Identification of Novel DNA Methylation Prognostic Biomarkers for AML With Normal Cytogenetics

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ABSTRACT

PURPOSE AML is a hematologic cancer that is clinically heterogeneous, with a wide range of clinical outcomes. DNA methylation changes are a hallmark of AML but are not routinely used as a criterion for risk stratification. The aim of this study was to explore DNA methylation markers that could risk stratify patients with cytogenetically normal AML (CN-AML), currently classified as intermediate-risk.

MATERIALS AND METHODS DNA methylation profiles in whole blood samples from 77 patients with CN-AML in The Cancer Genome Atlas (LAML cohort) were analyzed. Individual 5'-cytosine-phosphate-guanine-3' (CpG) sites were assessed for their ability to predict overall survival. The output was validated using DNA methylation profiles from bone marrow samples of 79 patients with CN-AML in a separate data set from the Gene Expression Omnibus.

RESULTS In the training set, using DNA methylation data derived from the 450K array, we identified 2,549 CpG sites that could potentially distinguish patients with CN-AML with an adverse prognosis (*intermediate-poor*) from those with a more favorable prognosis (*intermediate-favorable*) independent of age. Of these, 25 CpGs showed consistent prognostic potential across both the 450K and 27K array platforms. In a separate validation data set, nine of these 25 CpGs exhibited statistically significant differences in 2-year survival. These nine validated CpGs formed the basis for a combined prognostic biomarker panel, which includes an 8-CpG Somatic Panel and the methylation status of cg23947872. This panel displayed strong predictive ability for 2-year survival, 2-year progression-free survival, and complete remission in the validation cohort.

CONCLUSION This study highlights DNA methylation profiling as a promising approach to enhance risk stratification in patients with CN-AML, potentially offering a pathway to more personalized treatment strategies.

ACCOMPANYING CONTENT

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INTRODUCTION

AML is a hematologic cancer characterized by the accumulation of immature myeloid cells in the bone marrow and defective hematopoiesis.[1,2]

AML originates from an accumulation of genetic and epigenetic alterations in hematopoietic myeloid precursor cells, causing differentiation arrest and increased cell proliferation.[1,3,4] Genetic alterations, ranging from large chromosomal aberrations to recurrent mutations, have been reported to drive leukemogenesis in AML.[1,5-8] These alterations are used to classify patients with AML into favorable-, intermediate-, and adverse prognostic risk groups, according to the European Leukemia Net (ELN) genetic risk classification.[4,9-13]

In AML, epigenetic changes like histone modifications[14,15] and changes in micro RNA expression[16-18] are prevalent, with aberrant DNA methylation being the most characteristic.[3,19] DNA methylation, the addition of a methyl residue to a CG dinucleotide to form a 5-methylcytosine, regulates gene expression by altering the chromatin structure, DNA conformation, and DNA stability.[19-21] Patients with AML show distinct global patterns of DNA methylation, depending on their cytogenetic and genetic alterations.[22,23] Furthermore, studies describing the prognostic utility of methylation signatures in AML support the notion that DNA methylation is a hallmark of this disease.[3,24-29] Although the mechanisms underpinning aberrant DNA methylation remain elusive, potential factors such as mutations in epigenetic modifying enzymes,[15] age-associated changes in DNA

CONTEXT

Key Objective

AML is a hematologic cancer with heterogeneous clinical outcomes requiring improved risk stratification tools.

Knowledge Generated

This study explores DNA methylation markers for stratifying patients with cytogenetically normal AML (CN-AML), typically categorized as intermediate-risk. We identified nine 5′-cytosine-phosphate-guanine-3′ sites as potential prognostic biomarkers that distinguished patients with CN-AML with an adverse prognosis (intermediate-poor) from those with more favorable (intermediate-favorable) prognosis independent of age.

Relevance *(F.P.-Y. Lin)*

This study has identified potential new prognostic signatures of DNA methylation that may prompt the development of pragmatic assays and prospective investigations, thereby aiding further risk stratification in this intermediate-risk AML patient group.*

*Relevance section written by *JCO Clinical Cancer Informatics* Associate Editor Frank P.-Y. Lin, PhD, MBChB, FRACP, FAIDH.

methylation,[30] and global changes in chromatin configuration[31] may contribute to the abnormal methylation patterns found in AML.

Despite recent advances in diagnosis and treatment of AML, cure rates for both children and adults remain unsatisfactory.[2,32] Current treatment options for AML include standard chemotherapy, hematopoietic stem-cell transplant, and palliative care.[4] The ELN genetic risk classification is widely accepted by researchers and physicians and is used to guide treatment decisions. Within the ELN stratification, the intermediate-risk group is especially heterogeneous, representing patients with diverse molecular alterations and clinical outcomes. The largest subgroup of intermediate-risk patients is those with no cytogenetic alterations/cytogenetically normal AML (CN-AML), representing about 50% of all patients with AML.[1,5] Moreover, most of the patients reclassified by the recently improved 2022 ELN risk stratification were allocated to the intermediate- and adverse-risk groups, but its complexity precludes its full use in clinical practice.[33] Therefore, risk classification of patients with AML, importantly regarding CN-AML, still needs refinement[4,9,10] because of the urgent need for novel prognostic biomarkers to improve risk stratification and facilitate therapeutic decision making for these patients.

