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Applicability of 2022 classifications of acute myeloid leukemia in the real-world setting

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Abstract:
The increasing knowledge of molecular genetics of acute myeloid leukemia (AML) urged the update of previous diagnostic and prognostic schemes, which resulted in the development, in 2022, of the World Health Organization (WHO), the International Consensus Classification (ICC) and the new European LeukemiaNet (ELN) recommendations. We aimed to provide a real-world application of the new models, to unravel differences and similarities, and to test their implementation in clinical AML diagnosis. A total of 1001 patients diagnosed with AML were re-classified according to the new schemes. The overall diagnostic changes between the WHO 2016, compared to WHO 2022 and ICC classifications were 22.8% and 23.7%, respectively, with a 13.1% difference in patients' distribution between ICC and WHO 2022. The 2022 ICC "not otherwise specified" and WHO "defined by differentiation" AML categories shrank as compared to WHO 2016 (24.1% and 26.8% respectively, vs 38.7%), particularly due to an expansion of the myelodysplasia (MDS)-related group. Of 397 patients with a MDS-related AML according to the ICC, 55.9% were defined by the presence of a MDS-related karyotype. The overall re-stratification between ELN 2017 and 2022 was 12.9%. The 2022 AML classifications led to a significant improvement of diagnostic schemes. In the real-world setting, conventional cytogenetics, usually rapidly available and less expensive than molecular characterization, stratified 56% of secondary AML, still maintaining a powerful diagnostic role. Considering the similarities between WHO and ICC diagnostic schemes, a tentative to generate a unified model is desirable.

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Data Sharing

Data used for this study can be found at github for open-source access (https://github.com/ardadurmaz/aml), public available source (BEAT-AML Master trial) and any additional information is available upon request to the corresponding author.

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Abstract

The increasing knowledge of molecular genetics of acute myeloid leukemia (AML) urged the update of previous diagnostic and prognostic schemes, which resulted in the development, in 2022, of the World Health Organization (WHO), the International Consensus Classification (ICC) and the new European LeukemiaNet (ELN) recommendations. We aimed to provide a real-world application of the new models, to unravel differences and similarities, and to test their implementation in clinical AML diagnosis. A total of 1001 patients diagnosed with AML were re-classified according to the new schemes. The overall diagnostic changes between the WHO 2016, compared to WHO 2022 and ICC classifications were 22.8% and 23.7%, respectively, with a 13.1% difference in patients' distribution between ICC and WHO 2022. The 2022 ICC "not otherwise specified" and WHO "defined by differentiation" AML categories shrank as compared to WHO 2016 (24.1% and 26.8% respectively, vs 38.7%), particularly due to an expansion of the myelodysplasia (MDS)-related group. Of 397 patients with a MDS-related AML according to the ICC, 55.9% were defined by the presence of a MDS-related karyotype. The overall re-stratification between ELN 2017 and 2022 was 12.9%. The 2022 AML classifications led to a significant improvement of diagnostic schemes. In the real-world setting, conventional cytogenetics, usually rapidly available and less expensive than molecular characterization, stratified 56% of secondary AML, still maintaining a powerful diagnostic role. Considering the similarities between WHO and ICC diagnostic schemes, a tentative to generate a unified model is desirable.

Key points

- ICC and the 2022 WHO diagnostic classifications of acute myeloid leukemia present major similarities in real-word.
- Conventional cytogenetics, usually rapidly available and low-cost, can stratify about 56% of secondary acute myeloid leukemia cases.

Introduction

Diagnosis and treatment of acute myeloid leukemia (AML) require an integrated approach that takes into account clinical and laboratory characteristics, morphologic evaluation of bone marrow and peripheral blood, flow cytometry, cytogenetic and molecular analyses.[1] The recent advances of molecular medicine have unveiled the clinical utility of genomic profiles, which urged the necessity to integrate these information also into the daily practice for both diagnostic and prognostic purposes. In 2022, two updated classifications and a new prognostic stratification of AML have been proposed, including the 5th edition of The World Health Organization (WHO) classification of tumors,[2] The International Consensus Classification (ICC)[3] of Myeloid Neoplasms and Acute Leukemias, and the European LeukemiaNet (ELN) recommendations for AML prognosis.[4]

Among the main innovations introduced by both diagnostic classifications, the blast threshold required for AML diagnosis is certainly one of the most notable. The limit of 20% blasts was still used for the majority of AML categories in the 2016 WHO classification[5] (except for *RUNX1::RUNX1T1*-AML, *CBFB::MYH11*-AML, and acute promyelocytic leukemia, APL). This threshold has been discarded in the group with AML-defining recurrent genetic abnormalities according to the 2022 WHO, whereas ICC still requires 10%.[3,6] For all other AML subtypes, including myelodysplasia (MDS) related AML (AML-MR), the 20% blast cutoff is maintained. ICC also introduced the MDS/AML sub-entity in cases with 10-20% blasts, to indicate a fading of the diagnostic boundaries between MDS and AML when judged solely based on blast proportion, thereby emphasizing the increasing importance of molecular footprints to define the various nosologic subtypes.[3,6] One of the major updates of 2022 classifications is the more precise and objective definition of secondary AML. In this context, patients' clinical history is added as a disease attribute in ICC, and includes "therapy-related" AML, or a prior diagnosis of MDS or MDS/MPN. Germline predisposition is also considered as a diagnostic qualifier, and due to its relevant frequency has been confirmed as a patient and disease feature requiring specific definition.[3] Furthermore, the prior AML with MDS-related changes (MRC) is now better defined by a specific genomic signature, and referred to as AML-MR within the WHO 2022 classification. Conversely, ICC identifies multiple new subcategories: "AML with myelodysplasia-related cytogenetic abnormalities" (AML-MDSk), "AML with MDS-related gene mutations" (AML-MDSgene) and "AML with mutated *TP53*" (AML-*TP53*).[6,7]

