LETTER

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A 17-gene stemness score for rapid determination of risk in acute leukaemia

Stanley W. K. Ng[1]*, Amanda Mitchell[2]*, James A. Kennedy[2,3,4]*, Weihsu C. Chen[2], Jessica McLeod[2], Narmin Ibrahimova[2], Andrea Arruda[2], Andreea Popescu[2], Vikas Gupta[2,3,4], Aaron D. Schimmer[2,3,4,5], Andre C. Schuh[2,3,4], Karen W. Yee[2,3,4], Lars Bullinger[6], Tobias Herold[7,8], Dennis Görlich[9], Thomas Büchner[10]‡, Wolfgang Hiddemann[7,8], Wolfgang E. Berdel[10], Bernhard Wörmann[11], Meyling Cheok[12], Claude Preudhomme[13], Hervé Dombret[14], Klaus Metzeler[7,8], Christian Buske[15], Bob Löwenberg[16], Peter J. M. Valk[16], Peter W. Zandstra[1], Mark D. Minden[2,3,4,5]§, John E. Dick[2,17]§ & Jean C. Y. Wang[2,3,4]§

Refractoriness to induction chemotherapy and relapse after achievement of remission are the main obstacles to cure in acute myeloid leukaemia (AML)[1]. After standard induction chemotherapy, patients are assigned to different post-remission strategies on the basis of cytogenetic and molecular abnormalities that broadly define adverse, intermediate and favourable risk categories[2,3]. However, some patients do not respond to induction therapy and another subset will eventually relapse despite the lack of adverse risk factors[4]. There is an urgent need for better biomarkers to identify these high-risk patients before starting induction chemotherapy, to enable testing of alternative induction strategies in clinical trials[5]. The high rate of relapse in AML has been attributed to the persistence of leukaemia stem cells (LSCs), which possess a number of stem cell properties, including quiescence, that are linked to therapy resistance[6–10]. Here, to develop predictive and/or prognostic biomarkers related to stemness, we generated a list of genes that are differentially expressed between 138 LSC$^+$ and 89 LSC$^-$ cell fractions from 78 AML patients validated by xenotransplantation. To extract the core transcriptional components of stemness relevant to clinical outcomes, we performed sparse regression analysis of LSC gene expression against survival in a large training cohort, generating a 17-gene LSC score (LSC17). The LSC17 score was highly prognostic in five independent cohorts comprising patients of diverse AML subtypes ($n = 908$) and contributed greatly to accurate prediction of initial therapy resistance. Patients with high LSC17 scores had poor outcomes with current treatments including allogeneic stem cell transplantation. The LSC17 score provides clinicians with a rapid and powerful tool to identify AML patients who do not benefit from standard therapy and who should be enrolled in trials evaluating novel upfront or post-remission strategies.

To derive an LSC-based biomarker, 83 cell samples obtained from 78 AML patients (Extended Data Fig. 1a) were sorted into fractions based on expression of CD34 and CD38, and LSC activity in each fraction was assessed by xenotransplantation into NOD.*Prkdc*^{scid}.*Il2rg*^{null} (NSG) mice (Extended Data Fig. 1b). Consistent with previous reports, the majority of CD34$^+$ and a minority of CD34$^-$ fractions contained LSCs[11,12]. However, LSCs were detected in fractions of all CD34/CD38 phenotypes (Extended Data Fig. 1c, d), underscoring the importance of performing functional assays to define LSC activity.

Each of the functionally defined 138 LSC$^+$ and 89 LSC$^-$ fractions was subjected to gene expression (GE) analysis. By comparing GE profiles of LSC$^+$ and LSC$^-$ fractions, a list of differentially expressed (DE) genes was obtained; 104 genes exhibited $\geq$2-fold expression level differences ($P < 0.01$; Extended Data Fig. 1e and Extended Data Table 1). We defined an LSC$^+$ reference profile as the average expression levels of these 104 genes in the LSC$^+$ fractions. There was a strong correlation between engraftment ability of individual cell fractions and their GE similarity to the LSC$^+$ reference profile, as well as to the global GE profiles of normal haematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) from human umbilical cord blood[13] (Fig. 1a, b). Conversely, GE similarity to the LSC$^+$ reference profile was anti-correlated with the global GE patterns of mature myeloid cell types, including granulocytes and monocytes[14] (Fig. 1b). These findings suggest that the 104 genes most DE between LSC$^+$ and LSC$^-$ cell populations are associated with stem cell transcriptional programs that are shared between LSC and normal HSCs/MPPs.

To extract the core transcriptional components of stemness that relate to clinical outcomes across a broad spectrum of AML patient subtypes, we interrogated a large data set of 495 patients (Gene Expression Omnibus (GEO) accession GSE6891 (ref. 15)), in which 89 of the 104 DE LSC genes were captured. Expression of the 89 genes in these unfractionated patient samples was variable and showed a similar pattern of correlation to the LSC$^+$ reference profile, as did the sorted LSC$^+$ and LSC$^-$ fractions (Fig. 1c), suggesting that LSC-associated GE programs are detectable at the bulk cell level. We applied a statistical regression algorithm based on the least absolute shrinkage and selection operator (LASSO)[16,17] to relate GE to patient survival in this training cohort, using either the full list of 89 LSC genes or the subset of 43 genes more highly expressed in LSC$^+$ fractions. Analysis of the latter subset yielded an optimal 17-gene signature (LSC17 score), which could be calculated for each patient as the weighted sum of expression of the 17 genes (Fig. 1c). High LSC17 scores were strongly associated with poor overall survival (OS) and event-free survival (EFS) (Extended

[1]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario M5G 1A1, Canada. [2]Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario M5G 2M9, Canada. [3]Division of Medical Oncology and Hematology, Department of Medicine, University Health Network, Toronto, Ontario M5G 2M9, Canada. [4]Department of Medicine, University of Toronto, Toronto, Ontario M5G 1A1, Canada. [5]Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 1A1, Canada. [6]Department of Internal Medicine III, University Hospital of Ulm, 89081 Ulm, Germany. [7]Department of Internal Medicine III, University of Munich, 81377 Munich, Germany. [8]German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany. [9]Institute of Biostatistics and Clinical Research, University of Münster, 48149 Münster, Germany. [10]Department of Medicine, Hematology and Oncology, University of Münster, 48149 Münster, Germany. [11]Department of Hematology, Oncology and Tumor Immunology, Charité University Medicine, Campus Virchow, 10117 Berlin, Germany. [12]Jean-Pierre AUBERT Research Center UMR-S 1172, Institute for Cancer Research Lille, 59045 Lille, France. [13]University Hospital of Lille, Center of Pathology, Laboratory of Hematology, 59037 Lille, France. [14]Saint-Louis Hospital, Department of Hematology, University of Paris Diderot, 75010 Paris, France. [15]Comprehensive Cancer Center Ulm, Institute of Experimental Cancer Research, University Hospital of Ulm, 89081 Ulm, Germany. [16]Department of Hematology, Erasmus University Medical Centre, 3015 CE Rotterdam, the Netherlands. [17]Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5G 1A1, Canada.

*These authors contributed equally to this work.

§These authors jointly supervised this work.

‡Deceased.

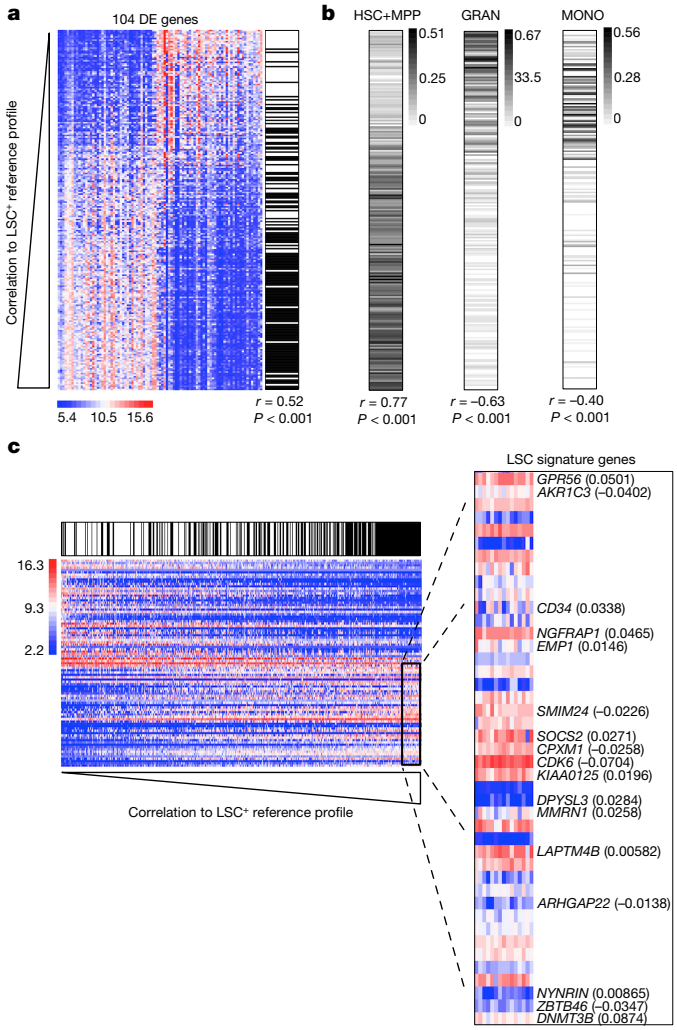

Figure 1 | Analysis of LSC-specific GE identifies an optimal 17-gene prognostic signature. a, GE patterns of the top 104 genes (columns) DE between 138 LSC^+ and 89 LSC^- cell fractions (rows). Horizontal black and white bars denote LSC^+ and LSC^- fractions, respectively. r = correlation coefficient between engraftment status and similarity to the LSC^+ reference profile. **b**, Similarity of global GE of each cell fraction to that of stem-cell-enriched cell populations (HSCs plus MPPs) and mature myeloid cell populations (granulocytes, GRAN; monocytes, MONO) from human umbilical cord blood. r = correlation coefficient between similarity to the LSC^+ reference profile and similarity to the cell types indicated. **c**, GE patterns of 89/104 DE genes captured in the GSE6891 data set. The relative ordering of the 89 genes is the same as in **b** (rotated counterclockwise 90°). Vertical black and white bars denote samples with LSC17 scores above and below the median, respectively. The 17 signature genes are depicted in the magnified view on the right (regression coefficients in parentheses).

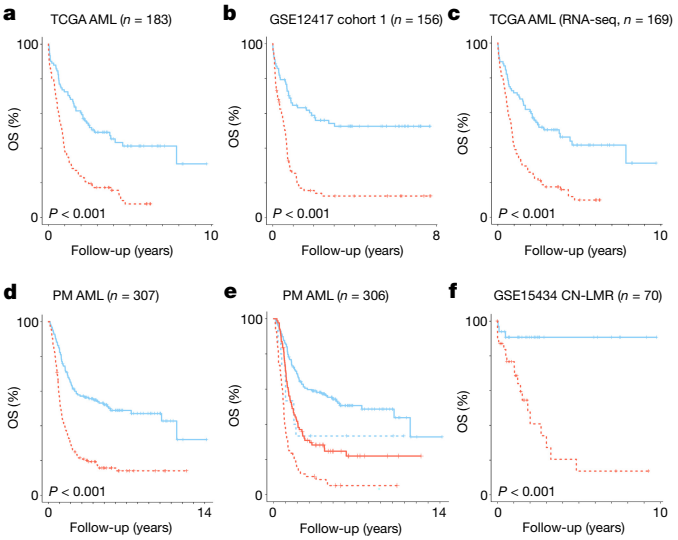

Figure 2 | LSC signature scores are associated with OS in multiple independent AML cohorts across different GE measurement platforms. a–**d**, Kaplan–Meier estimates of OS, according to LSC17 scores calculated using microarray (**a**, **b**), RNA-seq (**c**), or NanoString (**d**) GE data sets. **e**, Kaplan–Meier estimates of OS according to LSC17 score and whether or not CR was achieved after initial therapy (no CR, dotted lines; CR, solid lines). **f**, Kaplan–Meier estimates of OS, according to LSC3 scores calculated using microarray GE data. For all panels, OS of patients with scores above and below the median in each cohort are shown by red and blue lines, respectively.

Data Fig. 1f). In addition, patients with high LSC17 scores had significantly higher percentages of bone marrow blasts at diagnosis, a higher incidence of the *FLT3* internal tandem duplication mutation (*FLT3*-ITD) and adverse cytogenetics, higher rates of relapse, and lower response rates to standard induction chemotherapy, reflecting a link between LSC-associated GE programs and clinical outcomes.

