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A 6-gene leukemic stem cell score identifies high risk pediatric acute myeloid leukemia

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Abstract

Recently, mRNA-expression signature enriched in LSCs was used to create a 17-gene leukemic stem cell (LSC17) score predictive of prognosis in adult AML. By fitting a Cox-LASSO regression model to the clinical outcome and gene-expression levels of LSC enriched genes in 163 pediatric participants of the AML02 multi-center clinical trial (NCT00136084), we developed a 6-gene LSC score of prognostic value in pediatric AML (pLSC6). In the AML02 cohort, the 5-year event-free survival (EFS) of patients within low-pLSC6 group (n=97) was 78.3 (95% CI=70.5–86.9%) as compared to 34.5(95% CI=24.7–48.2 %) in patients within high-pLSC6 group (n=66 subjects), p<0.00001. pLSC6 remained significantly associated with EFS and overall survival (OS) after adjusting for induction 1-MRD status, risk-group, *FLT3*-status, WBC-count at diagnosis and age. pLSC6 formula developed in the AML02 cohort was validated in the pediatric AML-TARGET project data (n=205), confirming its prognostic value in both single-predictor and multiple-predictor Cox regression models. In both cohorts, pLSC6 predicted outcome of transplant

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patients, suggesting it as a useful criterion for transplant referrals. Our results suggest that pLSC6 score holds promise in redefining initial risk-stratification and identifying poor risk AML thereby providing guidance for developing novel treatment strategies.

Introduction

Resistant and relapsed disease remain the most prevalent forms of failure in both pediatric and adult AML. Persistence of leukemic stem cells (LSCs) is a primary cause of AML relapse(1, 2). LSCs are also associated with drug resistance. Thus, there is an unmet and urgent need to identify and quantify LSCs to predict prognosis and improve risk classification in AML.

Recently, Ng et al, 2016(3) identified 48 genes that were over-expressed in LSC enriched cell population (LSC positive) as compared to LSC-negative cell fractions. A sparse Cox model regression analysis of the mRNA expression of 43 of the 48 LSC enriched genes with survival data from adult AML patients was used to develop and validate a 17-gene stemness score (LSC17) that was predictive of outcome in adult AML patients(3).

The LSC17 was developed using adult outcome data and though the LSC enriched genes may overlap between adult and pediatric AML, the prognosis of pediatric AML is typically much better than that of adult AML(4). In this study, we applied the regression modeling to the mRNA expression of LSC enriched genes and outcome data for the multicenter pediatric AML02 clinical trial (NCT00136084(5)) and developed a six-gene LSC score for pediatric AML (pLSC6). We confirmed the predictive power of pLSC6 in a separate cohort of pediatric AML patients with publicly available data from the pediatric AML TARGET project (https://ocg.cancer.gov/programs/target/target-publication-guidelines).

Materials and methods

Patients

The pediatric AML LSC score was defined using data from 163 patients treated on the multicenter AML02 clinical trial (ClinicalTrials.gov Identifier: NCT00136084) with Affymetrix U133A microarray gene expression data obtained from diagnostic bone marrow specimens (Supplementary Table S1). Details of study design and clinical outcome were described elsewhere(5). Patients with t[8;21], inv[16], or t[9;11] chromosome abnormalities were classified as low-risk AML. High-risk AML classification included presence of −7, *FLT3*-ITD mutation, t[6;9], acute megakaryoblastic leukemia (AMKL), treatment-related AML, or AML arising from MDS. Absence of low or high-risk group features was classified as standard-risk AML. Patients were randomized to receive high (3 g/m^2, given every 12 h on days 1, 3 and 5) or low dose (100 mg/m^2 given every 12 h on days 1–10) cytarabine along with daunorubicin and etoposide as a first course of chemotherapy with subsequent treatment tailored to response and risk classification. MRD positivity was defined as one or more leukemic cell per 1000 mononuclear bone-marrow cells (i.e., ≥0.1%). Event free survival (EFS) was defined as the time from study enrollment to induction failure, relapse, secondary malignancy, death, or study withdrawal for any reason, with event-free patients

censored on the date of last follow-up. OS was defined as the time from study enrollment to death, with living patients censored on the date of last follow-up(5). St. Jude Institutional Review Board approved the study, and informed consent was obtained from parents/guardians and consents/assents from the individuals.

The validation cohort included 205 patients from Children's Oncology Group (COG) AAML0531(6) (NCT00372593) and AAML03P1 (NCT0070174) protocols(7) with RNA seq and clinical outcome data available through the TARGET project database (https://ocg.cancer.gov/programs/target/target-publication-guidelines).

Gene Expression Profiling

Gene expression profiling of leukemic blasts obtained at diagnosis in the AML02 discovery cohort was performed using GeneChip® Human Genome U133A [Affymetrix, Santa Clara, CA] as described previously(8). The MAS 5.0 algorithm was used to obtain normalized gene expression signals. All the gene expression data was $\log_2$ transformed before analysis. For the validation cohort, we downloaded publicly available RPKM (Reads per kilo base of transcript per million mapped reads data from TARGET database. We included 205 patients from AAML0531 and AAML03P1 clinical trials with gene expression data available from diagnostic specimens (RNAseq data from specimen obtained at relapse were not included in this analysis). We used log2(RPKM+1) values for subsequent statistical analysis, it should be noted that TARGET dataset was enriched for patients with poor outcome.

Development of Pediatric LSC score signature

Figure 1 illustrates the overall study design and implementation. Among the 48 LSC enriched genes previously identified by Ng and colleagues(3), 32 were represented in the AML02 U133A microarray expression data set. We fit a least absolute shrinkage and selection operator (LASSO) Cox regression model, as implemented in glmnet package of the R3.4.1 statistical software (www.r-project.org), to the expression of 32 genes and the event-free survival data of the AML02 study. LASSO regression penalizes the data fitting criteria in a way that eliminates less informative predictor variables to yield simpler and more interpretable models. To evaluate the variability and reproducibility of the LASSO Cox regression model estimates, we repeated the LASSO Cox regression fitting process for each of 1,000 leave-10%-out cross-validation evaluations. We chose to retain genes with non-zero coefficient estimates in at least 950 of these 1,000 evaluations. For each of these genes, the final model coefficient was the average of the coefficient estimates obtained for the set of cross-validation evaluations. We further utilized recursive partitioning survival model, as implemented in the rpart package, to dichotomize pLSC6 scores into "low" and "high" score groups to simplify reporting and graphing the association of pLSC6 with survival outcomes.

