Real-world validation study of the LSC17 score for risk prediction in patients with newly diagnosed acute myeloid leukemia

Tracy Murphy,[1°] Boyang Zhang,[2] Tong Zhang,[1,3,4] Ian King,[1,3,4] Jose-Mario Capo-Chichi,[1,3,4] Vikas Gupta,[1,5,6] Dawn Maze,[1,5,6] Caroline J. McNamara,[1,5,6] Mark D. Minden,[1,5,6] Aaron D. Schimmer,[1,5,6] Andre C. Schuh,[1,5,6] Hassan Sibai,[1,5,6] Karen W.L. Yee,[1,5,6] Andrea Arruda,[1] Zhibin Lu,[1] Dina Khalaf,[7] Chantal Rockwell,[8] Brian Leber,[7,9] Mitchell Sabloff,[8] Anne Tierens,[1,3,4] Tracy L. Stockley,[1,3,4] Steven M. Chan,[1,5,6] Stanley W.K. Ng[1,2] and Jean C.Y. Wang[1,5,6]

[1]Princess Margaret Cancer Center, University Health Network, Toronto, Ontario, Canada; [2]Department of Biological Chemistry, School of Medicine, University of California, Irvine (UCI), Irvine, CA, USA; [3]Laboratory Medicine Program, University Health Network, Toronto, Ontario, Canada; [4]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [5]Division of Medical Oncology and Hematology, Department of Medicine, University Health Network, Toronto, Ontario, Canada; [6]Department of Medicine, University of Toronto, Toronto, Ontario, Canada; [7]Juravinski Cancer Center, Hamilton, Ontario, Canada; [8]The Ottawa Blood Disease Center, The Ottawa Hospital, Ottawa, Ontario, Canada and [9]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

[°]*Current address: Hematology Department, University Hospital Galway, Galway, Ireland*

Correspondence: J.C.Y. Wang
jean.wang@uhn.ca

S.W.K. Ng
stanlewn@uci.edu

Received: March 7, 2025.
Accepted: July 1, 2025.
Early view: July 10, 2025.

https://doi.org/10.3324/haematol.2025.287777

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license

Abstract

Acute myeloid leukemia (AML) patients exhibit diverse molecular and cytogenetic changes with heterogeneous outcomes. The functionally-derived LSC17 gene expression score has demonstrated strong prognostic significance in retrospective analyses of adult and pediatric AML cohorts, where above-median scores are associated with worse outcomes compared to below-median scores in intensively-treated patients. Here we used a laboratory-developed clinically-validated NanoString-based LSC17 assay to test the prognostic value of the LSC17 score in a prospective multicenter study of 276 newly-diagnosed AML patients. Each patient's score was classified as high or low by comparison to a previously-established reference score. In the entire cohort, a high LSC17 score was associated with poor risk features, including advanced age and unfavorable genetic mutations. In the subset of 190 patients treated intensively, a high LSC17 score was associated with lower remission rates (63% *vs*. 94% after induction; *P*<0.0001), presence of measurable residual disease (46% *vs*. 10%; *P*<0.0001), and shorter overall survival (OS, 606 days *vs*. not reached; *P*=0.0004; hazard ratio [HR] =2.16; 95% confidence interval [CI]: 1.39-3.35) and relapse-free survival (RFS, 541 days *vs*. not reached; *P*=0.001; HR=1.99; 95% CI: 1.29-3.08). In multivariable analysis considering age, white blood cell count and European LeukemiaNet 2022 risk groups, the LSC17 score remained an independent predictor of RFS and OS. Allogeneic stem cell transplantation improved OS for patients with a high but not a low LSC17 score. This study establishes the real-world value of the LSC17 score as a robust tool for risk assessment in AML and paves the way for its incorporation into routine clinical practice.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignancy with multiple subtypes and variable clinical outcomes driven by disease characteristics as well as the clinical status of the patient.[1 2,3] While genomic classification has further rationalized risk stratification in AML, many challenges remain.[4] The accurate assessment of survival outcomes in AML subtypes driven by various combinations of driver mutations and cytogenetic abnormalities presents a challenge to the treating physician.[5]

AML is sustained by a rare subpopulation of leukemia stem cells (LSC) believed to drive therapy resistance and relapse.[6,7] The LSC17 gene expression score was developed based on functionally-defined LSC populations across the spectrum of AML subtypes.[8] In multiple independent

retrospective cohorts, the LSC17 score has been found to robustly discriminate between patients with significantly different outcomes.[9-12] Higher-than-median LSC17 scores were associated with poor treatment response and survival outcomes in both uni- and multi-variable survival analyses, independent of commonly used prognostic factors including cytogenetic and molecular risk groups.

The LSC17 score provides clinicians with a rapid and powerful tool for upfront risk stratification. A key advantage of the LSC17 test over other cytogenetic or genomic analysis typically performed for AML is its rapid turnaround time (24-48 hours on a NanoString platform), which permits risk determination for initial therapy decisions soon after diagnosis. To enable broad implementation of the score for risk assessment of newly-diagnosed AML patients, we previously developed and validated a NanoString-based assay in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory. Using this assay, scores for individual patients can be classified in real time as high *versus* low by comparison to a previously-established reference score, allowing for upfront risk-adapted management decisions.[13]

To test the prognostic power of LSC17 scores measured using this clinical assay, we designed a prospective feasibility study of unselected, newly-diagnosed patients from multiple cancer centers across Ontario. The key aim of this trial was to assess the ability of the LSC17 score to predict clinical outcomes when classified prospectively against a previously-established reference score rather than through a retrospective dichotomized median-split analysis. Clinical data was also collected to understand how the LSC17 score relates to contemporary risk stratification tools including the 2022 European LeukemiaNet (ELN) risk classification as well as recurrent AML driver mutations. In addition, treatment outcomes including measurable residual disease (MRD) monitoring post induction were assessed and correlated with LSC17 scores.

