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THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

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The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues was last updated in 2008. Since then, there have been numerous advances in the identification of unique biomarkers associated with some myeloid neoplasms and acute leukemias, largely derived from gene expression analysis and next-generation sequencing that can significantly improve the diagnostic criteria as well as the prognostic relevance of entities currently included in the WHO classification and that also suggest new entities that should be added. Therefore, there is a clear need for a revision to the current classification. The revisions to the categories of myeloid neoplasms and acute leukemia will be published in a monograph in 2016 and reflect a consensus of opinion of hematopathologists, hematologists, oncologists, and geneticists. The 2016 edition represents a revision of the prior classification rather than an entirely new classification and attempts to incorporate new clinical, prognostic, morphologic, immunophenotypic, and genetic data that have emerged since the last edition. The major changes in the classification and their rationale are presented here. (*Blood*. 2016; 127(20):2391-2405)

Introduction

In collaboration with the Society for Hematopathology and the European Association for Haematopathology, the World Health Organization (WHO) published the third and fourth editions of the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, in 2001 and 2008, respectively, as part of a series of *WHO Classification of Tumours* "blue book" monographs. In the spring of 2014, a clinical advisory committee (CAC) composed of ~100 pathologists, hematologists, oncologists, and geneticists from around the world convened to propose revisions to the fourth edition of the classification. The revision of the fourth edition follows the philosophy of the third and fourth editions to incorporate clinical features, morphology, immunophenotyping, cytogenetics, and molecular genetics to define disease entities of clinical significance. The fourth edition of the classification of hematopoietic and lymphoid tissues was the second volume of the WHO "blue book" tumor series, and the series publication is still in progress. A fifth edition series cannot begin until the fourth edition series is completed; but after 8 years of information and experience that have emerged from scientific and clinical studies, a revision of these criteria for hematopoietic and lymphoid neoplasms was felt to be necessary and timely. In relation to myeloid neoplasms and acute leukemia, this revision has been influenced by several factors including the following:

1. The discovery of recently identified molecular features has yielded new perspectives regarding diagnostic and prognostic markers that provide novel insights for the understanding of the pathobiology of these disorders.
2. Improved characterization and standardization of morphological features aiding in the differentiation of disease groups, particularly of the *BCR-ABL1*$^{-}$ myeloproliferative neoplasms (MPNs), has increased the reliability and reproducibility of di-agnoses.
3. A number of clinical-pathological studies have now validated the WHO postulate of an integrated approach that includes hematologic, morphologic, cytogenetic, and molecular genetic findings.

For these reasons, the fourth edition is being updated, but this 2016 classification is not a major overhaul of the disease categories. Rather, it is intended to incorporate new knowledge of these disorders obtained since the 2008 publication and is a revision of that classification. The purpose of this report is to summarize the major changes in the revised WHO classification of myeloid neoplasms and acute leukemia and to provide the rationale for those changes. Table 1 lists the major subtypes of myeloid neoplasms and acute leukemias according to the updated (2016) WHO classification.

Myeloproliferative neoplasms

The categories of MPNs have not significantly changed since the 2008 fourth edition of the classification, but discoveries of new mutations and improved understanding of the morphologic features of some entities have impacted the diagnostic criteria for the disease entities.

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Table 1. WHO classification of myeloid neoplasms and acute leukemia

WHO myeloid neoplasm and acute leukemia classification
Myeloproliferative neoplasms (MPN)
Chronic myeloid leukemia (CML), *BCR-ABL1*$^{+}$
Chronic neutrophilic leukemia (CNL)
Polycythemia vera (PV)
Primary myelofibrosis (PMF)
PMF, prefibrotic/early stage
PMF, overt fibrotic stage
Essential thrombocythemia (ET)
Chronic eosinophilic leukemia, not otherwise specified (NOS)
MPN, unclassifiable
Mastocytosis
Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1,* or with *PCM1-JAK2*
Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement
Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement
Myeloid/lymphoid neoplasms with *FGFR1* rearrangement
Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
Chronic myelomonocytic leukemia (CMML)
Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*$^{-}$
Juvenile myelomonocytic leukemia (JMML)
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
MDS/MPN, unclassifiable
Myelodysplastic syndromes (MDS)
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS)
MDS-RS and single lineage dysplasia
MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood
Myeloid neoplasms with germ line predisposition
Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
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*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1);*DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); *BCR-ABL1*
MPAL with t(v;11q23.3); *KMT2A* rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS
B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Mastocytosis, however, is no longer considered a subgroup of the MPNs due to its unique clinical and pathologic features, ranging from indolent cutaneous disease to aggressive systemic disease, and is now a separate disease category in the classification.

With regard to chronic myeloid leukemia (CML), BCR-ABL1^{+}, most cases of CML in chronic phase can be diagnosed from peripheral blood (PB) findings combined with detection of t(9;22)(q34.1;q11.2) or, more specifically, *BCR-ABL1* by molecular genetic techniques. However, a bone marrow (BM) aspirate is essential to ensure sufficient material for a complete karyotype and for morphologic evaluation to confirm the phase of disease.[1,2] In the era of tyrosine-kinase inhibitor (TKI) therapy, newly diagnosed patients may have a nearly normal lifespan, but regular monitoring for *BCR-ABL1* burden and for evidence of genetic evolution and development of resistance to TKI therapy is essential to detect disease progression.[3,4] Although the accelerated phase (AP) of CML is becoming less common in the era of TKI therapy, there are no universally accepted criteria for its definition. The criteria for AP in the revised WHO classification include hematologic, morphologic, and cytogenetic parameters which are supplemented by additional parameters usually attributed to genetic evolution,[5] and manifested by evidence of resistance to TKIs (see Table 2). These latter "response to TKI therapy" criteria for AP are considered as "provisional" until further supported by additional data. Diagnosis of blast phase (BP) still requires either at least 20% blasts in the blood or BM or the presence of an extramedullary accumulation of blasts. However, because the onset of lymphoid BP may be quite sudden, the detection of any bona fide lymphoblasts in the blood or marrow should raise concern for a possible impending lymphoid BP, and prompt additional laboratory and genetic studies to exclude this possibility.

