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Validation of ICC hierarchical classification in secondary AML

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Key Points

- MDS/AML with MDR mutations display overall different mutation profiles and improved prognosis, when compared to corresponding AML subtype.
- ELN 2022 applied to secondary AML stratified post-MDS AML in adverse risk (84.1%), whereas t-AML showed a more balanced risk distribution.

Secondary acute myeloid leukemia (AML) comprises heterogeneous entities, unified by poor prognosis. We evaluated the associations of genetic profiles with blast counts and patients' history in a cohort of 924 patients with myelodysplastic syndrome (MDS)/AML or AML, classified according to the International Consensus Classification (ICC). The cohort included 109 patients with "mutated TP53," 497 with "myelodysplasia-related (MDR) gene mutation," 93 with "MDR-cytogenetic abnormality," 77 were therapy-related, and 136 controls, categorized as "not otherwise specified" (NOS) AML. Exploring the ICC hierarchy, AML and MDS/AML categories with "mutated TP53" and "MDR-cytogenetic abnormality" presented similar biology and prognosis, irrespective of blast counts. Conversely, in MDS/AML with "MDR gene mutation" and NOS, profiles significantly differed from AML and were characterized by a higher number of mutations in *STAG2*, *SRSF2*, *ASXL1* and *TET2*. This corresponded to improved survival in MDS/AML vs AML (MDR-gene mutation: median overall survival 24.8 vs 13.6 months, $P < .0001$; and NOS: 49.9 vs 19.2 months, $P = .028$). Within each ICC-defined AML category, a prior MDS history vs de novo onset did not impact on patients' prognosis. We then analyzed secondary AML, defined by "prior MDS or MDS/MPN" or "therapy-related" (t-AML), as diagnostic qualifiers. According to European LeukemiaNet (ELN) 2022, AML progressing from MDS or MDS/myeloproliferative neoplasm (MPN) (AML post-MDS) mostly clustered in the adverse-risk group (84.1%), whereas t-AML showed more heterogeneous ELN profiles (12.9% favorable, 33.8% intermediate, and 53.3% adverse risk) reflecting diverse overall survival. Our findings underscore that genetic features and the ICC classification reliably capture disease biology, refine risk stratification, and ultimately guide treatment decisions in most secondary AML and MDS/AML.

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Data used for this study can be found in GitHub for open-source access at https://github.com/ardadurmaz/aml and publicly available sources (BEAT-AML Master trial, EuroMDS).

Additional information is available from the corresponding author, Enrico Attardi (enrico.attardi@uniroma2.it), upon request.

The full-text version of this article contains data supplement.

Introduction

Secondary acute myeloid leukemias (AML) represent a challenging subgroup of AML, characterized by poor prognosis and complexity in patient management. Historically, this subgroup includes AML that progresses from myelodysplastic syndrome/neoplasm (MDS) or MDS/myeloproliferative neoplasm (MPN), as well as therapy-related AML, arising after exposure to cytotoxic chemotherapy and/or radiotherapy. Although important similarities in the new 2022 diagnostic classifications of AML (5th edition of World Health Organization [WHO] and the International Consensus Classification [ICC])[1,2] have been recently recognized by diverse validation studies,[3,4] the different approaches to define AML ontogenesis (primary vs secondary) remain a pressing issue. In contrast to 2016 WHO AML classification,[5] both ICC and WHO 2022 classifications eliminated multilineage dysplasia and balanced cytogenetic abnormalities as diagnostic criteria. A previous history of MDS or MDS/MPN, the presence of specific cytogenetic abnormalities, and mutations in 8 myelodysplasia-related (MDR) genes (*ASXL1*, *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and *ZRSR2*) defines AML myelodysplasia-related (AML-MR) by WHO 2022. ICC encourages a diagnostic hierarchical structure, whereby molecular testing (AML with "mutated TP53" and "MDR gene mutation," same genes of WHO with the addition of *RUNX1*) supersedes cytogenetics (AML with "MDR cytogenetic abnormality").

An element of discrepancy of 2022 classifications pertains to the biological boundaries between MDS and AML, with the new MDS/AML category introduced by ICC, defined by 10% to 19% blasts. This subgroup largely overlaps with MDS with excess blasts 2 and MDS with increased blast 2 according to the 4th and 5th WHO editions, respectively, possibly representing a continuum with AML-MR. The new MDS/AML group also opens the possibility for these patients to be eligible for both MDS and AML trials. Myeloid neoplasms with "mutated TP53" deserve special mention, since they are characterized by an overall unfavorable prognosis, with somatic *TP53* mutation at variant allele frequency (VAF)>10% defining MDS/AML or AML, depending on the blast count.

Both 2022 classifications also established a list of qualifiers, including previous exposure to cytotoxic therapy (expanded to encompass immune interventions and Poly (ADP-ribose) polymerase inhibitors, PARP) to define therapy-related AML (AML after cytotoxic therapy according to WHO 2022), as well as AML secondary to germ line predisposition. AML progression from MDS or MDS/MPN is also a diagnostic qualifier by ICC.

We were interested in the application of the ICC classification to a large group of AML MDR and MDS/AML, to evaluate the impact of the 20% blast threshold boundary and the "previous MDS or MDS/MPN history." In the second part of this study, we focused on the analysis of biologic profiles and prognostic implications of AML with "previous MDS or MDS/MPN history" vs "therapy-related."

Methods

Patients' characteristics

A total of 924 patients with AML and MDS/AML, of a median age of 69 years (interquartile range, 59.2-75.8), treated between 2012 and 2024 were included in this study. Patient data were collected via chart review across 5 academic centers[1]: Tor Vergata University, Rome, Italy[2]; the Humanitas Cancer Center, Milan, Italy[3]; University of Naples Federico II, Naples, Italy[4]; AO Ordine Mauriziano Torino, Turin, Italy[5]; the Cleveland Clinic Foundation, Cleveland, Ohio, and public databases including additional Cleveland Clinic and BEAT-AML cohorts.[6-9] Patients' characteristics and treatment are shown in Table 1.

