Title

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Authors

Farrar, Jason
Smith, Jenny
Othus, Megan
et al.

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Long Noncoding RNA Expression Independently Predicts Outcome in Pediatric Acute Myeloid Leukemia

Jason E. Farrar, MD[1]; Jenny L. Smith, MS[2]; Megan Othus, PhD[3]; Benjamin J. Huang, MD[4]; Yi-Cheng Wang, MS[5]; Rhonda Ries, MA[2]; Tiffany Hylkema, BS[2]; Era L. Pogosova-Agadjanyan, BS, BA[2]; Sneha Challa, MS[2]; Amanda Leonti, MS[2]; Timothy I. Shaw, PhD[6]; Timothy J. Triche Jr, PhD[7]; Alan S. Gamis, MD, MPH[8]; Richard Aplenc, MD, PhD, MSCE[9]; E. Anders Kolb, MD[10]; Xiaotu Ma, PhD[11]; Derek L. Stirewalt, MD[2]; Todd A. Alonzo, PhD[5,12]; and Soheil Meshinchi, MD, PhD[2,13]

PURPOSE Optimized strategies for risk classification are essential to tailor therapy for patients with biologically distinctive disease. Risk classification in pediatric acute myeloid leukemia (pAML) relies on detection of translocations and gene mutations. Long noncoding RNA (lncRNA) transcripts have been shown to associate with and mediate malignant phenotypes in acute myeloid leukemia (AML) but have not been comprehensively evaluated in pAML.

METHODS To identify lncRNA transcripts associated with outcomes, we evaluated the annotated lncRNA landscape by transcript sequencing of 1,298 pediatric and 96 adult AML specimens. Upregulated lncRNAs identified in the pAML training set were used to establish a regularized Cox regression model of event-free survival (EFS), yielding a 37 lncRNA signature (lncScore). Discretized lncScores were correlated with initial and postinduction treatment outcomes using Cox proportional hazards models in validation sets. Predictive model performance was compared with standard stratification methods by concordance analysis.

RESULTS Training set cases with positive lncScores had 5-year EFS and overall survival rates of 26.7% and 42.7%, respectively, compared with 56.9% and 76.3% with negative lncScores (hazard ratio, 2.48 and 3.16; $P < .001$). Pediatric validation cohorts and an adult AML group yielded comparable results in magnitude and significance. lncScore remained independently prognostic in multivariable models, including key factors used in preinduction and postinduction risk stratification. Subgroup analysis suggested that lncScores provide additional outcome information in heterogeneous subgroups currently classified as indeterminate risk. Concordance analysis showed that lncScore adds to overall classification accuracy with at least comparable predictive performance to current stratification methods that rely on multiple assays.

CONCLUSION Inclusion of the lncScore enhances predictive power of traditional cytogenetic and mutation-defined stratification in pAML with potential, as a single assay, to replace these complex stratification schemes with comparable predictive accuracy.

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INTRODUCTION

Iterative refinements in risk stratification have been a cornerstone for improved outcomes in children with leukemia. The criteria used in stratifying pediatric acute myeloid leukemia (pAML) have evolved substantially in recent years, driven by better recognition of recurrent molecular changes that modify biology and response to chemotherapy (Data Supplement, online only).[1-5] Inclusion of minimal residual disease (MRD) assessment of induction response[6] and recent identification of high-risk immunophenotypes[7] have added additional features for stratification and prognostication.

Prognostic classification by coding gene expression has been studied in acute myeloid leukemia (AML) for nearly 20 years.[8-12] Although such studies have been important for better understanding of AML biology, stratification by gene expression patterns has been slow to penetrate clinical practice and is not widely used in pAML. Among several reasons for this failure, including challenges in reproducibility and ease of assay performance, a key problem has been that these classifiers have not yielded additional information compared with traditional testing.[13]

More recent efforts have focused on broadening the definition of gene expression to include nonprotein coding transcripts, including both micro-RNA and long noncoding RNA (lncRNA) expression.[14] lncRNAs are defined as transcripts longer than 200 base pairs that lack protein coding potential.[15] They are widely expressed in eukaryotes and increasingly recognized as critical mediators of diverse processes in normal development and differentiation including transcriptional regulation, chromatin architectural reorganization, modulation of translation, and post-translational modifications.[16]

ASSOCIATED CONTENT

See accompanying article on page 3059

Data Supplement

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

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CONTEXT

Key Objective

Does long noncoding RNA (lncRNA) expression in pediatric acute myeloid leukemia offer improved performance compared with conventional clinical risk classification criteria?

Knowledge Generated

We define and validate the lncScore, a 37-gene–based lncRNA expression classifier that yields comparable predictive performance to traditional cytogenetic and molecular testing, while also uncovering new predictive information not available in current techniques.

Relevance *(S. Bhatia)*

The lncScore as a single assay carries the potential to replace the current complex stratification schemes without losing predictive accuracy.*

*Relevance section written by *JCO* Associate Editor Smita Bhatia, MD, MPH, FASCO.

Altered lncRNA expression is implicated in a variety of disease processes, including neoplasia.[17] lncRNA are characteristically differentially expressed during normal and malignant hematopoiesis.[18-21] Recent studies have demonstrated novel prognostic potential using lncRNA expression in adult AML[22-27]; however, their relevance in pediatric disease is not well established.

We investigated the expression of lncRNA in childhood AML, testing its utility for risk characterization. We define a lncRNA-based expression classifier that, as a single assay, has comparable predictive performance to complex modern, multiassay-based stratification procedures, while also adding novel prognostic information uncaptured by current techniques. This work demonstrates that lncRNA-based risk stratification could augment or replace current stratification schemes to yield less complex and more precise risk stratification of childhood AML.

METHODS

AML Cohorts

We assayed 68 normal bone marrow and 1,299 AML cases from Children's Oncology Group (COG) studies (CCG-2961,[28] AAML03P1,[29] AAML0531,[30] and AAML1031[31]) by RNA-seq. Most samples (1,060, 82%) come from AAML1031, representing all cases from that trial where high-quality RNA was available; the remainder were selected from prior studies and were enriched for high-risk features. An additional unselected set comprising 96 adult AML specimens treated on SWOG Cancer Research Network trials S9031,[32] S9333,[33] S0112,[34] and S0106[35] (ClinicalTrials.gov identifier: NCT01503541) was examined for further validation, representing all cases with rRNA depleted RNA-seq data available for study. Written informed consent for biological correlative studies was obtained from participants during enrollment in the parent clinical trials, which were conducted in accordance with the Declaration of Helsinki. The Fred Hutchinson Cancer Research Center Institutional Review Board and the COG Myeloid Biology Committee approved and oversaw the conduct of this study.

Methods for RNA sequencing, transcript quantification, revised risk classification, training/validation set randomization, and lncScore model generation are outlined in the Data Supplement.

Outcome Analyses

Analyses of the association between lncScore and survival outcomes were performed using Kaplan-Meier estimates and the log-rank test. Hazard ratios (HRs) and associated CI were estimated in single and multivariable models by Cox proportional hazards regression. Predictive performance was assessed by concordance index,[36] with submodel comparisons performed using the method of Uno.[37] *P*-values < 0.05 were considered significant. Event-free survival (EFS) was defined as the time from enrollment to first event (relapse, induction failure, or death) or last follow-up. Overall survival (OS) was defined as the time from study enrollment to death or last follow-up. Relapse rate (RR) was defined as the time from end of induction to relapse or last follow-up. Post-induction disease-free survival (DFS) and OS were defined starting from the end of induction cycle 1 through these end points, respectively.

