated with worse outcome.[4]

As in younger patients, older patients with cytogenetically normal (CN) AML represent the largest AML subset.[5] This group is molecularly heterogeneous.[6,7] To date, however, the prognostic significance of molecular genetic alterations has been studied most extensively in younger (< 60 years) patients.[6-8] Recently, some, but not all, of these markers have also been shown to impact on outcome of older (≥ 60 years) CN-AML patients. For example,

NPM1 mutations,[9] and lower expression levels of the *BAALC* and *ERG*[10] genes have been associated with favorable outcome, whereas *FLT3* internal tandem duplication (*FLT3*-ITD)[11] and *WT1* mutations[12] have been shown to confer adverse prognosis in older patients, as they do in younger patients. However, to our knowledge, no study has investigated the prognostic impact of meningioma 1 (*MN1*) gene expression levels exclusively in CN-AML patients aged 60 years of age and older.

The *MN1* gene is localized at human chromosome band 22q12 and encodes a transcriptional coregulator.[13] *MN1* is involved in myeloid malignancies as a fusion partner of the *ETV6* gene in the recurrent translocation t(12;22)(p13;q11)[14] and has been shown to be overexpressed in subsets of AML.[15,16] We and others have shown that high *MN1* expression levels are prognosticators for poor outcome in younger CN-AML patients.[17,18]

With the hope to better predict the course of the disease, adjust therapeutic approaches, and improve outcome, we explored herein the prognostic significance of *MN1* expression in older de novo CN-AML patients. We have also analyzed genome-wide gene- and microRNA-expression profiles associated with *MN1* expression in these patients, to gain insights into *MN1*-associated disease.

Methods

Patients and treatment

Pretreatment bone marrow (BM) samples of 140 adults 60 years of age or older with de novo CN-AML and material available were analyzed for *MN1* expression. The patients were enrolled on Cancer and Leukemia Group B (CALGB) front-line intensive cytarabine/daunorubicin-based treatment protocols (for protocol details see supplemental Methods, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Institutional Review Board–approved, written informed consent for participation in these studies was obtained from all patients in accordance with the Declaration of Helsinki.

Cytogenetics and additional molecular markers

Pretreatment cytogenetic analyses of BM were performed by CALGB-approved institutional cytogenetic laboratories as part of CALGB 8461, a prospective cytogenetic companion study, and the results reviewed centrally.[19,20] For a case to be considered CN, at least 20 metaphase cells had to be analyzed and the karyotype found to be normal.[20]

The presence or absence of *FLT3*-ITD mutations in the tyrosine kinase domain of the *FLT3* gene (*FLT3*-TKD) and mutations in the *CEBPA*, *IDH1*, *IDH2*, *NPM1*, *TET2*, and *WT1* genes was determined centrally in pretreatment samples as described previously.[9,11,12,21-28] The expression levels of the *BAALC* and *ERG* genes in peripheral blood were also assessed centrally in pretreatment samples as previously described.[10,29-32] *miR-181a* expression was evaluated as previously described.[33]

RNA extraction and real-time RT-PCR to measure *MN1* expression levels

Preparation of pretreatment BM samples and the analysis of *MN1* expression were performed as previously described.[18] Briefly, total RNA was extracted using Trizol reagent, and complementary DNA was synthesized from total RNA. Quantitative real-time RT-PCR amplifications of *MN1* and *ABL1* were performed using standard curves. *MN1* copy numbers were normalized to *ABL1* copy numbers.

Gene- and microRNA-expression profiling

For gene- and microRNA-expression profiling, total RNA was extracted from pretreatment BM or blood mononuclear cells. Gene- and microRNA-expression profiling was performed using the Affymetrix U133 plus Version 2.0 array (Affymetrix) and The Ohio State University custom microRNA array (OSU_CCC Version 4.0), respectively, as previously reported[9] and detailed in supplemental Methods.

Definition of clinical end points and statistical analysis

The main objective of this study was to evaluate the prognostic value of *MN1* expression on clinical outcome in older de novo CN-AML patients. For these patients, the median *MN1/ABL1* copy number value was chosen to define the low and high *MN1* expressers. This cut-off was based on the trend in overall survival (OS) of patients divided into quartiles by *MN1* level values; patients in the first 2 quartiles had a better outcome than patients in quartiles 3 and 4 (*P* = .04 test for trend).[34]

Definitions of clinical end points (ie, CR, disease-free survival [DFS], OS, and event-free survival [EFS]) are provided in supplemental Methods. Associations between patients with low or high expression of *MN1* for baseline demographic, clinical, and molecular features were compared using the Fisher exact and Wilcoxon rank-sum tests for categorical and continuous variables, respectively. Estimated probabilities of DFS, OS, and EFS were calculated using the Kaplan-Meier method, and the log-rank test evaluated differences between survival distributions. Multivariable analyses are detailed in supplemental Methods. Briefly, 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

Table 1. Clinical and molecular characteristics according to *MN1* expression status in CN-AML patients 60 years of age or older

