original reports

CD123 Expression Is Associated With High-Risk Disease Characteristics in Childhood Acute Myeloid Leukemia: A Report From the Children's Oncology Group

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abstract

PURPOSE Increased CD123 surface expression has been associated with high-risk disease characteristics in adult acute myeloid leukemia (AML), but has not been well-characterized in childhood AML. In this study, we defined CD123 expression and associated clinical characteristics in a uniformly treated cohort of pediatric patients with newly diagnosed AML enrolled on the Children's Oncology Group AAML1031 phase III trial (NCT01371981).

MATERIALS AND METHODS AML blasts within diagnostic bone marrow specimens (n = 1,040) were prospectively analyzed for CD123 protein expression by multidimensional flow cytometry immunophenotyping at a central clinical laboratory. Patients were stratified as low-risk or high-risk on the basis of (1) leukemia-associated cytogenetic and molecular alterations and (2) end-of-induction measurable residual disease levels.

RESULTS The study population was divided into CD123 expression–based quartiles (n = 260 each) for analysis. Those with highest CD123 expression (quartile 4 [Q4]) had higher prevalence of high-risk *KMT2A* rearrangements and *FLT3*-ITD mutations ($P < .001$ for both) and lower prevalence of low-risk t(8;21), inv(16), and *CEBPA* mutations ($P < .001$ for all). Patients in lower CD123 expression quartiles (Q1-3) had similar relapse risk, event-free survival, and overall survival. Conversely, Q4 patients had a significantly higher relapse risk (53% *v* 39%, $P < .001$), lower event-free survival (49% *v* 69%, $P < .001$), and lower overall survival (32% *v* 50%, $P < .001$) in comparison with Q1-3 patients. CD123 maintained independent significance for outcomes when all known contemporary high-risk cytogenetic and molecular markers were incorporated into multivariable Cox regression analysis.

CONCLUSION CD123 is strongly associated with disease-relevant cytogenetic and molecular alterations in childhood AML. CD123 is a critical biomarker and promising immunotherapeutic target for children with relapsed or refractory AML, given its prevalent expression and enrichment in patients with high-risk genetic alterations and inferior clinical outcomes with conventional therapy.

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ASSOCIATED CONTENT

Appendix

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

CD123 is the alpha chain of the interleukin 3 receptor (IL-3Rα) and plays an important role in the production and function of hematopoietic cells.[1] IL-3 cytokine binding to IL-3α recruits the common beta chain to form the heterodimeric IL-3R and induces downstream JAK/STAT, Ras/MAPK, and PI3K signaling.[2] CD123 is expressed on normal plasmacytoid dendritic cells, basophils, monocytes, and eosinophils and in a variety of hematologic malignancies.[3] The majority of data describing the expression of CD123 have been in adult leukemias, where it has been shown to be expressed at high levels on B-acute lymphoblastic leukemia, acute myeloid leukemia (AML), blastic plasmacytoid dendritic cell neoplasm, and hairy cell leukemia.[4-8] CD123 expression in adults with AML has been associated with higher rates of chemoresistance and high-risk genetic alterations, particularly *FLT3* internal tandem duplication (*FLT3*-ITD).[9,10] In addition, CD123 expression has been found at high levels on leukemic stem cells, a small and cell cycle–dormant population that likely contributes to chemoresistance and relapse.[5,11]

The high prevalence of CD123 expression in AML and its association with high-risk genetic alterations and chemoresistant disease have generated robust interest in CD123-targeting therapeutic strategies. Significant antileukemia efficacy has been observed in preclinical

CONTEXT

Key Objective

CD123 is the alpha chain of the interleukin 3 receptor and is overexpressed in a variety of adult hematologic malignancies, including acute myeloid leukemia (AML). Within the context of the Children's Oncology Group protocol AAML1031, a phase III randomized trial of the proteasome inhibitor bortezomib in combination with conventional chemotherapy, we sought to characterize the incidence and prognostic significance of CD123 expression in pediatric AML.

Knowledge Generated

CD123 expression is directly associated with high-risk cytogenetic and molecular alterations in pediatric AML. High CD123 expression is associated with inferior outcomes, and this association carries independent prognostic significance.

Relevance

High CD123 expression is an important biomarker of chemoresistance and relapse risk in pediatric AML. Incorporation into risk stratification should be considered, and this emphasizes the importance of continuous development of CD123-targeting strategies.

models of AML treated with a variety of CD123-targeted agents, such as fusion proteins, monoclonal antibodies, antibody-drug conjugates, bispecific antibodies, and chimeric antigen receptor T-cell immunotherapies.[12-24] On the basis of these exciting preclinical results, several of these strategies have recently advanced to clinical investigation via early phase trials.[25]

The incidence and prognostic significance of CD123 expression in pediatric AML have not yet been defined. In this study, we quantified CD123 cell surface expression by flow cytometry analysis of 1,040 diagnostic specimens from children, adolescents, and young adults with newly diagnosed AML uniformly treated on a large international phase III clinical trial. We further correlated CD123 expression levels with sentinel AML-associated genetic alterations and clinical outcomes. We herein report that highest CD123 expression is enriched in pediatric patients with high-risk cytogenetic or molecular alterations and is associated with inferior event-free survival (EFS) and overall survival (OS).

MATERIALS AND METHODS

Patients and Treatment

Pediatric patients with newly diagnosed AML enrolled on the Children's Oncology Group (COG) AAML1031 phase III clinical trial (NCT01371981) were eligible for this subanalysis. Details of this trial have been previously described.[26] The study was approved by local Human Investigations Committees, and investigators obtained informed consent from each participant or each participant's guardian. Briefly, AAML1031 tested the efficacy of the addition of the proteasome inhibitor bortezomib in a randomized fashion to a four-cycle multiagent chemotherapy backbone. Low-risk patients received four courses of multiagent chemotherapy with induction 1, induction 2, intensification 1, and intensification 2 cycles. Patients stratified as high-risk because of high-risk AML-associated lesions and/or positive end-of-induction 1 (EOI1) multidimensional flow cytometric (MDF) measurable residual disease (MRD; $> 0.1\%$) received three courses of chemotherapy followed by best allogeneic donor hematopoietic stem-cell transplantation. Consenting patients with *FLT3*-ITD AML nonrandomly received the multitarget tyrosine kinase inhibitor sorafenib in combination with chemotherapy. Morphologic, cytogenetic, molecular genetic, and MDF analyses of diagnostic AML specimens were performed in central reference laboratories according to study guidelines. Research-level next-generation sequencing was also performed in a subset of samples.

