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REVIEW

The International Consensus Classification of acute myeloid leukemia

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Abstract

Acute myeloid leukemias (AMLs) are overlapping hematological neoplasms associated with rapid onset, progressive, and frequently chemo-resistant disease. At diagnosis, classification and risk stratification are critical for treatment decisions. A group with expertise in the clinical, pathologic, and genetic aspects of these disorders developed the International Consensus Classification (ICC) of acute leukemias. One of the major changes includes elimination of AML with myelodysplasia-related changes group, while creating new categories of AML with myelodysplasia-related cytogenetic abnormalities, AML with myelodysplasia-related gene mutations, and AML with mutated *TP53*. Most of recurrent genetic abnormalities, including mutations in *NPM1*, that define specific subtypes of AML have a lower requirement of ≥ 10% blasts in the bone marrow or blood, and a new category of MDS/AML is created for other case types with 10–19% blasts. Prior therapy, antecedent myeloid neoplasms or underlying germline genetic disorders predisposing to the development of AML are now recommended as qualifiers to the initial diagnosis of AML. With these changes, classification of AML is updated to include evolving genetic, clinical, and morphologic findings.

Keywords Acute myeloid leukemia · Genetic abnormalities · Classification

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease with a wide variety of clinical presentations, morphological features, and immunophenotypes. The revised 4th edition World Health Organization (WHO) classification of acute myeloid leukemia (AML), developed in 2016 and published in book form in 2017, recognized subtypes of AML based on clinical, morphologic, and genetic features [1]. The WHO AML classification has its origins in the morphologic French-American-British (FAB) classification, but evolved to define disease categories based on cytogenetic abnormalities, mutational profile, and patient history (prior MDS, MDS/MPN, MPN, or cytotoxic therapy), with a complex hierarchy of assigning each AML case to a unique disease category [2]. The specific subgroups included AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related myeloid neoplasms, and AML, not otherwise specified. Each disease category within the AML group attempted to define entities with similar biologic features associated with similar patient outcomes. One of the goals of the recently developed International Consensus Classification (ICC) of acute leukemias was to move to

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a more genetically defined classification while working in collaboration with clinicians through the Clinical Advisory Committee (CAC) process [3]. The ICC AML classification has been informed by the rapid evolution of molecular genetics into an indispensable diagnostic discipline that has brought about major advances in our understanding of the molecular landscape of AML.

TP53 is one of the most frequently mutated genes across all cancer types. AML patients with *TP53* mutations have a nearly uniformly poor prognosis, suggesting a biologically homogeneous single group, yet in the 2016 WHO classification were included under therapy-related AML (t-AML), AML not otherwise specified (AML-NOS), pure erythroid leukemia, and AML with myelodysplasia-related changes (AML-MRC) groups [1]. In the updated ICC classification, AML with mutated *TP53* is now recognized as a separate single entity [3]. A history of prior therapy or progression from myelodysplastic syndrome (MDS) or a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) is now applied as qualifiers to the diagnosis rather than as specific disease categories to reduce confusion caused by the substantial overlap of prior AML categories. The category of AML-MRC is eliminated. Most of the cases previously included in this subtype are now allocated to the new subgroups of AML with myelodysplasia-related cytogenetic abnormalities and AML with mutated *TP53* and AML with myelodysplasia-related gene mutations.

A number of genetic abnormalities, many of which are a result of recurring chromosome abnormalities, are used to define distinct disease entities and in the prior WHO classification included three specific subtypes of AML that did not require a minimal blast percentage. These included AMLs with t(8;21)(q22;q22.1), inv(16)(p13.1q22), t(16;16)(p13.1;q22), and t(15;17)(q24;q21). The ICC expands the category that may be diagnosed as AML with < 20% blasts to encompass additional recurring genetic abnormalities and also *NPM1* and in-frame bZIP *CEBPA* mutations and requires a blast count of ≥ 10%. The remaining AML subtypes retain the 20% or more blast requirement. MDS cases with 10–19% blasts are now diagnosed as MDS/AML, reflecting the diagnostic continuum between AML and MDS and clinical and genetic heterogeneity among individual patients with these lower blast counts.