RESULTS

lncRNA Is Differentially Expressed Between pAML and Normal Bone Marrow

We assessed the relationship between lncRNA expression and outcomes in pAML specimens sequenced as a part of the Target Pediatric AML Initiative.[5,14,38,39] The cohort initially consisted of 1,299 pediatric patients with de novo AML treated on four COG phase III trials. Patient characteristics are summarized in Table 1. Revision of risk classification from prior study definitions to current standards defined a large proportion of high-risk patients (Data Supplement). Overall outcomes for this cohort, according to

TABLE 1. Clinical Features of lncRNA Groups

	Full Cohort	Train	Validation 1	Validation 2	
Covariate	**n = 1,298, No. (%)**	**n = 780, No. (%)**	**n = 260, No. (%)**	**n = 258, No. (%)**	***P* (χ^2)**
Sex					
Male	667 (51.4)	400 (51.3)	142 (54.6)	125 (48.4)	.37
Female	631 (48.6)	380 (48.7)	118 (45.4)	133 (51.6)	
Age, years					
<3	325 (25)	193 (24.7)	68 (26.1)	64 (24.8)	.93
3-14	652 (50.3)	394 (50.5)	132 (50.8)	126 (48.8)	
≥15	321 (24.7)	193 (24.7)	60 (23.1)	68 (26.4)	
Study					
AAML03P1[a]	28 (2.2)	17 (2.2)	3 (1.2)	8 (3.1)	.76
AAML0531[a]	187 (14.4)	111 (14.2)	42 (16.2)	34 (13.2)	
AAML1031[b]	1,060 (81.7)	637 (81.7)	211 (81.2)	212 (82.2)	
CCG-2961[c]	23 (1.8)	15 (1.9)	4 (1.5)	4 (1.6)	
Study-defined initial risk (CFM)					
High	202 (15.6)	126 (16.1)	39 (15)	37 (14.3)	.98
Intermediate	693 (53.4)	410 (52.6)	140 (53.8)	143 (55.4)	
Low	389 (29.9)	235 (30.1)	79 (30.4)	75 (29.1)	
Unknown	14 (1.1)	9 (1.2)	2 (0.8)	3 (1.2)	
Updated initial risk definition (CFM)					
High	532 (41)	321 (41.2)	108 (41.5)	103 (39.9)	.98
Intermediate	344 (26.5)	203 (26)	70 (26.9)	71 (27.5)	
Low	422 (32.5)	256 (32.8)	82 (31.5)	84 (32.6)	
FLT3-ITD					
Yes	205 (15.8)	125 (16)	38 (14.6)	42 (16.3)	.94
<0.1	49 (3.8)	27 (3.5)	10 (3.8)	12 (4.7)	
No	1,043 (80.4)	627 (80.4)	212 (81.5)	204 (79.1)	
Unknown	1 (0.1)	1 (0.1)			
NPM1 mutation					
Yes	107 (8.2)	66 (8.5)	19 (7.3)	22 (8.5)	.86
No	1,188 (91.5)	712 (91.3)	241 (92.7)	235 (91.1)	
Unknown	3 (0.2)	2 (0.8)		1 (0.4)	
CEBPA mutation					
Yes	68 (5.3)	41 (5.3)	15 (5.8)	12 (4.7)	.85
No	1,230 (94.7)	739 (94.7)	245 (94.2)	246 (95.3)	
MRD after first induction course					
No	815 (62.8)	496 (63.6)	162 (62.3)	157 (60.9)	.83
Yes	360 (27.7)	213 (27.3)	75 (28.9)	72 (27.9)	
Unknown	123 (9.6)	71 (9.1)	23 (8.9)	29 (11.2)	
Complete remission after first induction course					
Yes	954 (73.5)	575 (73.7)	193 (74.2)	186 (72.1)	.36
No	300 (23.1)	176 (22.6)	59 (22.7)	65 (25.2)	
Death	19 (1.5)	10 (1.3)	3 (1.2)	6 (2.3)	
Unevaluable	25 (1.9)	19 (2.4)	5 (1.9)	1 (0.4)	

(continued on following page)

TABLE 1. Clinical Features of lncRNA Groups (continued)

Covariate	Full Cohort n = 1,298, No. (%)	Train n = 780, No. (%)	Validation 1 n = 260, No. (%)	Validation 2 n = 258, No. (%)	*P* (χ^2)
Complete remission after second induction course					
Yes	1,053 (81.1)	640 (82.1)	205 (78.8)	208 (80.6)	.88
No	136 (10.5)	79 (10.1)	32 (12.3)	25 (9.7)	
Death	23 (1.8)	12 (1.5)	5 (1.9)	6 (2.3)	
Unevaluable	86 (6.6)	49 (6.3)	18 (6.9)	19 (7.4)	
Stem-cell transplant in first remission					
No	957 (73.7)	573 (73.5)	191 (73.5)	193 (74.8)	.24
Yes	245 (18.9)	158 (20.8)	44 (16.9)	43 (16.7)	
Unknown	96 (7.4)	49 (6.3)	25 (9.5)	22 (8.5)	
Major group (for randomization blocks)					
KMT2A-r	306 (23.5)	187 (23.9)	61 (23.5)	58 (22.5)	1
RUNX1-RUNX1T1	149 (11.5)	90 (11.5)	30 (11.5)	29 (11.2)	
Other AML	148 (11.4)	86 (11)	31 (11.9)	31 (12)	
CBFB-MYH11	107 (8.2)	63 (8.1)	22 (8.5)	22 (8.5)	
NUP98-NSD1	100 (7.7)	60 (7.7)	20 (7.7)	20 (7.8)	
FLT3-ITD	93 (7.2)	57 (7.3)	17 (6.5)	19 (7.4)	
NPM1	72 (5.5)	44 (5.6)	13 (5)	15 (5.8)	
CEBPA	59 (4.5)	36 (4.6)	12 (4.6)	11 (4.3)	
CBFA2T3-GLIS2	38 (2.9)	23 (2.9)	7 (2.7)	8 (3.1)	
DEK-NUP214	37 (2.9)	20 (2.6)	9 (3.5)	8 (3.1)	
ETS-*ETV6*	33 (2.5)	20 (2.6)	7 (2.7)	6 (2.3)	
NUP98-KDM5A	30 (2.3)	19 (2.4)	5 (1.9)	6 (2.3)	
Rare (individually <2%)	126 (9.7)	75 (9.6)	26 (10)	25 (9.7)	
ETS-other	25 (1.9)	16 (2)	4 (1.5)	5 (1.9)	NA
Monosomy7	20 (1.5)	11 (1.4)	6 (2.3)	3 (1.2)	
NUP98-other	20 (1.5)	11 (1.4)	5 (1.9)	4 (1.6)	
KAT6A	17 (1.3)	11 (1.4)	2 (0.8)	4 (1.6)	
MLLT10	17 (1.3)	10 (1.3)	4 (1.5)	3 (1.2)	
RBM15-MKL1	10 (0.8)	5 (0.6)	2 (0.8)	3 (1.2)	
NPM1-MLF1	8 (0.6)	6 (0.8)	1 (0.4)	1 (0.4)	
RUNX1-CBFA2T3	9 (0.7)	5 (0.6)	2 (0.8)	2 (0.8)	
White blood cell count at presentation					
Median WBC (range)	25.9 (0.2-918.5)	25.9 (0.6-918.5)	24.8 (0.2-648.2)	26.8 (0.7-860)	.59 (Kruskal-Wallis)

Abbreviations: AML, acute myeloid leukemia; CFM, cytogenetic/fusion/molecular risk; HR, hazard ratio; lncRNA, long noncoding RNA; MRD, minimal residual disease.