Risk Stratification

Cytogenetic and molecular abnormalities and EOI1 MRD levels were used to stratify the study population into risk groups. The low-risk group included patients with t(8;21) with *RUNX1-RUNXT1* fusion, inv(16)/t(16;16) with *CBFB-MYH11* fusion, *NPM1* (*nucleophosmin 1*) mutations, or *CEBPA* mutations without *FLT3*-ITD alterations. The high-risk group included patients with a high allelic ratio (> 0.4) *FLT3*-ITD, monosomy 5, del(5q), or monosomy 7. Patients with noninformative cytogenetic and molecular abnormalities who were MRD-negative or MRD-positive at EOI1 were classified as low-risk or high-risk, respectively. Recent refinement of risk stratification includes the addition of multiple high-risk markers and has been incorporated into the successor COG AAML1831 trial (NCT04293562).[25,27-30] This refinement is based on inferior outcomes on predecessor trials and independent observations from other collaborative groups. Examples include high-risk *KMT2A* rearrangements (5-year EFS 23% ± 9%) and *CBFA2T3-GLIS2* (10% ± 13%), both of which had poor outcomes on AAML1031. Both risk stratification systems were used for analyses (Appendix Table A1, online only).

Assessment of CD123 Expression

Specimens were processed and analyzed by MDF using a difference from normal flow cytometric technique, as

previously described.[31] CD123 immunophenotyping was performed using a PE-conjugated anti-CD123 antibody (clone 9F5; BD Biosciences, Franklin Lakes, NJ) with quantification of mean fluorescence intensity (MFI) and conversion to a molecules per cell metric using CD4 MFI on normal T cells as a reference, as previously described.[32]

Statistical Analyses

Outcomes data for patients enrolled on AAML1031 were analyzed with a data cutoff date of March 31, 2019. The Kaplan-Meier method was used to estimate OS and EFS. OS was defined as the time from study entry to death from any cause or date of last follow-up in surviving patients. EFS was defined as the time from study entry until induction failure, relapse, or death. Complete remission was defined as a bone marrow aspirate containing $< 5\%$ blasts by morphologic analysis and without evidence of extramedullary disease. The cumulative incidence of relapse risk (RR) was calculated from EOI1 for patients in complete remission to relapse where deaths without a relapse were considered competing events. The significance of predictor variables was tested using the log-rank test for OS and EFS and Gray's test for RR.[33] The Cox proportional hazards model was used to estimate hazard ratios (HRs) for survival outcomes. Competing risk regression models were used to estimate HRs for analyses of RR. Both models were used for univariable and multivariable analyses. The Kruskal-Wallis test was used for comparison of continuous variables, and the chi-squared test was used to test for significance of observed differences in proportions. A P value $< .05$ was considered statistically significant. Statistical analyses were performed using Statistical Analysis Software (SAS) 9.4 (SAS Institute Inc, Cary, NC).

RESULTS

Study Population

A total of 1,400 pediatric patients with newly diagnosed AML were enrolled on AAML1031 from July 6, 2011, to July 18, 2014. Diagnostic bone marrow specimens were available for 1,040 patients (78.7%) and were analyzed by MDF for CD123 cell surface protein expression. Clinical characteristics of treated patients are detailed in Appendix Table A2 (online only).

CD123 Expression Levels and Disease Characteristics

For the purposes of clinical correlation, the study population was divided into quartiles (n = 260 patients per quartile) on the basis of flow cytometric quantification of CD123 expression (Appendix Fig A1, online only). The median CD123 molecules per cell were 557.5 (range, 121.2-781.7) for quartile 1 (Q1), 1,015.1 (range, 784.8-1,312) for Q2, 1,660.4 (range, 1,315-2,169.5) for Q3, and 2,963.3 (range, 2,181.6-11,726.1) for Q4 (Appendix Fig A2, online only). For purposes of analyses, patients in the highest CD123 expression quartile (Q4) were compared with the lower expression quartiles (Q1-Q3), as previously described for other AML cell surface markers.[34,35] CD123 expression levels were correlated with clinical and disease characteristics across the four quartiles, and sex, race, ethnicity, and age were determined to be similar among the cohorts. Diagnostic peripheral white blood cell counts and bone marrow blast percentages were highest in the Q4 patients (Appendix Table A2).

Cytogenetic and molecular data for the AAML1031 protocol–defined risk stratification were available for 1,030 (99%) and 1,040 (100%) patient samples, respectively. Among genetically low-risk patients, prevalence of t(8;21) (Q1-3 18.3% *v* Q4 3.2%; $P < .001$) and inv(16) (Q1-3 11.4% *v* Q4 4.8%; $P = .002$) was inversely associated with CD123 expression. Similarly, prevalence of *CEBPA* mutations (Q1-3 8.1% *v* Q4 0.4%; $P < .001$) was inversely associated with CD123 expression, whereas *NPM1* mutations occurred at similar rates across all CD123 quartiles (Fig 1). Among genetically high-risk patients, a direct association was observed between *FLT3*-ITD (Q1-3 8.6% *v* Q4 34.2%; $P < .001$) and CD123 expression. Conversely, no association between the CD123 expression level and monosomy 7 or monosomy 5/del(5q) was detected (Appendix Table A2). Other cytogenetic abnormalities associated with higher CD123 expression included t(6;9) with *DEK-NUP214* fusion (Q1-3 1.2% *v* Q4 3.1%; $P = .035$) and *KMT2A* rearrangements (Q1-3 19.6% *v* Q4 31.8%; $P < .001$). Further subanalysis of specific *KMT2A* fusion partners by the AAML1831 protocol–defined risk stratification demonstrated greater association of high-risk *KMT2A* rearrangements with highest CD123 expression (Q1-3 7.2% *v* Q4 15.0%; $P < .001$; Fig 1).

DNA-based next-generation sequencing was performed for 977 (94%) available diagnostic specimens. These molecular data were combined with clinical cytogenetic data to classify patients by the AAML1831 risk stratification. Patients with *NUP98* rearrangements occurred in all CD123 quartiles, but prevalence of the most common *NUP98-NSD1* fusion was higher in Q4 (10.8% in Q4 *v* 3.0% in Q1-3; $P < .001$), whereas the second most common *NUP98-KDM5A* fusion occurred more frequently in Q1-3 (1.9% in Q1-3 *v* 0% in Q4; $P = .017$). There was a nonstatistically significant trend of *CBFA2T3-GLIS2* fusion specimens (n = 21), as more frequent in Q1 (2.6% in Q1-3 *v* 0.8% in Q4; $P = .098$; Fig 2). As previously described, *NUP98-KDM5A* and *CBFA2T3-GLIS2* samples were strongly associated with the acute megakaryoblastic leukemia (AMKL) subtype.[36] AMKL, in general, was associated with low expression of CD123 (5.6% in Q1-3 *v* 1.2% in Q4; $P = .004$). There was no apparent association between CD123 expression and *ETV6* (n = 22) or *MECOM* (n = 6) alterations although analysis was limited by small numbers (Appendix Table A2).