Statistical analysis

Survival analyses were performed using survival and survminer packages in R3.4.1. Event-free survival (EFS) and overall survival (OS) probabilities were estimated using the Kaplan-Meier method and Cox proportional hazard models was used to compare the survival curves of patients within pLSC6 score groups as well as the association between each individual prognostic factor and survival outcomes. Multivariable Cox proportional hazard model was

used to evaluate the independent prognostic effect of the study covariates. Firth-penalized Cox regression was used to avoid monotone likelihoods and stabilize results for some analyses with small sample sizes as described in the supplementary note 1 (9, 10). Wilcoxon rank-sum or Kruskal-Wallis tests was used for continuous variable comparisons between/among patient subgroups. Chi-square or fisher exact tests were used for testing association between categorical variables. A bootstrap procedure(11), described in detail in the supplementary note 2, was used to compute a confidence interval for a ratio of hazard ratios (RHR) statistic comparing the strength of association of survival with LSC6 scores to that of LSC17. The evaluation of pLSC6 score was performed as a continuous variable as well as after dichotomization for showing the survival curves. All statistical analyses were performed using R software (www.r-project.org). The modified R script codes are available at GitHub (https://github.com/Abdelrahman-Elsayed/kit-nfold-cv-glmnet/blob/master/kit-nfold-cv-glmnet-v0.R).

Results

Expression of six leukemic stemness genes defines a LSC6 score of prognostic value

We found that 32 of 48 genes that Ng et al(3) identified as over-expressed in LSCs were represented on the U133A microarray mRNA expression array. LASSO Cox regression model was used to model EFS with mRNA expression data (32 LSC genes) as predictors in 163 pediatric AML patients (model-development cohort) treated on AML02(5). Six genes were identified as important in at least 950 of 1,000 leave-10%-out cross-validation replications of this analysis (Supplementary Figure 1). This rigorous model-development process defined a six-gene pediatric LSC score (pLSC6) which was computed for each patient using gene expression weighted by the regression coefficients as defined in the equation pLSC6 = (DNMT3B x 0.189)+(GPR56 × 0.054)+(CD34 × 0.0171)+(SOCS2 × 0.141)+ (SPINK2 × 0.109)+(FAM30A × 0.0516).

Each unit increase in pLSC6 was associated with a 4.34-fold increase in the rate of EFS events ($p < 0.00001$, 95% CI = 2.58–7.31) in a simple single-predictor Cox regression model. Recursive-partitioning Cox regression model was used to dichotomized pLSC6 with patients classified into low-pLSC6 score group (n=97 patients, 60%) or high-pLSC6 score group (n= 66 patients; 40%). Comparison of patient characteristics between low-pLSC6 and high-pLSC6 groups within AML02 is provided in Supplementary Table S2, initial risk group assignment, cytogenetics and *FLT3* status demonstrated significant difference by pLSC6 group classification.

The five-year EFS of patients with low-pLSC6 score was 78.3 (95% CI = 70.5–86.9) while that of patients with high-pLSC6 score was 34.5% (95% CI = 24.7–48.2); HR=4.14 (95% CI=2.46–6.98; $p<0.0001$, Figure 2A). Further high-pLSC6 score was predictive of inferior OS in AML02 cohort (HR=5.18, 95% CI=2.67–10.1; $p<0.0001$, Figure 2B).

In subset of patients (n=55), RNAseq data was available, we computed the pLSC6 score with the RNA-seq data using the coefficients defined above. The RNA-seq PLSC6 score strongly correlated with the U133A_pLSC6 score (Spearman R = 0.591; $p = 3.24 \times 10^{-6}$; Supplementary Figure 2A). RT-PCR based quantification on subset of patients (n=14) within

low and high pLSC6 score groups also demonstrated significant correlation between the pLSC6 derived using U133 or RT-PCR (Spearman R=0.82; p=0.00029).

Validation of pLSC6 as a Prognostic Score

To validate the prognostic value of pLSC6 in pediatric AML, we used the equation defined above to compute pLSC6 values in an independent cohort of 205 pediatric AML TARGET patients with clinical outcome and mRNA-seq expression data (model-validation cohort). We noted that the distribution of pLSC6 values for the TARGET model-validation cohort had a very similar shape as that of the pLSC6 values for the AML02 model-discovery cohort (Supplementary Figure 3; QQ plot). In a simple single-predictor cox model fit to the TARGET validation-cohort data, each unit increase in pLSC6 associated with a 1.91-fold increase in the rate of EFS failure events ($p < 0.0001$; 95% CI = 1.48–2.46). Recursive partitioning resulted in similar dichotomization of the TARGET as observed in AML02 cohort with 60% of patients (n= 126) patients within in low-pLSC6 group and 40% of patients (n=79) classified into high-pLSC6 group (patient characteristics by LSC6 group summarized in Supplementary Table S2). In the TARGET cohort, the five-year EFS of those with low-pLSC6 was 49.2 (95% CI = 41.1 – 58.9), compared with 13.7 (95% CI = 7.85 – 23.95) for those with high-pLSC6 (HR=2.86, 95% CI=2.02–4.04, $p<0.0001$, Figure 2C). Patients within high-pLSC6 also demonstrated inferior OS as compared to low-pLSC6 group within Target cohort (HR=2.81, 95% CI=1.85–4.28; $p<0.0001$, Figure 2D). Table 1. provides summary of univariate Cox regression results for association between study covariates with event free survival (EFS) and overall survival (OS) in AML02 the model-development and TARGET the model-validation cohorts.

pLSC6 is an independent prognostic factor in the AML02 and TARGET cohorts

We found that pLSC6 provided prognostic information beyond that provided by minimal residual disease (MRD) and molecular risk classification in both the AML02 and TARGET cohorts. pLSC6 differed across molecular risk classification ($p<0.0001$ in both cohorts; Supplementary Figure 4) and was strongly associated with MRD in both cohorts (AML02 cohort, $p <0.0001$, and Target cohort, $p =0.001$; Figure 3A and 3B respectively). Nevertheless, pLSC6 provided additional prognostic information beyond that available from these two factors widely used in clinical practice. In the AML02 cohort, the five-year EFS was 80.8 ± 4.6% in MRD- patients with low-pLSC6 score; 58.8 ± 11.9% in MRD+ patients with low- pLSC6 score, 55 ± 11.1% in MRD- patients with high-pLSC6 score, and 24.1 ± 6.4% in MRD+ patients with high-pLSC6 scores. A Cox model with dichotomized pLSC6 score and MRD as predictors found that high-pLSC6 score is associated with a 2.67-fold increased rate of EFS failure (95% CI= 1.48- 4.81, $p = 0.001$) and 3.31-fold increased rate of death (95% CI = 1.58- 6.97, $p = 0.0015$) relative to low-pLSC6 score in the AML02 cohort holding MRD constant. A similar model found high-pLSC6 score associated with a 2.38-fold increase in EFS failure rate (95% CI = 1.64–3.46, $p < 0.0001$) and 2.72-fold increase in death rate (95% CI = 1.71–4.36, $p < 0.0001$) in the TARGET cohort. Figure 3 shows EFS and OS in both AML02 (Figure 3C and 3D) and Target cohorts (Figure 3E and 3F) by pLSC6 and MRD status. These results indicate that pLSC6 provides additional prognostic information not captured by MRD.