For the first part of the study, we excluded 12 patients with AML-defining genetic abnormalities and, without applying any additional biological assumptions, 77 therapy-related AML patients. The remaining cohort (n = 835) was then assigned to the ICC categories "mutated TP53," "MDR gene mutation," "MDR cytogenetic abnormality," and "not otherwise specified" (NOS) (Figure 1). In the second part of the study, the diagnostic qualifiers of previous history of MDS or MDS/MPN (AML progressing from MDS or MDS/MPN [AML post-MDS], n = 188) or of a cytotoxic therapy for a previous tumor (therapy-related AML [t-AML], n = 77) were analyzed. Here, all ICC categories were included, independent of genetics.

At the time of initial MDS or AML diagnosis, all patients underwent bone marrow aspiration, conventional cytogenetics, and next generation sequencing (NGS) analyses for diagnostic purposes. The proportion of blasts was evaluated on bone marrow smears according to standard guidelines.[10] AML were defined by blast counts ≥20%, whereas MDS/AML included patients with 10% to 19% blasts in peripheral blood or bone marrow, according to ICC.[2]

Informed consent was obtained from patients in accordance with the ethical principles set forth by the Declaration of Helsinki, and the study was approved by the institutional review board of the participating institutions.

Table 1. Patients' characteristics at study entry

	Patients n = 924
Male : female ratio, M|F	1.5
Median age, y (IQR)	69 (59.2-75.8)
Age >65 y, n (%)	562 (60.8%)
MDS/AML (blast count 10%-19%), n (%)	374 (40.5%)
AML (blast count ≥20%) n (%)	550 (59.5%)
Prior history of MDS or MDS/MPN, AML post-MDS, n (%)	188 (20.4%)
Therapy-related AML, t-AML, n (%)	77 (8.3%)
Survival outcome available, n (%)	873 (94.5%)
Treatment information available, n (%)	774 (83.8%)
Intensive chemotherapy, n (%)	393 (50.8%)
Nonintensive therapy*, n (%)	268 (34.6%)
Palliative care, n (%)	169 (21.8%)
Hematopoietic stem cell transplant, n (%)	198 (29.7%)

IQR, interquartile range.
*Therapy with hypomethylating agents (azacitidine or decitabine), venetoclax, and AG-221.

Diagnostic procedures

Following morphologic evaluation of bone marrow or peripheral blood smears for blast counts, cytogenetic analysis was performed using standard chromosome banding techniques, and documented in compliance with the International System for Human Cytogenomic Nomenclature recommendations.[11] Evaluation of at least 20 metaphases was required. The mutational status of a list of 9 MDR genes according to the ICC (*ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and *ZRSR2*) and *TP53* was studied, as well as that of other 19 commonly mutated myeloid genes: *ABL1*, *BRAF*, *CALR*, *CBL*, *CEBPA*, *CSF3R*, *DNMT3A*, *ETV6*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *NPM1*, *PTPN11*, *SETBP1*, *TET2*, and *WT1*. Mutations were detected using NGS, with a variant calling at a ≥2% threshold. The variant allele frequency (VAF%) was collected for MDR genes and *TP53* mutations.

According to ICC,[2] multihit *TP53* status was defined as: 2 distinct *TP53* mutations (each VAF >10%), a single *TP53* mutation with 17p deletion on cytogenetics or complex karyotype involving chromosome 17p, or *TP53* mutation VAF of >50%. Data on copy-neutral loss of heterozygosity at the 17p *TP53* locus were not available.

Statistical analysis

Patients' characteristics stratified by MDS and AML type were summarized by descriptive statistics of median and interquartile range (continuous variables) or frequencies and percentages (categorical variables). The association between categorical variables in contingency tables was evaluated using Fisher exact test and Pearson $\chi 2$ test. The number of mutations and cytogenetic profiles were compared between AML subtypes (AML vs MDS/AML) using odds ratios with corresponding 95% confidence intervals. The odds ratios were visually represented using a forest plot. 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 and the pairwise log-rank test when >2 estimated curves were compared. The distribution of patients among the categories identified by the European LeukemiaNet (ELN) 2017 and 2022 classifications was studied using contingency tables, in which both absolute frequencies and percentages related to them were reported.[12,13] Univariate Cox models were created to evaluate the prognostic abilities of ELN 2022. Statistical analysis was performed using R software (version 4.3.0).

All tests were 2-sided, with $P < .05$ indicating a statistically significant difference.

Results

Evaluating the impact of 20% blast threshold: comparing the genetic profiles and outcome of AML vs MDS/AML ICC categories

Following the ICC hierarchical algorithm, we evaluated the uniqueness of the MDS/AML categories comparing their genetic profiles to that of AML, and excluding those with AML-defining recurring genetic abnormalities (patient distribution is shown in Figure 1). Minor differences were observed between AML and MDS/AML in the "mutated TP53" and "MDR cytogenetic abnormality" categories. In the "mutated TP53" category, AML vs MDS/AML presented as expected a significantly higher frequency of chromosome 17 abnormalities (27.0% vs 8.6%; $P = .027$),[14] and also a higher frequency of *DNMT3A* mutations (24.0% vs 0%; $P = .001$) (Figure 2B; supplemental Table 1A). Considering the "MDR cytogenetic abnormality" category, the only significant differences were observed for del(5q)/ t(5q)/add(5q) (23.0% vs 50%; $P = .010$; Figure 2D; supplemental Table 1C) and del(20q) (1.5% vs 18.0%, $P = .009$), which were less frequent in AML vs MDS/AML.

