Perspective

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

Daniel A. Arber¹, Attilio Orazi², Robert Hasserjian³, Jürgen Thiele⁴, Michael J. Borowitz⁵, Michelle M. Le Beau⁶, Clara D. Bloomfield⁷, Mario Cazzola⁸, James W. Vardiman⁶

¹. Stanford University, Stanford, CA
². Weill Cornell Medical College, New York, NY
³. Massachusetts General Hospital, Boston, MA
⁴. University of Cologne, Cologne, Germany
⁵. John Hopkins Medical Institutions, Baltimore, MD
⁶. University of Chicago, Chicago, IL
⁷. The Ohio State University, Columbus, OH
⁸. Department of Molecular Medicine, University of Pavia, and Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Short title: 2016 WHO Classification of myeloid neoplasms

Corresponding author:

Daniel A. Arber, MD
Department of Pathology
Stanford University
300 Pasteur Dr. H1401 M/C 5627
Stanford, CA 94305, USA

Scientific category: Myeloid Neoplasia

Abstract: 174 words
Text: 5960 words
References: 134
Abstract
The World Health Organization (WHO) Classification of Tumours of the Haematopoietic 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 (NGS) 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.

Introduction
In collaboration with the Society for Hematopathology and the European Association of Haematopathology, the World Health Organization (WHO) published the 3rd and 4th editions of a Classification of Tumors of the Haematopoietic and Lymphoid Tissues, in 2001 and 2008, respectively, as part of a series of WHO Classification of Tumors “blue book” monographs. In the spring of 2014, a clinical advisory committee (CAC) composed of approximately 100 pathologists, hematologists, oncologists, and geneticists from around the world convened to propose revisions to the 4th edition of the classification. The revision of the 4th edition follows the philosophy of the 3rd and 4th editions to incorporate clinical features, morphology, immunophenotyping, cytogenetics and molecular genetics to define disease entities of clinical significance. The 4th edition of the Classification of Haematopoietic and Lymphoid Tissues was the second volume offer the WHO “blue book” tumor series, and the series publication is still in progress. A 5th edition series cannot begin until the 4th 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–negative myeloproliferative neoplasms (MPNs), has increased the reliability and reproducibility of diagnoses.

(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 4th 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 4th 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.

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.

**Chronic myeloid leukemia, BCR-ABL1–positive (CML)**

Most cases of CML in chronic phase can be diagnosed from peripheral blood 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 life span, 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 \(BCR-ABL1\)–negative 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 association with chronic neutrophilic leukemia (CNL) (see also section on myelodysplastic/myeloproliferative neoplasms [MDS/MPN])\(^9\).
3. Polycythemia vera (PV) is possibly under-diagnosed using the hemoglobin levels published in the 4th edition, and of 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 inter-observer 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, 4, 5, 6 and 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.

**Mastocytosis**

As mentioned above, 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 and these are incorporated into the text of the monograph. Table 9 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 rearrangements 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;q24.1); PCM1-JAK2 as a new provisional entity. 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 (ALL) and responds to JAK inhibition. Other JAK2-rearranged neoplasms, e.g. 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-ALL, and these are best considered as BCR-ABL1-like B-ALL, a new provisional category of B-lymphoblastic leukemia/lymphoma.

**Myelodysplastic/myeloproliferative neoplasms**

The MDS/MPN category was introduced in the 3rd edition to include myeloid neoplasms with clinical, laboratory, and morphologic features that overlap between myelodysplastic syndromes (MDS) and MPN. 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.

