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Additional gene mutations may refine the 2017 European LeukemiaNet classification in adult patients with *de novo* acute myeloid leukemia aged <60 years

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Abstract

The European LeukemiaNet (ELN) recommendations for diagnosis and management of acute myeloid leukemia (AML) have become an important tool to assess patients' prognosis and guide treatment. We tested the prognostic impact of the 2017 ELN classification in a large cohort of 863 AML patients aged <60 years similarly treated on Cancer and Leukemia Group B/Alliance for Clinical Trials in Oncology studies. Based on multivariable models within each ELN genetic-risk group, we identified additional gene mutations that may refine the 2017 ELN risk classification. *BCOR*- or *SETBP1*-mutated Favorable-risk patients with non-core-binding-factor AML and *IDH*-mutated Adverse-risk patients had Intermediate-risk outcomes. Outcomes of *NPM1*/*WT1* co-mutated patients and those of *ZRSR2*-mutated patients resembled outcome of Adverse-risk

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patients. Moreover, *FLT3*-ITD^{high} allelic ratio conferred adverse rather than Intermediate-risk irrespective of the *NPM1* mutation status, and *DNMT3A* mutations associated with very poor survival. Applying these refinements reclassified 9% of current Favorable risk patients and 53% of current Intermediate-risk patients to the Adverse-risk group, with similar poor survival as current Adverse-risk patients. Furthermore, 4% of current Favorable-risk patients and 9% of Adverse-risk patients were reclassified to the Intermediate-risk group.

Introduction

The first edition of the European LeukemiaNet (ELN) recommendations for diagnosis and management of acute myeloid leukemia (AML) in adults,[1] authored by a panel of international experts, was published in 2010 and was incorporated into patient care. The 2010 ELN recommendations contained a standardized system for reporting cytogenetic and molecular alterations in studies that correlated such genetic findings with treatment outcomes of AML patients to facilitate meaningful comparisons among clinical studies.[1] Subsequently, several studies have established the clinical utility of the 2010 ELN genetic-risk classification.[2–4] The need for such a classification has been evident because of the heterogeneity of outcomes of AML patients, with only 35–40% of patients aged <60 years achieving long-term survival.[5] Importantly, the increasing knowledge about molecular features of AML, including gene mutations, gene-expression changes and epigenetic modifications, highlights the heterogeneity of the disease and helps identify patients that are likely to do well or, conversely, do poorly with standard chemotherapy.[6–11] Moreover, these molecular markers may be potential targets for anti-leukemic therapies and outcome of these patients may change with time following introduction of new agents.[12,13] Following the advances in understanding of the molecular landscape of AML, the ELN expert panel revised their classification in 2017, and, among other changes, added additional gene markers to the 2017 ELN genetic-risk categories.[14]

The aim of our study was to test how well the 2017 ELN classification stratifies AML patients into genetic-risk categories in, to our knowledge, the hitherto largest group of *de novo* adult AML patients aged <60 years with centrally reviewed cytogenetics who were treated with intensive cytarabine/daunorubicin-based chemotherapy. In addition, the patients were molecularly analyzed using a panel of 81 cancer- and leukemia-associated genes, which enabled us to investigate additional genetic markers that might refine the current ELN classification. Given the integration of the 2017 ELN classification into practice and study protocols, improvements to the classification may lead to earlier identification of patients that are unlikely to respond to standard treatment and may need different induction therapy, or of patients that are likely to experience relapse after initial response and need additional postremission therapy in first complete remission (CR).

Patients and methods

Patients, treatment, and cytogenetic studies

Pretreatment bone marrow (BM) or blood samples containing ≥20% leukemic blasts were obtained from 863 adults aged <60 years who were diagnosed with *de novo* AML

(excluding acute promyelocytic leukemia). Patients with AML evolving from an antecedent hematologic disorder and those with treatment-related AML were excluded. The patients were treated on Cancer and Leukemia Group B (CALGB) trials[15–22] described in the Supplementary Information. Because most CALGB treatment protocols did not allow performing allogeneic stem cell transplantation (allo-SCT) in first CR on study and patients receiving allo-SCT had to be taken off protocol, which resulted in either lack of or incomplete follow-up data, we excluded all patients who received allo-SCT from the current study. CALGB is now part of Alliance for Clinical Trials in Oncology (Alliance). Cytogenetic analyses of pretreatment BM and/or blood samples were performed by CALGB/Alliance-approved institutional laboratories, and results confirmed by central karyotype review.[23] Patients provided study-specific written informed consent to participate in treatment studies, CALGB 8461 (cytogenetic studies), CALGB 9665 (leukemia tissue banking) and CALGB 20202 (molecular studies). Study protocols were in accordance with the Declaration of Helsinki and approved by the institutional review boards at each center.

Statistical analysis

Definitions of the clinical endpoints–CR, disease-free survival (DFS) and overall survival (OS)–are provided in the Supplementary Information. Clinical and biological characteristics were compared using the Fisher's exact and Wilcoxon rank-sum tests for categorical and continuous variables, respectively. For CR, we calculated *P*-values using Fisher's exact test. For time-to-event analyses, we calculated survival estimates using the Kaplan-Meier method and compared groups using the log-rank test (*P*-values presented in the Figures with Kaplan-Meier curves).[24] A limited backward selection technique was used to build the final multivariable models for achievement of CR, DFS and OS.[25] We used logistic regression for modeling CR and Cox proportional hazard regression for modeling DFS and OS for univariable and multivariable outcome analyses. For a given gene mutation to be considered as a possible addition to the current risk group-defining markers, it had to significantly impact on outcome in at least one of the multivariable analyses. Furthermore, the CR rate or 3-year DFS or OS rates of patients carrying such mutation would have to be similar to those of patients belonging to a different risk-group in the 2017 ELN classification. The dataset was locked on January 10, 2019. Data collection and statistical analyses were performed by the Alliance Statistics and Data Center using SAS 9.4 and TIBCO Spotfire S+ 8.2.

Molecular analyses

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, San Diego, CA; see Supplementary Information for details).[26] The presence or absence of *FLT3* internal tandem duplications (*FLT3*-ITD), as well as quantification of the *FLT3*-ITD to *FLT3* wild-type allelic ratio (low/no vs high defined as ratio ≥0.5), were determined as previously described.[27] In addition, biallelic *CEBPA* mutations were determined by Sanger sequencing,[28] bringing the total number of genes analyzed in our study to 81.

Results

Clinical characteristics and outcome of AML patients according to the 2017 ELN classification

The pretreatment characteristics of 863 patients classified into the 2017 ELN genetic-risk groups are depicted in Table 1. The median age of all patients was 45 years (range, 17–59 years) and 45% of patients were women. Almost one-half (49%) of the patients belonged to the ELN Favorable-risk group, whereas 22% and 29% of patients belonged to the Intermediate- and Adverse-risk groups, respectively.