In recent years, data have emerged that suggest the need for revisions to the diagnostic criteria for the *BCR-ABL1*$^{-}$ MPNs,[6] as many new findings have been demonstrated to have diagnostic and/or prognostic importance:

1. The discovery of novel molecular findings in addition to *JAK2* and *MPL* mutations, in particular the *CALR* mutation, provide proof of clonality, diagnostic importance, and influence prognosis.[7,8]
2. The *CSF3R* mutation is strongly associated with chronic neutrophilic leukemia (CNL) (see also "Myelodysplastic/myeloproliferative neoplasms").[9]

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Table 2. Criteria for CML, accelerated phase

CML, accelerated phase criteria	
Any 1 or more of the following hematologic/cytogenetic criteria or response-to-TKI criteria:	
• Persistent or increasing WBC (>10 × 10^9/L), unresponsive to therapy	**"Provisional" response-to-TKI criteria**
• Persistent or increasing splenomegaly, unresponsive to therapy	• Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response* to the first TKI) or
• Persistent thrombocytosis (>1000 × 10^9/L), unresponsive to therapy	• Any hematological, cytogenetic, or molecular indications of resistance to 2 sequential TKIs or
• Persistent thrombocytopenia (<100 × 10^9/L) unrelated to therapy	• Occurrence of 2 or more mutations in *BCR-ABL1* during TKI therapy
• 20% or more basophils in the PB	
• 10%-19% blasts† in the PB and/or BM	
• Additional clonal chromosomal abnormalities in Ph^+ cells at diagnosis that include "major route" abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2	
• Any new clonal chromosomal abnormality in Ph^+ cells that occurs during therapy	

Large clusters or sheets of small, abnormal megakaryocytes, associated with marked reticulin or collagen fibrosis in biopsy specimens may be considered as presumptive evidence of AP, although these findings are usually associated with 1 or more of the criteria listed above.

*Complete hematologic response: WBC, <10 × 10^9/L; platelet count, <450 × 10^9/L, no immature granulocytes in the differential, and spleen nonpalpable.

†The finding of bona fide lymphoblasts in the blood or marrow, even if <10%, should prompt concern that lymphoblastic transformation may be imminent and warrants further clinical and genetic investigation; 20% or more blasts in blood or BM, or an infiltrative proliferation of blasts in an extramedullary site is CML, blast phase.

3. Polycythemia vera (PV) is possibly underdiagnosed using the hemoglobin levels published in the fourth edition, and the utility of BM morphology as a reproducible criterion for the diagnosis of PV is recognized.[8,10,11]
4. It is necessary to differentiate "true" essential thrombocythemia (ET) from prefibrotic/early primary myelofibrosis (prePMF) by, among other features, the morphologic findings in the BM biopsy, including the lack of reticulin fibrosis at onset, and this distinction has prognostic implications.[12-14]
5. The minor clinical criteria in prePMF that may have a major impact not only on accurate diagnosis but also on prognosis need to be explicitly defined.[14,15]
6. Standardized morphologic criteria of MPNs are important to enhance interobserver reproducibility of morphologic diagnoses (which currently demonstrates consensus rates ranging between 76% and 88%, depending on the study design).[12,13,16-18]

The revised criteria for CNL, PV, ET, PMF, and prePMF are listed in Tables 3-7 in addition to a slightly modified grading of reticulin and collagen BM fibers (Table 8). It is important to emphasize that an accurate histologic diagnosis has been proven to be key to predict prognosis in this group of diseases.[13]

Mastocytosis

As mentioned, mastocytosis is no longer listed under the broad heading of MPNs. Major advances in the understanding of mastocytosis have been made since the 2008 classification,[19] and these are incorporated into the text of the monograph. Table 9[20] lists the 2016 categories of mastocytosis, which includes a shortening of the name of the 2008 category of "systemic mastocytosis with associated clonal hematological non-mast-cell lineage disease (SH-AHNMD)" to the 2016 category of "systemic mastocytosis with an associated hematological neoplasm (SM-AHN)." In many cases, the AHN is an aggressive neoplasm that must be treated and the diagnosis should clearly and separately indicate the presence of this disorder in a distinct diagnosis line.

Myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or with *PCM1-JAK2*

The criteria for the diagnosis of the eosinophilia-related proliferations associated with specific molecular genetic changes are retained in the classification, although it is noted that eosinophilia may be absent in a subset cases. In the 2016 revision (Table 10), this disease group will incorporate the myeloid neoplasm with t(8;9)(p22;p24.1);*PCM1-JAK2* as a new provisional entity.[21,22] This rare entity is characterized by a combination of eosinophilia with BM findings of left-shifted erythroid predominance, lymphoid aggregates, and often myelofibrosis, at times mimicking PMF. It can also rarely present as T- or B-lymphoblastic leukemia (acute lymphoblastic leukemia [ALL]) and responds to JAK inhibition.[23] Other *JAK2*-rearranged neoplasms, for example, t(9;12)(p24.1;p13.2);*ETV6-JAK2* and t(9;22)(p24.1;q11.2);*BCR-JAK2* may have similar features, but are uncommon and are not currently included as distinct entities. Moreover, *ETV6-JAK2* and *BCR-JAK2*–rearranged neoplasms present primarily as B-cell ALL (B-ALL), and these are best considered as *BCR-ABL1*–like B-ALL, a new provisional category of B-lymphoblastic leukemia/lymphoma.[22]

Myelodysplastic/myeloproliferative neoplasms

The myelodysplastic syndrome (MDS)/MPN category was introduced in the third edition to include myeloid neoplasms with clinical, laboratory, and morphologic features that overlap between MDS and MPN.[24] Based on accumulated scientific evidence, a provisional entity within the MDS/MPN unclassifiable group, refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T), has been accepted as a full entity, now termed MDS/MPN with ring sideroblasts and thrombocytosis in the 2016 revision. The 2016 revised criteria for diseases in this category are summarized in Tables 11-14.[25]

In MDS/MPN, the karyotype is often normal or shows abnormalities in common with MDS. Targeted sequencing of genes mutated in

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Table 3. Diagnostic criteria for CNL

CNL diagnostic criteria
1. PB WBC $\geq 25 \times 10^9$/L
Segmented neutrophils plus band forms ≥80% of WBCs
Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) <10% of WBC
Myeloblasts rarely observed
Monocyte count $<1 \times 10^9$/L
No dysgranulopoiesis
2. Hypercellular BM
Neutrophil granulocytes increased in percentage and number
Neutrophil maturation appears normal
Myeloblasts <5% of nucleated cells
3. Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PV, ET, or PMF
4. No rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or *PCM1-JAK2*
5. Presence of *CSF3R* T618I or other activating *CSF3R* mutation
or
In the absence of a *CSFR3R* mutation, persistent neutrophilia (at least 3 mo), splenomegaly and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies

myeloid neoplasms detects mutations in a high proportion of cases of chronic myelomonocytic leukemia (CMML) as well as other MDS/MPN patients.[26] The most commonly mutated genes in CMML are *SRSF2*, *TET2*, and/or *ASXL1* (>80% of cases).[26,27] Other mutations which occur at lower frequency include *SETBP1*, *NRAS/KRAS*, *RUNX1*, *CBL*, and *EZH2*.[28,29] They can be helpful adjunct studies in difficult cases, particularly given the frequently normal karyotype of CMML, but should not be used alone as proof of neoplasia because some of these mutations occur in healthy older patients as so-called clonal hematopoiesis of indeterminate potential (CHIP)[30,31] (for further discussion, see "Myelodysplastic syndromes"). *ASXL1* is a predictor of aggressive disease behavior and has been incorporated into a prognostic scoring system for CMML alongside karyotype and clinicopathologic parameters.[27] Of note, *NPM1* mutation is seen in a rare subset of CMML (3%-5%) and appears also to herald a more aggressive clinical course.