When stratified on the basis of the AAML1031 risk classification, 373 (35.9%) patients were classified as low-risk, 154 (14.8%) patients were classified as high-risk, and 513

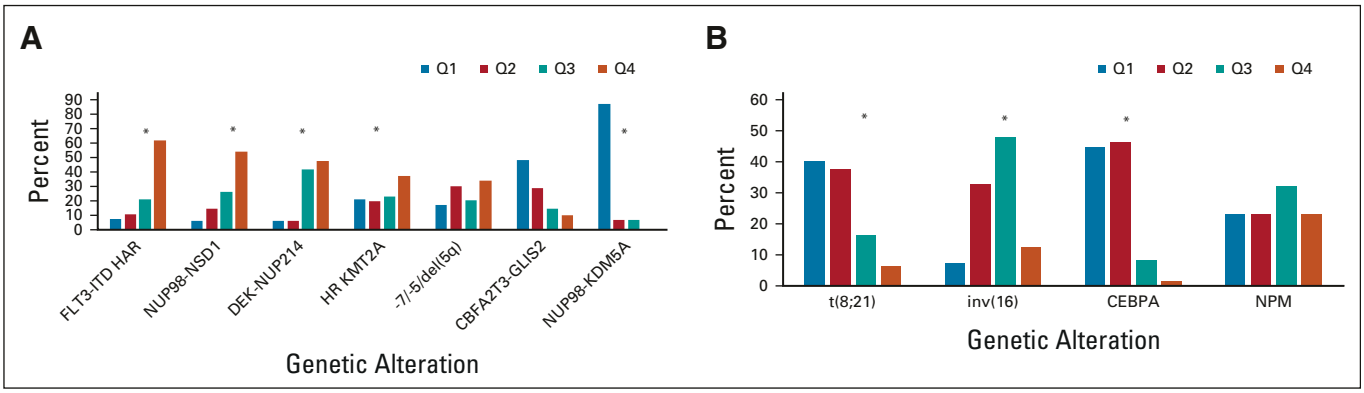

FIG 1. Prevalence of select (A) high-risk and (B) low-risk genetic features on the basis of the CD123 quartile. *$P < .05$ for Q4 versus Q1-3 by proportional comparison. HAR, high allelic ratio; HR, high-risk; Q, quartile.

(49.3%) patients had noninformative cytogenetic or molecular alterations that led to final risk classification by EOI1 MRD by MDF. Q4 contained a higher prevalence of high-risk alterations (Q4 33.1% *v* Q1-3 8.7%; $P < .001$) and lower prevalence of low-risk alterations (Q4 13.8% *v* Q1-3 43.2%; $P < .001$; Table 1). This significance was maintained when patients with noninformative alterations were further risk stratified on the basis of EOI1 MRD (Table 1). When all contemporary markers were incorporated and the updated AAML1831-based risk classification was applied, a higher proportion of patients were considered high-risk. Q4 maintained a higher prevalence of high-risk alterations (Q4 55.2% *v* Q1-3 26.4%; $P < .001$) and lower prevalence of low-risk alterations (Q4 19.4% *v* Q1-3 46.3%; $P < .001$; Table 1).

CD123 Expression Levels and Clinical Outcomes

CD123 expression was initially analyzed as a continuous variable and found to be associated with inferior clinical outcomes at increasing levels of expression. When CD123 expression was divided into quartiles and associated with clinical outcomes, the lower expressing quartiles (Q1, Q2, and Q3) clustered together and were therefore consolidated (Q1-3) to permit further comparisons with the highest CD123-expressing quartile (Q4; Appendix Fig A3, online only).

There was no difference in a patient's ability to achieve a morphologic or MRD-negative remission on the basis of the CD123 quartile (Table 2). Despite similar induction responses between quartile groups, the 5-year OS (48.9% ± 6.9% in Q4 *v* 68.5% ± 3.5% in Q1-3; $P = < .001$) and 5-year EFS (32.3% ± 6.2% in Q4 *v* 50.1% ± 3.66% in Q1-3; $P < .001$) were lowest in Q4, corresponding also with highest 5-year RR from EOI1 (53% ± 7.6% in Q4 *v* 38.7 ± 4.12% in Q1-3; $P < .001$; Figs 2A-2C). These differences were largely driven by the inferior outcomes of AAML1031-defined low-risk patients with high CD123 expression (Figs 2D-2F), as high-risk patients had analogous outcomes regardless of the quartile (Figs 2G-2I). These differences became less apparent but maintained significance when the AAML1831-based risk classification was applied and patients with contemporary high-risk alterations were reallocated for analysis (Fig 3). Importantly, high CD123 expression did not abrogate the favorable EFS conveyed by low-risk cytogenetic alterations, including t(8;21) (50% ± 35% in Q4 *v* 66% ± 8% in Q1-3; $P = .439$) and inv(16) (50% ± 29% in Q4 *v* 58% ± 11% in Q1-3; $P = .383$).

Given the detected significant association between CD123 quartiles and clinical outcomes, we performed Cox regression analyses to evaluate the impact of CD123 quartiles as a predictor of clinical outcomes in the context of contemporary prognostic features. AAML1831-defined risk groups were used as a covariate in both univariable (Table 3) and multivariable models (Appendix Table A3, online only). In the univariable model, high CD123 expression (Q4) was a significant prognostic factor for inferior OS (HR = 1.73; $P < .001$), EFS (HR = 1.59; $P < .001$), and RR (HR = 1.57; $P < .001$). In a multivariable model that included all known contemporary prognostic features, high CD123 expression retained prognostic significance for OS (HR = 1.43; $P = .005$), EFS (HR = 1.51; $P < .001$), and RR (HR = 1.42; $P = .009$).