AML with myelodysplasia-related gene mutations

Defined by mutations in ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2

AML with myelodysplasia-related changes was an attempt to identify a subgroup of AML featuring older age, lower blast counts, lower remission rate, and shorter overall survival. In the 2016 WHO classification, the category of AML-MRC applied to patients with AML who have 20% or more blasts in the blood or bone marrow and who meet any of the following criteria: a history of MDS or MDS/MPN, such as chronic myelomonocytic leukemia (CMML); an MDS-related cytogenetic abnormality; or multilineage dysplasia in 50% or more of two or more cell lines in the absence of *NPM1* or biallelic *CEBPA* mutations [1]. The classification of AML-MRC overlaps somewhat with the traditional term "secondary AML," which includes patients with AML that develops from an antecedent hematologic disorder (including MDS and MDS/MPN), as well as those with therapy-related AML that develops after prior cytotoxic therapy, radiotherapy, or immunosuppressive therapy. The AML-MRC group included a variety of cytogenetic abnormalities, including complex karyotypes (defined as three or more unrelated abnormalities) and other specified unbalanced and balanced abnormalities and excluded cases of therapy-related AML and AML with recurrent genetic abnormalities, such as t(8;21), inv(3), and t(6;9), the latter two of which may have multilineage dysplasia [4]. Whether the presence of dysplastic morphologic features alone warrants classification into a group associated with a poor prognosis has been controversial and several studies showed conflicting results as to whether dysplastic morphology is independently prognostic or merely reflects underlying adverse cytogenetics and/or mutation profile [5–9]. Although not part of the 2016 disease definition, various gene mutations are more commonly associated with AML-MRC, including mutations of *ASXL1, TP53*, and *U2AF1*, and could have accounted for prognostic significance within this group.

In the past decade, next-generation sequencing (NGS) technology has expanded our understanding of AML and revealed common genetic mutations with important roles in pathogenesis and prognosis [10]. Based on these results, European Leukemia Net (ELN) incorporated NGS data to develop a risk stratification system used in AML management [11]. Lindsley et al. [12] found that the presence of a mutation in *SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR*, or *STAG2* was > 95% specific for the diagnosis of secondary AML. Papaemmanuil et al. [13] confirmed lower survival rates and higher relapse rates in patients with chromatin-spliceosome gene mutations (including *SRSF2, ASXL1, STAG2*) as compared to other subgroups. A lower response rate and worse survival were also described in patients with mutations in *SRSF2, U2AF1,* and *ASXL1* by Gao et al. [14]. Baer et al. [15] identified a mutational pattern including *SRSF2, U2AF1, SF3B1, ASXL1, EZH2, BCOR,* and *STAG2* that allowed to distinguish AML-MRC from non-AML-MRC patients. Overall survival was inferior in patients harboring these mutations, irrespective of whether they were classified as AML-MRC or not. Furthermore, the

molecular AML-MRC-like pattern was identified in over 10% of patients not classified as MRC per WHO criteria but who experienced a similarly poor overall survival, suggesting the definition of AML-MRC should be expanded to include this molecularly determined subset as well. Based on this data, the ICC created this new category and eliminated AML-MRC defined solely by morphologic dysplasia and merged the prior category of AML with mutated *RUNX1* into this group (Fig. 1). *RUNX1* mutations in AML were already well known to be associated with prior therapy (especially radiation) and prior MDS [16, 17].

AML with myelodysplasia-related cytogenetic abnormalities

- Defined by detecting a complex karyotype (≥ 3 unrelated clonal chromosomal abnormalities in the absence of other class-defining recurring genetic abnormalities), 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), or idic(X)(q13) clonal abnormalities

By definition, patients with a 2016 WHO diagnosis of AML-MRC have a high frequency of adverse cytogenetics, including complex karyotypes [18]. One study reported that 262 patients with AML included 57% who were 75 years or older, 53% had poor-risk cytogenetics, and approximately one-third were reported to have had antecedent MDS [19]. A separate study also identified antecedent MDS or MDS/MPN and de novo AML with MDS-related cytogenetics as conferring a worse prognosis compared with patients with AML-MRC who had a diagnosis based on multilineage dysplasia [20].

Rogers et al. [21] reported that most cases of AML-MRC were associated with adverse genetic abnormalities, particularly –5/del(5q), –7/del(7q), and/or complex karyotype (CK). The presence of a complex karyotype (CK), defined as ≥ 3 chromosomal abnormalities, comprises 10 to 12% of all acute myeloid leukemia (AML) patients and constitutes