Chronic myelomonocytic leukemia

A diagnosis of CMML requires both the presence of persistent PB monocytosis $\geq 1 \times 10^9$/L and monocytes accounting for ≥10% of the white blood cell (WBC) differential count. Due to the discovery of molecular and clinical differences between the so-called "proliferative type" of CMML (WBC count $\geq 13 \times 10^9$/L) and the "dysplastic type" (WBC $<13 \times 10^9$/L), particularly those differences related to aberrancies in the RAS/MAPK signaling pathways,[32-34] the separation of CMML into these subtypes is warranted. In addition, blast percentage maintains clear prognostic importance in CMML as initially suggested in the third edition and later confirmed in the fourth edition. Recent evidence has shown that a more precise prognostication can be obtained with 3 blast-based groupings: CMML-0, a category for cases with <2% blasts in PB and <5% blasts in BM; CMML-1 for cases with 2% to 4% blasts in PB and/or 5% to 9% blasts in BM; and CMML-2 for cases with 5% to 19% blasts in PB, 10% to 19% in BM, and/or when any Auer rods are present.[33,35] The revision incorporates the CMML-0 category into the classification scheme. In view of the importance of separating promonocytes (blast equivalent cells) from monocytes, which can have abnormal features in CMML, precise morphologic evaluation is essential, with the appropriate integration of flow cytometry immunophenotyping and cytogenetic and molecular genetic testing. Because other disorders must be excluded before a diagnosis of CMML can be made, *BCR-ABL1* rearrangement should be excluded in all cases and *PDGFRA*, *PDGFRB*, *FGFR1* rearrangements or *PCM1-JAK2* fusions excluded if eosinophilia is present. A prior well-documented diagnosis of a MPN would also generally exclude CMML or another type of MDS/MPN.[36,37]

Atypical CML, *BCR-ABL1*$^-$

The rare MDS/MPN subtype atypical CML (aCML) is now better characterized molecularly and can be more easily separated from CNL, a rare subtype of MPN similarly characterized by neutrophilia. Although CNL is strongly associated with the presence of *CSF3R* mutations, these appear to be very rare in aCML (<10%).[38] Conversely, aCML is associated with *SETBP1* and/or *ETNK1* mutations in up to a third of cases.[28,39,40] The so-called MPN-associated driver mutations (*JAK2*, *CALR*, *MPL*) are typically absent in aCML.

Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis

The criteria for MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T; previously known as RARS-T) include thrombocytosis ($\geq 450 \times 10^9$/L) associated with refractory anemia, dyserythropoiesis in the BM with ring sideroblasts accounting for 15% or more of erythroid precursors, and megakaryocytes with features resembling those in PMF or ET. After the discovery that MDS/MPN-RS-T is frequently associated with mutations in the spliceosome gene *SF3B1* (which in turn are associated with the presence of ring sideroblasts), there is now enough evidence to support MDS/MPN-RS-T as a full entity.[41-44] In MDS/MPN-RS-T, *SF3B1* is often comutated with *JAK2* V617F or less frequently (<10%) with *CALR*, or *MPL* genes, thus providing a biological explanation for the true hybrid nature of this rare myeloid neoplasm. Unlike MDS with ring sideroblasts (see

Table 4. WHO criteria for PV

WHO PV criteria
Major criteria
1. Hemoglobin >16.5 g/dL in men
Hemoglobin >16.0 g/dL in women
or,
Hematocrit >49% in men
Hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†

*More than 25% above mean normal predicted value.

†Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

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Table 5. WHO criteria for ET

WHO ET criteria
Major criteria
1. Platelet count ≥450 × 10^9/L
2. BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR*, or *MPL* mutation
Minor criterion
Presence of a clonal marker or absence of evidence for reactive thrombocytosis
Diagnosis of ET requires meeting all 4 major criteria or the first 3 major criteria and the minor criterion

"Myelodysplastic syndromes"), the number of ring sideroblasts required for a diagnosis of MDS/MPN-RS-T is not altered by the presence or absence of a mutation in *SF3B1*. Because of changes in the MDS terminology (see "Myelodysplastic syndromes"), the name RARS-T was changed to MDS/MPN-RS-T.

Juvenile myelomonocytic leukemia

Juvenile myelomonocytic leukemia (JMML) is an aggressive clonal hematopoietic disorder of infancy and early childhood characterized by an excessive proliferation of cells of monocytic and granulocytic lineages that is included as a MDS/MPN subtype.[45,46] Approximately 90% of patients carry either somatic or germ line mutations of *PTPN11*, *KRAS*, *NRAS*, *CBL*, or *NF1*. These genetic aberrations are largely mutually exclusive and activate the RAS/MAPK pathway. The clinical and pathological findings of JMML are not substantially changed from the current WHO fourth edition (2008). However, molecular diagnostic parameters have been refined. The updated diagnostic findings are listed in Table 14.

Myelodysplastic syndromes

The MDS are a group of clonal BM neoplasms characterized by ineffective hematopoiesis, manifested by morphologic dysplasia in hematopoietic cells and by peripheral cytopenia(s). The revised classification introduces refinements in morphologic interpretation and cytopenia assessment and addresses the influence of rapidly accumulating genetic information in MDS diagnosis and classification.

Cytopenia is a "sine qua non" for any MDS diagnosis and in prior classifications, MDS nomenclature included references to "cytopenia" or to specific types of cytopenia (eg, "refractory anemia"). However, the WHO classification relies mainly on the degree of dysplasia and blast percentages for disease classification and specific cytopenias have only minor impact on MDS classification. Moreover, the lineage(s) manifesting significant morphologic dysplasia frequently do not correlate with the specific cytopenia(s) in individual MDS cases.[47-49] For these reasons, the terminology for adult MDS has changed to remove terms such as "refractory anemia" and "refractory cytopenia" and replaces them with "myelodysplastic syndrome" followed by the appropriate modifiers: single vs multilineage dysplasia, ring sideroblasts, excess blasts, or the del(5q) cytogenetic abnormality (see Table 15). There are no changes to childhood MDS; refractory cytopenia of childhood remains as a provisional entity within this category.

