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Received: April 19, 2019.
Accepted: August 13, 2019.
Pre-published: August 14, 2019.
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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:

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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/m² 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. 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. 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. 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. Patients enrolled on CALGB 9621 were treated similarly to those on CALGB 19808, as previously reported. 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/m²/d) with or without oblimersen. 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. 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. Patients enrolled on CALGB 10603 were treated with cytarabine and daunorubicin followed by consolidation with HiDAC with or without midostaurin. 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. 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. Patients on CALGB 11002 received decitabine with or without addition of the proteasome inhibitor bortezomib, for both induction and postremission therapy.

**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.

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 \(\text{FLT3}\) internal tandem duplications (\(\text{FLT3}\)-ITDs) was performed as previously described.\(^{17}\) In addition to the 80 gene sequencing panel, testing for \(\text{CEBPA}\) 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 \(\text{CEBPA}\) mutations were considered to be \(\text{CEBPA}\)-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 \(\geq 1.5 \times 10^9/\text{L}\) and platelets \(>100 \times 10^9/\text{L}\)), and no circulating leukemic blasts or evidence of extramedullary leukemia, all of which had to persist for \(\geq 4\) 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), European 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.
Supplementary References

1) Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. N Engl J Med. 1994;331(14):896–903.

2) Schiffer CA, Davis RB, Schulman P, et al. Intensive post remission therapy of acute myeloid leukemia (AML) with cytoxan/etoposide (CY/VP16) and diazaquone/mitoxantrone (AZQ/MITO). Blood. 1991;78(suppl):460 (abstract 1829).

3) Moore JO, Dodge RK, Amrein PC, et al. Granulocyte-colony stimulating factor (filgrastim) accelerates granulocyte recovery after intensive postremission chemotherapy for acute myeloid leukemia with aziridinyl benzoquinone and mitoxantrone: Cancer and Leukemia Group B study 9022. Blood. 1997;89(3):780–788.

4) Cassileth PA, Harrington DP, Appelbaum FR, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. New Engl J Med. 1998;339(23):1649–1656.

5) Moore JO, George SL, Dodge RK, et al. Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone as postremission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B study 9222. Blood. 2005;105(9):3420–3427.

6) Kolitz JE, George SL, Dodge RK, et al. Dose escalation studies of cytarabine, daunorubicin, and etoposide with and without multidrug resistance modulation with PSC-833 in untreated adults with acute myeloid leukemia younger than 60 years: final induction results of Cancer and Leukemia Group B study 9621. J Clin Oncol. 2004;22(21):4290–4301.

7) Marcucci G, Moser B, Blum W, et al. A phase III randomized trial of intensive induction and consolidation chemotherapy ± oblimersen, a pro-apoptotic Bcl-2 antisense oligonucleotide in untreated acute myeloid leukemia patients >60 years old. J Clin Oncol. 2007;25(suppl):360s (abstract 7012).
8) Attar EC, Johnson JL, Amrein PC, et al. Bortezomib added to daunorubicin and cytarabine during induction therapy and to intermediate-dose cytarabine for consolidation in patients with previously untreated acute myeloid leukemia age 60 to 75 years: CALGB (Alliance) study 10502. J Clin Oncol. 2013;31(7):923–929.

9) Blum W, Sanford BL, Klisovic R, et al. Maintenance therapy with decitabine in younger adults with acute myeloid leukemia in first remission: A phase 2 Cancer and Leukemia Group B study (CALGB 10503). Leukemia. 2017;31(1):34–39.

10) Stone RM, Berg DT, George SL, et al. Granulocyte-macrophage colony-stimulating factor after initial chemotherapy for elderly patients with primary acute myelogenous leukemia. N Engl J Med. 1995;332(25):1671–1677.

11) Kolitz JE, George SL, Marcucci G, et al. P-glycoprotein inhibition using valspodar (PSC-833) does not improve outcomes for patients under age 60 years with newly diagnosed acute myeloid leukemia: Cancer and Leukemia Group B study 19808. Blood. 2010(9):116:1413–1421.

12) Lee EJ, George SL, Caligiuri M, et al. Parallel phase I studies of daunorubicin given with cytarabine and etoposide with or without the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age or older with acute myeloid leukemia: results of Cancer and Leukemia Group B study 9420. J Clin Oncol. 1999;17(9):2831–2839.

13) Baer MR, George SL, Caligiuri MA, et al. Low-dose interleukin-2 immunotherapy does not improve outcome of patients age 60 years and older with acute myeloid leukemia in first complete remission: Cancer and Leukemia Group B study 9720. J Clin Oncol. 2008;26(30):4934–4939.