Patients classified in the Adverse-risk group were predominantly male (male vs female, 62% vs 38%, P=0.01), whereas the Favorable- and Intermediate-risk groups had similar male to female ratios (53% vs 47% and 49% vs 51%, respectively). Adverse-risk group patients presented with lower white blood cell (WBC) counts compared with Favorable- and Intermediate-risk patients (Adverse-risk, median WBC 20.7 $\times 10^9$/l vs 24.2 and 28.6 $\times 10^9$/l respectively, P=0.01; Table 1).

With respect to clinical outcome, the CR rate of our patient cohort was 76%. There were 38% of patients disease-free and 44% of patients alive 3 years after diagnosis, with median DFS and OS of 1.3 years and 2.0 years, respectively. As expected, the patient outcomes differed according to the ELN genetic-risk groups to which the patients were assigned (Table 1). The CR rate of Favorable-risk patients was 92%, compared with 77% and 48% CR rates of Intermediate-risk and Adverse-risk patients (P<0.001). Fifty-three percent of patients belonging to the Favorable-risk group were disease-free and 64% were alive 3 years after diagnosis, compared with 22% disease-free and 31% alive patients in the Intermediate-risk group, and only 10% and 19%, respectively, of patients classified in the Adverse-risk group (both P<0.001).

Mutational landscape of AML patients

We detected 2354 mutations, with an average of 3 mutations per patient (range, 0–9). The frequencies of gene mutations detected in ≥2% of patients in the entire cohort are provided in Supplementary Table S1. Forty-four genes were mutated in ≥2% of patients in one of the ELN groups (Supplementary Table S1). In addition to the ELN risk group-defining mutations [Favorable-risk, biallelic *CEBPA* mutations and *NPM1* mutations with no *FLT3*-ITD or low *FLT3*-ITD allelic ratio (*FLT3*-ITD^{low}); Intermediate-risk, *NPM1* and *FLT3*-ITD with high allelic ratio (*FLT3*-ITD^{high}); Adverse-risk, *FLT3*-ITD^{high} and *NPM1* wild-type, *RUNX1*, *ASXL1* (in the absence of favorable genetic features) and *TP53*],[14] the frequencies of several additional gene mutations differed between the ELN genetic-risk groups (Supplementary Table S1, Figure 1).

In the Favorable-risk group, *DNMT3A* and *NRAS* mutations were frequently found, in 24% and 20% of patients, respectively (Supplementary Table S1, Figure 1). In the Intermediate-risk group, mutations in *DNMT3A* were the most frequent mutations (33%) that were observed aside from the group-defining mutations outlined above, followed by mutations in *IDH2* and *WT1*, which were present in 13% and 12% of Intermediate-risk patients, respectively. In the Adverse-risk group, mutations in *DNMT3A* (found in 14% of patients),

NRAS (found in 11% of patients) as well as *IDH2* and *BCOR* (both detected in 9% of patients) were the most frequent besides risk-group defining mutations indicated above.

We also assessed frequencies of mutations categorized into functional groups in the 2017 ELN genetic-risk categories (Supplementary Table S2). RAS pathway mutations were more frequently found in the Favorable-risk group than in the Intermediate-risk and Adverse-risk groups (Favorable, 35%, Intermediate, 19%, Adverse, 24%; $P<0.001$, 3-way comparison). Mutations in kinase and methylation-related genes were most frequently observed in the Intermediate-risk group (Favorable, 33% and 39%, Intermediate, 56% and 51%, Adverse, 35% and 27%, respectively; $P<0.001$ for each 3-way comparison). Mutations in genes encoding for spliceosomes, transcription factors and tumor suppressors (Favorable, 8%, 23% and 10%, Intermediate, 11%, 9% and 14%, Adverse, 20%, 36% and 27%, respectively; $P<0.001$ for each 3-way comparison) were more common in the Adverse-risk group compared with the Favorable and Intermediate-risk groups.

Impact of gene mutations on the outcome of patients classified according to the 2017 ELN genetic-risk groups

We next evaluated whether any of the detected gene mutations associated with better or worse outcomes within each 2017 ELN genetic-risk group and might potentially be used to refine the current ELN classification. We performed univariable and multivariable outcome analyses for the achievement of CR, DFS and OS within each of the 2017 ELN genetic-risk groups and identified gene mutations within each group that associated with outcome in multivariable analyses (Table 2). Within the Favorable-risk group outcome analyses were performed separately for core-binding factor AML (CBF-AML) patients and non-CBF-AML patients.

Within the non-CBF-AML patients of the Favorable-risk group, the presence of *WT1* and *ZRSR2* mutations associated with lower odds of achieving a CR ($P=0.01$, odds ratio [OR]=0.28; $P=0.03$, OR=0.24, respectively). Mutations in *BCOR* ($P=0.02$, hazard ratio [HR]=2.61), *IDH1* ($P=0.02$, HR=1.67), *PTPN11* ($P=0.04$, HR=1.54), *SETBP1* ($P=0.03$, HR=2.27), *WT1* ($P=0.01$, HR=1.91) and *ZRSR2* ($P=0.04$, HR=2.12) associated with a worse DFS, whereas mutations in *BCOR* ($P=0.03$, HR=2.57), *IDH1* ($P=0.007$, HR=1.81), *SETBP1* ($P=0.01$, HR=2.38), *WT1* ($P<0.001$, HR=2.78), and *ZRSR2* ($P=0.005$, HR=2.48) associated with a shorter OS. Within the CBF-AML patients of the Favorable-risk group, the presence of *KIT* mutations associated with shorter DFS ($P<0.001$, HR=3.31) and OS ($P=0.01$, HR=2.12).

In the Intermediate-risk group, mutations in *WT1* associated with lower odds of achieving a CR ($P=0.02$, OR=0.31). Mutations in *DNMT3A* and *WT1* associated with a shorter DFS ($P<0.001$, HR=3.02; and $P=0.01$, HR=2.15, respectively) and OS ($P<0.001$, HR=1.85; and $P=0.005$, HR=1.98, respectively). Among patients belonging to the Adverse-risk group, those harboring *IDH2* mutations had longer OS ($P=0.03$, HR=0.57) than patients with wild-type *IDH2*. The presence of *NRAS* mutations associated with reduced DFS ($P<0.001$, HR=3.32) and OS ($P=0.008$, HR=1.74), and *TET2*-mutated patients had shorter OS ($P=0.009$, HR=1.94) than patients without these mutations. Not a single *NRAS*- or *TET2*-

mutated patient belonging to the Adverse-risk group was disease-free after one year, and no patient was still alive 3 years after diagnosis of AML.