Conversely, multiple differences were detected in the "MDR gene mutation" and NOS categories (Figure 2C-E). In "MDR gene mutation" category, significantly higher frequencies in *FLT3* (including internal tandem duplication, ITD and tyrosine kinase domain, TKD), *DNMT3A*, *CSF3R*, *IDH1*, *IDH2* and *BCOR* mutations were observed in AML vs MDS/AML (Figure 2C; supplemental Table 1B). Notably, median clone sizes of *STAG2* and *RUNX1* mutations were significantly larger in AML vs MDS/AML (*STAG2* median VAF: 0.68 vs 0.40, $P = .028$; *RUNX1* median VAF: 0.41 vs 0.28, $P = .007$). Similarly, "NOS AML," were defined by higher frequency of non-bZIP *CEBPA*, *FLT3*, *IDH2*, and *WT1* mutations, whereas MDS/AML showed higher frequencies of *TET2* mutations (Figure 2D; supplemental Table 1D).

These data were reflected by the analysis of outcome (Figure 2F-I). Indeed, OS was longer in "MDR gene mutation" MDS/AML vs AML (median OS: 24.8 vs 13.6 months, respectively, $P < .0001$; Figure 2G) and in NOS categories (median OS: 49.9 vs 19.2 months, respectively; $P = .028$; Figure 2I). No survival

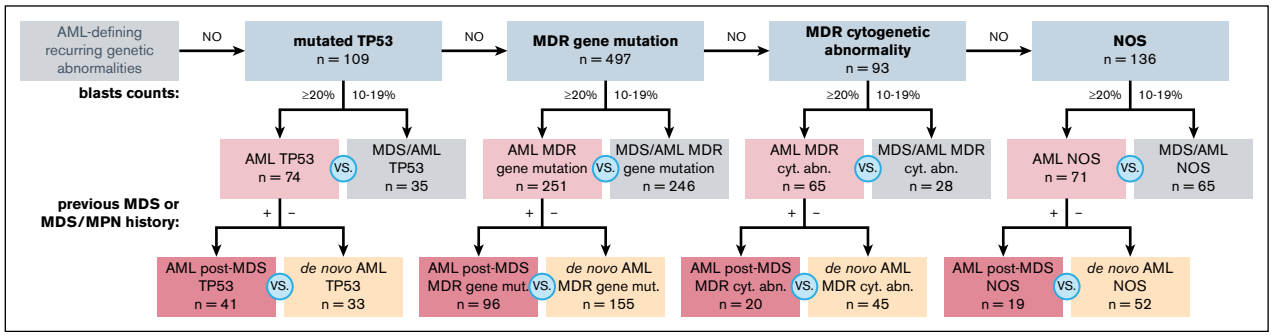

Figure 1. Distribution of patients with AML and MDS/AML included in the study, according to 2022 ICC hierarchical clustering. The figure illustrates the first part of the study. A total of 835 patients were assigned to the ICC categories "mutated TP53," "MDR gene mutation," "MDR cytogenetic abnormality" and NOS AML and MDS/AML. AML with a "defining recurring genetic abnormality" were excluded, as well as t-AML patients. Numbers are indicated for ICC categories. The number of patients with a previous history of MDS or MDS/MPN is indicated for each subgroup. Abs, abnormalities; cyt, cytogenetic; mut, mutation.