DISCUSSION

In this study, we report MDF quantification of surface CD123 expression on leukemia cells from children, adolescents, and young adults with newly diagnosed AML enrolled on the COG AAML1031 study. Our study used a uniform approach to sample processing and analysis and, to our knowledge, is the largest of those defining CD123 in patients with AML. We observed that highest CD123 expression was directly associated with high-risk leukemia-associated genetic alterations and inferior clinical outcomes. These results are concordant with previous studies of CD123 expression in adults with AML, with highest CD123 expression levels enriched in patients with *FLT3*-ITD AML or high wild-type FLT3 protein expression.[8,9,37-42]

We report previously unknown differences in CD123 expression in pediatric patients with t(8;21), inv(16), t(6;9), *KMT2A* rearrangements, *NUP98* fusions, and *CEBPA*

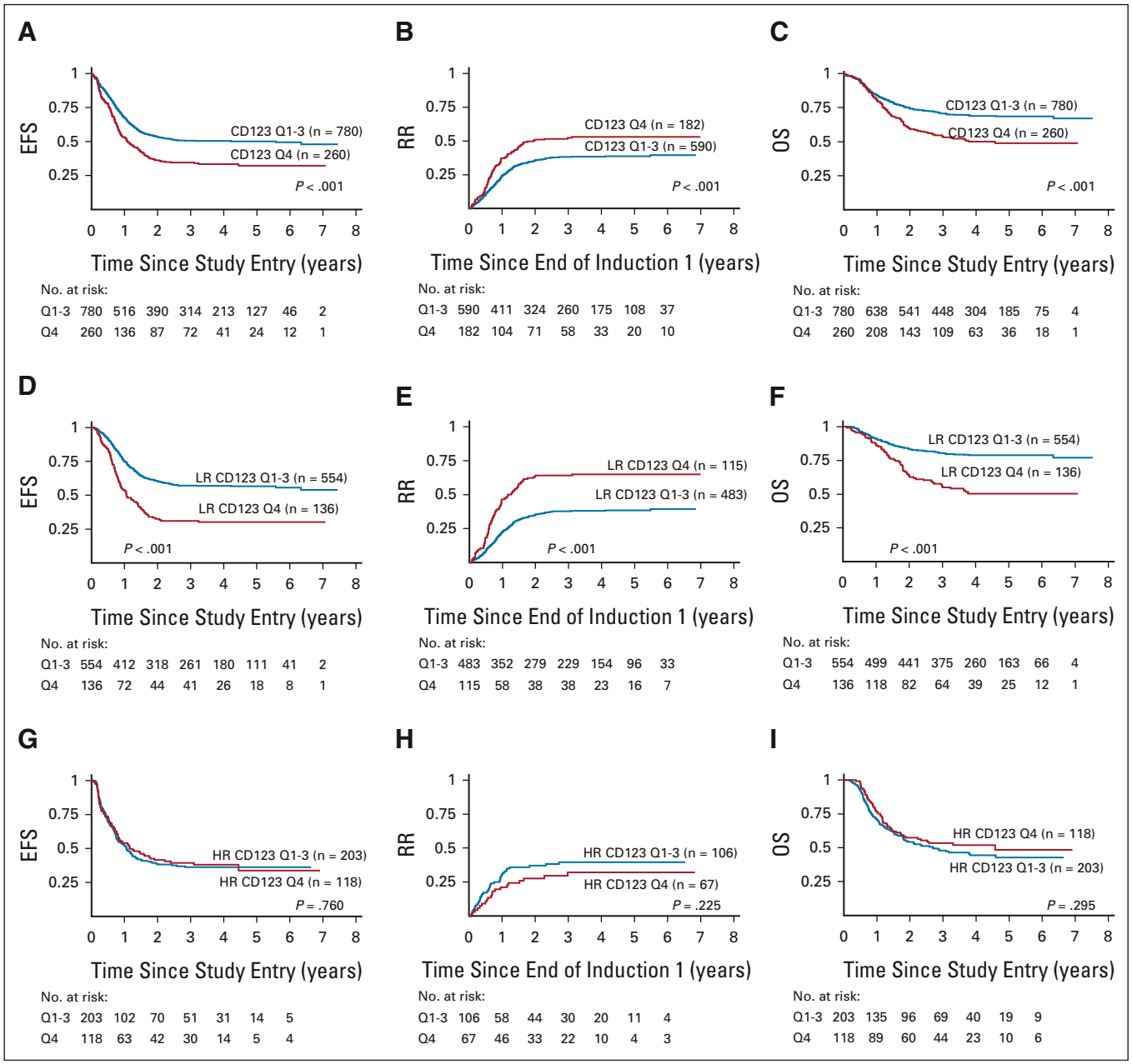

FIG 2. Correlation of clinical outcomes with CD123 expression on the basis of protocol-defined risk classification: (A) EFS (n = 1,040), (B) RR (n = 772), (C) OS (n = 1,040) from study entry stratified by CD123 expression quartiles, (D) EFS from study entry for low-risk (LR) patients, (E) RR for LR patients, (F) OS for LR patients, (G) EFS from study entry for high-risk (HR) patients, (H) RR for HR patients, and (I) OS for HR patients. Q4 versus 1-3 comparison by log-rank and Gray's tests. EFS, event-free survival; OS, overall survival; Q, quartile; RR, relapse risk.

mutations, which may reflect the inherent biology of pediatric versus adult AML. An example of this is the relatively even distribution of *NPM1*-mutant pediatric patients that we detected across the four CD123 quartiles in contrast to adult patients in whom *NPM1*-mutant AML has been associated with increased CD123 expression.[39,41,43,44] The relatively low prevalence of somatic *NPM1* mutations in pediatric AML and the lower association with normal karyotypes may partially explain why we did not detect a significant difference in CD123 expression in *NPM1*-mutated AML.[45-49] Other explanations for differing observations include cohort sample size, sample type (eg, bone marrow *v* peripheral blood), relative mutation frequency in children versus adults, method of CD123 expression assessment (eg, flow cytometry *v* immunohistochemistry), characterization of CD123 status (eg MFI *v* percent of positive cells), and statistical analysis methodology (eg, CD123 as a continuous variable *v* categorical).