One of the biggest challenges in this category is separating MDS from reactive causes of cytopenia and dysplasia. Although the threshold to define dysplasia will remain as 10% dysplastic cells in any hematopoietic lineage, it is recognized that dysplasia in excess of 10% may occur in some normal individuals and even more frequently in nonneoplastic causes of cytopenia.[50,51] Moreover, identification of dysplasia is not always reproducible among even experienced hematopathologists.[52,53] For these reasons, possible reactive etiologies of dysplasia should always be carefully considered prior to making a diagnosis of MDS, particularly when the dysplasia is subtle and limited to 1 lineage. Some dysplastic changes, particularly the presence of micromegakaryocytes (which can be highlighted by immunostaining for megakaryocyte markers in the BM trephine), are relatively specific for myelodysplasia and have high reproducibility.[53]

Table 6. WHO criteria for prePMF

WHO prePMF criteria
Major criteria
1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1*, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for *BCR-ABL1*+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of minor reactive BM reticulin fibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 × 10^9/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

‡Minor (grade 1) reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

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Table 7. WHO criteria for overt PMF

WHO overt PMF criteria
Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*$^{+}$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq 11 \times 10^9$/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

‡BM fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

The myeloblast percentage, as determined by counting well-prepared, cellular BM aspirate smears and/or touch preparations and a PB smear, remains critical in defining the WHO MDS categories and as risk strata in the Revised International Prognostic Scoring System (IPSS-R).[54] The presence of 1% blasts in the PB, with <5% BM blasts, defines 1 type of MDS, unclassifiable (MDS-U). However, because 1% blasts may not be reproducible as a single observation, this finding must now be demonstrated on at least 2 separate occasions in order to diagnose MDS-U according to this criterion. There is a major change in the diagnostic criteria for myeloid neoplasms with erythroid predominance (erythroid precursors ≥50% of all BM cells). In the updated classification, the denominator used for calculating blast percentage in all myeloid neoplasms is all nucleated BM cells, not just the "nonerythroid cells." This will result in most cases previously diagnosed as the erythroid/myeloid subtype of acute erythroid leukemia now being classified as MDS with excess blasts, as discussed in "AML, not otherwise specified" (see Table 16).

Despite the lowering of the neutropenia prognostic threshold in the IPSS-R to 0.8×10^9/L,[54] the WHO thresholds defining cytopenia will remain as in the original IPSS (hemoglobin, <10 g/dL; platelets, $<100 \times 10^9$/L; absolute neutrophil count, $<1.8 \times 10^9$/L); a diagnosis of MDS may be made in rare cases with milder levels of cytopenia, but at least 1 cytopenia must be present in order to make the diagnosis. It should be noted that some ethnic groups may have a reference range for normal absolute neutrophil count that is lower than 1.8×10^9/L, and thus caution should be exercised in interpreting neutropenia if it is the only cytopenia. MDS-U will continue to include cases with single lineage dysplasia or isolated del(5q) and pancytopenia, but in such cases all PB counts must be below the WHO thresholds given in this paragraph.

The same cytogenetic abnormalities listed in the 2008 WHO classification[55] remain MDS-defining in a cytopenic patient, even in the absence of diagnostic morphologic dysplasia. In such cases, the abnormality must be demonstrated by conventional karyotyping, not by fluorescence in situ hybridization (FISH) or sequencing technologies. The presence of +8, −Y, or del(20q) is not considered to be MDS-defining in the absence of diagnostic morphologic features of MDS. In spite of the increased knowledge of the prognostic importance of genetic findings in MDS, del(5q) remains as the only cytogenetic or molecular genetic abnormality that defines a specific MDS subtype. Based on recent data showing no adverse effect of 1 chromosomal abnormality in addition to the del(5q),[56-58] the entity MDS with isolated del(5q) may be diagnosed if there is 1 additional cytogenetic abnormality besides the del(5q), unless that abnormality is monosomy 7 or del(7q). Even though cytogenetic findings are not used to define other specific subtypes of MDS, they are strongly correlated with prognosis, as reflected in the 5 cytogenetic prognostic groups in the IPSS-R scheme[54,58]; thus, a complete BM karyotype remains a critical test in any newly diagnosed MDS case.

As with all the other myeloid neoplasms, a large amount of data has recently become available on recurring mutations in MDS. Targeted sequencing of a limited number of genes can detect mutations in 80% to 90% of MDS patients; the most commonly mutated genes in MDS are *SF3B1*, *TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *TP53*, and *EZH2*.[59,60] Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of apparently healthy older individuals without MDS, so-called "clonal hematopoiesis of indeterminate potential" (CHIP).[30,31,61] Although some patients with CHIP subsequently develop MDS, the natural history of this condition is not yet fully understood; thus, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia, where these mutations may be commonly found.[62] Further study is required to determine the optimal management and monitoring of such patients and to investigate possible links between specific mutations, mutant allele

Table 8. Grading of myelofibrosis

Myelofibrosis grading	
MF-0	Scattered linear reticulin with no intersections (crossovers) corresponding to normal BM
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis*
MF-3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers consistent with collagen, usually associated with osteosclerosis*

Semiquantitative grading of BM fibrosis (MF) with minor modifications concerning collagen and osteosclerosis. Fiber density should be assessed only in hematopoietic areas.

*In grades MF-2 or MF-3 an additional trichrome stain is recommended.

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Table 9. WHO classification of mastocytosis

WHO mastocytosis classification
1. Cutaneous mastocytosis (CM)
2. Systemic mastocytosis
a. Indolent systemic mastocytosis (ISM)*
b. Smoldering systemic mastocytosis (SSM)*
c. Systemic mastocytosis with an associated hematological neoplasm (SM-AHN)†
d. Aggressive systemic mastocytosis (ASM)*
e. Mast cell leukemia (MCL)
3. Mast cell sarcoma (MCS)

*These subtypes require information regarding B and C findings for complete diagnosis,[20] all of which may not be available at the time of initial tissue diagnosis.

†This category is equivalent to the previously described "systemic mastocytosis with an associated clonal hematological non-mast cell lineage disease (SM-AHNMD)." AHNMD and AHN can be used synonymously.

fraction, or mutation combinations and subsequent development of bona fide MDS.[63] Rare cases of familial MDS are associated with germ line mutations, which can be investigated by sequencing non-MDS patient tissue.

The number and types of specific mutations are strongly associated with disease outcome in MDS, and the addition of mutation data improves the prognostic value of existing risk-stratification schemes in MDS.[64,65] *TP53* mutation is associated with aggressive disease in MDS in general[66] and appears to predict poorer response to lenalidomide in patients with del(5q).[67-69] Evaluation for *TP53* mutation is recommended in patients with MDS with isolated del(5q) to help identify an adverse prognostic subgroup in this generally favorable prognosis MDS entity.

