Mutations associated with a 17-gene leukemia stem cell score and the score's prognostic relevance in the context of the European LeukemiaNet classification of acute myeloid leukemia

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Supplementary Appendix

Mutations associated with a 17-gene leukemia stem cell score and its prognostic relevance in the context of the European LeukemiaNet classification for acute myeloid leukemia

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Supplementary Methods

Participating Institutions

The following Cancer and Leukemia Group B (CALGB)/Alliance for Clinical Trials in Oncology (Alliance) institutions participated in this study and contributed at least five patients. For each of these institutions, the current or last principal investigator and the cytogeneticists who analyzed the cases are listed as follows:

The Ohio State University Medical Center, Columbus, OH: Claire F. Verschraegen, Karl S. Theil, Diane Minka and Nyla A. Heerema; North Shore University Hospital, Manhasset, NY: Daniel R. Budman, Prasad R. K. Koduru, Ayala Aviram-Goldring and Chandrika Sreekantaiah; Wake Forest University School of Medicine, Winston-Salem, NC: Heidi Klepin, P. Nagesh Rao, Wendy L. Flejter and Mark Pettenati; Dana Farber Cancer Institute, Boston, MA: Harold J. Burstein, Ramana V. Tantravahi, Cynthia C. Morton and Paola Dal Cin; Washington University School of Medicine, St. Louis, MO: Nancy L. Bartlett, Michael S. Watson, Eric C. Crawford, Jaime Garcia-Heras, Peining Li and Shashikant Kulkarni; Roswell Park Cancer Institute, Buffalo, NY: Ellis G. Levine and AnneMarie W. Block; University of Chicago Medical Center, Chicago, IL: Hedy L. Kindler, Diane Roulston, Katrin M. Carlson, Yanming Zhang and Michelle M. LeBeau; Duke University Medical Center, Durham, NC: Jeffrey Crawford, Sandra H. Bigner, Mazin B. Qumsiyeh, John Eyre and Barbara K. Goodman; University of Iowa Hospitals, Iowa City, IA: Daniel A. Vaena and Shivanand R. Patil; University of North Carolina, Chapel Hill, NC: Thomas C. Shea and Kathleen W. Rao; University of Maryland Greenebaum Cancer Center, Baltimore, MD: Martin J. Edelman, Joseph R. Testa, Maimon M. Cohen, Judith Stamberg and Yi Ning; Ft. Wayne Medical Oncology/Hematology, Ft. Wayne, IN: Sreenivasa Nattam and Patricia I. Bader; Christiana Care Health Services, Inc., Newark, DE: Gregory Masters, Digamber S. Borgaonkar, Jeanne

M. Meck, and Kathleen Richkind; Dartmouth Medical School, Lebanon, NH: Konstantin Dragnev, Doris H. Wurster-Hill and Thuluvancheri K. Mohandas; University of Vermont Cancer Center, Burlington, VT: Elizabeth F. Allen and Mary Tang; Weill Medical College of Cornell University, New York, NY: Scott Tagawa, Ram S. Verma, Prasad R.K. Koduru and Susan Mathew; Rhode Island Hospital, Providence, RI: Howard Safran, Teresita Padre-Mendoza, Hon Fong L. Mark, Shelly L. Kerman and Aurelia Meloni-Ehrig; Mount Sinai School of Medicine, New York, NY: Lewis R. Silverman and Vesna Najfeld; Western Pennsylvania Hospital, Pittsburgh, PA: John Lister and Gerard R. Diggans; SUNY Upstate Medical University, Syracuse, NY: Stephen L. Graziano, Larry Gordon and Constance K. Stein; Long Island Jewish Medical Center, Lake Success, NY: Daniel R. Budman, Prasad R. K. Koduru, Ayala Aviram-Goldring and Chandrika Sreekantaiah; Moores University of California San Diego Cancer Center, San Diego, CA: Barbara A. Parker, Renée Bernstein and Marie L. Dell'Aquila; University of Massachusetts Medical Center, Worcester, MA: William V. Walsh, Philip L. Townes, Vikram Jaswaney, Kathleen Richkind, Patricia Miron and Michael J. Mitchell; Eastern Maine Medical Center, Bangor, ME: Thomas H. Openshaw and Laurent J. Beauregard; University of Minnesota, Minneapolis, MN: Bruce A. Peterson, Diane C. Arthur and Betsy A. Hirsch; University of California at San Francisco, San Francisco, CA: Charalambos Andreadis and Kathleen E. Richkind; Walter Reed National Military Medical Center, Bethesda, MD: Mary Kwok, Digamber S. Borgaonkar and Kathleen E. Richkind; University of Alabama at Birmingham, Birmingham, AL: Robert Diasio and Andrew J. Carroll; Virginia Commonwealth University, Richmond, VA: Steven Grossman and Colleen Jackson-Cook; University of Puerto Rico, San Juan, Puerto Rico: Eileen I. Pacheco, Leonard L. Atkins, Cynthia C. Morton and Paola Dal Cin; University of Illinois, Chicago, IL: Arkadiusz Z. Dudek, Maureen M. McCorquodale, Kathleen E. Richkind and Valerie Lindgren; Massachusetts General Hospital, Boston, MA: David Ryan, Justin Gainor, Cynthia