Characteristic	Low *MN1* (n = 70)	High *MN1* (n = 70)	*P*
Age, y			.57
Median	66	69	
Range	60-81	60-81	
Sex, no. (%) of males	40 (57)	32 (46)	.24
Race, no. (%)			.49
White	66 (96)	63 (93)	
Nonwhite	3 (4)	5 (7)	
Hemoglobin, g/dL			.16
Median	9.1	9.4	
Range	5.4-13.6	6.0-13.1	
Platelets, × 10⁹/L			.43
Median	63	72	
Range	20-271	11-850	
WBC count, × 10⁹/L			.15
Median	33.8	21.5	
Range	1.0-450.0	1.0-434.1	
Blood blasts, %			1.0
Median	45	49	
Range	0-96	0-99	
BM blasts, %			.32
Median	71	64	
Range	15-97	7-96	
Extramedullary involvement, no. (%)	19 (28)	15 (22)	.55
***FLT3*-ITD, no. (%)**			.59
Present	22 (31)	26 (37)	
Absent	48 (69)	44 (63)	
***FLT3*-TKD, no. (%)**			.08
Present	10 (14)	3 (4)	
Absent	60 (86)	67 (96)	
***CEBPA*, no. (%)**			.14
Mutated	6 (9)	13 (19)	
Single mutated	5	7	
Double mutated	1	6	
Wild-type	64 (91)	57 (81)	
***IDH1*, no. (%)**			.30
Mutated	10 (14)	6 (9)	
Wild-type	59 (86)	63 (91)	
***IDH2*, no. (%)**			.54
IDH2-mutated	13 (19)	17 (24)	
R140-*IDH2*-mutated	12	13	
R172-*IDH2*-mutated	1	4	
Wild-type	56 (81)	53 (76)	
***NPM1*, no. (%)**			< .001
Mutated	55 (79)	26 (37)	
Wild-type	15 (21)	44 (63)	
***TET2*, no. (%)**			.85
Mutated	19 (28)	18 (26)	
Wild-type	49 (72)	51 (74)	
***WT1*, no. (%)**			1.0
Mutated	3 (4)	3 (4)	
Wild-type	67 (96)	67 (96)	
***BAALC* expression,* no. (%)**			< .001
Low	48 (73)	24 (34)	
High	18 (27)	46 (66)	
***ERG* expression,* no. (%)**			.23
Low	36 (55)	30 (43)	
High	30 (45)	40 (57)	
***miR-181a* expression (continuous)**			.04
Median (log expression units)	11.89	12.23	
Range	9.06-15.43	8.81-14.66	
ELN risk group,† no. (%)			.03
Favorable	40 (57)	26 (37)	
Intermediate-I	30 (43)	44 (63)	

*The median expression value was used as a cutpoint.

†Favorable risk group consists of patients with *CEBPA* mutations or those who are *FLT3*-ITD-negative and harbor *NPM1* mutations. Intermediate-I genetic group is composed of patients who are not in the Favorable group (ie, those with wild-type *CEBPA* and wild-type *NPM1* with or without *FLT3*-ITD or mutated *NPM1* with *FLT3*-ITD).
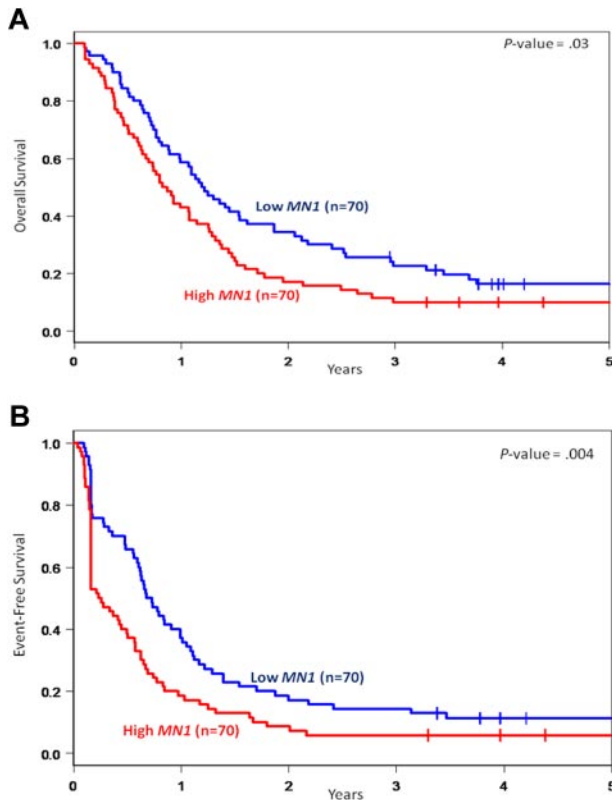
Figure 1. Outcome of CN-AML patients 60 years of age or older with respect to **MN1** expression. (A) OS. (B) EFS.

EFS to evaluate the impact of low or high expression of *MN1* 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% confidence intervals (CIs) were examined.

For the gene- and microRNA-expression profiling, summary measures of gene and microRNA expression were computed, normalized, and filtered (supplemental Methods). *MN1*-associated signatures were derived by comparing gene and microRNA expression between low and high *MN1* expressers. 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 constituted the signatures.

All analyses were performed by the CALGB Statistical Center.

Results

Associations of *MN1* expression with clinical and molecular characteristics and clinical outcome in older CN-AML patients

At diagnosis, the low and high *MN1* expresser groups did not differ significantly with regard to any of the clinical pretreatment characteristics. However, low *MN1* expression was associated with mutated *NPM1* ($P < .001$), lower *BAALC* expression levels ($P < .001$), and lower *miR-181a* expression levels ($P = .04$), as well as a trend for the presence of *FLT3*-TKD ($P = .08$; Table 1).

