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ORIGINAL REPORT

Combined Molecular and Clinical Prognostic Index for Relapse and Survival in Cytogenetically Normal Acute Myeloid Leukemia

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

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ABSTRACT

Purpose
Cytogenetically normal (CN) acute myeloid leukemia (AML) is the largest and most heterogeneous cytogenetic AML subgroup. For the practicing clinician, it is difficult to summarize the prognostic information of the growing number of clinical and molecular markers. Our purpose was to develop a widely applicable prognostic model by combining well-established pretreatment patient and disease characteristics.

Patients and Methods
Two prognostic indices for CN-AML (PINA), one regarding overall survival (OS; $PINA_{OS}$) and the other regarding relapse-free survival (RFS; $PINA_{RFS}$), were derived from data of 572 patients with CN-AML treated within the AML Cooperative Group 99 study (www.aml-score.org).

Results
On the basis of age (median, 60 years; range, 17 to 85 years), performance status, WBC count, and mutation status of *NPM1*, *CEBPA*, and *FLT3*-internal tandem duplication, patients were classified into the following three risk groups according to $PINA_{OS}$ and $PINA_{RFS}$: 29% of all patients and 32% of 381 responding patients had low-risk disease (5-year OS, 74%; 5-year RFS, 55%); 56% of all patients and 39% of responding patients had intermediate-risk disease (5-year OS, 28%; 5-year RFS, 27%), and 15% of all patients and 29% of responding patients had high-risk disease (5-year OS, 3%; 5-year RFS, 5%), respectively. $PINA_{OS}$ and $PINA_{RFS}$ stratified outcome within European LeukemiaNet genetic groups. Both indices were confirmed on independent data from Cancer and Leukemia Group B/Alliance trials.

Conclusion
We have developed and validated, to our knowledge, the first prognostic indices specifically designed for adult patients of all ages with CN-AML that combine well-established molecular and clinical variables and that are easily applicable in routine clinical care. The integration of both clinical and molecular markers could provide a basis for individualized patient care through risk-adapted therapy of CN-AML.

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INTRODUCTION

Pretreatment cytogenetic abnormalities have been shown to be the strongest prognostic factors for overall survival (OS) in patients with de novo acute myeloid leukemia (AML).[1] Patients with core binding factor leukemia [t(8;21), inv(16), or t(16;16)] belong to a favorable cytogenetic risk group, whereas patients without these aberrations but a complex karyotype (≥ three abnormalities) or monosomy 7, inv(3)(q21q26), t(6;11)(q27;q23), t(6;9)(p23;q34), or t(11;19)(q23;p13.1) have an adverse cytogenetic risk.[1] The intermediate cytogenetic risk group comprises the large group of patients with cytogenetically normal AML (CN-AML) and patients with t(9;11), trisomy 8 (+8), or other chromosomal changes.[1,2]

In recent years, a growing number of molecular markers have been described to further characterize CN-AML. Gene mutations with a favorable prognostic impact have been found in the nucleophosmin gene (*NPM1*) and the CCAAT/enhancer binding protein α gene (*CEBPA*).[3,4] Only patients with biallelic *CEBPA* (bi*CEBPA*) mutations show a

prolonged survival, whereas a monoallelic *CEBPA* mutation (mo*CEBPA*) has no impact on prognosis compared with wild-type *CEBPA* (wt*CEBPA*).[3,5,6] Internal tandem duplications (ITDs) in the fms-related tyrosine kinase 3 gene (*FLT3*-ITD) confer a negative prognostic effect on survival.[7-10]

In 2010, an international expert panel (European LeukemiaNet [ELN]) established a new classification for AML based on molecular and cytogenetic markers; CN-AML was subdivided into two genetic groups, favorable and intermediate-I.[11] In addition to genetic parameters, clinical factors such as patient age at diagnosis have been shown to influence outcome.[12,13]

For the practicing clinician, it is difficult to summarize and interpret the prognostic information provided by the large panel of individual risk markers. Thus, we decided to develop and validate easily clinically applicable prognostic indices for CN-AML (PINA), in patients of all ages, with respect to OS ($PINA_{OS}$) and relapse-free survival (RFS; $PINA_{RFS}$) by combining routinely available and well-established molecular and clinical characteristics. The new prognostic indices should be able to be easily adopted in routine practice for prognostication and guidance of risk-adapted postremission therapy in analogy to the Euro score[14] in chronic myelogenous leukemia or the mantle cell lymphoma international prognostic index.[15]

PATIENTS AND METHODS

Patients

After written informed consent, 783 patients with newly diagnosed CN-AML were treated on the randomized multicenter German AML Cooperative Group 99 (AMLCG99) trial comparing thioguanine, cytarabine, and daunorubicin plus high-dose cytarabine and mitoxantrone (HAM) versus HAM-HAM as induction therapy.[16] Further details on the AMLCG99 study are provided in the Data Supplement. We included in this study the 669 patients with CN-AML with a complete mutation status of *NPM1*, *FLT3*-ITD, and *CEBPA*.

Clinical and Molecular Markers

We investigated the following clinical characteristics evaluated at diagnosis and available from the trial documentation: WBC count, platelet count, hemoglobin level, lactase dehydrogenase level, peripheral-blood blasts, bone marrow blasts, de novo AML versus non–de novo AML, Eastern Cooperative Oncology Group performance status,[17] sex, and age.