The updated 2022 ELN recommendations differ from the previous 2017 edition[8] for the abrogation of the prognostic role previously assigned to *FLT3*-ITD allelic ratio (AR), based on the recognition of the favorable impact of FLT3-inhibitors on the prognosis of *FLT3*-ITD positive AML, now all included in the intermediate ELN risk group.[4] A further addition is the acknowledgement of the unfavorable prognostic role of the expanded list of MDS-related gene mutations, including *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*, now defining an adverse risk ELN group.

The impact of these innovations on clinical practice is not known yet.[9] Certainly, the amount of information required for the diagnostic and prognostic definitions, implies a strong collaboration between clinicians and hematology laboratories. Besides, every patient should have access to standardized molecular tests, and this may not be readily feasible in all parts of the world.[10] Molecular testing upon cytogenetics is particularly encouraged by the ICC hierarchical structure, whereby AML-TP53 precedes AML-MDSgene and the latter supersedes the AML-MDSk category. To investigate the clinical impact of the 2022 editions of the AML classification and prognostication systems, we reclassified a cohort of 1001 AML patients, previously diagnosed and stratified according to WHO 2016 and ELN 2017 criteria. Our primary objective was to provide a real-world application of the new AML classifications, testing the improved disease definition as compared to previous versions, and to validate the 2022 ELN prognostic stratification.

Patients and Methods

Patient characteristics

A total of 1001 AML patients were included in this study after obtaining written informed consent. The main inclusion criterion was a diagnosis of AML according to 2016 WHO classification. Patients previously classified as MDS, with blast count in the 10-19% range were not included in the present analysis. Patients data were collected through an International collaboration, including: i) Tor Vergata University, Rome, Italy (34 cases); ii) the Humanitas Cancer Center, Milan, Italy (88 cases); iii) the Cleveland Clinic Foundation, Ohio, USA (466 cases); iv) publicly available source (the BEAT AML Master Trial, 413 cases), and patients were treated during the 2012-2022 time period.[11,12] With the exception of BEAT AML Master Trial, data was obtained retrospectively via chart review. Characteristics of Cleveland Clinic and BEAT AML cohorts have been made publicly available elsewhere (https://github.com/ardadurmaz/aml). Biological samples and chart review for this study were obtained in accordance with the Declaration of Helsinki Principles and local Ethics Committee's approval. At the time of initial AML diagnosis, all patients underwent bone marrow aspirate, conventional cytogenetics and Next Generation Sequencing (NGS) analyses, according to standard guidelines.

All samples were evaluated for the mutational status of a list of commonly mutated myeloid genes, including *ASXL1, BCOR, CEBPA, EZH2, FLT3, NPM1, RUNX1, SF3B1, SRSF2, STAG2, TP53, U2AF1* and *ZRSR2*. Mutations were detected using NGS with a variant calling at a ≥2% threshold.[11,12] *FLT3*-ITD mutation burden was assessed either by capillary electrophoresis (CE), as allelic ratio (AR), or by NGS as Variant Allelic Frequency (VAF), in order to group patients according to ELN 2017.[8,13] Cytogenetic analysis was performed using standard chromosome banding techniques, and documented in compliance with ISCN recommendations.[14] Evaluation of at least 20 metaphases was required.[15] Patient clinical characteristics are shown in **Table 1**, while genetic subgrouping is detailed in **Fig 1**.

Patients were treated according to local protocols, including conventional chemotherapy (688 patients), non-intensive therapy (144 patients) and best supportive care (37 patients). Tyrosine-kinase inhibitors were used, alone or in combination with chemotherapy, in 11.2% of cases. Overall, the median follow-up time was 37.6 months (interquartile range, 14.7-73.1) from initial diagnosis.

Statistical analysis

Patients' characteristics were summarized by descriptive statistics of median and range (continuous variables) or by frequencies and percentages (categorical variables).

The distribution of patients among the categories identified by the ELN classifications was studied using contingency tables, in which both absolute frequencies and percentages related to them were reported. The association between categorical variables in contingency tables was evaluated by Pearson's chi-squared test. The number of patients re-classified from WHO 2016 to WHO 2022, and to ICC 2022 was compared using a two-sample z-test for equality of proportions with continuity correction.

Overall survival (OS) estimations with 95% confidence intervals were computed using the Kaplan-Meier method and survival curves were compared using the Log-Rank test.