In MDS/MPN, the karyotype is often normal or shows abnormalities in common with MDS. Targeted sequencing of genes mutated in myeloid neoplasms detects mutations in a high proportion of cases of chronic myelomonocytic leukemia (CMML) as well as other MDS/MPN patients. The most commonly mutated genes in CMML are SRSF2, TET2, and/or ASXL1 (>80% of cases). Other mutations which occur at lower frequency include SETBP1, NRAS/KRAS, RUNX1, CBL, and EZH2. 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) (for further discussion, see the section on MDS). ASXL1 is a predictor of aggressive disease behavior and has been incorporated into a prognostic scoring system for CMML alongside karyotype and clinicopathologic parameters. 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 ≥1×10^9/L and monocytes accounting for ≥10% of the white blood cell differential count. Due to the discovery of molecular and clinical differences between the so-called "proliferative type" of CMML (white blood cell count [WBC] ≥ 13 x 10^9/L) and the "dysplastic type" (WBC <13 x 10^9/L), particularly those differences related to aberrancies in the RAS/MAPK signaling pathways, the separation of CMML into these subtypes is warranted. In addition, blast percentage maintains clear prognostic importance in CMML as initially suggested in the 3rd edition and later confirmed in the 4th edition. Recent evidence has shown that a more precise prognostication can be obtained with three blast-based groupings: CMML-0, a category for cases with <2% blasts in PB and <5% blasts in BM; CMML-1 for cases with 2–4% blasts in PB and/or 5–9% blasts in BM; and CMML-2 for cases with 5–19% blasts in PB, 10–19% in BM, and/or when any Auer rods are present. 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 PDGFRα, PDGFRβ, FGFRI rearrangements or PCM1-JAK2 fusions excluded if eosinophilia is present. A prior well-documented diagnosis of an MPN would also generally exclude CMML or another type of MDS/MPN.

**Atypical CML, BCR-ABL1-negative (aCML)**

The rare MDS/MPN subtype aCML is now better characterized molecularly and can be more easily separated from CNL, a rare subtype of MPN similarly characterized by neutrophilia. While CNL is strongly associated with the presence of CSF3R mutations, these appear to be very rare in aCML (<10%) \(^{36}\). Conversely, aCML is associated with SETBP1 and/or ETNK1 mutations in up to a third of cases \(^{26,37,38}\). 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 (>450 x 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 \(^{39-42}\). In MDS/MPN-RS-T, SF3B1 is often co-mutated 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 discussion of MDS below), 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 below), the name RARS-T was changed to MDS/MPN-RS-T.

**Juvenile myelomonocytic leukemia (JMML)**

Juvenile myelomonocytic leukaemia 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. \(^{43,44}\) Approximately 90% of patients carry either somatic or germline mutations of PTPN11, KRAS, NRAS, CBL or NF1. These genetic aberrations are largely mutually exclusive and activate the RAS/mitogen-activated protein kinase (MAPK)
pathway. The clinical and pathological findings of JMML are not substantially changed from the current WHO 4th 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 (e.g. "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. 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 versus 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 non-neoplastic causes of cytopenia. Moreover, identification of dysplasia is not always reproducible among even experienced hematopathologists. 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 one 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.
The myeloblast percentage, as determined by counting well-prepared, cellular BM aspirate smears and/or touch preparations and a peripheral blood smear, remains critical in defining the WHO MDS categories and as risk strata in the Revised International Prognostic Scoring System (IPSS-R)\textsuperscript{52}. The presence of 1% blasts in the PB, with <5% BM blasts, defines one 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 two 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 \( \geq 50\% \) of all BM cells). In the updated classification, the denominator used for calculating blast percentage in all myeloid neoplasms is all nucleated bone marrow cells, not just the "non-erythroid 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 below in the section on acute myeloid leukemia (AML), not otherwise specified (NOS) (see Table 16).

Despite the lowering of the neutropenia prognostic threshold in the IPSS-R to 0.8 \( \times 10^9/L \)\textsuperscript{52}, the WHO thresholds defining cytopenia will remain as in the original IPSS (hemoglobin <10g/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 one 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 \( \times 10^9/L \), 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 above.