We evaluated the association of the LSC17 score with survival in three independent AML cohorts (one from The Cancer Genome Atlas (TCGA)[18], two from GEO accession GSE12417 (ref. 19)). In the TCGA AML cohort ($n = 183$), patients with a high LSC17 score had significantly shorter OS than patients with a low score (Fig. 2a and Extended Data Table 2; hazard ratio (HR) = 2.62; $P < 0.001$). This survival difference was also found for the subset of cytogenetically normal (CN)-AML patients ($n = 83$) (Extended Data Fig. 2a; median OS 10.4 versus 24.1 months; HR = 2.06; $P = 0.006$). Similar results were observed in the two CN-AML cohorts from GSE12417 (cohort 1: Fig. 2b, HR = 3.16, $P < 0.001$; cohort 2: Extended Data Fig. 2b, HR = 2.66, $P = 0.002$; Extended Data Table 3). In GSE12417 cohort 1, a high LSC17 score was associated with shorter OS regardless of whether or not complete remission (CR) was achieved (Extended Data Fig. 2c; CR: median OS 9.8 months versus not reached; HR = 3.20; $P < 0.001$; no CR: median OS 2.0 versus 2.5 months; HR = 1.58; $P = 0.14$). As in the training cohort, high LSC17 scores were significantly associated with adverse cytogenetic and molecular features, failure to achieve CR, and shorter EFS and relapse-free survival (RFS) (Extended Data Fig. 2d–g and Extended Data Tables 2, 3). When applied to RNA-sequencing (RNA-seq) data for the TCGA cohort, the LSC17 score remained highly associated with outcome (full cohort: Fig. 2c, HR = 2.48, $P < 0.001$; CN-AML subset: Extended Data Fig. 2h, HR = 2.38, $P = 0.001$), demonstrating robustness across technology platforms. In multivariate survival analysis using Cox proportional hazards (CPH) models, the LSC17 score retained significant prognostic value in all tested cohorts independent of known predictors of outcome including patient age, presenting white blood cell (WBC) count, cytogenetic risk group, type of AML (*de novo* versus secondary), and the presence of *FLT3*-ITD and *NPM1* mutations (Extended Data Table 4a).

Recent studies mapping the mutational landscape of AML have identified additional recurrent mutations that carry independent prognostic information[20]. Of our validation cohorts, extensive mutational profiling data was available only for the TCGA AML cohort. In this data set, six mutations frequently found in AML occurred in at least three patients and were also significantly associated with OS as single factors in univariate survival analysis. However, in a multivariate CPH model that included all six of these mutations as well as common clinical parameters (age, WBC count, cytogenetic risk group), only *DNMT3A* retained prognostic significance when the LSC17 score was included in the model, whereas the LSC17 score remained a strong and significant independent prognostic factor (Extended Data Table 5a). Recently, a comprehensive genomic classification scheme was reported and was shown to be more accurate for patient risk stratification than

the European LeukaemiaNet risk group definitions[3,21]. When this new scheme was applied to the TCGA AML cohort, inclusion of the LSC17 score in multivariate CPH models significantly improved the overall strength of association of the model with patient OS (Extended Data Table 5b, $P < 0.001$, likelihood ratio test), and the LSC17 score itself remained statistically significant. Three of the fourteen subgroups in the new genomic classification scheme are less well characterized ('driver mutations but not class-defining', 'no detected driver mutations', and 'meeting criteria for 2 or more subgroups'); patients in these groups had similar survival. The LSC17 score was able to discriminate between shorter and longer OS in the combined subset of patients falling into these three subgroups, and thus refines this state-of-the-art genomics classification scheme (Extended Data Fig. 2i and Extended Data Table 5c).

The LSC17 score displayed superior prognostic accuracy when tested against other published LSC signatures derived from GE analysis of cell populations defined phenotypically or by multidimensional mass cytometry[22,23], or generated based on epigenetic differences between a small number of functionally tested cell populations[24]. These other signatures, like our previously reported 42-gene LSC signature[11], are lists of DE genes generated by comparing cellular phenotypes or functional states without further statistical analysis of the contribution of each gene to explaining patient outcome. When tested in three independent cohorts (GSE12417 CN-AML cohorts 1 and 2, and TCGA AML), these other signatures were prognostic in some cases as single factors or when controlling for common clinical covariates. However, when the LSC17 score was incorporated in multivariate analysis, they were no longer significantly associated with survival, whereas the LSC17 score remained highly prognostic (Extended Data Table 6).

To develop a clinically applicable GE-based diagnostic test, we turned to the NanoString platform, which is reproducible, cost effective, and has a rapid turnaround time of 24–48 h (ref. 25). We designed a custom NanoString assay and generated GE data for 307 AML patients treated at the Princess Margaret (PM) Cancer Centre. A high LSC17 score was associated with known adverse prognostic features including older age, high initial WBC count, and unfavourable cytogenetics (Extended Data Table 7a). Consistent with our findings using microarray and RNA-seq GE data, patients with high LSC17 scores had significantly shorter OS than patients with low scores (Fig. 2d and Extended Data Table 7a; HR = 2.73; $P < 0.001$); this was true regardless of whether or not remission was achieved after primary induction therapy (Fig. 2e; CR: median OS 18.9 versus 90.3 months; HR = 2.18; $P < 0.001$; no CR: median OS 10.5 versus 20.7 months; HR = 2.16; $P = 0.02$). Similarly, a high LSC17 score was associated with shorter EFS and RFS (Extended Data Fig. 2j–m). The association between a high LSC17 score and shorter OS was also observed in the subset of patients with CN-AML (Extended Data Fig. 2n; median OS 13.7 versus 65.7 months; HR = 2.64; $P < 0.001$). Importantly, in multivariate survival analysis including established risk factors, the LSC17 score retained independent prognostic value in both the full cohort as well as in the CN-AML subset (Extended Data Table 4a). Together, these results demonstrate the broad applicability and strong prognostic value of the LSC17 score on the clinically serviceable NanoString platform.

Allogeneic stem cell transplantation (aSCT) has strong anti-leukaemic effects; however, potential benefits can be offset by considerable transplant-related mortality and thus the procedure is generally reserved for patients with a higher risk of relapse on the basis of available risk features (for example, cytogenetics, assessment of minimal residual disease)[26]. Inclusion of aSCT as a time-dependent covariate in the PM AML cohort (univariate Mantel–Byar analysis; Fig. 3a) did not demonstrate a significant impact of aSCT on OS for either high- or low-score patients (high LSC17 score, $P = 0.20$; low LSC17 score, $P = 0.06$). Furthermore, a high LSC17 score was associated with shorter OS, irrespective of whether or not patients underwent aSCT (aSCT: median OS 11.7 versus 28.4 months for high versus low LSC17 score, respectively; HR = 2.14; $P = 0.005$; no aSCT: median OS

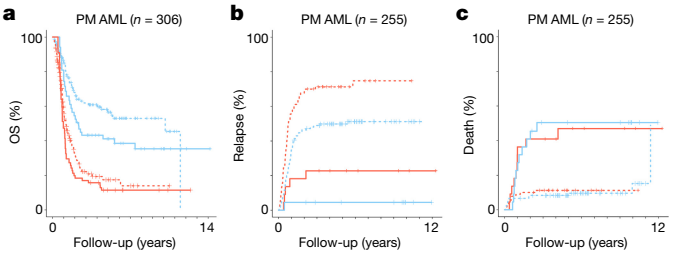

Figure 3 | Impact of aSCT on patient outcome. a, Simon and Makuch estimates of OS, according to LSC17 scores computed using NanoString GE data and whether or not patients received aSCT, **b**, **c**, Time from CR1 to first relapse (**b**) or death (**c**) as competing risks, as estimated by cumulative incidence analysis, according to LSC17 scores calculated using NanoString GE data. In all panels, red and blue lines show patients with scores above and below the median in each set, respectively, while solid and dotted lines denote patients who did and did not undergo aSCT, respectively.

14.7 versus 123.3 months; HR = 2.99; $P < 0.001$). The LSC17 score retained prognostic value when adjusted for common clinical factors in multivariate Andersen–Gill models (aSCT: HR = 2.00, $P = 0.04$; no aSCT: HR = 2.63, $P < 0.001$). Similar results were observed in the subset of CN-AML cases (Extended Data Fig. 2o), and in the analysis of EFS and RFS (data not shown).

We also examined the cumulative incidence of the competing risks of relapse and death from time of first CR (CR1) in patients who did or did not undergo aSCT in the PM AML cohort. A high LSC17 score was associated with earlier relapse in the subset of patients who did not undergo aSCT, in both univariate (Fig. 3b, sub-distribution HR (SHR) = 1.92; Gray's test $P < 0.001$; median time to relapse 9.31 versus 65.2 months) and Fine–Gray multivariate analysis (SHR = 1.85, $P = 0.003$). aSCT reduced the risk of relapse, although small patient numbers precluded seeing a statistically significant difference between high- and low-score patients (SHR = 5.26; $P = 0.09$). However, the reduction in relapse risk was offset by a significantly greater risk of death in both high- and low-score groups compared to patients who did not undergo aSCT (Fig. 3c; $P < 0.001$). Indeed, the risk of death after aSCT versus risk of relapse without aSCT was very similar for low-score patients. Thus, the LSC17 score will aid in defining which patients should undergo aSCT.

The LSC17 score was initially trained using clinical data from the GSE6891 data set, which included only a small group ($n = 44/495$, 9%) of CN-AML patients classified as low molecular risk (CN-LMR, defined as the presence of *NPM1* mutation and no *FLT3*-ITD); as such, survival differences within this small patient subset might not have been captured optimally by the statistical regression algorithm applied to the entire cohort. We therefore retrained the 17 LSC signature genes against OS for only the CN-LMR cases in GSE6891 and identified an optimized, reweighted sub-signature in which only 3 of the 17 genes contributed to the calculated score (LSC3). A high LSC3 score identified patients with poor outcome in an independent cohort of CN-LMR patients (GEO accession GSE15434 (ref. 27)) (Fig. 2f and Extended Data Table 7b; HR = 8.41; $P < 0.001$), and was strongly associated with shorter survival in the subset of 29 CN-LMR cases in the PM cohort analysed by NanoString methodology (Extended Data Fig. 2p; median OS 39.2 months versus not reached, HR = 3.65, $P = 0.05$), retaining independent prognostic value in multivariate analysis (Extended Data Table 4b). These findings demonstrate the feasibility of optimizing the LSC17 score for selected patient subsets.

We next tested the ability of the LSC17 score to predict therapy resistance (defined as failure to achieve CR after initial induction)[5], as this remains one of the primary barriers to cure. In the PM AML cohort, the LSC17 score as a single continuous variable was more predictive of therapy resistance than cytogenetic risk (area under the receiver operating characteristic curve (AUROC) = 0.78 versus 0.70). In multivariate logistic regression models that also considered age, WBC

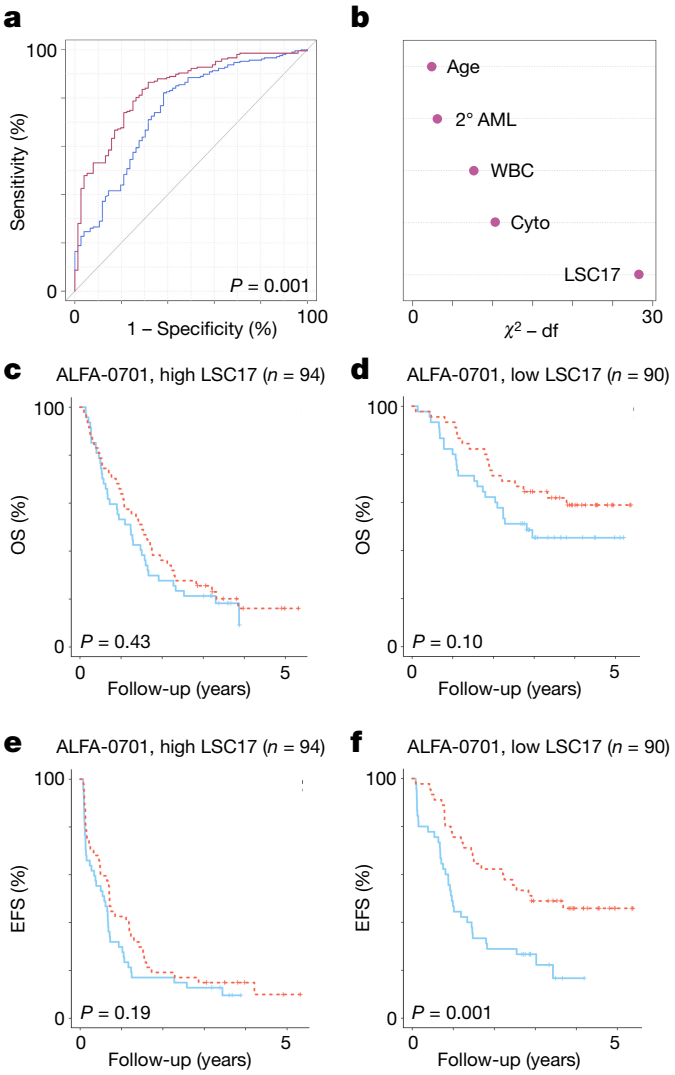

Figure 4 | LSC17 score predicts therapy response. a, Receiver operating characteristic (ROC) curves for prediction of initial therapy resistance, using logistic regression models that include age, WBC count, cytogenetic risk (Cyto), and *de novo* versus secondary AML (2° AML) as covariates, with (maroon line) or without (blue line) LSC17 score. **b**, Significance (chi-squared statistic) of each covariate for prediction of therapy resistance in the multivariate model that includes LSC17 scores. df, degrees of freedom. **c**–**f**, Kaplan–Meier estimates of OS (**c**, **d**) or EFS (**e**, **f**) for patients with high (**c**, **e**) or low (**d**, **f**) LSC17 scores treated with standard chemotherapy with (red lines) or without (blue lines) addition of GO.

count, cytogenetic risk and *de novo* versus secondary AML, inclusion of the LSC17 score markedly improved predictive ability (Fig. 4a; AUROC = 0.82 versus 0.73, increased sensitivity = 3.38%, increased specificity = 9.20%), and LSC17 score was the most significant covariate as measured by the Wald chi-squared statistic (Fig. 4b). Multivariate models that included either cytogenetic risk or continuous LSC17 score had comparable predictive value for therapy resistance (with LSC17: AUROC = 0.79 versus 0.73, increased sensitivity = 2.10%, increased specificity = 5.71%, $P = 0.12$). As the LSC17 score was trained to associate with OS, we tested whether reweighting the 17 genes to predict treatment response directly would result in even stronger predictive ability, using a random 50:50 split of the PM cohort for training and testing. Indeed, the retrained response score had better predictive value as a single factor than the unadjusted LSC17 score (AUROC = 0.81 versus 0.78). These results demonstrate that the LSC17 score improves the ability to predict therapy resistance in newly diagnosed AML patients.