TABLE 1. Risk Group Stratification by CD123 Expression Quartiles

Risk Group	Q1 (260), No. (%)	Q2 (260), No. (%)	Q3 (260), No. (%)	Q4 (260), No. (%)	Q1-3 (780), No. (%)	Q1-3 *v* Q4 *P*
AAML1031 cytogenetic/molecular risk group						
Noninformative	136 (52.3)	109 (41.9)	130 (50.0)	138 (53.1)	375 (48.1)	.719
Low	110 (42.3)	129 (49.6)	98 (37.7)	36 (13.8)	337 (43.2)	< .001
High	14 (5.4)	22 (8.5)	32 (12.3)	86 (33.1)	68 (8.7)	< .001
AAML1031-defined risk group[a]						
Low	191 (74.6)	198 (78.6)	165 (66.3)	136 (53.5)	554 (73.2)	< .001
High	65 (25.4)	54 (21.4)	84 (33.7)	118 (46.5)	203 (26.8)	

	Q1 (249), No. (%)	Q2 (249), No. (%)	Q3 (251), No. (%)	Q4 (252), No. (%)	Q1-3 (749), No. (%)	Q1-3 *v* Q4 *P*
AAML1831 cytogenetic/molecular risk group						
Noninformative	79 (31.7)	55 (22.1)	70 (27.9)	64 (25.4)	204 (27.1)	.623
Low	109 (43.8)	134 (53.8)	104 (41.4)	49 (19.4)	347 (46.3)	< .001
High	61 (24.5)	60 (24.1)	77 (30.7)	139 (55.2)	198 (26.4)	< .001
AAML1831-defined risk group[a]						
Low	165 (66.3)	172 (69.1)	144 (57.4)	100 (39.7)	481 (64.2)	< .001
High	84 (33.7)	77 (30.9)	107 (42.6)	152 (60.3)	268 (35.8)	

Abbreviations: EOI1, end-of-induction 1; MRD, measurable residual disease; Q, quartile.
[a]Incorporates the presence or absence of MRD at EOI1.

We also report that higher CD123 expression is associated with inferior outcomes. This association can partially be attributed to the enrichment of patients with high-risk cytogenetic and molecular markers within Q4. However, when outcome measures for the highest CD123 expression group (Q4) were analyzed within the context of a Cox regression analysis, the HRs observed were significant in both the univariable and multivariable models. This result suggests that high C123 expression is an independent predictor of outcomes in pediatric patients with AML. A total of 50 patients (4.8%) on AAML1031 had high CD123 expression in the presence of noninformative cytogenetic and molecular abnormalities and therefore did not receive treatment intensification. The validity of using CD123 as an independent prognostic marker and the benefit of subsequent treatment intensification, including allocation to hematopoietic stem-cell transplantation, will need to be confirmed on prospective clinical trials.

There is supporting evidence that CD123 may play a protective role in the development and maintenance of AML. Preclinical studies have shown that coincubation of human AML cells in vitro with IL-3 increases their proliferation, which can also be abrogated with the antibody-based IL-3 blockade.[19,50] Several groups have also reported cytokine secretion (including IL-3) directly from AML cells, which suggests an autocrine pathway for leukemia cell

TABLE 2. Induction Response and Clinical Outcomes by CD123 Expression Quartiles

Response	Q1 (260), No. (%)	Q2 (260), No. (%)	Q3 (260), No. (%)	Q4 (260), No. (%)	Q1-3 (780), No. (%)	Q1-3 *v* Q4 *P*
Response by EOI1						
CR	198 (78.0)	199 (78.7)	193 (75.4)	182 (71.9)	590 (77.3)	.082
No CR	56 (22.0)	54 (21.3)	63 (24.6)	71 (28.1)	173 (22.7)	
Not evaluable	6	7	4	7	17	
EOI1 MRD						
Negative	174 (69.3)	184 (76.3)	170 (70.2)	163 (67.9)	528 (71.9)	.234
Positive	77 (30.7)	57 (23.7)	72 (29.8)	77 (32.1)	206 (28.1)	

Abbreviations: CR, complete remission; EOI1, end-of-induction 1; MRD, measurable residual disease; Q, quartile.