Although a number of studies have explored DNA methylation profiles in patients with AML,[29,34-52] epigenetic biomarkers are still not considered in AML risk stratification and clinical decision making. In this context, the present study aimed to explore whole-genome DNA methylation patterns in CN-AML to identify novel DNA methylation biomarkers that could refine prognostic risk stratification for these patients.

MATERIALS AND METHODS

Data Sets

Whole-genome DNA methylation data from peripheral blood samples of 77 patients with CN-AML (The Cancer Genome Atlas [TCGA] AML [LAML] cohort) were imported from TCGA Consortium database, through the Xena platform at the University of California, Santa Cruz,[53] on January 19, 2019.[54] Methylation data were derived from *Illumina Infinium HumanMethylation450* and *Illumina Infinium HumanMethylation27* platforms, comprising 485,577 and 27,578 5′-cytosine-phosphate-guanine-3′ (CpG) sites, respectively. Methylation values are represented as beta values (β) ranging from 0 (unmethylated) to 1 (fully methylated). Mutation data were derived from the Illumina Genome Analyzer system and were imported from the Xena platform as a binary matrix of gene-level mutation calls. Patients with French-American-British-M3/acute promyelocytic leukemia (APL) were removed to avoid bias in the survival analysis since new therapeutics for APL have improved survival in these patients.[55]

Validation data were obtained from the GSE32251 data set (version: January 2, 2015),[40] available on Gene Expression Omnibus, comprising primary bone marrow samples from 79 patients with de novo CN-AML. The methylation data in GSE32251 were derived from the *Illumina Infinium HumanMethylation27 platform*. The *HumanMethylation450 and HumanMethylation27* platforms use the same nomenclature, allowing for direct comparison of individual CpG sites across platforms.

Biomarker Identification

We used a three-step algorithm for prognostic CpG site identification, starting with outlier and missing data

removal using the boxplot and listwise or case deletion methods, respectively. Next, we applied the maximally selected rank statistics method, using the maxstat R package, to identify a β-value cut point that divided the data set into two subgroups with maximal survival difference for each CpG site.[56] Kaplan-Meier survival curves were derived on the basis of each β-value cut point and compared by the log-rank test or by the two-stage test when intersection of the survival curves was detected.[57] Only CpG sites showing statistically significant differences in subgroup survival ($P < .05$) were selected for further analysis. In the third step, the survival curve selection algorithm was repeated using age-stratified groups, consisting of patients younger and older than 60 years. To reduce the impact of age-related DNA methylation changes, only CpG sites demonstrating prognostic significance in both younger and older patients were retained. CpG sites with statistically significant age differences between the two survival groups (Wilcoxon-Mann-Whitney; $P < .05$) were also excluded.

Calculating Methylation Differences Between Survival Groups

For each putative biomarker, methylation values for the survival subgroups were compared. Data normality was determined using the Shapiro-Wilk method. For normally distributed data, methylation differences were evaluated using the parametric t-test. When variances were unequal, as assessed by the Levene test, a Welch-Satterthwaite adjustment was used. For non-normally distributed data, the Wilcoxon-Mann-Whitney test was used. Only CpG sites with a $|\triangle\beta| \geq .2$ between survival subgroups were selected for further analysis.

Potential Prognostic Biomarker Validation

The potential biomarkers identified in the 450K array platform were tested, using the same cut points, in the 27K array data set of the 77 patients in the TCGA-LAML cohort. We focused exclusively on the CpGs with prognostic value in both arrays. Biomarkers identified in the training data set were validated in the GSE32251 data set using the same methylation cut points. Differences in 2-year survival were assessed for each site using a Fisher's exact test.

CpG Signature for Prognosis Prediction

To evaluate the potential of a multiprobe panel to predict prognosis in CN-AML, the prognostic biomarkers were divided into two subpanels: the 8-CpG Somatic Panel and the methylation status of cg23947872. A Cox proportional-hazards model was performed for each patient in the validation cohort, using the number of hypermethylated probes in each panel as the predictor variable and 2-year survival and 2-year progression-free survival (PFS) as outcomes. The model was used to predict the survival probability of each patient in the validation cohort at 2-years, according to the formula:

$$P(2-\text{year survival}) = \exp(-\text{expected}),$$

where *expected* corresponds to the expected number of deaths. Differences in survival probability between the predicted and real event data were compared using the Wilcoxon-Mann-Whitney test. The assumption of proportional hazards for the two covariates was tested by analyzing the Schoenfeld residuals with time.

Statistical Analysis

Statistical analyses were performed using R (versions 3.5.2-4.1.2), with a significance threshold at $P < .05$. A false discovery rate <5% was considered statistically significant for both the *t*-test and the Mann-Whitney test.