We also used a data set from the ALFA-0701 trial[28,29] (Extended Data Table 8a) to test the ability of the LSC17 score to predict response to gemtuzumab ozogamicin (GO), a drug–antibody conjugate shown to improve survival when added to standard induction chemotherapy. A higher LSC17 score was associated with shorter OS irrespective of treatment arm (Extended Data Fig. 2q; median OS 15.4 versus 46.2 months; HR = 2.45; $P < 0.001$). Notably, patients with low but not high LSC17 scores benefited from addition of GO to standard chemotherapy, with longer OS, EFS and RFS (OS: Fig. 4c, d, median not reached versus 34.3 months, HR = 0.60, $P = 0.11$; EFS: Fig. 4e, f, median 35.4 versus 11.7 months, HR = 0.42, $P = 0.001$; RFS: Extended Data Fig. 2r, s, median not reached versus 16.4 months, HR = 0.53, $P = 0.03$; Extended Data Table 8b). These data suggest that the LSC17 score could be used to facilitate more rational use of GO in patients most likely to benefit, while sparing high-score patients who do not derive benefit any potential toxicities.

Many cancer biomarkers rely on mutational profiling. However, the high degree of molecular complexity in AML presents a considerable challenge to clinical implementation of such approaches[18,21,30]. The strong prognostic value of the LSC17 score across the spectrum of AML genotypes suggests that perturbations caused by driver mutations coalesce on alterations in stemness properties, and that the LSC17 score is able to distil these downstream consequences. A high LSC17 score probably reflects biological properties of LSCs that confer resistance to standard AML therapy. The LSC17 NanoString assay will allow rapid risk assessment at diagnosis, enabling recommendation of more intensified investigational therapies to be directed to high-score patients predicted to have resistant disease, while sparing low-score patients unnecessary added toxicity. Furthermore, our analysis of the ALFA-0701 trial data demonstrates the utility of the LSC17 score as a tool for patient selection, and can probably be extended to additional patient cohorts treated with other experimental therapies as data becomes available in future studies. Finally, the LSC3 score will allow evaluation of the possible benefits of post-remission therapy or aSCT in CR1 for high-risk CN-LMR patients, or could be used in upfront therapy decisions at centres where CN-LMR patients can be rapidly identified by molecular diagnostics. Overall, incorporation of the LSC17 and LSC3 scores into risk determination algorithms for newly diagnosed AML patients will facilitate the development and clinical testing of novel anti-leukaemia therapies in the ongoing effort to prevent relapse and increase cure rates.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Ferrara, F. & Schiffer, C. A. Acute myeloid leukaemia in adults. *Lancet* **381,** 484–495 (2013).
2. Grimwade, D. *et al.* Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* **116,** 354–365 (2010).
3. Döhner, H. *et al.* Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood* **115,** 453–474 (2010).
4. Röllig, C. *et al.* Long-term prognosis of acute myeloid leukemia according to the new genetic risk classification of the European LeukemiaNet recommendations: evaluation of the proposed reporting system. *J. Clin. Oncol.* **29,** 2758–2765 (2011).
5. Walter, R. B. *et al.* Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center. *Leukemia* **29,** 312–320 (2015).
6. Kreso, A. & Dick, J. E. Evolution of the cancer stem cell model. *Cell Stem Cell* **14,** 275–291 (2014).
7. Saito, Y. *et al.* Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Sci. Transl. Med.* **2,** 17ra9 (2010).
8. Li, L. *et al.* SIRT1 activation by a c-MYC oncogenic network promotes the maintenance and drug resistance of human FLT3-ITD acute myeloid leukemia stem cells. *Cell Stem Cell* **15,** 431–446 (2014).

9. Fong, C. Y. *et al.* BET inhibitor resistance emerges from leukaemia stem cells. *Nature* **525,** 538–542 (2015).
10. Lechman, E. R. *et al.* miR-126 regulates distinct self-renewal outcomes in normal and malignant hematopoietic stem cells. *Cancer Cell* **29,** 214–228 (2016).
11. Eppert, K. *et al.* Stem cell gene expression programs influence clinical outcome in human leukemia. *Nature Med.* **17,** 1086–1093 (2011).
12. Sarry, J. E. *et al.* Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rγc-deficient mice. *J. Clin. Invest.* **121,** 384–395 (2011).
13. Laurenti, E. *et al.* The transcriptional architecture of early human hematopoiesis identifies multilevel control of lymphoid commitment. *Nature Immunol.* **14,** 756–763 (2013).
14. Novershtern, N. *et al.* Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell* **144,** 296–309 (2011).
15. Verhaak, R. G. *et al.* Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. *Haematologica* **94,** 131–134 (2009).
16. Friedman, J., Hastie, T. & Tibshirani, R. Regularization paths for generalized linear models via coordinate descent. *J. Stat. Softw.* **33,** 1–22 (2010).
17. Simon, N., Friedman, J., Hastie, T. & Tibshirani, R. Regularization paths for Cox's proportional hazards model via coordinate descent. *J. Stat. Softw.* **39,** 1–13 (2011).
18. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. *N. Engl. J. Med.* **368,** 2059–2074 (2013).
19. Metzeler, K. H. *et al.* An 86-probe-set gene-expression signature predicts survival in cytogenetically normal acute myeloid leukemia. *Blood* **112,** 4193–4201 (2008).
20. Grimwade, D., Ivey, A. & Huntly, B. J. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood* **127,** 29–41 (2016).
21. Papaemmanuil, E. *et al.* Genomic classification and prognosis in acute myeloid leukemia. *N. Engl. J. Med.* **374,** 2209–2221 (2016).
22. Levine, J. H. *et al.* Data-driven phenotypic dissection of AML reveals progenitor-like cells that correlate with prognosis. *Cell* **162,** 184–197 (2015).
23. Gentles, A. J., Plevritis, S. K., Majeti, R. & Alizadeh, A. A. Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. *J. Am. Med. Assoc.* **304,** 2706–2715 (2010).
24. Jung, N., Dai, B., Gentles, A. J., Majeti, R. & Feinberg, A. P. An LSC epigenetic signature is largely mutation independent and implicates the HOXA cluster in AML pathogenesis. *Nature Commun.* **6,** 8489 (2015).
25. Geiss, G. K. *et al.* Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nature Biotechnol.* **26,** 317–325 (2008).
26. Cornelissen, J. J. *et al.* The European LeukemiaNet AML Working Party consensus statement on allogeneic HSCT for patients with AML in remission: an integrated-risk adapted approach. *Nature Rev. Clin. Oncol.* **9,** 579–590 (2012).
27. Kohlmann, A. *et al.* Gene expression profiling in AML with normal karyotype can predict mutations for molecular markers and allows novel insights into perturbed biological pathways. *Leukemia* **24,** 1216–1220 (2010).
28. Castaigne, S. *et al.* Effect of gemtuzumab ozogamicin on survival of adult patients with *de-novo* acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet* **379,** 1508–1516 (2012).
29. Hills, R. K. *et al.* Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol.* **15,** 986–996 (2014).
30. Klco, J. M. *et al.* Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. *J. Am. Med. Assoc.* **314,** 811–822 (2015).

Supplementary Information is available in the online version of the paper.

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Author Contributions S.W.K.N. developed the signature derivation workflow, identified, refined and validated prognostic and predictive signatures, designed the custom NanoString assay, processed and analysed GE data, and performed statistical analyses and bioinformatics. A.M., W.C.C., J.M. and A.P. carried out functional xenograft transplantation, RNA extraction for GE analysis, and provided technical support for experiments. J.A.K., N.I., A.A., V.G., A.D.S., A.C.S., K.W.Y. and M.D.M. provided clinical annotations for the PM AML cohort. M.D.M. provided PM AML samples. S.W.K.N., J.C.Y.W., J.E.D. and M.D.M. interpreted the data. W.H., W.E.B., B.W., T.B., D.G., L.B., K.M., T.H. and C.B. provided clinical annotations for the GSE15434 and GSE12417 data sets. M.C., C.P. and H.D. provided GE and clinical data for the ALFA-0701 trial cohort. P.J.M.V. and B.L. provided clinical annotations for the GSE6891 data set. J.C.Y.W. and J.E.D. supervised the study. S.W.K.N. and J.C.Y.W. wrote the paper. A.M., J.A.K., P.W.Z., J.E.D. and M.D.M. revised the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.C.Y.W. (jwang@uhnresearch.ca).

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METHODS

Patient samples. All biological samples were collected with informed consent according to procedures approved by the Research Ethics Board of the University Health Network (UHN; REB# 01-0573-C) and viably frozen in the PM Leukaemia Bank. No statistical methods were used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment.

Xenotransplantation assays. Eighty-three clinical samples (81 peripheral blood (PB), 1 bone marrow (BM), 1 peritoneal fluid) obtained from 78 patients were stained with the following antibodies (all from BD, dilution 1:100, catalogue number in parentheses): anti-CD3-FITC (349201), anti-CD34-APC (340441), anti-CD38-PE (347687). Each sample was sorted on a FACSAria III (BD Biosciences) into four fractions based on CD34/CD38 expression; a total of 227 fractions with sufficient cell numbers were tested in xenotransplantation assays. The clinical characteristics of these patients are provided in Extended Data Fig. 1a. Sixty-two samples were diagnostic, 16 were obtained following relapse and 5 after unsuccessful induction treatment. Paired diagnosis–relapse samples were included from 3 patients. Diagnosis samples from BM and PB of 1 patient were included. Two PB relapse samples collected at different times from 1 patient were included.

Animal experiments were performed in accordance with institutional guidelines approved by the UHN Animal Care Committee. Eight to 12-week-old female NSG mice were sublethally irradiated (225 cGy) 24 h before intrafemoral injection of sorted AML cell fractions. Mice were killed 12 weeks post-transplant and human cell engraftment in the injected right femur was assessed by flow cytometry using human-specific antibodies (all used at 1:100 dilution, all from BD unless stated otherwise, catalogue number in parentheses): anti-CD3-FITC (349201), anti-CD19-PE (349209), anti-CD33-PE-Cy5 (Beckman Coulter PNIM2647U), anti-CD45-APC (340943), anti-CD38-PE-Cy7 (1:200, 335790) and anti-CD34-APC-Cy7 (custom made by BD). AML grafts were defined as $\geq$0.1% human $CD45^+CD3^-$ cells, with $\geq$90% CD33 expression. Sorted fractions were defined as LSC^+ if transplanted cells generated an AML graft in 1 or more mice; the remaining fractions were defined as LSC^-. All flow cytometric analysis was performed on a BD LSR II.

GE profiling of cell fractions. RNA was extracted using Qiagen RNeasy mini kits (catalogue 74106) and was subjected to GE analysis using Illumina HumanHT-12 v4 microarrays to investigate ~47,000 targets corresponding to ~30,000 genes. The resultant fluorescence intensity profiles were subjected to variance stabilization and robust spline normalization using the lumi 2.16.0 R package[31]. All data was put into the log base-2 scale. Differential GE analysis was performed using the limma 3.20.9 package[32] in R. Specifically, Smyth's moderated *t*-test was used with Benjamini–Hochberg multiple testing correction to compare GE profiles of LSC^+ versus LSC^- fractions. Relative proportions of GE programs of stem/progenitor and mature cell types purified from human umbilical cord blood (GEO accessions GSE42414 (ref. 13) and GSE24759 (ref. 14)) composing AML GE profiles were assessed using the Perturbation model[33].

Signature training. For signature development, we used published GE profiles of diagnostic samples obtained from 537 patients with *de novo* AML treated with curative intent (GSE6891). Clinical annotations for 521 cases were provided by the authors[15]. Of these, we removed 23 cases of myelodysplastic syndrome refractory anaemia with excess blasts (MDS-RAEB), 2 cases due to missing WBC count data, and 1 because there was no raw GE data available for download, leaving 495 cases for analysis (Extended Data Fig. 1f). The GE data from this study were generated using Affymetrix Human Genome (HG) U133 Plus 2.0 GeneChips. The probes available on this array capture 89 of the 104 LSC associated genes (43 of the 48 enriched in LSC^+ cell fractions) (Extended Data Table 1). Raw Affymetrix CEL files were imported using the affy 1.42.3 R package[34] and processed with the gcrma 2.36.0 package[35] in R, using version 17 of the custom chip definition files (CDF) for the HG-U133 Plus 2.0 platform from the University of Michigan[36].

For each gene, the probeset with the highest average GE in the training data was selected to represent that gene. To extract a core subset of genes from among the 43 that were more highly expressed in LSC^+ cell fractions that best explained patient outcomes in the training cohort, we used a linear regression technique based on the LASSO algorithm as implemented in the glmnet 1.9-8 R package[16,17], while enabling leave-one-out cross-validation to fit a Cox regression model. A minimal subset of 17 genes was selected whose weighted combined GE (LSC17 score) was highly correlated to survival outcomes in the training cohort.