pLSC6 also provides prognostic information not captured by molecular risk classification. Within each risk group in both cohorts, high-pLSC6 score patient had worse prognosis than low-pLSC6 score patients. In the AML02 cohort, Cox models with dichotomized pLSC6 score and molecular risk group (low, standard and high) found that high-pLSC6 score associated with worse EFS (HR = 3.45; 95 CI = 1.83–4.51; p = 0.0001) and OS (HR = 3.93; 95%CI = 1.78 – 8.72; p = 0.0007). In the TARGET cohort, similar results were obtained for EFS (HR = 2.16; CI = 1.47 – 3.17; p < 0.0001) and OS (HR = 2.03; CI = 1.26 – 3.26; p = 0.004). Of special interest, pLSC6 is significantly associated with EFS in standard risk patients of the AML02 cohort (HR= 2.86; 95% CI=1.29 – 6.33, p=0.009) and of the TARGET cohort (HR= 2.04; 95% CI=1.28 – 3.24, p=0.002), Figure 4. Thus, pLSC6 may help to better risk-stratify standard risk patients.

Even after adjusting for risk group, MRD, *FLT3* status, diagnostic WBC count, and age, dichotomized pLSC6 remained an independent predictor of worse EFS and OS in both cohorts (Figure 5).

Finally, pLSC6 was significantly associated with outcome within four of the five major treatment arms represented in the AML02 and TARGET data sets. Single-predictor Cox regression models indicate that each unit increase of the pLSC6 score associated with worse EFS in the low-dose ara-C arm of AML02 (HR = 4.15, 95% CI: 2.02, 8.52; p = 0.0001), the high-dose ara-C arm of AML02 (HR = 4.54; 95% CI: 2.05, 10.06; p = 0.0002), the AAML03P1 protocol (HR = 2.1; 95% CI: 1.10, 4.02; p = 0.025), and the standard arm of AAML0531 in the TARGET cohort (HR = 2.02; 95% CI: 1.31, 3.14;p p = 0.0016). In the GO arm of AAML0531, each unit increase in pLSC6 showed a non-significant association with worse EFS (HR=1.36; 95% CI: 0.86, 2.14; p = 0.18). Similar results were obtained for OS. Each unit increase in pLSC6 score was significantly associated with worse OS in the low-dose ara-C arm of AML02 (HR = 4.3; 95% CI = 1.87, 10.04; p = 0.0006), the high-dose ara-C arm of AML02 (HR = 7.07, 95% CI: 2.55–19.62; p = 0.0002), and the standard arm of the AAML0531 protocol (HR = 1.76, 95% CI: 1.03, 2.98; p = 0.037). Each unit increase in pLSC6 had a marginally significant association with worse OS in the GO arm of AAML0531 (HR = 1.64, 95% CI: 0.96, 2.79; p = 0.069) and the AAML03P1 protocol (HR = 2.3, 95% CI: 0.99, 5.38; p = 0.053).

pLSC6 may identify the best candidates for transplant

Among standard and high-risk patients of both the AML02 and TARGET cohorts, transplant was associated with better outcomes compared to chemotherapy alone for patients with low-pLSC6 score, but transplant and chemotherapy alone showed similarly dismal outcomes for patients with high-pLSC6 score (Figure 6A–D). Among low-pLSC6 score patients, transplant was associated with a statistically suggestive and clinically substantial improvement in EFS in the AML02 cohort (HR = 0.18; 95% CI: 0.001, 1.40; p = 0.12) and an improvement that was both clinically substantial and statistically significant in the TARGET cohort (HR = 0.14; 95% CI: 0.015,0.54; p = 0.002). Also, among low PLSC6 score patients, those with transplants had notably better OS in the AML02 cohort (HR = 0.30; 95% CI: 0.002, 2.52; p = 0.34) and significantly better OS in the TARGET cohort (HR = 0.28; 95% CI: 0.03, 1.15; p = 0.08). In contrast, our data indicates that transplant may not

provide a clinical benefit for patients with high-pLSC6 scores. Among AML02 patients with high-pLSC6 scores, Cox regression modeling found that transplant had a non-significant association with slightly *worse* EFS (HR = 1.22, 95% CI: 0.6, 2.44; p = 0.56) and OS (HR = 1.43, 95% CI: 0.71, 2.85; p = 0.30; Supplementary Note 1). Likewise, among TARGET patients with high-LSC6 scores, Cox regression modeling found that transplant had a non-significant association with slightly *worse* EFS (HR = 1.08, 95% CI: 0.50, 2.14; p = 0.83) and OS (HR = 1.16; 95% CI: 0.48, 2.52; p = 0.72; Supplementary Note 1). These results suggest that pLSC6 may identify patients who are most likely to benefit from transplant. However, before pLSC6 is used to identify transplant candidates in clinical practice, additional research is needed to confirm that it is predictive of transplant outcomes and establish the assay and threshold to be used to identify patients that would benefit from transplant.

Comparison of pLSC6 with LSC17

We further performed quantitative comparison between pLSC6 and LSC17 as predictors of EFS and OS in the pediatric AML TARGET cohort by computing 95% bootstrap confidence intervals (95% BCI) for the ratio of hazard ratios (RHR; Supplementary Note 2) expressed as the hazard ratio for the interquartile range of pLSC6 relative to the hazard ratio for the interquartile range of LSC17. RHR=1 indicates that pLSC6 and LSC17 have the same strength of association with the survival outcome; RHR>1 indicates that pLSC6 has a stronger association with survival than LSC17; and RHR<1 indicates that pLSC6 has a weaker association with survival than LSC17. In the TARGET cohort, the association of pLSC6 with EFS was 1.21 times stronger than the association of pLSC17 with EFS (RHR=1.21; 95% BCI = 0.95, 1.57). pLSC6 and LSC17 had a similar strength of association with OS (RHR = 1.18; 95% BCI = 0.90, 1.56).

Additionally, within the TARGET cohort, though LSC17 was not significantly associated with induction 1 MRD (p=0.44), pLSC6 was significantly associated with induction 1 MRD (p<0.0001), Supplementary Figure 5. Although these results suggest that pLSC6 may be a better predictor of EFS for pediatric AML than LSC17, this comparison is confounded by the cohorts included. Given the limitation of this retrospective analysis, further research is needed to more precisely compare the performance of these two scores before establishing a simpler predictor that may be useful in clinical practice.