With regard to MDS with ring sideroblasts (MDS-RS), recurrent mutations in the spliceosome gene *SF3B1* are frequent in MDS and are associated with the presence of ring sideroblasts. A change in the classification of MDS is the inclusion now of MDS cases with ring sideroblasts and multilineage dysplasia, lacking excess blasts or an isolated del(5q) abnormality, into the category of MDS-RS. This change is based largely on the link between ring sideroblasts and an *SF3B1* mutation, which appears to be an early event in MDS pathogenesis, manifests a distinct gene expression profile, and correlates with a favorable prognosis.[42,44,70-72] Recent studies have shown that in cases of MDS with any ring sideroblasts, the actual percentage of ring sideroblasts is not prognostically relevant.[73] Thus, in the revised classification, if an *SF3B1* mutation is identified, a diagnosis of MDS-RS may be made if ring sideroblasts comprise as few as 5% of nucleated erythroid cells, whereas at least 15% ring sideroblasts are still required in cases lacking a demonstrable *SF3B1* mutation. MDS-RS cases will be subdivided into cases with single lineage dysplasia (previously classified as refractory anemia with ring sideroblasts) and cases with multilineage dysplasia (previously classified as refractory cytopenia with multilineage dysplasia). Although MDS-RS cases lacking *SF3B1* mutation appear to have an adverse prognosis compared with those with the mutation, the role of multilineage dysplasia vs the *SF3B1* mutation in influencing outcome in MDS-RS remains controversial.[72,73]

Myeloid neoplasms with germ line predisposition

Although most cases of MDS or acute leukemia are sporadic diseases, it is becoming clear that a subgroup of cases is associated with germ line mutations and is familial.[74] A major change to the 2016 revision of the WHO classification is the addition of a section on myeloid neoplasms with germ line predisposition, which includes cases of MDS, MDS/MPN, and acute leukemias that occur on the background of a predisposing germ line mutation. The presence of the specific underlying genetic defect or predisposition syndrome should be noted as part of the diagnosis. Of note, germ line genetic aberrations are not unique to the patient with MDS or acute leukemia and should raise awareness of the need to screen family members for these aberrations. The major categories of such familial cases are summarized in Table 17.

Acute myeloid leukemia

AML with recurrent genetic abnormalities

The WHO continues to define specific acute myeloid leukemia (AML) disease entities by focusing on significant cytogenetic and molecular genetic subgroups. A large number of recurring, balanced cytogenetic abnormalities are recognized in AML, and most of those that are not formally recognized by the classification are rare.[75] The most common of these rare abnormalities that occur in pediatric patients are summarized in supplemental Table 1 (available on the *Blood* Web site), but these will not represent new disease categories. Minor refinements related to updates in gene names (such as the change from *MLL* to *KMT2A*) are included as well as recognition that the inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) does not represent a fusion gene, but repositions a distal *GATA2* enhancer to activate *MECOM* expression and simultaneously confer *GATA2* haploinsufficiency.[76,77] In order to stress the significance of the *PML-RARA* fusion, which may be cryptic or result

Table 10. Molecular genetic abnormalities in myeloid/lymphoid neoplasms associated with eosinophilia

Disease	Presentation	Genetics	Treatment
PDGFRA	Eosinophilia ↑Serum tryptase ↑Marrow mast cells	Cryptic deletion at 4q12 *FIP1L1-PDGFRA,* at least 66 other partners	Respond to TKI
PDGFRB	Eosinophilia Monocytosis mimicking CMML	t(5;12)(q32;p13.2) *ETV6-PDGFRB,* at least 25 other partners	Respond to TKI
FGFR1	Eosinophilia Often presents with T-ALL or AML	Translocations of 8p11.2 *FGFR1*-various partners	Poor prognosis; do not respond to TKI
PCM1-JAK2	Eosinophilia Rarely presents with T-LBL or B-ALL Bone marrow shows left-shifted erythroid predominance and lymphoid aggregates	t(8;9)(p22;p24.1) *PCM1-JAK2*	May respond to JAK2 inhibitors

↑, Increased.

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Table 11. Diagnostic criteria for CMML

CMML diagnostic criteria
• Persistent PB monocytosis ≥1 × 10^9/L, with monocytes accounting for ≥10% of the WBC count
• Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PMF, PV, or ET*
• No evidence of *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement or *PCM1-JAK2* (should be specifically excluded in cases with eosinophilia)
• <20% blasts in the blood and BM†
• Dysplasia in 1 or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and
• An acquired clonal cytogenetic or molecular genetic abnormality is present in hemopoietic cells‡
or
• The monocytosis (as previously defined) has persisted for at least 3 mo and
• All other causes of monocytosis have been excluded

*Cases of MPN can be associated with monocytosis or they can develop it during the course of the disease. These cases may simulate CMML. In these rare instances, a previous documented history of MPN excludes CMML, whereas the presence of MPN features in the BM and/or of MPN-associated mutations (*JAK2*, *CALR*, or *MPL*) tend to support MPN with monocytosis rather than CMML.

†Blasts and blast equivalents include myeloblasts, monoblasts, and promonocytes. Promonocytes are monocytic precursors with abundant light gray or slightly basophilic cytoplasm with a few scattered, fine lilac-colored granules, finely distributed, stippled nuclear chromatin, variably prominent nucleoli, and delicate nuclear folding or creasing. Abnormal monocytes, which can be present both in the PB and BM, are excluded from the blast count.

‡The presence of mutations in genes often associated with CMML (eg, *TET2*, *SRSF2*, *ASXL1*, *SETBP1*) in the proper clinical contest can be used to support a diagnosis. It should be noted however, that many of these mutations can be age-related or be present in subclones. Therefore, caution would have to be used in the interpretation of these genetic results.

from complex cytogenetic rearrangements other than t(15;17)(q24.1; q21.2), acute promyelocytic leukemia (APL) with this fusion is renamed as APL with *PML-RARA*. Finally, a new provisional category of AML with *BCR-ABL1* is added to recognize these rare de novo AML cases that may benefit from TKI therapy.[78,79] Although the diagnostic distinction between de novo AML with *BCR-ABL1* and blast transformation of CML may be difficult without adequate clinical information, the significance of detecting this targetable fusion is felt to warrant a provisional disease category. Preliminary data suggest that deletion of antigen receptor genes (IGH, TCR), *IKZF1* and/or *CDKN2A* may support a diagnosis of de novo disease vs BP of CML.[80]