With a median follow-up for living patients of 4.0 years (range, 3-11.6 years) and for those who did not have an event of 4.2 years (range, 3.3-11.6 years), low *MN1* expressers had a higher CR rate (80% vs 53%, $P = .001$) and longer OS ($P = .03$; Figure 1A) and EFS ($P = .004$; Figure 1B) than high *MN1* expressers (Table 2). We did not observe a significant difference in DFS between high and low *MN1* expressers ($P = .29$; Table 2)

In a multivariable model for CR, *MN1* expression was a strong prognostic factor ($P = .01$), when controlling for *BAALC* expression ($P < .001$) and WBC ($P = .01$; Table 3). In a multivariable analysis for EFS, *MN1* expression remained prognostic ($P = .03$), after adjustment for *BAALC* expression ($P = .002$), WBC ($P < .001$), and platelets ($P = .002$; Table 3). The risk of having an event (ie, induction failure, relapse, or death) for low *MN1* expressers was half that for high expressers (hazard ratio [HR] = 0.54; 95% CI, 0.34-0.86). However, *MN1* expression did not remain an important predictor in a multivariable model for OS.

Prognostic impact of *MN1* expression by 60 to 69 years of age and 70 years of age or older subgroups

We recently reported that the prognostic significance of *FLT3*-ITD and *NPM1* mutations in older adults differed between patients 60-69 years of age and those 70 years of age or older, with the adverse impact of *FLT3*-ITD being found mostly in the former[11] and the favorable impact of *NPM1* mutations in the latter.[9] Therefore, we analyzed the prognostic impact of *MN1* expression in these 2 age subgroups (Table 2). Low *MN1* expression was associated with higher CR rates both in patients 60-69 years of age (80% vs 54%, $P = .03$) and in those 70 years of age or older (81% vs 51%, $P = .03$). In contrast, a significantly longer OS ($P = .006$; 3-year OS rates, 31% vs 9%) and EFS ($P = .007$; 3-year EFS rates, 27% vs 6%) and a trend toward longer DFS ($P = .09$; 3-year DFS rates, 33% vs 11%) were observed only in patients 70 years of age and older (Table 2).

MN1 expression status remained independently associated with probability of achieving a CR for both age subgroups (60-69 years of age, $P = .02$, data not shown; 70 years of age or older, $P = .02$, Table 3), with no other variable remaining in the final model. Concerning patients 70 years of age or older (Table 3), low *MN1* expressers had almost 4 times greater odds of attaining a CR (odds ratio [OR] = 3.97; 95% CI, 1.22-12.90; Table 3). When we considered OS and EFS in this age group, we found an interaction between *MN1* expression and *NPM1* mutation status. The favorable impact of low *MN1* expression on OS and EFS appeared to be limited to patients who simultaneously carried an *NPM1* mutation ($P = .04$ and $P = .02$, respectively; Table 3), whereas there was no significant difference in OS or EFS between low and high *MN1* expressers with wild-type *NPM1* ($P = .58$ and $P = .87$, respectively; Table 3).

Taking into account the aforementioned OS and EFS interaction and the fact that we previously reported that the impact of *NPM1* mutations on outcome was much stronger in the 70 years of age or older subgroup,[9] we examined the relationship between *NPM1* mutation status and *MN1* expression status within this patient subgroup more closely. Among CN-AML patients 70 years of age or older, those with *NPM1* mutations who had low *MN1* expression had a trend for better CR rates ($P = .15$) and significantly longer DFS ($P = .003$), OS ($P = .002$; Figure 2A), and EFS ($P = .002$; Figure 2B) compared with the 3 other molecular subsets combined (ie, low *MN1* expressers with wild-type *NPM1*, high *MN1* expressers with mutated *NPM1*, and high *MN1* expressers with wild-type *NPM1*).

Prognostic impact of *MN1* expression within the ELN classification

Recently, the European LeukemiaNet (ELN) guidelines classified CN-AML patients into Favorable or Intermediate-I genetic groups based on the mutational status of the *CEBPA*, *NPM1*, and *FLT3*

Table 2. Outcomes according to *MN1* expression status in all CN-AML patients 60 years of age or older and, separately, in those 60-69 years of age and 70 years of age or older

Outcome	Low *MN1*	High *MN1*	P*	OR/HR (95% CI)
All patients	n = 70	n = 70		
CR rate, no. (%)	56 (80)	37 (53)	.001	3.57 (1.68, 7.56)
DFS			.29	0.79 (0.51, 1.23)
Median, y	0.9	0.6		
Disease-free at 3 y, % (95% CI)	18 (9-29)	11 (3-23)		
OS			.03	0.68 (0.48, 0.96)
Median, y	1.2	0.8		
Alive at 3 y, % (95% CI)	23 (14-33)	10 (4-18)		
EFS			.004	0.50† (0.33, 0.76)
Median, y	0.7	0.2		
Event-free at 3 y, % (95% CI)	14 (7-23)	6 (2-13)		
Patients 60-69 y	n = 44	n = 35		
CR rate, no. (%)	35 (80)	19 (54)	.03	3.28 (1.22, 8.81)
DFS			.82	0.94 (0.52, 1.69)
Median, y	0.7	0.5		
Disease-free at 3 y, % (95% CI)	9 (2-21)	11 (2-28)		
OS			.44	0.83 (0.52, 1.33)
Median, y	1.1	0.8		
Alive at 3 y, % (95% CI)	18 (9-31)	11 (4-24)		
EFS			.11	0.69 (0.44, 1.10)
Median, y	0.6	0.2		
Event-free at 3 y, % (95% CI)	7 (2-17)	6 (1-17)		
Patients 70 y or older	n = 26	n = 35		
CR rate, no. (%)	21 (81)	18 (51)	.03	3.97 (1.22, 12.90)
DFS			.09	0.54 (0.27, 1.10)
Median, y	1.3	0.7		
Disease-free at 3 y, % (95% CI)	33 (15-53)	11 (2-30)		
OS			.006	0.46 (0.26, 0.81)
Median, y	2.0	0.9		
Alive at 3 y, % (95% CI)	31 (15-49)	9 (2-21)		
EFS			.007	0.48 (0.27, 0.84)
Median, y	1.0	0.3		
Event-free at 3 y, % (95% CI)	27 (12-44)	6 (1-17)		

OR indicates the odds of achieving a CR for low *MN1* vs high *MN1* expressers; HR, the hazard of having an event for low *MN1* vs high *MN1* expressers; and CI, confidence interval.