The LSC17 score is calculated for each patient as a linear combination of GE of these 17 genes weighted by regression coefficients that were estimated from the training data as follows: LSC17 score = (*DNMT3B* × 0.0874) + (*ZBTB46* × −0.0347) + (*NYNRIN* × 0.00865) + (*ARHGAP22* × −0.0138) + (*LAPTM4B* × 0.00582) + (*MMRN1* × 0.0258) + (*DPYSL3* × 0.0284) + (*KIAA0125* × 0.0196) + (*CDK6* × −0.0704) + (*CPXM1* × −0.0258) + (*SOCS2* × 0.0271) + (*SMIM24* × −0.0226) + (*EMP1* × 0.0146) + (*NGFRAP1* × 0.0465) + (*CD34* × 0.0338) + (*AKR1C3* × −0.0402) + (*GPR56* × 0.0501). As above- and below-median scores in the training cohort were associated with adverse and favourable cytogenetic risk, respectively, a median threshold was used to discretize scores into high and low groups.

An optimized sub-signature was identified by applying the above described regression procedure to CN-LMR cases from GSE6891 with OS >30 days (n = 44), while restricting the analysis to the LSC17 genes. A new equation resulted for computing CN-LMR patient-specific risk scores: LSC3 score = (*DPYSL3* × 0.3) + (*AKR1C3* × −0.0477) + (*NYNRIN* × 0.194).

Similarly, a retrained treatment response score comprising 6 of the LSC17 genes was derived by applying the above described regression workflow to a randomly chosen half of the PM AML cohort: initial induction response score = −6.58 + (*MMRN1* × 0.0442) + (*KIAA0125* × 0.0814) + (*CD34* × 0.104) + (*GPR56* × 0.208) + (*LAPTM4B* × 0.168) + (*NYNRIN* × 0.121).

Signature testing: microarray data processing and analysis. The LSC17 score was initially validated against three published clinically annotated AML cohorts with available microarray GE data (one from TCGA[18] and two from GSE12417 (ref. 19)), while the LSC3 score was tested on an independent CN-LMR subset from GSE15434 (ref. 27). Treatment protocols and the criteria used for cytogenetic/molecular risk classification for each cohort have been previously described[18,19,27]. Raw Affymetrix CEL files (generated on the HG-U133 Plus 2.0 array) containing GE data of a cohort of *de novo* AML patients of all cytogenetic risk groups[18] along with clinical data were downloaded from the TCGA AML data portal (n = 183; Extended Data Table 2). Raw Affymetrix CEL files (generated on the HG-U133 A, B, and Plus 2.0 arrays) containing GE data for two independent cohorts of CN-AML cases[19] were downloaded (GSE12417) and clinical annotations were provided by the authors. Of the 163 GE profiles in GSE12417 CN-AML cohort 1, we removed 2 PB, 1 MDS-RAEB, and 4 other cases with missing clinical data, leaving 156 for analysis (Extended Data Table 3a). For this cohort, the GE data generated on the HG-U133 A and B arrays were merged. The same inclusion criteria for analysis were applied to the 79-patient GSE12417 CN-AML cohort 2, leading to the removal of 1 MDS, 5 PB, and 3 other cases due to missing clinical data, leaving 70 for analysis (Extended Data Table 3b). A data set of 70 CN-LMR HG-U133 Plus 2.0 array profiles was downloaded (GSE15434), with clinical data provided by the authors[27]. In addition, clinical and HG-U133 Plus 2.0 microarray GE data for AML patients treated in the ALFA-0701 trial were provided by the authors[28] (Extended Data Table 8a). All microarray data were normalized as described for the training data set (GSE6891). Signature scores (LSC17 or LSC3) were calculated for each patient in the validation cohorts using the linear equations derived during signature training and a median threshold.

Signature testing: RNA-seq data processing and analysis. One-hundred and sixty-nine patients in the TCGA AML cohort had both microarray and RNA-seq GE data available. The Illumina GA-IIX RNA-seq profiles normalized to reads per kilobase of transcript per million mapped reads (RPKM) were downloaded from the TCGA AML data portal. A value of 1 was added to the RPKM values before applying a log-transformation to the base-2 scale. For each gene, the entry with the maximum mean GE in the data set was used for computing LSC17 scores.

NanoString assay design and GE profiling. We submitted the 17 Affymetrix probeset identifiers associated with the LSC17 score (Extended Data Table 1), along with reference genes chosen to cover a wide range of expression levels in AML[37], to NanoString Technologies[25] for custom codeset creation. The 100 base pair (bp) NanoString probes were fabricated to overlap or be proximal to the corresponding Affymetrix probe target regions. On each 12-lane NanoString cartridge implementing this codeset design, a single lane was reserved for a control composed of an equal parts mixture of 26 synthetic 100 bp DNA oligonucleotides designed to resemble the target transcripts (Integrated DNA Technologies, 1.8 pM per oligonucleotide), against which the GE across all cartridges was normalized to minimize inter-cartridge variability as done by others[38,39]. For each of the remaining 11 lanes, 100 ng, 150 ng, or 250 ng of RNA per sample (5 μl) was incubated with 20 μl of reporter probe and 5 μl of capture probe mix (supplied by the manufacturer) at 65 °C for 16 to 24 h for hybridization on the nCounter Prep Station (version 4.0.11.1). After hybridization, excess probes were washed out using a 2-step magnetic bead-based purification strategy according to the manufacturer's protocol, and purified target/probe complexes were immobilized on the NanoString cartridge for data collection. Transcript counts were determined using the nCounter Digital Analyzer (version 2.1.2.3) at the high-resolution setting. Specifically, digital images were processed with final barcode counts tabulated in reporter code count (RCC) output files.

Signature testing: NanoString data processing and analysis. The NanoString assay was performed using RNA from bulk mononuclear cells obtained from 307 banked diagnostic samples collected from patients treated at PM with curative intent between 1999 and 2012 (Extended Data Table 7a). Patients were excluded if they received any cytoreductive treatment other than hydroxyurea or died within one month of starting therapy. RCC files containing raw transcript counts from each cartridge were analysed using the nSolver analysis software (version 2.0.72)

for quality control (QC) and normalization purposes using default settings for GE analysis. Specifically, RCC files for each cartridge along with a reporter library file containing codeset probe annotations were imported into nSolver. The software was used to normalize the captured transcript counts to the geometric mean of the reference genes included in our assay and the codeset's internal positive controls, and to check for imaging, binding, positive spike-in, and normalization quality. The control lane of each cartridge was processed in the same manner as the RNA lanes using the nSolver software without normalization to reference genes.

The output files from nSolver were read into R for further QC, normalization, and data processing. An RNA input correction step was used to adjust the GE counts of each cartridge to the reference amount of 100 ng RNA. The control lanes for cartridges 1 to 3 were used as blank lanes to estimate per-probe background noise. None of the signature or reference probes exhibited high background counts (that is, <3 standard deviations (s.d.) above the geometric mean of the codeset's 8 internal negative control probes) and thus no background subtraction was required. In lanes where RNA was present, all signature and reference probe counts were well above 3 s.d. over background. The coefficient of variation (CV; s.d. divided by mean GE) and maximum fold change (MFC; maximum divided by minimum GE) were used to quantify GE variation. All reference probes had lower CV and MFC values compared to signature probes and most codeset controls while spanning a sufficiently large range of signature probe GE.

To batch-correct control oligonucleotide counts, multiplicative corrective constants were computed and applied to each batch of control lanes according to the oligonucleotide preparation schedule. Specifically, the oligonucleotide counts of each batch of control lanes were scaled by a ratio of geometric means between the oligonucleotide counts in each batch and that of all control lanes. We next used the batch-corrected control lanes to minimize inter-cartridge technical variation in RNA counts. The geometric mean of the corrected oligonucleotide counts in the control lane of cartridge 5 (arbitrarily chosen) was divided by the same summary value corresponding to each of the other cartridges to produce per-cartridge scaling factors. The RNA and oligonucleotide counts of each cartridge were then adjusted using these factors by means of multiplication, thereby minimizing batch induced GE variation. A final round of normalization to the reference genes was then performed by adjusting the GE counts in all 307 RNA lanes in the data set using a ratio of geometric means between the reference GE counts in each cartridge and that of all cartridges. The fully normalized GE counts were $\log_2$-transformed after incrementing by 1. Signature scores (LSC17 or LSC3) were computed for each patient using the scaled data.

PM cohort treatment details. All patients received induction chemotherapy with a 3+7 backbone (daunorubicin 60 mg/m^2 intravenously (i.v.) daily $\times$ 3 d + cytarabine (ara-C) 200 mg/m^2 i.v. daily $\times$ 7 d. A minority of patients were enrolled in clinical trials employing 3+7 with GO ($n=7$) or midostaurin ($n=3$). Barring contraindications, patients achieving CR went on to receive two cycles of consolidation chemotherapy with daunorubicin 45 mg/m^2 i.v. on days 1–2 + ara-C 3 g/m^2 every 12 h on days 1, 3, 5). Patients with core binding factor leukaemia received one cycle of this consolidation followed by two cycles of ara-C (3 g/m^2 every 12 h on days 1, 3, 5). For APL patients, induction and the first consolidation cycle included all-*trans* retinoic acid (ATRA) 45 mg/m^2 daily $\times$ 28 d, daunorubicin 60 mg/m^2 i.v. daily $\times$ 3 d and ara-C 100 mg/m^2 i.v. daily $\times$ 7 d. The second consolidation cycle included ATRA $\times$ 28 d, daunorubicin 45 mg/m^2 i.v. daily on days 1–3 and ara-C 1.5 g/m^2 every 12 h on days 1, 3, 5. For patients $\geq$60 years of age with WBC count <10, ara-C was omitted from induction and consolidation. For APL patients with initial WBC count <10, maintenance therapy consisted of ATRA 45 mg/m^2/d $\times$ 7 d on alternating weeks $\times$ 9 months. For all others, maintenance involved 21 monthly cycles of 6-mercaptopurine 75 mg/m^2/d daily for 21 d and methotrexate 20 mg/m^2/d once weekly; every other cycle included ATRA 45 mg/m^2/d $\times$ 14 d. aSCT was performed in CR1 for high-risk patients, typically those with secondary AML, adverse cytogenetics, or normal karyotype with poor prognostic molecular features.

Statistical analysis. All statistical analyses were performed in R 3.1.0 (ref. 40). The Spearman rank method of correlation was used unless specified otherwise. Various two-tailed tests were used to evaluate the differences in baseline clinical characteristics between patients with high versus low LSC17 scores. OS was defined as the time from AML diagnosis until death from any cause or last clinical follow-up. EFS was defined as the time from AML diagnosis until an event (that is, induction failure, relapse or death from any cause) or last follow-up. RFS was defined as the time from CR1 until relapse or death (regardless of cause) or last clinical follow-up[41]. Univariate survival analysis was performed using the Kaplan–Meier and CPH models and a median threshold, with comparisons performed using Mantel–Cox log-rank tests. For multivariate analyses, covariates for CPH models included LSC17 or LSC3 score, as well as established risk factors (for example, age and WBC count at diagnosis, *de novo* versus secondary AML, cytogenetic risk group, and *NPM1* and *FLT3*-ITD mutational status). 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 HRs, while violation of the proportional hazards assumption was detected by examining Schoenfeld residuals, and eliminated by setting offending parameters as stratifying variables in the model as done by others[18,19]. Cumulative incidence analysis of relapse and death as competing risks was assessed using Gray (univariate) and Fine–Gray (multivariate) methods[42,43], as implemented in the cmprsk[44] and riskRegression[45] R packages in the EZR software[46]. The impact of aSCT on OS, EFS and RFS, was assessed by encoding transplant as a time-dependent covariate in uni- and multi-variate Mantel–Byar[47] and Andersen–Gill[48] models, respectively, where univariate results were visualized using Simon–Makuch plots[49]. All survival analyses were performed using the survival 2.38-1 R package[50]. In comparing the LSC17 score to other reported signatures, custom CDFs were used to summarize microarray probe expression for each gene, unless specified otherwise.

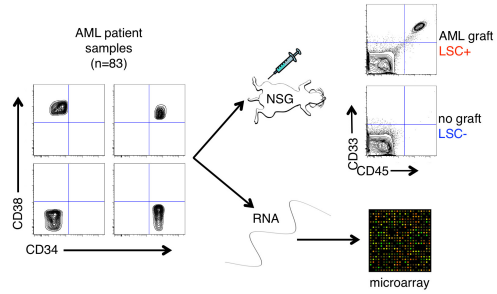
In analyses assessing prediction of treatment response, uni- and multi-variate logistic regression models were used with the bootstrap-adjusted AUROC metric to determine the ability of various parameters to predict initial induction response. The rms 4.4-1 R package[51] was used for logistic regression analysis, while the pROC 1.8 and PredictABEL 1.2-2 R packages were used for ROC curve analyses[52,53]. Relative importance of individual covariates in multivariate logistic regression models was estimated by examining the partial Wald Chi-squared statistic as done by others[5].

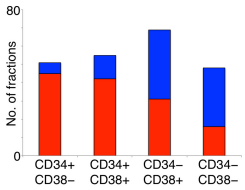
Data availability. All raw and normalized GE data that support the findings of this study have been deposited in the GEO SuperSeries under accession number GSE76009 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76009).