Two univariate Cox models were created to compare the prognostic ability of the ELN 2017 and ELN 2022 classifications. The Akaike Information Criterion (AIC) was used to compare the two models' goodness of fit to the data. All tests were two-sided with $p<0.05$ indicating a statistically significant difference.

Statistical analysis was performed using R software.[16]

Results

Distribution of AML subsets according to ICC and 2022 WHO classifications

To validate the diagnostic accuracy of the 2022 AML classifications, we re-classified a cohort of 1001 patients, according to the 2022 WHO and ICC models.[2,3] **Table 2** shows the main differences among WHO 2016, WHO 2022, and ICC classifications, while **Fig 1** shows the distribution of the diagnostic subcategories according to the different classifications. In general, the overall shift of diagnostic categories was 22.8% and 23.7% between WHO 2016 versus WHO 2022 and ICC classifications, respectively, whereas this was 13.1% between ICC and WHO 2022. The categories defined "not otherwise specified" (NOS) by ICC and "defined by differentiation" (DD) according to WHO 2022 significantly shrank as compared to WHO 2016 (24.1% and 26.8% respectively, vs 38.7%, $p<0.0001$), particularly due to an expansion of MDS-related categories (see below). According to the ICC, older patients were more frequently diagnosed with a *TP53* or MDS-gene mutated AML (**Fig S1**).

Setting the WHO 2016 as a backbone to compare the shift of nosologic categories (**Fig 1B**), the number of *KMT2A*-rearranged (*KMT2A*-R) and *MECOM*-R AML was lower in ICC, compared to WHO 2022 (5.0% vs 6.0%), but still higher than that in WHO 2016 (3.3%, **Fig S2**) due to a precise definition of *KMT2A* and *MECOM* atypical rearrangements in ICC. The *CEBPA*-mutated AML category, which included 1.6% cases with biallelic-*CEBPA* (*CEBPA*bi) mutations in WHO 2016, included 1.4% *CEBPA*bZIP mutated cases by ICC. This category was wider in the WHO 2022[2] because of the inclusion of both *CEBPA*bi and *CEBPA*bZIP mutations (2.1%). *RUNX1*-mutated AML, indicated by WHO 2016 as a provisional entity, was eliminated in the new classifications. As a result, 82 *RUNX1*-mutated AML were re-classified by WHO 2022 as follows: 74.4% AML-MR, 22.0% AML-DD, 2.4% AML-*CEBPA*, and 1.2% AML "*MECOM* with extended rearrangements" (AML-*MECOM*-RE). In the ICC classification, 92.7% of cases were classified as AML-MDSgene, 3.7% AML-*TP53,* 2.4% AML-*CEBPA*bZIP and 1.2% AML-*MECOM*-R.

Molecular profile vs cytogenetics for the diagnosis of secondary AML (sAML)

According to the different criteria proposed by the 2 schemes (**Table 2**), we re-classified the subgroups of sAML (**Fig 1**). The 184 cases previously defined AML-MRC increased to 353 AML-MR according to WHO 2022 (35.3% of our cohort), while ICC criteria led to identification of 312 MDS-related (AML-MDSgene, 23.8% and AML-MDSk, 7.4%), and 85 *TP53*-mutated AML (8.5%).
Only 4 WHO 2022 AML-MR subjects were defined "NOS" according to the ICC, due to the presence of the 11q- and 13q- cytogenetic abnormalities (each in 2 patients), not included in the ICC AML-MDSk category (**Fig S3**). On the other side, 90.6% of ICC AML-*TP53* fell in the WHO 2022 AML-MR group, because of the concomitant presence of a complex karyotype in 81.8% of cases, a MDS-related gene mutation in 7.8%, a MDS-related cytogenetic abnormality or both a cytogenetic and a molecular alteration in 10.4% of patients.

We then explored whether a reversed ICC hierarchical approach, with cytogenetics first and NGS as a second step, could also be used to assign patients to the different subgroups. Out of 397 patients with a MDS-related AML according to ICC, 55.9% of cases were defined by the presence of a MDS-related karyotype, including those grouped by ICC in the AML-*TP53*, AML-MDSgene and AML-MDSk categories (**Fig 2A**).

When considering AML with normal karyotype (NK, n=452), 34.5% presented at least one ICC MDS-related gene mutation, but these mutations had a diagnostic relevance in only 26.3% of cases according to the ICC hierarchical algorithm (**Fig 2B**). Furthermore, 34.5% presented also at least one additional AML-diagnostic gene mutation (*NPM1*, 30.1%, *CEBPA*bZIP, 2.4% and *TP53*, 2.0%). Therefore, NGS refined the diagnosis in 60.8% and 58.0% of NK AML cases based on ICC and WHO 2022, respectively.

Risk stratification according to ELN

According to the ELN 2017 prognostic stratification, AML patients (n=953 pts, excluding 48 patients with APL) were divided into 3 subgroups: 25.8% favorable, 37.1% intermediate and 37.1% adverse. By applying the ELN 2022 criteria, where *FLT3*-ITD mutations invariably identify intermediate-risk, and 9 somatic mutations define the unfavorable risk (**Fig 3A**), 21.9% of patients fell in the favorable, 32.9% in the intermediate, and 45.2% in the adverse risk groups ($p<0.05$ for the favorable and intermediate, $p<0.0005$ for the adverse risk group). Overall, the changes in re-stratification between the ELN 2016 and the ELN 2022 shifted 12.9% of patients.