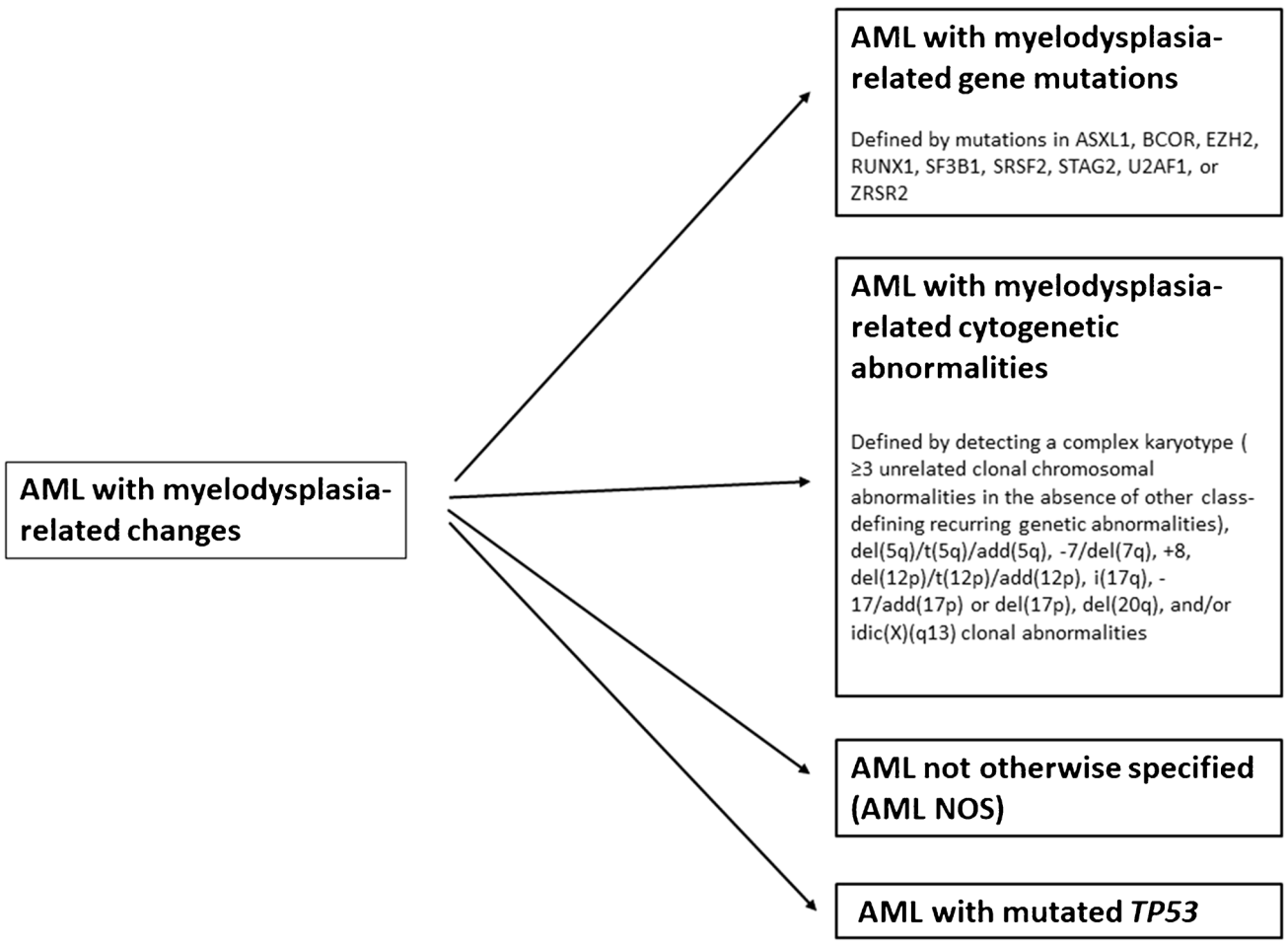

Fig. 1 AML with myelodysplasia-related changes group is now re-classified as new categories of AML with myelodysplasia-related cytogenetic abnormalities, AML with myelodysplasia-related gene mutations, and AML with mutated *TP53*

the second largest cytogenetic subset of AML patients (after those with normal karyotype). In the ELN classification, a complex karyotype is defined as three or more chromosomal abnormalities in the absence of the WHO-designated recurring translocations or inversions, such as t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3), or t(3;3), whereas the UK National Cancer Research Institute Adult Leukemia Working Group requires four or more chromosomal abnormalities as an adverse risk factor [22]. Other studies have shown that AML with monosomal karyotype (MK) presented with a significantly worse overall survival, disease-free survival, and complete response rate and predominantly was subclassified as AML-MRC, suggesting MK as a possible stronger adverse prognostic factor than the traditionally defined CK [23–25]. This ICC category retains the prior unbalanced cytogenetic abnormalities of AML-MRC, but now adds + 8 and del(20q) for such subclassification because, in the context of an increase in blast cells, they are considered myelodysplasia-related even if they may occur in non-MDS settings when blast cells are not increased.

AML with recurrent genetic abnormalities

This group has been expanded to include additional variant translocations involving *RARA*, *KMT2A*, and *MECOM* and other genetically related entities (see Table 1 and Table 2). The number of blasts required for a diagnosis of AML in the presence of recurrent genetic abnormalities is ≥ 10% for the entire group with exception of AML with t(9;22)(q34.1;q11.2): the latter still requires at least 20% blasts to avoid potential overlap with the progression of chronic myeloid leukemia. Compared with patients with myeloid blast transformation of CML, patients with AML with t(9;22)(q34.1;q11.2) have less frequent splenomegaly, lower peripheral blood basophilia, and lower bone marrow cellularity and myeloid-to-erythroid ratio [26]. Despite these differences,