∗*P* values for categorical variables are from Fisher exact test. *P* values for time-to-event variables are from the log-rank test (OS, DFS, and EFS).

†Does not meet the proportional hazards assumption, HR reported at 3 months.

genes.[8] The ELN Favorable genetic group is composed of CN-AML patients with *CEBPA* mutation and/or *NPM1* mutation without *FLT3*-ITD, whereas the Intermediate-I genetic group encompasses all other CN-AML patients (ie, CN-AML patients with wild-type *CEBPA* and either *NPM1* mutation with *FLT3*-ITD or wild-type *NPM1* with or without *FLT3*-ITD). We thus investigated the prognostic impact of *MN1* expression within these ELN genetic groups. Among the 140 patients, 66 were in the Favorable genetic group and 74 in the Intermediate-I genetic group. Lower *MN1* expression levels were found more frequently in the Favorable than Intermediate-I group patients (57% vs 43%, *P* = .03; Table 1).

Within the ELN Favorable group, we observed no significant differences in CR rates (*P* = .24), DFS (*P* = .84), OS (*P* = .81), or EFS (*P* = .69) between low and high *MN1* expressers (Table 4). In contrast, within the Intermediate-I genetic group, CN-AML patients with low *MN1* expression had better CR rates (77% vs 43%, *P* = .008), a trend toward longer DFS (*P* = .15; 3-year DFS rates, 13% vs 0%), and significantly longer OS (*P* = .05; 3-year OS rates, 10% vs 2%) and EFS (*P* = .003; 3-year EFS rates, 10% vs 0%; Table 4). The CR rate of 77% in patients with low *MN1* expression in the Intermediate-I genetic group was comparable to CR rates of patients with both low and high *MN1* expression in the ELN Favorable group (83% and 69%, respectively). Likewise, the EFS of low *MN1* expressers in the Intermediate-I genetic group was not significantly different from the EFS of patients in the ELN Favorable genetic group (Table 4; Figure 3B).

Because each of the ELN genetic groups is composed of specific molecular subsets, the ELN guidelines recommend reporting outcome measures also by these specific subsets. There was no impact of *MN1* expression on either of the 2 CN-AML molecular subsets within the ELN Favorable genetic group (data not shown). The situation was different when we analyzed the impact of *MN1* expression in the 3 molecular subsets composing the Intermediate-I genetic group. As seen in supplemental Table 1, all patients in the subset characterized by wild-type *NPM1* genes and the presence of *FLT3*-ITD had high *MN1* expression, thus precluding assessment of the prognostic significance of *MN1* expression in this subset. Of the remaining 2 subsets, significant differences in CR rates, OS, and EFS between the low and high *MN1* expressers were observed only among patients with mutated *NPM1* who harbored *FLT3*-ITD, whereas these outcome measures did not differ significantly in the subset encompassing patients with wild-type *NPM1* and no *LT3*-ITD (supplemental Table 1). However, because the numbers of patients in each subset composing the ELN Intermediate-I genetic group were relatively small (29, 11, and 34 patients, respectively), our analyses

Table 3. Multivariable regression analysis for outcome according to the *MN1* expression status in all older patients with de novo CN-AML, and in those 70 years of age or older

End point	Variables in final models	OR/HR	95% CI	P
All patients				
CR*	*MN1*, low vs high	3.16	1.29, 7.70	.01
	BAALC, low vs high	4.39	1.85, 10.44	< .001
	WBC, each 2-fold increase	0.76	0.62, 0.93	.01
EFS†	*MN1*, low vs high	0.54	0.34, 0.86	.03¶
	BAALC, low vs high	0.43	0.27, 0.68	.002¶
	WBC, each 2-fold increase	1.22	1.09, 1.35	< .001¶
	Platelets, each 50-unit increase	1.15	1.05, 1.25	.002
Patients 70 y or older				
CR‡	*MN1*, low vs high	3.97	1.22, 12.90	.02
OS§	Interaction of *MN1* and *NPM1*			.29
	Mutated *NPM1*:	0.41	0.17, 0.97	.04
	MN1, low vs high			
	Wild-type *NPM1*:			.58
	MN1, low vs high			
EFS‖	Interaction of *MN1* and *NPM1*			.14
	Mutated *NPM1*:	0.37	0.15, 0.88	.02
	MN1, low vs high			
	Wild-type *NPM1*:			.87
	MN1, low vs high			

OR > 1 (< 1) indicates higher (lower) CR rate for the higher values of the continuous variables and the first category listed for the categorical variables. HR > 1 (< 1) indicates higher (lower) risk for an event for the higher values of the continuous variables and the first category listed for the categorical variables.

*Variables considered in the model based on univariable analyses were *MN1* expression (high vs low; median cut), *BAALC* expression (high vs low; median cut), *FLT3*-ITD (positive vs negative), *IDH2* (mutated vs wild-type), *NPM1* (mutated vs wild-type), WBC (continuous, log base 2), and platelets (continuous, 50-unit increase).