Gene mutations identified in multivariable models may refine the 2017 ELN classification

To test whether inclusion of the gene mutations identified in the multivariable models might refine the ELN genetic risk classification, we compared the outcomes of patients harboring these gene mutations with the outcomes of patients belonging to another ELN risk-group (e.g., CR, DFS and OS of Favorable-risk patients with a given mutation were compared with the CR, DFS and OS of patients in the Intermediate- and Adverse-risk categories). In the non-CBF-AML Favorable-risk group, the CR rates of patients harboring mutations in *WT1* were similar to the CR rates of patients belonging to the Intermediate group (Figure 2a; *WT1*-mutated Favorable-risk vs Intermediate-risk, 75% vs 77%, *P*=0.81). With respect to DFS, non-CBF-AML Favorable-risk patients with mutations in *BCOR*, *SETBP1*, or *WT1* had similar DFS to that of the Intermediate-risk patients (3-year rates, *BCOR*-mutated Favorable-risk vs Intermediate-risk, 29% vs 22%; *P*=0.72; *SETBP1*-mutated Favorable-risk vs Intermediate-risk, 20% vs 22%, *P*=0.88; *WT1*-mutated Favorable-risk vs Intermediate-risk, 24% vs 22%, *P*=0.72). The DFS of patients with *ZRSR2* mutations actually resembled those of the Adverse-risk group (*ZRSR2*-mutated Favorable-risk vs Adverse-risk, 11% vs 10%, *P*=0.33).

With respect to OS, again the 3-year rates of Favorable-risk *BCOR*-, *SETBP1*- and *WT1*-mutated patients closely resembled those of Intermediate-risk patients (3-year rates, *BCOR*-mutated 38% vs 32%, *P*=0.91; *SETBP1*-mutated 45% vs 32%, *P*=0.67; *WT1*-mutated 29% vs 32%, *P*=0.69), whereas *ZRSR2*-mutated patients had OS similar to OS of the Adverse-risk group (23% vs 19%, *P*=0.46). Among the CBF-AML patients in the Favorable-risk group, those harboring *KIT* mutations had a DFS similar to DFS of the Intermediate-risk patients (3-year rates, 24% vs 22%, *P*=0.62; Figure 2b). However, the OS of *KIT*-mutated Favorable-risk CBF-AML patients was significantly better than OS of Intermediate-risk patients (3-year rates, 53% vs 32%, *P*=0.01), suggesting that the former should remain classified as having Favorable-risk.

In the ELN Intermediate-risk group, patients harboring *WT1* mutations had CR rates, DFS and OS that were similar to those of ELN Adverse-risk patients (CR rates, 58% vs 48%, *P*=0.40; 3-year DFS rates, 14% vs 10%, *P*=0.80; 3-year OS rates, 19% vs 19%, *P*=0.99; Figure 2c). Of the 14 *WT1*-mutated patients who achieved a CR, all but one experienced relapse of their disease. Furthermore, *DNMT3A*-mutated patients in the ELN Intermediate-risk group had DFS and OS that resembled those of Adverse-risk patients (3-year DFS rates, 10% vs 10%, *P*=0.67; 3-year OS rates, 19% vs 19%, *P*=0.28). Patients classified in the ELN Adverse-risk group who harbored *IDH2* mutations had outcome similar to that of Intermediate-risk patients with regard to CR rates (65% vs 77%, *P*=0.30), DFS (20% vs 22%, *P*=0.48) and OS (30% vs 32%, *P*=.89; Figure 2d), which supports their inclusion in the Intermediate-risk group.

High allelic ratio of *FLT3*-ITD confers short DFS and OS irrespective of co-occurring *NPM1* mutation

The inclusion of *FLT3*-ITD allelic ratio as a criterion for genetic-risk group assignment (with only high allelic ratio being considered as an adverse prognosticator) was one of the major changes in the 2017 ELN classification update. Patients with mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} are now classified as having Favorable genetic risk, whereas wild-type *NPM1* patients without *FLT3*-ITD or with *FLT3*-ITD^{low}, and *NPM1*-mutated patients with *FLT3*-ITD^{high} belong to the Intermediate-risk group. Finally, patients who harbor *FLT3*-ITD^{high} without concomitant *NPM1* mutation are classified as having Adverse-risk. To test the validity of these criteria in our data, we assessed the prognostic impact of *NPM1* mutations within the *FLT3*-ITD-negative/*FLT3*-ITD^{low} patient group, as well as the impact of *NPM1* mutations on outcome of *FLT3*-ITD^{high} patients.

Among *FLT3*-ITD-negative/*FLT3*-ITD^{low} patients, those harboring a *NPM1* mutation had a higher CR rate (88% vs 74%, $P<0.001$), and a longer OS (3-year OS rates, 60% vs 45%, $P<0.001$) than patients with wild-type *NPM1* (Figure 3a). There was no significant difference in DFS between *NPM1*-mutated and *NPM1* wild-type patients. Within the *FLT3*-ITD^{high} cohort, patients harboring a *NPM1* mutation had higher CR rates than those without a *NPM1* mutation (81% vs 51%, $P=0.003$). However, there was no significant difference in either DFS or OS between patients with and without *NPM1* mutations (Figure 3b), indicating that the negative prognostic impact of *FLT3*-ITD^{high} may outweigh the positive prognostic impact of *NPM1* mutations with respect to those survival endpoints.

Co-occurrence of *WT1* and *NPM1* mutations confers especially poor outcome

We analyzed the prognostic impact of combinations of mutations in the *WT1* and *NPM1* genes in patients classified in the non-CBF-AML Favorable-risk or Intermediate-risk groups. Patients with *WT1* mutations had lower CR rates than those with wild-type *WT1* regardless of whether they had *NPM1* mutations (*WT1*-mutated/*NPM1*-mutated, 69%; *WT1*-mutated/*NPM1* wild-type, 68%; *WT1* wild-type/*NPM1*-mutated, 90%; *WT1* wild-type/*NPM1* wild-type, 83%; $P=0.001$). However, the adverse prognostic impact of *WT1* mutations with respect to DFS and OS was dependent on coexisting *NPM1* mutations (Figure 4). The outcome of *WT1*-mutated/*NPM1*-mutated patients was, unexpectedly, much worse than that of *WT1*-mutated/*NPM1*-wild-type patients: the former had 3-year DFS rates of 5% versus 46% ($P=0.008$) and 3-year OS rates of 9% versus 47% ($P=0.002$). Thus, in addition to the *FLT3*-ITD high allelic ratio, the coexistence of *WT1* and *NPM1* mutations considerably alters the positive prognostic impact of *NPM1* mutations, making patients harboring such a combination of mutations candidates for more aggressive treatment.

