Guidelines for

Myeloid and Histiocytic/Dendritic Neoplasms

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The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/ Dendritic Neoplasms

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- The aim of this paper is to provide an overview of the new edition of the WHO classification for myeloid and histiocytic/dendritic tumours.
- The last edition of the haematolymphoid classification dates back to 2008 and was revised in 2017. The application of this classification is predicated on integrating morphologic (cytology and histology), immunophenotypic, molecular and cytogenetic data.
- Where possible, a triad of attributes was systematically applied and ncluded: lineage + dominant clinical attribute + dominant biologic attribute.
- **Lineage attribution** rests on immunophenotyping with flow cytometry and/or immunohistochemistry. **Dominant clinical attributes** are general features of the untreated disease and include descriptors such as acute, chronic, cytopenia(s)(myelodysplasia) and cytosis(es) (myeloproliferation). **Most biologic attributes** include gene fusions, rearrangements, and mutations.
- Fusions are part of the nomenclature of types/subtypes when the identities of both implicated genes are required or often desirable criteria for diagnosis (e.g., PML::RARA the new designation of gene fusions using double colon marks (::)

CLONAL HAEMATOPOIESIS

- Clonal haematopoiesis (CH) refers broadly to the presence of a population of cells derived from a mutated multipotent stem/progenitor cell harbouring a selective growth advantage in the absence of unexplained cytopenias, haematological cancers, or other clonal disorders.
- Association with
- increased overall mortality, cardiovascular diseases, and myeloid malignancies. More specific emerging associations, such as those characterizing the VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic UBA1 mutations) syndrome represent manifestations of the interplay between inflammation and CH/myeloid neoplasia that are being gradually uncovered.
- Inclusion of CH in the classification represents a key inaugural effort to define and codify such myeloid precursor lesions.

CLONAL HAEMATOPOIESIS-CHIP & CCUS

Clonal haematopoiesis of indeterminate potential (CHIP) defined as a term referring specifically to CH

- harbouring somatic mutations of myeloid malignancy-associated genes detected in the blood or bone marrow at a variant allele fraction (VAF) of ≥ 2% (≥4% for X-linked gene mutations in males)in individuals without a diagnosed haematologic disorder or unexplained cytopenia
- The significance of variants detected at lower levels is unclear at Present
- SF3B1, TET 2, ASXL1, DNMT3A, U2AF1, TP53, BCOR ;Mutations detected by Cytogenetics/FISH/SEQUENCING
- **Clonal cytopenia of undetermined significance (CCUS)** is definedas CHIP detected in the presence of one or more persistent cytopenias that are otherwise unexplained by haematologic or non-haematologic conditions and that do not meet diagnostic criteria for defined myeloid neoplasms.
- **Cytopenia definitions** are harmonized for CCUS, MDS, and MDS/MPN; they include
- Hb <13g/dL in males and <12 g/dL in females for anaemia,
- Absolute neutrophil count <1.8 ×109/L for leukopenia, and

platelets <150 ×109/L for thrombocytopenia

Previously Threshold : Hb < 10, ANC < 1.8, Plt < 100

Myeloproliferative Neoplasms

- The main types remain largely unchanged from the prior edition
- Initial diagnostic evaluation of MPN continues to depend on close correlation between clinical features, molecular diagnostics, and usually morphologic evaluation of a trephine bone marrow biopsy

Table 1. Myeloproliferative neoplasms.

- Chronic myeloid leukaemia
- Polycythaemia vera
- Essential thrombocythaemia
- Primary myelofibrosis
- Chronic neutrophilic leukaemia
- Chronic eosinophilic leukaemia
- **Juvenile myelomonocytic leukaemia**
- Myeloproliferative neoplasm, **not otherwise specified**

Chronic myeloid leukaemia

- **Risk factors are refined, and**
- **Accelerated phase is no longer required**
- Chronic myeloid leukaemia (CML) is defined by the BCR::ABL1fusion resulting from t(9;22)(q34;q11).
- The natural history of untreated CML before the introduction of targeted tyrosine kinase inhibitors (TKI) was biphasic or triphasic: an initial indolent CP followed by a blast phase (BP), with or without an intervening accelerated phase (AP). With TKI therapy and careful disease

monitoring, the incidence of progression to advanced phase disease has decreased, and the 10-year overall survival rate for CML is 80-90%.