Table 1 Classification of acute myeloid leukemia (AML) with percentage of blasts required for diagnosis

AML with recurrent genetic abnormalities	Blast % requirement
• Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)/*PML*::*RARA*	≥ 10%
• APL with other RARA rearrangements	≥ 10%
• AML with t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*	≥ 10%
• AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*	≥ 10%
• AML with t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*	≥ 10%
• AML with other *KMT2A* rearrangements[1]	≥ 10%
• AML with other *MECOM* rearrangements[2]	≥ 10%
• AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*; *MECOM*(*EVI1*)	≥ 10%
• AML with t(9;22)(22)(q34.1;q11.2)/BCR::ABL1	≥ 20%
• AML mutated bZIP *CEBPA*	≥ 10%
• AML with mutated *NPM1*	≥ 10%
• AML with mutated *TP53*	≥ 20%

[1]Includes AMLs with: t(4;11)(q21.3;q23.3)/*AFF1*::*KMT2A*; t(6;11)(q27;q23.3)/*AFDN*::*KMT2A*

[2]t(10;11)(p12.3;q23.3)/*MLLT10*::*KMT2A*; t(10;11)(q21.3;q23.3)/*TET1*::*KMT2A*; t(11;19)(q23.3;p13.1)/*KMT2A*::*ELL*; t(11;19)(q23.3;p13.3)/*KMT2A*::*MLLT1*

[3]Includes AMLs with: t(2;3)(p11 ~ 23;q26.2)/*MECOM*::?; t(3;8)(q26.2;q24.2)/*MYC*, *MECOM*; t(3;12)(q26.2;p13.2)/*ETV6*::*MECOM*; t(3;21)(q26.2;q22.1)/*MECOM*::*RUNX1*

Table 2 Rare recurring translocations in acute myeloid leukemia

Acute myeloid leukemia (AML) with other rare recurring translocations	Blast % requirement
AML with t(1;3)(p36.3;q21.3)/*PRDM16*::*RPN1*	≥ 10%
AML with t(3;5)(q25.3;q35.1)/*NPM1*::*MLF1*	≥ 10%
AML with t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP*	≥ 10%
AML (megakaryoblastic) with t(1;22)(p13.3;q13.1)/*RBM15*::*MRTF1**	≥ 10%
AML with t(5;11)(q35.2;p15.4/ *NUP98*::*NSD1**	≥ 10%
AML with t(11;12)(p15.4;p13.3)/*NUP98*::*KMD5A**	≥ 10%
AML with *NUP98* and other partners*	≥ 10%
AML with t(7;12)(q36.3;p13.2)/*ETV6*::*MNX1**	≥ 10%

*Occurs predominantly in children

some cases will only become apparent as blast transformation when they recur with the chronic phase of CML. Cases with < 10% blasts and *PML::RARA*, *RUNX1::RUNXT1*, and *CBFB::MYH11* rearrangements are exceedingly rare. In such cases, careful attention should be paid to including blast equivalents in the blast count and confirming the cytogenetic findings, particularly if detected initially at a low level. Many of these cases likely represent early AML and could be treated as such if clinically indicated, particularly in the absence of MDS morphologic features. Patients with < 10% blasts and any of the other AML-associated genetic findings including *KMT2A* translocations, inv(3)/t(3;3), and *CEBPA* mutations should continue to be classified as MDS until further data can be accumulated as to the clinical behavior and optimal treatment approach of these rare cases. About 2% of APL cases are characterized by atypical rearrangements, where *RARA* is fused to partners other than *PML* or in which the translocation involves other members of the RAR superfamily. The *MDS1/EVI1* complex (*MECOM*) is located on chromosome 3q26. EVI1 is a nuclear transcription factor and represents a proto-oncogene playing an important role in leukemogenesis in myeloid malignancies [27]. *MECOM* rearrangements cause EVI1 overexpression, which leads to global distortion of hematopoiesis and adverse prognosis in AML. Summerer et al. [28] and Rogers et al. [21] found in a study of 120 patients that the conventional classification of patients with myeloid neoplasms carrying *MECOM* rearrangements into MDS or AML applying a threshold of 20% bone marrow blasts does not reflect genetic profiles or clinical outcome.