14) Uy GL, Mandrekar SJ, Laumann K, et al. A phase 2 study incorporating sorafenib into the chemotherapy for older adults with FLT3-mutated acute myeloid leukemia: CALGB 11001. Blood Adv. 2017;1(5):331–340.
15) Roboz GJ, Mandrekar SJ, Desai P, et al. A randomized trial of 10 days of decitabine alone or with bortezomib in previously untreated older patients with acute myeloid leukemia: CALGB 11002 (Alliance). Blood Adv. 2018;2(24):3608–3617.

16) Mrózek K, Carroll AJ, Maharry K, et al. Central review of cytogenetics is necessary for cooperative group correlative and clinical studies of adult acute leukemia: the Cancer and Leukemia Group B experience. Int J Oncol. 2008;33(2):239–244.

17) Whitman SP, Archer KJ, Feng L, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a Cancer and Leukemia Group B study. Cancer Res. 2001;61(19):7233–7239.

18) Marcucci G, Maharry K, Radmacher MD, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B study. J Clin Oncol. 2008;26(31):5078–5087.

19) Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.

20) Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–447.

21) Vittinghoff E, Glidden DV, Shiboski SC, McCulloch CE. Regression Methods in Biostatistics: Linear, Logistic, Survival and Repeated Measures Models. Springer: New York, NY, USA, 2005.

22) Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53(282):457–481.
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<sup>low</sup>, n (%) | 17-gene<sup>high</sup>, n (%) |
|-----------------|-------------------------------|-------------------------------|
| **Younger Patients** |                               |                               |
| 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)                       |
| (n=403)         |                               | (n=326)                       |
| **Older Patients** |                               |                               |
| 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)                         |
| (n=64)          |                               | (n=141)                       |
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) |     |
| Complete remission, %              | 44                         | 58  | 58  | 64  | 0.82|
| Disease-free survival              |                            |     |     |     |     |     |
| Median, years                      | 3.5                        | 0.5 | 0.5 | 0.5 | 0.15|
| % Disease-free at 3 years          | 50                         | 2   | 15  | 14  |     |
| 95% confidence interval            | 6-84                       | 0-10 | 6-28 | 2-37 |     |
| Overall survival                   |                            |     |     |     |     |     |
| Median, years                      | 0.4                        | 0.7 | 0.8 | 1.0 | 0.14|
| % 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\_low gene (n=467) | 17\_high gene (n=467) | P    |
|---------------|----------------------|------------------------|------|
| AKT1, n (%)   | Mutated 1 (0) 461 (100) | 4 (1) 451 (99) | 0.21 |
|               | Wild-type 460 (100) | 452 (99) |                  |
| ARAF, n (%)   | Mutated 2 (0) 460 (100) | 3 (1) 452 (99) | 0.68 |
|               | Wild-type 445 (95) | 418 (90) |                  |
| ASXL1, n (%)  | Mutated 22 (5) 445 (95) | 49 (10) 418 (90) | 0.001 |
|               | Wild-type 443 (95) | 418 (90) |                  |
| ATM, n (%)    | Mutated 1 (0) 461 (100) | 4 (1) 451 (99) | 0.21 |
|               | Wild-type 460 (100) | 452 (99) |                  |
| AXL, n (%)    | Mutated 9 (2) 453 (98) | 4 (1) 451 (99) | 0.26 |
|               | Wild-type 450 (99) | 452 (99) |                  |
| BCOR, n (%)   | Mutated 20 (4) 442 (96) | 32 (7) 423 (93) | 0.09 |
|               | Wild-type 446 (97) | 445 (98) |                  |
| BCRORL1, n (%)| Mutated 16 (3) 446 (97) | 10 (2) 445 (98) | 0.32 |
|               | Wild-type 444 (97) | 444 (97) |                  |
| BRAF, n (%)   | Mutated 4 (1) 458 (99) | 1 (0) 454 (100) | 0.37 |
|               | Wild-type 454 (99) | 450 (99) |                  |
| BRD4, n (%)   | Mutated 4 (1) 458 (99) | 5 (1) 450 (99) | 0.75 |
|               | Wild-type 458 (99) | 450 (99) |                  |
| BRNIP3, n (%) | Mutated 6 (1) 456 (99) | 11 (2) 444 (98) | 0.23 |
|               | Wild-type 456 (99) | 444 (98) |                  |
| BTK, n (%)    | Mutated 1 (0) 461 (100) | 3 (1) 452 (99) | 0.37 |
|               | Wild-type 460 (100) | 452 (99) |                  |
| CBL, n (%)    | Mutated 7 (2) 455 (98) | 12 (3) 443 (97) | 0.25 |
|               | Wild-type 455 (98) | 443 (97) |                  |
| CCND1, n (%)  | Mutated 3 (1) 459 (99) | 1 (0) 454 (100) | 0.62 |
|               | Wild-type 459 (99) | 454 (100) |                  |
| CCND2, n (%)  | Mutated 7 (2) 455 (98) | 1 (0) 454 (100) | 0.07 |
|               | Wild-type 455 (98) | 454 (100) |                  |
| Biallelic CEBPA, n (%) | Mutated 61 (18) 272 (82) | 5 (1) 399 (99) | <0.001 |
|               | Wild-type 391 (81) | 404 (87) |                  |
| Gene          | Mutated | Wild-type | p-value |
|--------------|---------|-----------|---------|
| CSNK1A1      | 0 (0)   | 1 (0)     | 0.50    |
|              | 462 (100) | 454 (100) |         |
| CTNNB1       | 0 (0)   | 2 (0)     | 0.25    |
|              | 462 (100) | 453 (100) |         |
| DNMT3A       | 76 (16) | 137 (30)  | <0.001  |
|              | 386 (84) | 318 (70)  |         |
| ETV6         | 10 (2)  | 14 (3)    | 0.42    |
|              | 452 (98) | 441 (97)  |         |
| EZH2         | 16 (3)  | 12 (3)    | 0.57    |
|              | 446 (97) | 443 (97)  |         |
| FBXW7        | 0 (0)   | 1 (0)     | 0.49    |
|              | 462 (100) | 451 (100) |         |
| FLT3-ITD     | 65 (15) | 149 (34)  | <0.001  |
|              | 380 (85) | 291 (66)  |         |
| FLT3-TKD     | 45 (10) | 29 (6)    | 0.07    |
|              | 412 (90) | 420 (94)  |         |
| GATA1        | 1 (0)   | 0 (0)     | 1.00    |
|              | 461 (100) | 455 (100) |         |
| GATA2        | 34 (7)  | 15 (3)    | 0.008   |
