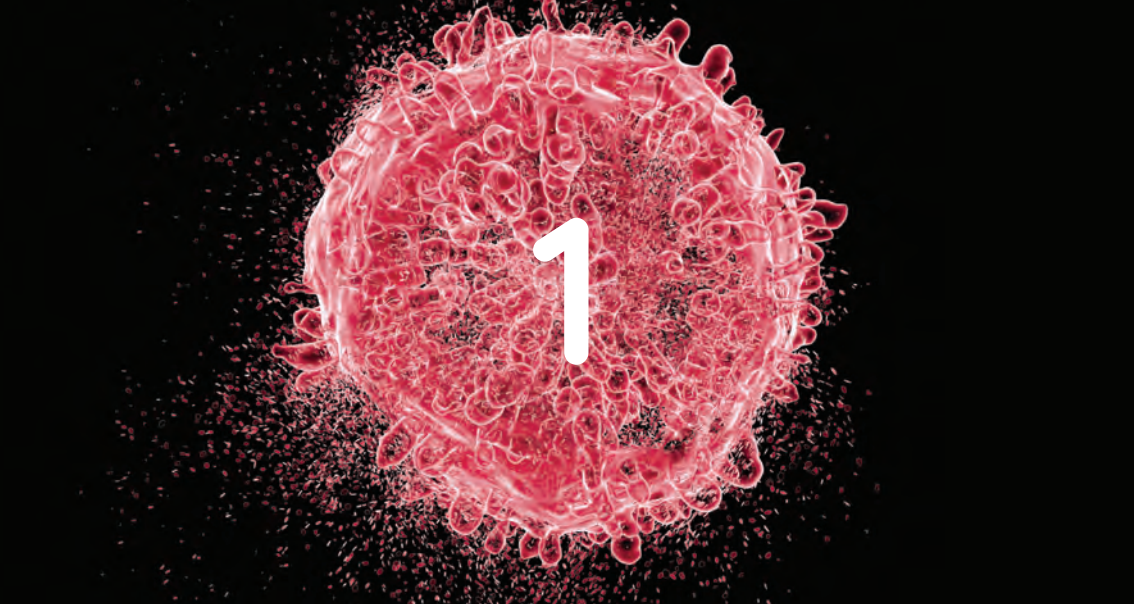

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Identification of high risk-AML patients

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Introduction

In recent years there has been substantial progress in our knowledge of disease risk associated with acute myeloid leukemia (AML), which may affect routine clinical practice. These advances include insights into the clinical value of genomic abnormalities for diagnosis and prognosis, the clinical significance of inherited predisposition to AML, technological advancements in the quantitative assessment of measurable residual disease (MRD) and their utility for assessing therapeutic response and disease risk.[1-5] Somatic mutations drive the development of AML. Although the epigenetic state of leukemia cells, the bone marrow microenvironment, the health of normal hematopoietic cells, and other features are important for the disease biology,

and for determining disease-associated risk, somatic mutations can be assessed readily in a routine clinical setting.[1-5] Leukemia develops from the serial acquisition of somatic mutations in hematopoietic stem and progenitor cells. Initiating mutations may lead to an expanded cloning of cells that is apparent in the peripheral blood, termed clonal hematopoiesis, a common premalignant state that increases in prevalence with age. Although some mutations, such as those in *DNMT3A*, *TET2*, and *ASXL1*, are more common in clonal hematopoiesis and appear to be relatively early events in leukemogenesis, others tend to be acquired later in the course of leukemia development, including mutations in *FLT3*, *NRAS*, and *RUNX1*. The combinations of mutations that ultimately drive leukemogenesis are affected by biological cooperativity and mutual exclusivity between mutated genes.[1-5] The International Consensus Classification (ICC) of AML that updated the prior revised 4th edition of the WHO Classification recently introduced changes in the blast thresholds and new genetic entities that define AML, further expanding the spectrum of classification identified by cytogenetic and mutational profiles, and providing a basis on which to improve the identification of homogeneous groups of patients with high-risk disease (Table 1.I).[1] New data has emerged that prompted an adjustment of the risk classification based on recurrent chromosomal abnormalities and gene mutations. In addition to baseline genetic characterization, the importance of response to initial therapy and the assessment of early MRD in individual risk assignment are now well recognized. Accordingly, a patient with favorable-risk AML may be re-classified as intermediate-risk or vice versa in clinical practice, based on the presence or absence of MRD, respectively. The International Expert Panel of European LeukemiaNET recently updated the 2017 recommendations on the diagnosis, risk assessment and management of AML in adults (Table 1.II).[3, 4] The most important changes made to the previous risk classification are: 1) The FLT3-ITD Allelic Ratio is no longer considered in the risk classification, and consequently, all AML with *FLT3-ITD* are now categorized in the intermediate-risk group, irrespective of the Allelic Ratio or concurrent presence of *NPM1* mutation; 2) AML with myelodysplasia-related gene mutations is now categorized in the adverse-risk group – these mutations, typically associated with AML following an antecedent hematologic disease, are also prevalent in *de-novo* AML, and indicate adverse risk even in the absence of myelodysplasia-related cytogenetic abnormalities; 3) the presence of adverse-risk cytogenetic abnormalities in *NPM1*-mutated AML now defines adverse risk; 4) in-frame mutations of the *CEBPA* gene, irrespective of

Table 1.I. AML and related neoplasms and acute leukemias of ambiguous lineage according to ICC (International Consensus Conference) 2022 Classification.

AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)
APL with t(15;17)(q24.1;q21.2)/*PML::RARA*
AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*
AML with t(6;9)(p22.3;q34.1)/*DEK::NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)*
AML with other rare recurring translocations
AML with mutated *NPM1*
AML with in-frame bZIP mutated *CEBPA*
AML with t(9;22)(q34.1;q11.2)/BCR::ABL1a
Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10-19% blasts in BM or PB)
AML with mutated *TP53*
AML with myelodysplasia-related gene mutations
Defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*
AML with myelodysplasia-related cytogenetic abnormalities*
AML not otherwise specified (NOS)
Myeloid sarcoma
Myeloid proliferations related to Down Syndrome
Transient abnormal myelopoiesis associated with Down Syndrome

Myeloid leukemia associated with Down Syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
MPAL with t(9;22)(q34.1;q11.2)/*BCR::ABL1*
MPAL with t(v;11q23.3)/*KMT2A* rearranged
MPAL, B/myeloid, not otherwise specified
MPAL, T/myeloid, not otherwise specified

Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities. Unbalanced clonal 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); and/or idic(X)(q13).

*Cytogenetic abnormalities sufficient for the diagnosis of AML with MDS-related cytogenetic abnormalities and the absence of other AML-defining disease categories.

BM: bone marrow; PB: peripheral blood.

their occurrence as biallelic or monoallelic mutations are categorized in the favorable-risk group; and 5) hyperdiploid karyotypes with multiple trisomies (or polysomies) are no longer considered complex karyotypes or adverse-risk.[1, 5] In this book chapter, we will adopt the definition of high-risk AML according to ICC 2022 and ELN 2022 Criteria.

AML with myelodysplasia-related gene mutations and/or cytogenetic abnormalities

The 2016 WHO Classification of myeloid neoplasms recognized an AML category called "acute myeloid leukemia with myelodysplasia-related changes (AML-MRC)," which included patients with AML that develops after myelodysplastic syndromes (MDS) or MDS/myeloproliferative neoplasms (MPN), AML with multilineage dysplasia, and *de-novo* AML

Table 1.II. European LeukemiaNet (ELN) Risk Classification by genetics at initial diagnosis.

Risk category
Favorable
t(8;21)(q22;q22.1)/*RUNX1::RUNX1T*
inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
Mutated *NPM1* without *FLT3-ITD*
bZIP in-frame mutated *CEBPA*
Intermediate
Mutated *NPM1* with *FLT3-ITD*
Wild-type *NPM1* with *FLT3-ITD*
t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*
Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse
t(6;9)(p23;q34.1)/*DEK::NUP214*
t(v;11q23.3)/*KMT2A*-rearranged
t(9;22)(q34.1;q11.2)/*BCR::ABL1*
t(8;16)(p11;p13)/*KAT6A::CREBBP*
inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)*
t(3q26.2;v)/*MECOM(EVI1)*-rearranged
-5 or del(5q); -7; -17/abn(17p)
Complex karyotype
Monosomal karyotype
Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*
Mutated *TP53*

with certain MDS-related cytogenetic abnormalities.[1, 5] This 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.[1-5] It has been estimated that AML-MRC represents up to 48% of all adult AML cases. Outcomes for patients with AML-MRC, or more generally those with secondary AML, following conventional combination chemotherapy, are poor compared with many other AML subtypes, with lower remission rates and shorter overall survival. Given the high-risk nature of AML-MRC, a clear understanding of the AML-MRC diagnosis and appropriate treatment options is important to help improve outcomes.[6-9] According to the 2016 WHO Classification, the AML-MRC designation applies 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 lineages (*i.e.*, dysgranulopoiesis, dyserythropoiesis, or dysmegakaryopoiesis) in the absence of *NPM1* or *CEBPA* mutations.[1, 5] AML-MRC includes a variety of cytogenetic abnormalities, including complex karyotypes (defined as three or more unrelated abnormalities, not including core binding factor rearrangements and the *PML/RARA* rearrangement), and other specified unbalanced and balanced abnormalities. AML-MRC is also characterized by a relatively high frequency of *ASXL1* mutations (35% of patients) and low frequencies of *FLT3* and *DNMT3A* mutations. Patients with *de-novo* AML-MRC tend to have a higher frequency of *TP53* mutations, and those with antecedent MDS or MDS/MPN had a higher frequency of *SETBP1*, *RUNX1*, and *SRSF2* mutations compared with the other AML categories; patients with AML-MRC tended to have a lower frequency of *SF3B1* mutations.[6-9] Patients with a known history of MDS or MDS/MPN are the easiest to diagnose, as they can be diagnosed based on clinical history. In patients without evidence of a previous chronic myeloid neoplasm, the assessment of multilineage dysplasia requires a skilled hematopathologist who is comfortable with the evaluation of dysplastic features, as well as adequate aspirate samples to judge morphologic changes and sufficient residual hematopoietic precursors to confidently comment on dysplastic features in 50% or more of the cells.[6-9] For these reasons, the 2022