In addition to AML, *NPM1* mutation has been detected in MDS (2%) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) (3%), with MDS/MPN represented by mainly chronic myelomonocytic leukemia (CMML). Blasts in *NPM1* mutated cases often show monocytic differentiation (Fig. 2). One of the largest multicenter cohorts of myeloid neoplasms with mutated *NPM1* and less than 20% blasts was reported by Patel et al. [29] and showed that they occurred in younger patients and were more commonly associated with a normal karyotype when compared with comparable *NPM1* wild-type myeloid neoplasms. The mutation landscape was also different, as *NPM1*-mutated MN more commonly showed *DNMT3A* and *PTPN11* mutations, while *ASXL1*, *RUNX1*, *TP53*, *IDH1*, *IDH2*, *FLT3*, *NRAS*, and *KRAS* mutations were less common than in the *NPM1* wild-type neoplasms [21]. When looking at the clinical treatment and outcomes, most patients with *NPM1*-mutated MN (73%) were treated with hypomethylating agents (HMA) upfront, and 39% progressed to AML at a median time of 5.2 months. As a contrast, none of the patients treated with intensive induction chemotherapy progressed to AML. These data suggest that a more intensive chemotherapy upfront may benefit patients with *NPM1*-mutated myeloid neoplasms [29]. Montalban-Bravo et al. [30] reported similar observations in a smaller patient cohort of 31 patients. Based on these studies, the ICC classification has lowered the required blast for AML with mutated *NPM1* to 10%, similar to the cutoff for AML with recurring cytogenetic abnormalities.

AML with in-frame bZIP CEBPA mutations

CEBPA is a single-exon gene located on chromosome 19.q13.1 that encodes for CCAAT/enhancer-binding protein-α, a lineage-specific basic leucine zipper (bZIP) transcription factor required to form myeloid progenitors from multipotent hematopoietic stem cells [31, 32]. The most common *CEBPA* mutations in AML are N-terminus frameshift mutations that result in premature truncation of the p42 isoform while preserving the expression of the p30 isoform. Other *CEBPA* mutations are usually in-frame insertions or deletions at the C-terminus basic leucine zipper region that interfere with DNA binding and dimerization [33]. *CEBPA* mutation is found in 7–16% of adults and 4.5–15% of pediatric AML patients. *CEBPA* mutations in AML have been associated with FAB M1 and M2 morphology, high CD34 expression on blasts, and predominance in the normal karyotype. A favorable prognosis was

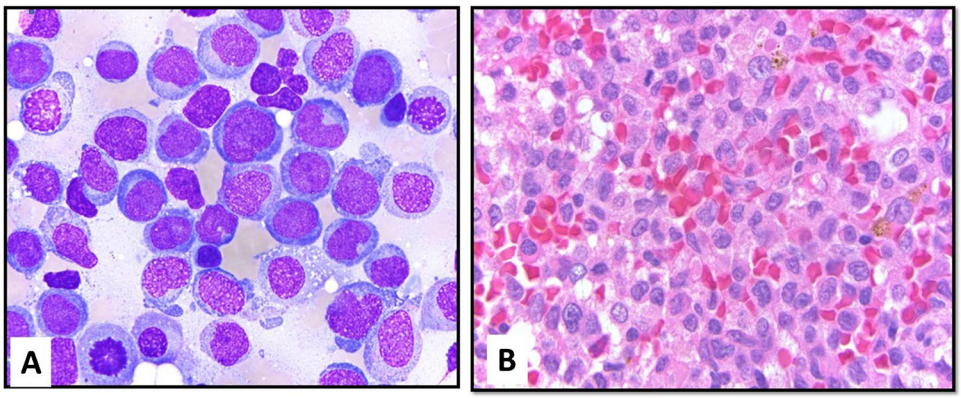

Fig. 2 AML with mutated *NPM1* showing blasts with moderate cytoplasm and variably prominent nucleoli in the aspirate smears (**A**) and biopsy (**B**)

previously noted in patients with biallelic or double mutations, while patients with monoallelic *CEBPA* mutations (CEBPAsm) did not differ in their response to treatment from AML patients with wild-type CEBPA [34]. This finding led to the inclusion of biallelic *CEBPA* mutations as an independent entity in the 2016 WHO classification, as well as a favorable prognostic group in the ELN2017 recommendations. However, the impact of monoallelic *CEBPA* mutations has now been investigated in more detail in a few studies. In a study of 4708 adults with AML, Taube et al. [35] found that *CEBPA* mutations that are in-frame mutations affecting the basic leucine zipper region (bZIP) confer a favorable outcome, irrespective of their occurrence as biallelic (CEBPAbi) or single mutation (CEBPAsm). These patients present at a younger age, with higher white blood cell counts and higher frequency of *GATA2* mutations, and are able to achieve high complete remission rates and long median event-free and overall survival. Their findings are in line with a recent report in 2958 pediatric AML cases also demonstrating that *CEBPA* bZIP domain mutations are associated with favorable clinical outcomes, regardless of mono- or biallelic mutational status [36]. Based on this data, the ICC created the category of AML with in-frame bZIP *CEBPA* mutations; other types of *CEBPA* mutations, whether monoallelic or biallelic, are not considered as a distinct disease group.