C. Morton and Paola Dal Cin; University of Tennessee Cancer Center, Memphis, TN: Harvey B. Niell and Sugandhi A. Tharapel.

Patients and treatment

We investigated 934 adult patients with *de novo* acute myeloid leukemia (AML) who were enrolled on CALGB/Alliance study protocols and received treatment, as detailed below. Patients were excluded from outcome analyses if they received allogeneic hematopoietic stem cell transplantation in first complete remission (CR).

All patients gave written informed consent for participation in the studies. All study protocols were in accordance with the Declaration of Helsinki and approved by Institutional Review Boards at each treatment center. Patients were treated on CALGB/Alliance protocols CALGB 8525 (n=33), 8621 (n=1), 8721 (n=1), 8821 (n=3), 8923 (n=7), 9022 (n=5), 9120 (n=1), 9222 (n=56), 9420 (n=9), 9621 (n=107), 9720 (n=79), 10201 (n=67), 10502 (n=22), 10503 (n=233), 10603 (n=55), 11001 (n=5), 11002 (n=7), and 19808 (n=243).

Patients enrolled on CALGB 8525 were treated with induction chemotherapy consisting of cytarabine and daunorubicin, and were randomly assigned to consolidation with or without 3g/m^2 cytarabine followed by maintenance treatment.[1] The patient enrolled on CALGB 8621 received high-dose cytarabine (HiDAC) for seven days in combination with mitoxantrone for the first three days. The patient enrolled on CALGB 8721 received two courses of treatment with HiDAC plus asparaginase on days 1 and 8. After induction consisting of cytarabine in combination with daunorubicin, the patients enrolled on CALGB 8821 received intensive post remission therapy with cyclophosphamide/etoposide and diazaquone/mitoxantrone.[2] Patients on CALGB 8923 were treated with induction therapy consisting of cytarabine and daunorubicin and were randomly assigned to receive postremission therapy with cytarabine

alone or in combination with mitoxantrone. Patients enrolled on CALGB 9022 received induction chemotherapy consisting of cytarabine in combination with daunorubicin followed by consolidation with one cycle of HiDAC, a cycle of cyclophosphamide and etoposide, and one cycle of mitoxantrone and diaziquone.[3] The patients enrolled on CALGB 9120 received standard induction chemotherapy. After CR had been achieved, idarubicin (two days) and cytarabine (five days) were administered. The patients received a single course of high-dose cytarabine.[4] Patients enrolled on CALGB 9222 received induction chemotherapy consisting of cytarabine in combination with daunorubicin followed by consolidation with one cycle of HiDAC. Different doses of mitoxantrone were explored, and the consolidation treatment was randomized to three cycles of monotherapy with HiDAC or consolidation with one cycle of HiDAC, a cycle of cyclophosphamide and etoposide, and one cycle of mitoxantrone and diaziquone.[5] Patients on CALGB 9420 and 9720 received induction chemotherapy consisting of cytarabine in combination with daunorubicin and etoposide, with PSC-833 (valspodar) or without PSC-833.[12,13] Patients enrolled on CALGB 9621 were treated similarly to those on CALGB 19808, as previously reported.[6] Patients on CALGB 9720 received a single cytarabine/daunorubicin consolidation course and were randomly assigned to low-dose recombinant interleukin-2 maintenance therapy or none. Patients on CALGB 10201 received induction chemotherapy consisting of cytarabine and daunorubicin, with or without the BCL2 antisense oblimersen sodium. The consolidation included two cycles of cytarabine ($2g/m^2/d$) with or without oblimersen.[7] For patients on CALGB 10502, bortezomib was added to both induction consisting of cytarabine and daunorubicin and to consolidation with two cycles of intermediate-dose cytarabine.[8] Patients enrolled on CALGB 10503 were assigned to receive induction chemotherapy consisting of cytarabine, daunorubicin, and etoposide. Upon achievement of CR, patients received HiDAC and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by autologous peripheral HSCT. Patients not eligible for HSCT received HiDAC. After intensification,