DNMT3A mutations negatively impact DFS and OS, but not achievement of CR, in Intermediate-risk patients

Our investigation of the association of *DNMT3A* mutations with outcome of patients belonging to the Intermediate-risk group revealed that *DNMT3A*-mutated patients had a CR rate of 83% (compared with 73% of *DNMT3A* wild-type patients, $P=0.21$), but that 85% of those *DNMT3A*-mutated patients who achieved a CR relapsed. Both DFS (3-year rates, 10% vs 29%, $P<0.001$), and OS (3-year rates, 19% vs 37%, $P=0.002$) of *DNMT3A*-mutated

patients were shorter than those of *DNMT3A* wild-type patients. Consequently, *DNMT3A*-mutated patients without Favorable-risk markers should be considered as having Adverse-risk because of their high relapse rate and poor DFS and OS, despite high likelihood of achieving a CR. Thus, early allo-SCT may be useful for these *DNMT3A*-mutated patients.

Suggested refinement of the 2017 ELN classification by inclusion of additional molecular markers

Based on the results of our multivariable analyses, as well as analysis of prognostic significance of co-occurring mutations, we propose possible refinement of criteria used for risk group assignment (Table 3). We propose that only those *NPM1*-mutated non-CBF-AML patients who harbor neither *FLT3*-ITD^{high} nor a *WT1* mutation should be considered as having a Favorable risk. In fact, *FLT3*-ITD^{high} patients should always be considered as Adverse-risk, regardless of their *NPM1* mutation status. In addition, we identified *ZRSR2* mutations as a new marker associated with Adverse-risk outcome. Furthermore, *DNMT3A* mutations in the absence of Favorable-risk markers were associated with very short DFS and OS rates, and may also be considered as Adverse-risk.

Patients originally classified in the non-CBF-AML 2017 Favorable-risk group who harbored mutations in *BCOR* or *SETBP1*, and Adverse-risk patients with *IDH2* mutations had outcomes that resembled those of patients currently classified in the Intermediate-risk group.

Application of the aforementioned changes to the 2017 ELN non-CBF-AML Favorable-risk group resulted in reclassification of 19 (4%) patients to the revised Intermediate-risk group, and 33 patients (9%) to the revised Adverse-risk group. Notably, of the 189 patients originally classified in the Intermediate-risk group, more than half were reclassified as having Adverse-risk when using the suggested refinement (n=100, 53%). The re-classification of the majority of Intermediate-risk patients was due to either the presence of *FLT3*-ITD^{high} (n=70) and/or *DNMT3A* mutation (n=60), while the presence of either *WT1* or *ZRSR2* mutations each accounted for the change of 12% of patients. Conversely, 23 (9%) patients were transferred from the 2017 ELN Adverse-risk group to the modified Intermediate-risk group. Clinical outcome of patients in the refined 2017 ELN classification is shown in Supplementary Table S3, and the Kaplan-Meier curves are shown in Figure 5a. For comparison, Figure 5b contains Kaplan-Meier curves depicting DFS and OS of patients classified according to the original 2017 ELN classification.

As a result of the aforementioned reclassification of patients, the 3-year DFS and OS rates of 371 patients constituting the revised Favorable-risk group were slightly better than the respective DFS (3-year rates: 57% vs 53%) and OS (3-year rates: 69% vs 64%) of 423 patients classified in the original 2017 ELN Favorable-risk group. Likewise, the outcome of 131 patients in the new Intermediate-risk group also improved in comparison with that of the original Intermediate-risk group (n=189) with regard to both DFS (3-year rates: 32% vs 22%) and OS (3-year rates: 41% vs 31%). In contrast, DFS (10% vs 10%) and OS (19% vs 19%) of the original (n=251) and revised (n=361) Adverse-risk groups were identical (Table 1 and Supplementary Table S3).

Discussion

The determination of genetic risk in AML patients is a moving target. First, because new gene mutations constantly add new information to the prognostic landscape.[6,29–32] Second, a risk classification is based on a standardized treatment received by the analyzed patient group, which means that molecular markers previously considered as adverse or favorable may lose their prognostic significance when novel targeted therapies are used, as exemplified by *FLT3* inhibitors,[33] or tyrosine kinase inhibitors targeting *KIT* mutations in CBF-AML.[34]

Continuous updating of genetic-risk classifications is of special importance for patients that are classified as having Favorable risk, because they respond relatively well to standard chemotherapy, and thus are not being considered for allo-SCT in first CR nor for alternative treatment strategies. Our analysis of a relatively large panel of recurrent mutations in adult AML suggests several gene mutations and mutation combinations as possible important refinements of the existing ELN classification.

Our data suggest *WT1* mutations as a possibly important additional marker that should be considered in *NPM1*-mutated patients, since the presence of both these mutations negatively affects the patients' DFS and OS. The presence of *WT1* mutations has been associated with poor outcomes of AML patients in most,[35–38] but not all,[39] studies. However, our data suggest that *WT1* mutations should be considered in the context of *NPM1* mutation status, since it was coexistence of both mutations, not the presence of *WT1* mutations alone, that was associated with the worst outcome. Although *NPM1*-mutated patients receiving standard chemotherapy who also harbored a *WT1* mutation had a CR rate of 69% and thus might be considered good candidates for standard consolidation treatment, only 5% of those patients remained disease-free and only 9% were alive 3 years after diagnosis. However, given the relatively small numbers of patients with both *NPM1* and *WT1* mutations, these findings should be corroborated by future studies. This suggests that patients with *WT1* and *NPM1* mutations may be good candidates for early allo-SCT. Similarly, *DNMT3A*-mutated patients, who currently belong to the Intermediate-risk group, have a high likelihood of responding to initial treatment, but 84% of them experienced relapse of their disease and had short DFS and OS similar to patients classified in the Adverse-risk group.

Our findings that the presence of a high *FLT3*-ITD allelic ratio outweighs the positive prognostic impact of *NPM1* mutations, and should be considered as portending Adverse-risk regardless of co-occurring mutations, is in agreement with Boddu *et al.*[40] who found no significant difference in survival of mutated vs wild-type *NPM1* patients when comparing patients with high vs those with low *FLT3*-ITD allelic ratio. However, in contrast to Boddu *et al.*[40] we did see an impact of *NPM1* mutations in the cohort of patients with *FLT3*-ITD$^{low/no}$. Interestingly, our analyses also suggested a prognostic importance of less common gene mutations in the *BCOR*, *SETBP1* and *ZRSR2* genes. While Papaemmanuil *et al.*[6] suggested that AML with chromatin remodeling–spliceosome mutations constitutes a distinct subgroup, very little is known about the possible prognostic impact of *SETBP1* mutations in AML.[41] Thus, our results showing prognostic impact of less common gene mutations should be confirmed by further studies.

The only patients reclassified from a prognostically worse ELN risk group to a better one were Adverse-risk patients harboring *IDH2* mutations whose outcome was similar to that of Intermediate-risk patients. Most patients were *FLT3*-ITD$^{low/no}$, and a complex karyotype was detected in only 7 of 23 (30%) patients in this group. The association of *IDH2* mutations with an improved OS was previously reported by Patel *et al.*[7] both in the entire cohort of AML patients they analyzed and in patients with Intermediate-risk. However, this suggested risk-group refinement by *IDH2*, as well as the known importance of *FLT3*-ITD^{high}, have to be considered in view of the FDA-approved inhibitors (e.g., addition of midostaurin to induction chemotherapy for *FLT3*-mutated patients, and/or *FLT3*- or *IDH*-directed targeted therapies), which may only be the beginning of changes in current risk-stratification approaches in the era of increasing targeted therapy options.