Cytogenetic and molecular analyses were performed on bone marrow aspirates. The cytogenetic diagnosis of CN-AML was based on analyses of ≥ 20 metaphases and performed according to the International System for Human Cytogenetic Nomenclature guidelines.[18] For molecular characterization, mutations of *NPM1*,[19] *FLT3*-ITD,[8,20] mo*CEBPA*,[3,21] and bi*CEBPA*[3,21] were analyzed as previously described.

Outcome Parameters

OS was calculated from pretreatment random assignment to death from any cause. RFS was determined for responders from the first day of complete remission (CR) until relapse or death from any cause. Patients alive were censored for OS at last follow-up date, and patients in CR were censored for RFS at last disease assessment. In 124 patients who had undergone allogeneic transplantation according to the study protocol, OS and RFS were censored at the start of transplantation.

Statistical Analyses

Univariable and multivariable Cox proportional hazards regression model analyses were performed for OS and RFS. After including all candidate variables in one multivariable Cox regression model, we identified the independent prognostic factors by backward elimination using an exclusion significance level of 1% for the Wald statistic. Because of the known interacting effects of *NPM1* mutation and *FLT3*-ITD on OS and RFS,[22] we included the interaction term (simultaneous mutation of *NPM1* and *FLT3*-ITD) in addition to *NPM1* and *FLT3*-ITD mutation status.

The prognostic scores were defined as the weighted sums of the independent prognostic factor values, weighted with their regression coefficients from the final Cox models. By establishing two cutoff values for each prognostic score, three risk groups were defined. We used the minimal *P* value approach[23] to define risk groups with the greatest survival differences as measured by the log-rank statistic, controlling for a comparable separation of adjacent risk groups.

Internal validation was performed by a bootstrap procedure.[24] Nine hundred ninety-nine bootstrap replications were carried out to get estimates of hazard ratios (HRs) between risk groups corrected for overfitting. External validation was performed using data of 529 patients with CN-AML treated on Cancer and Leukemia Group B (CALGB) front-line trials (details in Data Supplement)[25-32] by means of Kaplan-Meier estimates, log-rank tests, and Cox regression. Further details on statistical methods are provided in the Data Supplement.

Table 1. Multivariable Cox Regression Models for OS and RFS

Independent Prognostic Factors	OS				RFS			
	Weight*	Hazard Ratio	95% CI	*P*	Weight*	Hazard Ratio	95% CI	*P*
Within *FLT3*-ITD–negative group: *NPM1* positive *v* negative	−1.20	0.3	0.2 to 0.4	< .001	−1.61	0.2	0.1 to 0.3	< .001
Within *FLT3*-ITD–positive group: *NPM1* positive *v* negative	−0.31	0.7	0.5 to 1.2	.19	−0.37	0.7	0.4 to 1.2	.17
Within *NPM1*-negative group: *FLT3*-ITD positive *v* negative	−0.26	0.8	0.5 to 1.2	.25	−0.44	0.6	0.4 to 1.1	.11
Within *NPM1*-positive group: *FLT3*-ITD positive *v* negative	0.62	1.9	1.3 to 2.7	.001	0.80	2.2	1.5 to 3.4	< .001
Interaction *NPM1* and *FLT3*-ITD: *NPM1* positive/*FLT3*-ITD positive *v* *NPM1* negative or *FLT3*-ITD negative	0.89	2.4	1.4 to 4.2	.002	1.24	3.5	1.8 to 6.6	< .001
bi*CEBPA* *v* mo/wt*CEBPA*	−1.30	0.3	0.1 to 0.6	< .001	−1.34	0.3	0.1 to 0.5	< .001
WBC (10^6/L, per 10-fold increase)	0.57	1.8	1.4 to 2.2	< .001	0.54	1.7	1.3 to 2.2	< .001
Age, years (per increase of 10 years)	0.44	1.5	1.4 to 1.7	< .001	0.18	1.2	1.1 to 1.3	.004
ECOG performance status 2-4 *v* 0-1	0.40	1.5	1.2 to 1.9	.001	—	—	—	—

Abbreviations: bi*CEBPA*, biallelic *CEBPA* mutation; ECOG, Eastern Cooperative Oncology Group; *FLT3*-ITD, internal tandem duplication of the *FLT3* gene; mo*CEBPA*, monoallelic *CEBPA* mutation; *NPM1*, nucleophosmin; OS, overall survival; PINA, prognostic index in cytogenetically normal acute myeloid leukemia; RFS, relapse-free survival; wt*CEBPA*, wild-type *CEBPA*.

*Weights in $PINA_{OS}$ and $PINA_{RFS}$ scores as determined by the regression coefficients. WBC count, platelet count, hemoglobin level, lactase dehydrogenase level, bone marrow blasts, de novo versus non–de novo acute myeloid leukemia, performance status, sex, age, and mutations of *NPM1*, *FLT3*-ITD, and *CEBPA* were included in the Cox regression models for backward elimination. The analyses were performed using 572 patients for OS and 381 patients for RFS who had data for all these variables (Data Supplement). Characteristics of the patients used for multivariable regression are described in the Data Supplement.