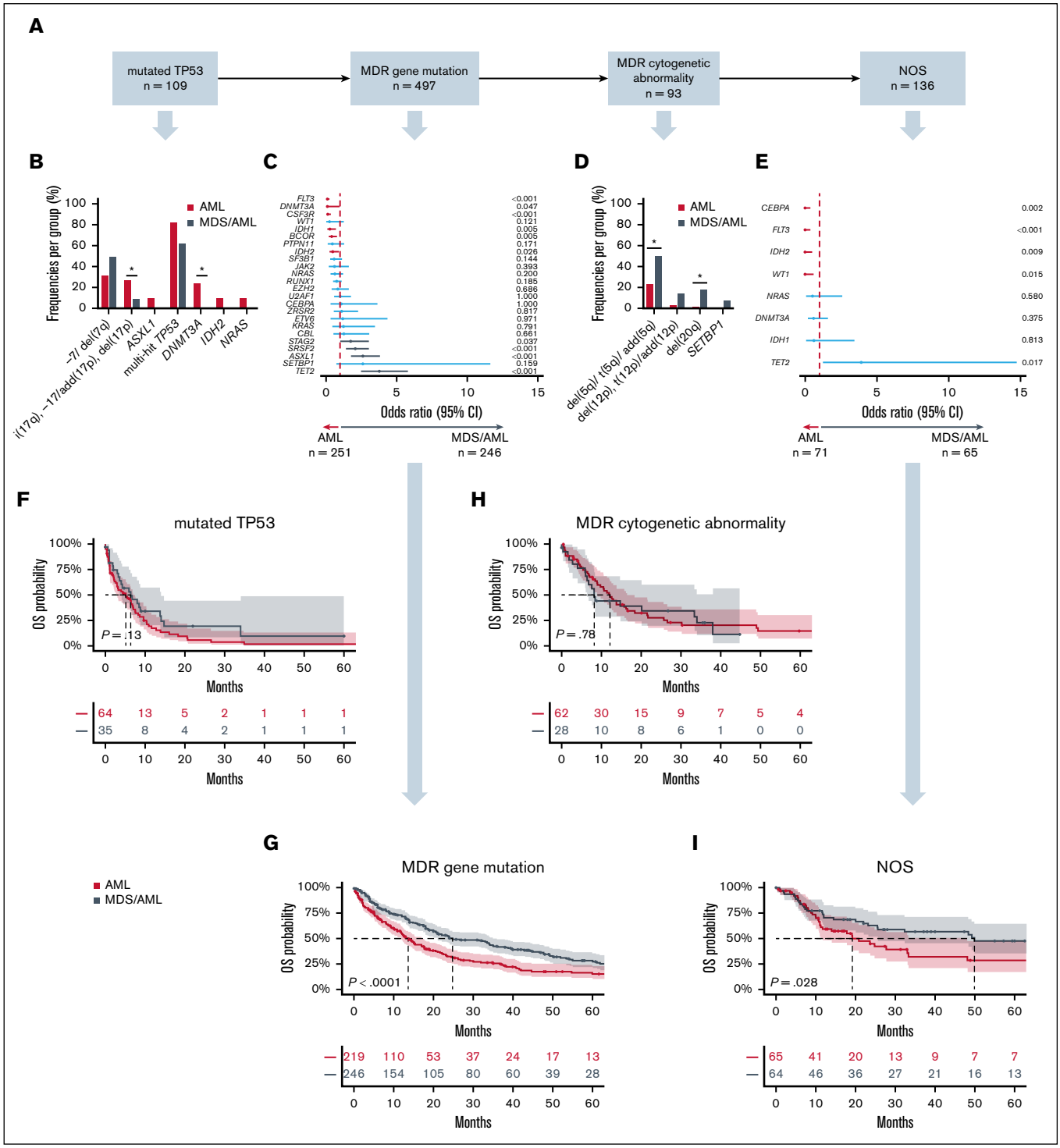

Figure 2. Genomic profiles and outcome of patients with AML vs MDS/AML according to ICC hierarchical classification. Comparison related to biological profile (B-E) and outcome (F-I) of MDS/AML and AML within each ICC category, according to the 20% blast threshold. (A) Patient distribution following the ICC diagnostic algorithm. (B,D) Bar-plots showing differences in mutated genes with $P < .1$. The * indicates genes with $P < .05$ (full data shown in supplemental Table 2). (C,E) Forest plots presenting univariate analyses and odds ratio (OR) for the association of somatic mutations with MDS/AML vs AML groups (statistical significance studied by Fisher exact test). Red color indicates genes enriched in AML, gray indicates genes enriched in MDS/AML, and blue indicates non-significant associations. (F-I) Kaplan-Meier curves showing survival estimates of patients with AML vs MDS/AML. Numbers of patients at risk are indicated below the curves and are color coded. *P* values were obtained by log-rank test. Groups are color coded: gray color indicates MDS/AML (blast, 10%-19%), whereas red indicates AML (blast >20%).