RESULTS

Identification of Prognostic CpG Sites

DNA methylation changes are common in patients with CN-AML[34] and might have prognostic value. We assessed genome-wide DNA methylation patterns in blood samples from 77 patients with CN-AML using data from the TCGA LAML cohort.[3] For each CpG site, we identified optimal methylation cut points, using maximally selected rank statistics, that resulted in a significant survival difference between patients on each side of the cut point, thereby creating two subgroups of patients, one with a better prognosis (*intermediate-favorable*) and the other with worse prognosis (*intermediate-poor*; Data Supplement, Table S1). Since advanced age is an independent risk factor for poor survival in CN-AML,[9] only CpG sites that showed prognostic value in both younger and older patients were considered informative. Using this strategy, we identified 2,549 differentially methylated CpG sites that were associated with prognosis in patients with CN-AML and for which a beta-methylation difference of at least .2 ($|\Delta\beta| \geq .2$) existed between the *intermediate-favorable* and *intermediate-poor* subgroups (Data Supplement, Table S1).

To enhance the robustness and generalizability of our study, we focused exclusively on CpG sites that showed prognostic potential in both the 450K and 27K array platforms within the same TCGA LAML cohort of patients with CN-AML. Of the 2,549 prognostic CpG sites identified, 158 were available for analysis in the 27K array platform, 25 of which demonstrated prognostic potential in both platforms (Data Supplement, Table S2). To validate the identified CpG sites, we applied the same β-value cutoffs to methylation data from a separate data set (GSE32251), which comprised 79 primary bone marrow samples from patients with de novo CN-AML. Of the 25 prognostic CpG sites identified in the LAML cohort, nine were able to segregate the patients with CN-AML in the validation data set into subgroups with statistically significant differences in 2-year survival (Fisher's exact test; $P < .05$; Table 1). We refer to these nine validated CpG sites as prognostic CpG sites. Kaplan-Meier

TABLE 1. Validated Prognostic CpG Sites in Patients With CN-AML

CpG Site	Chromosome	Gene	CpG Location[a]	*P*[b]	Δβ[c]	HR[d]
cg09096950	chr11	*UBE4A*	TSS1500	<.001	.34	0.34
cg23947872	chrX	*MTMR1*	Body	.003	−.57	2.37
cg13015534	chr17	*ST6GALNAC1*	First exon (50%); 5′UTR (50%)	.003	.37	0.37
cg03112433	chr7	*CDK14*	TSS200	.005	.47	0.46
cg02981703	chr1	*CA6*	TSS200	.006	.30	0.40
cg12941369	chr3	*PDCD6IP*	TSS1500	.006	.36	0.47
cg14611112	chr9	*LCN6*	TSS1500	.010	.32	0.48
cg10635061	chr2	*FHL2*	First exon (50%); 5′UTR (50%)	.013	.32	0.44
cg17890764	chr3	*ITIH4*	TSS200	.013	.37	0.49

Abbreviations: 5′UTR, within the 5′ untranslated region, between the TSS and the ATG start site; ATG, start codon; Body, between the ATG and stop codon, irrespective of the presence of introns, exons, TSS, or promoters; CN-AML, cytogenetically normal AML; CpG, 5′-cytosine-phosphate-guanine-3′; HR, hazard ratio; TSS, transcriptional start site; TSS1500, 200-1,500 bases upstream of the TSS; TSS200, 0-200 bases upstream of the TSS.
[a]More than one feature may be described because of splice variants.
[b]The *P* value corresponds to the difference in survival between the intermediate-favorable and intermediate-poor subgroups as assessed by the log-rank or two-stage test (if survival curves intersected).
[c]Calculated as the difference between the average β-value in the intermediate-favorable and intermediate-poor subgroups.
[d]HR with hypomethylation as reference. HR >1 indicates that hypermethylation increases hazard.

survival curves for the prognostic CpG sites display distinct survival outcomes between the intermediate-favorable and intermediate-poor subgroups (Fig 1; Data Supplement, Fig S1). All prognostic CpG sites were located in a protein-coding gene. Eight of the prognostic CpG sites were hypomethylated in the intermediate-poor subgroup, whereas one was hypermethylated (Fig 2).

We examined the distribution of males and females within the subgroups generated by each of the nine prognostic CpGs. We observed no statistically significant differences in the eight CpGs located in somatic chromosomes (Fisher's exact test; $P > .05$). However, cg23947872, located on chromosome X, stratified the patients into groups with different proportions of males and females (Fisher's exact test; $P < .001$; Data Supplement, Table S3).

We analyzed the blast count percentages in both blood and bone marrow and found no statistically significant differences between the subgroups generated by each of the nine prognostic CpGs (Wilcoxon-Mann-Whitney; $P > .05$).

Most prognostic CpGs were in 5' regulatory sequences of their associated protein-coding genes. Only one site was located in the gene body (Table 1).

Survival Prediction Using the Prognostic CpG Sites

To assess whether the nine prognostic CpGs could be used collectively to predict survival, we performed a multivariate Cox proportional hazard analysis using the number of hypermethylated probes (relative to their prespecified cut points) in each one of two predictor variables: (1) 8-CpG Somatic Panel (cg12941369, cg03112433, cg09096950, cg17890764, cg10635061, cg02981703, cg14611112, cg13015534) and (2) the methylation status of cg23947872.