patients received the DNA methyltransferase inhibitor decitabine for maintenance.[9] Patients enrolled on CALGB 10603 were treated with cytarabine and daunorubicin followed by consolidation with HiDAC with or without midostaurin.[10] Patients enrolled on CALGB 19808 were randomly assigned to receive induction chemotherapy with cytarabine, daunorubicin, and etoposide with or without PSC-833, a multidrug resistance protein inhibitor.[11] On achievement of CR, patients were assigned to intensification with high-dose cytarabine and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by autologous peripheral blood HSCT. For patients treated on CALGB 11001, sorafenib was added to the induction and consolidation treatment consisting of daunorubicin and cytarabine and consolidation with HiDAC, followed by sorafenib maintenance.[14] Patients on CALGB 11002 received decitabine with or without addition of the proteasome inhibitor bortezomib, for both induction and postremission therapy.[15]

Cytogenetic and molecular analyses

Cytogenetic analyses of pretreatment bone marrow and/or blood samples were performed by institutional laboratories approved by the CALGB/Alliance using unstimulated short-term (24- or 48-hour) cultures. For the karyotype to be determined as normal, at least 20 bone marrow metaphase cells had to have been analyzed and no clonal abnormality found. Cytogenetic results were confirmed by central karyotype review.[16]

The mutational status of 80 protein coding genes was determined centrally at The Ohio State University by targeted amplicon sequencing using the MiSeq platform (Illumina) Briefly, variants were excluded if they occurred with variant allele fractions (VAFs) of <0.10; were sequenced to a depth of <15 reads; occurred only in one read direction if sequenced in both directions; if the region contained many variants with low quality scores; or if they occurred in all analyzed samples including run controls. In addition, samples with high

background noise were entirely excluded from analysis. Samples were considered non-evaluable for a specific gene if ≥85% of the amplicons covering the target regions within the coding sequence of the gene were sequenced to a depth of <15 reads. Testing for the presence or absence of *FLT3* internal tandem duplications (*FLT3*-ITDs) was performed as previously described.[17] In addition to the 80 gene sequencing panel, testing for *CEBPA* mutations was performed with Sanger sequencing as previously described,[18] thus resulting in a total of 81 genes whose mutational status were assessed in our study. In accordance with the revision of the WHO classification of myeloid neoplasms and acute leukemia,[19] only patients with biallelic *CEBPA* mutations were considered to be *CEBPA*-mutated.

Definition of clinical endpoints and statistics

Clinical endpoints were defined according to generally accepted criteria.[1,20] A CR was defined as recovery of morphologically normal bone marrow and blood counts (i.e., neutrophils ≥1.5 x 10^9/L and platelets >100 x 10^9/L), and no circulating leukemic blasts or evidence of extramedullary leukemia, all of which had to persist for ≥4 weeks. DFS was measured from the date of achievement of a CR until the date of relapse or death from any cause; patients not known to have relapsed or died at last follow-up were censored on the date they were last examined. OS was measured from the date of diagnosis to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive.

Baseline clinical, biological characteristics, and CR were compared using the Fisher's exact and Wilcoxon rank-sum tests for categorical and continuous variables, respectively.[21] Estimated probabilities of DFS and OS were calculated using the Kaplan-Meier method,[22] and the log-rank test evaluated differences between survival distributions.