Discussion

Unique properties of leukemic stem cells (LSCs) such as quiescence, self-renewal and chemo-resistance contributes to relapse and treatment failure in AML patients. Though efforts for identification and quantification for LSCs has been a prime area of research to advance AML treatment strategies, this is still not used in clinical setting(12–15). Recently Ng et al(3), defined and validated prognostic value of a leukemic stemness score derived from expression levels of 17 genes (LSC17) score in adult AML patient cohorts(3). This LSC17 signature demonstrated potential for risk stratification and identification for high risk adult AML patients. A follow-up study in the pediatric AML cohort- ELAM02, with the assumption that pediatric and adult LSCs share common gene expression programs further

demonstrated prognostic value of LSC17(16). Future studies directed towards generation of gene-expression profiles in fraction of pediatric $CD34^{+}CD38^{-}$ LSC cells is warranted to further refine the gene-expression score specific to pediatric AML.

Given that cure rates with standard intensive chemotherapy vary between pediatric vs. adult AML patients(4) in the present study, we utilized the genes reported to be enriched in the LSC+ cell fraction(3) and applied regression model against event free survival (EFS) in a pediatric AML cohort-AML02, the model-generation cohort. A six-gene LSC score of prognostic significance to pediatric AML was defined and designated as pLSC6. pLSC6 though had different coefficients as compared to the original LSC17 score equation, shared 4 genes DNMT3B, GPR56, CD34 and SOCS2 with LSC17. Two new genes, SPINK2 and FAM30A were part of the pLSC6 score. Of these genes DNMT3B codes for DNA methyltransferase B and its prognostic and biological significance has been previously established in adult AML(17) and recently our results demonstrate its prognostic value in pediatric AML(18). Specifically, we have shown that DNMT3B methylation levels correlate with its expression levels which was further associated with global methylation burden of leukemic cells. Further, we observed that DNMT3B expression, methylation and genome-wide methylation burden was significantly associated with survival in pediatric AML(18). GPR56, a G protein coupled receptor has been established as a novel LSC marker and high-GPR56 levels have been shown to associate with poor clinical outcomes in AML patients(19). GPR56 has been also been associated with HOXA9 induced leukemeogenesis(20) as well as is a marker of EVI1-high-AML(21). Among other genes, CD34 a cell surface antigen is a marker for hematopoietic stem cells and hematopoietic progenitor cells. SOCS2 codes for suppressor of cytokine signaling-2, which negatively regulates cytokine signaling and has been associated with poor outcome as well as poor risk group features in pediatric children's oncology group AAML03P1 cohort(22). SPINK2, codes for a serine-proteinase inhibitor Kazal-type 2, its role in AML or cancer is not yet established. However, high SPINK2 expression has been reported in leukemia cell lines and it is speculated that SPINK2 potentially interacts with cancer related proteinase essential for tumor progression(23). FAM30A is an RNA gene affiliated with a non-coding RNA and at present its role in AML is not known.

We acknowledge that generation of enriched gene expression profile from pediatric LSC would be most appropriate for developing pediatric specific score. Nonetheless evaluation using previously reported list of LSC enriched genes and statistical rigor, our results demonstrate prognostic value of pLSC6 score driven by expression levels of 6 genes in prediction of induction 1 MRD, EFS and OS in two independent pediatric AML patient cohorts. It should be noted that despite Target cohort being enriched in poor performing AML we observed consistent and significant association of pLSC6 with outcome. Further pLSC6 score holds prognostic value in redefining high and low risk AML patients who are currently assigned to the standard risk group category. This will be a significant step forward in developing treatment regimens for poor performing standard-risk group patients based on pLSC6 scores. MRD levels hold- significant prognostic implication in AML and are being used for risk stratification of patients after initial response to chemotherapy(24, 25). However, limitations associated with its routine use include the lack of standardized methods for MRD detection, precise timing for determination of MRD, and well-established

universally defined thresholds. Our results show that a pLSC6 not only predicts MRD status post induction 1 but also provides prognostic value beyond MRD status and initial risk group classification, which are the two most commonly used methods utilized for monitoring response. Our results further suggest that risk stratification using pLSC6 can identify patients within high-pLSC6 score category who are less likely to benefit from transplant and thus may be candidates for investigational therapies.

In summary, we believe that pLSC6 score generated specifically using outcome data from pediatric AML holds promise in redefining initial risk stratification and identifying poor risk AML. pLSC6 score enhances clinical utility by reducing the panel of genes without losing the effectiveness of the predictive power thus providing potential for developing guidance for developing novel treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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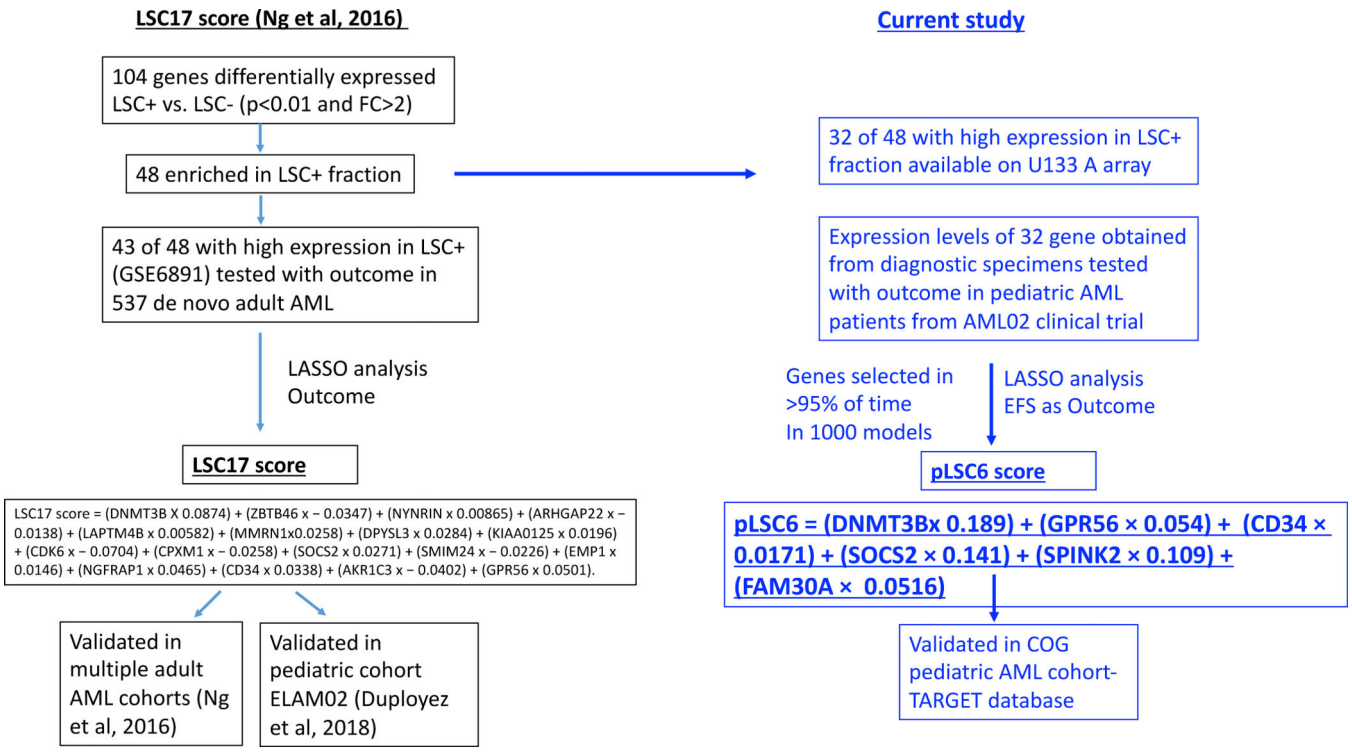