31. Du, P., Kibbe, W. A. & Lin, S. M. lumi: a pipeline for processing Illumina microarray. *Bioinformatics* **24,** 1547–1548 (2008).
32. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **43,** e47 (2015).
33. Qiao, W. *et al.* PERT: a method for expression deconvolution of human blood samples from varied microenvironmental and developmental conditions. *PLOS Comput. Biol.* **8,** e1002838 (2012).
34. Gautier, L., Cope, L., Bolstad, B. M. & Irizarry, R. A. affy—analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* **20,** 307–315 (2004).
35. Wu, J., Irizarry, R., MacDonald, J. & Gentry, J. Gcrma: background adjustment using sequence information. R package version 2.36.0 (2016).
36. Dai, M. *et al.* Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. *Nucleic Acids Res.* **33,** e175 (2005).
37. Macrae, T. *et al.* RNA-seq reveals spliceosome and proteasome genes as most consistent transcripts in human cancer cells. *PLoS ONE* **8,** e72884 (2013).
38. Scott, D. W. *et al.* Determining cell-of-origin subtypes of diffuse large B-cell lymphoma using gene expression in formalin-fixed paraffin-embedded tissue. *Blood* **123,** 1214–1217 (2014).
39. Nielsen, T. *et al.* Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. *BMC Cancer* **14,** 177 (2014).
40. R Development Core Team. *A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2014).
41. Cheson, B. D. *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.* **21,** 4642–4649 (2003).
42. Gray, R. J. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann. Stat.* **16,** 1141–1154 (1988).
43. Fine, J. P. & Gray, R. J. A proportional hazards model for the subdistribution of a competing risk. *J. Am. Stat. Assoc.* **94,** 496–509 (1999).
44. Gray, B. cmprsk: subdistribution analysis of competing risks. R package version 2.2-7 (2014).
45. Gerds, T. A. & Scheike, T. H. riskRegression: risk regression for survival analysis. R package version 0.0.8 (2016).
46. Kanda, Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.* **48,** 452–458 (2013).
47. Mantel, N. & Byar, D. Evaluation of response-time data involving transient states: an illustration using heart transplant data. *J. Am. Stat. Assoc.* **69,** 81–86 (1974).
48. Andersen, P. & Gill, R. D. Cox's regression model for counting processes: a large sample study. *Ann. Stat.* **10,** 1100–1120 (1982).
49. Simon, R. & Makuch, R. W. 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.* **3,** 35–44 (1984).
50. Therneau, T. M. & Grambsch, P. M. *Modeling Survival Data: Extending the Cox Model* (Springer, 2000).
51. Harrell, F. E. Jr. rms: regression modeling strategies. R package version 4.4-1 (2016).
52. Robin, X. *et al.* pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* **12,** 77 (2011).
53. Kundu, S., Aulchenko, Y. S., van Duijn, C. M. & Janssens, A. C. PredictABEL: an R psackage for the assessment of risk prediction models. *Eur. J. Epidemiol.* **26,** 261–264 (2011).

a

	n=78
Female Sex [*n* (%)]	27 (34.6)
Age at AML Diagnosis [years]	
median (range)	61.6 (20.1-86.2)
***De novo* vs. Secondary AML [*n* (%)]**	
De novo AML	54 (69.2)
Secondary AML / t-AML	23 (29.4)
CMML	1 (1.3)
PB WBC count at diagnosis ($x10^9$/L)	
Median (range)	49.7 (1.3-606)
BM blast % at diagnosis	n=60
Median (range)	80 (23-96)
Karyotype [n (%)]	
Normal karyotype	32 (41.0)
Abnormal karyotype	35 (44.9)
Not available	11 (14.1)
MRC cytogenetic class at diagnosis [n (%)]	
Favorable	0 (0)
Intermediate	46 (59.0)
Adverse	21 (26.9)
Not available	11 (14.1)
AML subtypes [n (%)]	
APL	0 (0)
Non-APL	78 (100)
CN AML- *NPM1* [n (%)]	n=32
NPM1 mutation	18 (56.3)
No *NPM1* mutation	10 (31.3)
Not available	4 (12.5)
CN AML- *FLT3*-ITD [n (%)]	n=32
FLT3-ITD positive	16 (50)
FLT3-ITD negative	14 (43.8)
Not available	2 (6.3)





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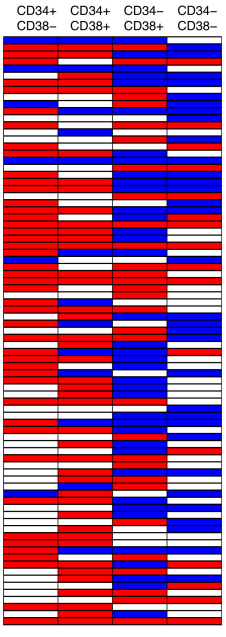

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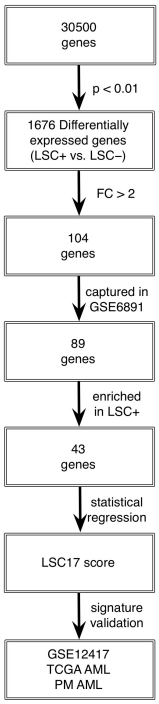
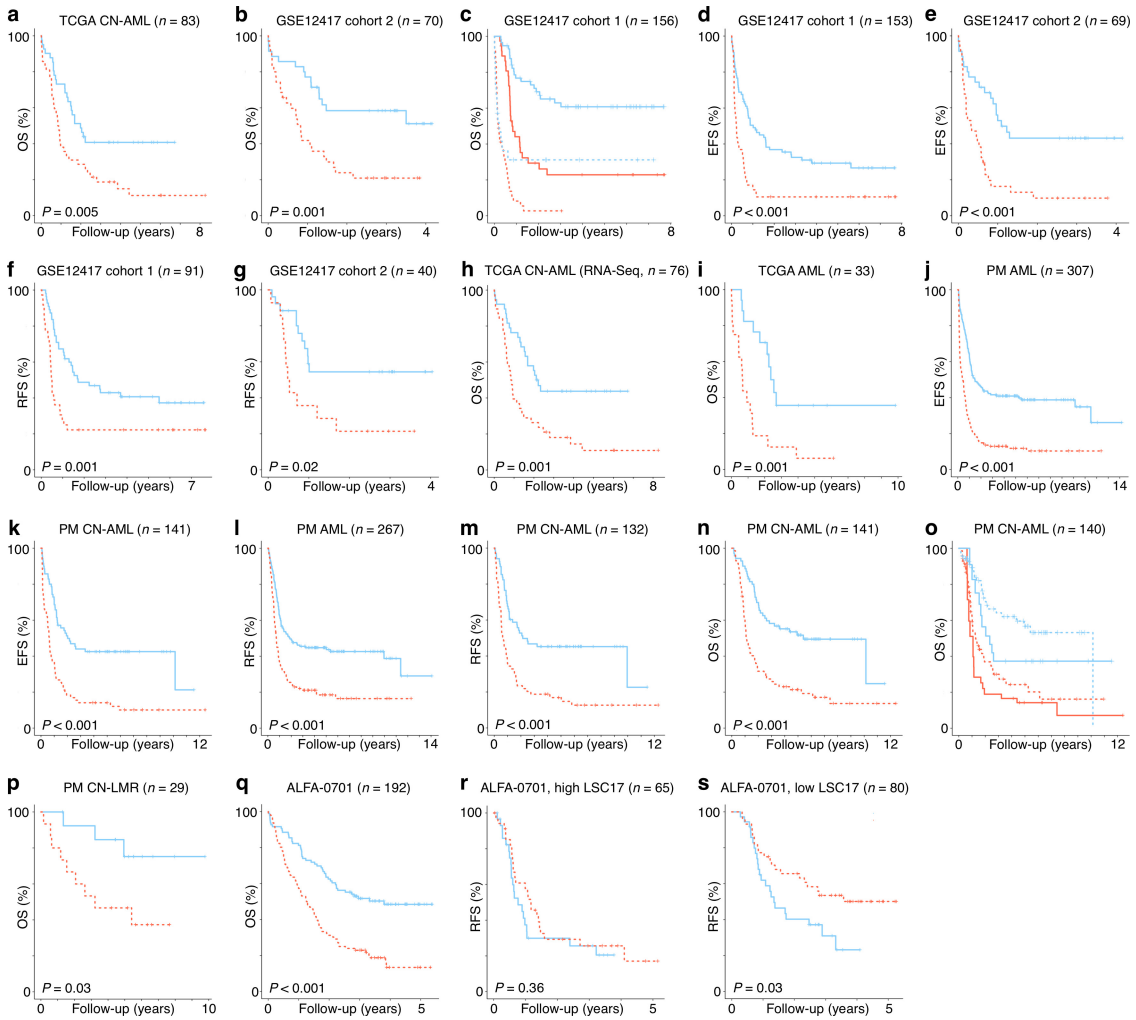


f

Characteristic	GSE6891 Training Cohort (*n*=495)	High LSC17 score subset (*n*=248)	Low LSC17 score subset (*n*=247)	P-value
Female Sex [*n* (%)]	252 (50.9)	132 (53.2)	120 (48.6)	0.34‡
Age at AML Diagnosis [years]				
median (range)	46 (15-77)	47 (15-77)	45 (15-77)	0.33†
***De novo* vs. Secondary AML [*n* (%)]**				
De novo	495 (100)	248 (100)	247 (100)	N/A
Secondary / t-AML	0 (0)	0 (0)	0 (0)	
PB WBC count at diagnosis ($x10^9$/L)				
median (range)	31 (0.30-510)	32 (0.8-278)	30 (0.3-510)	0.82*
Blast % at diagnosis ($x10^9$/L)				
Median BM Blasts (range)	66 (0-98)	71 (0-98)	60 (0-98)	0.004*
Cytogenetic risk class at diagnosis [n (%)]¶	n=483	n=241	n=242	
Favorable	99 (20.5)	7 (2.9)	92 (38)	<0.001‖
Intermediate	302 (62.5)	171 (71)	131 (54.1)	
Adverse	82 (17)	63 (26.1)	19 (7.85)	
Karyotype [n (%)]	n=494		n=246	
Normal karyotype	210 (42.5)	118 (47.6)	92 (37.4)	0.02‡
Abnormal karyotype	284 (57.5)	130 (52.4)	154 (62.6)	
AML subtypes [n (%)]				
APL	23 (4.65)	4 (1.61)	19 (7.69)	0.001‖
Non-APL	472 (95.4)	244 (98.4)	228 (92.3)	
CN AML- *NPM1* [n (%)]	n=210	n=118	n=92	
NPM1 mutation	124 (59)	63 (53.4)	61 (66.3)	0.08‡
No *NPM1* mutation	86 (41)	55 (46.6)	31 (33.7)	
CN AML- *FLT3*-ITD [n (%)]	n=210	n=118	n=92	
FLT3-ITD positive	93 (44.3)	71 (60.2)	22 (23.9)	<0.001‡
FLT3-ITD negative	117 (55.7)	47 (39.8)	70 (76.1)	
Treatment Response [n (%)]				
Complete Response	394 (79.6)	175 (70.6)	219 (88.7)	<0.001‡
No Response	101 (20.4)	73 (29.4)	28 (11.3)	
Events [n (%)]				
Relapse	202 (40.8)	117 (47.2)	85 (34.4)	0.005‡
Survival Parameters [days]				
Median Overall Survival	576	318	3960	<0.001§
Median Event-Free Survival	3678	3003	3732	0.01§

Extended Data Figure 1 | Overview of LSC signature training and testing. a, Clinical characteristics of the 78 patients analysed by xenotransplantation and microarray GE analysis. CMML, chronic myelomonocytic leukaemia; t-AML, therapy-associated AML; CN, cytogenetically normal. **b**, Schematic of the experimental protocol. **c**, **d**, Summary of functionally defined LSC^+ and LSC^- fractions in each phenotypic cell population as a whole (**c**) and for each patient (**d**). Red and blue denote LSC^+ and LSC^-, respectively. In **d**, each row represents fractions sorted from one patient sample. White boxes denote fractions that were not included in the analysis due to insufficient cell numbers for xenotransplantation and/or insufficient RNA. **e**, Strategy used to identify and test the 17 LSC signature genes. **f**, Key clinical characteristics of the GSE6891 signature training cohort. **P* value calculated using the Wilcoxon rank-sum test; †*P* value calculated using the Student's *t*-test; ‡*P* value calculated using Pearson's chi-squared test; §*P* value calculated using log-rank test; ||*P* value calculated using Fisher's exact test; ¶cytogenetic risk groups were defined as per GSE6891 investigators[15].



Extended Data Figure 2 | LSC17 and LSC3 scores are associated with survival in multiple AML cohorts. a–n, **q**, Kaplan–Meier estimates of OS, EFS or RFS according to LSC17 scores in various patient cohorts, as indicated. In **c**, patients were also analysed according to whether or not CR was achieved after initial treatment (no CR, dotted lines; CR, solid lines). **i**, The subset of patients in the TCGA AML cohort with no clear genomic classification as defined previously[21]. **o**, Simon and Makuch estimates of OS, according to LSC17 scores and whether or not patients received aSCT (no aSCT, dotted lines; aSCT, solid lines). **p**, Kaplan–Meier estimates of OS of CN-LMR patients, according to LSC3 scores. In **a–q**, patients with scores above and below the median in each cohort are shown by red and blue lines, respectively. **r**, **s**, Kaplan–Meier estimates of RFS for patients with high (**r**) or low (**s**) LSC17 scores treated with standard chemotherapy with (red lines) or without (blue lines) addition of GO.