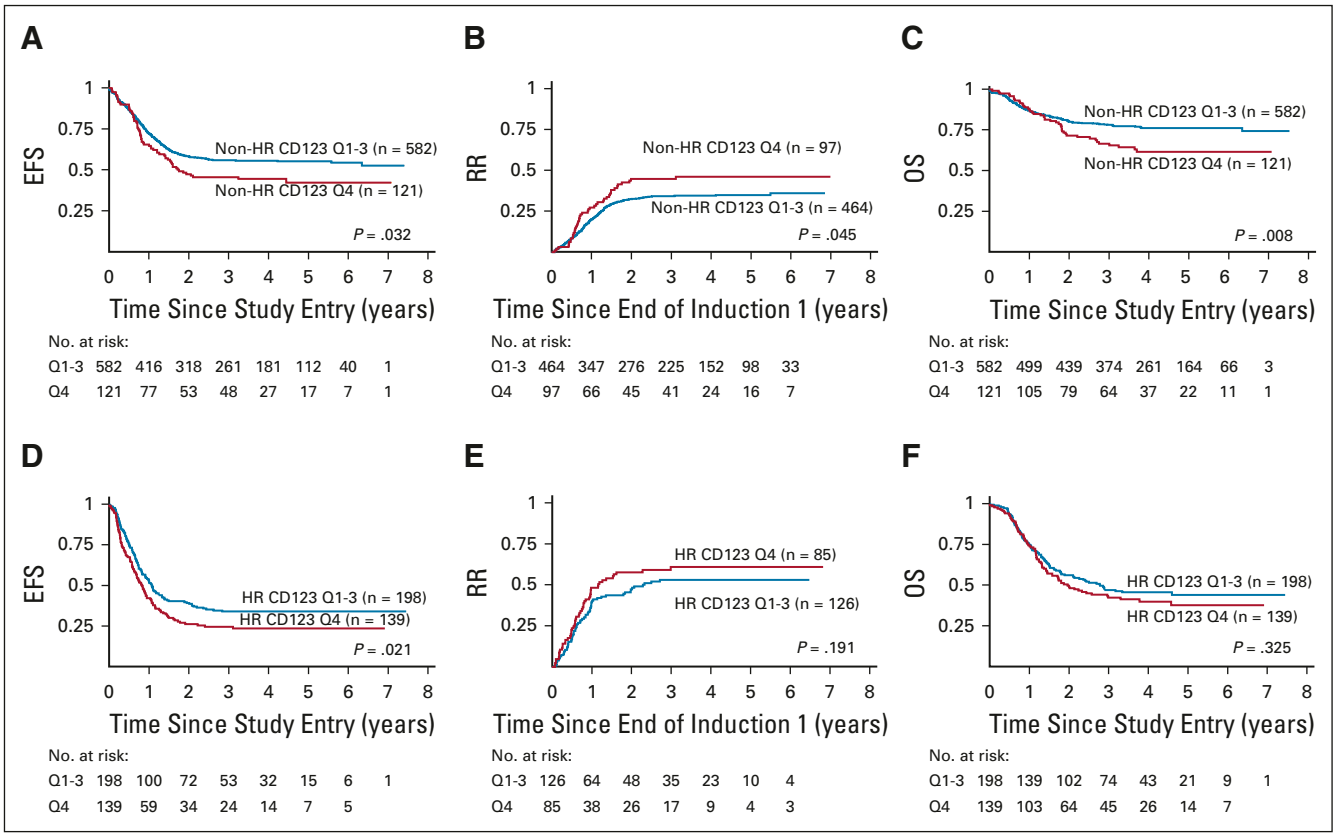

FIG 3. Correlation of clinical outcomes with CD123 expression on the basis of the contemporary AAML1831-based cytogenetic and molecular risk factors: (A) EFS from study entry for non–high-risk (non-HR) patients, (B) RR for non-HR patients, (C) OS for non-HR patients, (D) EFS from study entry for high-risk (HR) patients, (E) RR for HR patients, and (F) OS for HR patients. Q4 versus 1-3 comparison by log-rank and Gray's tests. EFS, event-free survival; OS, overall survival; Q, quartile; RR, relapse risk.

maintenance and/or proliferation.[50] Testa et al[8] showed that CD123-overexpressing AML blasts exhibit increased proliferation and resistance to apoptosis and constitutive and IL-3–inducible STAT5 phosphorylation. On the basis of these data, they hypothesized that CD123/IL3-R overexpression could confer a growth advantage. This prediction was further supported by their observation of greater leukocytosis and inferior clinical outcomes in patients with CD123-overexpressing AML versus those with lower expression.[8] More recently, Wittwer et al showed that higher CD123 expression is associated with reduced *CXCR4* RNA and CXCR4 protein expression, which is essential for hematopoietic stem-cell homing to the bone marrow and maintenance of this niche. Genetic deletion or pharmacologic inhibition of CXCR4 further led to AML cellular egress from the bone marrow, implicating potential interplay between CXCR4 and CD123 in AML cells.[51] In addition, it is plausible that the reported high CD123 expression on AML leukemic stem cells could lead to high CD123-expressing bulk

TABLE 3. Cox Univariable Regression Analysis of CD123 Expression (Q1-3 *v* Q4) and Other Prognostic Factors

	OS				EFS				RR from CR1			
Variable	**No.**	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***
CD123 expression												
Q1-3	780	1			780	1			590	1		
Q4	260	1.73	1.39 to 2.15	< .001	260	1.59	1.33 to 1.91	< .001	182	1.57	1.23 to 2	< .001
AAML1831 cytogenetic/molecular risk group												
Standard	268	1			268	1			191	1		
Low	396	0.35	0.25 to 0.48	< .001	396	0.46	0.36 to 0.57	< .001	345	0.39	0.29 to 0.51	< .001
High	337	1.69	1.32 to 2.16	< .001	337	1.32	1.08 to 1.61	.007	211	1.07	0.82 to 1.4	.624

Abbreviations: EFS, event-free survival; HR, hazard ratio; OS, overall survival; Q, quartile; RR, relapse risk.

AML cells that may represent a more immature developmental arrest phase and confer greater chemoresistance.[52-54]

A notable exception to our study's correlation between high CD123 expression and high-risk genetic features was the lack of enrichment of *CBFA2T3-GLIS2* and *NUP98-KDM5A* subtypes in the CD123 Q4. These pediatric-specific AML fusions are associated with strikingly poor outcomes and correlate with both young age at diagnosis and the non–Down syndrome-associated AMKL subtype.[28,36,55-59] In general, the pediatric AMKL specimens included in our study had low expression of CD123 and were enriched in Q1. These results are consistent with the reported observation that CD123 is not normally expressed on megakaryocytic progenitors despite expression on mature megakaryocytes.[60] It is thus plausible that children with AMKL may not benefit greatly from new CD123-targeting immunotherapies, but this question will require more formal study via clinical trials.

The primary limitation of this study is its retrospective design. This limitation is largely overcome by the large sample size and homogenously treated population on a randomized controlled trial, including centralized testing of CD123 expression. Our study was also limited by our lack of ability to characterize CD123 on relapsed disease. Although previous studies have shown that CD123 expression is durable at relapse, this will be confirmed in future planned studies, including assessment for possible CD123 splice variants.[52,61]

In summary, our data indicate that CD123 expression is directly associated with high-risk genetic factors and inferior clinical outcomes in pediatric patients with AML and may serve as an important biomarker of chemoresistance and RR. We posit that patients with high CD123 expression will likely be enriched in relapse populations suitable for early phase clinical trial investigation of CD123-targeting immunotherapies. Trials integrating detailed cytogenetic and molecular genetic analyses with sophisticated MDF immunophenotyping technologies will continue to improve our understanding of the potential significance of cell surface antigen expression levels and may further refine childhood AML risk stratification.[62] These findings also have important implications for the design and interpretation of current and planned pediatric phase I/2 clinical trials testing antibody-based or cellular CD123-targeted immunotherapies and may help to identify patients most likely to benefit from these new treatment approaches.

AFFILIATIONS

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

CD123 Expression is Associated With High-Risk Disease Characteristics in Childhood Acute Myeloid Leukemia: A Report From the Children's Oncology Group

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APPENDIX

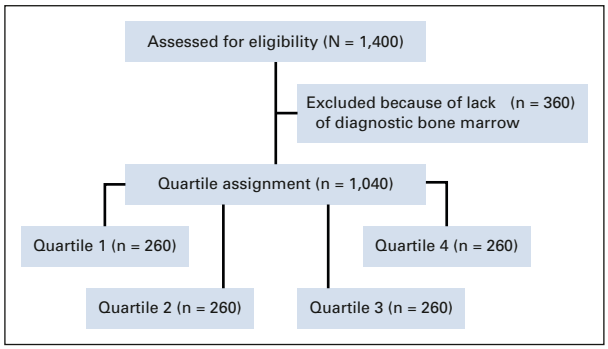

FIG A1. CONSORT diagram.