- The designation of AP has thus become less relevant, **where resistance stemming from ABL1 kinase mutations and/or additional cytogenetic abnormalities** and the development of BP represent key disease attributes.
- Accordingly, AP is omitted in the current classification in favour of an emphasis on high risk features associated with CP progression and resistance to TKI.
- **Criteria for BP**
- (1) ≥20% myeloid blasts in the blood or bone marrow; or (2) the presence of an extramedullary proliferation of blasts; or (3) the presence of increased lymphoblasts in peripheral blood or bone marrow.
- The optimal cutoff for lymphoblasts and the significance of low-level B-lymphoblasts remain unclear and require additional studies

Diagnostic criteria for BCR::ABL1-neg Myeloproliferative neoplasms

- **PV**
- Major diagnostic criteria for the diagnosis of PV include elevated haemoglobin concentration and/or haematocrit, accompanied by trilineage hyperplasia (panmyelosis), with pleomorphicmature megakaryocytes in the bone marrow, and JAK2 p.V617F or JAK2 exon 12 mutations.
- As the determination of increased red cell mass with 51Cr-labeled red cells has become uncommon in routine clinical practice, it has been removed as a diagnostic criterion.
- **ET & PMF**
- Criteria not changed
- While JAK2, CALR, and MPL mutations are considered driver events, mutations in other genes – particularly TET2, ASXL1, and DNMT3A – are found in over half of patients with MPN.
- Mutations affecting splicing regulators (SRSF2, SF3B1, U2AF1, ZRSR2) and other regulators of chromatin structure, epigenetic functions and cellular signaling (e.g., EZH2, IDH1, IDH2, CBL, KRAS, NRAS, STAG2, TP53) are less common. These additional mutations are more frequent in PMF and advanced disease compared to PV and ET, and some are known to correlate with a poorer prognostic risk (e.g., EZH2, IDH1, IDH2, SRSF2, U2AF1, and ASXL1 mutations in PMF)

Chronic neutrophilic leukaemia (CNL)

- a BCR::ABL1-negative MPN characterized by sustained peripheral blood neutrophilia(white blood cell count (WBC) ≥ 25 × 109/ L, with ≥80% segmented neutrophils and bands), bone marrow hypercellularity due to neutrophilic granulocyte proliferation, and hepatosplenomegaly.
- CSF3R mutations are common in this disease and detected in >60% of cases

Chronic Eosinophilic Leukemia

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- Eosinophilia > 1.5
- WHO Criteria for CML, PV, ET,PMF, CMML, CNL Atypical CML not met
- No rearrangement of PDGFRA,PDGFRB or FGFR1and no PCM1 JAK2, ETV6 JAK2 or BCR-JAK 2 fusion
- Blasts < 20% in PB or BM and inv 16 or diagnostic features of AML are absent
- Clonal cytogenetic abnormality or blasts >2% in PB or >5% in BM

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- (1) the time interval required to define sustained hypereosinophilia is reduced from 6 months to 4 weeks;
- (2) addition of requirement for both clonality and abnormal bone marrow morphology (e.g., megakaryocytic or erythroid dysplasia); and
- (3)elimination of increased blasts (≥2% in peripheral blood or 5-19% in bone marrow) as an alternative to clonality.
- These criteria improve the distinction between CEL and entities such as Idiopathic hypereosinophilic syndrome and hypereosinophilia of unknown significance
- As the criteria of CEL and its place relative to other disorders with eosinophilia have become well characterized, the qualifier “not otherwise specified” is no longer needed and has been omitted from the name.

JMML

- Recognized as myeloproliferative neoplasm of early childhood with frequent association with germline pathogenic gene variants
- Juvenile myelomonocytic leukaemia (JMML) is a haematopoietic stem cell-derived myeloproliferative neoplasm of early childhood.
- The pathogenetic mechanism in at least 90% of cases involves unchecked activation of the RAS pathway.
- A diagnosis of JMML can be made by combining clinical, laboratory, and molecular criteria. The genetic background of JMML plays a major role in risk stratification and therapeutic approaches, with cases initiated by somatic mutations involving PTPN11 and germline pathogenic variants associated with neurofibromatosis type 1 being the most aggressive types, while some cases associated with pathogenic germline CBL variants undergoing occasionally spontaneous remission.
- The inclusion of JMML under MPN reflects its molecular pathogenesis and underscores the virtual absence of stigmata of bona fide myelodysplastic neoplasia in this disease.