AML with mutated TP53

Alterations involving the *TP53* locus are complex and include gene deletions and loss of heterozygosity as well as single nucleotide and insertion/deletion mutations [37]. Most cancer-associated *TP53* mutations, including those in AML, are missense mutations that involve the DNA-binding domain (DBD) [38]. Mutant premature termination codons and frameshift mutations result in strong disruption of p53 function, whereas the impact of mutations resulting in a single amino-acid substitution or deletion is dependent on their position within the DBD. Among patients with a new diagnosis of AML, at least 10% will have disease-harboring mutations in *TP53* but up to 30% in certain subpopulations such as those with secondary AML, therapy-related AML, or acute erythroid leukemia [39–42]. *TP53* mutation is considered to be in the adverse prognostic group of the 2017 ELN classification [43], and recent data suggest that *TP53*-mutated AML confers a particularly poor prognosis compared with other ELN adverse cases, with a 2-year median overall survival of only 12.8% even when intensively treated [44]. The dismal effect of *TP53* on patient outcome appears to transcend both blast count and disease ontogeny, with equally poor outcomes whether patients present as MDS or AML, and whether the disease is therapy-related or clinically de novo [45].

The clinical impact of the *TP53* alteration in AML and MDS depends on whether the allelic disruption is monoallelic or biallelic, which determines the amount of functional TP53 protein present. Analyses of patients with *TP53*-mutated MDS demonstrated that approximately 40% of the population harbors disease with a copy-neutral loss of heterozygosity, which, based on the predicted absence of the functional TP53 protein, was significantly associated with inferior survival; conversely, patients with a monoallelic loss of *TP53* behave similarly to patients with *TP53* wild-type disease [46]. Some studies have suggested that higher *TP53* VAF (>40%) was also associated with shorter survival, although this has not been found in other studies of *TP53*-mutated AML [47, 48]. While multi-hit *TP53* mutation is required for MDS with mutated *TP53*, in AML and MDS/AML with mutated *TP53*, any pathogenic *TP53* mutation VAF of ≥10% is considered sufficient. Pure erythroid leukemia (PEL) which has a high prevalence of at least two TP53 abnormalities (both mutations and aberrant or deleted chromosome 17p) in >90% of cases [40] is now classified as AML with mutated *TP53* if it meets PEL criteria, which has remained same as was previously defined in WHO classification (1).

Diagnostic qualifiers

To reduce confusion caused by the substantial overlap of prior AML categories, the classification now identifies ontological associations as qualifiers to the diagnosis rather than as specific disease categories. All AML cases that are therapy-related should be qualified as such by entering a "therapy-related" statement after the diagnosis. Although it remains important to recognize therapy-relatedness of myeloid neoplasms, the first priority is to classify the disease according to its morphologic and genetic features. The single gene mutation or gene fusion categories will now take precedent over the myelodysplasia-related gene mutation and the myelodysplasia-related cytogenetic groups, though such findings may again impact prognosis in the genetic groups and should be noted in the diagnosis. After excluding all other genetic categories, some cases will remain unclassified and those will continue to be diagnosed as AML, NOS. Similar modifiers should be used for cases that progress from MDS and from MDS/MPN or are associated with germline predisposition (see Table 3). In the hierarchical ICC AML classification, the presence of an AML-defining recurrent genetic abnormality (as listed in Tables 1 and 2) would take the first precedent, followed by myelodysplasia-related mutations and myelodysplasia-related cytogenetic abnormalities. Both *NMP1* and *TP53* mutated AML are considered AML with recurrent genetic abnormalities (based on gene mutations) and, if present together, should be listed as such.

Table 3 Diagnostic qualifiers that should be used following acute myeloid leukemia diagnosis

Diagnostic qualifiers	
Therapy-related	Prior chemotherapy, radiotherapy, immune interventions
Progressing from myelodysplastic syndrome	MDS should be confirmed by standard diagnostics
Progressing from myelodysplastic/myeloproliferative neoplasm	MDS/MPN should be confirmed by standard diagnostics
Germline predisposition	Germline mutations

If *TP53* mutation is present along with myelodysplasia-related gene mutations, a diagnosis of AML with mutated *TP53* should be made.

Diagnostic evaluation

A thorough patient history and relevant clinical data, including a physical examination, imaging findings, and blood laboratory values, should be obtained [49]. Cytogenetics and next-generation sequencing should be utilized to investigate the genomic features of each new patient's AML. ELN genetic risk stratification can then be employed to assess prognosis and has been adopted by multiple society consensus guidelines including the NCCN.