[a]AAML0531: five cycles of chemotherapy ± gemtuzumab ozogamicin; stem cell transplant in first remission for high-risk patients; AAML03P1 was the nonrandomized feasibility pilot to AAML0531.

[b]AAML1031: four to five cycle chemo ± bortezomib; stem cell transplant for high risk in CR1; +sorafenib for *FLT3*-ITD.

[c]CCG-2961: idarubicin as induction anthracycline; random assignment to second high-intensity course with DCTE-I/R versus fludarabine; random assignment to interleukin-2 maintenance.

a modern definition of presenting risk by cytomolecular features (cytogenetic/fusion/molecular risk, CFM) and by final risk (FR), a definition that incorporates presenting risk and postinduction MRD determination,[31] are illustrated in Figures 1A-1D.

The study population was divided into training (n = 781), validation 1 (n = 260), and validation 2 (n = 258) groups using a randomization scheme blocked for key molecular features. There were no significant differences in the distribution of these features among the groups (Table 1). After

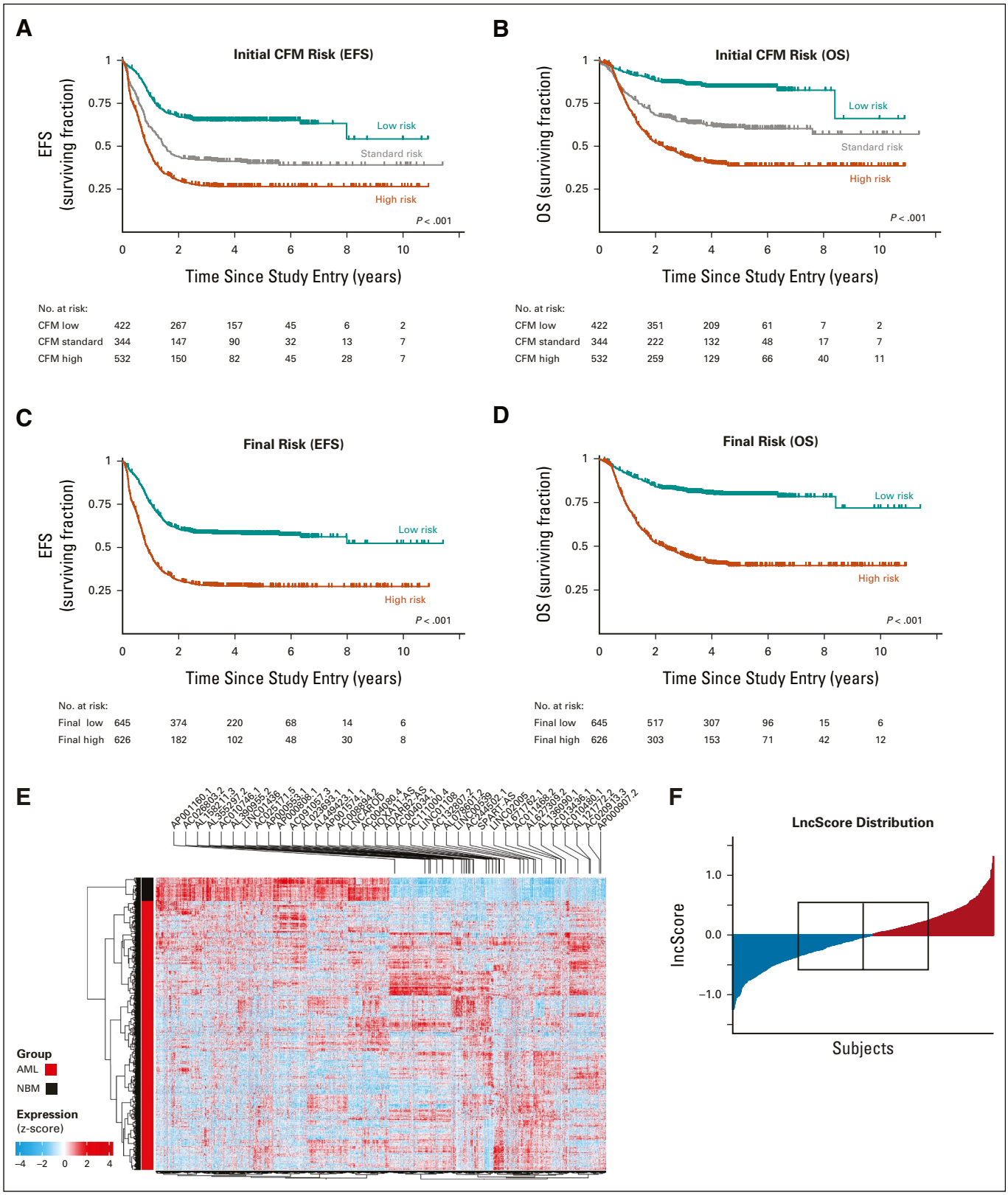

FIG 1. Identification of a lncRNA signature associated with outcome in pediatric AML. Overall treatment outcomes of the 1,298-subject study cohort after reclassification to a current schema for initial risk ([A] CFM EFS; [B] CFM OS) and postinduction risk determination ([C] EFS for FR; [D] OS by FR classification). (E) Differential analysis of annotated lncRNA expression in pAML training set data compared with normal bone marrow–identified 1,346 lncRNAs with altered expression (adjusted *P*-value of <0.05, >2-fold change in average expression). We trained a (continued on following page)