Methods

Patients referred to three leukemia treating centers (Princess Margaret [PM] Cancer Center, Juravinski Cancer Center and the Ottawa Blood Disease Center) from June 2017 to April 2020 were approached to participate in the study. Biological samples were collected with informed consent according to procedures approved by the Research Ethics Board of each center and viably frozen in the PM Leukemia Bank. Baseline demographics and disease characteristics were recorded for all patients. Standard diagnostic criteria including molecular testing and cytogenetics were collected from all patients (*Online Supplementary Figure S1*). *FLT3*-internal tandem duplication (ITD) testing was performed according to local institutional protocols. Patients who were *FLT3*-ITD negative with *NPM1* mutation were classified as favorable risk; patients who were *FLT3*-ITD positive with or without *NPM1* mutation were classified as intermediate risk according to ELN 2022 criteria.[5] Targeted next-generation sequencing (NGS) using a custom myeloid gene panel on the Illumina sequencing platform was performed on the entire cohort of patients using 50 ng of DNA isolated from peripheral blood or bone marrow samples collected at the time of diagnosis.[14,15]

Samples for measurement of LSC17 score were collected in a 2.5 mL RNA Paxgene© tube at the time of routine diagnostic bone marrow aspiration or blood testing from patients with a suspected diagnosis of AML and were sent to the Advanced Molecular Diagnostics Laboratory (AMDL) for testing. The sample was analyzed on a certified NanoString platform using a standardized bioinformatics pipeline that had been developed in the previously published validation study.[13] Individual LSC17 scores were classified as high or low based on comparison to a previously-established reference score.

Patients were treated as per the current standard of care or physician's choice at each participating site as directed by current risk stratification methods or clinical assessment at the time of diagnosis. The majority of patients received induction with 7+3. Within this group, 29 of 39 *FLT3*-mutated patients also received midostaurin. At PM, FLAG-Ida was the induction regimen of choice in high-risk patients with therapy-related AML and those with antecedent hematological disorders or complex cytogenetics. The LSC17 score result was not revealed to either the patient or treating physician. For those patients who were treated within clinical trials, dose modifications were made in accordance with guidelines of the specific clinical trial protocols and were not altered by the LSC17 score status.

MRD monitoring of post-induction bone marrow samples was performed by flow cytometry for a subset of patients at PM. A standardized three-tube ten-color leukemia panel (*Online Supplementary Table S1*) was applied to assess leukemia-associated and differences-from-normal immunophenotypes.[16] The antibody tubes include three core markers (CD45, CD34 and CD33) in addition to lineage and maturation markers allowing the detection of cross-lineage antigen expression, asynchronous, and altered antigen expression across the different lineages. Bone marrow samples were processed within 24 hours after collection using a stain, lyse and wash technique as previously described.[16] A minimum of 250,000 events were acquired on a Navios cytometer TM (Beckman Coulter, Miami, FL, USA). List mode files were analyzed using Kaluza software 1.3 (Beckman Coulter) or Infinicyt Software 1.7 (Cytognos, Salamanca, Spain). Aberrant antigen expression was documented in the diagnostic samples and electronically saved in reference images with the analyzed data files. All clones exceeding 10% of the total leukemic cells were monitored in the follow-up samples. MRD was calculated as percentage of viable cells, determined by light scatter features, based on a minimum of 50 clustered events. All samples with >0.1%

leukemic events were considered as MRD positive.
Clinical outcome measures including complete remission (CR or CRi), relapse-free survival (RFS), and overall survival (OS) were defined by the IWG and 2022 ELN AML criteria.[5,17] One hundred and ninety and 168 patients had available data for OS and RFS analysis, respectively. Additional details on chemotherapy regimens, sequencing techniques, mutation calling and MRD flow cytometry analysis are provided in the *Online Supplementary Appendix*.

Statistical analysis

Pre-study sample size analysis suggested that 150 patients were required to demonstrate a hazard ratio for death of 2.3 between patients with a high or low LSC17 score (α=0.05 and power =0.8). Comparison of pretreatment clinical and disease characteristics between the high- and low-score groups was performed using the Wilcoxon-Mann-Whitney test for continuous variables and the χ^2 test for categorical variables. For categorical variables where any group size was less than five, Fisher's exact test was used instead of the χ^2 test. Univariable OS and RFS curves were computed using the Kaplan-Meier method, and differences between groups of patients with high or low LSC17 scores were tested using the log-rank test. A *P* value of <0.05 was considered significant.
Uni- and multi-variable survival analyses were performed using Cox proportional hazards models, with comparisons performed using Mantel-Cox log-rank and Wald tests. For multivariable analyses, covariables for Cox proportional hazards models included LSC17 score as well as established risk factors (age, white blood cell [WBC] count at diagnosis, *de novo versus* AML with antecedent hematological disorders [AHD]/therapy-related (t-)AML, and ELN 2022 risk group). The intermediate-risk subgroup was used as a reference against which other risk subgroups were compared, unless specified otherwise. Wald's test was used to evaluate the significance of hazard ratios (HR). Competing risk analyses were performed using the cmprsk package.[18] The impact of allogeneic stem cell transplantation (aSCT) on OS was assessed by encoding transplantation as a time-dependent covariable in uni- and multi-variable Mantel-Byar and time-dependent Cox regression models, respectively, where univariable results were visualized using Simon-Makuch plots.[19-21] Time-dependent analyses were performed using the EZR R package.[22] All survival analyses were performed using the survival R package.[23] Comparative analyses between full and null models with and without the LSC17 score, respectively, were performed using the likelihood ratio test.
Accuracy of MRD prediction was evaluated using multivariable logistic regression models built using the rms R package along with the bootstrap-adjusted area under the receiver operator curve (AUROC) metric. The pROC R package was used for receiver operator curve analyses.[24] Relative importance of individual covariables in multivariable logistic regression models was estimated by examining the partial Wald χ^2 statistic as done by others.[25]
Data was processed and analyzed using MedCalc (version 18.6) or Prism (version 7.05 for Windows) software and in R statistical programming environment as described.