Although the WHO authors struggled with how to incorporate the recent discoveries in gene mutations in AML,[81-83] the text for all disease categories is expanded to discuss the prognostic significance of various gene mutations and their frequency in the different AML subtypes. An updated table further summarizes the various genes mutated in AML and their significance (supplemental Table 2). The finding that the improved prognosis associated with AML with mutated *CEBPA* is associated with biallelic, but not single, mutations of the gene[84-88] has resulted in a change in that disease definition to require biallelic mutations. Additionally, due to the lack of prognostic significance of multilineage dysplasia in patients without MDS-associated cytogenetic findings and with a mutation of *NPM1* or biallelic mutation of *CEBPA*,[89-91] these mutations now supersede the presence of multilineage dysplasia in the classification. Finally, a provisional category of AML with mutated *RUNX1* has been added to the classification for cases of de novo AML with this mutation that are not associated with MDS-related cytogenetic abnormalities. This new provisional disease category appears to represent a biologically distinct group with a possibly worse prognosis than other AML types.[92-95]

AML with myelodysplasia-related changes

The category of AML with myelodysplasia-related changes has been retained, but is refined to better incorporate cases with features suggesting a poor prognosis. As mentioned, the presence of multilineage dysplasia alone will not classify a case as AML with myelodysplasia-related changes when a mutation of *NPM1* or biallelic mutation of *CEBPA* is present.[89-91] In cases lacking these mutations, the morphologic detection of multilineage dysplasia (defined as the presence of 50% or more dysplastic cells in at least 2 cell lines) remains a poor prognostic indicator and is sufficient to make a diagnosis of AML with myelodysplasia-related changes.[90,96,97] A history of MDS remains as an inclusion criterion for this category as does the presence of an MDS-related cytogenetic abnormality with 1 exception: del(9q) has been removed as a defining cytogenetic abnormality for AML with myelodysplasia-related changes because of its association with *NPM1* or biallelic *CEBPA* mutations[98,99] and its apparent lack of prognostic significance in those settings. Table 18 lists the cytogenetic abnormalities that now define AML with myelodysplasia-related changes.

Therapy-related myeloid neoplasms

Therapy-related myeloid neoplasms (t-MNs) remain as a distinct category in the classification for patients who develop myeloid neoplasms following cytotoxic therapy. The t-MNs may be further subdivided as therapy-related MDS or AML (t-MDS or t-AML), but the associated cytogenetic abnormality, which is important for determining therapy and prognosis, should be identified in the final diagnosis. A number of t-MN cases have been shown to have germ line mutations in cancer susceptibility genes; careful family histories to uncover cancer susceptibility are warranted in t-MN patients.[100]

AML, not otherwise specified

Although the subcategories of AML, not otherwise specified (NOS) lack prognostic significance when cases are classified based on *NPM1* mutation and *CEBPA* biallelic mutation status,[101] the CAC agreed to keep the AML, NOS subcategories with only a single change: the subcategory of acute erythroid leukemia, erythroid/myeloid type (previously defined as a case with ≥50% BM erythroid precursors and ≥20% myeloblasts among nonerythroid cells) has been removed from the AML category. In the new classification, myeloblasts are always counted as a percentage of total marrow cells and the majority of

Table 12. Diagnostic criteria for aCML, *BCR-ABL1*$^-$

aCML diagnostic criteria
• PB leukocytosis due to increased numbers of neutrophils and their precursors (promyelocytes, myelocytes, metamyelocytes) comprising ≥10% of leukocytes)
• Dysgranulopoiesis, which may include abnormal chromatin clumping
• No or minimal absolute basophilia; basophils usually <2% of leukocytes
• No or minimal absolute monocytosis; monocytes <10% of leukocytes
• Hypercellular BM with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
• <20% blasts in the blood and BM
• No evidence of *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement, or *PCM1-JAK2*
• Not meeting WHO criteria for *BCR-ABL1*$^+$ CML, PMF, PV, or ET*

*Cases of MPN, particularly those in accelerated phase and/or in post-polycythemic or post-essential thrombocythemic myelofibrosis, if neutrophilic, may simulate aCML. A previous history of MPN, the presence of MPN features in the BM and/or MPN-associated mutations (in *JAK2*, *CALR*, or *MPL*) tend to exclude a diagnosis of aCML. Conversely, a diagnosis of aCML is supported by the presence of *SETBP1* and/or *ETNK1* mutations. The presence of a *CSF3R* mutation is uncommon in aCML and if detected should prompt a careful morphologic review to exclude an alternative diagnosis of CNL or other myeloid neoplasm.

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Table 13. Diagnostic criteria for MDS/MPN with ring sideroblasts and thrombocytosis

MDS/MPN diagnostic criteria
• Anemia associated with erythroid lineage dysplasia with or without multilineage dysplasia, ≥15% ring sideroblasts,* <1% blasts in PB and <5% blasts in the BM
• Persistent thrombocytosis with platelet count ≥450 × 10^9/L
• Presence of a *SF3B1* mutation or, in the absence of *SF3B1* mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features†
• No *BCR-ABL1* fusion gene, no rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*; or *PCM1-JAK2*; no (3;3)(q21;q26), inv(3)(q21q26) or del(5q)‡
• No preceding history of MPN, MDS (except MDS-RS), or other type of MDS/MPN

*At least 15% ring sideroblasts required even if *SF3B1* mutation is detected.

†A diagnosis of MDS/MPN-RS-T is strongly supported by the presence of *SF3B1* mutation together with a mutation in *JAK2* V617F, *CALR*, or *MPL* genes.

‡In a case which otherwise fulfills the diagnostic criteria for MDS with isolated del (5q)-no or minimal absolute basophilia; basophils usually <2% of leukocytes.

such cases have <20% total blast cells and are now classified as MDS (usually MDS with excess blasts). This change was based on the close biologic relationship of erythroid/myeloid type acute erythroid leukemia to MDS in terms of its clinical presentation, morphologic features, and genetic abnormalities, as well as the low reproducibility of nonerythroid blast counts and an attempt to achieve uniformity in expressing blast percentages across all myeloid neoplasms.[102-106] Cases with ≥50% or more erythroid cells and ≥20% total myeloblasts usually meet criteria for AML with myelodysplasia-related changes and should be diagnosed as such; cases with ≥20% total myeloblasts not meeting criteria for AML with myelodysplasia-related changes or AML with recurrent genetic abnormalities should be categorized as 1 of the other subtypes of AML, NOS. Pure erythroid leukemia remains as an AML, NOS subtype and is now the only type of acute erythroid leukemia. Table 16 summarizes the current diagnostic approach to neoplastic marrow specimens with 50% or more erythroid precursors.

Myeloid sarcoma

Myeloid sarcoma remains in the classification as a unique clinical presentation of any subtype of AML. Myeloid sarcoma may present de novo, may accompany PB and marrow involvement, may present as relapse of AML, or may present as progression of a prior MDS, MPN, or MDS/MPN.[107] Although listed separately in the classification, cases of myeloid sarcoma without evidence of marrow disease should be investigated comprehensively so that they can be classified into a more specific AML subtype.