†Variables considered in the model based on univariable analyses were *MN1* expression (high vs low; median cut), *BAALC* expression (high vs low; median cut), *ERG* expression (high vs low; median cut), *FLT3*-ITD (positive vs negative), *IDH2* (mutated vs wild-type), *NPM1* (mutated vs wild-type), *WT1* (mutated vs wild-type), WBC (continuous, log base 2), and platelets (continuous, 50-unit increase).

‡Variables considered in the model based on univariable analyses were *MN1* expression (high vs low; median cut), *BAALC* expression (high vs low; median cut), platelets (continuous, 50-unit increase), and *NPM1* (mutated vs wild-type).

§Variables considered in the model based on univariable analyses were *MN1* expression (high vs low; median cut), *BAALC* expression (high vs low; median cut), *IDH1* (mutated vs wild-type), *NPM1* (mutated vs wild-type), and platelets.

‖Variables considered in the model based on univariable analyses were *MN1* expression (high vs low; median cut), *BAALC* expression (high vs low; median cut), *IDH2* (mutated vs wild-type), *NPM1* (mutated vs wild-type), and platelets.

¶Does not meet the proportional hazards assumption. For EFS, the HR for *BAALC*, high vs low (median cut), *MN1*, high vs low (median cut), and WBC are reported at 3 months.

should be considered preliminary and of a descriptive nature, and the results have to be confirmed by larger studies.

Genome-wide gene-expression profiling

To gain insights into the biology of older CN-AML patients differentially expressing *MN1*, we derived a genome-wide gene expression signature. The *MN1*-associated gene expression signature consisted of 507 probe sets, representing 323 annotated genes (Figure 4).

In low *MN1* expressers, 258 probe sets, representing 158 genes, were found underexpressed, and 249 probe sets, representing 164 genes, overexpressed compared with high *MN1* expressers. The probe set representing *MN1* was among the most underexpressed probe sets in the low *MN1*-expressing patients, corroborating the quantification of *MN1* expression obtained by real-time RT-PCR (Figure 4). All microarray gene expression data are available on ArrayExpress under accession number E-TABM-1189.

Consistent with our observation in younger patients,[18] patients with low *MN1* expression had lower expression of genes previously associated with worse outcome in AML, such as *BAALC*,[19,29,30] the surface marker *CD200*,[35] the growth factor *HGF*,[36] and *CD34*, as well as the adhesion molecule *CD44*, a key regulator of AML leukemic stem cells necessary for the stem cells to interact with their microenvironment (Figure 4).[37,38] We also observed lower expression of *ABCB1* (*MDR1*), a gene encoding the multidrug resistance protein, whose high expression also has been associated

with worse outcome in older AML patients.[39] Furthermore, patients with low *MN1* expression had lower expression of *AKT3*, a member of the *AKT* kinase family, which has a central role in cell proliferation, survival, and drug resistance in AML,[40] and of the transcription factor *STAT5B*. Indeed, Heuser et al[41] previously showed that STAT5 signaling is critical for leukemia stem cell self-renewal in an *MN1* and *HOXA9*-expressing leukemia model.

Highly expressed in low *MN1*-expressing patients were the *HOXA* and *HOXB* cluster genes, as well as the *HOX* cofactor *MEIS1*, which are important for developmental processes and hematopoietic stem cell function (Figure 4).[42] We also observed higher expression of the tumor suppressor *TP53BP2*, which is known to interact with and inhibit the antiapoptotic protein BCL2.[43]

Genome-wide microRNA expression profiling

To further elucidate the biologic features associated with low *MN1* expression, we derived a microRNA expression signature. The *MN1*-associated microRNA expression signature was composed of 20 probes (Figure 5), 13 of which, representing 9 microRNAs, were underexpressed, and 7, representing 7 microRNAs, overexpressed in low *MN1* expressers compared with high *MN1* expressers. All microRNA data are available on ArrayExpress under accession number E-TABM-1190.

In the low *MN1*-expressing patients, we found *miR-126* and its passenger strand *miR-126** among the most underexpressed microRNAs, which is consistent with our previous findings in
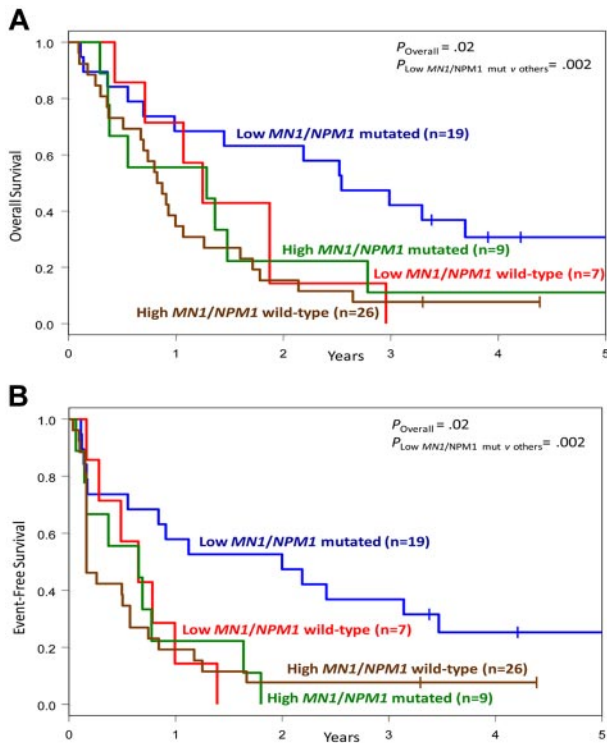
Figure 2. Outcome of CN-AML patients 70 years of age or older with respect to *MN1* expression and *NPM1* mutation status. (A) OS. (B) EFS.