Figure 1: Overall schema of study.

Left panel summarizes previous study by Ng et al, 2016 reporting LSC17 and a follow up validation of adult LSC17 in pediatric AML by Duployez et al, 2018. Right panel provides summary of the strategy utilized in the current study to establish a pediatric specific LSC score consisting of 6 genes designated as **pLSC6**.

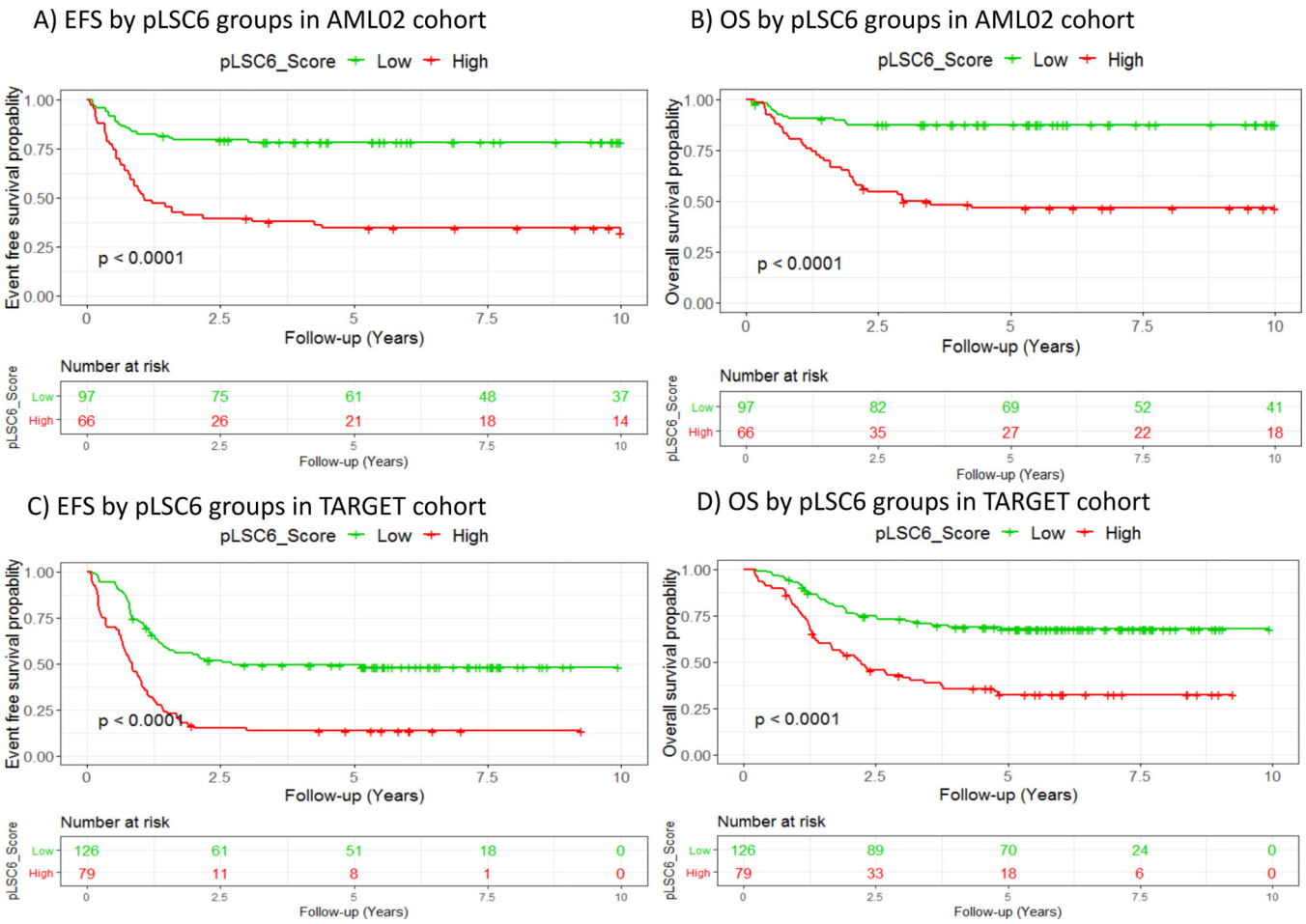

Figure 2: Pediatric LSC6 (pLSC6) score based on six stem cell genes (DNMT3B, GPR56 and CD34, SOCS2, SPINK2 and FAM30A) predicts clinical outcomes in two independent cohorts of pediatric AML – AML02 and TARGET.

Based on recursive portioning cutoff, Patients were categorized according to their pLSC6 scores into two groups; low (green color; around 60% of AML02, TARGET patients) and high (red color; around 40% of the patients). High pLSC6 scores predicts poor event free survival (A, C) and overall survival (B, D) in AML02 and TARGET cohorts respectively. Number of patients at risk during follow up period of 10 years is given and P-values are based on Cox-hazard models.