Importantly, our study did not include testing for minimal residual disease (MRD). Given the substantial body of evidence about the impact of MRD on survival of AML patients, this represents a notable limitation to our analyses, which needs to be addressed in future studies. In summary, our study provides a comprehensive analysis of prognostic markers in younger adults with AML treated with standard chemotherapy without allo-SCT within the framework of the 2017 ELN genetic-risk classification. Our results suggest a refinement of this classification by including additional gene mutations, which has led to identification of patients that may need more intensive treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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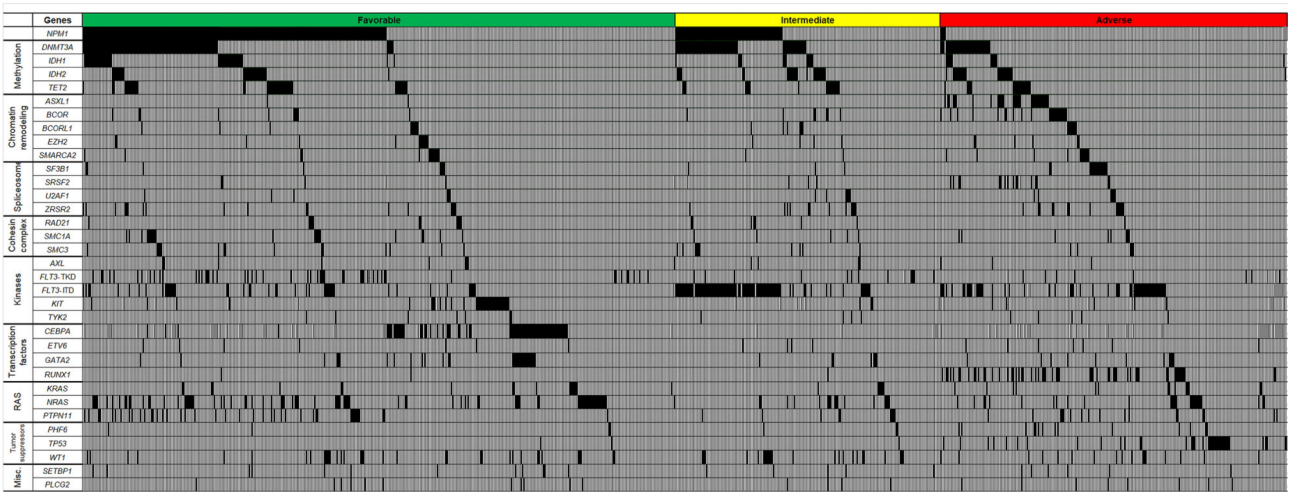

Figure 1.

Oncoprint of genes most frequently mutated in younger acute myeloid leukemia patients categorized into genetic-risk groups according to the 2017 European LeukemiaNet (ELN) classification (green color, Favorable-risk; yellow, Intermediate-risk; red, Adverse-risk). Each column represents an individual patient. Black color indicates genes found to be mutated, light gray indicates wild-type status of the gene, and dark gray indicates missing values.

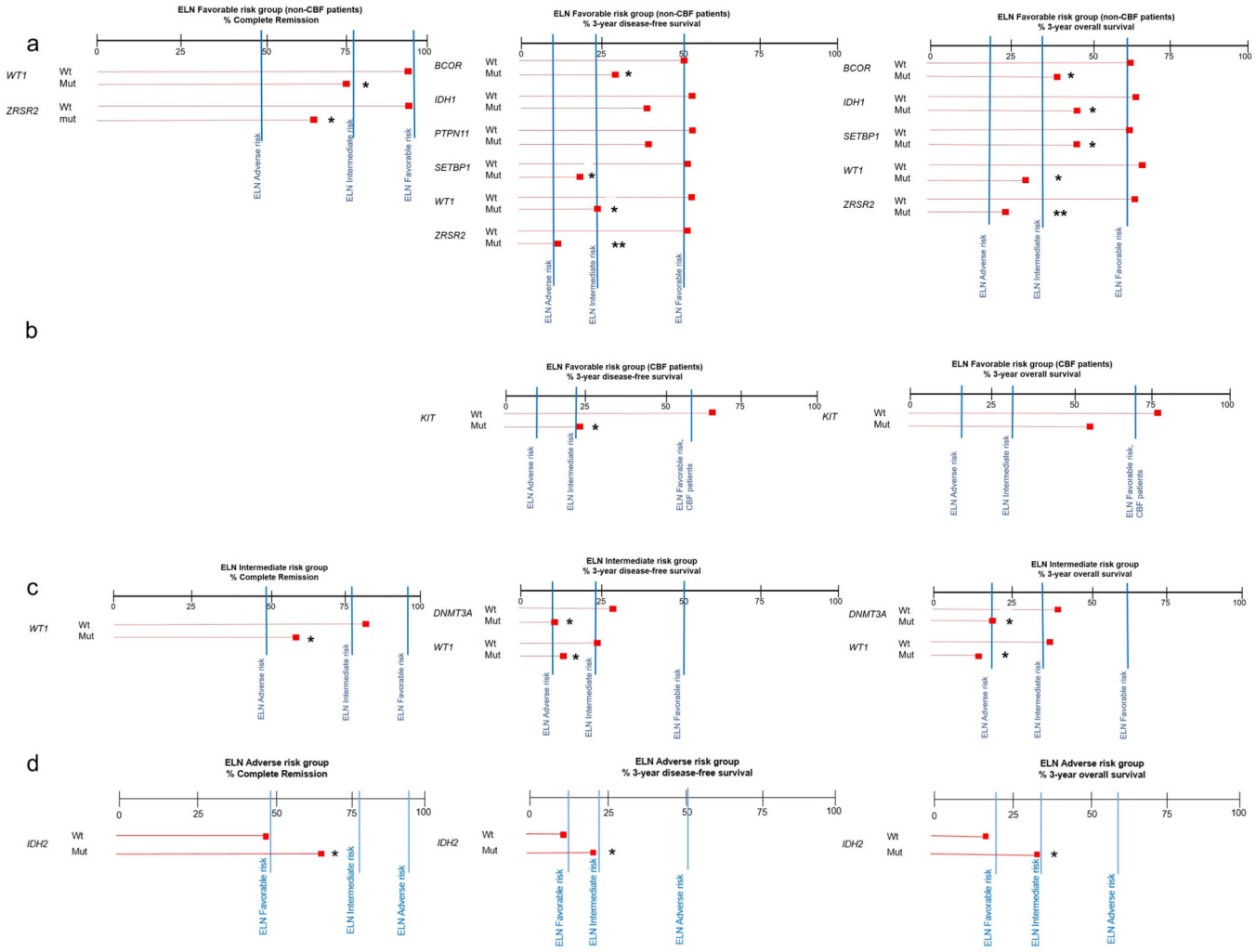

Figure 2.