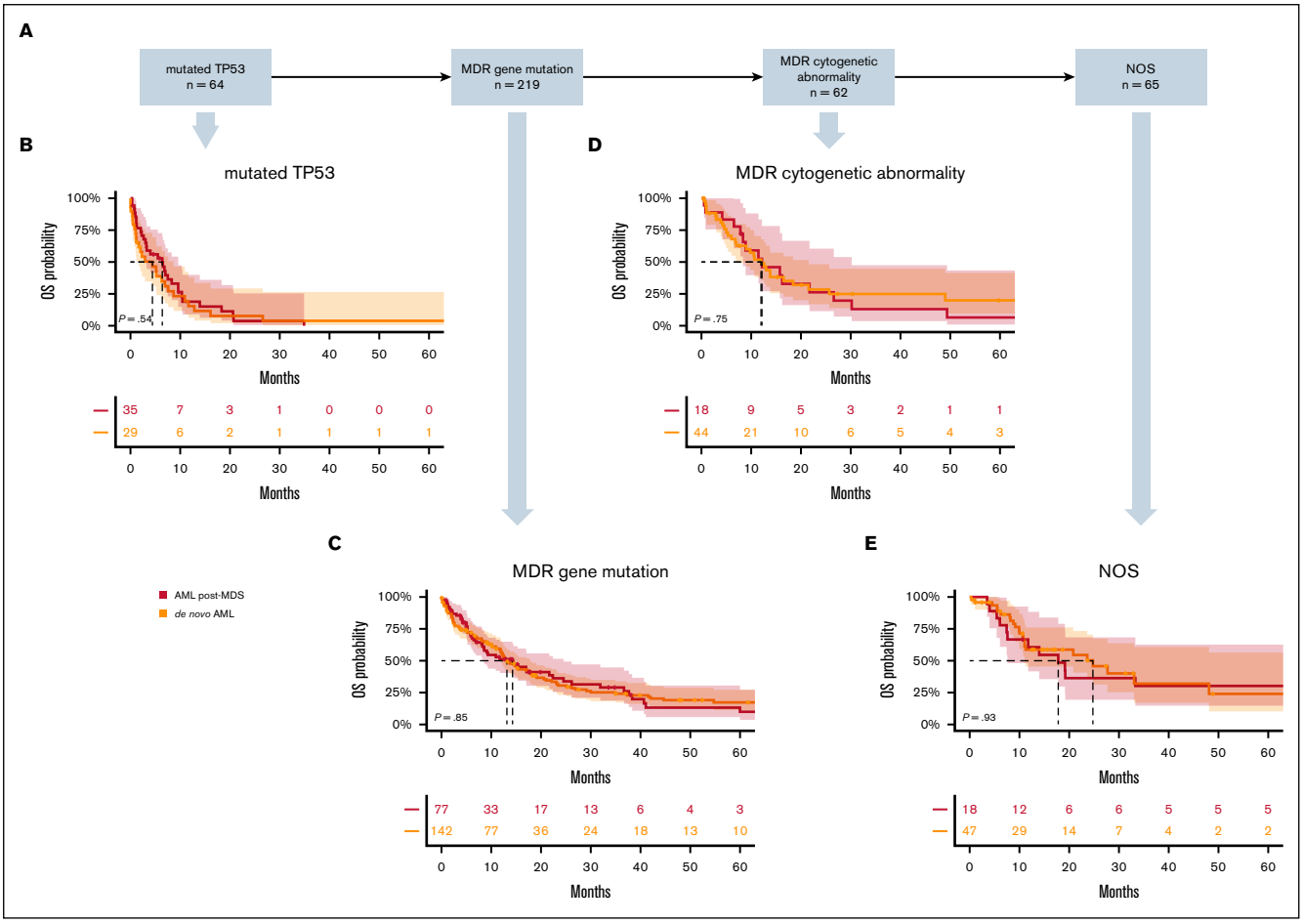

Figure 3. Outcome according to patients' history (AML progressing from MDS vs de novo AML). Outcome comparison of AML with and without the diagnostic qualifier "prior MDS or MDS/MPN" within each ICC category. (A) Patient distribution following the ICC diagnostic algorithm. (B-E) Kaplan-Meier curve showing survival estimates of patients with AML post-MDS vs de novo AML (pAML). Numbers of patients at risk are indicated below the curves and are color coded. *P* values were obtained by log-rank test. Groups are color coded: orange color indicates de novo AML, whereas dark red indicates AML with prior history of MDS or MDS/MPN.

differences according to blast counts were observed according to the "mutated TP53" and "MDR cytogenetic abnormality" MDS/AML vs AML categories (median OS in "mutated TP53": 6.4 vs 5.2 months, $P = .130$; median OS in "MDR cytogenetic abnormality": 8.2 vs 12.2 months, $P = .780$; Figure 2F,H).

Although the prognostic value of MDR gene mutations has been established within the homonymous category, we questioned whether the presence of additional MDR cytogenetic abnormalities confers further prognostic value (supplemental Figures 1 and 2). We demonstrated that MDR cytogenetic abnormalities can further stratify the "MDR gene mutations" MDS/AML subgroup, but not the AML counterpart.

Characterization of secondary AML based on clinical history (post-MDS) vs genetically-defined secondary AML

We then evaluated the diagnostic and prognostic role of the previous history of MDS or MDS/MPN (AML post-MDS, n = 176 patients), comparing them to patients with a de novo onset (n = 285) among the ICC categories (Figure 1). In general, AML post-MDS and de novo AML were characterized by similar mutational profiles (supplemental Table 2A-D). The only exceptions were higher frequencies in AML post-MDS of *ASXL1* (48.0% vs 25% in de novo AML; $P = .012$), *SF3B1* mutations (19.0% vs 7.1% in de novo AML; $P = .005$; supplemental Table 2B) and complex karyotypes (75.0% vs 44.0%, in de novo AML $P = .023$; supplemental Table 2C).

In this line, no differences in outcome were observed in AML post-MDS vs de novo AML within all the ICC categories (median OS in "mutated TP53": 6.4 vs 4.4 months, $P = .540$; median OS in "MDR gene mutation": 14.3 vs 13.2 months, $P = .850$; 12-months OS in "MDR cytogenetic abnormality": 12.2 vs 12.0 months, $P = .750$; median OS in NOS: 17.8 vs 24.8 months, $P = .930$; Figure 3). Bold values represent the statistically significant results ($P < .05$).

Diagnostic and prognostic classification of AML post-MDS vs therapy-related AML

We then analyzed the genetic profiles and outcome of patients with AML with a previous history of MDS or MDS/MPN (AML post-MDS, n = 188) vs those with previous exposure to cytotoxic therapy (t-AML, n = 77). When compared to t-AML, AML post-MDS were