Juvenile myelomonocytic leukaemia

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Table 5.03 Diagnostic criteria for juvenile myelomonocytic leukaemia; modified from Locatelli F and Niemeyer CM {23

Clinical and haematological criteria (all 4 criteria are required)
- Peripheral blood monocyte count ≥ 1 × 10^9/L
- Blast percentage in peripheral blood and bone marrow of < 20%
- Splenomegaly
- No Philadelphia (Ph) chromosome or *BCR-ABL1* fusion

Genetic criteria (any 1 criterion is sufficient)
- Somatic mutation[a] in *PTPN11, KRAS* or *NRAS*
- Clinical diagnosis of neurofibromatosis type 1 or *NF1* mutation
- Germline *CBL* mutation and loss of heterozygosity *of CBL*[b]

Other criteria
Cases that do not meet any of the genetic criteria above must meet the following criteria in addition to the clinical and haematological criteria above:
- Monosomy 7 or any other chromosomal abnormality
  or
- ≥ 2 of the following:
  - Increased haemoglobin F for age
  - Myeloid or erythroid precursors on peripheral blood smear
  - Granulocyte-macrophage colony-stimulating factor (also called CSF2) hypersensitivity in colony assay
  - Hyperphosphorylation of STAT5

[a] If a mutation is found in *PTPN11, KRAS* or *NRAS* it is essential to consider that it might be a germline mutation and the diagnosis of transient abnormal myelopoiesis of Noonan syndrome must be considered.
[b] Occasional cases have heterozygous splice-site mutations.

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- Updates to diagnostic criteria include: (1) exclusion of
- KMT2A rearrangements; (2) elimination of monosomy 7 as a cytogenetic criterion; and, (3) emphasizing the significance of diagnostic molecular studies, particularly those aimed at demonstrating RAS pathway activation.

MASTOCYTOSIS

- Mastocytosis comprises rare heterogeneous neoplasm characterized by an accumulation of abnormal mast cells in various organs or tissues, typically driven by constitutive activation of the KIT receptor.
- Significant comorbidities include IgE dependent allergies, vitamin D deficiency, and psychiatric, psychological or mental problems.
- The classification continues to recognize three disease types: systemic mastocytosis (SM),cutaneous mastocytosis (CM) and mast cell sarcoma (MCS)
- A somatic point mutation in the KIT gene at codon 816 is detected in >90% of patients with SM. Other rare activating KIT alterations include mutations in the extracellular (e.g., deletion of codon 419 on exon 8 or A502_Y503dup in exon 9), transmembrane (e.g., NM_000222:KIT p.F522C), or juxtamembrane (e.g.,NM_000222:KIT p.V560G) domains, detected in <1% of advanced SM cases but enriched in cases of indolent SM.

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Table 3.01 Classification of mastocytosis variants

Cutaneous mastocytosis
- Urticaria pigmentosa/maculopapular cutaneous mastocytosis
- Diffuse cutaneous mastocytosis
- Mastocytoma of skin

Systemic mastocytosis
- Indolent systemic mastocytosis[a] (including the bone marrow mastocytosis subtype)
- Smouldering systemic mastocytosis[a]
- Systemic mastocytosis with an associated haematological neoplasm[b]
- Aggressive systemic mastocytosis[a]
- Mast cell leukaemia

Mast cell sarcoma

[a] The complete diagnosis of these variants requires information regarding B and C findings (Table 3.04, p. 66), all of which may not be available at the time of initial tissue diagnosis.
[b] This variant is equivalent to the previously described entity 'systemic mastocytosis with an associated clonal haematological non–mast cell lineage disease', and the terms can be used synonymously.

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Table 2. Mastocytosis types and subtypes.

Cutaneous mastocytosis
Urticaria pigmentosa/Maculopapular cutaneous mastocytosis
Monomorphic
Polymorphic
Diffuse cutaneous mastocytosis
Cutaneous mastocytoma
Isolated mastocytoma
Multilocalized mastocytoma
Systemic mastocytosis
Bone marrow mastocytosis
Indolent systemic mastocytosis
Smoldering systemic mastocytosis
Aggressive systemic mastocytosis
Systemic mastocytosis with an associated haematologic neoplasm
Mast cell leukemia
Mast cell sarcoma

Note: Well-differentiated systemic mastocytosis (WDSM) represents a morphologic variant that may occur in any SM type/subtype, including mast cell leukaemia.

MASTOCYTOSIS

Table 3.02 Diagnostic criteria for cutaneous and systemic mastocytosis

Cutaneous mastocytosis

Skin lesions demonstrating the typical findings of urticaria pigmentosa/maculopapular cutaneous mastocytosis, diffuse cutaneous mastocytosis or solitary mastocytoma, and typical histological infiltrates of mast cells in a multifocal or diffuse pattern in an adequate skin biopsy[a].
In addition, features/criteria sufficient to establish the diagnosis of systemic mastocytosis must be absent {1567,4105,4107}. There are three variants of cutaneous mastocytosis (see Table 3.01).

Systemic mastocytosis

The diagnosis of systemic mastocytosis can be made when the major criterion and at least 1 minor criterion are present, or when ≥3 minor criteria are present.