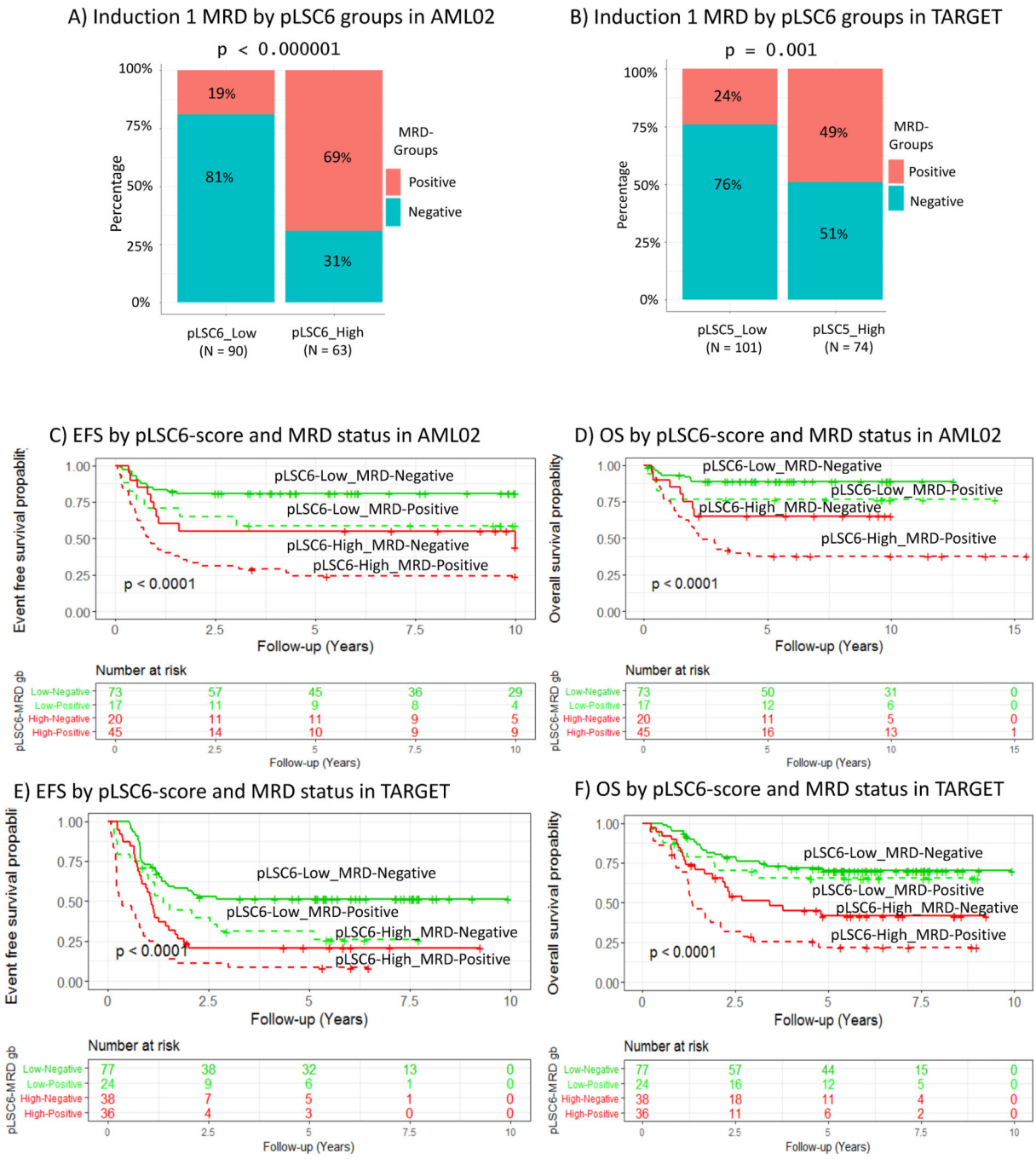

Figure 3: Pediatric LSC6 (pLSC6) score and MRD status after induction I course of treatment.

Patients found positive for residual leukemic cells after induction 1 course of treatment (MRD-IND1 > 0.1%) had statistically significant higher distribution in the pLSC6 high score group as compared to the low pLSC6 score group in AML02 (A) and TARGET (B) cohorts. P-value based on Chi-square test. Event free survival (C and E) and overall survival (D and F) probabilities by pLSC6 score and MRD status in AML02 (C and D) and TARGET cohorts (E and F) respectively. Green color represents patients with low pLSC6 scores while

red represent patients with high pLSC6. Solid lines represent MRD-ve patients and dashed lines for MRD+ve patients.