Results

Patient characteristics

All patients in this study underwent LSC17 score testing as part of a prospective validation study or enrollment in a prospective trial between June 2017 and April 2020. Overall, 391 patients were recruited to the study, of which 115 were excluded due to an alternative (non-AML) diagnosis. Of the remaining 276 patients, 190 received curative-intent induction chemotherapy. Demographic and disease characteristics of the study cohort are described in the *Online Supplementary Table S2*.
The median age of the cohort was 67 years (interquartile range, 57-73 years). The median age of patients with a high LSC17 score was higher than those with a low score (69 *vs*. 63.5 years; *P*=0.0003). There were no sex differences between low and high LSC17 score patients. Seventy-four percent of patients had *de novo* AML. More patients in the high LSC17 score group had t-AML (diagnosed on clinical grounds) or AML with AHD compared to the low-score group, however this difference was not statistically significant (28% *vs*. 24%; *P*=0.55).

A high LSC17 score is associated with poor risk features at diagnosis

In the entire cohort of 276 AML patients, 174 patients (63%) had a high LSC17 score. WBC count (4.2 *vs*. 10.2×10^9/L; *P*=0.0003), absolute neutrophil count (ANC; 0.8 *vs*. 1.3×10^9/L; *P*= 0.02) and peripheral blood blast count (0.5 *vs*. 1.9×10^9/L; *P*=0.008) were lower in the high LSC17 score group compared to the low score group. Red cell distribution width (RDW) was higher in the high LSC17 score group (17.7 *vs*. 17.1; *P*=0.008). The remaining blood count parameters at diagnosis were not statistically different between patients with high and low scores.
A high LSC17 score was associated with myelodysplasia-related changes (MRC) in the bone marrow (33% *vs*. 14%; *P*=0.0006). A low LSC17 score was associated with favorable risk according to cytogenetic risk group and ELN 2022 classification (*Online Supplementary Table S2*). Conversely, a high LSC17 score was associated with poor risk cytogenetics (38% *vs*. 13%; *P*<0.0001) and adverse risk according to ELN 2022 (75% *vs*. 26%; *P*<0.0001). Median LSC17 scores correlated with ELN 2022 risk categories, rising with increased clinical risk (Figure 1A; *P*<0.0001 for all pairwise comparisons). When considered in the smaller cohort of 190 patients who received induction chemotherapy, these risk associations remained statistically significant (*Online*

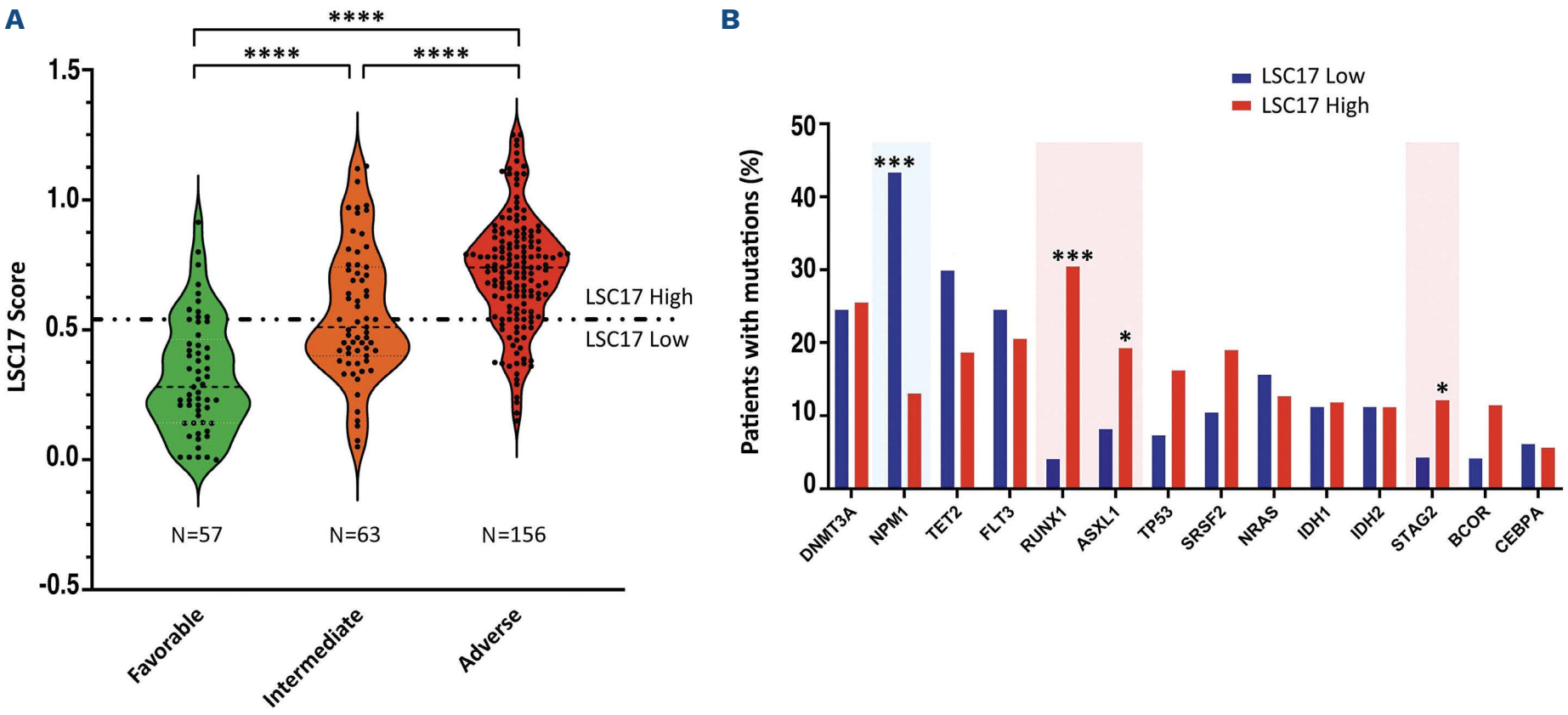

Figure 1. A high LSC17 score is associated with poor risk features at diagnosis. (A) Violin plot of LSC17 scores according to patients' European LeukemiaNet 2022 risk classification. The dot-dash line indicates the reference LSC17 score of 0.51, which was used to classify individual patient scores as high *versus* low. ****P<0.0001 by unpaired t test. (B) Bar graphs illustrating the percentage of patients with low or high LSC17 scores bearing mutations in the indicated genes. *P<0.05; ***P<0.001 comparing patients with low *versus* high LSC17 scores.