The same cytogenetic abnormalities listed in the 2008 WHO Classification\textsuperscript{53} 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 one chromosomal abnormality in addition to the del(5q)\textsuperscript{54-56}, the entity, MDS with isolated del(5q),
may be diagnosed if there is one 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; 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 80-90% of MDS patients; the most commonly mutated genes in MDS are SF3B1, TET2, SRSF2, ASXL1, DNMT3A, RUNX1, U2AF1, TP53, and EZH2. 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). 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. Further study is required to determine the optimal management and monitoring of such patients and to investigate possible links between specific mutations, mutant allele fraction, or mutation combinations and subsequent development of bona fide MDS. Rare cases of familial MDS are associated with germline 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. TP53 mutation is associated with aggressive disease in MDS in general and appears to predict poorer response to lenalidomide in patients with del(5q). 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.

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. Recent studies have shown that in cases of MDS with any ring sideroblasts, the actual percentage of ring sideroblasts is not prognostically relevant. 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, while 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 to those with the mutation, the role of multilineage dysplasia versus the SF3B1 mutation in influencing outcome in MDS-RS remains controversial.

Myeloid Neoplasms with Germline Predisposition

While most cases of MDS or acute leukemia are sporadic diseases, it is becoming clear that a subgroup of cases is associated with germline mutations and is familial. A major change to the 2016 revision of the WHO classification is the addition of a section on myeloid neoplasms with germline predisposition, which includes cases of MDS, MDS/MPN and acute leukemias that occur on the background of a predisposing germline mutation. The presence of the specific underlying genetic defect or predisposition syndrome should be noted as part of the diagnosis. Of note, germline 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 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. The most common of these rare abnormalities that occur in pediatric patients are summarized in Supplemental Table 1, 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)(q21.3;q26.2) 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. In order to stress the significance of the PML-RARA fusion, which may be cryptic or result 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 tyrosine kinase inhibitor therapy. While 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 versus blast phase of CML.

While the WHO authors struggled with how to incorporate the recent discoveries in gene mutations in AML, 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 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, 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.

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 above, 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. In cases lacking these mutations, the morphologic detection of multilineage dysplasia (defined as the
presence of 50% or more dysplastic cells in at least two cell lines) remains a poor prognostic indicator and is sufficient to make a diagnosis of AML with myelodysplasia-related changes. A history of MDS remains as an inclusion criterion for this category as does the presence of an MDS-related cytogenetic abnormality with one 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 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 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 germline mutations in cancer susceptibility genes; careful family histories to uncover cancer susceptibility are warranted in t-MN patients.

**AML, not otherwise specified**

Although the subcategories of AML, NOS lack prognostic significance when cases are classified based on NPM1 mutation and CEBPA biallelic mutation status, 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 non-erythroid 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 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 non-erythroid blast counts and an attempt to achieve uniformity in expressing blast percentages across all myeloid neoplasms. 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 one 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 peripheral blood and marrow involvement, may present as relapse of AML, or may present as progression of a prior MDS, MPN, or MDS/MPN.\(^{105}\) 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.\(^{106,107}\) Both are usually megakaryoblastic proliferations, with TAM occurring at birth or within days of birth and resolving in one to two months and myeloid leukemia occurring later, but usually in the first three 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.\(^{108}\)

**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\(^ {109,110}\). 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\(^ {111,112}\).

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 two 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 MPO is the only myeloid-associated feature. Multiparameter flow cytometry is the method of choice for recognizing MPAL; even when there are not two 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 classification and these are discussed in more detail below. In addition, the classification of hypodiploid B-ALL now highlights the unique association between low hypodiploid ALL and TP53 mutations that are often constitutional.

**B-ALL with intrachromosomosal amplification of chromosome 21 (iAMP21).**

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 extra copies on a single abnormal chromosome 21 in metaphase FISH). 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.

**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, though different algorithms applied to
the same sets of cases did not classify all cases the same way\textsuperscript{119}. 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 ($EPOR$)\textsuperscript{120}. Cases with $CRLF2$ translocations are often associated with $JAK$ gene mutations and are particularly common in children with Down syndrome\textsuperscript{121}. 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 $JAK2$\textsuperscript{122}. 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\textsuperscript{123}.