$PINA_{OS}$ score =

-1.20 (if *NPM1*+, otherwise 0)-0.26 (if *FLT3*-ITD+, otherwise 0)
+0.89 (if *NPM1*+ and *FLT3*-ITD+, otherwise 0)-1.30 (if bi*CEBPA*, otherwise 0)
+0.57 x $\log_{10}$WBC [10^6/L]+0.044 x age [years]+0.40 (if ECOG 2-4, otherwise 0)

LowR $PINA_{OS}$	$PINA_{OS}$ score	< 4.0
IntR $PINA_{OS}$	$PINA_{OS}$ score	≥ 4.0 and < 5.4
HiR $PINA_{OS}$	$PINA_{OS}$ score	≥ 5.4

$PINA_{RFS}$ score =

-1.61 (if *NPM1*+, otherwise 0)-0.44 (if *FLT3*-ITD+, otherwise 0)
+1.24 (if *NPM1*+ and *FLT3*-ITD+, otherwise 0)-1.34 (if bi*CEBPA*, otherwise 0)
+0.54 × $\log_{10}$WBC [10^6/L]+0.018 × age [years]

LowR $PINA_{RFS}$	$PINA_{RFS}$ score	< 2.0
IntR $PINA_{RFS}$	$PINA_{RFS}$ score	≥ 2.0 and < 2.9
HiR $PINA_{RFS}$	$PINA_{RFS}$ score	≥ 2.9

Fig 1. Definition of $PINA_{OS}$ and $PINA_{RFS}$. bi*CEBPA*, biallelic *CEBPA* mutation; ECOG, Eastern Cooperative Oncology Group performance status; *FLT3*-ITD+, presence of an internal tandem duplication of the *FLT3* gene; HiR, high risk; IntR, intermediate risk; LowR, low risk; *NPM1*+, mutation in the nucleophosmin gene; OS, overall survival; PINA, prognostic index in cytogenetically normal acute myeloid leukemia; RFS, relapse-free survival.

RESULTS

Patient Characteristics and Outcome

We used data from 669 patients with CN-AML treated in the AMLCG99 trial, excluding 114 patients with CN-AML without information about *FLT3*-ITD or mutations of *NPM1* or *CEBPA* (Data Supplement). Median age was 60 years (range, 17 to 85 years). Patient characteristics, treatment, and clinical outcome are provided in the Data Supplement. Between the 669 selected patients and the 114 nonselected patients, there was no difference in OS (median, 1.9 *v* 1.6 years, respectively; $P = .31$) or RFS (median, 1.5 *v* 1.4 years, respectively; $P = .58$). The results of univariable analyses for OS and RFS are provided in the Data Supplement.

Prognostic Factors and Risk Stratification for OS ($PINA_{OS}$)

Multivariable analyses identified the following characteristics as independent adverse prognostic factors for OS: wild-type *NPM1* (*NPM1* negative) among *FLT3*-ITD–negative patients (HR, 3.3; $P < .001$), *FLT3*-ITD positive among *NPM1*-mutated patients (HR, 1.9; $P = .001$), wt*CEBPA* or mo*CEBPA* (HR, 3.7; $P < .001$), higher WBC count (HR, 1.8; $P < .001$), higher age (HR, 1.5; $P < .001$), and Eastern Cooperative Oncology Group performance status ≥ 2 (HR, 1.5; $P = .001$; Table 1, Data Supplement). *NPM1* and *FLT3*-ITD mutation status showed interacting effects on OS ($P = .002$). Between the 572 patients included in multivariable Cox regression for OS and the 97 excluded patients, there was a tendency toward shorter OS in the excluded patients (median, 2.1 years *v* 1.0 year, respectively; $P = .066$) but comparable RFS (median, 1.5 *v* 1.5 years, respectively; $P = .55$).

By $PINA_{OS}$ (Fig 1), we defined three groups of low-risk (LowR; 29% of patients), intermediate-risk (IntR; 56% of patients), and high-risk (HiR; 15% of patients) patients with 5-year OS rates of 74%, 28%, and 3%, respectively (overall $P < .001$; Fig 2A). The HRs for IntR versus LowR and HiR versus IntR were 4.1 (95% CI, 2.7 to 5.6) and 2.3 (95% CI, 1.8 to 2.9), respectively (Data Supplement). Similar results were obtained without censoring for allogeneic transplantation.

Prognostic Factors and Risk Stratification for RFS ($PINA_{RFS}$)

Among the patients who achieved a CR, the following characteristics were independent adverse prognostic factors for RFS: *NPM1* negative among *FLT3*-ITD–negative patients (HR, 5.0; $P < .001$), *FLT3*-ITD positive among *NPM1*-mutated patients (HR, 2.2; $P < .001$), wt*CEBPA* or mo*CEBPA* (HR, 3.8; $P < .001$), higher WBC count