Multiparameter flow cytometry (FCM) is important for lineage assignment of newly diagnosed acute leukemia. In AML, FCM is necessary to confirm acute myeloid leukemia with minimal differentiation and to detect monocytic differentiation. Characteristic findings in AML with main specific genetic abnormalities have been described and summarized in the WHO 2008 and 2016 publications [1, 50]. AML with *NPM1* mutation has been at first described as APL-like due to CD34 and HLA-DR negative blast populations (1) but it has been immunophenotypically characterized in detail in more recent publications. Three subtypes of marker expression have been described: blastic, myelomonocytic, and purely monocytic [51, 52]. The presence of at least a small monocytic population and CD11c positive blast population in *NPM1* + AML makes the *quick* FCM differentiation between APL and *NMP1* + AML easy. In AML with monocytic differentiation, there is a sequence of antigen expression (including progressive acquisition of CD14, CD35, and decrease of HLA-DR on CD64-positive and CD300-negative cells) that is extremely helpful for precise identification of the stage of the maturational arrest of monocytic-lineage blast cells and promonocytes, as well as the maturation status of residual monocytic cells, whenever they are also present. Increasing reactivity for CD36, CD35, and CD14 is observed in maturing promonocytes. Fully mature monocytes are characterized by their reactivity forCD300e, at a stage where CD14 and CD35 expression have already reached their highest levels, after a slight decrease in HLA-DR expression. Therefore, the expression of CD64 by blast cells that retain HLA-DR expression, even in the absence of positivity for CD34 and CD117, allows early discrimination between monocytic-lineage AML and other AML subtypes [53].

Like AML with *RUNX1*::*RUNX1T1*, RUNX1-mutated AML has been associated with mixed phenotype acute leukemia (MPAL) features [54] and with the expansion of the plasmacytoid dendritic cell compartment [55, 56].

AML with myelodysplasia-related genetic abnormalities (both chromosomal abnormalities and gene mutations) often carry immunophenotypic features like those described in MDS [57–59] (Fig. 3). The simultaneous occurrence of several mutations and heterogeneous complex karyotypes makes it difficult to characterize specific patterns associated with specific mutations. However, high expression of CD34 and aberrant expression of CD7 have been associated with *TP53* mutation [60]. CD14 expression in the granulocytic compartment [61] and increased CD45RA expression in the CD34 + /CD38 stem cell compartment have been described in cases with chromosome 7 abnormalities [62].

FCM immunophenotyping is also of importance to detect MPAL characteristics of blast populations. The MPAL criteria for lineage assignment are still a matter of some debate [63–66]. With the new definition of AML and MDS/AML with myelodysplasia-related genetic changes, many cases previously assigned to the MPAL category with predominant myeloid blast populations will now be diagnosed as AML based on cytogenetic or mutation findings or MDS/AML. However, one should be aware of MPAL cases with predominant lymphatic populations that may carry similar genetic abnormalities (especially in the pediatric population) [67].

While historically there has been concern that treatment delays to wait for genomic tests could harm patient outcomes, there is data to suggest this is not the case. In a study of 599 French patients (40% were aged ≥ 60 years), a delay in treatment with a median time-to-treatment of 8 days was not associated with early death, complete response (CR) rate, or OS in multivariable analysis [68]. More recently, a preliminary analysis of the Beat AML Master trial of 395 patients aged ≥ 60 years of age demonstrated the feasibility of obtaining cytogenetic and NGS testing to target induction therapy [69]. Approximately 95% of patients had these tests completed within 7 days and the median OS for patients assigned to three investigational substudies on the basis of genetic features was significantly longer than those who