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\textsuperscript{119}.

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

Although there has been considerable investigation into genetic mechanisms of T-ALL over the past decade, with the ability to identify non-overlapping genetic subgroups of T-ALL that can, to some extent, be matched to stages of differentiation\textsuperscript{124}, 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, one subset with unique biology is recognized as a new provisional entity (see below). Indolent T-lymphoblastic proliferation, which was briefly mentioned in the 4\textsuperscript{th} edition classification, is now a more readily recognized non-neoplastic entity that may mimic T-lymphoblastic lymphoma\textsuperscript{125}. 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, non-aberrant 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

This leukemia has a unique immunophenotypic and genetic makeup indicating only limited early T-cell differentiation, with retention of some myeloid and stem cell characteristics at both the immunophenotypic and genetic level\textsuperscript{126-129}. By definition, blasts in ETP ALL express CD7 but lack CD1a and CD8, and are positive for one or more of the myeloid/stem cell markers CD34, CD117, HLADR, CD13, CD33, CD11b or CD65\textsuperscript{126}. 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 $\textit{FLT3}$, $\textit{NRAS/KRAS}$, $\textit{DNMT3A}$, $\textit{IDH1}$, and $\textit{IDH2}$, are reported at high frequency in ETP ALL\textsuperscript{127,128}, while more typical T-ALL-associated mutations such as activating mutations in $\textit{NOTCH1}$ or mutations in $\textit{CDKN1A/2}$ are infrequent\textsuperscript{129}. While initial small series of ETP ALL suggested that outcome was very poor\textsuperscript{126,130}, more recent larger series with more effective therapy showed either a small but statistically non-significant difference in outcome\textsuperscript{131}, or, in the largest series to date, no prognostic significance\textsuperscript{132}.

Authorship

All authors were involved in the writing and editing of the manuscript.

Conflict of interest statement

None of the authors have any conflict of interest to disclose in regards to the current manuscript.

Acknowledgement: The authors acknowledge the unrestricted financial support of the Clinical Advisory Committee meeting held in Chicago, IL, March 31-April 1, 2014 from the following organizations: American Society of Hematology, Joseph Carreras Foundation, Fondazione Italiana Linfomi (FIL), Leukemia Clinical Research Foundation, University of Chicago Comprehensive Cancer Center, Beckman Coulter Corporation, Celgene Corporation, Dako, Genentech Corporation, Incyte Corporation, Leica Corporation, Millennium Pharmaceuticals, Pharmacyclics, Seattle Genetics Corporation, Sysmex Corporation, and Ventana Medical Systems, Inc, a member of the Roche Group.
References:


26. Meggendorfer M, Bacher U, Alpermann T, et al. SETBP1 mutations occur in 9% of MDS/MPN and in 4% of MPN cases and are strongly associated with atypical CML, monosomy 7, isochromosome i(17)(q10), ASXL1 and CBL mutations. *Leukemia.* 2013;27(9):1852-1860.


### Table 1. WHO classification of myeloid neoplasms and acute leukemia

#### Myeloproliferative neoplasms (MPN)
- Chronic myeloid leukemia, *BCR-ABL1*–positive
- Chronic neutrophilic leukemia
- Polycythemia vera
- Primary myelofibrosis (PMF)
  - Primary myelofibrosis, prefibrotic/early stage
  - Primary myelofibrosis, overt fibrotic stage
- Essential thrombocythemia
- Chronic eosinophilic leukemia, not otherwise specified (NOS)
- Myeloproliferative neoplasm, unclassifiable

#### Mastocytosis

#### Myeloid/lymphoid neoplasms with eosinophilia and abnormalities 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
- Atypical chronic myeloid leukemia, *BCR-ABL1*–negative
- Juvenile myelomonocytic leukemia
- Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable