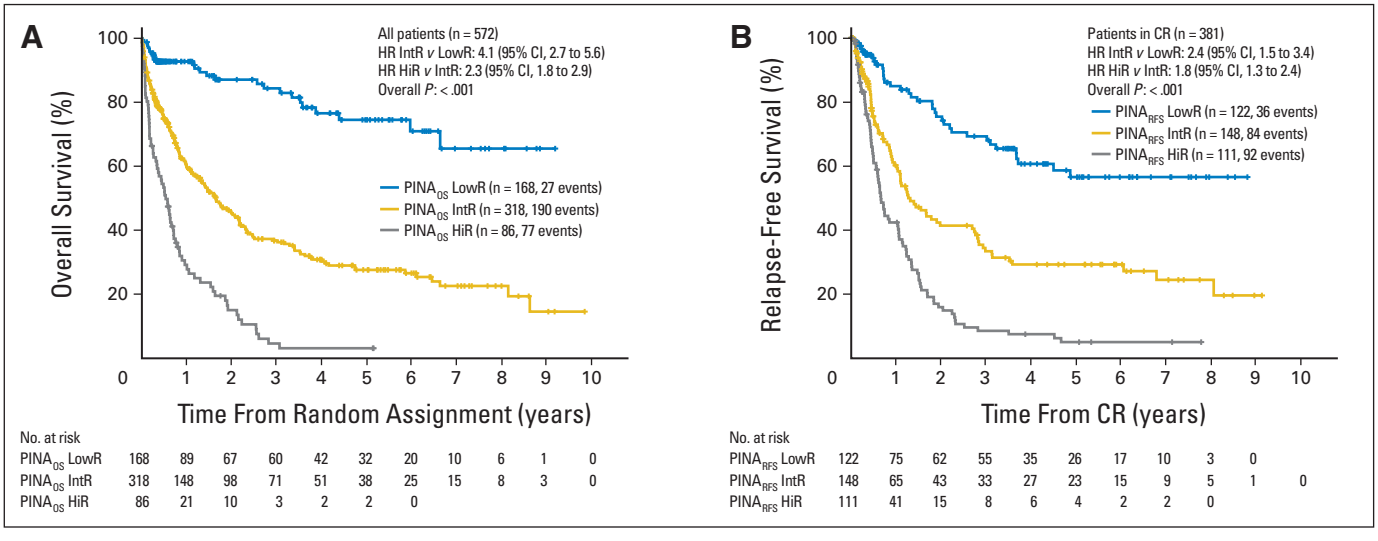

Fig 2. Outcome of patients with cytogenetically normal acute myeloid leukemia (CN-AML) according to the new prognostic indices in the German AML Cooperative Group (AMLCG) cohort. (A) Overall survival (OS) according to the prognostic index for CN-AML for OS ($PINA_{OS}$). (B) Relapse-free survival (RFS) according to the prognostic index for CN-AML for RFS ($PINA_{RFS}$). The hazard ratios (HRs) were corrected for overfitting; see Data Supplement. For patient selection, see Data Supplement. In the AMLCG cohort, the median $PINA_{OS}$ score was 4.5 (range, 2.1 to 6.3), and the median $PINA_{RFS}$ score was 2.5 (range, 0.8 to 4.0). CR, complete remission; HiR, high risk; IntR, intermediate risk; LowR, low risk; PINA, prognostic index in cytogenetically normal acute myeloid leukemia.