Multivariable logistic regression models were generated for attainment of CR, and multivariable proportional hazards models were constructed for DFS and OS using a limited backwards elimination procedure. Variables considered for model inclusion were: 17-gene leukemia stem cell (LSC) score (high *versus* low), age (as a continuous variable, in 10-year increments), sex (male *versus* female), race (white *versus* non-white), white blood cell count [(WBC) as a continuous variable, in 50-unit increments], hemoglobin (as a continuous variable, in 1-unit increments), platelet count (as a continuous variable, in 50-unit increments), extramedullary involvement (present *versus* absent), Europrean LeukemiaNet (ELN 2017 risk categories (Intermediate-risk *versus* Favorable-risk and Adverse-risk *versus* Favorable-risk), *BCOR* mutations (mutated *versus* wild-type), *BCORL1* mutations (mutated *versus* wild-type), *DNMT3A* mutations (mutated *versus* wild-type), *ETV6* mutations (mutated *versus* wild-type), *EZH2* mutations (mutated *versus* wild-type), tyrosine kinase domain mutation in the *FLT3* gene [(*FLT3*-TKD) present *versus* absent], *GATA2* mutations (mutated *versus* wild-type), *IDH1* mutations (mutated *versus* wild-type), *IDH2* mutations (mutated *versus* wild-type), *KRAS* mutations (mutated *versus* wild-type), *NRAS* mutations (mutated *versus* wild-type), *PHF6* mutations (mutated *versus* wild-type), *PTPN11* mutations (mutated *versus* wild-type), *RAD21* mutations (mutated *versus* wild-type), *SETBP1* mutations (mutated *versus* wild-type), *SF3B1* mutations (mutated *versus* wild-type), *SMARCA2* mutations (mutated *versus* wild-type), *SMC1A* mutations (mutated *versus* wild-type), *SMC3* mutations (mutated *versus* wild-type), *SRSF2* mutations (mutated *versus* wild-type), *TET2* mutations (mutated *versus* wild-type), *U2AF1* mutations (mutated *versus* wild-type), *WT1* mutations (mutated *versus* wild-type), *ZRSR2* mutations (mutated *versus* wild-type), *ERG* expression levels (high *versus* low) and *BAALC* expression levels (high *versus* low). For *ERG* and *BAALC* the median expression value was used as the cut point to divide patients into high and low expressers. Only markers with at least eight mutated

patients in each 17-gene LSC score group (high/low) were included the multivariable modeling. Variables significant at $\alpha=0.2$ from the univariable analyses were considered for multivariable analyses. For the time-to-event endpoints, the proportional hazards assumption was checked for each variable individually.

All analyses were performed by the Alliance Statistics and Data Center on a database locked on July 5, 2018 using SAS 9.4 and TIBCO Spotfire S+ 8.2.

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Tables and Figures

Supplementary Table S1. Comparison of Cancer and Leukemia Group B/Alliance for Clinical Trials in Oncology treatment trials' for patients with acute myeloid leukemia with a low and those with a high 17-gene leukemia stem cell score.

Protocol number	17-gene^{low}, n (%)	17-gene^{high}, n (%)
Younger Patients	**(n=403)**	**(n=326)**
8525	15 (4)	11 (3)
8721	0 (0)	1 (0)
8821	1 (0)	1 (0)
9022	3 (1)	2 (1)
9120	1 (0)	0 (0)
9222	31 (8)	25 (8)
9621	55 (14)	52 (16)
19808	146 (36)	97 (30)
10503	131 (33)	102 (31)
10603	20 (5)	35 (11)
Older Patients	**(n=64)**	**(n=141)**
8525	1 (2)	6 (4)
8621	0 (0)	1 (1)
8821	0 (0)	1 (1)
8923	4 (6)	3 (2)
9420	4 (6)	5 (4)
9720	27 (42)	52 (37)
10201	16 (25)	51 (36)
10502	8 (13)	14 (10)
11001	2 (3)	3 (2)
11002	2 (3)	5 (4)

Supplementary Table S2. Comparison of clinical outcomes of younger adult patients (aged <60 years) enrolled onto Cancer and Leukemia Group B/Alliance for Clinical Trials in Oncology treatment trials.

Endpoint	Protocols						
	8525 (n=26)	**9222 (n=56)**	**9621 (n=107)**	**10503 (n=233)**	**10603 (n=55)**	**19808 (243)**	***P***
Complete remission, %	69	68	80	76	69	79	0.24
Disease-free survival							0.79
Median, years	0.8	1.0	1.6	1.9	1.4	1.3	
% Disease-free at 3 years	28	34	42	42	42	40	
95% confidence interval	10-49	20-49	31-52	35-49	26-57	33-47	
Overall survival							0.36
Median, year	1.5	2.2	2.0	2.8	1.5	2.0	
% Alive at 3 years	27	46	45	49	42	43	
95% confidence interval	12-44	33-59	35-54	42-54	29-54	37-49	

Supplementary Table S3. Comparison of clinical outcomes of older patients (aged ≥60 years) enrolled onto Cancer and Leukemia Group B/Alliance for Clinical Trials in Oncology treatment trials.