Supplementary Table S3).

Association of the LSC17 score with recurrent myeloid mutations was analyzed for mutations occurring with a frequency of greater than 5% in the study cohort. Patients with a low LSC17 score were more likely to have mutations in *NPM1*, while *RUNX1, ASXL1* and *STAG2* mutations were seen more frequently in patients with a high LSC17 score (Figure 1B; *Online Supplementary Table S4*).

A high LSC17 score is associated with worse treatment response and presence of measurable residual disease

To assess the ability of the LSC17 score to predict treatment response, we considered only those patients who received induction chemotherapy (N=190). Patients with a high LSC17 score were less likely to achieve remission after one cycle of induction chemotherapy (Table 1; 63% *vs*. 94%; P<0.0001) although there was no difference in CR rate after two cycles (Table 1; 11% *vs*. 4%; P=0.09). When considering different induction regimens, the same pattern of response was seen in the group of patients who received 7+3-based induction (*Online Supplementary Table S5*). However, patients with high LSC17 scores who underwent induction with a FLAG-Ida regimen had similar response rates to patients with low LSC17 scores, although the cohort size was limited (N=38) (*Online Supplementary Table S5*)

Results of MRD testing by flow cytometry were available for 140 patients (77%). Patients with a high LSC17 score were more likely to have MRD at the time of remission compared to low-score patients (Table 1; 46% *vs*. 10%; P<0.0001). Indeed, patients with a high LSC17 score were less likely to achieve MRD-negative remission regardless of the treatment regimen received, although this did not reach statistical significance in the smaller group of patients treated with FLAG-Ida (*Online Supplementary Table S5*; 30/52 [58%] *vs*. 54/59 [92%]; P<0.0001 for 7+3; 8/18 [44%] *vs*. 9/11 [82%]; P=0.06 for FLAG-Ida). The ability of the LSC17 score to predict MRD was evaluated using multivariable logistic regression models. Addition of the

Table 1. Summary of outcomes in intensively-treated acute myeloid leukemia patients who had remission assessment (N=182).

	LSC17 score low N=82	LSC17 score high N=100	*P*
Remission after induction cycle 1, N (%)			
Yes	77 (94)	63 (63)	<0.0001****
No	5 (6)	37 (37)	
CR, N (%)			
Yes	79 (96)	89 (89)	0.09
No	3 (4)	11 (11)	
MRD, N (%)			
Positive	7 (10)	32 (46)	<0.0001****
Negative	63 (90)	38 (54)	

CR: complete remission; MRD measurable residual disease (determined by flow cytometry). ****P value extremely significant.

LSC17 score to a model that included age, AML type (*de novo vs*. AHD/t-AML) and ELN 2022 risk groups as covariables significantly improved the MRD predictive accuracy (Figure 2A; *P*<0.01, likelihood ratio test). Furthermore, the LSC17 score was by far the most important predictor of MRD (χ^2=11.2; *P*<0.001) over ELN 2022 adverse risk (χ^2=5.4; *P*=0.02) (Figure 2B; *Online Supplementary Table S6*). Thus, measurement of the LSC17 score provides a valuable tool for upfront prediction of MRD following conventional intensive chemotherapy.

A high LSC17 score is associated with inferior survival outcomes

Survival outcomes were assessed for patients who received induction chemotherapy (N=190). In the entire cohort, irrespective of the induction regimen received, patients with high LSC17 scores had worse overall survival (OS, 606 days *vs*. not reached; Figure 3A, *P*=0.0004; HR=2.16; 95% confidence interval [CI]: 1.39-3.35) and relapse-free survival (RFS, 541 days *vs*. not reached; Figure 3B; *P*=0.001; HR=1.99; 95% CI: 1.29-3.08) compared to patients with low LSC17 scores. These findings were also true for the subgroups of patients treated with either 7+3 or FLAG-Ida (*Online Supplementary Figure S2*), and for both younger (age ≤60) and older (age >60) patients (*Online Supplementary Figure S3*), although not statistically significant for RFS in the younger group. Within individual ELN 2022 risk groups, the LSC17 score had a trend towards significant sub-stratification in the intermediate-risk group for OS (*Online Supplementary Figure S4*; *P*=0.14; HR=2.01; 95% CI: 0.79-5.11). For RFS, this difference was statistically significant in the intermediate group (*Online Supplementary Figure S5*; *P*=0.02; HR=2.66; 95% CI: 1.16-6.1), while trending towards significance in the other groups.

In multivariable survival analysis using a Cox proportional hazards model without the LSC17 score, patient age (*P*=0.006) and ELN 2022 adverse risk (*P*=0.01) were prognostic for OS (Table 2). Importantly, when the LSC17 score was added to the model, only the score and age retained significant prognostic value (*P*=0.005). Moreover, the model with the LSC17 score included fit the data significantly better than the null model as determined via the likelihood ratio test (*P*<0.001). The LSC17 score was also significantly associated with RFS in multivariable survival analysis (*Online Supplementary Table S7*). Multivariable analysis of OS and RFS within the subgroup of 150 patients who underwent 7+3 induction therapy showed similar results (*Online Supplementary Tables S8, S9*). For both OS and RFS, addition of the LSC17 score significantly improved the model, and the score was an independent predictor of both OS (*P*=0.04) and RFS (*P*=0.03).