The multivariate Cox proportional hazard model was globally statistically significant when examined by three tests: likelihood ratio test ($P < .001$), Wald test ($P < .001$), and score log-rank statistics ($P < .001$). The individual covariates were also statistically significant within the multivariate model (Table 2). The assumption of proportional hazards was tested and was nonsignificant for each of the two covariates and globally.

To assess the role of sex as a confounding factor, we compared survival rates between males and females in the discovery cohort and found no significant differences (log-rank; $P = .11$). To further control for sex as a confounder, we added sex as a covariate in a second Cox proportional hazard model. Sex as a covariate was not a significant predictor of survival ($P = .24$) and slightly reduced global statistical significance of the model. The 8-CpG Somatic Panel maintained statistical significance ($P = .008$), and cg23947872 shifted to borderline significance ($P = .051$).

The probability of survival at 2 years for each patient in the validation cohort was then calculated according to the model (Data Supplement, Table S4), and differences in expected survival were compared with real event data (Fig 3).

Predicted survival probabilities were significantly higher among patients who survived at least 2 years (Wilcoxon-Mann-Whitney; $P < .001$), in patients who achieved complete remission (Wilcoxon-Mann-Whitney; $P = .032$), and in patients who reached 2-year PFS (Wilcoxon-Mann-Whitney; $P < .001$).

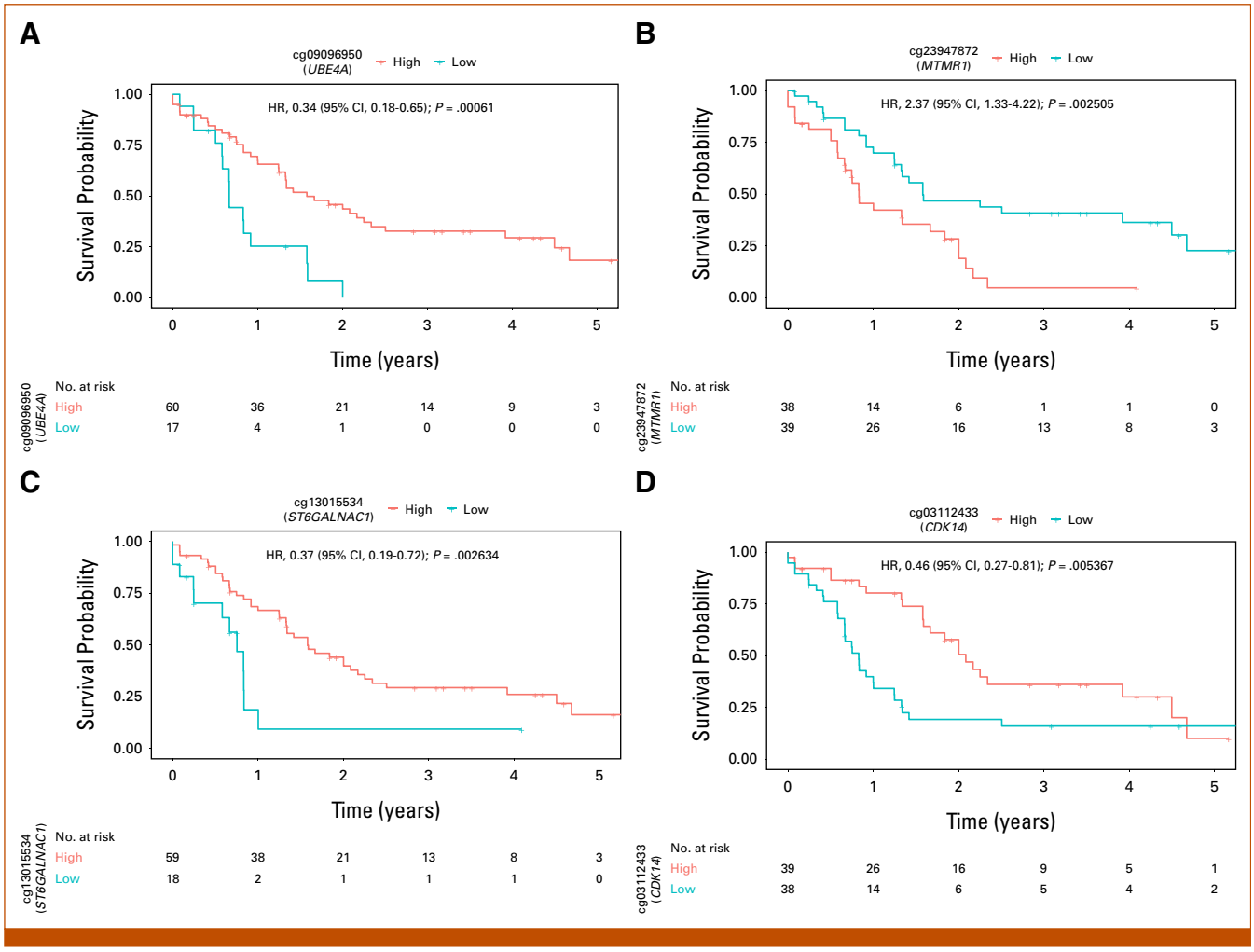

FIG 1. Kaplan-Meier survival curves for four of the validated prognostic CpG sites, comparing high with low methylation. Data are shown for the training cohort: (A) cg09096650, (B) cg23947872, (C) cg13015534, and (D) cg03112433. Related RefSeq genes are described in parentheses. CpG, 5′-cytosine-phosphate-guanine-3′; HR, hazard ratio; RefSeq, Reference Sequence.