Endpoint	Protocols				
	9420 (n=9)	**9720 (n=79)**	**10201 (n=67)**	**10502 (n=22)**	***P***
Complete remission, %	44	58	58	64	0.82
Disease-free survival					0.15
Median, years	3.5	0.5	0.5	0.5	
% Disease-free at 3 years	50	2	15	14	
95% confidence interval	6-84	0-10	6-28	2-37	
Overall survival					0.14
Median, years	0.4	0.7	0.8	1.0	
% Alive at 3 years	22	14	10	32	
95% confidence interval	3-51	7-22	5-19	14-51	

Supplementary Table S4. Comparison of gene mutations between acute myeloid leukemia patients with a high and those with a low 17-gene leukemia stem cell score.

Gene	17-gene^{low} (n=467)	17-gene^{high} (n=467)	*P*
AKT1, n (%)			0.21
Mutated	1 (0)	4 (1)	
Wild-type	461 (100)	451 (99)	
ARAF, n (%)			0.68
Mutated	2 (0)	3 (1)	
Wild-type	460 (100)	452 (99)	
ASXL1, n (%)			0.001
Mutated	22 (5)	49 (10)	
Wild-type	445 (95)	418 (90)	
ATM, n (%)			0.21
Mutated	1 (0)	4 (1)	
Wild-type	461 (100)	451 (99)	
AXL, n (%)			0.26
Mutated	9 (2)	4 (1)	
Wild-type	453 (98)	451 (99)	
BCOR, n (%)			0.09
Mutated	20 (4)	32 (7)	
Wild-type	442 (96)	423 (93)	
BCORL1, n (%)			0.32
Mutated	16 (3)	10 (2)	
Wild-type	446 (97)	445 (98)	
BRAF, n (%)			0.37
Mutated	4 (1)	1 (0)	
Wild-type	458 (99)	454 (100)	
BRD4, n (%)			0.75
Mutated	4 (1)	5 (1)	
Wild-type	458 (99)	450 (99)	
BRINP3, n (%)			0.23
Mutated	6 (1)	11 (2)	
Wild-type	456 (99)	444 (98)	
BTK, n (%)			0.37
Mutated	1 (0)	3 (1)	
Wild-type	461 (100)	452 (99)	
CBL, n (%)			0.25
Mutated	7 (2)	12 (3)	
Wild-type	455 (98)	443 (97)	
CCND1, n (%)			0.62
Mutated	3 (1)	1 (0)	
Wild-type	459 (99)	454 (100)	
CCND2, n (%)			0.07
Mutated	7 (2)	1 (0)	
Wild-type	455 (98)	454 (100)	
Biallelic *CEBPA*, n (%)			<0.001
Mutated	61 (18)	5 (1)	
Wild-type	272 (82)	399 (99)	