In competing risk analysis considering death and relapse as competing risks, none of the tested covariables retained significant prognostic value in a Cox regression model (*Online Supplementary Table S10*). However, in a multivariable Fine-Gray model of competing risks, the LSC17 score along with age and AHD/t-AML but not ELN 2022 risk retained significant prognostic value (*Online Supplementary Table*

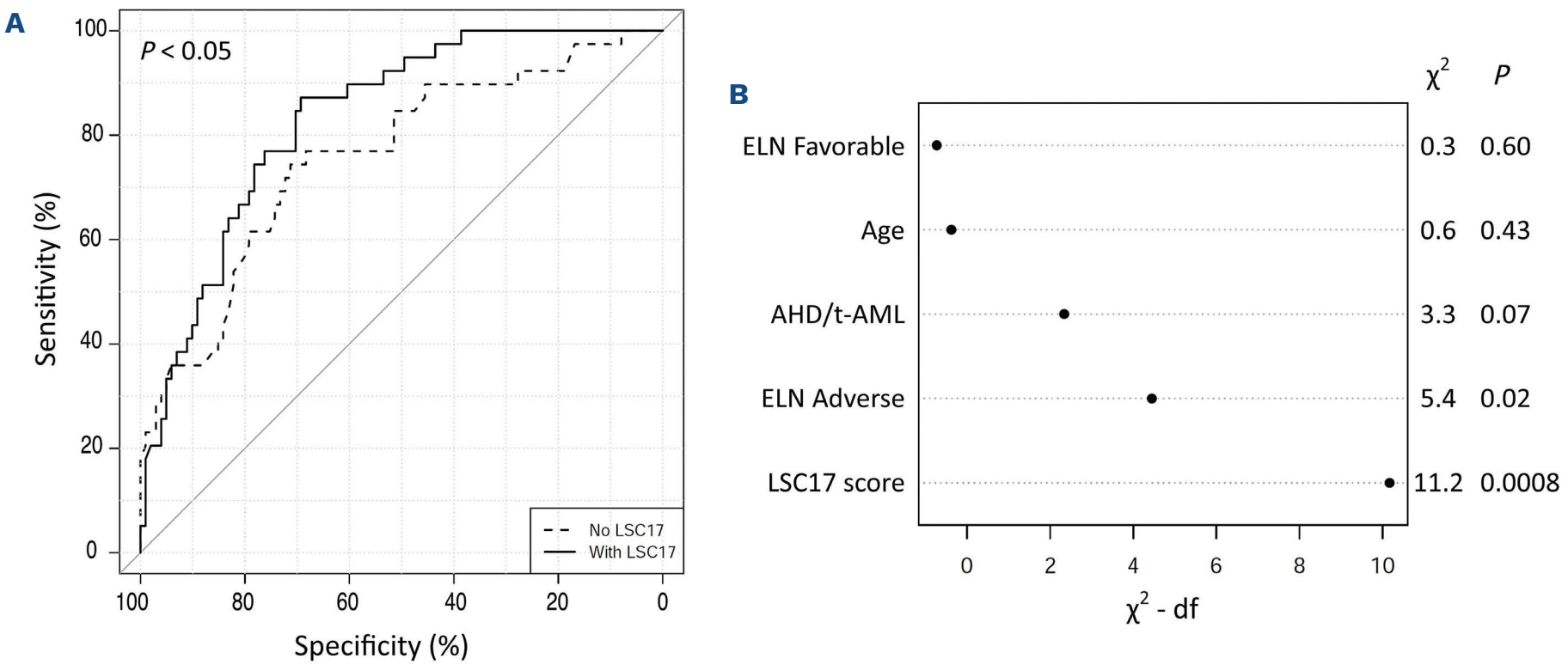

Figure 2. A high LSC17 score is associated with measurable residual disease at remission. (A) Receiver operating characteristic (ROC) curve comparing the performance of 2 logistic regression models for predicting measurable residual disease (MRD). The dashed curve represents the model without LSC17 score, accounting for age, European LeukemiaNet (ELN) 2022 risk group (favorable, intermediate as baseline, or adverse) and acute myeloid leukemia (AML) type (*de novo* as baseline *vs*. AML with antecedent hematologic disorder [AHD] or therapy-related AML [t-AML]) as predictors (area under the curve [AUC] =0.734). The solid curve represents the model with LSC17 score included (AUC=0.804). *P* value, likelihood ratio test. (B) Plot demonstrating the relative importance of variables included in the MRD prediction model corresponding to the solid line in Figure 2A.

S11), with the LSC17 score having the highest HR (*P*=0.02; HR=3.07; 95% CI: 1.20-7.84).

Allogeneic stem cell transplantation improves survival of patients with a high LSC17 score

aSCT is used to treat AML patients with a high risk of relapse based on current risk classification. In our cohort of intensively-treated patients, 30 of 79 (38%) low LSC17 score patients who achieved CR and 58 of 89 (65%) high score patients proceeded to aSCT. Inclusion of aSCT as a time-dependent covariable (Mantel-Byar analysis with univariable Cox regression analysis) did not demonstrate a significant impact of aSCT on OS for patients with a low LSC17 score (Figure 4A; *P*=0.38; HR=0.67; 95% CI: 0.28-1.63). In contrast, patients with a high LSC17 score had significantly improved OS following aSCT (Figure 4B; *P*=0.005; HR=0.43; 95% CI: 0.24-0.78). These findings were also true in the subgroup of patients over the age of 60 (*Online Supplementary Figure S6*).

In multivariable survival analysis using time-dependent Cox regression models with and without the LSC17 score, aSCT was strongly protective and AHD/t-AML was associated with worse OS in both models (Table 3). The LSC17 score was strongly prognostic (*P*=0.01; HR= 3.7; 95% CI: 1.36-10.02) and addition of the score significantly improved the predictive model (*P*=0.01; likelihood ratio test). Furthermore, the borderline statistical significance of ELN risk groups was further reduced when the LSC17 score was added, suggesting that