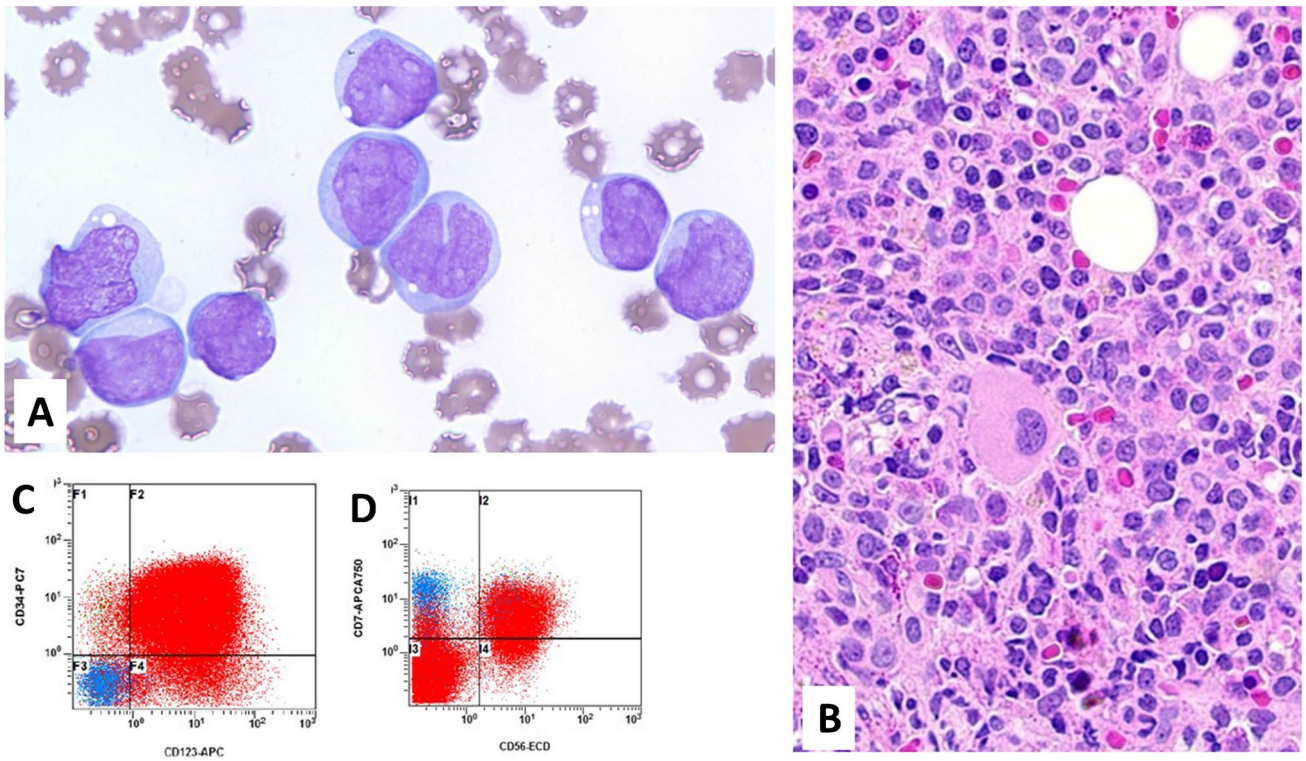

Fig. 3 AML with myelodysplasia-related gene mutations (*BCOR* mutation) showing large blasts with moderate cytoplasm and prominent nucleoli (**A**) and scattered hypolobated megakaryocytes (**B**). Flow cytometry plots (**C** and **D**) shows that these blasts express CD34, CD123, CD7, and CD56

received standard-of-care treatment (12.8 vs. 3.9 months) [69]. Therefore, the more focused molecular approach proposed by the ICC is feasible and should allow for improved patient outcomes.

Measurable residual disease

The term "minimal residual disease" was replaced by "measurable residual disease" (MRD) in 2018 by the European Leukemia Net (ELN) AML expert panel and the new term is applied in studies by others [70, 71]. Irrespective of the methodology employed, MRD is a strong predictor of relapse and shorter survival in AML patients [71]. ELN recommendations based on a Delphi poll on both flow cytometry (FCM) and molecular MRD detection were recently updated [72]. In FCM, the application of a multiparameter approach with the integration of the diagnostic leukemia-associated immunophenotype (LAIP) and different from normal (DfN) aberrant immunophenotype approaches is recommended, ideally with a possibility to compare the follow-up sample with the diagnostic sample analyzed with the same panel. A combination of LAIP and DfN approaches makes it possible to detect both residual and new emergent leukemic clones. To reliably use flow MRD for clinical decision-making, the lower limit of detection (LLOD) and lower limit of quantification (LLOQ) should be reported for each MRD measurement [72]. Since relapses occur also in MRD-negative patients, further research is focusing on measuring the frequency and targeting of residual leukemic stem cells, which are a subpopulation of CD34 + /CD38- leukemic cells capable of self-renewal and may be resistant to therapy [73].

In molecular MRD detection, real-time quantitative polymerase chain reaction (RT-qPCR) for detection of common fusion transcripts [73] and mutations in genes such as *NPM1, FLT3*-ITD, *CEBPA, IDH1, IDH2, KIT, RAS, RUNX1,* and *TP53* or *WT1* gene overexpression is considered as the "gold standard" [74]. A method that is gaining increased interest is droplet digital (dd)PCR that does not require a standard curve, measures the absolute number of the molecule of interest, and can be applied to detect and quantify the level of individual somatic gene mutations [75]. ddPCR is limited by the requirement for mutation-specific primers and probes and is therefore most suitable for monitoring patients with highly recurrent mutations such as those in *NPM1, IDH1, IDH2,* and *FLT3*. The use of high-sensitivity, error-corrected next-generation sequencing (NGS) in MRD detection overcomes some limitations of ddPCR and can detect mutations in any targeted gene

allowing for MRD detection in nearly all AML patients. While previously too expensive or logistically complicated to implement, these methods are now entering clinical use. NGS-based MRD methods are challenged by the inability to distinguish between leukemia-related somatic mutations and persistent clonal hematopoiesis (generally persistent mutations in *DNMT3A, TET2,* or *ASXL1*—so-called DTA mutations) as well as the necessity to consider germline mutations in genes that may be involved in leukemogenesis [72]. These challenges must be overcome before NGS-MRD can be recommended as a stand-alone MRD technique [72].

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Declarations

Conflict of interest The authors declare no competing interests.

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