All AML classified as "adverse risk" by ELN 2017 did not change risk class in the 2022 edition (**Fig 3B**), with the exception of 2 patients (one with high *FLT3*-ITD AR, and the other one with *RUNX1* and a monoallelic-*CEBPA*-bZIP mutations). Of the ELN 2017 intermediate risk group, 77.4% remained in this category, while 0.8% and 21.8% moved into the favorable and adverse risk groups, respectively (**Fig 3B**). Of 77 AML with an ELN 2017 intermediate karyotype, 76 switched to adverse risk in the 2022 revision due to the presence of at least one MDS-related gene mutation, while one presented an atypical *MECOM* rearrangement.

Looking at the ELN 2017 favorable risk-group, 83.3% of AML were confirmed favorable also by ELN 2022, whereas 15.5% and 1.2% were up-staged into the intermediate and adverse ELN 2022 risk groups. Considering the AML re-categorized as intermediate according to ELN 2022, 34 out of 38 were diagnosed as AML-*NPM1*/*FLT3*-ITD "low", while the remaining 4 patients presented *CEBPA*bi mutations not involving the bZIP domain.

As to outcomes, both ELN editions confirmed their stratification capability, without significant differences in their prognostic power, as defined by AIC (**Table S2**). A total of 688 patients treated using conventional chemotherapy was evaluated, and the 24-month overall survival (OS) was 53.6% and 59.7% in favorable risk, 49.7% and 49.0% in intermediate risk, and 27.0% and 30.0% in adverse risk AML, for ELN 2017 and ELN 2022, respectively (**Fig 3C**). Re-stratifying according to ELN 2022 the 192 patients classified as ELN 2017 favorable risk and treated with conventional chemotherapy, the difference in OS between favorable and intermediate risk groups was statistically significant ($p<0.01$), while there was no survival difference between patients re-classified as adverse risk, deriving from the ELN 2017 intermediate risk (**Fig 3D**). On the other hand, both ELN 2017 and ELN 2022 did not stratify patients treated with non-intensive therapies (**Fig S5**).

We also focused on the heterogeneous group of patients with NK and adverse mutations according to ELN. Specifically, patients treated with intensive regimens with multiple MDS-related gene mutations had significantly worse outcomes when compared to those harboring only one MDS-related gene lesion (**Fig 3E**).

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Discussion

In 2022, two new AML classifications have been proposed as a response to the new molecular advances in the field. Our extensive real-word reclassification of 1001 cases confirmed the crucial role of the newly added molecular alterations for an accurate AML diagnosis. Indeed, NGS precisely categorized AML cases previously falling into the NOS "basket category", mainly by expanding the MDS-related group (82.7% in the WHO 2022 MR category, and 78.7% in the MDS-gene/*TP53* ICC categories).[17,18] This notwithstanding, while a prominent role of NGS analysis is given by the ICC hierarchic structure, it must be recognized that this may not be readily available in all Centers.

Herein, we will discuss some prototypic examples. Looking at the rarer AML subtypes, *KMT2A* and *MECOM* atypical rearrangements and *CEBPA*bZIP single-mutation expanded the AML-defining disease categories in both 2022 classifications. Given the importance of these abnormalities, their correct identification becomes essential. One of the most important difference between the 2022 classifications is the introduction of AML-*TP53* disease category in the ICC, because of the negative prognostic role of bi-allelic *TP53* mutations (or ≥ 10% VAF) regardless of blast counts.[7,19] We found that about 90% of AML with *TP53* mutations are included in the AML-MR according to WHO 2022, due to the coexistence of cytogenetic alterations, especially complex karyotype (81.8% of cases), and/or accompanying somatic MDS-related gene mutations (**Fig S3**).[7] In this context, 9.4% of AML-*TP53*, according to the ICC, would not emerge based on WHO 2022 rules. Harmonization between the two new classifications towards the diagnostic role of *TP53* in AML is crucial, especially once a TP53-targeted therapy will hopefully be available.[20]

Another difference is represented by the case of *RUNX1*-mutated AML, which is an AML-defining mutation by ICC, but not by 2022 WHO, and prognostically unfavourable by ELN 2022. However, of 76 *RUNX1*-mutated AML in our cohort, classified as AML-MDSgene by ICC, 77.6% were included in the WHO 2022 AML-MR category due to the presence of at least one additional MDS-related gene mutation.[19] Given the association of *RUNX1* mutations with sAML and with evolution of bone marrow failure syndromes, consideration of these mutations in the diagnostic process of AML may need to be re-considered.[21,22]