Line graphs showing the impact of gene mutations found to be prognostically significant in multivariable models for **a** Non-core-binding factor acute myeloid leukemia (non-CBF-AML) patients in the 2017 European LeukemiaNet (ELN) Favorable-risk group, **b** CBF-AML Favorable-risk patients, **c** Intermediate-risk patients and **d** Adverse-risk patients on complete remission rates (left panel), 3-year disease-free survival rates (middle panel) and 3-year overall survival rates (right panel). The percentages are depicted for both wild-type (wt) and mutated (mut) patients, relative to the median rates according to the ELN genetic-risk classification (blue vertical bars). * mutated versus Intermediate-risk, $P \leq 0.05$ (adjusted for multiple comparisons); ** mutated versus Adverse-risk, $P \leq 0.05$ (adjusted for multiple comparisons).

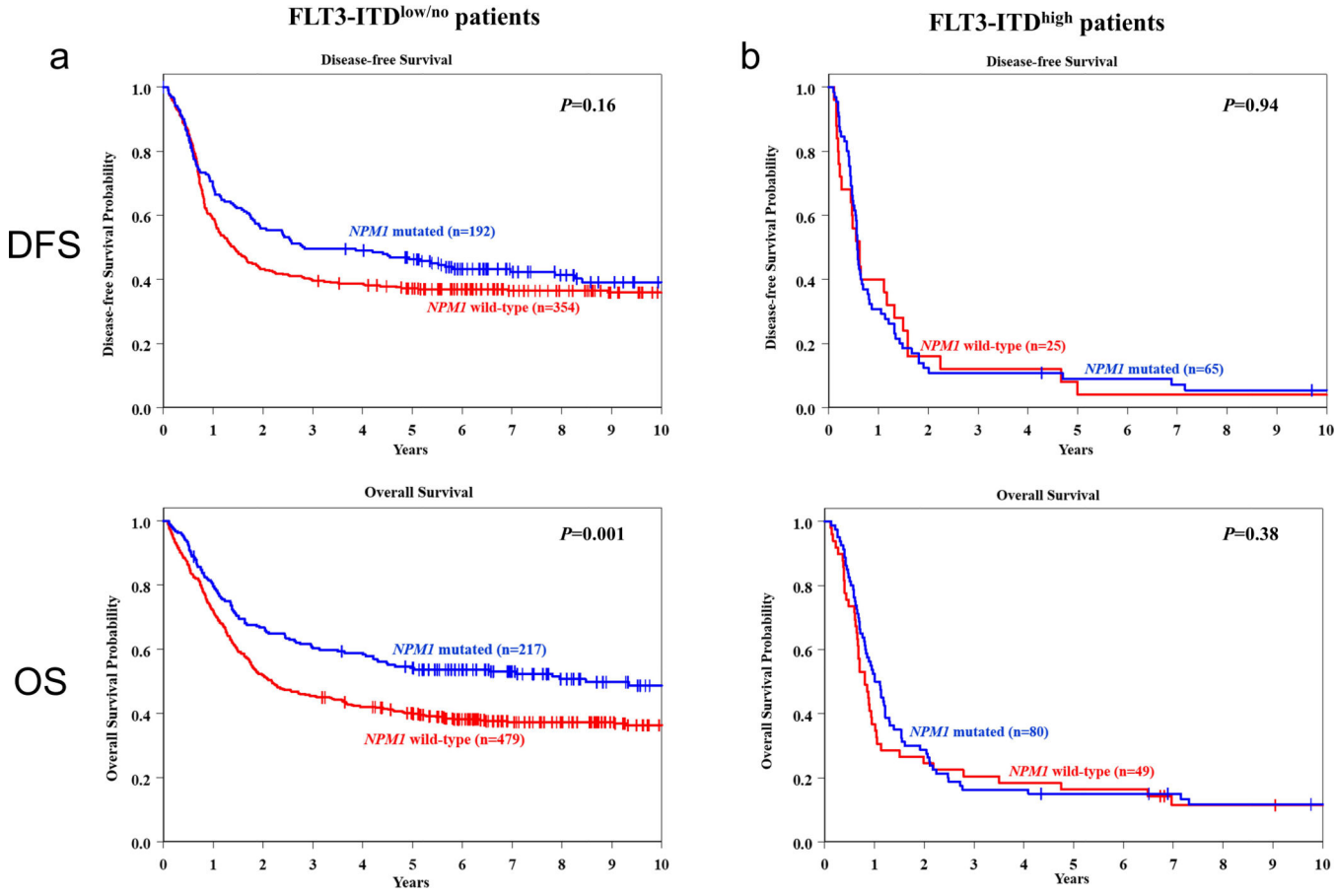

Figure 3.

a Disease-free survival (DFS, upper graph) and overall survival (OS, lower graph) of *NPM1*-mutated (blue line) and *NPM1*-wild-type (red line) patients who were either *FLT3*-ITD-negative or harbored *FLT3*-ITD^{low}. **b** DFS (upper graph) and OS (lower graph) of *NPM1*-mutated (blue line) and *NPM1*-wild-type (red line) patients who harbored *FLT3*-ITD^{high}.

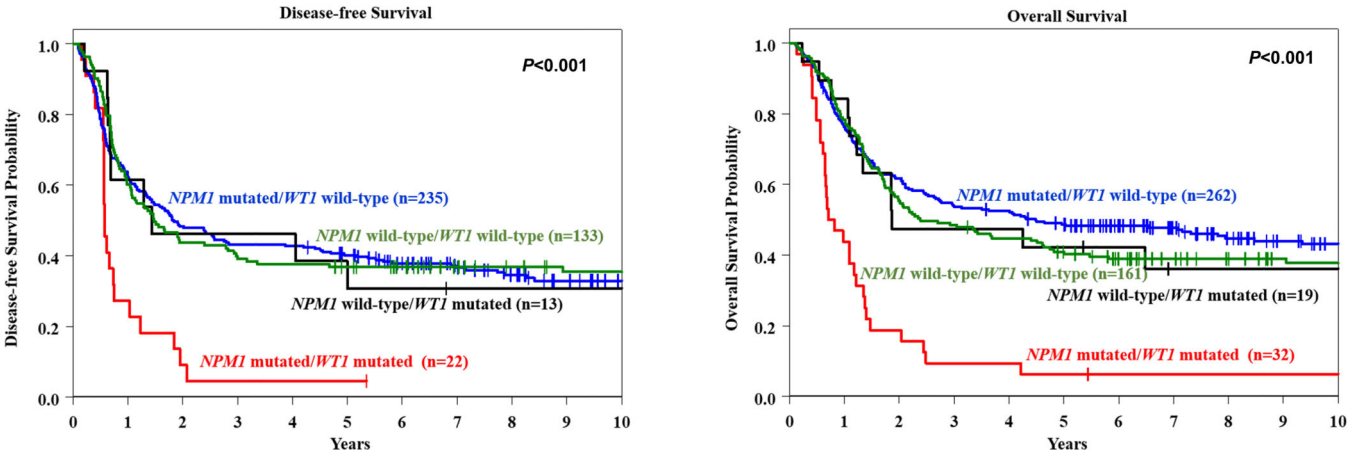

Figure 4.