Receiver operating characteristics curves were generated using the model's expected survival probabilities and real event data from the validation cohort (Fig 4). The model accurately predicted complete remission (AUC = 0.7), 2-year PFS (AUC = 0.81), and 2-year survival (AUC = 0.81), further validating the prognostic value of the model.

Both the 8-CpG Somatic Panel and the methylation status of cg23947872 were able to independently predict 2-year survival and 2-year PFS (Wilcoxon-Mann-Whitney; $P < .05$), but not complete remission.

Impact of Key Mutations on Survival Prediction

It has been previously reported that somatic mutations are important drivers of leukemogenesis. Several key mutations, such as *FLT3*, *NPM1*, *ASXL1*, *RUNX1*, *CEBPA*, *TP53*, *IDH1*, *IDH2*, and *DNMT3A*, have been shown to have clinical relevance and affect the prognosis of patients with AML. We examined the presence of these mutations on the intermediate-favorable and intermediate-poor subgroups generated by each prognostic CpG, in the TCGA LAML cohort. Fisher's exact test was performed to ascertain if the frequency of each mutation differed significantly between the two subgroups. We found that seven prognostic CpGs divided the patients into subgroups with different proportions of at least one of the following mutations: *DNMT3A*, *FLT3*, *NPM1*, *CEBPA*, and *IDH2* (Fisher's exact test; $P < .05$; Data Supplement, Table S5).

Considering these results, we tested the impact of these four mutations on the survival of the 77 studied patients with CN-AML. The presence of mutations on *FLT3*, *IDH2*, or *DNMT3A* was associated with worse prognosis (two-stage; $P < .05$), whereas mutations on *CEBPA* did not significantly affect survival. As such, to assess the potential confounding effects of the mutation status of *FLT3*, *DNMT3A*, and *IDH2*,