Myeloid proliferations of Down syndrome

The myeloid proliferations of Down syndrome include transient abnormal myelopoiesis (TAM) and myeloid leukemia associated with Down syndrome.[108,109] Both are usually megakaryoblastic proliferations, with TAM occurring at birth or within days of birth and resolving in 1 to 2 months and myeloid leukemia occurring later, but usually in the first 3 years of life with or without prior TAM and persisting if not treated. The myeloid neoplasms of Down syndrome have a similar behavior that is independent of blast cell count and these are not subclassified into MDS or AML. Both TAM and myeloid leukemia associated with Down syndrome are characterized by *GATA1* mutations and mutations of the JAK-STAT pathway, with additional mutations identified in the myeloid leukemia cases.[110]

Acute leukemias of ambiguous lineage

No new entities will be defined within this subgroup of acute leukemias. However, several studies have been published since the 2008 classification that have confirmed both the clinical relevance of the entity and its subdivision into genetic subgroups.[111,112] Although data are still preliminary, it appears that mixed phenotype acute leukemia (MPAL) with the t(9;22) can respond favorably to treatment that includes a TKI.[113,114]

The small list of specific lineage markers useful for defining MPAL is unchanged (Table 19), but it is now emphasized that in cases in which it is possible to resolve 2 distinct blast populations, it is not necessary that the specific markers be present, but only that each individual population would meet a definition for either a B, T, or myeloid leukemia. Similarly, cases of ALL or AML in which a diagnosis of MPAL is not being considered do not need to meet the more strict MPAL criteria in order to assign lineage; these criteria do not universally apply for the diagnosis of AML or ALL, but only for MPAL. It is also now recognized that some cases of otherwise typical B-ALL with homogeneous expression of lymphoid markers on a single blast population may express low-level myeloperoxidase using immunophenotypic methods without other evidence of myeloid differentiation. Because the clinical significance of this finding has not yet been established, it is recommended that care be taken before making a diagnosis of B/myeloid MPAL when low-intensity myeloperoxidase (MPO) is the only myeloid-associated feature. Multiparameter flow cytometry is the method of choice for recognizing MPAL; even when there are not 2 distinctly separable populations, most cases of MPAL will show heterogeneity of expression of some antigens such that MPO expression will be expressed on the subset of blasts that show relatively brighter expression of myeloid markers and lower intensity of B-cell–associated markers.

B-cell lymphoblastic leukemia/lymphoma (B-ALL)

Two important new provisional entities with recurrent genetic abnormalities have been recognized and incorporated into the

Table 14. Diagnostic criteria for JMML

JMML diagnostic criteria
I. Clinical and hematologic features (all 4 features mandatory)
• PB monocyte count ≥1 × 10^9/L
• Blast percentage in PB and BM <20%
• Splenomegaly
• Absence of Philadelphia chromosome (*BCR/ABL1* rearrangement)
II. Genetic studies (1 finding sufficient)
• Somatic mutation in *PTPN11** or *KRAS** or *NRAS**
• Clinical diagnosis of NF1 or *NF1* mutation
• Germ line *CBL* mutation and loss of heterozygosity of CBL†
III. For patients without genetic features, besides the clinical and hematologic features listed under I, the following criteria must be fulfilled:
• Monosomy 7 or any other chromosomal abnormality or at least 2 of the following criteria:
• Hemoglobin F increased for age
• Myeloid or erythroid precursors on PB smear
• GM-CSF hypersensitivity in colony assay
• Hyperphosphorylation of STAT5

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*Germ line mutations (indicating Noonan syndrome) need to be excluded.

†Occasional cases with heterozygous splice site mutations.

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Table 15. PB and BM findings and cytogenetics of MDS

Name	Dysplastic lineages	Cytopenias*	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia (MDS-SLD)	1	1 or 2	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2 or 3	1-3	<15%/<5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)					
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1	1 or 2	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2 or 3	1-3	≥15%/≥5%†	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1-3	1-2	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality except −7 or del (7q)
MDS with excess blasts (MDS-EB)					
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
with 1% blood blasts	1-3	1-3	None or any	BM <5%, PB = 1%,‡ no Auer rods	Any
with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1-3	<15%§	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

*Cytopenias defined as: hemoglobin, <10 g/dL; platelet count, $<100 \times 10^9$/L; and absolute neutrophil count, $<1.8 \times 10^9$/L. Rarely, MDS may present with mild anemia or thrombocytopenia above these levels. PB monocytes must be $<1 \times 10^9$/L

†If *SF3B1* mutation is present.

‡One percent PB blasts must be recorded on at least 2 separate occasions.

§Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD.

classification and these are discussed in more detail in the following sections. In addition, the classification of hypodiploid B-ALL now highlights the unique association between low hypodiploid ALL and *TP53* mutations that are often constitutional.[115,116]

B-ALL with intrachromosomal amplification of chromosome 21. This leukemia is characterized by amplification of a portion of chromosome 21, characteristically detected by FISH with a probe for the *RUNX1* gene that reveals 5 or more copies of the gene (or 3 or more

Table 16. Diagnostic approach to myeloid neoplasms when erythroid precursors comprise ≥50% of BM nucleated cells

BM erythroid precursors	Myeloblast % of all cells in BM (or PB)	Prior therapy?	Recurring WHO genetic abnormality?	Meets criteria for AML-MRC?	Fourth edition diagnosis	Updated fourth edition diagnosis
≥50%	NA	Yes	NA	NA	Therapy-related myeloid neoplasm	Therapy-related myeloid neoplasm
≥50%	≥20%	No	Yes	NA	AML with recurring genetic abnormality	AML with recurring genetic abnormality
≥50%	≥20%	No	No	Yes	AML with myelodysplasia-related changes	AML with myelodysplasia-related changes
≥50%	≥20%	No	No	No	AML, NOS, acute erythroid leukemia (erythroid/myeloid type)	AML, NOS (non erythroid subtype)
≥50%	<20%, but ≥20% of nonerythroid cells	No	No*	NA	AML, NOS, acute erythroid leukemia (erythroid/myeloid subtype)	MDS†
≥50%	<20%, and <20% of nonerythroid cells	No	No*	NA	MDS†	MDS†
>80% immature erythroid precursors with ≥30% proerythroblasts	<20%	No	No*	NA	AML, NOS, acute erythroid leukemia (pure erythroid type)	AML, NOS, acute erythroid leukemia (pure erythroid type)

AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; NA, not applicable.

*Cases of AML t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*, AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11* or APL with *PML-RARA*, may rarely occur in this setting with <20% blasts and those diagnoses would take precedence over a diagnosis of AML, NOS, or MDS.