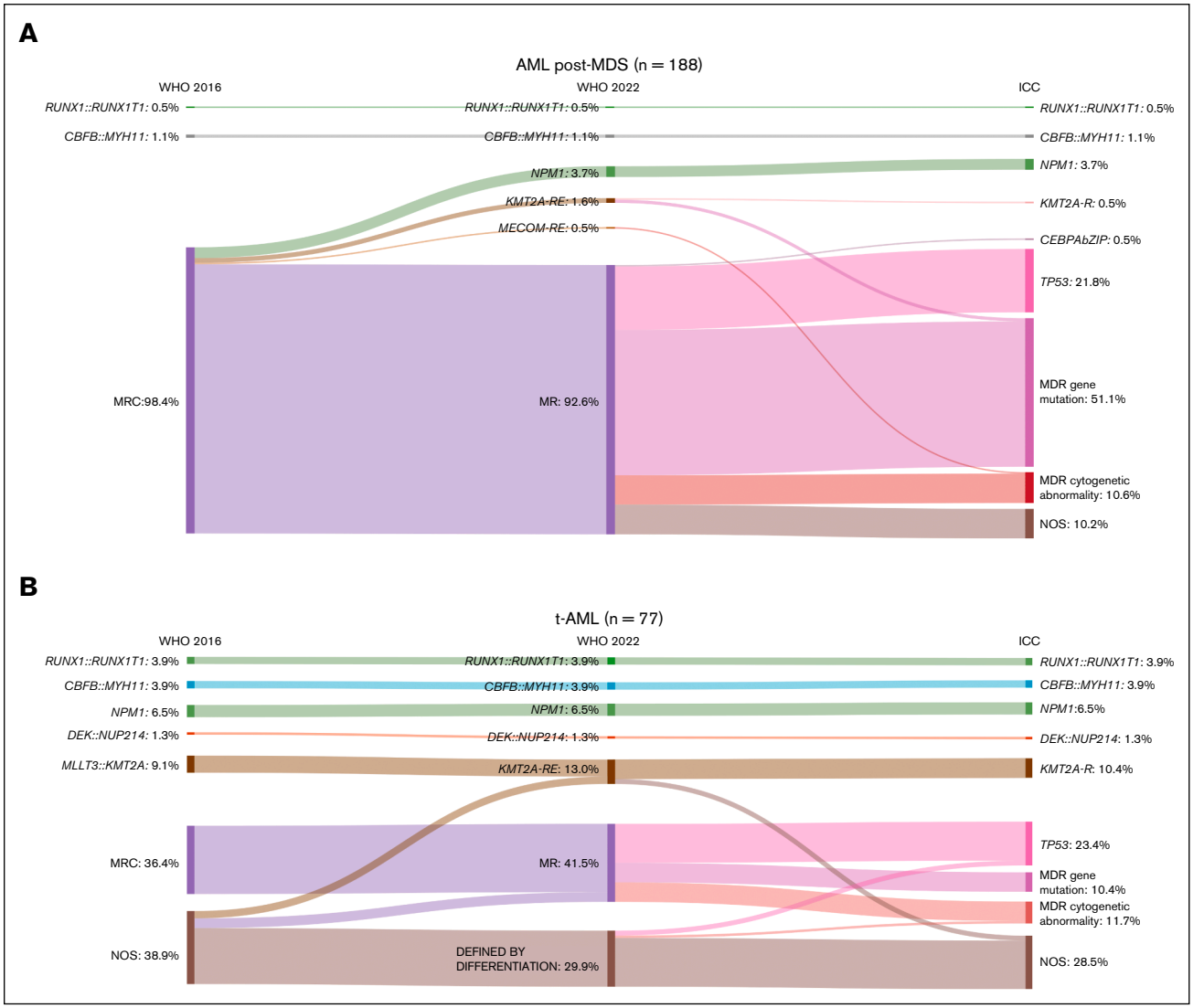

Figure 4. Diagnostic classification of secondary AML (post-MDS vs therapy-related). (A-B) The similarities and classification differences according to WHO 2016, 2022 and ICC across AML post-MDS (A) and t-AML (B) are shown using Sankey plots. *CEBPAbZIP*, AML with mutated CEBPAbZIP; *KMT2A-R*, *KMT2A* rearrangements; *KMT2A-RE*, *KMT2A* with extended rearrangements; *MECOM-RE*, *MECOM* with extended rearrangements; MRC, MDR changes; *NPM1*, AML with mutated NPM1; *TP53*, AML with mutated TP53.

characterized by a higher number of mutations (2.4 vs 1.4, $P < .001$), in particular involving *ASXL1* (24.0% vs 5.3%, $P < .001$), *RUNX1* (22.0% vs 2.6%, $P < .001$), *SF3B1* (10.0% vs 1.3%, $P = .015$), *SRSF2* (20.0% vs 5.3%, $P = .003$), *JAK2* (5.9% vs 0%, $P = .037$) and *TET2* (14.0% vs 2.6%, $P = .007$) genes (supplemental Table 3).

The majority of AML post-MDS fell into the AML-MRC WHO 2016 (98.4%) and AML-MR WHO 2022 (92.6%) categories (Figure 4A). Only a minor proportion presented recurrent cytogenetic abnormalities (*RUNX1*::*RUNX1T1* and *CBFB*::*MYH11*, n = 3), *NPM1* mutations (n = 7), and other disease-defining rearrangements.

Similarly, according to the hierarchical structure of ICC, 83.5% of AML post-MDS were defined by the presence of at least 1 MDR genetic alteration, namely "mutated TP53" (21.8%) or "MDR gene mutation" (51.1%) or "MDR cytogenetic abnormality" (10.6%) categories. A "defining recurrent genetic abnormality" was found in only 6.3% of AML post-MDS (12/188), whereas 10.2% of patients (19/188), were classified as NOS (Figure 4A).

Conversely, the t-AML subgroup showed greater diagnostic heterogeneity, with a higher prevalence of AML with "defining recurrent abnormality" (26.0% vs 6.4% in AML post-MDS, $P < .00001$; Table 2; Figure 4B). They also displayed higher frequencies of *KMT2A* rearrangements [13.0% vs 1.6% in post-MDS (WHO 2022) and 10.4% vs 0.5% (ICC), $P < .0001$ for both], and lower frequency of patients falling into the AML with "MDR gene mutation" ICC category (10.4% vs 51.1%, $P < .00001$).

We then evaluated the prognostic distribution of AML post-MDS vs t-AML, by applying ELN 2017 and 2022 stratifications, both conceived for patients receiving intensive therapy. The distribution of

Table 2. Comparison of AML progressing from MDS or MDS/MPN (AML post-MDS) and therapy-related AML (t-AML) according to ICC subcategories

ICC AML subcategories	AML post-MDS n = 188 (%)	t-AML n = 77 (%)	*P* value
AML with defining genetic abnormalities	12 (6.3%)	20 (26%)	**<.00001**
NPM1	7 (3.7%)	5 (6.5%)	.325
CBFB::MYH11	2 (1.1%)	3 (3.9%)	.129
RUNX1::RUNX1T1	1 (0.5%)	3 (3.9%)	**.043**
KMT2A rearrangements	1 (0.5%)	8 (10.4%)	**<.0001**
*CEBPA*bZIP	1 (0.5%)	0	-
DEK::NUP214	0	1 (1.3%)	-
AML with mutated TP53	41 (21.8%)	18 (23.4%)	.780
AML with MDR gene mutation	96 (51.1%)	8 (10.4%)	**<.00001**
AML with MDR cytogenetic abnormality	20 (10.7%)	9 (11.6%)	.804
AML NOS	19 (10.1%)	22 (28.6%)	**<.001**

Bold values represent the statistically significant results (<.05).