Kaplan-Meier curves depicting the disease-free (left panel) and overall survival (right panel) of *NPM1*-mutated/*WT1*-mutated patients (red line), *NPM1*-mutated/*WT1* wild-type patients (blue line), *NPM1* wild-type/*WT1*-mutated patients (black line), and *NPM1* wild-type/*WT1* wild-type patients (green line).

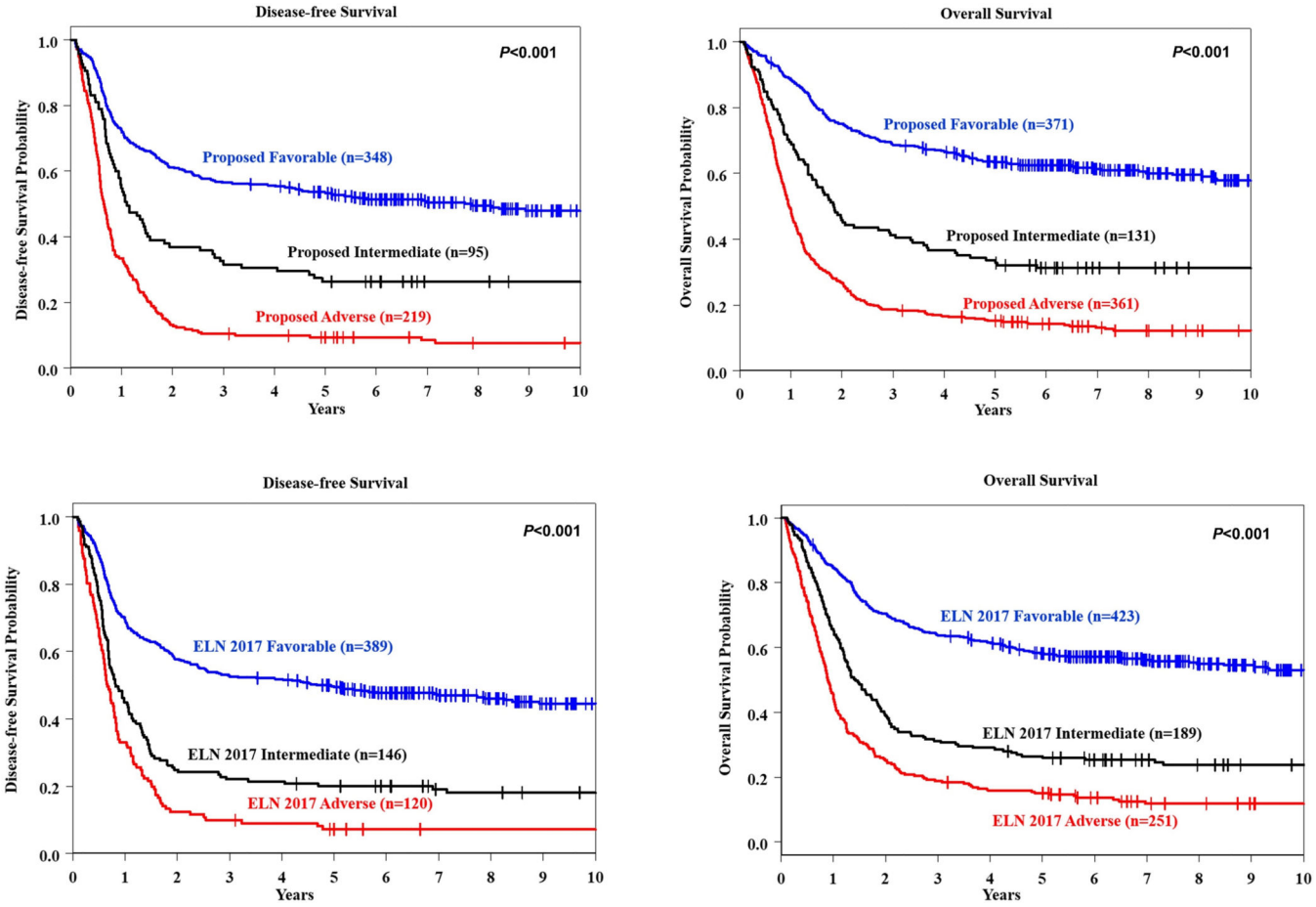

Figure 5.

a Kaplan-Meier curves depicting the disease-free (DFS, left panel) and overall survival (OS, right panel) of younger (aged <60 years) adult patients with acute myeloid leukemia classified into proposed Favorable-risk (blue line), Intermediate-risk (black) and Adverse-risk (red) 2017 European LeukemiaNet (ELN) groups after the proposed refinement of the ELN classification. **b** DFS (left graph) and OS (right graph) of younger patients classified into the original ELN genetic-risk groups.

Table 1.

Pretreatment clinical characteristics and outcome of younger patients with *de novo* acute myeloid leukemia assigned to the genetic-risk groups according to the 2017 ELN classification

Characteristic	All patients n=863	Favorable-risk n=423	Intermediate-risk n=189	Adverse-risk n=251	*P*-value[a]
Age, years					0.54
Median	45	44	44	46	
Range	17–59	17–59	17–59	17–59	
Sex, n (%)					0.01
Male	473 (55)	226 (53)	92 (49)	155 (62)	
Female	390 (45)	197 (47)	97 (51)	96 (38)	
Race, n (%)					0.27
White	723 (85)	360 (87)	154 (82)	209 (85)	
Nonwhite	124 (15)	53 (13)	33 (18)	38 (15)	
Hemoglobin, g/dl					0.37
Median	9.2	9.2	9.4	9.1	
Range	2.3–25.1	2.3–25.1	2.9–14.4	4.6–14.8	
Platelet count, x10^9/l					0.07
Median	53	49	56	58	
Range	4–648	4–648	9–445	8–341	
WBC count, x10^9/l					0.01
Median	24.3	24.2	28.6	20.7	
Range	0.4–475.0	0.4–475.0	0.9–308.8	0.6–320.0	
Blood blasts, %					0.05
Median	56	52	62	56	
Range	0–99	0–97	0–98	0–99	
Bone marrow blasts, %					<0.001
Median	66	63	74	67	
Range	0–97	2–97	10–96	0–97	
Extramedullary involvement, n (%)	222 (27)	127 (31)	46 (26)	49 (20)	0.001
Complete remission, n (%)	655 (76)	389 (92)	145 (77)	121 (48)	<0.001
Disease-free survival					<0.001
Median, years	1.3	4.7	0.8	0.7	
% Disease-free at 3 years (95% CI)	38 (34–42)	53 (48–57)	22 (16–29)	10 (5–16)	
Overall survival					<0.001
Median, years	2.0	12.4	1.4	0.9	