Major criterion

Multifocal dense infiltrates of mast cells (≥15 mast cells in aggregates) detected in sections of bone marrow and/or other extracutaneous organ(s)

Minor criteria

1. In biopsy sections of bone marrow or other extracutaneous organs,
   >25% of the mast cells in the infiltrate are spindle-shaped or have atypical morphology or
   >25% of all mast cells in bone marrow aspirate smears are immature or atypical.
2. Detection of an activating point mutation at codon 816 of *KIT* in the bone marrow, blood or another extracutaneous organ
3. Mast cells in bone marrow, blood or another extracutaneous organ express CD25, with or without CD2, in addition to normal mast cell markers[b].
4. Serum total tryptase is persistently >20 ng/mL, unless there is an associated myeloid neoplasm, in which case this parameter is not valid.

[a] This criterion applies to both the dense focal and the diffuse mast cell infiltrates in the biopsy.
[b] CD25 is the more sensitive marker, by both flow cytometry and immunohistochemistry.

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- Diagnostic criteria for SM have been modified. Namely, expression of CD30 and the presence of any KIT mutation causing ligand-independent activation have been accepted as minor diagnostic criteria. Basal serum tryptase level >20 ng/ml, which should be adjusted in case of hereditary alpha-tryptasaemia, is a minor SM criterion
- Classical B-findings ('burden of disease') and C-findings('cytoreduction-requiring') have undergone minor refinements.
- Most notably, NM_000222:KIT p.D816V mutation with VAF ≥ 10% in bone marrow cells or peripheral blood leukocytes qualifies as a B-finding.

MYELODYSPLASTIC NEOPLASMS New terminology and grouping framework

- The classification introduces the term myelodysplastic neoplasms (abbreviated MDS) to replace myelodysplastic syndromes, underscoring their neoplastic nature and harmonizing terminology with MPN.
- Additionally, the recommended threshold for dysplasia is set at 10% for all lineages.
- MDS entities are now grouped as those having defining genetic abnormalities and those that are morphologically defined.

MYELODYSPLASTIC NEOPLASMS New terminology and grouping framework

MDS with defining genetic abnormalities
MDS with low blasts and isolated 5q del(MDS-5q)
MDS with low blasts and SF3B1 mutation (MDS SF3B1)* **Detection of ≥15% ring sideroblasts may substitute for SF3B1 mutation.** **Acceptable related terminology: MDS with low blasts and ring sideroblasts.**
MDS with biallelic TP53 inactivation(MDS- bi TP53)

TP53 in MDS

- Pathogenic TP53 alterations of any type (sequence variations, segmental deletions and copy neutral loss of heterozygosity) are detected in 7-11% of MDS.
- Among these, about two-thirds of patients have multiple TP53 hits (multi-hit), consistent with biallelic TP53 alterations.
- Biallelic TP53 (biTP53) alterations may consist of multiple mutations or mutation with concurrent deletion of the other allele. This “multi-hit” mutational status results in a neoplastic clone that lacks any residual wild-type p53 protein.
- Clinical detection of biallelic TP53 alterations is based on sequencing analysis (covering at least exons 4 to 11) , often coupled with a technique to detect copy number status, usually fluorescence in situ hybridization with a probe set specific for the TP53 locus on 17p13.1 and/or array techniques (e.g., comparative genomic hybridization or single nucleotide polymorphism arrays)
- A TP53 VAF ≥ 50% may be regarded as presumptive (not definitive) evidence of copy loss on the trans allele or copy neutral loss of heterozygosity when a constitutional TP53 variant can be ruled out.
- When two or more TP53 mutations are detected, they usually affect both alleles and can be considered a multi-hit status.
- Over 90% of patients with MDS-biTP53 have complex, mostly very complex (>3), karyotype and thus are regarded as very high risk in IPSS-R

- Additional studies are needed to determine whether biTP53 status is per se AML-defining, a point for consideration in future editions. Notwithstanding, published data suggests that MDS-biTP53 may be regarded as AML-equivalent for therapeutic considerations

MDS Morphologically defined	blasts	WHO 4th Edition
MDS with low blasts (MDS LB)	<5% BM and <2% PB	MDS SLD & MDS-MLD
MDS, hypoplastic(MDS-h)* By definition, ≤25% bone marrow cellularity, age adjusted.		
MDS with increased blasts (MDS -IB)		
MDS -IB1	5-9% BM or 2-4% PB	MDS EB1
MDS-IB2	10-19% BM or 5-19% PB or Auer rods	MDS EB2
MDS with fibrosis(MDS-f)	5-19% BM ,2-19% PB	MDS –F -B