#### Myelodysplastic syndromes (MDS)
- MDS with single lineage dysplasia
- MDS with ring sideroblasts
  - MDS with ring sideroblasts and single lineage dysplasia
MDS with ring sideroblasts 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 germline predisposition

Acute myeloid leukemia and related neoplasms

Acute myeloid leukemia with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1); RUNXI-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 RUNXI

Acute myeloid leukemia with myelodysplasia-related changes

Therapy-related myeloid neoplasms

Acute myeloid leukemia, 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
- Myeloid leukemia associated with Down syndrome

Acute leukemias of ambiguous lineage

Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34.1;q11.2); BCR-ABL1
- Mixed phenotype acute leukemia with t(v;11q23.3); MLL rearranged
- Mixed phenotype acute leukemia, B/myeloid, NOS
Mixed phenotype acute leukemia, 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*
Table 2. Criteria for CML, Accelerated Phase
Any one or more of the following hematologic/cytogenetic criteria or response-to-TKI criteria:

- Persistent or increasing WBC (>10 x 10^9/L), unresponsive to therapy
- Persistent or increasing splenomegaly, unresponsive to therapy
- Persistent thrombocytosis (>1000 x 10^9/L), unresponsive to therapy
- Persistent thrombocytopenia (<100 x 10^9/L) unrelated to therapy
- 20% or more basophils in the peripheral blood
- 10-19% blasts ** in the peripheral blood and/or bone marrow
- 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

**Provisional” Response-to-TKI Criteria

- Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response *** to the first TKI) or
- Any hematological, cytogenetic or molecular indications of resistance to two sequential TKIs
- Occurrence of two or more mutations in BCR-ABL1 during TKI 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 one or more of the criteria listed above.

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

***Complete hematologic response: WBC <10 x 10^9/L, Platelet count <450 x 10^9/L, no immature granulocytes in the differential, and spleen non-palpable.

Table 3. Diagnostic criteria for chronic neutrophilic leukaemia (CNL)

1. Peripheral blood WBC ≥25 x 10^9/L
   - Segmented neutrophils plus band forms ≥80% of white blood cells
   - Neutrophil precursors (promyelocytes, myelocytes and metamyelocytes) <10% of WBC
   - Myeloblasts rarely observed
   - Monocyte count <1 x 10^9/L
   - No dysgranulopoiesis

2. Hypercellular bone marrow
   - Neutrophil granulocytes increased in percentage and number
   - Neutrophil maturation appears normal
   - Myeloblasts less than 5% of nucleated cells

3. Not meeting WHO criteria for BCR-ABL1 positive 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 months), 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

Table 4. WHO criteria for polycythemia vera (PV)

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. Bone marrow 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 JAK2V617F or JAK2 exon 12 mutation

Minor criterion:
Subnormal serum erythropoietin level

Diagnosis of PV requires meeting either all three major criteria, or the first two major criteria and the minor criterion**

* > 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)
### Table 5. WHO criteria for essential thrombocythemia (ET)

**Major criteria:**

1. Platelet count $> 450 \times 10^9$/L

2. Bone marrow 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 four major criteria or the first three major criteria and the minor criterion.**

---

From [www.bloodjournal.org](http://www.bloodjournal.org) by guest on November 16, 2017. For personal use only.
Table 6. WHO criteria for prefibrotic/early primary myelofibrosis (prePMF)

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 one of the following, confirmed in two consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis > 11 x 10^9/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range

Diagnosis of prePMF requires meeting all three major criteria, and at least one minor criterion

* see Table 8

**in the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (e.g. 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
Table 7. WHO criteria for overt primary myelofibrosis (PMF)

**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 one of the following, confirmed in two consecutive determinations:

a. Anemia not attributed to a comorbid condition
b. Leukocytosis ≥11 x 10⁹/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 three major criteria, and at least one minor criterion**

* see Table 8

** in the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (e.g. 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
Table 8. Grading of myelofibrosis: Semiquantitative grading of BM fibrosis (MF) with minor modifications concerning collagen and osteosclerosis*