Although the criteria to define AML-MR, based on previous history of MDS or MDS/MPN, were maintained by WHO 2022, the panel of MDS-related genes, together with MDS cytogenetic alterations, covers most of secondary AML, regardless of their previous history.[11] Indeed, the genetic signature of these entities appears similar to that of high-risk MDS, and generally of AML progressing from antecedent hematological disorders, as compared to *de novo* AML.[11,23,24] The diagnostic classifiers become also of prognostic significance as the MDS-gene signature identifies cases with adverse ELN risk, as shown by previous studies focusing on AML ontogeny.[11,17,25,26] While it has been shown that the time from AML diagnosis to treatment start does not significantly impact patients' outcome in clinically stable patients, the currently proposed

diagnostic algorithm posits some methodological and socio-economic challenges.[27] Indeed, it must be considered that NGS analysis may be not widely available, and often needs long turnaround times and prohibitive costs for some Centers. The main issue concerns MDS-related AML where the hierarchical approach, according to ICC, requires the availability of the mutation status, prior to or simultaneously with karyotype information, which is far from reality even in many experienced, "first-world" Centers. In NK AML, NGS indeed refined the diagnosis in 60.8% and 58.0% of cases, according to ICC and WHO 2022, respectively, while more than half AML MDS-related (56%) could be characterized by conventional cytogenetics, and stratified as adverse risk. Therefore, conventional cytogenetics demonstrated to be a useful tool, still able to discriminate a large number of patients in a fast, relatively low-cost and easier fashion. On the other hand, NGS confirms to be essential to correctly classify roughly 44% of MDS-related AML. In this context, concerted efforts are now needed to make NGS more cost effective and globally available, especially in cases where it changes the treatment approach. In this line, the appeal to dedicated referral Centers highly specialized in NGS diagnostics may help harmonize the process of AML diagnosis.

The ELN 2022 risk stratification, regardless of the relevant adjustments, did not improve the prognostic capability compared to the previous version in our patients. This may be related to the conceptually important, but overall not substantial changes between the two schemes, the heterogeneity of treatments adopted in our real-world cohort, and the use of strategies partly belonging to the pre-FLT3 inhibitors era.[28]

One of the most important updates in ELN 2022 is the reconsideration of *FLT3*-ITD AR, whose role has been abolished.[29] Therefore, the presence of mutated *FLT3*-ITD without any other adverse genetic abnormalities, defines the intermediate risk, regardless of *FLT3*-ITD AR and co-presence of *NPM1* mutations. In our cohort, the majority of patients considered ELN 2017 favorable risk who were re-stratified as ELN 2022 intermediate risk, consisted of *NPM1*-mutant/*FLT3*-ITD "low". The accuracy of ELN 2022 in identifying favorable-risk AML was confirmed by our real-word data, with the clinical consequence of avoiding patients' overtreatment. However, capillary electrophoresis continues to be recommended as the standardized diagnostic tool for *FLT3* mutations,[30] particularly for its quick turnaround, and in light of the indication for the treatment with FLT3-inhibitors such as midostaurin, as well as for its ability to detect longer size *FLT3*-ITDs, which are underestimated by NGS.[31]

A special mention deserves the newly-defined adverse-risk group, which was enriched with subjects with NK and at least one adverse mutation. Our data confirm a previous study reporting that patients with a single MDS-related gene mutation have a better overall survival compared to those with more than one alteration.[32] Thus, one could speculate whether or not having a single MDS-related gene and a NK is sufficient to decide upon treatment intensification, as also suggested by a recent ELN 2022 validation study.[33] Future ef-

296 forts focusing on the relation between the new prognostication system and various treatments (especially
297 HSCT) are warranted.

298 Our study presented some caveats. In particular, the retrospective and multicenter nature of our patient
299 cohort determined that some results may have been impacted by differences in healthcare systems and
300 practice, as well as the broad time range in patient enrollment. Furthermore, all AML cases were defined by
301 ≥20% blasts, excluding the new ICC MDS/AML category from the present evaluation.

302 In conclusion, the use of molecular data is now crucial to precisely diagnose and risk-stratify patients with
303 AML. More than a dozen genes have been incorporated within current diagnostic and prognostic schemes,
304 and genome scanning approaches have been proposed to capture the complexity of AML biology. However,
305 the high costs, the level of expertise in molecular biology and issues of standardization still represent
306 hurdles to provide equitable care for AML patients worldwide. Given the substantial similarities, the
307 conflicts between the newly competing diagnostic systems may be resolved, reaching an agreement on a
308 unified model, which must also take into account the available resources worldwide.
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Authorship Contributions

Contribution: E.A., A.S. and M.T.V. designed the study, interpreted the data, wrote the manuscript; B.B took part in manuscript writing; C.G. took part in data collection, edited the manuscript, helped in data interpretation, and gave helpful intellectual insights during the study; A.P. and M.C. and performed statistical analysis; T.O., E.F., S.T., M.D., M.R.P. and A.D. helped in data analysis and participated in data interpretation; H.A., V.V., J.P.M., M.G.D.P. and A.V. participated in samples and data collection, M.T.V. took responsibility for the integrity and the accuracy of the data presented; and all authors reviewed and approved the final version of this manuscript.

Disclosure of Conflicts of Interest

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Data sharing statement

Data used for this study can be found at github for open-source access (https://github.com/ardadurmaz/aml), public available source (BEAT-AML Master trial) and any additional information is available upon request to the corresponding author.

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Figure Legends

Fig 1. Patient's distribution according to WHO 2016, ICC and WHO 2022 diagnostic classifications.

A) distribution of 1001 AML patients according to the diverse classifications.