CSNK1A1,n (%)			0.50
Mutated	0 (0)	1 (0)	
Wild-type	462 (100)	454 (100)	
CTNNB1,n (%)			0.25
Mutated	0 (0)	2 (0)	
Wild-type	462 (100)	453 (100)	
DNMT3A,n (%)			<0.001
Mutated	76 (16)	137 (30)	
Wild-type	386 (84)	318 (70)	
ETV6, n (%)			0.42
Mutated	10 (2)	14 (3)	
Wild-type	452 (98)	441 (97)	
EZH2, n (%)			0.57
Mutated	16 (3)	12 (3)	
Wild-type	446 (97)	443 (97)	
FBXW7, n (%)			0.49
Mutated	0 (0)	1 (0)	
Wild-type	462 (100)	451 (100)	
FLT3-ITD, n (%)			<0.001
Present	65 (15)	149 (34)	
Absent	380 (85)	291 (66)	
FLT3-TKD, n (%)			0.07
Present	45 (10)	29 (6)	
Absent	412 (90)	420 (94)	
GATA1, n (%)			1.00
Mutated	1 (0)	0 (0)	
Wild-type	461 (100)	455 (100)	
GATA2, n (%)			0.008
Mutated	34 (7)	15 (3)	
Wild-type	428 (93)	440 (97)	
GSK3B, n (%)			0.21
Mutated	1 (0)	4 (1)	
Wild-type	461 (100)	451 (99)	
HIST1H1E,n (%)			0.75
Mutated	4 (1)	5 (1)	
Wild-type	458 (99)	450 (99)	
HNRNPK, n (%)			0.45
Mutated	2 (0)	4 (1)	
Wild-type	460 (100)	451 (99)	
IDH1, n (%)			0.08
Mutated	24 (4)	37 (8)	
Wild-type	438 (96)	418 (92)	
IDH2, n (%)			0.91
Mutated	41 (9)	42 (9)	
Wild-type	421 (91)	413 (91)	
IKZF1, n (%)			0.45
Mutated	6 (1)	9 (2)	
Wild-type	459 (99)	446 (98)	
IKZF3, n (%)			0.25
Mutated	3 (1)	0 (0)	
Wild-type	459 (99)	455 (100)	
IL7R, n (%)			0.50
Mutated	0 (0)	1 (0)	
Wild-type	462 (100)	453 (100)	

JAK1, n (%)			0.06
Mutated	9 (2)	2 (0)	
Wild-type	453 (98)	453 (100)	
JAK2, n (%)			0.45
Mutated	5 (1)	2 (0)	
Wild-type	443 (99)	432 (100)	
JAK3, n (%)			1.00
Mutated	5 (1)	4 (1)	
Wild-type	457 (99)	451 (99)	
KIT, n (%)			<0.001
Mutated	25 (6)	4 (1)	
Wild-type	404 (94)	414 (99)	
KLHL6, n (%)			1.00
Mutated	1 (0)	0 (0)	
Wild-type	461 (100)	455 (100)	
KMT2A, n (%)			0.04
Mutated	2 (0)	9 (2)	
Wild-type	460 (100)	446 (98)	
KRAS, n (%)			0.20
Mutated	12 (3)	19 (4)	
Wild-type	450 (97)	436 (96)	
MAPK3, n (%)			0.62
Mutated	1 (0)	2 (0)	
Wild-type	461 (100)	453 (100)	
MED12, n (%)			0.80
Mutated	9 (2)	7 (2)	
Wild-type	453 (98)	448 (98)	
MYD88, n (%)			1.00
Mutated	1 (0)	1 (0)	
Wild-type	461 (100)	454 (100)	
NF1, n (%)			0.05
Mutated	12 (4)	22 (8)	
Wild-type	299 (96)	262 (92)	
NOTCH1,n (%)			0.26
Mutated	4 (1)	8 (2)	
Wild-type	458 (99)	447 (98)	
NPM1, n (%)			0.14
Mutated	140 (30)	161 (35)	
Wild-type	321 (70)	299 (65)	
NRAS, n (%)			0.40
Mutated	72 (16)	61 (13)	
Wild-type	390 (84)	394 (87)	
PHF6, n (%)			0.28
Mutated	14 (3)	8 (2)	
Wild-type	448 (97)	447 (98)	
PIK3CD, n (%)			0.51
Mutated	6 (1)	3 (1)	
Wild-type	456 (99)	452 (99)	
PIK3CG, n (%)			1.00
Mutated	5 (1)	4 (1)	
Wild-type	457 (99)	451 (99)	
PLCG2, n (%)			0.10
Mutated	17 (4)	8 (2)	
Wild-type	445 (96)	447 (98)	