younger patients.[18] In addition, underexpressed were *miR-146a*, low expression of which has been associated with the 5q− syndrome,[44] and *miR-146b*. Furthermore, we observed a lower expression of *miR-30b*, whose amplification and overexpression have been linked to medulloblastoma.[45]

Consistent with the higher expression of *HOX* genes in low *MN1* expressers, we observed higher expression of the *HOX*-gene

embedded microRNAs *miR-10a* and *miR-10b*. We also observed higher expression of *let-7b*, a member of a known tumor-suppressor microRNA family, which has been found down-regulated in AML with favorable cytogenetics [ie, t(8;21), inv(16) and t(15;17)].[46] We observed higher expression of *miR-449a*, shown to target *HDAC1* and induce growth arrest in prostate cancer.[47]

Discussion

The majority of patients with AML are older than 60 years at diagnosis. Although our knowledge of molecular prognostic markers is most extensive in younger CN-AML patients,[7] recently there has been progress in our understanding of the role molecular alterations play in prognostication of older patients.[9-12,27,28] The main objective of this study was to elucidate the prognostic impact of *MN1* expression in older CN-AML patients, and to determine whether this knowledge can be integrated into the landscape of other established molecular markers.

We demonstrate here that CN-AML patients 60 years of age or older with low *MN1* expression have higher CR rates and that their OS and EFS are longer than those of patients with high *MN1* expression. However, we did not observe a significant difference in DFS, which is somewhat different from our findings in younger patients, where low *MN1* expression associated with higher CR rates and longer DFS, OS, and EFS.[18] This discrepancy might be related to the differences in the intensity of treatment regimens administered to the younger and older patients. As in younger CN-AML patients, we observed an association of low *MN1* expression with mutated *NPM1* and lower *BAALC* expression. In multivariable analyses, *MN1* expresser status remained a significant prognosticator for CR attainment, even in the context of other molecular markers, including *NPM1* mutation and *BAALC* expresser status. Indeed, the expresser status of *MN1* and *BAALC* were the only molecular markers associated with CR achievement

Table 4. Outcomes according to *MN1* expression in older CN-AML within the ELN genetic groups

End point	All	Low *MN1*	High *MN1*	P*	OR/HR (95% CI)
ELN Favorable group	n = 66	n = 40	n = 26		
CR, no. (%)	51 (77)	33 (83)	18 (69)	.24	2.10 (0.65, 6.72)
DFS				.84	
Median, y	0.9	1.0	0.7		1.07 (0.56, 2.03)
Disease-free at 3 y, % (95% CI)	22 (12-34)	21 (9-36)	22 (7-43)		
OS				.81	0.94 (0.54, 1.61)
Median, y	1.5	1.5	1.4		
Alive at 3 y, % (95% CI)	29 (18-40)	32 (19-47)	23 (9-40)		
EFS				.69	0.90 (0.53, 1.53)
Median, y	0.8	0.9	0.6		
Event-free at 3 y, % (95% CI)	17 (9-27)	18 (8-31)	15 (5-31)		
ELN Intermediate-I group	n = 74	n = 30	n = 44		
CR, no. (%)	42 (57)	23 (77)	19 (43)	.008	4.32 (1.54, 12.17)
DFS				.15	0.63 (0.33, 1.20)
Median, y	0.5	0.6	0.5		
Disease-free at 3 y, % (95% CI)	7(2-17)	13 (3-30)	0 (NA)		
OS				.05	0.61 (0.38, 1.00)
Median, y	0.7	0.9	0.7		
Alive at 3 y, % (95% CI)	5 (2-12)	10 (3-24)	2 (.1-10)		
EFS				.003	0.49 (0.30, 0.80)
Median, y	0.3	0.6	0.2		
Event-free at 3 y, % (95% CI)	4 (1-10)	10 (3-24)	0 (NA)		

OR indicates the odds of achieving a CR for low *MN1* vs high *MN1* expressers; HR, the hazard of having an event for low *MN1* vs high *MN1* expressers; CI, confidence interval; and NA, not applicable (CI could not be attained).

*P values for categorical variables are from Fisher exact test. P values for time-to-event variables are from the log-rank test (OS, DFS, and EFS).
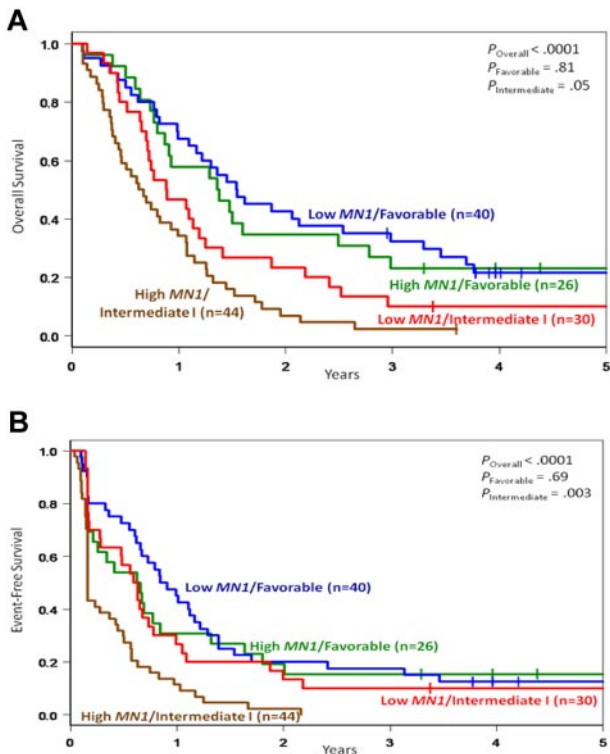
Figure 3. Outcome of CN-AML patients 60 years of age or older with respect to *MN1* expression within the ELN Favorable and ELN Intermediate-I genetic groups. (A) OS. (B) EFS.

in the patients investigated in the current study. We did not find an independent impact of *MN1* expression status on OS in the entire cohort of older patients that we analyzed, in contrast to younger patients.[18] This might be accounted for by differences in disease biology but might also be related to the lower intensity of postremission treatment administered to older AML patients compared with younger patients.