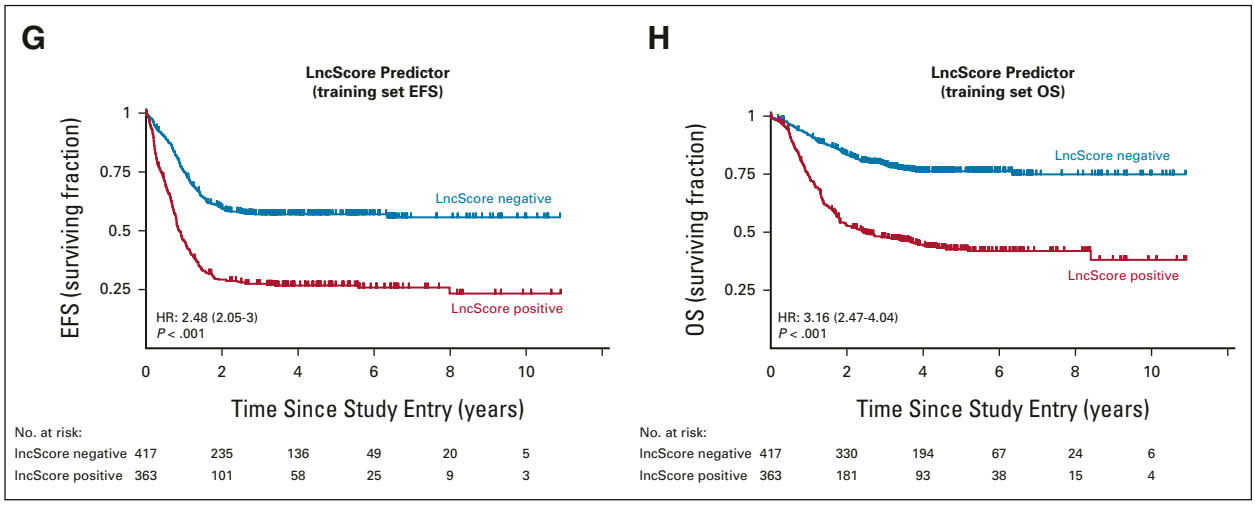

FIG 1. (Continued). Cox regression model for EFS on the aberrantly upregulated training set lncRNAs (right half of dendrogram) from training set data to identify 37 lncRNAs (labeled above) with significant effect on EFS. (F) The lncScore was calculated as the weighted sum after applying model coefficients to lncRNA expression estimates. In the training cohort set, lncScores ranged from –1.24 to 1.31 and were nearly centered at 0 (F; red, positive lncScore; blue, negative lncScore; boxed region indicates interquartile range and median). lncScores were strongly correlated with (G) event-free survival and (H) overall survival, with the HR for a positive score of 2.48 ($P < .001$) and 3.16 ($P < .001$), respectively. AML, acute myeloid leukemia; CFM, cytogenetic/fusion/molecular risk; EFS, event-free survival; FR, final risk; HR, hazard ratio; lncRNA, long noncoding RNA; NBM, normal bone marrow; OS, overall survival; pAML, pediatric acute myeloid leukemia.

derivation of the lncScore, one subject in the training set was determined to be ineligible and was removed from subsequent outcome analyses. A diagram for the investigations reported here is illustrated in the Data Supplement.

Identification and Validation of a 37-Gene lncRNA Score for pAML Outcome

We defined differentially expressed lncRNAs by comparison of AML data from the training set to bulk normal control bone marrow. Differential expression analysis of annotated lncRNA revealed 1,346 transcripts, with 647 upregulated in pAML (Fig 1E and Data Supplement). To determine whether lncRNA expression at the time of diagnosis was predictive of treatment outcome, we selected these upregulated lncRNAs for inclusion in a regularized Cox proportional hazards regression model of EFS. We limited analysis to upregulated genes in AML to best allow identification of lncRNAs that may be associated with disease progression and development, and whose expression may be identifiable as a detectable biomarker, rather than finding absence of expression in downregulated lncRNAs between normal and AML samples.

This approach defined a set of 37 lncRNAs (Data Supplement). We applied the trained model coefficients to the normalized lncRNA expression data (Data Supplement), producing a weighted sum of expression for each patient to create an expression score, which we term the lncScore. The distribution of lncScores revealed approximately equal numbers of patients with positive and negative scores in the training cohort, with values ranging from –1.24 to +1.31 (Fig 1F). lncScore was significantly predictive of both OS and EFS as a continuous variable (training set HR for OS, 3.67; 95% CI, 2.69 to 5.02; HR for EFS, 4.1; 95% CI, 3.27 to 5.14; all $P < .001$) and when discretized by quartile (Data Supplement). Since the median lncScore was close to 0, and clinical decision making revolves around identifying patients for early intensification by bone marrow transplantation, we dichotomized the training set cohort into those with positive or negative lncScores for further analysis. Comparison of these groups revealed positive lncScores had an EFS of 27% ± 5% at 5 years from diagnosis compared with 57% ± 5% for those with negative scores (HR, 2.48; 95% CI, 2.05 to 3; $P < .001$, Fig 1G and Data Supplement). lncScore was similarly predictive of OS (5-year OS, 43% ± 6% *v* 76% ± 4%; HR, 3.16; 95% CI, 2.47 to 4.04; $P < .001$; Fig 1H and Data Supplement).

We validated the association of lncScore with survival measures in two pediatric validation sets not used during lncRNA selection or survival model training, with one set reserved for potential model revision. As in the training cohort, both validation 1 and validation 2 cohorts showed similar distribution of lncScores across samples (median, –0.011 and –0.026; range, –1.10 to 1.27 and –1.44 to 1.01, respectively). The magnitude and significance level of predictive effect was comparable with the training set for both OS (HR, 2.87 and 3, respectively; $P < .001$) and EFS (HR, 2.38 and 2.36, respectively; $P < .001$; Figs 2A and 2B). These results suggested adequate predictive performance for the lncScore as initially derived from the training set. Without a need to hold an additional validation set in reserve for optimization, we subsequently combined the pAML validation 1 and 2 groups (n = 518) for increased power in multivariable analyses (Data Supplement).