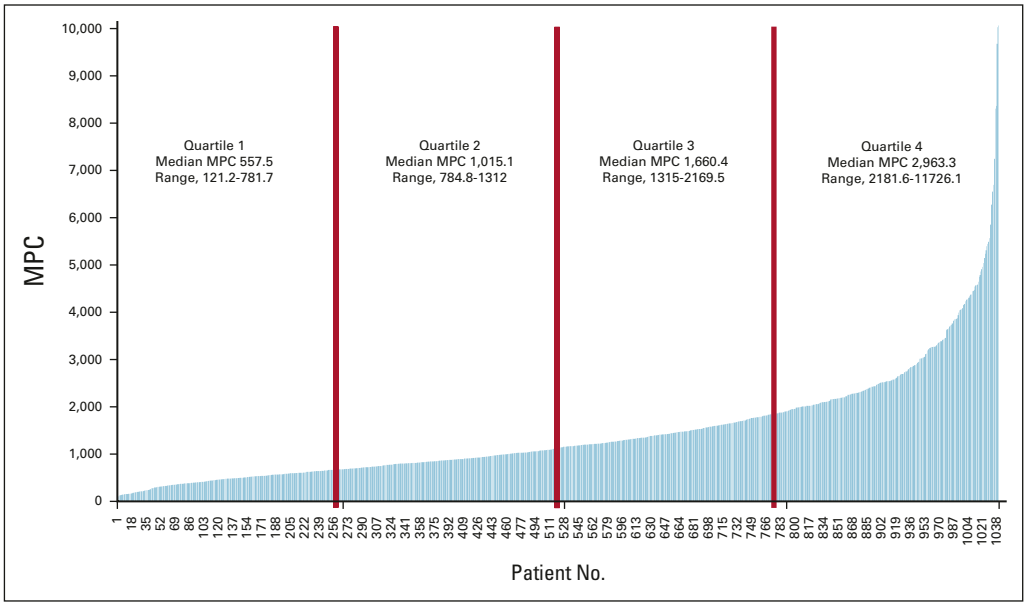

FIG A2. Flow cytometric quantification of surface CD123 expression on diagnostic AML bone marrow specimens from children, adolescents, and young adults enrolled on AAML1031. Data are displayed as the median number of molecules/cell in ascending order of CD123 expression (n = 1,040 specimens). *x*-axis depicts the definition of each quartile (n = 260 specimens/quartile) and relative specimen numbers. AML, acute myeloid leukemia; MPC, molecules per cell.

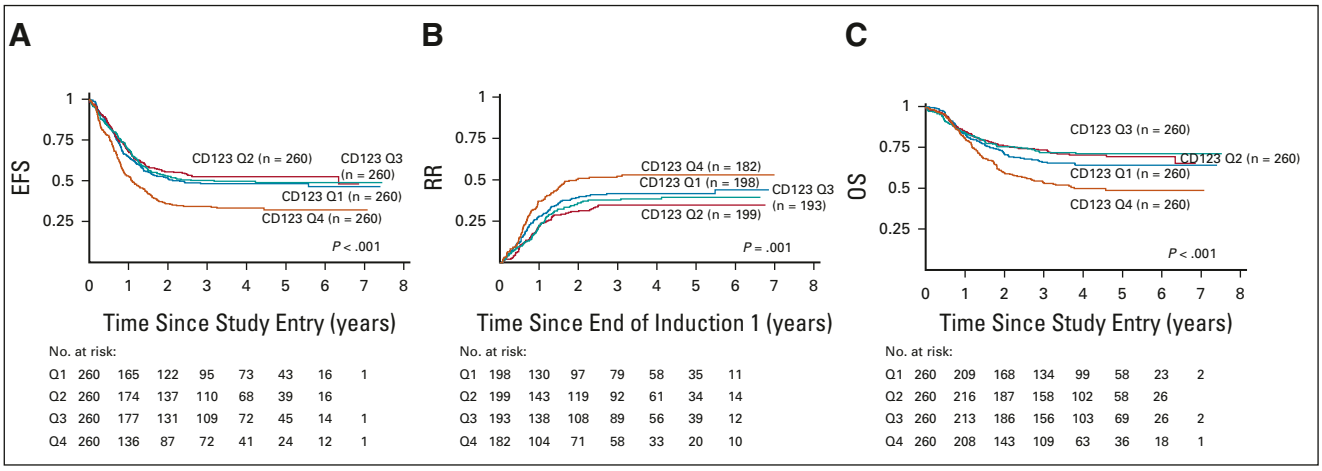

FIG A3. Correlation of clinical outcomes with CD123 expression quartiles: (A) EFS (n = 1,040), (B) RR (n = 772), and (C) OS (n = 1,040) from study entry stratified by CD123 expression quartiles. $P < .001$ for all Q4 versus Q3 versus Q2 versus Q1 by log-rank and Gray's tests. EFS, Event-free survival; OS, overall survival; Q, quartile; RR, relapse risk.

TABLE A1. Unfavorable and Favorable Prognostic Markers on the Basis of Clinical Trial

AAML1031 Risk Stratification		AAML1831 Risk Stratification	
Unfavorable	**Favorable**	**Unfavorable**	**Favorable**
Monosomy 7	t(8;21) (q21.2;q22) with *RUNX1-RUNX1T1* fusion	Monosomy 7	t(8;21) (q21.2;q22) with *RUNX1-RUNX1T1* fusion
Monosomy 5/5q–	inv(16)/t15;15) (p13.1q22.1) with *CBFB-MYH11* fusion	Monosomy 5/5q–	inv(16)/t15;15) (p13.1q22.1) with *CBFB-MYH11* fusion
FLT3/ITD+ with allelic ratios > 0.4%	*NPM1* mutation	*FLT3*/ITD+ with allelic ratios > 0.1%	*NPM1* mutation
	CEBPA mutation	t(6;9) (p22.3;q34.1) with *DEK-NUP214* fusion	*CEBPA* mutation (bZip domain)
		inv(3) (q21.3q26.2) (RPN1-MECOM)	
		t(3;21) (26.2;q22) (RUNX1-MECOM)	
		KMT2A rearrangements t(4;11) (q21;q23.3) [*KMT2A-AFF1*] t(10;11) (p12.3;q23.3) [*KMT2A-MLLT10*] t(10;11) (p12.1;q23.3) [*KMT2A-ABI1*] t(11;19) (q23.3;p13.3) [*KMT2A-MLLT1*] t(6;11) (q27;q23.3) [*KMT2A-AFDN]*	
		NUP98 rearrangements (chromosome 11p15)	
		ETV6 rearrangements (chromosome 12p13.2)	
		12p13.2 *(ETV6)* deletion	
		t(16;21) (p11.2;q22.2) [*FUS-ERG*]	
		t(3;5) (q25q34) [*NPM1-MLF1*]	
		inv(16) (p13.3q24.3) [*CBFA2T3-GLIS2*]	
		t(8;16) (p11.2;p13.3) [*KAT6A-CREBBP*]	
		10p12.3 *(MLLT10)* rearrangements	
		RAM phenotype	