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-0</td>
<td>Scattered linear reticulin with no intersections (cross-overs) corresponding to normal BM</td>
</tr>
<tr>
<td>MF-1</td>
<td>Loose network of reticulin with many intersections, especially in perivascular areas</td>
</tr>
<tr>
<td>MF-2</td>
<td>Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis**</td>
</tr>
<tr>
<td>MF-3</td>
<td>Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers consistent with collagen, usually associated with osteosclerosis**</td>
</tr>
</tbody>
</table>

* Fiber density should be assessed only in hematopoietic areas

** In grades MF-2 or MF-3 an additional trichrome stain is recommended
Table 9. WHO classification of mastocytosis

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 leukaemia (MCL)

3. Mast cell sarcoma (MCS)

*These subtypes require information regarding B and C findings for complete diagnosis, 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.
Table 10. Molecular genetic abnormalities in myeloid/lymphoid neoplasms associated with eosinophilia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Presentation</th>
<th>Genetics</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFRA</td>
<td>Eosinophilia</td>
<td>Cryptic deletion at 4q12</td>
<td>Respond to TKI</td>
</tr>
<tr>
<td></td>
<td>↑ Serum tryptase</td>
<td><em>FIP1L1-PDGFR</em>, at least 666 other partners</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Marrow mast cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGFRB</td>
<td>Eosinophilia</td>
<td>t(5;12)(q31~33;p12)</td>
<td>Respond to TKI</td>
</tr>
<tr>
<td></td>
<td>Monocytosis mimicking CMML</td>
<td><em>ETV6-PDGFR</em>, at least 25 other partners</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR1</td>
<td>Eosinophilia</td>
<td>Translocations of 8p11</td>
<td>Poor prognosis; do not respond to TKI</td>
</tr>
<tr>
<td></td>
<td>Often presents with T-ALL or AML</td>
<td><em>FGFR1</em>-various partners</td>
<td></td>
</tr>
<tr>
<td>PCMI-JAK2</td>
<td>Eosinophilia</td>
<td>t(8;9)(p22;p24.1)</td>
<td>May respond to JAK2 inhibitors</td>
</tr>
<tr>
<td></td>
<td>Often presents with T-LBL or B-ALL</td>
<td><em>PCMI-JAK2</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone marrow shows left-shifted erythroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>predominance and lymphoid aggregates</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↑, increased; TKI, tyrosine kinase inhibitors; CMML, chronic myelomonocytic leukemia; T-ALL, T lymphoblastic leukemia/lymphoma; AML, acute myeloid leukemia; B-ALL, B lymphoblastic leukemia/lymphoma
Table 11. Diagnostic criteria for chronic myelomonocytic leukaemia

- Persistent PB monocytosis $\geq 1 \times 10^9/L$, with monocytes accounting for $\geq 10\%$ of the white blood cell count
- Not meeting WHO criteria for BCR-ABL1-positive CML, primary myelofibrosis, polycythaemia vera or essential thrombocythaemia
- No evidence of PDGFA, PDGFRB or FGFR1 rearrangement or PCM1-JAK2 (should be specifically excluded in cases with eosinophilia)
- Fewer than 20% blasts in the blood and bone marrow
- Dysplasia in one 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 haemopoietic cells

OR

- the monocytosis (as previously defined) has persisted for at least 3 months 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, while the presence of MPN features in the bone marrow 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 grey or slightly basophilic cytoplasm with a few scattered, fine lilac-coloured 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 (e.g. 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.
Table 12. Diagnostic criteria for atypical chronic myeloid leukemia, BCR-ABL1 negative

- Peripheral blood 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
- Less than 20% blasts in the blood and bone marrow
- No evidence of PDGFRα, PDGFRβ, or FGFR1 rearrangement, or PCM1-JAK2
- Not meeting WHO criteria for BCR-ABL1-positive chronic myeloid leukaemia, primary myelofibrosis, polycythaemia vera or essential thrombocythaemia*