|              | 428 (93) | 440 (97)  |         |
| GSK3B        | 1 (0)   | 4 (1)     | 0.21    |
|              | 461 (100) | 451 (99)  |         |
| HIST1H1E     | 4 (1)   | 5 (1)     | 0.75    |
|              | 458 (99) | 450 (99)  |         |
| HNRNPK       | 2 (0)   | 4 (1)     | 0.45    |
|              | 460 (100) | 451 (99)  |         |
| IDH1         | 24 (4)  | 37 (8)    | 0.08    |
|              | 438 (96) | 418 (92)  |         |
| IDH2         | 41 (9)  | 42 (9)    | 0.91    |
|              | 421 (91) | 413 (91)  |         |
| IKZF1        | 6 (1)   | 9 (2)     | 0.45    |
|              | 459 (99) | 446 (98)  |         |
| IKZF3        | 3 (1)   | 0 (0)     | 0.25    |
|              | 459 (99) | 455 (100) |         |
| IL7R         | 0 (0)   | 1 (0)     | 0.50    |
|              | 462 (100) | 453 (100) |         |
| Gene   | Mutated | Wild-type | p-value |
|--------|---------|-----------|---------|
| JAK1   | 9 (2)   | 2 (0)     | 0.06    |
|        | 453 (98)| 453 (100)|         |
| JAK2   | 5 (1)   | 4 (1)     | 1.00    |
|        | 443 (99)| 450 (99)  |         |
| KIT    | 25 (6)  | 4 (1)     | <0.001  |
|        | 404 (94)| 414 (99)  |         |
| KLHL6  | 1 (0)   | 0 (0)     | 1.00    |
|        | 461 (100)| 455 (100)|         |
| KIT2A  | 2 (0)   | 9 (2)     | 0.04    |
|        | 460 (100)| 446 (98) |         |
| KRAS   | 12 (3)  | 19 (4)    | 0.20    |
|        | 450 (97)| 436 (96)  |         |
| MAPK3  | 1 (0)   | 2 (0)     | 0.62    |
|        | 461 (100)| 453 (100)|         |
| MED12  | 9 (2)   | 7 (2)     | 0.80    |
|        | 453 (98)| 448 (98)  |         |
| MYD88  | 1 (0)   | 1 (0)     | 1.00    |
|        | 461 (100)| 454 (100)|         |
| NF1    | 12 (4)  | 22 (8)    | 0.05    |
|        | 299 (96)| 262 (92)  |         |
| NOTCH1 | 4 (1)   | 8 (2)     | 0.26    |
|        | 458 (99)| 447 (98)  |         |
| NPM1   | 140 (30)| 161 (35)  | 0.14    |
|        | 321 (70)| 299 (65)  |         |
| NRAS   | 72 (16) | 61 (13)   | 0.40    |
|        | 390 (84)| 394 (87)  |         |
| PHF6   | 14 (3)  | 8 (2)     | 0.28    |
|        | 449 (97)| 447 (98)  |         |
| PIK3CD | 6 (1)   | 3 (1)     | 0.51    |
|        | 456 (99)| 452 (99)  |         |
| PIK3CG | 5 (1)   | 4 (1)     | 1.00    |
|        | 457 (99)| 451 (99)  |         |
| PLCG2  | 17 (4)  | 8 (2)     | 0.10    |
|        | 445 (96)| 447 (98)  |         |
| Gene     | Mutated | Wild-type | p-value |
|----------|---------|-----------|---------|
| PLEKHG5  | 0 (0)   | 460 (100) | 0.49    |
| PRKCB    | 6 (1)   | 456 (99)  | 0.29    |
| PRKD3    | 3 (1)   | 459 (99)  | 0.50    |
| PTEN     | 2 (0)   | 460 (100) | 0.50    |
| PTPN11   | 35 (8)  | 427 (92)  | 0.70    |
| RAD21    | 13 (3)  | 449 (97)  | 0.52    |
| RAF1     | 3 (1)   | 459 (99)  | 0.72    |
| RUNX1    | 36 (8)  | 427 (92)  | 0.002   |
| SAMHD1   | 3 (1)   | 459 (99)  | 0.22    |
| SETBP1   | 10 (2)  | 452 (98)  | 0.67    |
| SF1      | 2 (0)   | 460 (100) | 0.17    |
| SF3A1    | 1 (0)   | 461 (100) | 0.37    |
| SF3B1    | 11 (2)  | 451 (98)  | 0.05    |
| SMARCA2  | 9 (2)   | 453 (98)  | 1.00    |
| SMC1A    | 23 (5)  | 439 (95)  | 0.13    |
| SMC3     | 16 (3)  | 446 (97)  | 0.85    |
| SRSF2    | 23 (5)  | 436 (95)  | 0.02    |
| STAG2    | 5 (1)   | 457 (99)  | 0.009   |
| Gene  | Mutated | Wild-type | p-value |
|-------|---------|-----------|---------|
| SYK   | 2 (0)   | 5 (1)     | 0.28    |
|       | 460 (100)| 450 (99)  |         |
| TET2  | 47 (10) | 74 (16)   | 0.008   |
|       | 415 (90)| 381 (84)  |         |
| TGM7  | 3 (1)   | 0 (0)     | 0.25    |
|       | 457 (99)| 447 (100) |         |
| TP53  | 8 (2)   | 34 (7)    | <0.001  |
|       | 454 (98)| 421 (93)  |         |
| TYK2  | 6 (1)   | 9 (2)     | 0.45    |
|       | 456 (99)| 446 (98)  |         |
| U2AF1 | 11 (2)  | 18 (4)    | 0.19    |
|       | 451 (98)| 437 (96)  |         |
| WTT1  | 40 (9)  | 30 (7)    | 0.26    |
|       | 422 (91)| 425 (93)  |         |
| XPO1  | 1 (0)   | 4 (1)     | 0.21    |
|       | 461 (100)| 451 (99) |         |
| ZRSR2 | 19 (4)  | 27 (6)    | 0.23    |
|       | 443 (96)| 428 (94)  |         |
| Total number of mutations | 2 | 3 | <0.001 |
| Median | 0-8 | 0-9 | |
| Range  |         |         |         |

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.

| Endpoint | Younger patients (n=729) | Older patients (n=205) | P   |
|----------|--------------------------|------------------------|-----|
|          | 17-gene<sup>low</sup> LSC score (n=403) | 17-gene<sup>high</sup> LSC score (n=326) | | 17-gene<sup>low</sup> LSC score (n=64) | 17-gene<sup>high</sup> LSC score (n=141) | |
| ELN Group, n (%) | | | | | | |
| Favorable | 264 (68) | 78 (26) | <0.001 | 20 (36) | 23 (18) | 0.009 |
| 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.