B) relationship, overlaps and differences across different AML sub-types shown by Sankey plot. The overall proportion of genetically defined AML, according to the new classifications, is shown in brackets.

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; *MECOM*-R, *MECOM* rearrangements; *MECOM*-RE, *MECOM* with extended rearrangements; ICC, International Consensus Classification; *KMT2A*-R, *KMT2A* rearrangements; *KMT2A*-RE, *KMT2A* with extended rearrangements; MDSgene, AML with myelodysplasia-related gene mutations; MDSk, AML with myelodysplasia-related cytogenetic abnormalities; MPN, myeloproliferative neoplasm; MRC, myelodysplasia-related changes; MR, myelodysplasia-related; WHO, World Health Organization.

Fig 2. Distribution and outcome of AML patients according to their cytogenetic and molecular profile.

A) distribution of secondary AML (n= 397 AML with MDS related categories), according to the presence of MDS-related cytogenetic abnormalities. Each category was further stratified according to the hierarchical ICC classification system, shown in the outer circle (AML with *TP53* mutation, AML with myelodysplasia-related gene mutations, AML with myelodysplasia-related cytogenetic abnormalities, grey shades).

B) frequency of ICC diagnostic subcategories in AML patients with normal karyotype (n=452).

AML, acute myeloid leukemia; MDSgene, AML with myelodysplasia-related gene mutations; MDSk, AML with myelodysplasia-related cytogenetic abnormalities; NK, normal karyotype.

Fig 3. AML stratification according to the European LeukemiaNet 2017 and 2022.

A) stratification of patients with AML (n=953, excluding patients with acute promyelocytic leukemia).

B) relationship between risk groups as shown by Sankey plot, comparing the two ELN models.

C) Kaplan-Meier curves show survival estimates of AML patients treated with conventional chemotherapy according to ELN 2017 and 2022 (n=688 patients with available survival data).

D) overall survival of favorable (n=192) and intermediate (n=276) risk AML patients, according to ELN 2017, treated with conventional chemotherapy and re-stratified by ELN 2022. Among ELN 2017 favorable risk cases, 3 were re-stratified into the adverse risk group by ELN 2022, and were therefore not included. Furthermore, 2 patients belonging to the ELN 2017 intermediate risk category were not included, since they

were re-stratified into the ELN 2022 favorable risk group.

E) overall survival of patients with normal karyotype AML treated with conventional chemotherapy (n=74), classified as ICC AML-MDSgene category, and based on the presence of 1 or ≥2 MDS gene mutations.

Patients alive at last follow-up are censored. Numbers at risk are indicated below the curves and color-coded. P-values shown are the result of the Log-rank test. AML, acute myeloid leukemia; OS, overall survival.

Table 1. Patient characteristics

	AML patients (n = 1001)
Male : female ratio (M/F)	539/462
Median age, years (range)	61 (1-93)
Age < 65 years, n (%)	597 (59.7%)
Survival outcome available, n***** (%)	881 (92.4%)
Treatment available, n***** (%)	870 (91.3%)
Conventional chemotherapy	688 (72.2%)
Non-intensive therapy **	144 (15.1%)
Palliative care	37 (3.9%)
FLT3-inhibitors (alone or in combination with CTX)	107 (11.2%)
Hematopoietic stem cell transplantation	323 (33.9%)

"*" indicates "excluding patients with APL and those with not-available survival or treatment data".
AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; n, number, CTX: chemotherapy.

"**" indicates therapy with hypomethylating agents (azacitidine or decitabine), venetoclax and AG-221.

460 **Table 2. AML classifications**

461

AML	WHO 2016	ICC	WHO 2022
With defining genetic abnormalities[1]	*PML::RARA* (APL)§ *RUNX1::RUNX1T1*§ *CBFB::MYH11*§ *MLLT3::KMT2A* *MECOM* *BCR::ABL1* (provisional entity) *NPM1* ***CEBPA*bi** *DEK::NUP214* *RBM15::MKL1*	*RARA*-R (APL) *RUNX1::RUNX1T1* *CBFB::MYH11* *KMT2A*-R *MECOM*-R *BCR::ABL1* *NPM1* ***CEBPA*bZIP** *DEK::NUP214* other rare recurring translocations†	*PML::RARA* (APL) *RUNX1::RUNX1T1* *CBFB::MYH11* *KMT2A*-RE *MECOM*-RE *BCR::ABL1** *NPM1* ***CEBPA**** *DEK::NUP214* *RBM15::MRTFA*
Previous history of MDS, or MDS/MPN[2]	**MRC** disease defining	*diagnostic qualifiers*	**MR** disease defining
Myelodysplasia-related with defining cytogenetic abnormalities[2]	complex karyotype -7/del(7q) del(5q)/t(5q) i(17q)/t(17p) -13/del(13q) del(11q) del(12p)/t(12p) idic(X)(q13) **balanced abnormalities**	**MDSk** complex karyotype del(5q)/t(5q)/add(5q), -7/del(7q), **+8** del(12p)/t(12p)/add(12p), i(17q), -17/add(17p) or del(17p) **del(20q),** idic(X)(q13)	complex karyotype del5q or 5q loss -7, del7q or 7q loss **del11q** del12p or 12p loss **-13 or del13q** del17p or 17p loss or i17q idic(X)(q13)
Dysplasia[2]	>50% of cells of at least two lineages		
With defining mutations[2]	***RUNX1*** (provisional entity)	**MDSgene** *ASXL1, BCOR, EZH2,* ***RUNX1****,* *SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*	*ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*
***TP53*-mut**[3]		***TP53***	
Without defining genetic abnormalities[4]	Not otherwise specified (**NOS**)	Not otherwise specified (**NOS**)	Defined by differentiation (**DD**) (morphological classification)