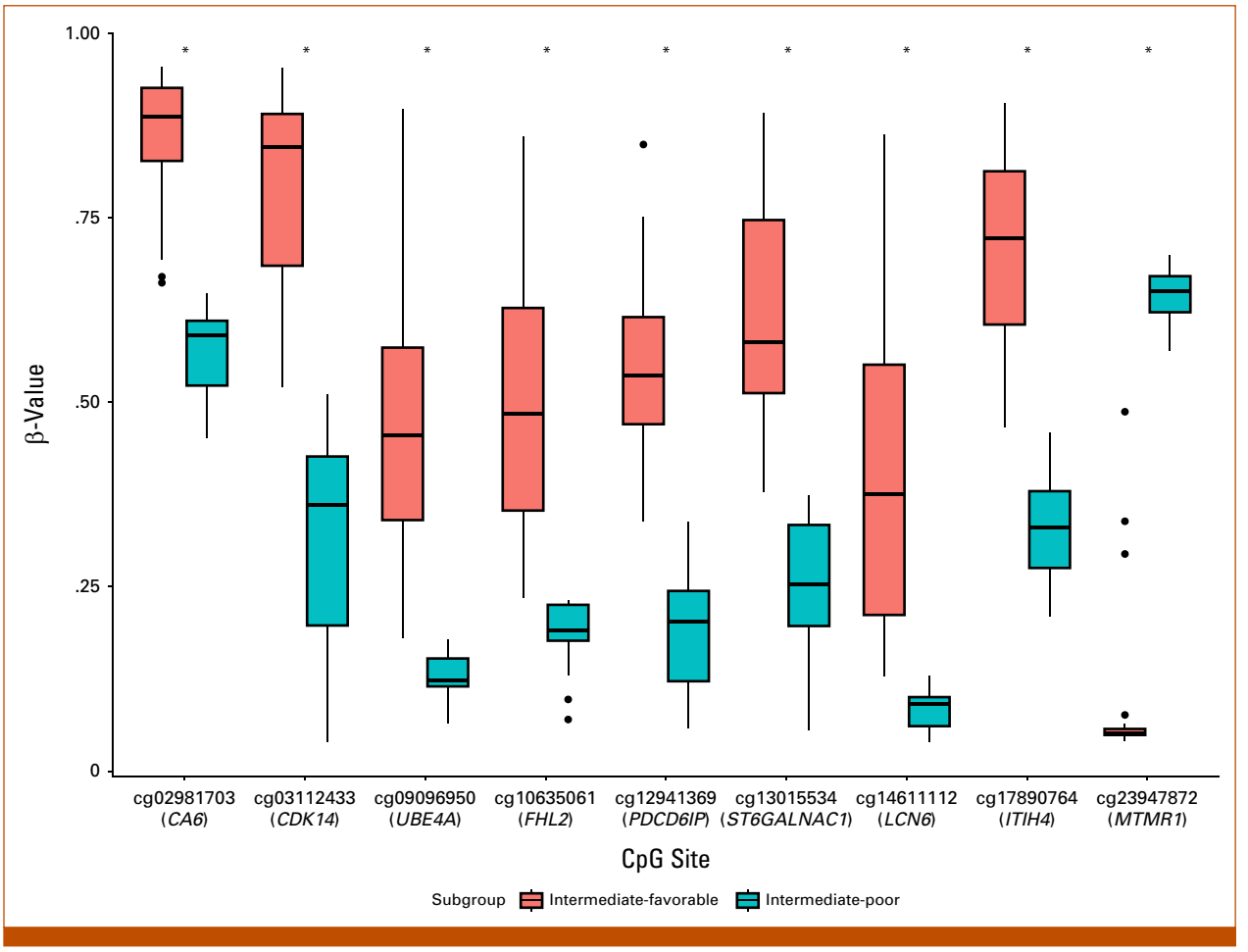

FIG 2. CpG methylation at prognostic CpG biomarkers. CpG methylation is represented by the β-value, which ranges from 0 to 1. The first eight CpG sites are hypomethylated in the *intermediate-poor* subgroup, and the last CpG site (cg23947872) is hypermethylated in the *intermediate-poor* subgroup. CpG, 5′-cytosine-phosphate-guanine-3′. *P < 0.0001.

we explored an adjusted model that included these as covariates. While this model adjustment slightly altered the significance levels of our CpG biomarkers, it did not considerably change the overall predictive power of the model. Notably, the methylation status of cg23947872 maintained its statistical significance (P = .017), reinforcing its potential as a robust biomarker. The 8-CpG Somatic Panel, however, showed reduced statistical significance (P = .07) in this adjusted model, suggesting a potential interaction with the mutations. Markedly, while this expanded model remained statistically significant globally (likelihood ratio test; P = .004, Wald test; P = .006, score log-rank statistics; P = .004), the increase in *P-values* indicates a marginally reduced overall model fit.

DISCUSSION

Given the heterogeneity of AML, especially in the intermediate-risk CN-AML group, current ELN classification falls short in outcome prediction.[58,59] In this study, blood samples from 77 patients with CN-AML were analyzed to identify potential prognostic methylation biomarkers. A total of 2,549 CpG sites were associated with prognosis among intermediate-risk patients in the training set, of which 158 were available for analysis in both 450K and 27K array platforms. Notably, 25 of these demonstrated consistent prognostic potential across both platforms. Using a separate cohort, nine of these 25 CpG sites were validated as

TABLE 2. Multivariate Cox Proportional Hazard Model Results

Variable	Beta Coefficient	HR (95% CI for HR)	*z* Value	Wald *P*
No. of hypermethylated probes in the 8-CpG somatic panel	−.22741	0.7966 (0.7065 to 0.8981)	−3.715	<.001
Methylation status of cg23947872	.66091	1.9366 (1.0758 to 3.4860)	2.204	.028

Abbreviations: CpG, 5′-cytosine-phosphate-guanine-3′; HR, hazard ratio.