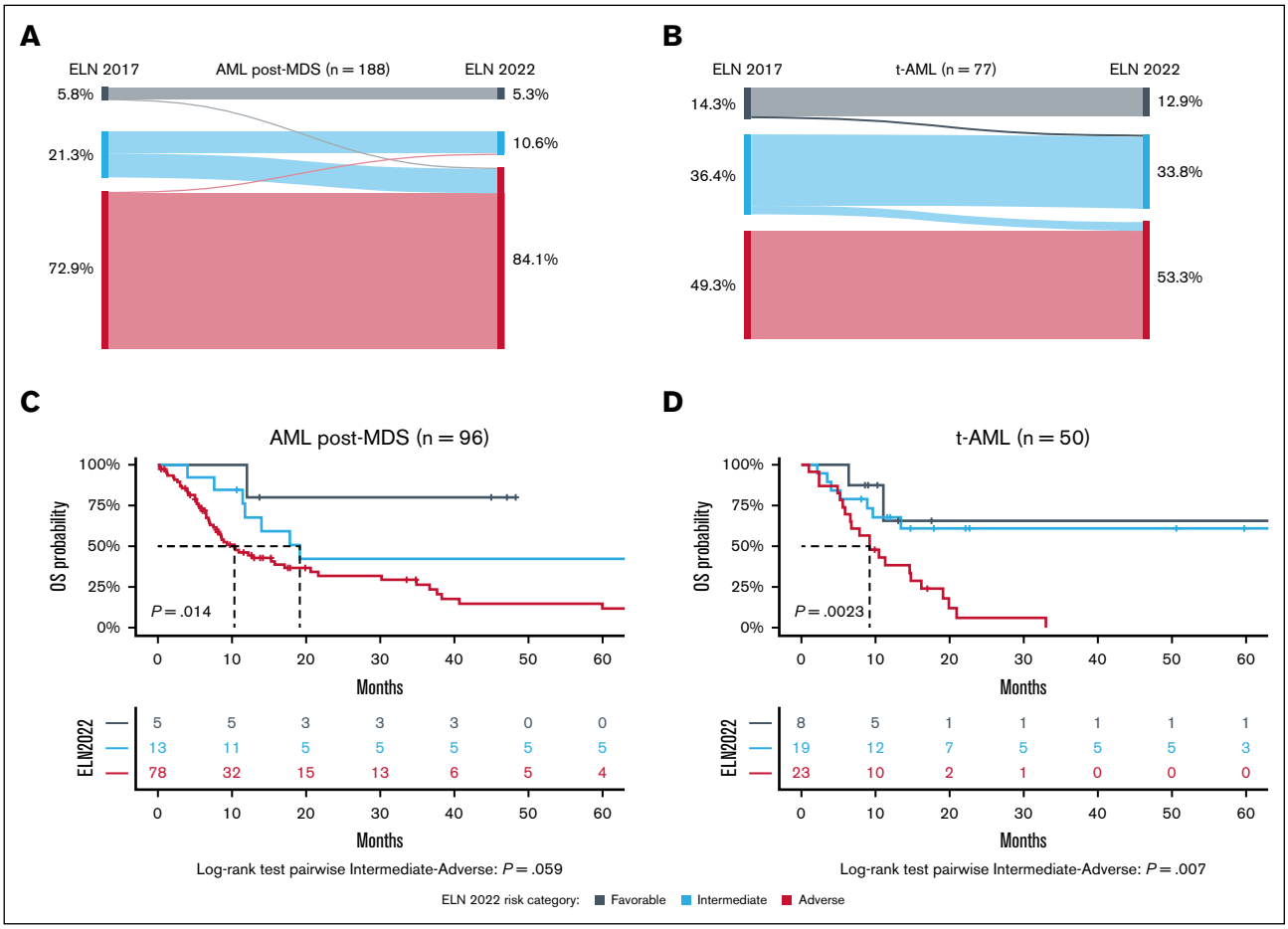

Figure 5. Stratification and outcome of secondary AML (post-MDS vs therapy-related). (A-B) Relationship between risk scoring of AML post-MDS (A) vs t-AML (B), comparing ELN 2017 and 2022 models shown using Sankey plots. (C-D) Survival estimates of patients with secondary AML (AML post-MDS, panel C and t-AML, panel D) treated with intensive chemotherapy and grouped according to ELN 2022 (n = 96 and 50 patients with available survival data). Numbers of patients at risk are indicated below the curves and are color coded: gray color indicates the favorable, blue indicates the intermediate, and red indicates the adverse prognostic risk group. *P* values were obtained by log-rank test. The log-rank test for pairwise intermediate vs adverse risk was *P* = .059 for AML post-MDS and .007 for t-AML.

subgroups was significantly different. However, although t-AML were nicely stratified according to ELN 2022, with 12.9% favorable, 33.8% intermediate, and 53.3% adverse risk (Figure 5A-B), in AML post-MDS, 84.1% of patients clustered in the adverse-risk group, with 11.1% of patients transitioning from ELN 2017 intermediate-risk.

We then analyzed patients survival by ELN 2022 in 146 patients treated with intensive chemotherapy. The difference in survival was significant in both patients with AML post-MDS and in t-AML ($P = .015$ and .0023, respectively; Figure 5C-D). In the pairwise analysis, survival was borderline significant in AML post-MDS for intermediate and adverse-risk groups (19.1 months of median OS in the intermediate group vs 10.3 in the adverse group, $P = .059$; Figure 5C). In t-AML, survival at 1 year was similar in favorable and intermediate ELN 2022 subgroups (65.6% vs 67.7%) and was poor in the adverse subgroup (median OS of intermediate-risk group not reached vs 9.2 months in the adverse-risk group, $P = .007$; Figure 5D). These data must be considered with caution, given the reduced t-AML sample size.

After restratifying AML post-MDS and in patients with t-AML treated with less intensive therapy by the 2024 ELN criteria, the difference in OS resulted statistically significant in the AML post-MDS group, but not in the t-AML probably due to the low sample size (supplemental Figures 3 and 4).