Table 6.01 Diagnostic criteria for myelodysplastic syndrome (MDS) entities

Entity name	Number of dysplastic lineages	Number of cytopenias[a]	Ring sideroblasts as percentage of marrow erythroid elements	Bone marrow (BM) and peripheral blood (PB) blasts	Cytogenetics by conventional karyotype analysis
MDS-SLD	1	1–2	<15% / <5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS-MLD	2–3	1–3	<15% / <5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS-RS					
MDS-RS-SLD	1	1–2	≥15% / ≥5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated del(5q)
MDS-RS-MLD	2–3	1–3	≥15% / ≥5%[b]	BM <5%, PB <1%, no Auer rods	Any, unless fulfils 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 loss of chromosome 7 or del(7q)
MDS-EB					
MDS-EB-1	1–3	1–3	None or any	BM 5–9% or PB 2–4%, BM <10% and PB <5%, no Auer rods	Any
MDS-EB-2	1–3	1–3	None or any	BM 10–19% or PB 5–19% or Auer rods, BM and PB <20%	Any
MDS-U					
with 1% blood blasts	1–3	1–3	None or any	BM <5%, PB = 1%[c], no Auer rods	Any
with SLD and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1–3	<15%[d]	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality[e]

MDS-EB, MDS with excess blasts; MDS-MLD, MDS with multilineage dysplasia; MDS-RS, MDS with ring sideroblasts; MDS-RS-MLD, MDS with ring sideroblasts and multilineage dysplasia; MDS-RS-SLD, MDS with ring sideroblasts and single lineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; MDS-U, MDS, unclassifiable; SLD, single lineage dysplasia.

[a] Cytopenias defined as haemoglobin concentration < 10 g/dL, platelet count < 100 × 10^9/L and absolute neutrophil count < 1.8 × 10^9/L, although MDS can present with mild anaemia or thrombocytopenia above these levels; PB monocytes must be < 1 × 10^9/L.
[b] If *SF3B1* mutation is present.
[c] 1% PB blasts must be recorded on ≥ 2 separate occasions.
[d] Cases with ≥ 15% ring sideroblasts by definition have significant erythroid dysplasia and are classified as MDS-RS-SLD.
[e] See Table 6.03, p. 104.

- The diagnostic criteria of MDS-5q have not changed
- While recognized as factors that may potentially alter the biology and/or prognosis of the disease, the presence of SF3B1 or a TP53 mutation(not multi-hit) does not per se override the diagnosis of MDS-5q
- Recent studies have identified MDS-SF3B1 as a distinct disease type that includes over 90% of MDS with ≥5% ring sideroblasts

The term MDS with low blasts and ring sideroblasts is retained as an acceptable alternative to be used for cases with wild-type SF3B1 and ≥15% ring sideroblasts**.**

Hypoplastic MDS is listed as a distinct MDS type in this edition. Long recognized as having distinctive features, MDS-h is associated with a T-cell mediated immune attack on haematopoietic stem and progenitor cells, along with oligoclonal expansion of CD8 + cytotoxic T-cells overproducing IFNγ and/or TNFα.

- Several features overlap across the triad of MDS-h, paroxysmal nocturnal haemoglobinuria (PNH) and aplastic anaemia (AA),including an association with CH .Many patients with MDS-h have sustainable responses to agents used in patients with AA (i.e., anti-thymocyte globulin, ATG). As such, an emphasis is placed on careful morphologic evaluation, typically requiring trephine biopsy evaluation in addition to evaluation of bone marrow smears and touch preparations, and detection of mutations and/or clonal cytogenetic abnormalities. Individuals with germline pathogenic variants in GATA2, DDX41, Fanconi anaemia (FA) or telomerase complex genes can have hypoplastic bone marrow and evolve to MDS and/or AML and do not respond to immunosuppressive treatment.
- Specifically, MDS,unclassifiable, which was present in the prior edition, is removed
- The boundary between MDS and AML is softened, but the 20% blast cutoff to define AML is retained
- Reassessment of the bone marrow blast percentage defining the boundary of MDS-IB2 and AML has been advocated for several cogent reasons and in view of novel therapeutic approaches that show efficacy in patients currently classified as MDS or AML with 10-30% myeloid blasts
- Salient practical challenges underpinning arguments for such a reassessment include:
- (1) any blast-based cutoff is arbitrary and cannot reflect the biologic continuity naturally inherent in myeloid pathogenic mechanisms;
- (2) blast enumeration is subject to sampling variations/error and subjective evaluation; and, (3) no gold standard for blast enumeration exists, and orthogonal testing platforms can and often do produce discordant results
- The pros and cons of merging MDS-IB2 with AML and adopting a 10% cutoff for what would be called MDS/AML were explored in multidisciplinary expert discussions and at editorial board meetings in the course of producing this classification.
- Lowering the blast cutoff to define AML was felt to suffer from the same challenges listed above and would merely replace one cutoff with another.