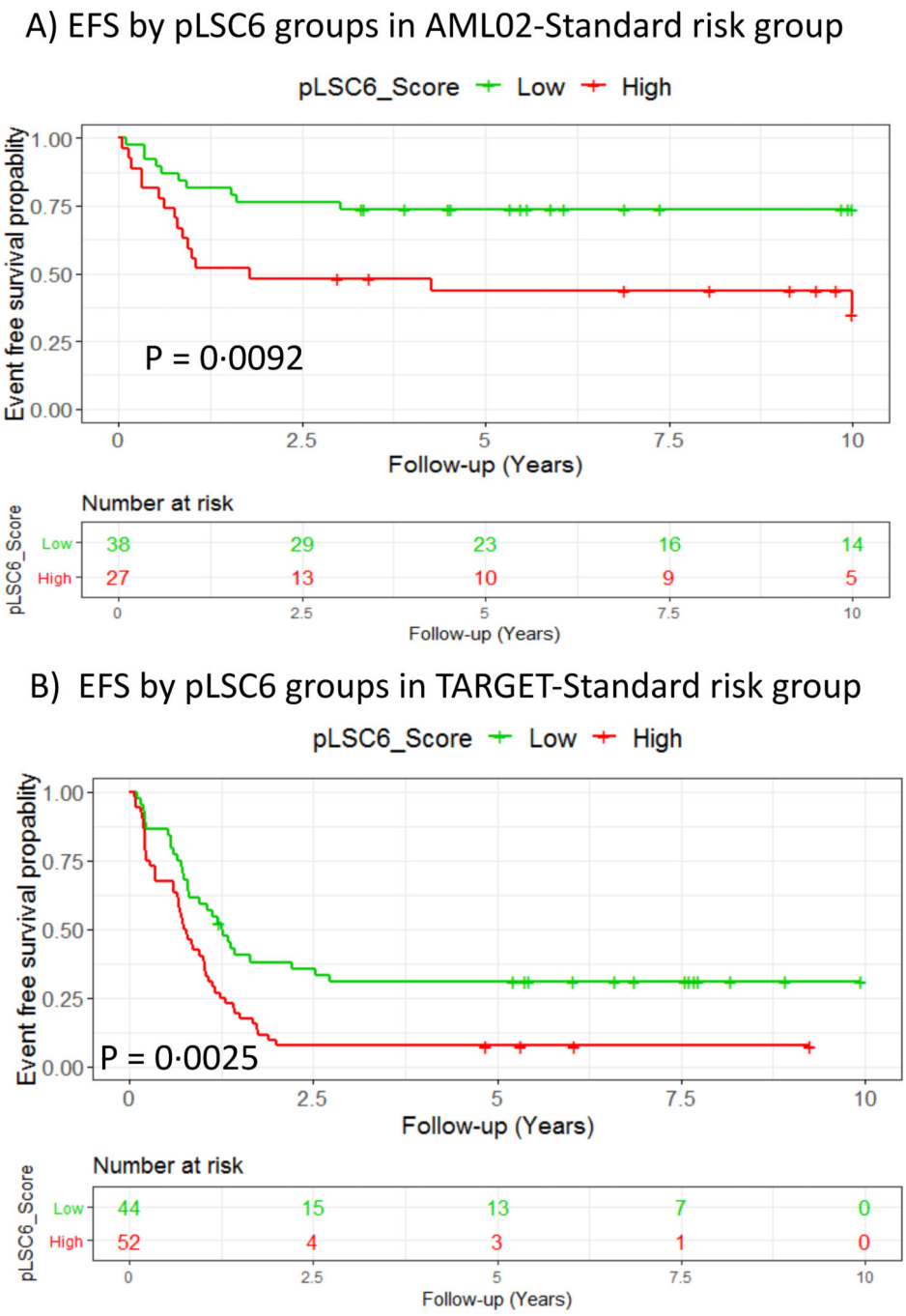

Figure 4: pLSC6 score subclassifies standard risk group patients by clinical outcome.

Kaplan-Meier estimates of EFS by high (red) or low (green) pLSC6 score groups in AML02 (A) and TARGET cohort; (B)

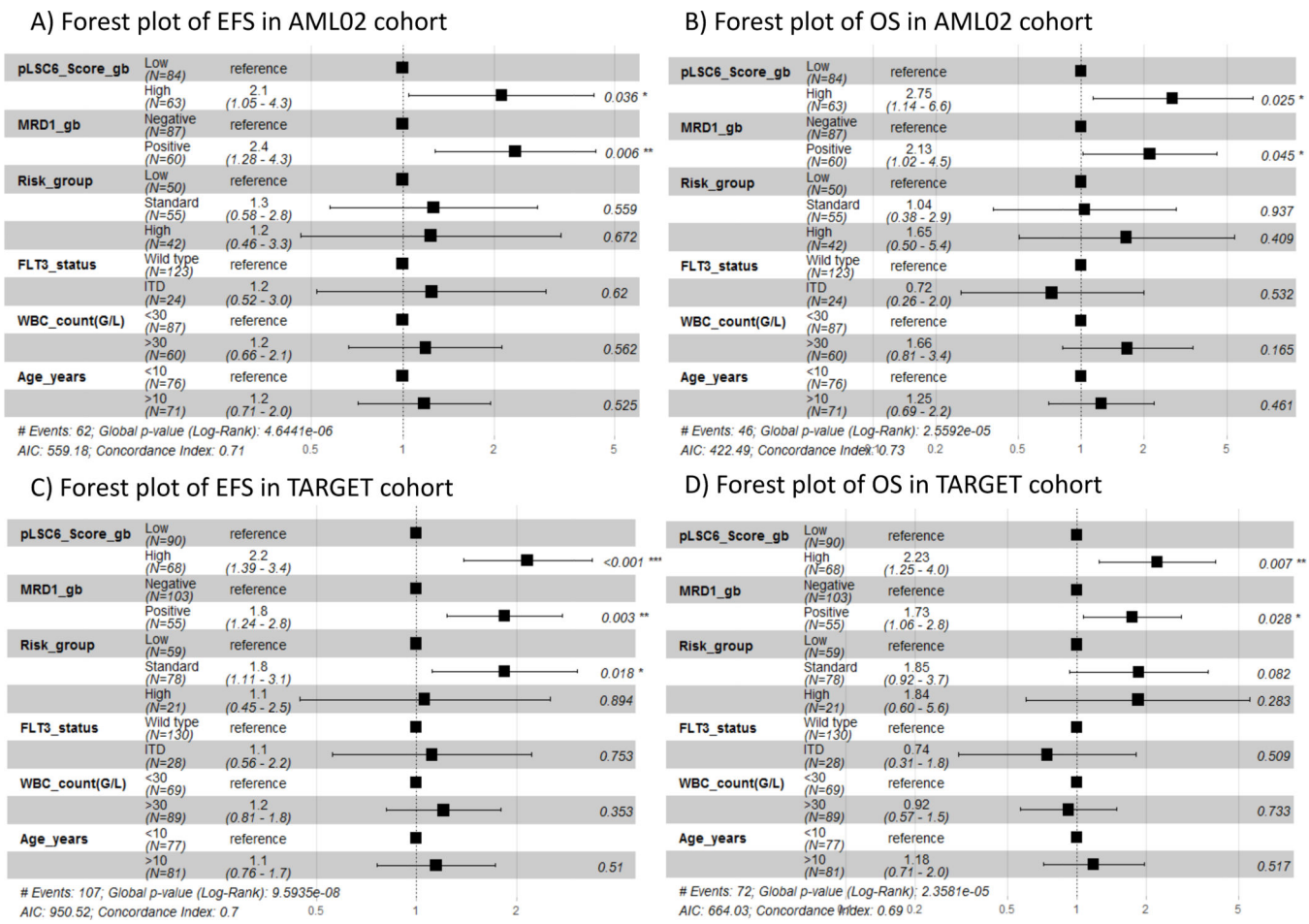

Figure 5: Forest plots of multivariable Cox-proportional hazard models showing pLSC6 score as an independent prognostic factor of EFS and OS in AML02 and Target cohorts.

Hazard ratios and 95% confidence intervals Cis are listed next to each variable for EFS (A, C) and OS (B, D) in AML02 and TARGET-AML cohorts, respectively. Within Forest plot, HR for each variable is depicted as a black box and 95% CI are shown as horizontal lines. The vertical line crossing the value of 1 represents non statistically significant effect, odds of less than one indicate better, whereas greater than 1 indicate worse effects.