Discussion

The definition of secondary AML has long been a challenge for hematologists and pathologists, significantly evolving over time. In this line, AML classifications increasingly recognized MDR genetic abnormalities over the years and more precisely defined MDR AML subtypes. The 20% blast border has also been part of this revision process, as the new MDS/AML category has been introduced by ICC.[2] To challenge this frame, we first interrogated the 20% blast threshold analyzing the differences between MDS/AML and AML across the ICC hierarchy. In the ICC categories "MDR gene mutation" and NOS, where NGS analysis plays a major role in biological characterization, the 20% blast threshold still acts as a watershed separating 2 distinct biological profiles and outcomes. In AML, the mutational landscape was dominated by AML-like genes, particularly *FLT3*, *IDH1*, *IDH2*, *CEBPA*, *DNMT3A*, and *WT1* mutations, in line with previous reports.[15,16] AML were also characterized by higher frequency of *CSF3R* mutations, consistent with its role in promoting a proliferative phenotype,[17] and *BCOR* mutations. Conversely, MDS/AML were enriched for *TET2*, *ASXL1*, *SRSF2*, and *STAG2* mutations, suggesting that the biological continuum between MDS, MDS/AML and secondary AML may encompass subgroups with distinct clonal dynamics.

In contrast, MDS/AML and AML with "mutated TP53" shared similar biological profiles and outcome, confirming earlier reports that they represent a single disease entity.[18] We observed a similar overlap in the category defined as "MDR cytogenetic abnormality," which was associated with a uniformly dismal prognosis in both MDS/AML and AML. In the diagnostic routine, information on karyotypes is often available in AML prior to NGS data and may help to promptly identify high-risk patients. We previously showed that 86.9% and 31.0% of patients with AML with "mutated TP53" and "MDR gene mutation," respectively, presented MDR cytogenetic abnormalities.[3] Our demonstration that the MDS/AML "MDR gene mutation" category can be further stratified by MDR cytogenetic abnormalities reinforces the clinical relevance of the 20% blast threshold and supports the legitimacy of dedicated MDS-specific prognostic systems.[19]

We then wondered whether a previous history of MDS could encompass genetic signatures in AML. For this reason, we compared AML post-MDS with de novo AML, according to ICC. Except for *ASXL1*, *SF3B1* mutations and complex karyotype (enriched in AML post-MDS), the frequencies of other MDR gene mutations, cytogenetic abnormalities and prognosis were similar in the anamnestically- vs genetically-defined secondary AML. This confirms that the ontogeny designation is not the real "watershed" of AML subcategories, and that the genetic signature plays a dominant role.[6,20,21] These findings are relevant, considering the availability of treatments released for t-AML or AML-MRC, such as the liposomal encapsulation of cytarabine and daunorubicin (CPX-351), which could be beneficial for primary AMLs with a MDR genetic profile.[22] Indeed, it has been shown that survival following treatment with CPX-351 was improved in patients with .AML and secondary-type mutations, vs other secondary AML.[23]

In the second part of our study, we aimed at validating the ICC diagnostic qualifiers of AML post-MDS and t-AML. Although these 2 groups are often considered as a single unfavorable entity, our data confirmed their distinct genetic background. The group of AML post-MDS was significantly enriched with MDR genetic alterations. This has important implications, as we demonstrated that even when excluding a history of MDS or MDS/MPN as a "classifier," over 80% of AML post-MDS fell within one of "mutated TP53," "MDR gene mutation," or "MDR cytogenetic abnormality" ICC categories. This finding also impacts on the prognostic risk stratification, as 84% of AML post-MDS fell into the ELN 2022 adverse-risk category. Although at the time of AML diagnosis, results of conventional karyotyping and NGS testing should be ideally available to make treatment decisions, a prior MDS history provides important and immediate insights into the AML subtype. This qualifier, despite not being a "classifier," may suggest unfavorable outcomes from the very first day of diagnosis.

On the other hand, t-AML displayed high heterogeneity in biological profile and prognosis and could be nicely stratified according to ELN 2022. This supports the idea that therapy-related AML should be approached as "second" neoplasms, rather than being categorized as "secondary" AML, and treated using standard approaches according to guidelines and patient fitness.[24-26] This is in line with recent evidence concerning *NPM1*-mutated AML, which shows similar genetic profile and survival, independent of de novo vs therapy-related ontogeny.[27] On the other hand, the high frequency of *TP53* mutations (23.4%) and *KMT2A* rearrangements (10.4%) in the t-AML group underscores the need to prioritize their detection using dedicated assays.[28]

This study has several limitations, including the low number of patients with t-AML, and the limited use of modern treatment

strategies. Indeed, the type of intensive chemotherapy received by our patients was 7+3 in most cases, and only few patients were treated with the VEN-AZA combination. In addition, we did not address the role of the third ICC diagnostic qualifier, namely "germ line predisposition," which require personalized strategies tailored to the specific germ line defect.[29,30]

Our findings highlight that blast count is a still valuable parameter, associated with biologically distinct disease categories in MDR-gene mutation and NOS AML categories. Molecular and cytogenetic alterations in secondary AML supersede a prior history of MDS or MDS/MPN or of cytotoxic therapy, as defined by ICC.

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Authorship

Contribution: E.A. and M.T.V. conceptualized the study; E.A. and M.T.V. contributed to writing (original draft preparation); M.C. and A.P. performed statistical analysis; L.G., A.S., E.F., F. Mallegni, F. Moretti, G.S., H.A., A.D., I.F., G.M., M.M., V.V., A.V., M.G.D.P., and C.G. performed data collection; J.P.M. provided data review and editing; M.T.V. contributed to writing (review and editing); and all authors have read and agreed to the published version of the manuscript.

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