To our knowledge, only one recent study of the prognostic significance of *MN1* expression in CN-AML included patients 60 years of age or older among those analyzed.[48] This study showed that high *MN1* expression was associated with lower probability of CR achievement and shorter relapse-free survival, OS, and EFS.[48] However, in contrast to our findings, *MN1* expression was not an independent prognostic factor in the entire cohort of 210 patients analyzed by Metzeler et al,[48] and the outcome data were not reported separately for a subgroup of 101 patients 60 years of age or older.[48]

Our group recently reported age-related differences with respect to the impact on outcome of 2 molecular markers in older CN-AML patients. We found a stronger impact of *NPM1* mutations in patients 70 years of age or older as opposed to those 60-69 years of age,[9] and a stronger impact of *FLT3*-ITD in patients 60-69 years of age as opposed to those 70 years of age and older.[11] The current study provides evidence that the prognostic impact of *MN1* expression is also influenced by the patients' age. Although low *MN1* expressers in both age subgroups had higher probability of achieving a CR, *MN1* expression was prognostic with respect to OS and EFS only in the subgroup 70 years of age or older. Moreover, our data suggest that our finding of better outcome associated with *NPM1* mutations in patients 70 years of age or older does not pertain to all such patients but mostly to those who, in addition to *NPM1* mutation, have low *MN1* expression. Conse-

quently, if our findings are confirmed and a standardized method of *MN1* expression quantification is established, testing for both *NPM1* mutations and *MN1* expression could be recommended to achieve the best prognostic stratification of CN-AML patients 60 years of age or older. The reasons for the age-related differences in the impact of *MN1* expression, *NPM1* mutations, or *FLT3*-ITD on outcome of older patients remain unknown.

Recently, the ELN expert panel proposed a novel risk classification for AML based on cytogenetics and molecular markers.[8] Within this classification, CN-AML patients are assigned to Favorable or Intermediate-I genetic groups based on the mutational status of the *CEBPA*, *NPM1*, and *FLT3* genes.[8] To evaluate whether determination of *MN1* expression levels can improve this classification, we analyzed the prognostic significance of *MN1* expression status separately within the ELN Favorable and the Intermediate-I genetic groups of CN-AML. In our patient cohort, *MN1* expression did not impact on outcome of the Favorable group. However, we observed a strong impact of *MN1* expression status on patients belonging to the Intermediate-I genetic group. Within this group, patients with low expression of *MN1* had better outcome than those with high *MN1* expression, and their CR rates and EFS were not significantly different from those in the ELN Favorable group. However, DFS and OS of low *MN1* expressers in the Intermediate-I genetic group were better than high *MN1* expressers, but not comparable with those in the ELN Favorable group. If our findings are confirmed, *MN1* expression status might become a molecular marker that will help refine the ELN classification. Furthermore, our analysis of molecular subsets within the Intermediate-I genetic group suggests that patients who benefit most from having low *MN1* expression are those who harbor both *NPM1* mutation and *FLT3*-ITD. This finding requires corroboration in a larger set of patients.