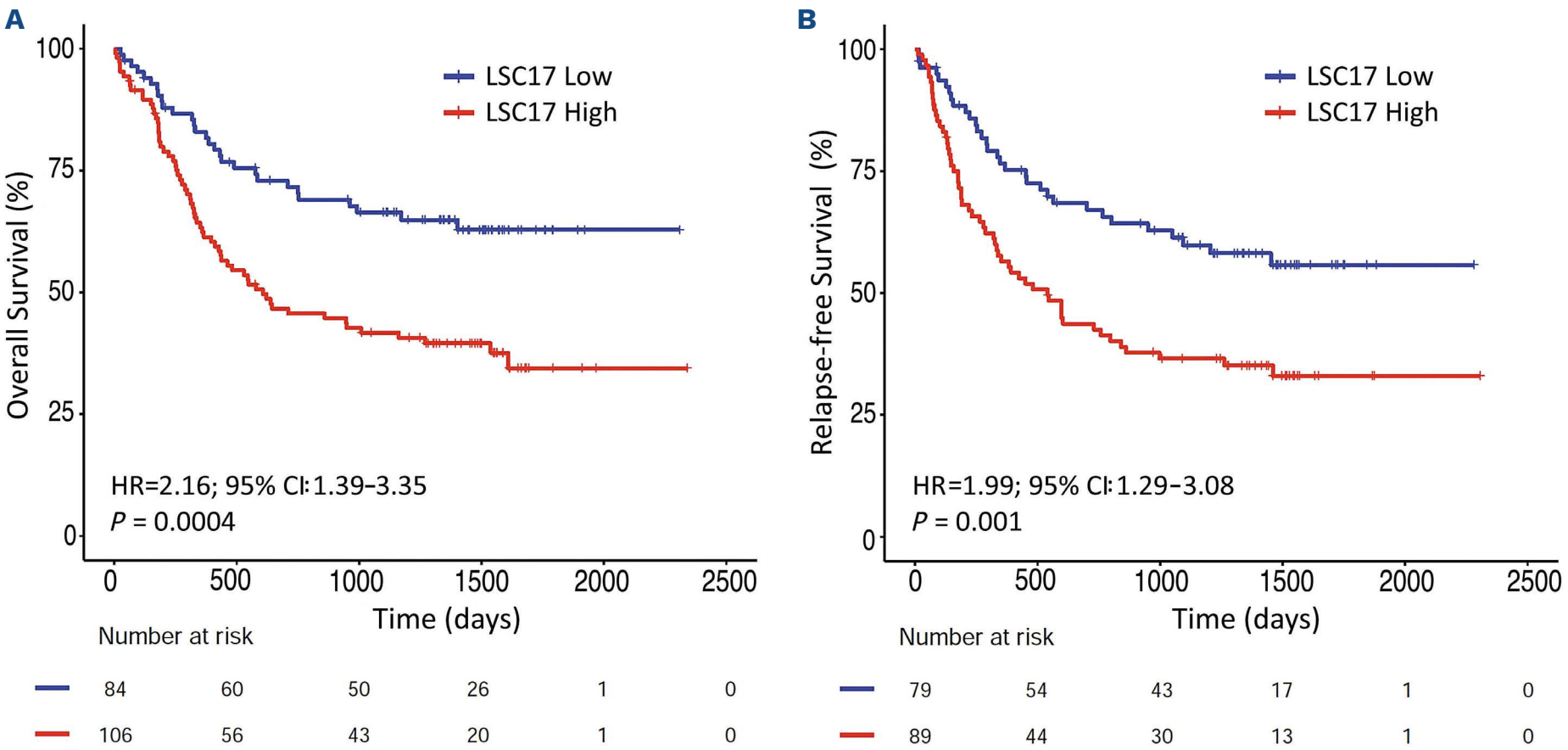

Figure 3. A high LSC17 score is associated with shorter survival in intensively-treated patients. Kaplan-Meier estimates of overall survival (N=190) (A) and relapse-free survival (N=168) (B) of acute myeloid leukemia patients who received induction chemotherapy, according to LSC17 scores measured using the clinical NanoString assay and classified by comparison to the reference score. In both panels, blue and red lines show patients with low and high LSC17 scores, respectively. HR: hazard ratio; CI: confidence interval.

Table 2. Multivariable analysis of overall survival.

Variable	Without LSC17 score			With LSC17 score		
	HR	95% CI	*P*	HR	95% CI	*P*
LSC17 score	-	-	-	3.51	1.47-8.36	0.005**
Age	1.03	1.01-1.05	0.006**	1.03	1.01-1.05	0.005**
WBC count	1.00	1.00-1.00	0.23	1.00	1.00-1.00	0.40
ELN 2022 favorable	0.78	0.39-1.56	0.48	1.06	0.51-2.20	0.87
ELN 2022 adverse	1.97	1.15-3.39	0.01*	1.67	0.97-2.89	0.07
AHD/t-AML	1.49	0.93-2.38	0.10	1.56	0.97-2.50	0.07

HR: hazard ratio; CI: confidence interval; WBC: white blood cell; ELN: European LeukemiaNet; AML: acute myeloid leukemia; AHD: AML with antecedent hematologic disorder; t-AML: therapy-related AML. *P* value calculated using the Wald test. *$P<0.05$; **$P<0.01$.

the score captures some of the risk information explained by ELN risk categories.

Discussion

This prospective study validates the robust prognostic value of the stemness-based LSC17 score as a clinical tool for predicting treatment response, MRD, and survival in AML patients. Previous studies examining the prognostic value of the LSC17 score have all involved retrospective cohort analyses with classification of high *versus* low scores based on a median split. Importantly, this is the first study in which unselected newly-diagnosed AML patients were classified as having a high or low LSC17 score based on comparison to an established reference score, a requirement for real-world application in the management of AML patients. Prospective validation of this clinical tool now enables upfront risk assessment and identification of high-risk patients who may benefit from alternative therapeutic approaches.