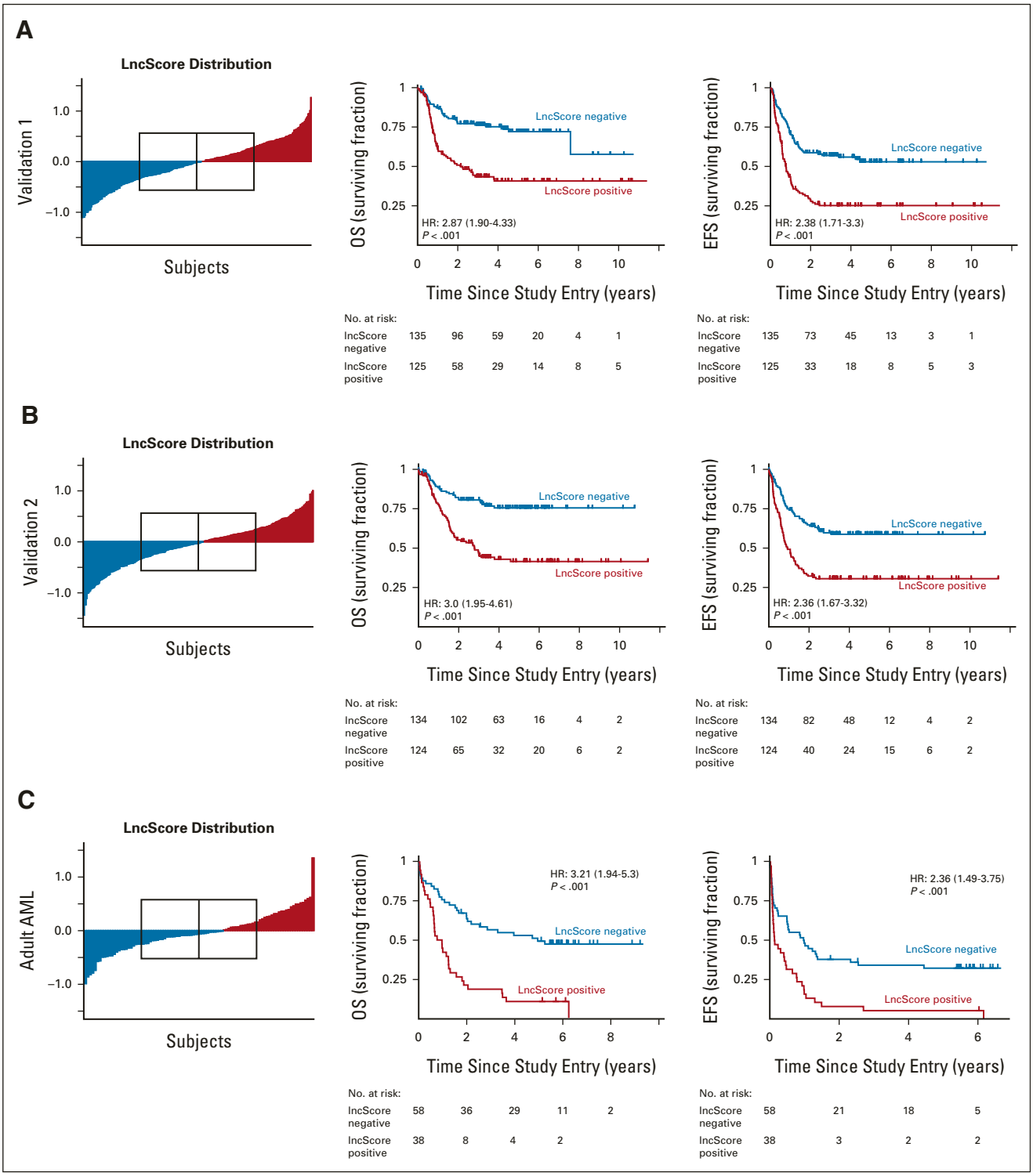

FIG 2. Validation of the prognostic significance of lncScore in independent pediatric and adult data sets. Independent univariable analysis in both (A) validation 1 and (B) validation 2 sets showed a comparable distribution of lncScores to that observed in the training set, with median scores nearly centered on 0 (red, positive lncScore; blue, negative lncScore; boxed region in the left-hand panels indicates interquartile range and median). Estimates of the HR for OS and EFS were comparable with those seen in the training set and remained highly significant ($P < .001$). (C) Application of lncScores to an independent adult RNA-seq data set demonstrated a similar distribution across samples, with slightly lower percentage of positive cases. Comparison of adult cases with positive versus negative lncScore shows a significant difference in both OS (HR, 3.21; 95% CI, 1.94 to 5.3; $P < .001$) and EFS (HR, 2.36; 95%, CI, 1.49 to 3.75; $P < .001$). AML, acute myeloid leukemia; EFS, event-free survival; HR, hazard ratio; OS, overall survival.

To establish generalizability, we tested whether the lncScore was predictive of adult AML outcomes in a technically and clinically distinctive data set. Evaluation of lncScores in 96 adult AML cases also showed a significant association for EFS (HR, 2.36; 95% CI, 1.49 to 3.75; $P < .001$) and OS (HR, 3.21; 95% CI, 1.94 to 5.3; $P < .001$; Fig 2C and Data Supplement). These results suggest that the lncScore is a robust and reproducible predictor of outcome with potential relevance across the AML age spectrum.

lncScore Is an Independent Predictor of pAML Outcome With Accuracy Comparable With Established Initial Risk Markers

To examine whether lncScore provides independent prognostic information, we performed multivariable analysis on OS and EFS from study enrollment and on RR from end induction. Including those factors defined at diagnosis that were identified as significant by univariable Cox regression in both training and validation sets (lncScore, CFM risk class, and presenting WBC count; Data Supplement), lncScore retained prognostic significance in the training set (HR of 2.07, 1.84, and 1.82 for OS, EFS, and RR from EOI1, respectively; all at $P < .001$; Data Supplement). Concordance statistics exceeded 66% for each survival metric (OS, 0.7; EFS, 0.667; RR, 0.679). Comparable results were observed in the combined validation group (HR OS, 1.75; $P = .001$; C-stat, 0.672; EFS, 1.56; $P = .001$; C-stat, 0.671; RR, 1.67; $P = .004$; C-stat, 0.689). Estimates of the concordance difference between submodels containing CFM classification compared with lncScore showed negligible differences, with large *P*-values for these comparisons in both validation and training sets. The full model containing lncScore, CFM, and WBC count slightly outperformed either submodel, but this effect was only statistically significant against the CFM comparison in the training group and lncScore comparison in validation set (Data Supplement). These results suggested that the lncScore provides comparable accuracy to traditional pretherapy classification metrics while having potential to add additional prognostic information.

lncScore Is Informative in Heterogeneous Cytogenetic Subgroups

To determine the distribution and prognostic contribution of lncScores across key presenting features, we analyzed lncScores in the context of upfront CFM classification and key fusions: *CBFA2T3-GLIS2, CBFB-MYH11, KMT2A*-r, *NUP98*-r, *RUNX1-RUNX1T1*, and those lacking a cytogenetic change or with rare fusions (none/other). Positive lncScores were most common among CFM high- and intermediate-risk group patients but infrequent among patients with low-risk disease (Fig 3A). lncScores similarly tracked with cytogenetic markers: few positive scores were detected among favorable translocations, but predominantly positive scores were seen in unfavorable groups such as *NUP98*-rearranged and *CBFA2T3-GLIS2* fusions. By contrast, lncScores were widely distributed in heterogeneous subgroups including cases with *KMT2A* fusions and those with rare or lacking a detectable fusion (none/other; Fig 3B).

We evaluated treatment outcomes in the *KMT2A*-rearranged and none/other groups to further examine the relationship between lncScore and subgroup outcomes. Survival analysis in *KMT2A* fusion cases in the validation cohort (N = 119) confirmed a marked separation observed in the training set (validation set 5-year OS, 81% ± 16% *v* 37% ± 10%; HR, 4.24; $P = .002$; 5-year EFS, 61% ± 19% *v* 28% ± 9%; HR, 2.5; $P = .007$; Figs 3C and 4A-4C Data Supplement) that was substantially better than the current standard for allocating to high- or intermediate-risk groups on the basis of fusion partner (Fig 3D and Data Supplement). Cases with rare fusions or lacking a fusion also showed a significant outcome association when stratified by lncScore (validation set 5-year OS, 68% ± 9% *v* 51% ± 11%; HR, 1.93; $P = .008$; 5-year EFS, 50% ± 10% *v* 33% ± 10%; HR, 1.76; $P = .003$; Fig 3E and Data Supplement). In aggregate, these data suggest that the lncScore may recapitulate prognostic information available from known high- and low-risk cytogenetic classes but adds additional information to the current standard classification model, particularly in heterogeneous groups presently classified as intermediate risk.