The molecular mechanisms by which *MN1* contributes to leukemia remain elusive. To gain deeper insights into the biology of the disease, we derived gene- and microRNA-expression signatures associated with *MN1* expression. The genome-wide microarray profiling supports the prognostic significance of low *MN1* expression levels by demonstrating concurrent underexpression of genes and microRNAs associated with biologic features of aggressive phenotypes. Not surprisingly, we found the gene expression signature associated with *MN1* expression derived in older CN-AML patients to be similar to the one we reported in patients younger than 60 years.[18] It is known that *MN1* expression not only negatively impacts on cell differentiation but also affects chemotherapy response,[49] which is in line with our finding of the importance of low *MN1* expression for CR achievement. Heuser et al[50] previously showed that genes that are associated with undifferentiated hematologic precursor cells also associate with a poor response to induction therapy. Consistently, in both younger and older patients, low *MN1* expression was associated with higher CR rates and lower expression of known adverse outcome predictors and of genes involved in chemotherapy resistance, such as *ABCB1*. These findings may, at least in part, explain the observed, independent association with better treatment response of low *MN1* expressers in older CN-AML. We also found overlapping microRNA expression features in younger and older patients with low *MN1* expression, including down-regulation of *miR-126* and *miR-130b*, and this may indicate that these microRNAs play an important role in modifying patients' response to therapy.
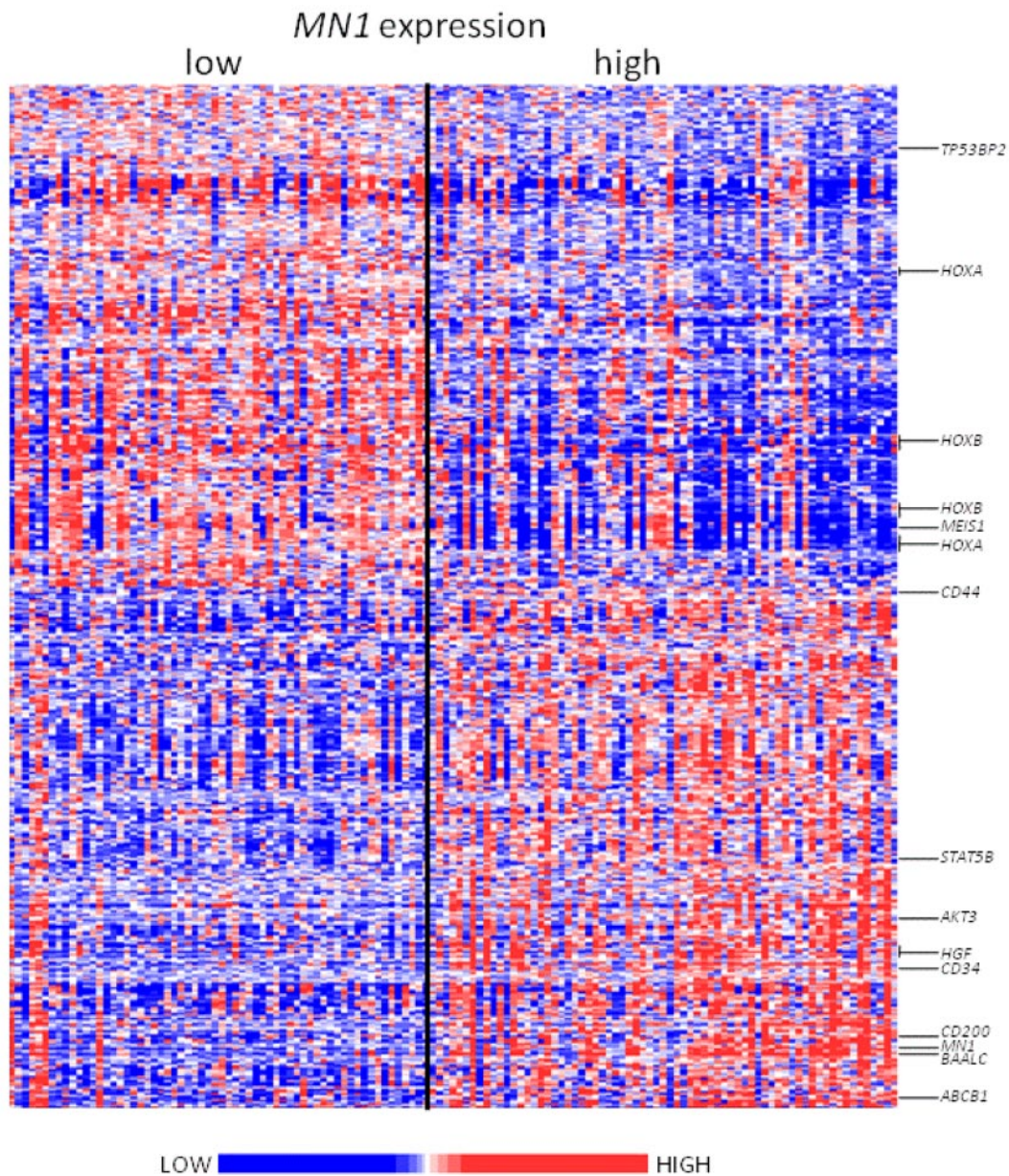
Figure 4. Heat map of the derived gene expression signature associated with *MN1* expression in the group of CN-AML patients 60 years of age or older. The patients are ordered from left to right by increasing expression of *MN1*. Expression values of the probe sets are represented by color: blue represents expression less than the median value for the given probe set; and red, expression greater than the median value for the given probe set. Up- and down-regulated genes that are mentioned in the text are indicated along the side.

In conclusion, we show that *MN1* expression is an important predictor of treatment response in older de novo CN-AML patients. Prognostic impact of *MN1* expression is especially strong in patients 70 years of age or older, and a combination of low *MN1* expression and mutated *NPM1* identifies a subset of these patients with a particularly good outcome. Furthermore, the gene- and microRNA-expression profiles we derived may help to shed light on the complex biology of *MN1*-associated disease. Once a standardized method of expression quantification is established (eg, by digital mRNA quantification technologies) and absolute cutpoints are defined, measurements of pretreatment *MN1* expression may be included in diagnostic panels and used to improve risk stratification of older CN-AML patients and to guide treatment decisions in clinical trials testing new agents targeting genes, such as *ABCB1* or even *MN1* itself.

Figure 5. Heat map and table of the derived microRNA expression signature associated with *MN1* expression in the group of CN-AML patients 60 years of age or older. (A) Heat map of the derived microRNA expression signature associated with *MN1* expression in the group of CN-AML patients 60 years of age or older. The patients are ordered from left to right by increasing expression of *MN1*. Expression values of the probe sets are represented by color: blue represents expression less than the median value for the given probe; and red, expression greater than the median value for the given probe. Up- and down-regulated genes and microRNAs are indicated along the side. (B) Tables showing fold changes of the microRNAs that form the signature with corresponding fold changes.

Authorship

Contribution: S.S., G.M., J.K., M.D.R., K. Mrózek, K. Maharry, M.A.C., and C.D.B. contributed to the design and analysis of this study and the writing of this manuscript; S.S., H.B., K.H.M., S.P.W., and Y.-Z.W. carried out laboratory-based research; J.E.K., M.D.R., and K. Maharry performed statistical analyses; G.M.,

B.L.P., M.R.B., J.E.K., A.J.C., R.A.L., M.A.C., and C.D.B. were involved directly or indirectly in care of patients and/or sample procurement; and all authors agreed on the final version.

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