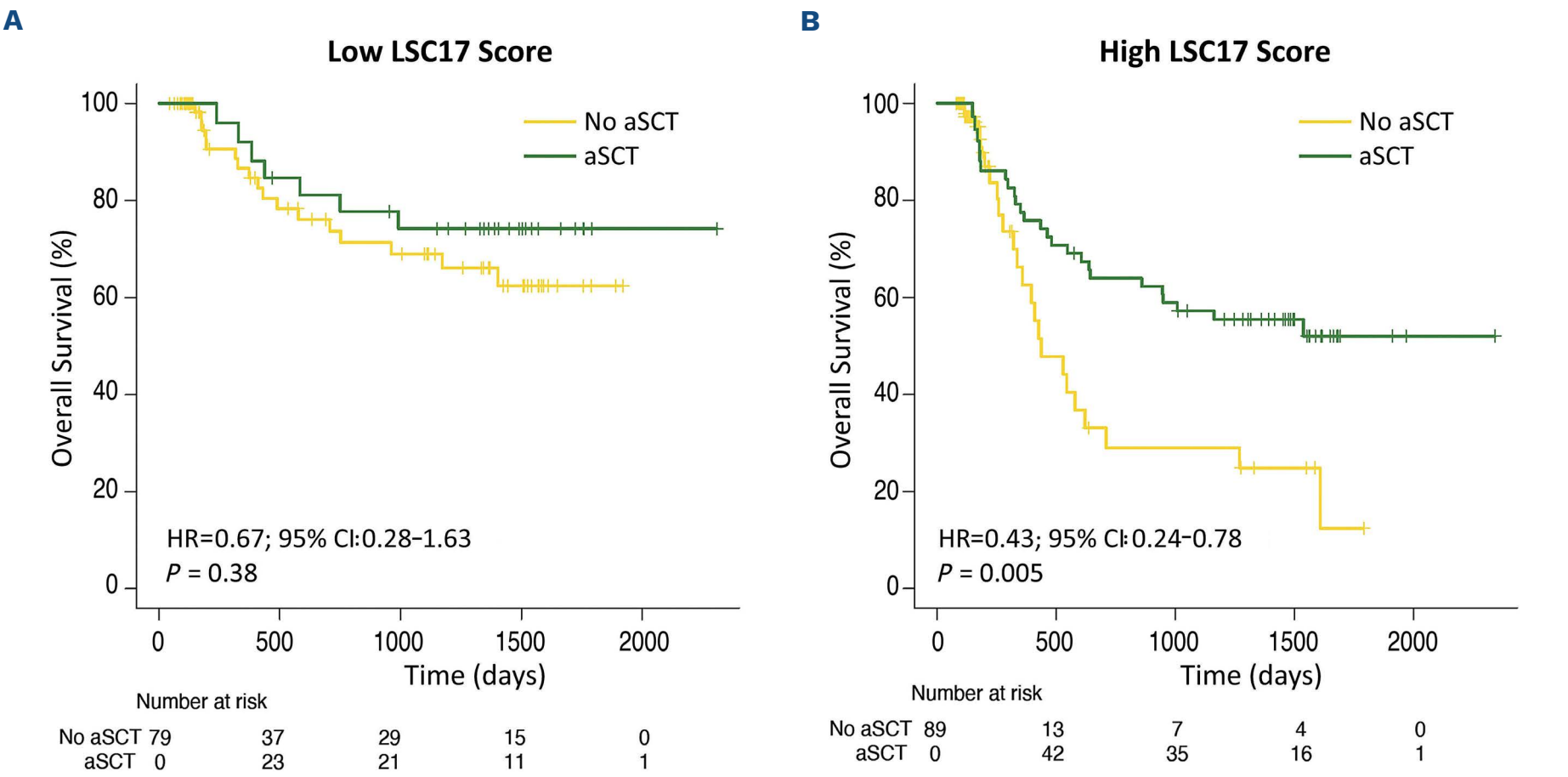

Figure 4. Patients with a high LSC17 score benefit from allogeneic stem cell transplantation. Simon and Makuch estimates of overall survival according to whether or not patients underwent allogeneic stem cell transplantation (aSCT), for patients with low (A) and high (B) LSC17 scores. In both panels, green and yellow lines show patients who did and did not undergo aSCT, respectively; HR: hazard ratio; CI: confidence interval.

Table 3. Multivariable analysis of overall survival including allogeneic stem cell transplantation as a time-dependent covariable.

Variable	Without LSC17 score			With LSC17 score		
	HR	95% CI	*P*	HR	95% CI	*P*
LSC17 Score	-	-	-	3.70	1.36-10.02	0.01*
aSCT	0.44	0.25-0.76	0.003**	0.40	0.23-0.69	0.001**
Age	1.02	1.00-1.04	0.14	1.02	0.99-1.04	0.18
WBC count	1.00	1.00-1.00	0.40	1.00	1.00-1.00	0.49
ELN 2022 favorable	0.49	0.23-1.05	0.07	0.66	0.30-1.48	0.32
ELN 2022 adverse	1.71	0.95-3.07	0.08	1.46	0.81-2.64	0.21
AHD/t-AML	2.14	1.24-3.69	0.006**	2.27	1.31-3.93	0.003**

HR: hazard ratio; CI: confidence interval; aSCT: allogeneic stem cell transplantation; WBC: white blood cell; ELN: European LeukemiaNet; AML: acute myeloid leukemia; AHD: AML with antecedent hematologic disorder; t-AML: therapy-related AML. *P* value calculated using the Wald test. *$P<0.05$; **$P<0.01$.

The LSC17 score differentiated survival outcomes most significantly in the intermediate risk group of the updated ELN 2022 classification, suggesting that the LSC17 score will be particularly valuable in guiding treatment decisions in this heterogeneous group of patients that typically represents approximately 30-40% of AML cohorts. The LSC17 score did not differentiate outcomes in the favorable or adverse ELN 2022 risk groups, although there was a trend towards worse outcomes in high-score patients in the adverse risk group. Additional studies with larger numbers of patients are needed to assess the impact of the LSC17 score in these risk groups. Nevertheless, multivariable analysis demonstrated that the LSC17 score provides prognostic value independent of existing risk classifiers including the ELN 2022 risk classification. Additionally, the LSC17 score is the strongest variable by far for predicting the presence of MRD following induction chemotherapy. Patients with a high LSC17 score were less likely to achieve MRD-negative remission than patients with a low LSC17 score; this was observed even in the subset of patients that underwent induction with FLAG-Ida despite similar initial response rates among patients with high and low LSC17 scores, highlighting the need for better upfront therapies for high-risk patients.

In the current study, patients with a high LSC17 score who underwent aSCT had significantly improved OS compared to those who did not, while low-score patients had similar outcomes regardless of aSCT. This result contrasts with our prior study in which high-score patients did not benefit and low score patients had worse outcomes following aSCT.[8] These differences likely reflect improvements in conditioning regimens and supportive care over time and underscore the importance of ongoing evaluation of prognostic tools in the context of evolving therapies. The survival benefit of aSCT was also observed in the subset of high-score patients over the age of 60. Thus, measurement of the LSC17 score can aid in decisions regarding whether or not to proceed with aSCT in this older demographic where the potential benefits must be weighed against the frequent presence of comorbidities. Notably, patients with a low LSC17 score had better outcomes in this study than patients with a high score even with aSCT, reinforcing the strong prognostic association of the score with survival. Future studies will be needed to determine whether more intensive or alternative induction regimens can improve outcomes post-aSCT in patients with high LSC17 scores.