Comparison and Integration of lncScore With Postinduction Prognostic Criteria

To test whether lncScores could replace or augment a modern stratification scheme, we compared lncScore predictions with updated FR determination. FR grouping within the context of current (AAML1831, ClinicalTrials.gov identifier: NCT04293562) and recent COG studies is a complex criterion incorporating cytologic and molecular classification at the time of presentation with induction response by assessment of MRD to determine length and intensity of consolidative therapy.[31,40]

Because FR is established after induction, we assessed OS and DFS after first induction course in a multivariable model including FR and lncScore, again comparing with single-term submodels. lncScore and FR class were both significant at $P < .001$ in multivariable Cox models of training and validation sets for both outcome measures (Table 2; Figs 4A-4C; Data Supplement). Point estimates for HR were slightly higher by lncScore than FR for all but training set OS, with all CI showing substantial overlap. Comparison of single term submodels of lncScore versus FR in both training and validation groups showed similar trends, slightly favoring lncScore, but with nonsignificant concordance differential estimates (validation set HR for OS, 3.41 *v* 3.19; C-index diff, –0.004; $P = .867$; validation set DFS HR 2.44 *v* 2.16; C-index diff, –0.014; $P = .483$; training set OS, 3.13 *v* 4.21; C-index diff, –0.016; $P = .7$; training set DFS HR, 2.5 *v* 2.29; C-index diff, –0.028; $P = .168$). As with initial risk features, a full model containing FR and lncScore outperformed either FR

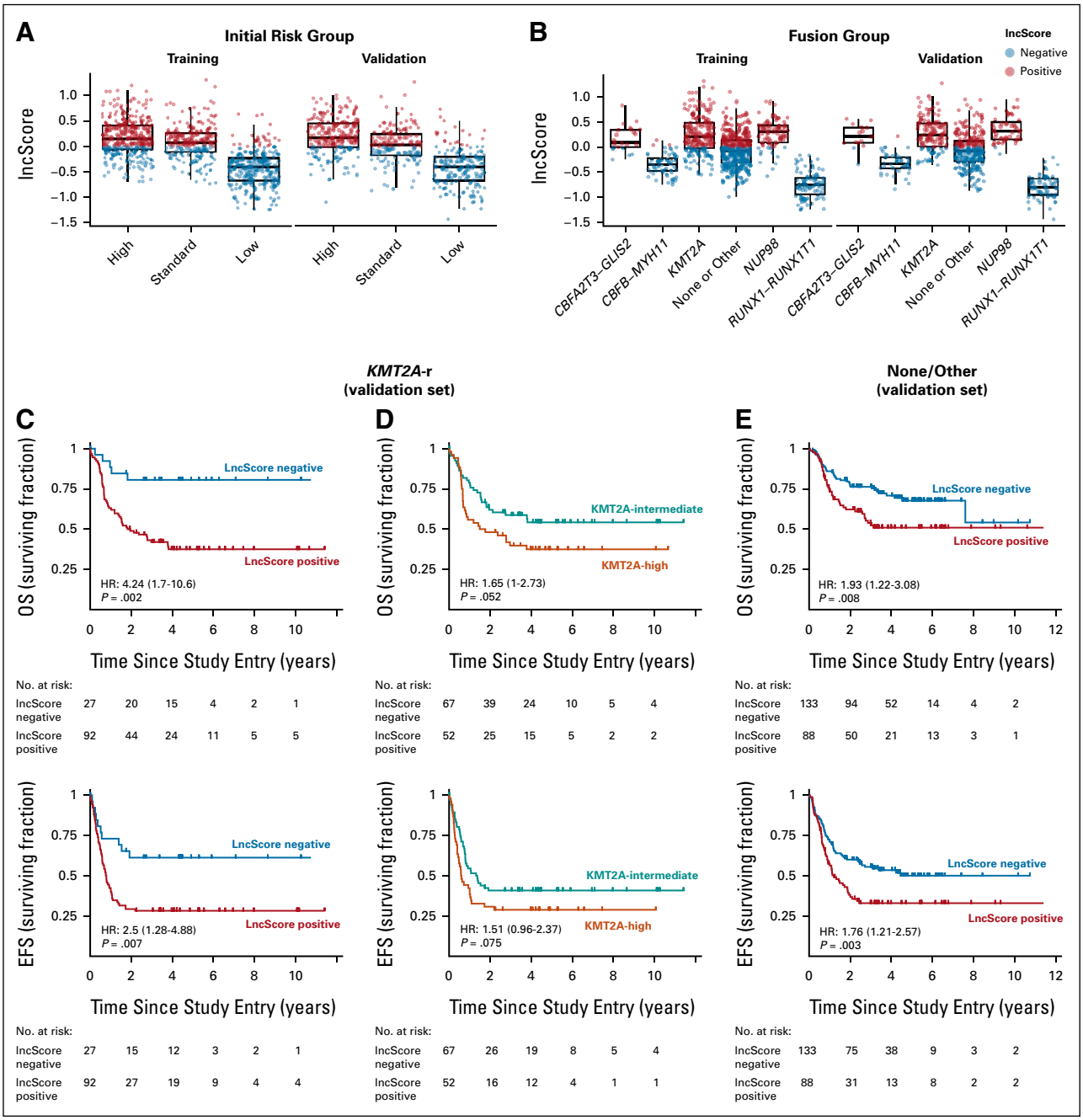

FIG 3. lncScore is informative in heterogeneous subgroups. (A) In both the training and validation pediatric data sets, unfavorable lncScores were most common in high initial-risk cases, mixed in intermediate-risk, but infrequent among low-risk cases. (B) When separated by fusion class, cases with higher-risk fusions including *CBFA2T3-GLIS2* and *NUP98* fusions showed nearly exclusively positive lncScores, whereas favorable-risk translocations showed the converse. Heterogeneous fusion groups including *KMT2A*-r and those with rare or lacking an identifiable fusion showed high levels of variability in lncScores. (C) Outcome differences in the *KMT2A*-r validation set were dramatic, with 81% versus 37% and 61% versus 28% 5-year survival for OS and EFS respectively (HR, 4.25; 95% CI, 1.7 to 10.6; $P < .001$; and 2.51; 95% CI, 1.29 to 4.89; $P < .001$). (D) Outcomes for these *KMT2A*-r validation set patients as stratified by fusion partner risk group are illustrated as contrast. (E) Cases with rare recurrent fusions or lacking a detectable fusion also showed significant outcome differences when stratified by lncScore (5-year OS, 67.7% *v* 48.4%; HR, 1.9; $P = .002$; 5-year EFS, 50.6% *v* 31.9%; HR, 1.8; $P = .001$). Training set cases with *KMT2A*-r or in the none/other fusion category showed similar findings, as illustrated in the Data Supplement. EFS, event-free survival; HR, hazard ratio; OS, overall survival.

or lncScore alone, with significant *P*-values in both possible concordance model comparisons for DFS and OS in the validation set (validation *P*-value range 0.008-<0.001; Table 2; Data Supplement). Together, these data suggest that lncScore, as a single assay, has comparable predictive performance to the currently used complex stratification