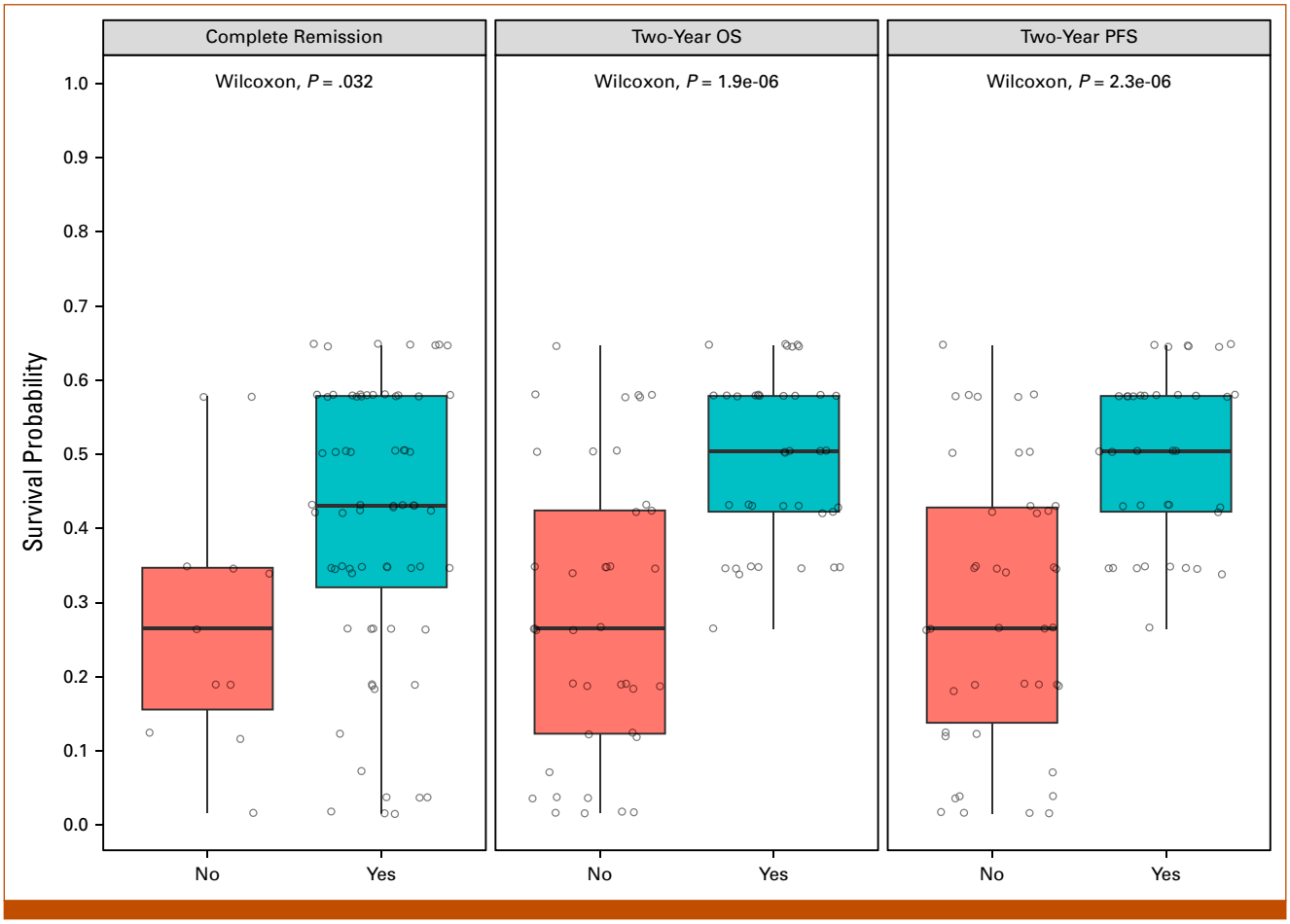

FIG 3. Predicted survival relative to actual survival. Each black circle represents a patient. In each panel, patients are subdivided according to whether they achieved a negative outcome (red) or positive outcome (blue). Predicted survival for each patient is shown on the *y*-axis. Differences in median predicted survival probabilities were tested for (1) complete remission, (2) 2-year OS, and (3) 2-year PFS using the Wilcoxon-Mann-Whitney test. OS, overall survival; PFS, progression-free survival.

prognostic biomarkers for CN-AML. The cross-platform and cross-cohort consistency of these CpG sites further demonstrates their reliability and potentially broader applicability in clinical settings. Nonetheless, it is important to note that different normalization methods across platforms could potentially affect the validation of the CpGs, thereby constituting a limitation of this analysis.

Hypomethylation was associated with poor prognosis in the eight prognostic CpGs located outside the X chromosome. Interestingly, the CpG located on the X chromosome exhibited the opposite behavior, where hypomethylation was linked to a favorable prognosis. The association between hypomethylation and poor outcomes also suggests that further study is required with respect to the impact of hypomethylating agents like decitabine and azacitidine on patients with AML since these are important treatment options for patients with AML who are not eligible for intensive chemotherapy.[4]

The nine individually validated prognostic CpGs were then combined into a unified prognostic biomarker panel. This integrative approach aimed to benefit from the collective predictive power of the nine CpGs, thus creating a more comprehensive tool for the assessment of CN-AML patient prognosis. The prognostic panel comprises two components: the 8-CpG Somatic Panel and the methylation status of cg23947872. Although both were able to individually predict 2-year survival and 2-year PFS in the validation cohort, together they form a substantially more robust prognostic biomarker capable of predicting not only more accurately 2-year survival (AUC = 0.81) and 2-year survival without recurrence (AUC = 0.81) but also complete remission (AUC = 0.7) in patients with CN-AML.

Several key mutations have been associated with both leukemogenesis and prognosis in patients with AML, of which some have already been incorporated into the ELN