TABLE A2. Disease Characteristics by CD123 Expression Quartiles

Disease Characteristic	Q1 (260)	Q2 (260)	Q3 (260)	Q4 (260)	Q1-3 (780)	Q1-3 *v* Q4 *P*
Sex, No. (%)						
Male	133 (51.2)	131 (50.4)	139 (53.5)	137 (52.7)	403 (51.7)	.774
Median age, years (range)	8.1 (0.03-24.96)	12.1 (0.38-29.21)	11.5 (0.08-27.31)	9.8 (0-29.55)	10.56 (0.03-29.21)	.523
Race, No. (%)						
American Indian or Alaska Native	2 (0.8)	1 (0.4)	2 (0.9)	4 (1.7)	5 (0.7)	.190
Asian	10 (4.2)	14 (6.0)	8 (3.5)	11 (4.6)	32 (4.6)	.988
Native Hawaiian or Other Pacific Islander	2 (0.8)	1 (0.4)	1 (0.4)	1 (0.4)	4 (0.6)	.778
Black or African American	27 (11.4)	31 (13.3)	34 (15)	39 (16.4)	92 (13.2)	.224
White	195 (82.6)	186 (79.8)	182 (80.2)	182 (76.5)	563 (80.9)	.143
Multiple races	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	.087
Unknown	24	27	33	22	84	
Ethnicity, No. (%)						
Hispanic or Latino	47 (18.5)	42 (16.8)	48 (19.3)	45 (18.1)	137 (18.2)	.966
Not Hispanic or Latino	207 (81.5)	208 (83.2)	201 (80.7)	204 (81.9)	616 (81.8)	
Unknown	6	10	11	11	27	
Cytogenetics, No. (%)						
Normal	62 (23.9)	64 (25.0)	62 (24.1)	76 (29.5)	188 (24.4)	.104
t(8;21) with *RUNX1-RUNXT1* fusion	59 (23.8)	52 (21.1)	24 (9.7)	8 (3.2)	135 (18.2)	< .001
inv(16) with *CBFB-MYH11* fusion	6 (2.4)	32 (13.0)	46 (18.5)	12 (4.8)	84 (11.3)	.003
KMT2A-rearranged	44 (16.9)	45 (17.4)	61 (23.7)	83 (31.8)	153 (19.6)	< .001
t(X;11) (q24;q23)	0 (0.0)	0 (0.0)	2 (0.8)	0 (0.0)	2 (0.3)	1.000
t(11;19) (q23;p13.1)	7 (2.8)	3 (1.2)	2 (0.8)	2 (0.8)	12 (1.6)	.537
t(1;11) (q21;q23)	2 (0.8)	0 (0.0)	1 (0.4)	1 (0.4)	3 (0.4)	1.000
t(9;11) (p22;q23)	10 (4.0)	14 (5.6)	18 (7.2)	31 (12.3)	42 (5.6)	< .001
t(10;11) (p12;q23)	0 (0.0)	4 (1.6)	3 (1.2)	5 (2.0)	7 (0.9)	.190
t(11;19) (q23;p13.3)	5 (2.0)	2 (0.8)	2 (0.8)	7 (2.8)	9 (1.2)	.141
t(6;11) (q27;q23)	3 (1.2)	4 (1.5)	7 (2.8)	5 (2.0)	14 (1.9)	1.000
t(6;9) with *DEK-NUP214* fusion	1 (0.4)	1 (0.4)	7 (2.8)	8 (3.2)	9 (1.2)	.048
Monosomy 7	2 (0.8)	5 (2.0)	4 (1.6)	8 (3.1)	11 (1.5)	.108
Del(7q)	8 (3.2)	9 (3.6)	15 (6.0)	7 (2.8)	32 (4.3)	.284
Monosomy 5/del(5q)	3 (1.2)	4 (1.6)	1 (0.4)	2 (0.8)	8 (1.1)	1.000
+8	19 (7.7)	19 (7.7)	34 (13.7)	32 (12.7)	72 (9.7)	.171
RBM15-MKL1	7 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	7 (0.9)	.203
Other abnormalities	72 (27.8)	39 (15.2)	25 (9.7)	39 (15.1)	136 (17.6)	.355
Unknown	1	4	3	2	8	
Mutations, No. (%)						
FLT3-ITD	19 (7.3)	33 (12.7)	45 (17.3)	98 (37.7)	97 (12.4)	< .001
CEBPA-mutant	28 (10.8)	29 (11.2)	5 (1.9)	1 (0.4)	62 (7.9)	< .001
NPM1-mutant	19 (7.3)	19 (7.3)	28 (10.8)	32 (12.3)	66 (8.5)	.066

(continued on following page)

TABLE A2. Disease Characteristics by CD123 Expression Quartiles (continued)

Disease Characteristic	Q1 (260)	Q2 (260)	Q3 (260)	Q4 (260)	Q1-3 (780)	Q1-3 *v* Q4 *P*
MECOM (*EVI1*) abnormality	1 (0.4)	2 (0.8)	1 (0.4)	2 (0.8)	4 (0.5)	.644
NUP98 rearrangements	19 (7.3)	11 (4.2)	15 (5.8)	30 (11.5)	45 (5.8)	.002
12p (*ETV6*) abnormality	4 (1.6)	5 (2.0)	7 (2.8)	6 (2.4)	16 (2.1)	.819
CBFA2T3-GLIS2	10 (4.0)	6 (2.4)	3 (1.2)	2 (0.8)	19 (2.5)	.095
WBC $\times 10^3$ μL median (range)	16.5 (0.6-918.5)	17.8 (0.6-523.7)	31.1 (0.6-712.7)	34.9 (0.8-549.9)	20.2 (0.6-918.5)	.016
BM blast, % (range)	60 (0-98)	70 (3-98)	67 (10-100)	78 (0-100)	66 (0-100)	< .001

Abbreviations: BM, bone marrow; Q, quartile.

TABLE A3. Cox Multivariable Regression Analysis of CD123 Expression (Q1-3 *v* Q4) and Other Prognostic Factors

	OS				EFS				RR from CR1			
Variable	**No.**	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***
CD123 expression												
Q1-3	707	1			707	1			557	1		
Q4	235	1.43	1.12 to 1.83	.005	235	1.51	1.23 to 1.85	< .001	169	1.42	1.09 to 1.85	.009

NOTE. Adjusted on the basis of the AAML1831 cytogenetic/molecular risk group, age group, MRD status, and *FLT3*-ITD high allelic ratio.
Abbreviations: EFS, event-free survival; HR, hazard ratio; MRD, measurable residual disease; OS, overall survival; Q, quartile; RR, relapse risk.