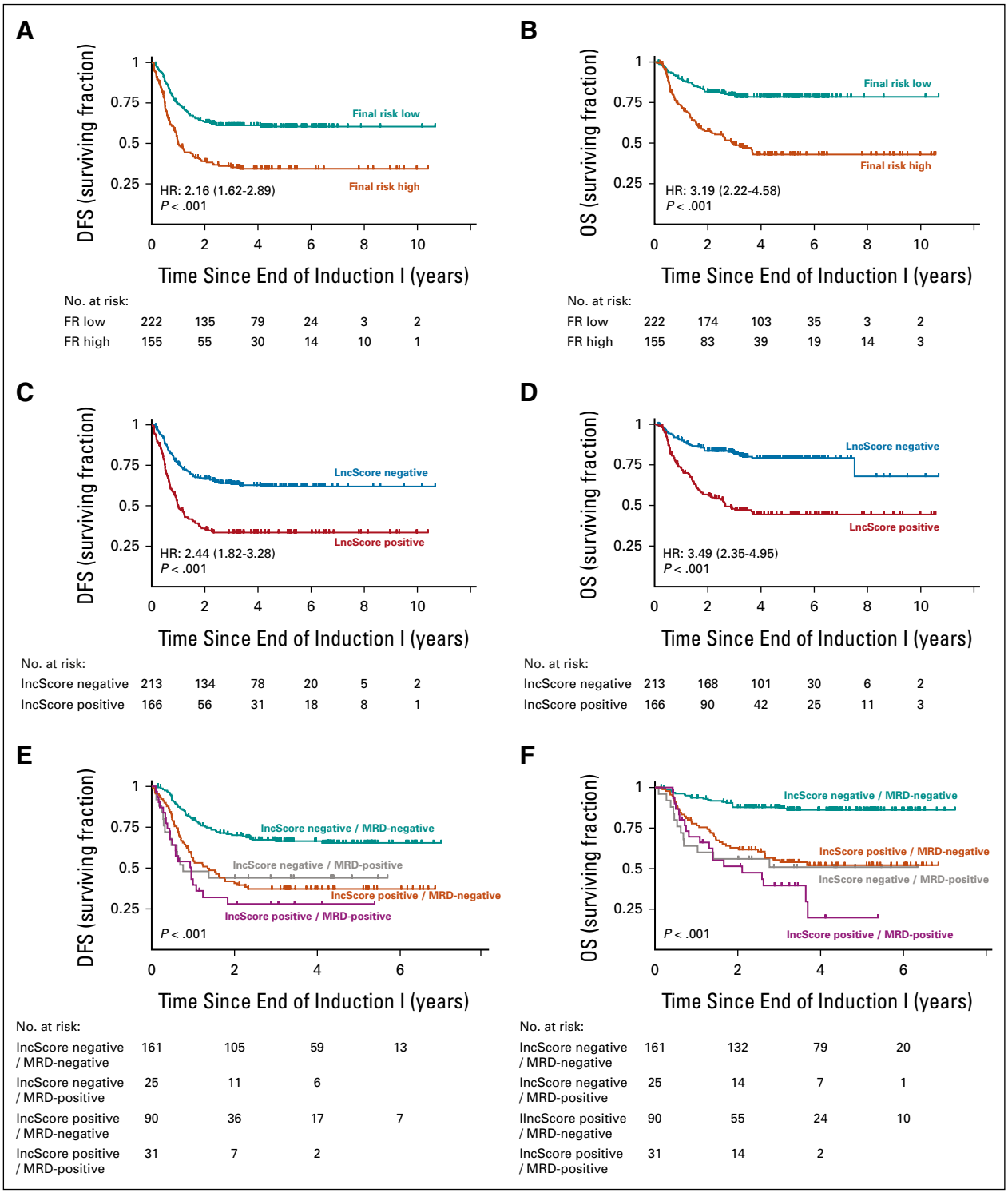

FIG 4. Assessment of lncScores with postinduction prognostic factors. Outcomes in the validation set by FR category as defined by current (AAML1831) stratification guidelines show (A) 59.1% versus 38.7% 5-year DFS (HR, 2.16; 95% CI, 1.62 to 1.89; $P < .001$) and (B) 78.4% versus 46.1% for OS (HR, 3.19; 95% CI, 2.22 to 4.58; $P < .001$). Comparison of cases binarized by lncScore shows comparable results, (C) with a 5-year DFS of 65.7% versus 35% (HR, 2.44; 95% CI, 1.82 to 3.28; $P < .001$) and (D) with a 5-year OS of 82% versus 45.8% (HR, 3.41; 95% CI, 2.35 to 4.95; $P < .001$; Data Supplement). Patients with negative lncScore and absence of detectable end-induction MRD comprise over half of cases and show historically excellent outcomes, with (E) a DFS of 65% ± 8% and (F) an OS of 86% ± 6% in the validation cohort. Patients with one or both markers positive showed poor outcomes, with DFS ranging from 28% to 44% and OS from 20% to 52%. DFS, disease-free survival; EFS, event-free survival; FR, final risk; HR, hazard ratio; MRD, minimal residual disease; OS, overall survival.

TABLE 2. Multivariable Analysis of Postinduction Risk (see also the Data Supplement)

	OS From EOI 1				DFS From EOI 1			
Model Comparison	**No.**	**HR**	**95% CI**	***P***	**No.**	**HR**	**95% CI**	***P***
Full model (~lncScore + FR)								
Inc group								
Negative	211	1			211	1		
Positive	166	2.438	1.62 to 3.67	<.001	166	2.004	1.45 to 2.77	<.001
Final risk group								
Low	222	1			222	1		
High	155	2.182	1.47 to 3.25	<.001	155	1.628	1.18 to 2.24	.003
C-statistics			0.683				0.640	
FR model								
Final risk group								
Low	222	1			222	1		
High	155	3.19	2.22 to 4.58	<.001	155	2.162	1.62 to 2.89	<.001
C-statistics			0.634			0.597		
lncScore model								
Inc group								
Negative	211	1			211	1		
Positive	166	3.409	2.35 to 4.95	<.001	166	2.443	1.82 to 3.28	<.001
C-statistics			0.645				0.613	

	Estimate	***P***	**Estimate**	***P***
C-Stats differential				
FR//-//lncScore	–0.004	.867	–0.014	.483
FR//-//Model	–0.046	**.008**	–0.041	**.005**
lncScore//-//Model	–0.042	**<.001**	–0.027	**<.001**

Abbreviations: DFS, disease-free survival; EOI 1, end of first induction; FR, final risk; HR, hazard ratio; OS, overall survival.

scheme, while addition of lncScore to current standards outperforms either approach alone.

Since lncScore does not include the induction response information that is nested in FR class, we tested inclusion of MRD with lncScore for postinduction outcome prediction. lncScores remained highly significant in both training and validation sets by multivariable regression (validation HR for DFS and OS, 2.19 and 2.83, respectively; both $P < .001$; Data Supplement). The effect of MRD was significant but more modest (validation set DFS HR, 1.89; $P < .001$; OS HR, 2.5; $P \leq 0.001$). The complete model including lncScore and MRD outperformed either factor alone, suggesting that inclusion of MRD indeed improves predictive accuracy of the lncScore. Practically, this approach defined a large group of patients (approximately 50%) with both negative lncScore and without MRD demonstrating historically excellent outcomes in pAML (validation set OS and DFS of 85% and 65%, respectively), while those with either or both markers positive showed markedly inferior outcomes (Figs 4E and 4F; Data Supplement).