Extended Data Table 1 | List of 104 DE LSC genes

Gene Symbol	Entrez ID	Illumina Probe ID*	Log_2 Fold Change†	P-value‡	Affymetrix Probeset ID§	Signature Gene
CD34	947	ILMN_1732799	2.15	<0.0001	209543_s_at	LSC17
SPINK2	6691	ILMN_1763516	1.99	<0.0001	206310_at	N/A
LAPTM4B	55353	ILMN_2101832	1.8	<0.0001	214039_s_at	LSC17
HOXA5	3202	ILMN_1753613	1.72	<0.0001	213844_at	N/A
GUCY1A3	2982	ILMN_1808590	1.62	<0.0001	229530_at	N/A
SHANK3	85358	ILMN_2317581	1.59	<0.0001	227923_at	N/A
ANGPT1	284	ILMN_1677723	1.51	<0.0001	205609_at	N/A
ARHGAP22	58504	ILMN_1676361	1.48	<0.0001	206298_at	LSC17
LOC284422	284422	ILMN_1774375	1.45	<0.0001	231982_at	LSC17
MYCN	4613	ILMN_2219767	1.41	<0.0001	209757_s_at	N/A
MAMDC2	256691	ILMN_1679391	1.4	<0.0001	228885_at	N/A
PRSSL1	400668	ILMN_1673605	1.4	<0.0001	N/A	N/A
KIAA0125	9834	ILMN_1707491	1.4	<0.0001	206478_at	LSC17
GPSM1	26086	ILMN_1709307	1.38	<0.0001	226043_at	N/A
HOXA9	3205	ILMN_1739582	1.38	<0.0001	N/A	N/A
MMRN1	22915	ILMN_1660114	1.36	<0.0001	205612_at	LSC17
FSCN1	6624	ILMN_1808707	1.32	<0.0001	210933_s_at	N/A
DNMT3B	1789	ILMN_2328972	1.31	<0.0001	220668_s_at	LSC17
HOXA6	3203	ILMN_1815570	1.28	<0.0001	208557_at	N/A
AIF1L	83543	ILMN_3246401	1.25	<0.0001	223075_s_at	N/A
SOCS2	8835	ILMN_1798926	1.24	<0.0001	203373_at	LSC17
CDK6	1021	ILMN_1802615	1.23	<0.0001	224851_at	LSC17
FAM69B	138311	ILMN_1757440	1.2	<0.0001	229002_at	N/A
NGFRAP1	27018	ILMN_2370091	1.2	<0.0001	217963_s_at	LSC17
C3orf54	389119	ILMN_1690454	1.2	<0.0001	229507_at	N/A
CPXM1	56265	ILMN_1712046	1.2	<0.0001	227860_at	LSC17
TNFRSF4	7293	ILMN_2112256	1.2	<0.0001	214228_x_at	N/A
ZBTB46	140685	ILMN_1710092	1.19	<0.0001	227329_at	LSC17
DPYSL3	1809	ILMN_1679262	1.16	<0.0001	201431_s_at	LSC17 & LSC3
NYNRIN	57523	ILMN_3236858	1.15	<0.0001	220911_s_at	LSC17 & LSC3
COL24A1	255631	ILMN_1810996	1.13	<0.0001	238732_at	N/A
FAM30A	29064	ILMN_3187535	1.11	<0.0001	N/A	N/A
C10orf140	387640	ILMN_3239861	1.1	<0.0001	N/A	N/A
SPNS2	124976	ILMN_3301749	1.07	<0.0001	225671_at	N/A
GPR56	9289	ILMN_2384122	1.07	0.00054	212070_at	LSC17
AKR1C3	8644	ILMN_1713124	1.06	<0.0001	209160_at	LSC17 & LSC3
FLT3	2322	ILMN_1766363	1.05	<0.0001	206674_at	N/A
TFPI	7035	ILMN_1707124	1.05	<0.0001	213258_at	N/A
KCNK17	89822	ILMN_1717702	1.04	<0.0001	224049_at	N/A
EPDR1	54749	ILMN_1675797	1.03	<0.0001	223253_at	N/A
C1orf150	148823	ILMN_1762204	1.02	<0.0001	N/A	N/A
BIVM	54841	ILMN_2214098	1.02	<0.0001	222761_at	N/A
H2AFY2	55506	ILMN_1705570	1.02	<0.0001	218445_at	N/A
VWF	7450	ILMN_1752755	1.02	0.000103	202112_at	N/A
EMP1	2012	ILMN_1801616	1.01	<0.0001	201324_at	LSC17
RAGE	5891	ILMN_1745282	1.01	<0.0001	205130_at	N/A
ATP8B4	79895	ILMN_1783956	1.01	<0.0001	220416_at	N/A
GATA2	2624	ILMN_2102670	1	<0.0001	209710_at	N/A
SLC25A37	51312	ILMN_1715969	-1.01	<0.0001	222528_s_at	N/A
SGK	6446	ILMN_3305938	-1.01	<0.0001	201739_at	N/A
LOC652694	652694	ILMN_1680274	-1.01	<0.0001	N/A	N/A
ITPR3	3710	ILMN_1815500	-1.02	<0.0001	201187_s_at	N/A
LOC654103	654103	ILMN_1802808	-1.02	<0.0001	N/A	N/A
CXCR4	7852	ILMN_1801584	-1.04	<0.0001	217028_at	N/A
FCRL3	115352	ILMN_1691693	-1.05	<0.0001	N/A	N/A
RBM38	55544	ILMN_2404049	-1.05	<0.0001	212430_at	N/A
LILRA5	353514	ILMN_2357419	-1.06	<0.0001	215838_at	N/A
IL18RAP	8807	ILMN_1721762	-1.06	<0.0001	207072_at	N/A
CCDC109B	55013	ILMN_1801766	-1.08	<0.0001	218802_at	N/A
ISG20	3669	ILMN_1659913	-1.09	<0.0001	33304_at	N/A
MTSS1	9788	ILMN_2073289	-1.09	<0.0001	203037_s_at	N/A
CECR1	51816	ILMN_1751851	-1.1	<0.0001	219505_at	N/A
ADAM19	8728	ILMN_1713751	-1.1	<0.0001	209765_at	N/A
FCGR2A	2212	ILMN_1666932	-1.11	<0.0001	N/A	N/A
AIM2	9447	ILMN_1681301	-1.11	<0.0001	206513_at	N/A
NPL	80896	ILMN_1782070	-1.14	<0.0001	223405_at	N/A
IL10RA	3587	ILMN_1652825	-1.15	<0.0001	204912_at	N/A
CTSL1	1514	ILMN_1812995	-1.16	<0.0001	202087_s_at	N/A
GNLY	10578	ILMN_1708779	-1.19	<0.0001	205495_s_at	N/A
CKAP4	10970	ILMN_1790891	-1.19	<0.0001	200999_s_at	N/A
ADM	133	ILMN_1708934	-1.19	<0.0001	202912_at	N/A
KLRB1	3820	ILMN_2079655	-1.19	<0.0001	214470_at	N/A
SLC15A3	51296	ILMN_2085862	-1.21	<0.0001	219593_at	N/A
FGR	2268	ILMN_1795158	-1.22	<0.0001	208438_s_at	N/A
FCRLA	84824	ILMN_1691071	-1.22	<0.0001	235372_at	N/A
IL2RB	3560	ILMN_1684349	-1.23	<0.0001	205291_at	N/A
CXCL16	58191	ILMN_1728478	-1.24	<0.0001	223454_at	N/A
SLC4A1	6521	ILMN_1772809	-1.24	<0.0001	205592_at	N/A
GZMH	2999	ILMN_1731233	-1.27	<0.0001	210321_at	N/A
FLJ22662	79887	ILMN_1707286	-1.27	<0.0001	218454_at	N/A
LOC647506	647506	ILMN_3240375	-1.28	<0.0001	N/A	N/A
GIMAP4	55303	ILMN_1748473	-1.29	<0.0001	219243_at	N/A
JAZF1	221895	ILMN_1682727	-1.32	<0.0001	225798_at	N/A
CTSH	1512	ILMN_2390853	-1.33	<0.0001	202295_s_at	N/A
GZMA	3001	ILMN_1779324	-1.35	<0.0001	205488_at	N/A
CHST15	51363	ILMN_1670926	-1.35	<0.0001	203066_at	N/A
AQP9	366	ILMN_1715068	-1.4	<0.0001	205568_at	N/A
CD247	919	ILMN_1676924	-1.41	<0.0001	210031_at	N/A
BCL6	604	ILMN_1737314	-1.42	<0.0001	203140_at	N/A
SLC7A7	9056	ILMN_1810275	-1.43	<0.0001	204588_s_at	N/A
E2F2	1870	ILMN_1777233	-1.45	<0.0001	228361_at	N/A
LOC647450	647450	ILMN_1699214	-1.45	<0.0001	N/A	N/A
GZMB	3002	ILMN_2109489	-1.47	<0.0001	210164_at	N/A
LOC652493	652493	ILMN_1739508	-1.61	<0.0001	N/A	N/A
HBM	3042	ILMN_2091454	-1.62	<0.0001	240336_at	N/A
CD14	929	ILMN_2396444	-1.74	<0.0001	201743_at	N/A
ALAS2	212	ILMN_2367126	-1.76	<0.0001	211560_s_at	N/A
HBB	3043	ILMN_2100437	-1.78	<0.0001	209116_x_at	N/A
LOC642113	642113	ILMN_1652199	-1.79	<0.0001	N/A	N/A
AHSP	51327	ILMN_1696512	-1.84	<0.0001	219672_at	N/A
FCN1	2219	ILMN_1668063	-1.85	<0.0001	205237_at	N/A
CD48	962	ILMN_2061043	-1.85	<0.0001	204118_at	N/A
HBA2	3040	ILMN_2127842	-2.06	<0.0001	N/A	N/A
HBA1	3039	ILMN_3240144	-2.07	<0.0001	N/A	N/A

*Illumina microarray probe IDs.
†Fold change LSC^+ compared to LSC^- GE profiles.
‡Student's *t*-test *P* values for fold changes.
§Affymetrix microarray probeset IDs.

Extended Data Table 2 | Clinical characteristics of the TCGA AML cohort

Characteristic	TCGA AML cohort	High LSC17 score subset	Low LSC17 score subset	P-value
	(*n*=183)	(*n*=92)	(*n*=91)	
Female Sex [*n* (%)]	85 (46.4)	39 (42.4)	46 (50.5)	0.33‡
Age at AML Diagnosis [years]				
median (range)	57 (18-88)	61 (21-88)	55 (18-82)	0.002†
PB WBC count at diagnosis (x10^9/L)				
median (range)	16.8 (0.5-298.4)	12.1 (0.5-297.4)	26.1 (0.6-298.4)	0.06*
Blast % at diagnosis (x10^9/L)				
Median BM Blasts (range)	72 (30-100)	73 (30-99)	72 (30-100)	0.97*
Median PB Blasts (range)	33 (0-98)	35.5 (0-98)	33 (0-97)	0.57*
AML subtypes [n (%)]				
APL	17 (9.29)	4 (4.35)	13 (14.3)	0.02‖
Non-APL	166 (90.7)	88 (95.7)	78 (85.7)	
Cytogenetic risk class at diagnosis [n (%)]¶				
Favorable	38 (20.8)	7 (7.61)	31 (34.1)	<0.001‖
Intermediate	105 (57.4)	53 (57.6)	52 (57.1)	
Adverse	40 (21.9)	32 (34.8)	8 (8.79)	
Karyotype [n (%)]	n=179	n=90	n=89	
Normal karyotype	83 (46.4)	36 (40)	47 (52.8)	0.11‡
Abnormal karyotype	96 (53.6)	54 (60)	42 (47.2)	
Molecular Risk at diagnosis [n (%)]	n=180	n=91	n=89	
Favorable	35 (19.4)	5 (5.49)	30 (33.7)	<0.001‖
Intermediate	98 (54.4)	50 (54.9)	48 (53.9)	
Adverse	47 (26.1)	36 (39.6)	11 (12.4)	
CN AML- *NPM1* [n (%)]	n=83	n=36	n=47	
NPM1 mutation	42 (50.6)	18 (50)	24 (51.1)	1.00‡
No *NPM1* mutation	41 (49.4)	18 (50)	23 (48.9)	
CN AML- *FLT3*-ITD [n (%)]	n=83	n=36	n=47	
FLT3-ITD positive	23 (27.7)	16 (44.4)	7 (14.9)	0.005‖
FLT3-ITD negative	60 (72.3)	20 (55.6)	40 (85.1)	
Survival Parameters [days]				
Median Overall Survival	492	303	1029	<0.001§

*P value calculated using the Wilcoxon rank-sum test.
†P value calculated using the Student's t-test.
‡P value calculated using the Pearson's chi-squared test.
§P value calculated using the log-rank test.
‖P value calculated using the Fisher's exact test.
¶Cytogenetic risk groups were defined by TCGA research network[18].