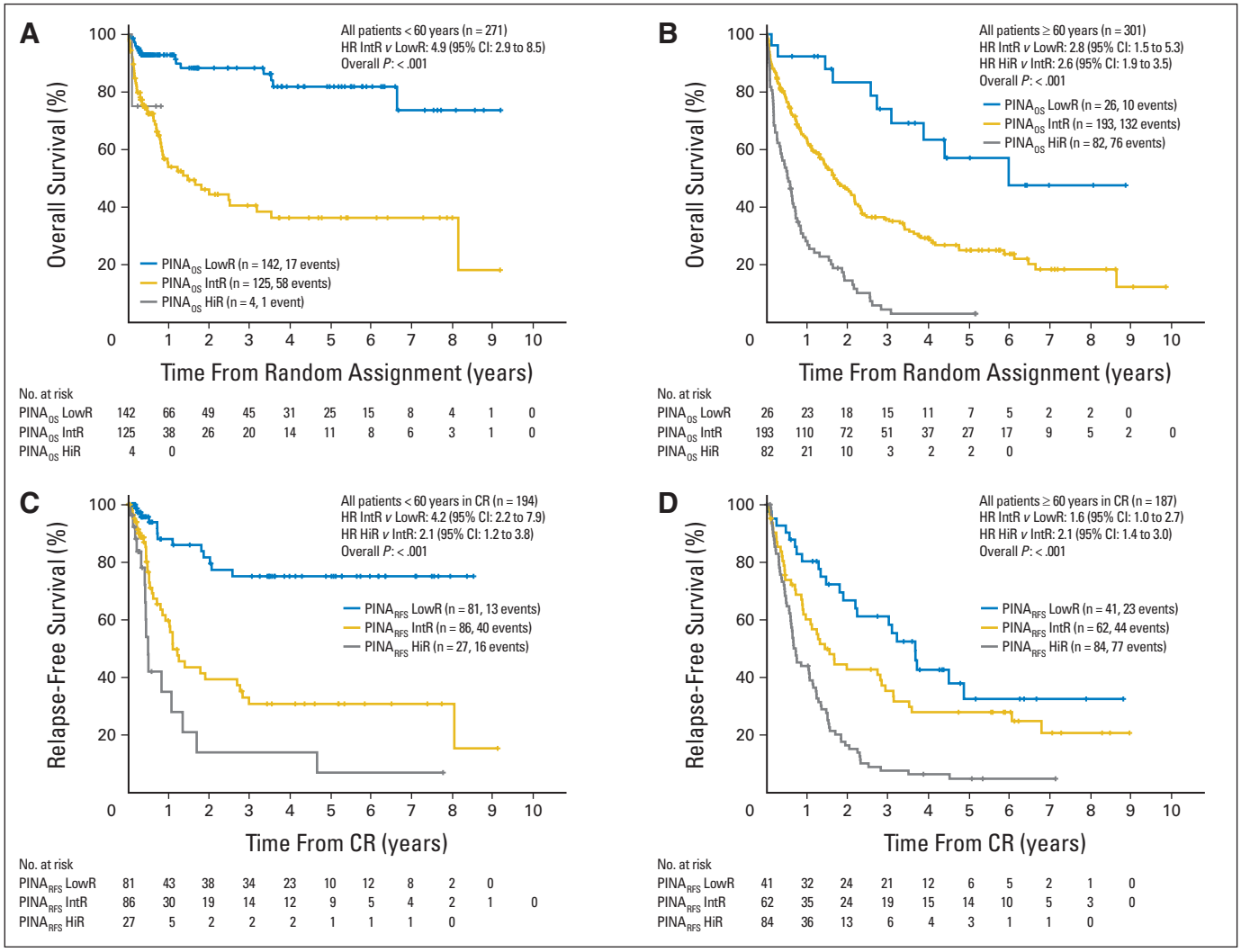

Fig 3. Outcome of patients with cytogenetically normal acute myeloid leukemia (CN-AML) according to the new prognostic indices stratified by age in the German AML Cooperative Group (AMLCG) cohort. (A) Overall survival (OS) according to the prognostic index for CN-AML for OS ($PINA_{OS}$) in patients younger than 60 years old. (B) OS according to the $PINA_{OS}$ in patients ≥ 60 years old. (C) Relapse-free survival (RFS) according to the prognostic index for CN-AML for RFS ($PINA_{RFS}$) in patients younger than 60 years old. (D) RFS according to the $PINA_{RFS}$ in patients ≥ 60 years old. In patients younger than 60 years old, the median $PINA_{OS}$ score was 3.9 (range, 2.1 to 5.6), and the median $PINA_{RFS}$ score was 2.2 (range, 0.8 to 3.7). In patients ≥ 60 years old, the median $PINA_{OS}$ score was 5.0 (range, 3.0 to 6.3), and the median $PINA_{RFS}$ score was 2.9 (range, 1.2 to 4.0). CR, complete remission; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk; PINA, prognostic index in cytogenetically normal acute myeloid leukemia.

(HR, 1.7; $P < .001$), and higher age (HR, 1.2; $P = .004$; Table 1, Data Supplement). *NPM1* and *FLT3*-ITD mutation status showed interacting effects on RFS ($P < .001$).

By $PINA_{RFS}$ (Fig 1), we defined LowR (32% of patients), IntR (39% of patients), and HiR (29% of patients) groups with 5-year RFS rates of 55%, 27%, and 5%, respectively (overall $P < .001$; Fig 2B). The HRs for IntR versus LowR and HiR versus IntR were 2.4 (95% CI, 1.5 to 3.4) and 1.8 (95% CI, 1.3 to 2.4), respectively (Data Supplement). Similar results were obtained without censoring for allogeneic transplantation.

Cumulative incidence of relapse analysis, treating relapse, death, and allogeneic transplantation in first CR as competing events, confirmed the prognostic value of $PINA_{RFS}$ for relapse after first CR (Data Supplement). At 5 years, patients in the LowR, IntR, and HiR $PINA_{RFS}$ groups showed cumulative incidence rates for relapse of 35%, 56%, and 72%, respectively (overall $P < .001$).

Prognostic Model in Patients Younger Than Age 60 Years and Age 60 Years or Older

Among patients younger than 60 years old, the $PINA_{OS}$ LowR group included 52% of patients; they had a 5-year OS rate of 82%. Forty-six percent of patients were of IntR; they had a 5-year OS rate of 36%. The HiR group included only four patients (Fig 3A). In patients ≥ 60 years old, among the 9% of LowR, 64% of IntR, and 27% of HiR patients according to $PINA_{OS}$, 5-year OS rates were 57%, 25%, and 3%, respectively (Fig 3B).

In patients younger than 60 years old, the $PINA_{RFS}$ distinguished LowR, IntR, and HiR groups comprising 42%, 44%, and 14% of patients, with 5-year RFS rates of 75%, 31%, and 7%, respectively (Fig 3C). In patients ≥ 60 years old, the $PINA_{RFS}$ distinguished LowR, IntR, and HiR groups comprising 22%, 33%, and 45% of patients, with 5-year RFS rates of 32%, 28%, and 5%, respectively (Fig 3D).