*Cases of myeloproliferative neoplasms (MPN), particularly those in accelerated phase and/or in post-polycythaemic or post-essential thrombocythaemic myelofibrosis, if neutrophilic, may simulate aCML. A previous history of MPN, the presence of MPN features in the bone marrow 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 chronic neutrophilic leukaemia or other myeloid neoplasm
Table 13. Diagnostic criteria for myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis

- Anaemia associated with erythroid lineage dysplasia with or without multilineage dysplasia, \( \geq 15\% \) ring sideroblasts*, <1% blasts in peripheral blood and <5% blasts in the bone marrow
- Persistent thrombocytosis with platelet count \( \geq 450 \times 10^9/L \)
- 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

*\( \geq 15\% \) 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
Table 14. Diagnostic Criteria for Juvenile Myelomonocytic Leukemia (modified from Locatelli & Niemeyer, 2014)

I. Clinical and hematologic features (all 4 features mandatory)
   - Peripheral blood monocyte count ≥ 1x10^9/L
   - Blast percentage in peripheral blood and bone marrow <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
   - Germline 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 peripheral blood smear
   - GM-CSF hypersensitivity in colony assay
   - Hyperphosphorylation of STAT5

*Germline mutations (indicating Noonan syndrome) need to be excluded
**Occasional cases with heterozygous splice site mutations
Table 15. Peripheral blood and BM findings and cytogenetics of myelodysplastic syndromes (MDS)

<table>
<thead>
<tr>
<th>Name</th>
<th>Dysplastic lineages</th>
<th>Cytopenias</th>
<th>Ring sideroblasts as % of marrow erythroid elements</th>
<th>Bone marrow (BM) and peripheral blood (PB) blasts</th>
<th>Cytogenetics by conventional karyotype analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS with single lineage dysplasia (MDS-SLD)</td>
<td>1</td>
<td>1 or 2</td>
<td>&lt;15% / &lt;5%(^2)</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS with multilineage dysplasia (MDS-MLD)</td>
<td>2 or 3</td>
<td>1-3</td>
<td>&lt;15% / &lt;5%(^2)</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS with ring sideroblasts (MDS-RS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS-RS with single lineage dysplasia (MDS-RS-SLD)</td>
<td>1</td>
<td>1 or 2</td>
<td>≥15% / ≥5%(^2)</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS-RS with multilineage dysplasia (MDS-RS-MLD)</td>
<td>2 or 3</td>
<td>1-3</td>
<td>≥15% / ≥5%(^2)</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>Any, unless fulfills all criteria for MDS with isolated del(5q)</td>
</tr>
<tr>
<td>MDS with isolated del(5q)</td>
<td>1-3</td>
<td>1-2</td>
<td>None or any</td>
<td>BM &lt;5%, PB &lt;1%, no Auer rods</td>
<td>del(5q) alone or with 1 additional abnormality except -7 or del(7q)</td>
</tr>
<tr>
<td>MDS with excess blasts (MDS-EB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 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%
  - MDS-defining abnormality
  - Auer rods
  - Any