Characteristic	All patients n=863	Favorable-risk n=423	Intermediate-risk n=189	Adverse-risk n=251	*P*-value[a]
% Alive at 3 years (95% CI)	44 (40–47)	64 (59–68)	31 (25–38)	19 (14–24)	

Abbreviations: CI, confidence interval; n, number; ELN, European LeukemiaNet; WBC, white blood cell.

[a] *P*-values for categorical variables are from Fisher's exact test, *P*-values for continuous variables are from the Wilcoxon rank sum test and they are comparing the three risk groups: Favorable, Intermediate and Adverse. *P*-values for the time to event variables are from the log-rank test and they are comparing the three risk groups: Favorable, Intermediate and Adverse.

Table 2.

Multivariable outcome analyses of younger patients with *de novo* acute myeloid leukemia assigned to the genetic-risk groups according to the 2017 ELN genetic risk classification

Variables in final model	Complete remission		Disease-free survival		Overall survival	
	OR (95% CI)	*P*[a]	HR (95% CI)	*P*[b]	HR (95% CI)	*P*[b]
Favorable-risk group[c]						
Non-CBF-AML patients						
BCOR, mutated vs wild-type			2.61 (1.14-6.00)	0.02	2.57 (1.12-5.89)	0.03
IDH1, mutated vs wild-type			1.67 (1.07-2.59)	0.02	1.81 (1.17-2.80)	0.007
PTPN11, mutated vs wild-type			1.54 (1.02-2.33)	0.04		
SETBP1, mutated vs wild-type			2.27 (1.10-4.66)	0.03	2.38 (1.20-4.73)	0.01
WT1, mutated vs wild-type	0.28 (0.10-0.75)	0.01	1.91 (1.14-3.20)	0.01	2.78 (1.72-4.50)	<0.001
ZRSR2, mutated vs wild-type	0.24 (0.07-0.89)	0.03	2.12 (1.03-4.37)	0.04	2.48 (1.31-4.69)	0.005
Age, 10-year increase					1.21 (1.03-1.42)	0.02
WBC count, 50-unit increase					1.19 (1.06-1.33)	0.004
CBF-AML patients						
KIT, mutated vs wild-type			3.31 (1.94-5.63)	<0.001	2.12 (1.18-3.81)	0.01
Intermediate-risk group[c]						
DNMT3A, mutated vs wild-type			3.02 (1.99-4.59)	<0.001	1.85 (1.31-2.62)	<0.001
WT1, mutated vs wild-type	0.31 (0.12-0.81)	0.02	2.15 (1.17-3.94)	0.01	1.98 (1.23-3.16)	0.005
Age, 10-year increase					1.31 (1.12-1.52)	<0.001
Hemoglobin level, 1-unit increase	1.24 (1.02-1.51)	0.03	0.85 (0.77-0.94)	0.002		
Platelet count, 50-unit increase			0.79 (0.68-0.93)	0.004	0.86 (0.75-0.98)	0.03
Sex, male vs female	0.33 (0.15-0.70)	0.004				
Adverse-risk group[c]						
IDH2, mutated vs wild-type					0.57 (0.34-0.95)	0.03
NRAS, mutated vs wild-type			3.32 (1.80-6.11)	<0.001	1.74 (1.15-2.61)	0.008
TET2, mutated vs wild-type					1.94 (1.18-3.18)	0.009

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Variables in final model	Complete remission		Disease-free survival		Overall survival	
	OR (95% CI)	*P*[a]	HR (95% CI)	*P*[b]	HR (95% CI)	*P*[b]
Age, 10-year increase					1.18 (1.04-1.33)	0.008
Hemoglobin level	1.24 (1.05-1.46)	0.01				
Platelet count, 50-unit increase			0.81 (0.69-0.96)	0.01		
Race, white vs nonwhite	0.32 (0.15-0.72)	0.005				
WBC count, 50-unit increase					1.20 (1.06-1.37)	0.006

Variables in the table are in alphabetical order and separated by gene mutations and other clinical characteristics. Multivariable analyses were done separately for CBF- and non-CBF patients within the Favorable-risk group.

Abbreviations: AML, acute myeloid leukemia, CI, confidence interval; CBF, core-binding factor; CR, complete remission; DFS, disease-free survival; ELN, European LeukemiaNet; HR, hazard ratio; OR, odds ratio; OS, overall survival; WBC, white blood cell.

NOTE. An OR <1 (>1) means lower (higher) CR rate for first category listed of a dichotomous variable or higher values of a continuous variable. A HR >1 (<1) corresponds to a higher (lower) risk for first category listed of a dichotomous variable or higher values of a continuous variable. A limited backward selection technique was used to build the final model for achievement of CR, DFS and OS. Variables considered in the model were variables that were significant at the likelihood ratio test *P*-value <0.20 from the univariable models (detailed in the Supplementary Information).

[a] *P*-values for CR attainment were determined by logistic regression.

[b] *P*-values for DFS and OS were determined using Cox proportional hazards regression.

[c] Please see Supplementary Material for variables considered in the multivariable models

Table 3

Proposed refinement of the 2017 European LeukemiaNet (ELN) risk classification by additional gene mutations

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}
	Mutated *NPM1* without *WT1* mutation
	Biallelic mutated *CEBPA*
Intermediate	Mutated *BCOR*[a] (without adverse-risk genetic lesions)
	Mutated *SETBP1*[a] (without adverse-risk genetic lesions)
	Mutated *IDH2*[b] (without adverse-risk genetic lesions)
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM (EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype, monosomal karyotype
	FLT3-ITD^{high} (irrespective of *NPM1* mutation status)[c]
	Mutated *NPM1* and mutated *WT1*[a]
	Mutated *DNMT3A*[a,b]
	Mutated *RUNX1*[b]
	Mutated *ASXL1*[b]
	Mutated *TP53*
	Mutated *ZRSR2*[a]

Indicated in red color are gene mutations identified in our models whose outcomes resembled those of the groups that they were now added to, and that may refine the current risk stratification

[a] Markers impacting on disease-free and overall survival, but not on the achievement of a complete remission.

[b] These markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

[c] *FLT3*-ITD^{high} is defined as ≥0.5 as per ELN guidelines.