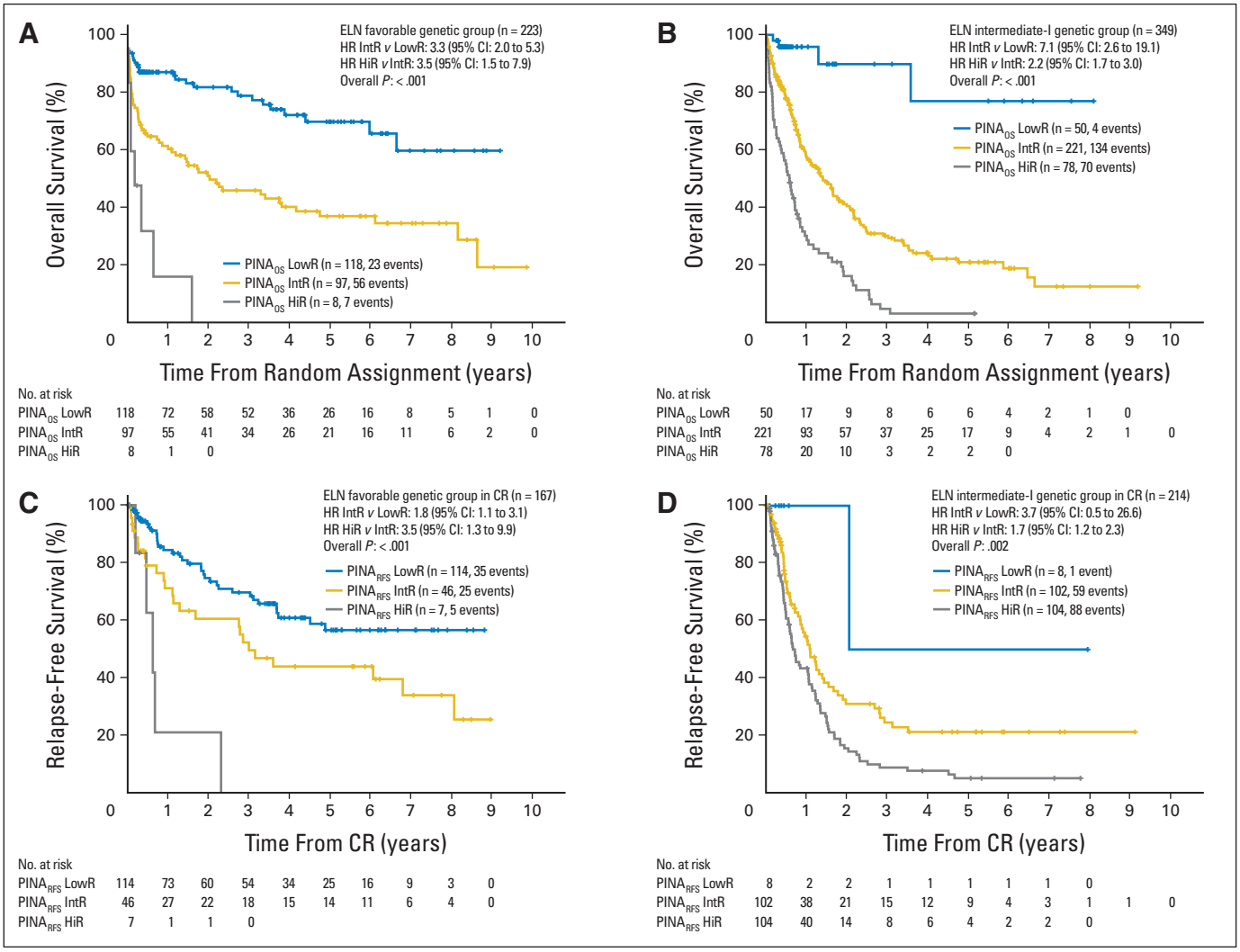

Fig 4. Outcome of patients with cytogenetically normal acute myeloid leukemia (CN-AML) according to the new prognostic indices among European LeukemiaNet (ELN) genetic groups in the German AML Cooperative Group (AMLCG) cohort. (A) Overall survival (OS) according to the prognostic index for CN-AML for OS ($PINA_{OS}$) in patients in the ELN favorable genetic group. (B) OS according to the $PINA_{OS}$ in patients in the ELN intermediate-I genetic group. (C) Relapse-free survival (RFS) according to the prognostic index for CN-AML for RFS ($PINA_{RFS}$) in patients in the ELN intermediate-I genetic group. CR, complete remission; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk; PINA, prognostic index in cytogenetically normal acute myeloid leukemia.

$PINA_{OS}$, $PINA_{RFS}$, and ELN Genetic Groups

In the ELN favorable genetic group, 53%, 43%, and 4% of patients were classified as LowR, IntR, and HiR according to the $PINA_{OS}$, respectively (Data Supplement); 5-year OS rates in these groups were 73%, 39%, and 0%, respectively (Fig 4A). In the ELN intermediate-I genetic group, 14%, 63%, and 22% of patients were classified as LowR, IntR, and HiR according to the $PINA_{OS}$, respectively; 5-year OS rates in these groups were 77%, 21%, and 3%, respectively (Fig 4B).

In the ELN favorable genetic group, 68%, 28%, and 4% of patients were classified as LowR, IntR, and HiR according to the $PINA_{RFS}$, respectively; 5-year RFS rates in these groups were 57%, 44%, and 0%, respectively (Fig 4C). In the ELN intermediate-I genetic group, 4%, 48%, and 49% of patients were classified as LowR, IntR, and HiR according to $PINA_{RFS}$, respectively; 5-year RFS rates in this groups were 50%, 21%, and 5%, respectively (Fig 4D).

External Validation

Patients in the validation cohort showed characteristics similar to the AMLGC99 analysis cohort. CALGB patients all had de novo AML and, compared with the patients in our study, showed a slightly higher frequency of *NPM1* mutations (61% *v* 52%, respectively) and *FLT3*-ITD (35% *v* 30%, respectively). None of the CALGB patients underwent allogeneic transplantation in first CR. An overview of patient selection, characteristics, treatment, and outcome is provided in the Data Supplement.