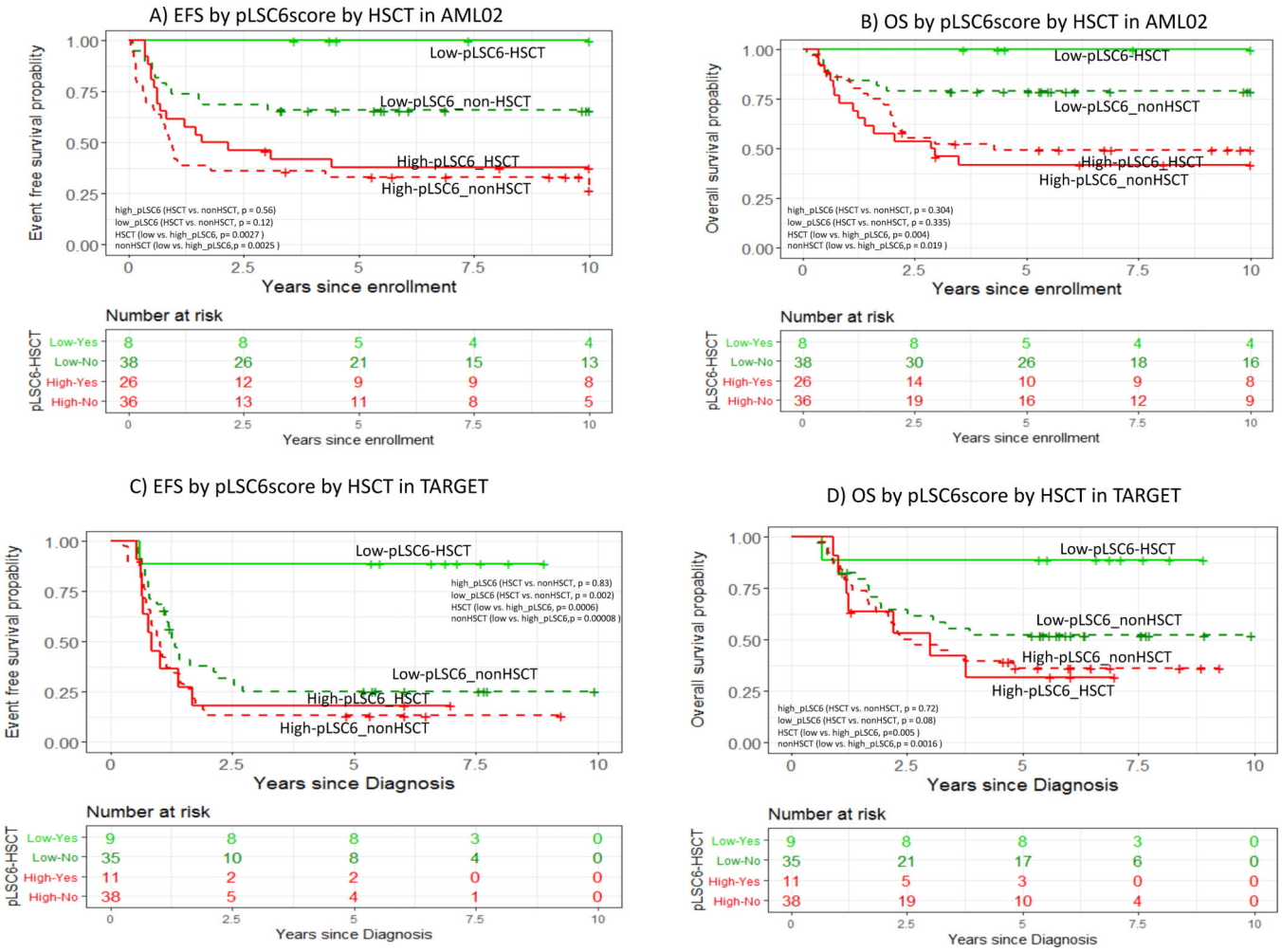

Figure 6:

Kaplan-Meier estimates of EFS (A, C) and OS (B, D) by pLSC6 score in standard and high-risk AML patients who did or did not receive hematopoietic stem cell transplantation (HSCT) in AML02 and TARGET cohorts, respectively. Green line: low pLSC6, red line :high pLSC6. Solid lines: HSCT and dashed lines: nonHSCT.

Table 1:

Univariate Cox regression results for association between study covariates with event free survival (EFS) and overall survival (OS) in AML02 and TARGET cohorts.

	Event free survival (EFS) time				Overall survival (OS) time			
	AML02		TARGET		AML02		TARGET	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Low-pLSC6	-	-	-	-	-	-	-	-
High-pLSC6	4.14 (2.46 – 6.98)	<0.0001	2.86 (2.02 – 4.04)	<0.0001	5.18 (2.67 – 10.1)	<0.0001	2.81 (1.85 – 4.28)	<0.0001
Low risk group	-	-	-	-	-	-	-	-
Standard risk group	1.99 (0.965 – 4.12)	0.062	2.95 (1.98 – 4.39)	<0.0001	2.27 (0.873 – 5.91)	0.093	3.01 (1.81 – 5.03)	<0.0001
High risk group	4.56 (2.29 – 9.05)	<0.0001	2.26 (1.24 – 4.14)	0.007	6.18 (2.55 – 14.99)	<0.0001	2.75 (1.31 – 5.76)	0.007
Cytogenetically normal	-	-	-	-	-	-	-	-
Inv(16)	0.143 (0.033 – 0.611)	0.009	0.926 (0.509 – 1.68)	0.8	0.0932 (0.012–0.701)	0.021	0.641 (0.267– 1.48)	0.298
t (8;21)	0.368 (0.139 – 0.977)	0.045	0.683 (0.376 – 1.24)	0.211	0.293 (0.085–0.998)	0.045	0.763 (0.366 –1.58)	0.469
t (9;11)	0.731 (0.276 – 1.94)	0.529	0.745 (0.315 – 1.76)	0.503	0.559 (0.164 – 1.91)	0.353	0.818 (0.284 –2.35)	0.711
11q23	0.835 (0.382 – 1.82)	0.65	2.09 (1.22 – 3.57)	0.006	0.828 (0.343 – 1.99)	0.674	1.91 (1.008 – 3.63)	0.047
Other aberrations	1.04 (0.573 – 1.89)	0.894	1.82 (1.15 – 2.86)	0.009	0.827 (0.4413– 1.66)	0.592	1.96 (1.15 – 3.35)	0.013
FLT3-WT	-	-	-	-	-	-	-	-
Mutation	1.05 (0.326 – 3.38)	0.934	0.91 (0.47 – 1.73)	0.76	0.493 (0.067 – 3.61)	0.486	0.56 (0.23 – 1.39)	0.21
FLT3-ITD	2.69 (1.54 – 4.71)	0.0005	1.45 (0.92 – 2.29)	0.11	2.74 (1.46 – 5.14)	0.002	1.36 (0.79 – 2.34)	0.27
WBC > 30(G/L)	1.18 (0.727 – 1.92)	0.5	0.95 (0.67 – 1.34)	0.78	1.36 (0.77 – 2.42)	0.287	0.76 (0.51 – 1.15)	0.19
BM Blast >70%	1.36 (0.813 – 2.26)	0.244	1.07 (0.76 – 1.5)	0.71	1.55 (0.852 – 2.83)	0.15	0.98 (0.65 – 1.48)	0.94
Peripheral Blast >50%	1.45 (0.889 – 2.36)	0.136	0.84 (0.59 – 1.18)	0.32	1.94 (1.08 – 3.48)	0.026	0.79 (0.53 – 1.21)	0.29
Age > 10 years	1.15 (0.707 – 1.87)	0.572	1.09 (0.78 – 1.52)	0.61	1.18 (0.669 – 2.09)	0.561	1.23 (0.82 – 1.86)	0.32

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