462

463 Major differences among classifications are highlighted in bold.

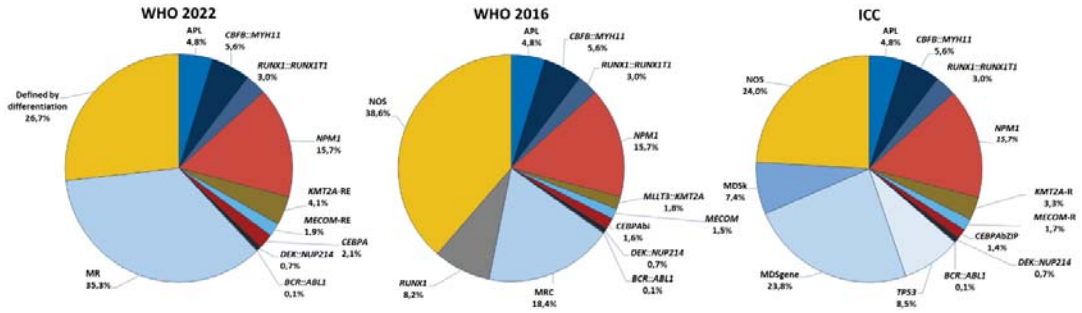
464 While for WHO 2016 a blast count ≥ 20% is required for AML diagnosis (with exceptions indicated by "§"), ICC
465 requires ≥ 10% blasts, and for WHO 2022 no minimal blast counts is required (with some exceptions, see "*"). A blast
466 count ≥ 20% is still required for groups 2,3,4. "§": no minimal blast count required; "*": blast count ≥ 20% still required;
467 "†": *PRDM16*::*RPN1*,*NPM1*::*MLF1*, *KAT6A*::*CREBBP*, *RBM15::MRTFA*, *NUP98* and other partners, *ETV6*::*MNX1*,
468 *PICALM::MLLT10*, *FUS::ERG*, *RUNX1::CBFA2T3*, *CBFA2T3::GLIS2.*

AML, acute myeloid leukemia; *MECOM*-R, *MECOM* rearrangements; *MECOM*-RE, *MECOM* with extended rearrangements; ICC, International Consensus Classification; *KMT2A*-R, *KMT2A* rearrangements; *KMT2A*-RE, *KMT2A* with extended rearrangements; MDS, myelodysplastic syndromes; MPN, myeloproliferative neoplasm; MRC, myelodysplasia-related changes; MR, myelodysplasia-related; mut, mutation; WHO, World Health Organization.

Figure 1

Figure 1. Patient's distribution according to WHO 2016, ICC and WHO 2022 diagnostic classifications.

A)



B)