- Further, an arbitrary cutoff of 10% blasts to define AML (even if qualified as MDS/AML or AML/MDS) carries a risk of overtreatment. Accordingly, a balanced approach was adopted by eliminating blast cutoffs for most AML types with defining genetic alterations but retaining a 20% blast cutoff to delineate MDS from AML.
- Notwithstanding, there was broad agreement that MDS-IB2 maybe regarded as AML-equivalent for therapeutic considerations and from a clinical trial design perspective when appropriate.

Childhood Myelodysplastic Neoplasms

Enhanced specificity of disease terminology introduced

Table 4. Childhood myelodysplastic neoplasms (MDS).

	Blasts
Childhood MDS with low blasts	<5% BM; <2% PB
Hypocellular	
Not otherwise specified	
Childhood MDS with increased blasts	5–19% BM; 2–19% PB

BM bone marrow, *PB* peripheral blood.

Childhood MDS with low blasts (cMDS-LB)

- replaces the former term “refractory cytopenia of childhood (RCC)”.
- It includes two subtypes: childhood MDS with low blasts, hypocellular; and, childhood MDS with low blasts, not otherwise specified (NOS).
- Exclusion of non-neoplastic causes of cytopenia such as infections, nutritional deficiencies, metabolic diseases, bone marrow failure syndromes (BMFS), and germline pathogenic variants remains an essential diagnostic prerequisite for childhood MDS with low blasts. Approximately 80% of cases show hypocellular bone marrow with features similar to severe aplastic anemia and other BMFS, requiring close morphologic examination to evaluate the distribution, maturation, and presence of dysplasia in haematopoietic lineages
- Cytogenetic abnormalities like monosomy 7, 7q deletion, or complex karyotype are associated with an increased risk of progression to AML and typically treated with haematopoietic stem cell transplantation, while cases with normal karyotype or trisomy 8 can have an indolent course.
- The qualifying term childhood MDS emphasizes that this category of myeloid neoplasms is biologically distinct from that seen in adults , underscoring the need to further elucidate its pathogenesis which remains incompletely understood

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- The qualifying term childhood MDS emphasizes that this category of myeloid neoplasms is biologically distinct from that seen in adults , underscoring the need to further elucidate its pathogenesis which remains incompletely understood

Childhood MDS with increased blasts (cMDS-IB)

- Childhood MDS with increased blasts (cMDS-IB) is defined as
- having ≥5% blasts in the bone marrow or ≥2% blasts in the peripheral blood.
- The genetic landscape of cMDS-IB and cMDS-LB is similar, and they both differ from MDS arising in adults.
- Acquired cytogenetic abnormalities and RAS-pathway mutations are more common in cMDS-IB compared to cMDS-LB

Myelodysplastic/myeloproliferative neoplasms

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- Chronic myelomonocytic leukaemia
- Atypical CML
- Juvenile myelomonocytric leukemia
- Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis
- Myelodysplastic/myeloproliferative neoplasm, unclassifiable

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- Chronic myelomonocytic leukaemia
- Myelodysplastic/myeloproliferative neoplasm with neutrophilia
- Myelodysplastic/myeloproliferative neoplasm with SF3B1 mutation and thrombocytosis
- Myelodysplastic/myeloproliferative neoplasm, not otherwise specified
- The term MDS/MPN with ring sideroblasts and thrombocytosis has been retained as an acceptable term to be used for cases with wild-type SF3B1 and ≥15% ring sideroblasts

CMML

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- 1-Persistent peripheral blood monocytosis > 1 x 109/l with monocytes > 10% leucocytes
- 2-WHO criteria for CML, PV ,ET ,PMF not met
- 3-No rearrangement of PDGFRA,PDGFRB,FGFR1 and no PCM1-JAK2(to be exclusded in cases of eosinophilia)
- 4-Blasts < 20% in PF and BM
- 5-Dysplasia involving > 1 myeloid lineages

Or

If dysplasia is minimal, criteria 1-4 are met and

There is an acquried clonal cytogenetic

or molecular abnormality

Or

Monocytosis > 3 months and all other causes

excluded

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Table 6. Diagnostic criteria of chronic myelomonocytic leukaemia.