PLEKHG5,n (%)			0.49
Mutated	0 (0)	1 (0)	
Wild-type	460 (100)	447 (100)	
PRKCB, n (%)			0.29
Mutated	6 (1)	2 (0)	
Wild-type	456 (99)	453 (100)	
PRKD3, n (%)			0.50
Mutated	3 (1)	5 (1)	
Wild-type	459 (99)	450 (99)	
PTEN, n (%)			0.50
Mutated	2 (0)	0 (0)	
Wild-type	460 (100)	455 (100)	
PTPN11, n (%)			0.70
Mutated	35 (8)	31 (7)	
Wild-type	427 (92)	424 (93)	
RAD21, n (%)			0.52
Mutated	13 (3)	9 (2)	
Wild-type	449 (97)	446 (98)	
RAF1, n (%)			0.72
Mutated	3 (1)	4 (1)	
Wild-type	459 (99)	451 (99)	
RUNX1, n (%)			0.002
Mutated	36 (8)	65 (14)	
Wild-type	427 (92)	394 (86)	
SAMHD1, n (%)			0.22
Mutated	3 (1)	7 (2)	
Wild-type	459 (99)	448 (98)	
SETBP1, n (%)			0.67
Mutated	10 (2)	12 (3)	
Wild-type	452 (98)	443 (97)	
SF1, n (%)			0.17
Mutated	2 (0)	6 (1)	
Wild-type	460 (100)	449 (99)	
SF3A1, n (%)			0.37
Mutated	1 (0)	3 (1)	
Wild-type	461 (100)	452 (99)	
SF3B1, n (%)			0.05
Mutated	11 (2)	22 (5)	
Wild-type	451 (98)	433 (95)	
SMARCA2,n (%)			1.00
Mutated	9 (2)	9 (2)	
Wild-type	453 (98)	446 (98)	
SMC1A, n (%)			0.13
Mutated	23 (5)	13 (3)	
Wild-type	439 (95)	442 (97)	
SMC3, n (%)			0.85
Mutated	16 (3)	14 (3)	
Wild-type	446 (97)	441 (97)	
SRSF2, n (%)			0.02
Mutated	23 (5)	41 (9)	
Wild-type	436 (95)	410 (91)	
STAG2, n (%)			0.009
Mutated	5 (1)	17 (4)	
Wild-type	457 (99)	438 (96)	

SYK, n (%)			0.28
Mutated	2 (0)	5 (1)	
Wild-type	460 (100)	450 (99)	
TET2, n (%)			0.008
Mutated	47 (10)	74 (16)	
Wild-type	415 (90)	381 (84)	
TGM7, n (%)			0.25
Mutated	3 (1)	0 (0)	
Wild-type	457 (99)	447 (100)	
TP53, n (%)			<0.001
Mutated	8 (2)	34 (7)	
Wild-type	454 (98)	421 (93)	
TYK2, n (%)			0.45
Mutated	6 (1)	9 (2)	
Wild-type	456 (99)	446 (98)	
U2AF1, n (%)			0.19
Mutated	11 (2)	18 (4)	
Wild-type	451 (98)	437 (96)	
WT1, n (%)			0.26
Mutated	40 (9)	30 (7)	
Wild-type	422 (91)	425 (93)	
XPO1, n (%)			0.21
Mutated	1 (0)	4 (1)	
Wild-type	461 (100)	451 (99)	
ZRSR2, n (%)			0.23
Mutated	19 (4)	27 (6)	
Wild-type	443 (96)	428 (94)	
Total number of mutations			<0.001
Median	2	3	
Range	0-8	0-9	

No mutation in the *BCL2*, *MAPK1*, *U2AF2* and *ZMYM3* genes were found in any patient.
n, number.

Supplementary Table S5. Classification of younger adult (aged <60 years) and older (aged ≥60 years) patients with acute myeloid leukemia according to the 2017 European LeukemiaNet (ELN) guidelines.

	Younger patients (n=729)			**Older patients (n=205)**		
Endpoint	**17-gene^{low} LSC score (n=403)**	**17-gene^{high} LSC score (n=326)**	***P***	**17-gene^{low} LSC score (n=64)**	**17-gene^{high} LSC score (n=141)**	***P***
ELN Group, n (%)			<0.001			0.009
Favorable	264 (68)	78 (26)		20 (36)	23 (18)	
Intermediate	56 (14)	96 (32)		13 (24)	23 (18)	
Adverse	67 (17)	123 (41)		22 (40)	79 (63)	

Supplementary Figure S1. Differences in outcome of older patients (aged ≥60 years) with acute myeloid leukemia according to the 17-gene leukemia stem cell (LSC) score, stratified by European LeukemiaNet (ELN) genetic risk classification. (A) Disease-free survival (DFS) and (B) overall survival (OS) of patients within the ELN Favorable-risk group according to the 17-gene LSC score. (C) DFS and (D) OS of patients within the ELN Intermediate-risk group according to the 17-gene LSC score. (E) DFS and (F) OS of patients within the ELN Adverse-risk group according to the 17-gene LSC score.