Extended Data Table 3 | Clinical characteristics of the GSE12417 CN-AML cohorts

a

Characteristic	GSE12417 CN-AML cohort 1	High LSC17 score subset	Low LSC17 score subset	P-value
	(*n*=156)	(*n*=78)	(*n*=78)	
Female Sex [*n* (%)]	84 (53.8)	39 (50)	45 (57.7)	0.42‡
Age at AML Diagnosis [years]				
median (range)	57 (17-83)	61 (20-81)	54.5 (17-83)	0.03†
***De novo* vs. Secondary AML [*n* (%)]**				
De novo	149 (95.5)	74 (94.9)	75 (96.2)	1.00‖
Secondary / t-AML	7 (4.49)	4 (5.13)	3 (3.85)	
PB WBC count at diagnosis (x10^9/L)				
median (range)	36.2 (0.095-486)	45.3 (0.9-486)	30.6 (0.095-289)	0.18*
BM blast % at diagnosis (x10^9/L)	n=153	*n*=77	*n*=76	
median (range)	85 (20-100)	90 (20-100)	80 (20-100)	0.04*
***NPM1* [n (%)]**				
NPM1 mutation	83 (53.2)	42 (53.8)	41 (52.6)	1.00‡
No *NPM1* mutation	73 (46.8)	36 (46.2)	37 (47.4)	
***FLT3*-ITD [n (%)]**				
FLT3-ITD positive	75 (48.1)	53 (67.9)	22 (28.2)	<0.001‡
FLT3-ITD negative	81 (51.9)	25 (32.1)	56 (71.8)	
Treatment Response [n (%)]				
Complete Response	94 (60.3)	37 (47.4)	57 (73.1)	0.001‡
No Response	62 (39.7)	41 (52.6)	21 (26.9)	
Survival Parameters [days]				
Median Overall Survival	294	223	not reached	<0.001§
Median Event-Free Survival	192 (n=153)	83 (n=76)	371 (n=77)	<0.001§
Median Relapse-Free Survival	384 (n=91)	178 (n=36)	627 (n=55)	0.001§

b

Characteristic	GSE12417 CN-AML cohort 2	High LSC17 score subset	Low LSC17 score subset	P-value
	(*n*=70)	(*n*=35)	(*n*=35)	
Female Sex [*n* (%)]	29 (41.4)	18 (51.4)	11 (31.4)	0.14‖
Age at AML Diagnosis [years]				
median (range)	62 (18-85)	62 (22-81)	62 (18-85)	0.15†
***De novo* vs. Secondary AML [*n* (%)]**				
De novo	62 (88.6)	28 (80)	34 (97.1)	0.05‖
Secondary / t-AML	8 (11.4)	7 (20)	1 (2.86)	
PB WBC count at diagnosis (x10^9/L)	*n*=68	*n*=34	n=34	
median (range)	15 (1-440.3)	14.0 (1-440.3)	17.8 (1-280)	0.85*
BM blast % at diagnosis (x10^9/L)	*n*=67	*n*=33	n=34	
median (range)	80 (18-97)	80 (18-95)	87.5 (20-97)	0.26*
***NPM1* [n (%)]**				
NPM1 mutation	36 (51.4)	13 (37.1)	23 (65.7)	0.03‖
No *NPM1* mutation	34 (48.6)	22 (62.9)	12 (34.3)	
***FLT3*-ITD [n (%)]**				
FLT3-ITD positive	19 (27.1)	14 (40)	5 (14.3)	0.03‖
FLT3-ITD negative	51 (72.9)	21 (60)	30 (85.7)	
Treatment Response [n (%)]	*n*=68	*n*=33		
Complete Response	43 (63.2)	15 (45.5)	28 (80)	0.005‖
No Response	25 (36.8)	18 (54.5)	7 (20)	
Survival Parameters [days]				
Median Overall Survival	500	301	not reached	0.001§
Median Event-Free Survival	243 (n=69)	120 (n=34)	398	<0.001§
Median Relapse-Free Survival	368 (n=40)	183 (n=14)	not reached (n=26)	0.02§

*P value calculated using the Wilcoxon rank-sum test.
†P value calculated using the Student's *t*-test.
‡P value calculated using the Pearson's chi-squared test.
§P value calculated using the log-rank test.
||P value calculated using the Fisher's exact test.

Extended Data Table 4 | Multivariate survival analysis of LSC17 and LSC3 scores

a

Overall Survival	TCGA AML (n=183)*		TCGA AML RNA-Seq (n=166)*	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 Score	2.50 (1.37-4.58)	0.002	1.91 (1.23–2.98)	0.003
Age	Stratifier§	N/A	1.03 (1.02-1.05)	<0.001
WBC count	1.01 (1.00-1.015)	0.004	1.01 (1.00-1.01)	<0.001
Favorable Cytogenetics	0.73 (0.36-1.49)	0.39	0.71 (0.37-1.36)	0.30
Adverse Cytogenetics	1.52 (0.80-2.89)	0.19	1.57 (1.02-2.41)	0.04
	TCGA CN-AML (n=83)*		TCGA CN-AML RNA-Seq (n=76)*	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 Score	5.32 (1.27-22.3)	0.02	2.44 (1.25-4.77)	0.008
Age	Stratifier§	N/A	1.02 (1.00-1.046)	0.02
WBC count	1.02 (1.00-1.03)	0.01	1.01 (1.00-1.01)	0.002
NPM1 Mutation	1.01 (0.26-3.90)	0.98	0.96 (0.53-1.74)	0.90
FLT3-ITD Mutation	5.23 (1.12-24.4)	0.03	1.28 (0.64-2.56)	0.48
	GSE12417 CN-AML Cohort 1 (n=156)*		GSE12417 CN-AML Cohort 2 (n=68)*	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 Score	2.45 (1.54-3.89)	<0.001	2.29 (1.08-4.84)	0.02
Age	1.02 (1.00-1.04)	0.009	1.03 (1.00-1.06)	0.04
WBC count	1.00 (1.00-1.00)	0.95	1.00 (1.00-1.00)	0.002
NPM1 Mutation	0.72 (0.47-1.10)	0.13	0.52 (0.25-1.08)	0.08
FLT3-ITD Mutation	1.88 (1.17-3.02)	0.009	1.08 (0.47-2.48)	0.85
Secondary / t-AML	1.49 (0.65-3.42)	0.34	0.56 (0.17-1.83)	0.34
	PM AML (n=284)*		PM CN-AML (n=85)*	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 Score	2.49 (1.78-3.48)	<0.001	2.02 (1.04-3.92)	0.03
Age	1.00 (0.99-1.01)	0.25	1.01 (0.98-1.03)	0.40
WBC count	1.00 (1.00-1.00)	0.003	1.00 (0.99-1.00)	0.11
NPM1 Mutation	N/A	N/A	0.44 (0.22-0.92)	0.02
FLT3-ITD Mutation	N/A	N/A	1.96 (0.97-3.95)	0.05
Favorable Cytogenetics	0.46 (0.26-0.79)	0.005	N/A	N/A
Adverse Cytogenetics	1.96 (1.33-2.91)	<0.001	N/A	N/A
Secondary / t-AML	2.39 (1.61-3.54)	<0.001	2.63 (1.15-6.01)	0.02

b

Overall Survival	GSE15434 CN-LMR (n=70)*		PM CN-LMR (n=29)*	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC3 Score	8.49 (2.46-29.3)	<0.001	6.30 (1.22-32.3)	0.02
Age	0.99 (0.94-1.04)	0.81	0.99 (0.93-1.05)	0.84
WBC count	N/A	N/A	1.01 (1.00-1.02)	0.02
Secondary / t-AML	1.19 (0.34-4.22)	0.78	11.5 (1.42-93.5)	0.02

t-AML, therapy-associated AML.
*Number of patients with full clinical annotation.
†95% confidence interval.
‡*P* value calculated using the Wald test.
§Age violated the proportional hazards assumption.

Extended Data Table 5 | The LSC17 score refines genomic classifications

a

TCGA AML (n=183)*	Univariate Analysis		Multivariate Analysis 1		Multivariate Analysis 2 (P<0.001)‖	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 Score	N/A	N/A	Not included in model	N/A	3.08 (1.56-6.06)	0.001
PML-RARA Mutation	0.30 (0.12-0.73), n=15	0.008	0.43 (0.10-1.74)	0.23	0.34 (0.08-1.47)	0.15
MYH11-CBFB Mutation	0.33 (0.12-0.90), n=10	0.03	0.40 (0.08-1.96)	0.26	0.27 (0.05-1.38)	0.11
FLT3 in-frame Mutation	5.98 (2.12-16.8), n=4	<0.001	4.78 (0.73-31.3)	0.10	7.75 (0.90-66.2)	0.06
DNMT3A Mutation	1.58 (1.07-2.32), n=45	0.02	1.74 (0.95-3.17)	0.07	1.92 (1.02-3.58)	0.04
RUNX1 Mutation	1.79 (1.05-3.03), n=17	0.03	1.44 (0.66-3.17)	0.35	1.08 (0.48-2.45)	0.84
TP53 Mutation	3.61 (2.09-6.22), n=16	<0.001	2.30 (0.92-5.71)	0.07	1.71 (0.66-4.38)	0.26
Age	N/A	N/A	Stratifier§	N/A	Stratifier§	N/A
WBC count	N/A	N/A	1.00 (1.00-1.01)	0.006	1.01 (1.00-1.01)	0.005
Favorable Cytogenetics	N/A	N/A	1.32 (0.43-4.06)	0.62	2.33 (0.68-7.92)	0.17
Adverse Cytogenetics	N/A	N/A	1.61(0.78-3.31)	0.19	1.40 (0.65-3.02)	0.39

b

Overall Survival TCGA AML (n=183)*	Multivariate Analysis 1		Multivariate Analysis 2 (P=0.001)‖	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 score	Not included in model	N/A	2.85 (1.47-5.52)	0.002
Age	Stratifier§	N/A	Stratifier§	N/A
WBC count	1.00 (1.00-1.01)	0.02	1.00 (1.00-1.01)	0.01
$CEBPA^{biallelic}$	0.86 (0.11-6.57)	0.89	1.45 (0.18-11.5)	0.72
Chromatin–spliceosome	1.80 (0.54-5.98)	0.33	1.00 (0.28-3.54)	0.99
$IDH2^{R172}$	<0.001 (0.00-inf)	0.99	<0.001 (0.00-inf)	0.99
inv(16)	1.08 (0.23-5.03)	0.91	0.82 (0.17-3.85)	0.80
MLL fusion	2.19 (0.51-9.35)	0.28	1.77 (0.39-7.89)	0.45
No class-defining drivers	2.19 (0.60-7.98)	0.23	1.54 (0.40-5.87)	0.52
No driver mutations	1.20 (0.16-8.60)	0.85	0.71 (0.09-5.34)	0.74
NPM1 mutation	2.21 (0.70-6.93)	0.17	1.65 (0.51-5.30)	0.39
t(8;21)	1.52 (0.21-10.8)	0.67	2.22 (0.29-16.5)	0.43
TP53–aneuploidy	4.53 (1.34-15.3)	0.01	2.12 (0.58-7.73)	0.25
2+ classes	3.26 (0.48-21.9)	0.22	1.70 (0.24-11.8)	0.59

c

Overall Survival TCGA AML (n=33)*	Multivariate Analysis 1		Multivariate Analysis 2 (P=0.01)‖	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High LSC17 score	Not included in model	N/A	3.33 (1.30-8.51)	0.01
Age	1.05 (1.02-1.09)	<0.001	1.04 (1.01-1.08)	0.006
WBC count	1.01 (1.00-1.02)	0.02	1.01 (1.00-1.02)	0.006
No driver mutations	0.54 (0.12-2.41)	0.42	0.34 (0.07-1.53)	0.16
2+ classes	1.84 (0.51-6.61)	0.34	1.19 (0.32-4.37)	0.78

b, Genomic classes were compared to t(15;17) in CPH models. **c**, Genomic classes were compared to the no class-defining drivers category[21] in CPH models. Patient scores above or below the median score of the entire TCGA AML cohort were designated as high or low LSC17 score, respectively.

*Number of patients with full clinical annotation.

†95% confidence interval.

‡*P* value was calculated using the Wald test.

§Age violated the proportional hazards assumption.

‖*P* value was calculated using the likelihood ratio test to assess model improvement by including LSC17.

Extended Data Table 6 | The LSC17 score improves survival association compared to other LSC signatures