ICC Classification introduced two distinct disease categories based on myelodysplasia-related genomic abnormalities, which can help clinicians to recognize these patients using more homogeneous and standardized criteria: 1) cases lacking *TP53* mutation, but with mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2* are categorized as "AML with myelodysplasia-related gene mutations" irrespective of any prior history of MDS AML with myelodysplasia-related gene mutations, and irrespective of any prior history of MDS – these mutations are closely associated with AML following prior MDS or MDS/MPN, and confer an adverse prognosis even if they occur in *de-novo* AML; and 2) the new category "AML with myelodysplasia-related cytogenetic abnormalities" includes cases previously classified as AML-MRC due to the presence of myelodysplasia-associated cytogenetic findings, but lacking *TP53* or myelodysplasia-related gene mutations.[1] Importantly, "AML with myelodysplasia-related gene mutations" is now categorized in the adverse-risk group by 2022 ELN Criteria. These mutations, typically associated with AML following an antecedent hematologic disease, are also prevalent in *de-novo* AML, and indicate adverse risk even in the absence of myelodysplasia-related cytogenetic abnormalities. Beyond the previously considered *ASXL1* and/or *RUNX1* genes, this category of myelodysplasia-related gene mutations now includes pathologic variants in at least one of the *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2* genes.[1]

The percentage of blasts cut-off to distinguish high-risk MDS *vs.* AML

Reassessment of the bone marrow blast percentage defining the boundary of high-risk MDS, and AML has been advocated for several cogent reasons, and in view of novel therapeutic approaches that show efficacy in patients currently classified as MDS or AML with 10-30% myeloid blasts. The pros and cons of merging high-risk MDS with AML and adopting a 10% cut-off were explored by ICC and WHO international expert panels.[6] In the 2022 ICC Classification, all recurrent genetic abnormalities that define specific subtypes of AML were considered to establish a diagnosis of AML if there are ≥10% blasts in the bone marrow or blood. The expert found that the clinical behavior of myeloid neoplasms with these rearrangements reflects

the specific genetic abnormality, even for cases presenting with <20% blasts. Although all other AML subtypes still require ≥20% blasts for diagnosis, a new category of myelodysplastic syndrome (MDS)/AML has been introduced in association with defined genomic abnormalities, to include cases with 10-19% blasts in the bone marrow or blood, and to recognize the fact that these cases lie on the border between AML and MDS in terms of biology and prognosis. As a practical consequence, patients diagnosed with MDS/AML should be eligible for either MDS or AML clinical trials and treatment approaches.[1] On the other hand, the 2022 WHO Classification of hematological neoplasms provided different recommendations. The expert panel found that lowering the blast cut-off to define AML would suffer from several challenges: 1) any blast-based cut-off is arbitrary and cannot reflect the biologic continuity naturally inherent in myeloid pathogenic mechanisms; 2) blast enumeration is subject to sampling variations/error and subjective evaluation; and 3) there is no gold standard for blast enumeration. Further, according to the WHO expert panel, an arbitrary cut-off of 10% blasts to define AML may carry a risk of overtreatment. A balanced approach was accordingly adopted by eliminating blast cut-offs for most AML types with defining genetic alterations but retaining a 20% blast cut-off to delineate MDS from AML. Notwithstanding, there was broad agreement that high risk MDS may be regarded as AML-equivalent for therapeutic considerations, and from a clinical trial design perspective when appropriate.[5]

AML with *TP53* mutations

TP53 is a critical tumor suppressor gene located on chromosome 17p13.1 that encodes the p53 protein, which, in response to cellular stress, including deoxyribonucleic acid (DNA) damage, increases in levels and ultimately induces the transcription of the genes responsible for DNA damage repair and cell cycle arrest/apoptosis, among other things. As a result, deficiency in the functional p53 protein predicted by mutations in or deletions of this "guardian of the genome" allows cells that would otherwise be destined for programmed cell death (apoptosis) to escape it and foster progression of the malignant disease.[10] At least 10% of patients with a new diagnosis of AML will have disease-harboring mutations in *TP53*, but up to 30% in certain subpopulations such as those with secondary AML or