In this prospective feasibility study, the LSC17 score was not revealed to the treating physicians, which precluded incorporation of the score into real-time decision making. Future studies are needed to assess the impact of LSC17-informed treatment decisions on patient outcomes, including the selection of standard induction *versus* alternative treatments and recommendations for aSCT. Additionally, incorporation of the LSC17 score as a correlative test in prospective clinical trials of novel therapies will aid in identifying those that are effective in reducing MRD and improving outcomes in high-risk patients.

The LSC17 has been demonstrated by several independent groups in retrospective analyses to provide robust risk assessment in both pediatric and adult AML. We previously showed that the protocol for the CLIA laboratory-developed NanoString-based LSC17 assay is easily established with highly reproducible results across independent laboratories, facilitating broad implementation.[13] Prospective validation of its prognostic value in individual newly-diagnosed AML patients now paves the way for incorporation of this tool into routine clinical practice to enable more effective risk-adapted treatment strategies to improve outcomes.

Disclosures

ADS has received research funding from Takeda Pharmaceuticals, BMS and Medivir AB; and consulting fees/honorarium from Takeda, Novartis, Jazz, and Otsuka Pharmaceuticals. ADS is named on a patent application for the use of DNT cells to treat AML. ADS is a member of the Medical and Scientific Advisory Board of the LLS of Canada. BL received consulting fees and honoraria from AbbVie, Alexion, AMGEN, Astellas, Astex, BMS/Celgene, Jazz, Janssen Novartis, Otsuka, Paladin, Pfizer, Roche, Treadwell, Gilead/KITE and Servier. There is an existing license agreement between Pfizer and UHN, and JCYW may be entitled to receive financial benefits from this license and in accordance with UHN's intellectual property policies. The remaining authors have no conflicts of interest to disclose.

Contributions

The study was designed by TM, SMC, and JCYW. LSC17 score assays were performed and analyzed by TZ, IK, JMCC, ZL and TLS. MRD analysis was completed by AT. VK, DM, CJM, MDM, ADS, ACS, HS, KWLY, DK, CR, BL, MS and SMC were involved in patient recruitment and clinical management. TM, BZ and SWKN carried out statistical analysis. TM, SWKN and JCYW wrote the manuscript. JCYW and SWKN supervised the study.

Acknowledgments

The research team would like to acknowledge patients and staff of all the treating centers for their contribution to this study.

Data-sharing statement

Detailed information for individual patients including clinical data, calculated LSC17 scores, treatment and mutational profiles has been provided in the Online Supplementary Appendix.

References

1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136-1152.
2. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36(7):1703-1719.
3. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.
4. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209-2221.
5. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
6. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328-333.
7. Zeng AGX, Bansal S, Jin L, et al. A cellular hierarchy framework for understanding heterogeneity and predicting drug response in acute myeloid leukemia. Nat Med. 2022;28(6):1212-1223.
8. Ng SWK, Mitchell A, Kennedy JA, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. Nature. 2016;540(7633):433-437.
9. Vasseur L, Fenwarth L, Lambert J, et al. LSC17 score complements genetics and measurable residual disease in acute myeloid leukemia: an ALFA study. Blood Adv. 2023;7(15):4024-4034.
10. Bill M, Nicolet D, Kohlschmidt J, et al. Mutations associated with a 17-gene leukemia stem cell score and the score's prognostic relevance in the context of the European LeukemiaNet classification of acute myeloid leukemia. Haematologica. 2020;105(3):721-729.
11. Kim DDH, Novitzky Basso I, Kim TS, et al. The 17-gene stemness score associates with relapse risk and long-term outcomes following allogeneic haematopoietic cell transplantation in acute myeloid leukaemia. EJHaem. 2022;3(3):873-884.
12. Duployez N, Marceau-Renaut A, Villenet C, et al. The stem cell-associated gene expression signature allows risk stratification in pediatric acute myeloid leukemia. Leukemia. 2019;33(2):348-357.
13. Ng SW, Murphy T, King I, et al. A Clinical laboratory-developed LSC17 stemness score assay for rapid risk assessment of acute myeloid leukemia patients. Blood Adv. 2021;6(3):1064-1073.
14. Spiegel JY, McNamara C, Kennedy JA, et al. Impact of genomic alterations on outcomes in myelofibrosis patients undergoing JAK1/2 inhibitor therapy. Blood Adv. 2017;1(20):1729-1738.
15. Thomas M, Sukhai MA, Zhang T, et al. Integration of technical, bioinformatic, and variant assessment approaches in the validation of a targeted next-generation sequencing panel for myeloid malignancies. Arch Pathol Lab Med. 2017;141(6):759-775.
16. Jafari K, Tierens A, Rajab A, Musani R, Schuh A, Porwit A. Visualization of cell composition and maturation in the bone marrow using 10-color flow cytometry and radar plots. Cytometry B Clin Cytom. 2018;94(2):219-229.
17. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol. 2003;21(24):4642-4649.
18. Gray R. cmprsk: Subdistribution analysis of competing risks. R package version 2.2-12 (2024), https://CRAN.R-project.org/package=cmprsk. Accessed on April 17, 2025.
19. Mantal N, Byar DP. Evaluation of response-time data involving transient states: an illustration using heart-transplant data. J Am Stat Assoc. 1974;69(345):81-86.
20. Andersen PK, Gill RD. Cox's regression model for counting processes: a large sample study. Ann Stat. 1982;10(4):1100-1120.
21. Simon R, Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurrence of an event: application to responder versus non-responder bias. Stat Med. 1984;3(1):35-44.
22. Kanda, Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.
23. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model statistics for biology and health. Springer New York; 2000.
24. Robin X, Turck N, Hainard A et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77.
25. Walter RB, Othus M, Burnett AK, et al. Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center. Leukemia. 2015;29(2):312-320.