†Classify based on myeloblast percentage of all BM cells and of PB leukocytes and other MDS criteria.

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Table 17. Classification of myeloid neoplasms with germ line predisposition

Myeloid neoplasm classification
Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction
AML with germ line *CEBPA* mutation
Myeloid neoplasms with germ line *DDX41* mutation*
Myeloid neoplasms with germ line predisposition and preexisting platelet disorders
Myeloid neoplasms with germ line *RUNX1* mutation*
Myeloid neoplasms with germ line *ANKRD26* mutation*
Myeloid neoplasms with germ line *ETV6* mutation*
Myeloid neoplasms with germ line predisposition and other organ dysfunction
Myeloid neoplasms with germ line *GATA2* mutation
Myeloid neoplasms associated with BM failure syndromes
Myeloid neoplasms associated with telomere biology disorders
JMML associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders
Myeloid neoplasms associated with Down syndrome*

*Lymphoid neoplasms also reported.

extra copies on a single abnormal chromosome 21 in metaphase FISH).[117,118] It occurs in about 2% of children with ALL, especially older children with low WBC counts. It is uncommon in adults. This new entity is associated with an adverse prognosis which can, to some extent, be overcome with more aggressive therapy.[117]

B-ALL with translocations involving tyrosine kinases or cytokine receptors ("BCR-ABL1–like ALL"). This newly recognized entity is assuming increasing importance because of its association with an adverse prognosis and responses of some cases to TKI therapies; however, it has been difficult to define in the clinical setting. It was originally described separately by different groups who demonstrated a series of cases of poor-prognosis childhood ALL with gene expression profiles similar to those seen in cases of ALL with *BCR-ABL1*,[119,120] though different algorithms applied to the same sets of cases did not classify all cases the same way.[121] Common features of *BCR-ABL1*–like ALL include translocations involving other tyrosine kinases, or alternatively translocations involving either the cytokine receptor-like factor 2 (*CRLF2*) or, less commonly, rearrangements leading to truncation and activation of the erythropoietin receptor (EPOR).[122] Cases with *CRLF2* translocations are often associated with *JAK* gene mutations and are particularly common in children with Down syndrome.[123] This translocation results in upregulation of the thymocyte stromal lymphopoietin receptor (TSLPR) gene product of *CRLF2* on leukemic cells that can readily be detected by flow cytometry.

The cases with translocations involving tyrosine kinase genes involve many different genes including *ABL1* (with partners other than *BCR*), as well as other kinases including *ABL2*, *PDGFRB*, *NTRK3*, *TYK2*, *CSF1R*, and *JAK2*.[124] Over 30 different partner genes have been described. Some patients, especially those with *EBF1-PDGFRB* translocations, have shown remarkable responses to TKI therapy, even after failing conventional therapy.[125]

Patients with *BCR-ABL1*–like ALL show a high frequency of loss of *IKZF1* and *CDKN2A/B*, but these deletions also occur in high frequency in other types of ALL as well.[121]

T-cell lymphoblastic leukemia/lymphoma (T-ALL)

Although there has been considerable investigation into genetic mechanisms of T-cell ALL (T-ALL) over the past decade, with the ability to identify nonoverlapping genetic subgroups of T-ALL that can, to some extent, be matched to stages of differentiation,[126] assays to measure these are not yet standard and the prognostic implications still controversial; thus, most differentiation stage subgroups are not formally included in the classification. However, 1 subset with unique biology is recognized as a new provisional entity (see next paragraph). Indolent T-lymphoblastic proliferation, which was briefly mentioned in the fourth edition classification, is now a more readily recognized nonneoplastic entity that may mimic T-lymphoblastic lymphoma.[127] It typically involves lymphoid tissue of the upper aerodigestive tract but may occur in other locations. Local recurrences are common and systemic dissemination is rare. Histologic examination of involved lymph nodes shows infiltration and sometimes replacement by proliferations of lymphoblasts that are less cytologically atypical than the usual T-lymphoblastic lymphoma. Although the blasts have an immature thymic phenotype that can be demonstrated by TdT staining in lymph nodes, the phenotype reflects a developmentally normal, nonaberrant phenotype and the proliferations are not clonal. These latter features allow this indolent entity to be distinguished from T-lymphoblastic lymphoma.

Early T-precursor (ETP) ALL leukemia has a unique immunophenotypic and genetic makeup indicating only limited

Table 18. Cytogenetic abnormalities sufficient to diagnose AML with myelodysplasia-related changes when ≥20% PB or BM blasts are present and prior therapy has been excluded

Cytogenetic abnormalities
Complex karyotype (3 or more abnormalities)
Unbalanced abnormalities
−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
t(11;16)(q23.3;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.2)
t(2;11)(p21;q23.3)
t(5;12)(q32;p13.2)
t(5;7)(q32;q11.2)
t(5;17)(q32;p13.2)
t(5;10)(q32;q21.2)
t(3;5)(q25.3;q35.1)

Table 19. Criteria for lineage assignment for a diagnosis of MPAL

Lineage assignment criteria
Myeloid lineage
MPO* (flow cytometry, immunohistochemistry, or cytochemistry)
or
Monocytic differentiation (at least 2 of the following: nonspecific esterase cytochemistry, CD11c, CD14, CD64, lysozyme)
T-lineage
Strong† cytoplasmic CD3 (with antibodies to CD3 ε chain)
or
Surface CD3
B-lineage
Strong† CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10
or
Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10

*See "Acute leukemias of ambiguous lineage" for caveats related to weaker antigen expression, or to expression by immunohistochemistry only.

†Strong defined as equal or brighter than the normal B or T cells in the sample.

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early T-cell differentiation, with retention of some myeloid and stem cell characteristics at both the immunophenotypic and genetic level.[128-131] By definition, blasts in ETP ALL express CD7 but lack CD1a and CD8, and are positive for 1 or more of the myeloid/stem cell markers CD34, CD117, HLADR, CD13, CD33, CD11b, or CD65.[128] They typically also express CD2 and cytoplasmic CD3 and may express CD4, but these are not part of the definition. CD5 is often negative and when positive is present on <75% of the blast population. Myeloid-associated gene mutations, such as *FLT3*, *NRAS/KRAS*, *DNMT3A*, *IDH1*, and *IDH2*, are reported at high frequency in ETP ALL,[129,130] whereas more typical T-ALL–associated mutations such as activating mutations in *NOTCH1* or mutations in *CDKN1/2* are infrequent.[131] Although initial small series of ETP ALL suggested that outcome was very poor,[128,132] more recent larger series with more effective therapy showed either a small but statistically nonsignificant difference in outcome,[133] or, in the largest series to date, no prognostic significance.[134]

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