Overall Survival	GSE12417 CN-AML Cohort 1 Univariate Analysis (n=156)*		GSE12417 CN-AML Cohort 2 Univariate Analysis (n=70)*		TCGA AML Univariate Analysis (n=183)*	
Covariate	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡	Hazard Ratio (95% CI)†	P-value‡
High SDPC Score	2.26 (1.50-3.40)	<0.001	1.43 (0.78-2.63)	0.24	0.96 (0.68-1.35)	0.82
High IFPC Score	2.18 (1.45-3.27)	<0.001	1.65 (0.89-3.06)	0.10	1.19 (0.85-1.68)	0.30
High Jung et al. Score	2.35 (1.56-3.54)	<0.001	1.45 (0.78-2.67)	0.23	2.02 (1.42-2.87)	<0.001
High Gentles et al. Score	2.15 (1.43-3.22)	<0.001	1.21 (0.66-2.23)	0.53	1.68 (1.18-2.38)	0.003
	Multivariate Analysis (P<0.001)‖		**Multivariate Analysis (P=0.02)‖**		**Multivariate Analysis (P=0.001)‖**	
Covariate	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**
High LSC17 Score	2.31 (1.42-3.76)	<0.001	2.33 (1.10-4.91)	0.02	2.67 (1.45-4.92)	0.001
High SDPC Score	1.20 (0.73-1.98)	0.46	0.53 (0.23-1.23)	0.14	0.71 (0.40-1.26)	0.24
Age	1.02 (1.00-1.03)	0.01	1.03 (1.00-1.07)	0.02	Stratifier§	N/A
WBC count	1.00 (1.00-1.00)	0.92	1.00 (1.00-1.00)	<0.001	1.01 (1.00-1.01)	0.006
Favorable Cytogenetics	N/A	N/A	N/A	N/A	0.84 (0.39-1.77)	0.64
Adverse Cytogenetics	N/A	N/A	N/A	N/A	1.70 (0.86-3.36)	0.12
Secondary / t-AML	1.54 (0.67-3.55)	0.30	0.50 (0.15-1.63)	0.25	N/A	N/A
FLT3-ITD Mutation	1.81 (1.11-2.94)	0.01	1.21 (0.51-2.83)	0.65	N/A	N/A
NPM1 Mutation	0.77 (0.48-1.23)	0.27	0.35 (0.14-0.87)	0.02	N/A	N/A
	Multivariate Analysis (P<0.001)‖		**Multivariate Analysis (P=0.03)‖**		**Multivariate Analysis (P=0.003)‖**	
Covariate	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**
High LSC17 Score	2.31 (1.44-3.71)	<0.001	2.17 (1.02-4.61)	0.04	2.47 (1.30-4.69)	0.005
High IFPC Score	1.28 (0.81-2.04)	0.28	1.40 (0.70-2.81)	0.33	1.03 (0.58-1.83)	0.91
Age	1.02 (1.00-1.03)	0.01	1.03 (1.00-1.06)	0.03	Stratifier§	N/A
WBC count	1.00 (1.00-1.00)	0.99	1.00 (1.00-1.00)	0.002	1.01 (1.00-1.01)	0.004
Favorable Cytogenetics	N/A	N/A	N/A	N/A	0.73 (0.35-1.49)	0.38
Adverse Cytogenetics	N/A	N/A	N/A	N/A	1.51 (0.79-2.89)	0.20
Secondary / t-AML	1.52 (0.66-3.48)	0.32	0.57 (0.17-1.86)	0.35	N/A	N/A
FLT3-ITD Mutation	1.75 (1.07-2.85)	0.02	0.95 (0.40-2.25)	0.92	N/A	N/A
NPM1 Mutation	0.71 (0.46-1.09)	0.12	0.52 (0.25-1.08)	0.08	N/A	N/A
	Multivariate Analysis (P=0.001)‖		**Multivariate Analysis (P=0.01)‖**		**Multivariate Analysis (P=0.01)‖**	
Covariate	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**
High LSC17 Score	2.30 (1.38-3.85)	0.001	3.24 (1.29-8.13)	0.01	2.25 (1.17-4.29)	0.01
High Jung et al. Score	1.14 (0.69-1.88)	0.59	0.59 (0.26-1.33)	0.20	1.37 (0.70-2.67)	0.35
Age	1.02 (1.00-1.04)	0.01	1.03 (1.00-1.06)	0.04	Stratifier§	N/A
WBC count	1.00 (1.00-1.00)	0.84	1.00 (1.00-1.00)	0.001	1.01 (1.00-1.01)	0.003
Favorable Cytogenetics	N/A	N/A	N/A	N/A	0.71 (0.34-1.46)	0.35
Adverse Cytogenetics	N/A	N/A	N/A	N/A	1.41 (0.73-2.74)	0.30
Secondary / t-AML	1.48 (0.64-3.40)	0.35	0.49 (0.15-1.63)	0.25	N/A	N/A
FLT3-ITD Mutation	1.82 (1.11-2.97)	0.01	1.01 (0.43-2.36)	0.96	N/A	N/A
NPM1 Mutation	0.73 (0.47-1.12)	0.15	0.50 (0.24-1.03)	0.06	N/A	N/A
	Multivariate Analysis (P=0.001)‖		**Multivariate Analysis (P=0.01)‖**		**Multivariate Analysis (P=0.009)‖**	
Covariate	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**	**Hazard Ratio (95% CI)†**	**P-value‡**
High LSC17 Score	2.17 (1.31-3.58)	0.002	2.56 (1.14-5.71)	0.02	2.27 (1.21-4.28)	0.01
High Gentles et al. Score	1.33 (0.83-2.11)	0.23	0.74 (0.34-1.59)	0.44	1.37 (0.79-2.38)	0.25
Age	1.02 (1.00-1.04)	0.008	1.03 (0.99-1.06)	0.05	Stratifier§	N/A
WBC count	1.00 (1.00-1.00)	0.74	1.00 (1.00-1.00)	0.002	1.00 (1.00-1.01)	0.003
Favorable Cytogenetics	N/A	N/A	N/A	N/A	0.70 (0.34-1.43)	0.33
Adverse Cytogenetics	N/A	N/A	N/A	N/A	1.41 (0.73-2.71)	0.29
Secondary / t-AML	1.49 (0.65-3.41)	0.34	0.48 (0.14-1.68)	0.25	N/A	N/A
FLT3-ITD Mutation	1.80 (1.12-2.91)	0.01	1.17 (0.49-2.79)	0.71	N/A	N/A
NPM1 Mutation	0.74 (0.48-1.13)	0.16	0.48 (0.22-1.03)	0.06	N/A	N/A

SDPC, surface-defined primitive cells[22]; IFPC, inferred functionally primitive cells[22].
*Number of patients with full clinical annotation.
†95% confidence interval.
‡*P* value was calculated using the Wald test.
§Age violated the proportional hazards assumption.
‖*P* value was calculated using the likelihood ratio test to assess model improvement by including LSC17.

Extended Data Table 7 | Clinical characteristics of the PM AML and GSE15434 CN-LMR AML cohorts

a

Characteristic	PM AML (*n*=307)	High LSC17 score (*n*=154)	Low LSC17 score (*n*=153)	P-value
Female Sex [*n* (%)]	148 (48.2)	77 (50)	71 (46.4)	0.60‡
Age at AML Diagnosis [years]				
median (range)	52 (18-81)	56 (18-81)	49 (20-81)	<0.001†
***De novo* vs. Secondary AML [*n* (%)]**				
De novo	268 (87.3)	130 (84.4)	138 (90.2)	0.17‡
Secondary / t-AML	39 (12.7)	24 (15.6)	15 (9.8)	
PB WBC count at diagnosis (x10^9/L)				
median (range)	17.6 (0.7-399)	12.2 (0.7-212)	26.8 (1.6-399)	<0.001*
BM blast % at diagnosis (x10^9/L)	n=284	n=142	n=142	
median (range)	80 (10-98)	80 (10-98)	80 (16-95)	0.05*
AML subtypes [n (%)]	n=251	n=126	n=125	
APL	12 (4.78)	7 (5.56)	5 (4)	0.77‖
Non-APL	239 (95.2)	119 (94.4)	120 (96)	
Karyotype [n (%)]	n=284	n=141	n=143	
Normal karyotype	141 (49.6)	61 (43.3)	80 (55.9)	0.04‡
Abnormal karyotype	143 (50.4)	80 (56.7)	63 (44.1)	
MRC Cytogenetic risk class at diagnosis [n (%)]	n=284	n=141	n=143	
Favorable	48 (16.9)	13 (9.22)	35 (24.5)	
Intermediate	196 (69)	91 (64.5)	105 (73.4)	<0.001‖
Adverse	40 (14.1)	37 (26.2)	3 (2.1)	
CN AML- *NPM1* [n (%)]	n=87	n=29	n=58	
NPM1 mutation	48 (55.2)	9 (31)	39 (67.2)	0.002‖
No *NPM1* mutation	39 (44.8)	20 (69)	19 (32.8)	
CN AML- *FLT3*-ITD [n (%)]	n=95	n=34	n=61	
FLT3-ITD positive	23 (24.2)	8 (23.5)	15 (24.6)	1.00‖
FLT3-ITD negative	72 (75.8)	26 (76.5)	46 (75.4)	
Treatment Response [n (%)]	n=306	n=153	n=153	
Complete Remission	223 (72.9)	85 (55.6)	138 (90.2)	<0.001‖
No Response	83 (27.1)	68 (44.4)	15 (9.8)	
Survival Parameters [n (%)]				
Median Overall Survival	671	400	2035	<0.001§
Median Event-Free Survival	301	161	541	<0.001§
Median Relapse-Free Survival	378 (n=267)	265 (n=118)	689 (n=149)	<0.001§

b

Characteristic	GSE15434 CN-LMR cohort (*n*=70)	High LSC3 score subset (*n*=35)	Low LSC3 score subset (*n*=35)	P-value
Female Sex [*n* (%)]	34 (48.6)	16 (45.7)	18 (51.4)	0.81‡
Age at AML Diagnosis [years]				
median (range)	54 (30-83)	57 (36-83)	51 (30-75)	0.08†
***De novo* vs. Secondary AML [*n* (%)]**				
De novo	65 (92.9)	32 (91.4)	33 (94.3)	1.00‖
Secondary / t-AML	5 (7.14)	3 (8.57)	2 (5.71)	
PB WBC count at diagnosis (x10^9/L)	n=45	n=21	n=24	
median (range)	17.9 (0.9-365)	29.4 (1.6-365)	14.4 (0.9-86)	0.11*
Blast % at diagnosis (x10^9/L)	n=69,67	n=35,33	n=34	
Median BM Blasts (range)	69 (0-95)	75 (0-95)	65.5 (14-95)	0.11*
Median PB Blasts (range)	18 (0-94)	60 (0-94)	9 (0-90)	0.003*
Karyotype [n (%)]				
Normal karyotype	70 (100)	35 (100)	35 (100)	1.00‖
Abnormal karyotype	0 (0)	0 (0)	0 (0)	
Treatment Response [n (%)]	n=39	n=20	n=19	
Complete Response	35 (89.7)	18 (90)	17 (89.5)	1.00‖
No Response	4 (10.3)	2 (10)	2 (10.5)	
CN AML- *NPM1* [n (%)]				
NPM1 mutation	70 (100)	35 (100)	35 (100)	1.00‖
No *NPM1* mutation	0 (0)	0 (0)	0 (0)	
CN AML- *FLT3*-ITD [n (%)]				
FLT3-ITD positive	0 (0)	0 (0)	0 (0)	1.00‖
FLT3-ITD negative	70 (100)	35 (100)	35 (100)	
Survival Parameters [days]				
Median Overall Survival	1767	679	not reached	<0.001§

*P value calculated using the Wilcoxon rank-sum test.
†P value calculated using the Student's *t*-test.
‡P value calculated using the Pearson's chi-squared test.
§P value calculated using the log-rank test.
||P value calculated using the Fisher's exact test.

Extended Data Table 8 | Clinical characteristics and multivariate survival analysis of the ALFA-0701 AML cohort

a

Characteristic	ALFA-0701 Trial Cohort	High LSC17 score subset	Low LSC17 score subset	P-value
	(*n*=192)	(*n*=96)	(*n*=96)	
Female Sex [*n* (%)]	98 (51)	45 (0.47)	53 (0.55)	0.31‡
Age at AML Diagnosis [years]				
median (range)	62.1 (50.1-70.8)	61.8 (50.1-70.8)	62.3 (50.5-70.7)	0.89†
***De novo* vs. Secondary AML [*n* (%)]**				
De novo	192 (100)	96 (100)	96 (100)	N/A
Secondary / t-AML	0 (0)	0 (0)	0 (0)	
PB WBC count at diagnosis (x10^9/L)	*n*=191	*n*=95		
median (range)	5.1 (0.15-210.6)	3.82 (0.5-210.6)	8.8 (0.15-187)	0.01*
Treatment arm (GO or standard)				
Gemtuzumab Ozogamicin	98 (0.51)	47 (0.49)	51 (0.53)	0.66‡
Standard treatment	94 (0.49)	49 (0.51)	45 (0.47)	
Cytogenetic risk class at diagnosis [n (%)]¶	*n*=175	*n*=91	*n*=84	
Favorable	4 (0.02)	0 (0)	4 (0.04)	
Intermediate	129 (0.73)	59 (0.64)	70 (0.83)	<0.001‖
Adverse	42 (0.24)	32 (0.35)	10 (0.12)	
Karyotype [n (%)]	*n*=173	*n*=91	*n*=82	
Normal karyotype	99 (0.57)	44 (0.48)	55 (0.67)	0.02‡
Abnormal karyotype	74 (0.42)	47 (0.51)	27 (0.33)	
AML subtypes [n (%)]				
APL	0 (0)	0 (0)	0 (0)	N/A
Non-APL	192 (100)	96 (100)	96 (100)	
CN AML- *NPM1* [n (%)]	*n*=99	*n*=44	*n*=55	
NPM1 mutation	44 (0.44)	16 (0.36)	28 (0.51)	0.001‡
No *NPM1* mutation	55 (0.55)	28 (0.63)	27 (0.49)	
CN AML- *FLT3*-ITD [n (%)]	*n*=99	*n*=44	*n*=55	
FLT3-ITD positive	24 (0.24)	17 (0.38)	7 (0.12)	0.08‖
FLT3-ITD negative	75 (0.75)	27 (0.61)	48 (0.87)	
Treatment Response [n (%)]				
Complete Response	132 (0.68)	61 (0.63)	71 (0.74)	0.16‡
No Response	60 (0.31)	35 (0.36)	25 (0.26)	
Events [n (%)]	*n*=145	*n*=65	*n*=80	
Relapse	90 (0.62)	46 (0.70)	44 (0.55)	0.07‡
Survival Parameters [days]				
Median Overall Survival	666	462	1387	<0.001§
Median Event-Free Survival	330	241	532	<0.001§
Median Relapse-Free Survival	504 (n=145)	362 (n=65)	902 (n=80)	<0.001§

b

ALFA-0701 Low LSC17 Score Subset	Event-Free Survival (n=90)#		Relapse-Free Survival (n=80)#	
Covariate	Hazard Ratio (95% CI)☆	P-value**	Hazard Ratio (95% CI)☆	P-value**
GO vs. Standard Treatment	0.34 (0.19-0.63)	<0.001	0.42 (0.21-0.83)	0.01
Age	1.04 (0.99-1.10)	0.09	1.03 (0.97-1.09)	0.31
WBC count	1.00 (0.99-1.01)	0.11	1.00 (0.99-1.01)	0.10
Favorable Cytogenetics	1.29 (0.39-4.23)	0.66	1.26 (0.30-5.31)	0.75
Adverse Cytogenetics	1.98 (0.82-4.77)	0.12	0.44 (0.06-3.31)	0.43

*P value calculated using the Wilcoxon rank-sum test.
†P value calculated using the Student's *t*-test.
‡P value calculated using the Pearson's chi-squared test.
§P value calculated using the log-rank test.
‖P value calculated using the Fisher's exact test.
¶Cytogenetic risk groups were defined by ALFA-0701 investigators[28].
#Number of patients with full clinical annotation.
☆95% confidence interval.
**P value was calculated using the Wald test.