Prerequisite criteria
1. Persistent absolute (≥0.5 × 10^9/L) and relative (≥10%) peripheral blood monocytosis.
2. Blasts constitute <20% of the cells in the peripheral blood and bone marrow.[a]
3. Not meeting diagnostic criteria of chronic myeloid leukaemia or other myeloproliferative neoplasms.[b]
4. Not meeting diagnostic criteria of myeloid/lymphoid neoplasms with tyrosine kinase fusions.[c]
Supporting criteria
1. Dysplasia involving ≥1 myeloid lineages.[d]
2. Acquired clonal cytogenetic or molecular abnormality.
3. Abnormal partitioning of peripheral blood monocyte subsets.[e]
Requirements for diagnosis
- Pre-requisite criteria must be present in all cases.
- If monocytosis is ≥ 1 × 10^9/L: one or more supporting criteria must be met.
- If monocytosis is ≥0.5 and <1 × 10^9/L: supporting criteria 1 and 2 must be met.
Subtyping criteria
- Myelodysplastic CMML (MD-CMML): WBC < 13 × 10^9/L
- Myeloproliferative CMML (MP-CMML): WBC ≥ 13 × 10^9/L
Subgrouping criteria (based on percentage of blasts and promonocytes)
CMML-1: <5% in peripheral blood and <10% in bone marrow
CMML-2: 5–19% in peripheral blood and 10–19% in bone marrow

[a]Blasts and blast equivalents include myeloblasts, monoblasts and promonocytes.
[b]Myeloproliferative neoplasms (MPN) can be associated with monocytosis at presentation or during the course of the disease; such cases can mimic CMML. In these instances, a documented history of MPN excludes CMML. The presence of MPN features in the bone marrow and/or high burden of MPN-associated mutations (*JAK2*, *CALR* or *MPL*) tends to support MPN with monocytosis rather than CMML.
[c]Criteria for myeloid/lymphoid neoplasms with tyrosine kinase fusions should be specifically excluded in cases with eosinophilia.
[d]Morphologic dysplasia should be present in ≥10% of cells of a haematopoietic lineage in the bone marrow.
[e]Based on detection of increased classical monocytes (>94%) in the absence of known active autoimmune diseases and/or systemic inflam-

- The blast-based subgroup of CMML-0 (<2% blasts in blood and <5% blasts in bone marrow) introduced in the previous edition has been eliminated in view of evidence that its addition provides no or limited prognostic significance
- Namely, the cutoff for absolute monocytosis is lowered from 1.0 ×109/L to 0.5 ×109/L to incorporate cases formerly referred to as oligomonocytic CMML. To enhance diagnostic accuracy when absolute monocytosis is ≥0.5 ×109/L but <1.0 ×109/L, detection of one of more clonal cytogenetic or molecular abnormality and documentation of dysplasia in at least one lineage are required.
- Abnormal partitioning of peripheral blood monocyte subsets is introduced as a new supporting criterion ..
- Two disease subtypes with salient clinical and genetic features are now formally recognized based on WBC:
  - Myelodysplastic CMML (MD-CMML) (WBC < 13 × 109 /L) and
  - Myeloproliferative CMML (MP-CMML) (WBC ≥ 13 × 109/L).
- MP-CMML is commonly associated with activating RAS pathway mutations and adverse clinical outcomes

ACUTE MYELOID LEUKAEMIA

- Foremost is the separation of AML with defining genetic abnormalities from AML defined by differentiation. The latter eliminates the previously confusing use of the term AML NOS, under which types based on differentiation were listed.
- Another key change, as indicated above, is the elimination of the 20% blast requirement for AML types with defining genetic abnormalities (with the exception of AML with BCR::ABL1 fusion and AML with CEBPA mutation)*. Removal of the blast cutoff requires correlation between morphologic findings and the molecular genetic studies to ensure that the defining abnormality is driving the disease pathology. This approach was deemed more appropriate than assigning another arbitrary lower bone marrow blast cutoff.
- A third component of the new structure is the introduction of a section on AML with other defined genetic alterations, a landing spot for new and/or uncommon AML subtypes that may (or may not) become defined types in future editions of the classification
- *AML with t8,21; AML with inv 16cand APML were considered to be acute leukemias without regard to blast count. It was controversial whether all cases with t 9,11, t6,9; inv3 or t 1,22 as well as AML with BCR ABL1 be characterized as AML when blasts < 20%
- AML with BCR::ABL1 and AML with CEBPA mutation are the only disease types with a defined genetic abnormality that require at least 20% blasts for diagnosis. The blast cutoff requirement is needed for the former to avoid overlap with CML. Distinguishing AML with BCR::ABL1 from initial myeloid blast phase of CML can be challenging, and additional evidence continues to be needed to better characterize this AML type.
- There is insufficient data to support any change in the blast cutoff criterion for AML with CEBPA mutation