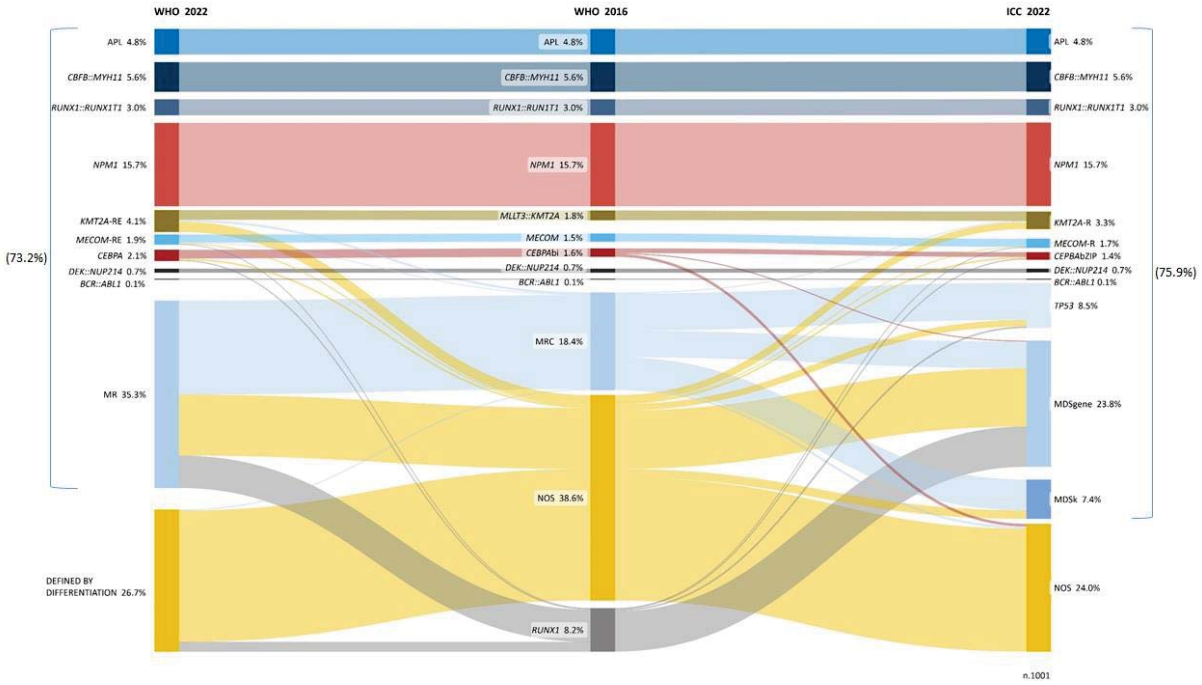
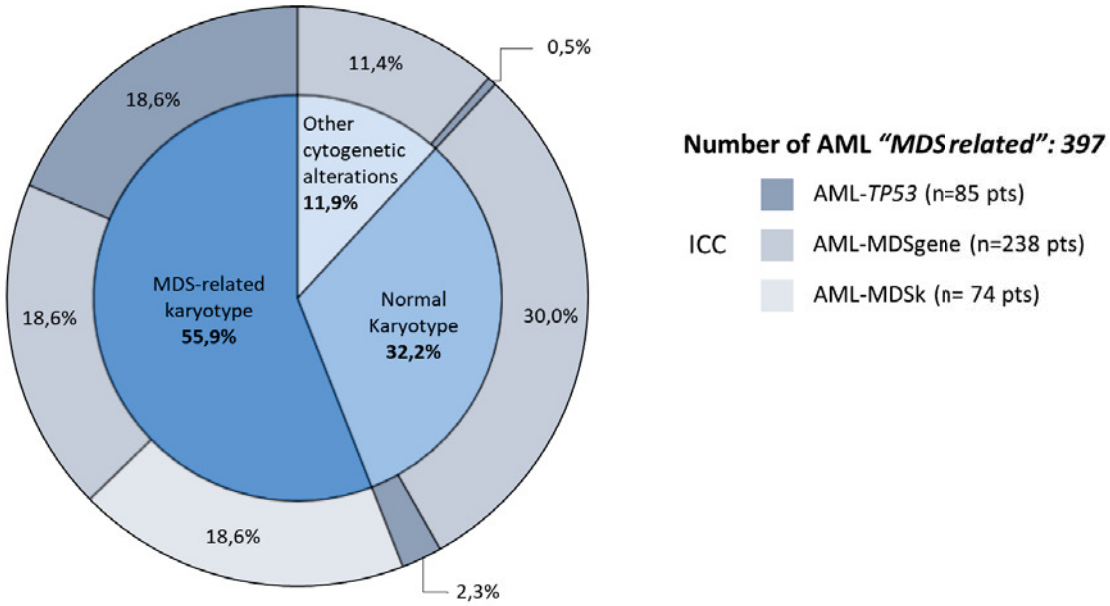


Figure 2. Distribution and outcome of AML patients according to their cytogenetic and molecular profile.

A)



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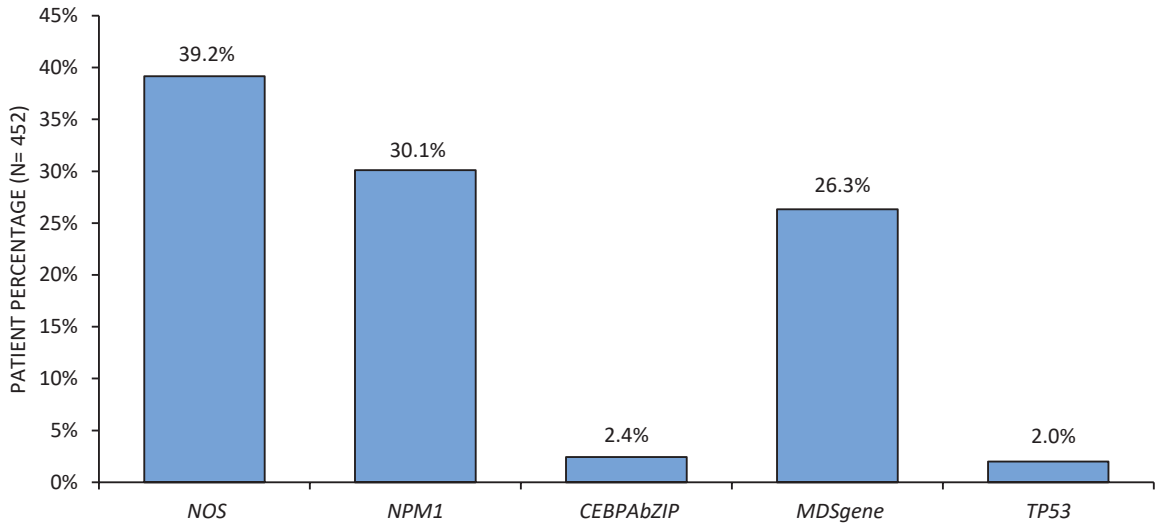

Figure 3

Figure 3. AML stratification according to the European LeukemiaNet 2017 and 2022.