In the validation cohort, 38%, 50%, and 12% of patients were classified as LowR, IntR, and HiR by $PINA_{OS}$, respectively; 5-year OS rates in these groups were 51%, 18%, and 3%, respectively. The HRs for OS comparing IntR versus LowR and HiR versus IntR were 2.6 (95% CI, 2.1 to 3.3) and 1.8 (95% CI, 1.4 to 2.4) respectively (Fig 5A). By $PINA_{RFS}$, 41%, 40%, and 19% of patients who achieved a CR were LowR, IntR, and HiR, respectively; 5-year RFS rates in these groups

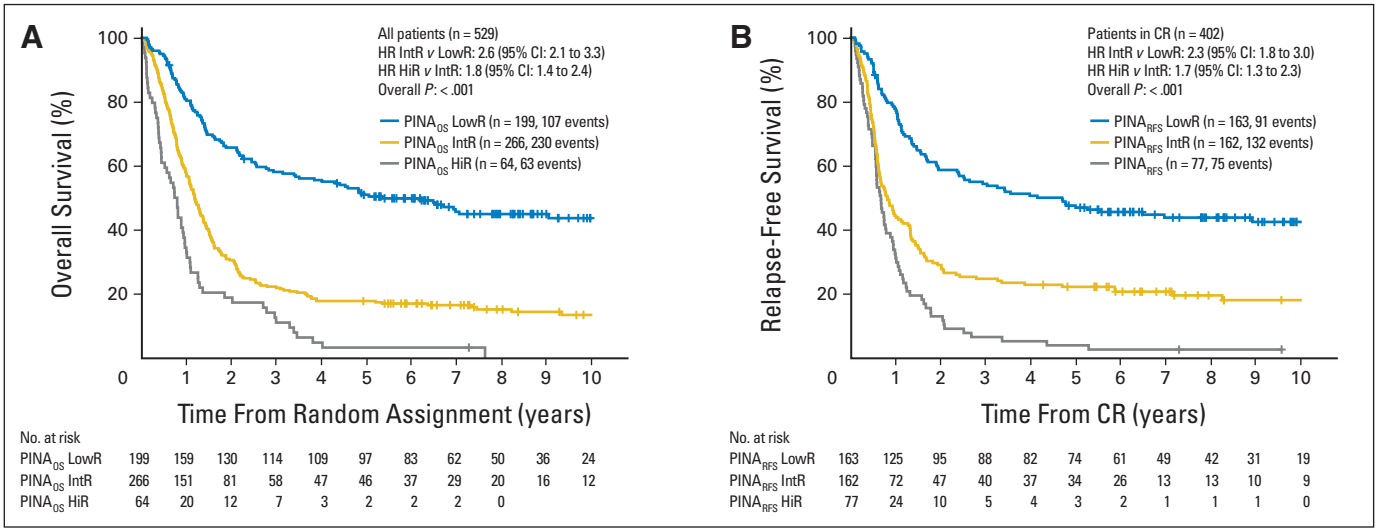

Fig 5. Validation of the prognostic indices for cytogenetically normal acute myeloid leukemia (CN-AML) for overall survival (OS; $PINA_{OS}$) and relapse-free survival (RFS; $PINA_{RFS}$) in an independent data set of patients with CN-AML from the Cancer and Leukemia Group B. (A) OS according to the $PINA_{OS}$. (B) RFS according to the $PINA_{RFS}$. In the validation cohort, the median $PINA_{OS}$ score was 4.3 (range, 1.7 to 6.9), and the median $PINA_{RFS}$ score was 2.0 (range, 0.6 to 4.0). CR, complete remission; HiR, high risk; HR, hazard ratio; IntR, intermediate risk; LowR, low risk.

were 47%, 22%, and 4%, respectively. The HRs comparing IntR versus LowR and HiR versus IntR were 2.3 (95% CI, 1.8 to 3.0) and 1.7 (95% CI, 1.3 to 2.3), respectively (Fig 5B). The external validation of $PINA_{OS}$ and $PINA_{RFS}$ in patients less than and $\geq$ 60 years old is provided in the Data Supplement.

DISCUSSION

In this study, we developed and validated two new prognostic indices that allow stratification of patients with CN-AML into subgroups with different OS ($PINA_{OS}$) or RFS ($PINA_{RFS}$) based on the combination of clinical and well-established molecular characteristics. Our results are derived from a large cohort of 669 patients with CN-AML treated homogeneously in a randomized clinical trial. We confirmed the validity of the new prognostic indices on a large, completely independent, and comparable CN-AML patient cohort from CALGB/Alliance trials.

$PINA_{OS}$ and $PINA_{RFS}$ have been developed in a well-characterized cohort of patients with CN-AML to specifically model prognosis in this clinically important entity. Our scores are based on the largest CN-AML cohort that has been used for such purposes so far. A number of other prognostic scoring systems for AML have been published.[33-39] In contrast to our PINA scores, other models that include both clinical data and molecular markers were restricted to patients $\geq$ 60 years[34,37-39] or $\leq$ 60 years[33,35,36] of age or were not specifically developed for CN-AML.[34-39] The only other risk score that has been developed specifically for CN-AML was reported from Damm et al[33] and was restricted to patients age $\leq$ 60 years. Krug et al[34] have developed a model for older patients with AML that is easily applicable in the clinic, but it applies primarily to the end points of CR rate and early death rate. In contrast, $PINA_{OS}$ and $PINA_{RFS}$ are the first prognostic indices developed for adult patients with CN-AML of all age groups. We demonstrate that our scores can be used for risk stratification in older and younger patients with CN-AML, both in our own data and in the external validation cohort. Therefore, in the context of CN-AML, our PINA indices might be more widely applicable than other scoring systems.