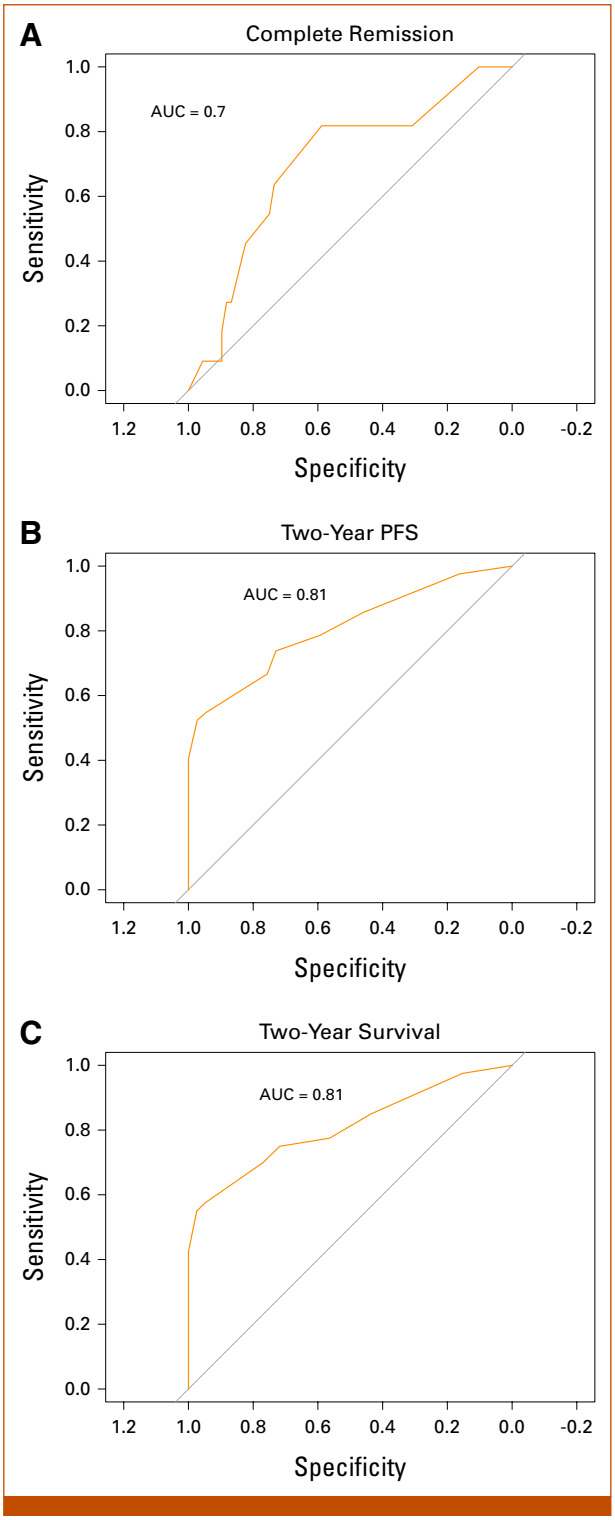

FIG 4. ROC curves of the model's predicted survival probabilities versus the observed outcomes in the validation cohort. Sensitivity is displayed on the *y*-axis, and specificity on the *x*-axis. The gray line represents the random classifier line. ROC curves are displayed for (A) complete remission, (B) 2-year survival, and (C) 2-year PFS. PFS, progression-free survival; ROC, receiver operating characteristic.

risk stratification.[13] These include *FLT3*, *NPM1*, *CEBPA*, *RUNX1*, *ASXL1*, and *TP53*. Other genes, such as *DNMT3A* and *IDH1/2*, that encode for enzymes involved in epigenetic processes are often mutated in patients with CN-AML.[60-62] As these mutations have been reported to have a potential impact on survival rates of patients with AML, they represent potential confounding factors in our prognostic model. Recognizing this, we examined how these key mutations were distributed between the intermediate-favorable and intermediate-poor subgroups, generated by each prognostic CpG. We observed that seven prognostic CpGs formed patients' subgroups with different proportions of at least one of *DNMT3A*, *FLT3*, *NPM1*, *CEBPA*, or *IDH2* mutations. *CEBPA* mutations were later found to have no influence on survival rates in the discovery cohort. To explore the potential confounding effects of *FLT3*, *DNMT3A*, and *IDH2* mutations, we generated a separate adjusted model including these as covariates. This adjustment did not severely affect the overall predictive power of the model, which remained statistically significant globally. Nonetheless, the 8-CpG Somatic Panel showed reduced statistical significance (P = .07), which potentially suggests some association with the mutations. Conversely, cg23947872 maintained its statistical significance (P = .017). Adding these mutations as covariates not only did not improve the predictive power of the model but slightly reduced the overall model fit. These findings not only underscore the potential of DNA methylation as a standalone prognostic tool in CN-AML but also acknowledge the complex interplay between genetic and epigenetic factors in this disease.

This 9-CpG methylation panel has the potential to be a valuable tool in predicting survival in patients with CN-AML and assisting clinicians in choosing appropriate therapies on the basis of the predicted outcomes.

Future studies should evaluate the value of this panel to identify which patients in the intermediate-risk group are more likely to benefit from an allogeneic hematopoietic cell transplantation, which is currently the only curative treatment approach for AML but has to be considered after a careful risk-benefit evaluation.[13]

In summary, DNA methylation has significant potential as a prognostic biomarker for patients with CN-AML, previously classified as intermediate-risk. Given the paucity of clinical specimens, only a subset of potential DNA biomarkers could be validated in this study; however, the panel of nine validated prognostic CpGs showed a high level of predictive ability with respect to clinical outcomes. Additional cohorts will be required to validate further potential markers and to identify the most strongly predictive subset.

AFFILIATIONS

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EQUAL CONTRIBUTION

C.C. and D.P. are co-first authors. M.T.F. and P.C.B. are co-senior authors.

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DATA SHARING STATEMENT

The data sets analyzed during the current study are available in TCGA and GEO repositories, available through https://xenabrowser.net/datapages/?cohort=TCGA%20Acute%20Myeloid%20Leukemia%20(LAML)&removeHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu%3A443, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32251.

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Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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