DISCUSSION

In this study, we identify and validate a 37-gene lncRNA-based classification system that improves upon state-of-the art predictive strategies to better differentiate pAML into lower- and higher-risk categories at risk for treatment failure. Our findings generally corroborate the results from studies of adult patients with AML in illustrating the relevance of lncRNA expression to outcome prediction.[22-27] While developed for pediatric disease, our study also suggests the lncScore may be predictive beyond pediatric AML to adult disease, although the adult sample examined here is limited in size.

Notably, none of the lncRNAs identified in the signature have been previously implicated in AML outcomes. Several factors may explain this discrepancy including unique biological differences of pediatric versus adult AML (including a substantially higher proportion of oncofusion-driven disease),[5] the relatively large size of the training data set used here, the sequencing chemistry—which used stranded sequencing after rRNA depletion rather than

poly(A) selection, allowing for the detection of both polyadenylated (poly-A) and non–poly-A lncRNAs[41] as well as antisense transcripts—and generally high coverage transcriptome sequencing, potentially allowing for better detection of weakly expressed lncRNAs. As in prior lncRNA studies, it is challenging to determine on the basis of available data whether individual lncRNA components serve as direct mediators of therapy resistance or are passengers marking a broader transcriptional milieu of resistance.[24] Consistent with the generally incomplete state of lncRNA annotation, many of the lncScore transcripts presently lack any functional annotation. Several, however, have potentially important direct roles in AML biology through *WNT*[42] signaling, *HOXA* cluster expression,[43] and stem-cell maintenance.[44] The marked improvement in predicting *KMT2A*-r case outcomes on the basis of lncScore, compared with fusion partner identity, is likely partially due to passenger effects, since transformed cell of origin has been demonstrated to significantly influence transcriptional patterns in *KMT2A*-r leukemia.[45] On the basis of the dramatic differences between these two predictive methods in *KMT2A*-r pAML, it seems plausible that lncScore encodes partner-gene and cell-of-origin information that is unavailable from *KMT2A* partner definition alone.

Several features of the lncScore make it favorable for future development and clinical application. Our selection of transcripts overexpressed relative to normal bone marrow leaves the assay less susceptible to sensitivity issues in partially diluted marrow samples. In four cohorts (training, validation 1, validation 2, and adult), the median lncScore score lay extremely close to 0, thus motivating our selection of 0 as an absolute cutpoint for dichotomization. This approach obviates the need for large reference data sets or concurrent controls for median definitions.

In addition to improving on state-of-the art prognostics, this study suggests that the lncScore offers comparable performance to modern stratification methods in a single assay. Both findings are of potential importance since prognostic classification schemes presently used in pAML leave room for predictive strengthening while posing substantial practical hurdles to execution; successful stratification is presently a logistical and interpretive challenge. For example, the schema currently used by COG requires bone marrow testing for numerous targeted gene assessments by Sanger sequencing and fragment length analysis technology, g-banded cytogenetics, interphase fluorescent in situ hybridization against numerous targets, immunophenotyping, as well as targeted DNA and RNA short-read sequencing to define rare fusion partners, potentially supplemented with directed quantitative polymerase chain reaction assays. This complexity is costly and burdensome even in high-volume centers and in patients with ample bone marrow for testing but becomes particularly problematic where limited marrow is available, in lower-volume centers that infrequently encounter patients with pAML, or in settings of limited medical resources. Hence, there is motivation to improve and simplify predictive testing. The inclusion of lncScore, as a standalone assay, or in combination with other sequencing analyses, is fully compatible with a broader move toward next-generation sequencing as an upfront diagnostic modality in AML.[46,47]

AFFILIATIONS

[1]Department of Pediatrics, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR
[2]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA
[3]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA
[4]Department of Pediatrics, Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA
[5]Children's Oncology Group, Monrovia, CA
[6]Department of Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL
[7]Center for Epigenetics, Van Andel Research Institute, Grand Rapids, MI
[8]Department of Pediatrics, Children's Mercy Hospitals and Clinics, Kansas City, MO
[9]Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA
[10]Nemours Center for Cancer and Blood Disorders and Alfred I. DuPont Hospital for Children, Wilmington, DE
[11]Department of Computational Biology, St Jude Children's Research Hospital, Memphis, TN
[12]Department of Population and Public Health Sciences, University of Southern California, Los Angeles, CA
[13]Department of Pediatrics, University of Washington, Seattle, WA

CORRESPONDING AUTHOR

Jason E. Farrar, MD, Department of Pediatrics, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, 1 Children's Way, MS 512-10, Little Rock 72202, AR; Twitter: @rarrafnosaj; e-mail: jefarrar@uams.edu.

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DATA SHARING STATEMENT

The covariates, outcomes, and lncRNA expression data supporting the development of this biomarker are included in the supplementary data files. The complete transcriptomic data are available in dbGaP as outlined in the Data Supplement methods section.

AUTHOR CONTRIBUTIONS

Conception and design: Jason E. Farrar, Jenny L. Smith, Benjamin J. Huang, Alan S. Gamis, E. Anders Kolb, Soheil Meshinchi
Financial support: Jason E. Farrar, Soheil Meshinchi
Administrative support: Jason E. Farrar, Soheil Meshinchi
Provision of study materials or patients: Richard Aplenc, Soheil Meshinchi
Collection and assembly of data: Jason E. Farrar, Jenny L. Smith, Megan Othus, Rhonda Ries, Tiffany Hylkema, Era L. Pogosova-Agadjanyan, Amanda Leonti, Alan S. Gamis, Richard Aplenc, Todd A. Alonzo
Data analysis and interpretation: Jason E. Farrar, Jenny L. Smith, Benjamin J. Huang, Yi-Cheng Wang, Rhonda Ries, Era L. Pogosova-Agadjanyan, Sneha Challa, Timothy I. Shaw, Alan S. Gamis, Richard Aplenc, E. Anders Kolb, Xiaotu Ma, Derek L. Stirewalt, Todd A. Alonzo, Soheil Meshinchi
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Long Noncoding RNA Expression Independently Predicts Outcome in Pediatric Acute Myeloid Leukemia

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Jason E. Farrar
Research Funding: Novartis

Megan Othus
Consulting or Advisory Role: Glycomimetics, Cascadia Labs, Merck, Daiichi Sankyo, Biosight
Other Relationship: Celgene, Glycomimetics

Tiffany Hylkema
Stock and Other Ownership Interests: Moderna Therapeutics, Quest Diagnostics

Sneha Challa
Employment: Fred Hutchinson Cancer Research Center
Research Funding: Fred Hutchinson Cancer Research Center

Timothy I. Shaw
Patents, Royalties, Other Intellectual Property: Timothy Shaw reports a patent application in CAR T cell therapy US20220267425A1, Timothy Shaw reports a pending patent application on software for survival analysis

Timothy J. Triche
Patents, Royalties, Other Intellectual Property: Inventor on patent licensed to Adela Bio (Inst)

Alan S. Gamis
Consulting or Advisory Role: Novartis

Richard Aplenc
Expert Testimony: Vorys, Sater, Seymour, and Pease LLP

E. Anders Kolb
This author is a member of the *Journal of Clinical Oncology* Editorial Board. Journal policy recused the author from having any role in the peer review of this manuscript.
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Derek L. Stirewalt
Stock and Other Ownership Interests: Yatiri Bio

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