### Refractory cytopenia of childhood
- 1-3
- 1-3
- None
- BM <5%, PB <2%
- Auer rods
- Any

---

1. Cytopenias defined as haemoglobin <10 g/dL, platelet count <100 x 10^9/L, and absolute neutrophil count <1.8 x 10^9/L; rarely, MDS may present with mild anaemia or thrombocytopenia above these levels. PB monocytes must be <1 x 10^9/L
2. If SF3B1 mutation is present.
3. 1% PB blasts must be recorded on at least two separate occasions.
4. Cases with ≥15% ring sideroblasts by definition have significant erythroid dysplasia, and are classified as MDS-RS-SLD
Table 16. Diagnostic approach to myeloid neoplasms when erythroid precursors comprise ≥50% of bone marrow (BM) nucleated cells.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50%</td>
<td>NA</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>Therapy-related myeloid neoplasm</td>
<td>Therapy-related myeloid neoplasm</td>
</tr>
<tr>
<td>≥50%</td>
<td>≥20%</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>AML with recurring genetic abnormality</td>
<td>AML with recurring genetic abnormality</td>
</tr>
<tr>
<td>≥50%</td>
<td>≥20%</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>AML with myelodysplasia-related changes</td>
<td>AML with myelodysplasia-related changes</td>
</tr>
<tr>
<td>≥50%</td>
<td>≥20%</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>AML, NOS, acute erythroid leukemia (erythroid/myeloid type)</td>
<td>AML, NOS (non erythroid subtype)</td>
</tr>
<tr>
<td>≥50%</td>
<td>&lt;20%, but ≥20% of non-erythroid cells</td>
<td>No</td>
<td>No*</td>
<td>NA</td>
<td>AML, NOS, acute erythroid leukemia (erythroid/myeloid subtype)</td>
<td>MDS**</td>
</tr>
<tr>
<td>≥50%</td>
<td>&lt;20%, and &lt;20% of non-erythroid cells</td>
<td>No</td>
<td>No*</td>
<td>NA</td>
<td>MDS**</td>
<td>MDS**</td>
</tr>
<tr>
<td>&gt;80% immature erythroid precursors with &gt;30% proerythroblasts</td>
<td>&lt;20%</td>
<td>No</td>
<td>No*</td>
<td>NA</td>
<td>AML, NOS, acute erythroid leukemia (pure erythroid type)</td>
<td>AML, NOS, acute erythroid leukemia (pure erythroid type)</td>
</tr>
</tbody>
</table>

* Cases of AML t(8;21)(q22;q22.1); RUNXI-RUNX1T1, AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 or acute promyelocytic leukemia with PML-RARA, may rarely occur in this setting with less than 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 peripheral blood leukocytes and other MDS criteria

Abbreviations:  BM, bone marrow; PB, peripheral blood; AML, acute myeloid leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; AML-NOS, acute myeloid leukemia, not otherwise specified; NA, not applicable; MDS, myelodysplastic syndrome
Table 17. Classification of Myeloid Neoplasms with Germline Predisposition

**Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction**
- AML with germline CEBPA mutation
- Myeloid neoplasms with germline DDX41 mutation* 

**Myeloid neoplasms with germline predisposition and pre-existing platelet disorders**
- Myeloid neoplasms with germline RUNX1 mutation* 
- Myeloid neoplasms with germline ANKRD26 mutation* 
- Myeloid neoplasms with germline ETV6 mutation* 

**Myeloid neoplasms with germline predisposition and other organ dysfunction**
- Myeloid neoplasms with germline GATA2 mutation 
- Myeloid neoplasms associated with BM bone marrow failure syndromes 
- Myeloid neoplasms associated with telomere biology disorders 
- Juvenile myelomonocytic leukemia associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders 
- Myeloid neoplasms associated with Down syndrome* 

* Lymphoid neoplasms also reported.
Table 18. Cytogenetic abnormalities sufficient to diagnose AML with myelodysplasia-related changes when $\geq$20% PB or BM blasts are present and prior therapy has been excluded.

- **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 mixed phenotype acute leukemia (MPAL)

**Myeloid lineage**  
Myeloperoxidase* (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 epsilon chain)  
  or  
Surface CD3

**B lineage**  
Strong** CD19 with at least one of the following strongly expressed: CD79a, cytoplasmic CD22 or CD10  
  or  
Weak CD19 with at least two of the following strongly expressed: CD79a, cytoplasmic CD22 or CD10

* see text for caveats related to weaker antigen expression, or to expression by IHC only

** strong defined as equal or brighter than the normal B or T cells in the sample
The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia

Daniel A. Arber, Attilio Orazi, Robert Hasserjian, Jürgen Thiele, Michael J. Borowitz, Michelle M. Le Beau, Clara D. Bloomfield, Mario Cazzola and James W. Vardiman