$PINA_{OS}$ and $PINA_{RFS}$ are AML prognostic scoring systems that further refine the current widely used standard guideline for AML diagnosis and management, the ELN classification.[11] According to the ELN classification, patients with CN-AML are divided into two different risk groups, the ELN favorable genetic group and the ELN intermediate-I genetic group. In contrast, the $PINA_{OS}$ and $PINA_{RFS}$ subdivide patients with CN-AML into three distinct groups. The classification into three risk groups instead of two has the advantage of separating patients with a very good prognosis and those with a very bad prognosis from an intermediate group of patients. This allows for clinical trials exploring treatment modifications in the extreme groups. In addition, by using $PINA_{OS}$ and $PINA_{RFS}$, patients in both ELN genetic groups (ELN favorable and ELN intermediate-I) could be further stratified. This shows that the integration of clinical and molecular data as assessed by our scores adds significant information to, and thus improves, the ELN classification with regard to treatment stratification. This may potentially translate into more risk-specific therapeutic approaches. For example, according to the ELN classification, 65% and 35% of patients less than 60 years old classified as LowR by $PINA_{OS}$ belong to the ELN favorable and ELN intermediate-I genetic groups, respectively. Five-year OS within the LowR group according to $PINA_{OS}$ in patients less than 60 years old was 82% and not significantly different between the ELN favorable and ELN intermediate-I genetic groups ($P = .541$). Thus, our data suggest that a subgroup of patients less than 60 years old currently classified as ELN intermediate-I might not benefit from allogeneic transplantation, which would currently be recommended according to the ELN guidelines.

Our scores are the first, to our knowledge, to integrate the established molecular markers of *NPM1*, *FLT3*-ITD, and *CEBPA* recognized by the current ELN and WHO classifications[40] together with clinical characteristics for risk stratification in patients with CN-AML of all age groups. Our models are exclusively based on routinely

available genetic and clinical characteristics, thus making them easily applicable during routine clinical practice. Of note, $PINA_{OS}$ is the only published risk model that considers the patient's performance status also in younger patients. Because performance status partly reflects the presence of comorbidities, $PINA_{OS}$ might prove to be valid in population-based cohorts not necessarily treated in trials, although this requires further validation. The PINA scores are also unique because they incorporate the type of *CEBPA* mutation, accounting for the fact that a favorable effect on prognosis has been demonstrated only for bi*CEBPA*, but not for mo*CEBPA*, mutations.[3,6]

In conclusion, with the $PINA_{OS}$ and $PINA_{RFS}$, we have developed and validated, to our knowledge, the first prognostic indices specifically designed for patients of all ages with CN-AML. $PINA_{OS}$ and $PINA_{RFS}$ are valid in patients less than 60 and $\geq$ 60 years old and further refine the generally used ELN genetic classification for CN-AML. Because the PINA scores are based on a combination of well-established and routinely available molecular markers and clinical parameters, they can be easily adopted in routine clinical care. Web-based calculators of both PINA indices are made available at http://www.aml-score.org.

In the context of the rapidly growing number of newly discovered AML markers with uncertain clinical relevance discovered by whole-genome sequencing, the $PINA_{OS}$, an integrative prognostic index based on currently well-established risk factors, could serve as a common benchmark for future molecularly based prognostic algorithms. Furthermore, $PINA_{RFS}$ can be applied as a tool to stratify postremission therapy according to relapse risk and chances of cure in future clinical studies.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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GLOSSARY TERMS

NPM1: gene coding for nucleophosmin (also called nucleolar phosphoprotein B23 [numatrin]), which is primarily localized in the nucleolus of the nucleus. It contains an N-terminus oligomerization domain, a metal-binding site, two acid-rich domains, and two nuclear localization signals in the C-terminus of the protein. It is an RNA-binding phosphoprotein involved in the assembly of ribosomal proteins into ribosomes and the transport of ribonucleoproteins between the cellular compartments. The gene is involved in several tumor-associated chromosome translocations.

bootstrap procedure: a nonparametric statistical method to estimate sampling distributions of an estimator by resampling with a replacement from the original sample. In prognostic research, the bootstrap helps to obtain an impression of the validity of predictions in new but similar patients.

Cox proportional hazards regression model: a statistical model for regression analysis of censored survival data, examining the relationship of censored survival distribution to one or more covariates. This model produces covariate coefficient estimates with their standard errors, hazard ratios, 95% CIs, and significance levels.

cumulative incidence of relapse (CIR): the use of competing risk analyses indicated in the presence of competing events (such as death and relapse); the Gray's test is a recommended method to compare cumulative incidence of relapse between groups.

external validation: the process of validating the classifier obtained from developmental studies using truly independent data external to the study used to develop the classifier. The objective of external validation is to determine whether use of a completely specified diagnostic classifier for therapeutic decision making in a defined clinical context results in patient benefit. For example, an independent validation study could be a prospective clinical trial in which patients are randomly assigned to treatment assignment without use of the classifier versus treatment assignment with the aid of the classifier.

overall survival: time from the date of entry into a study until the date of death from any cause.

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