AML with defining genetic abnormalities

- Acute promyelocytic leukaemia with PML::RARA fusion
- Acute myeloid leukaemia with RUNX1::RUNX1T1 fusion
- Acute myeloid leukaemia with CBFB::MYH11 fusion
- Acute myeloid leukaemia with DEK::NUP214 fusion
- Acute myeloid leukaemia with RBM15::MRTFA fusion
- Acute myeloid leukaemia with BCR::ABL1 fusion
- Acute myeloid leukaemia with KMT2A rearrangement
- Acute myeloid leukaemia with MECOM rearrangement
- Acute myeloid leukaemia with NUP98 rearrangement
- Acute myeloid leukaemia with NPM1 mutation
- Acute myeloid leukaemia with CEBPA mutation
- Acute myeloid leukaemia, myelodysplasia-related
- Acute myeloid leukaemia with other defined genetic alterations

Changes in 5th Edition

- AML with RBM15::MRTFA (formerly RBM15::MKL1) constitute distinct types in future editions. At present, subtypes under this heading include AML with rare genetic fusions. (Acute megakayoblastic with t 1;22)
- Three AML types with characteristic rearrangements involving KMT2A, MECOM, and NUP98 are recognized. A blast count under 20% is acceptable based on studies demonstrating that patients with <20% blasts (MDS) and any of these rearrangements have clinical features that resemble those with higher blast counts. It is important to note that rearrangements involving these three genes, particularly NUP98, may be cryptic on conventional karyotyping.
- AML with KMT2A rearrangement is the new term that replaces “AML with t(9;11)(p22;q23); KMT2A-MLLT3”.
- AML with other defined genetic alterations represents a landing spot for new, often rare, emerging entities whose recognition is desirable to determine whether they might

AML with KMT2A rearrangement

- AML with KMT2A rearrangement is the new term that replaces “AML with t(9;11)(p22;q23); KMT2A-MLLT3”.
- More than 80 KMT2A fusion partners have been described, with MLLT3, AFDN, ELL, and MLLT10 being most common.
- While not required, the identification of the fusion partner is desirable since it could provide prognostic information and may impact disease monitoring.
- Adult patients often present with high blast counts, usually with monocytic differentiation. In children particularly, AML with KMT2A::MLLT3 and KMT2A::MLLT10 show megakaryoblastic differentiation and/or low blast counts in bone marrow aspirate smears.

AML with defining genetic abnormalities

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- AML defined by mutations include AML with NPM1 and AML with CEBPA mutation. AML with NPM1 mutation can be diagnosed irrespective of the blast count, albeit again with emphasis on judicious clinicopathologic correlation. This approach aligns with data showing that cases previously classified as MDS or MDS/MPN with NPM1 progress to AML in a short period of time. Similar data have emerged from patients with CH who acquire NPM1 mutation.
- The definition of AML with CEBPA mutation has changed to include biallelic (biCEBPA) as well as single mutations located in the basic leucine zipper (bZIP) region of the gene (smbZIP-CEBPA).
- The favourable prognosis associated with smbZIP-CEBPA has been demonstrated in cohorts of children and adults up to 70 years old.
- RUNX1 mutations in AML overlap with such a broad range of defining molecular features that it was determined to lack enough specificity to define a standalone AML type.

Changes from5th Edition

- AML with mutated RUNX1 removed
- AML with biallelic CEBPA includes single mutations as well

AML, myelodysplasia-related (AML-MR).

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- 1. ≥20% blasts in PB or BM
- 2. Any of the fol
  - Hx of MDS or MDS/MPN
  - MDS related CTG abnormality
  - Mulitineage dysplasia*multilineage dysplasia alone is insufficient for Dx of AML MRC in de novo case of AML with mutated NPM1 or Bi allelic CEBPA
- 3. Absence of both of
  - Prior cytotoxic or radiation therapy for unrelated disease
  - Recurrent cytogenetic abnormality

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- defined as a neoplasm with ≥20% blasts expressing a myeloid immunophenotype and harboring specific cytogenetic and molecular abnormalities associated with MDS, arising de novo or following a known history of MDS or MDS/MPN.
- Key changes include:
- (1) removal of morphology alone as a diagnostic premise to make a diagnosis of AML-MR;
- (2) update of defining cytogenetic criteria; and,
- (3) introduction of a mutation-based definition based on a set of 8 genes – SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, STAG2, > 95% of which are present specifically in AML arising post MDS or MDS/MPN

AML-MR

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Table 8.04 Cytogenetic abnormalities sufficient for the diagnosis of acute myeloid leukaemia with myelodysplasia-related changes when ≥20% peripheral blood or bone marrow blasts are present and prior therapy has been excluded

Complex karyotype (≥3 abnormalities)

Unbalanced abnormalities

- Loss of chromosome 7 or del(7q)
- del(5q) or t(5q)
- Isochromosome 17q or t(17p)
- Loss of chromosome 13 or del(13q)
- del(11q)
- del(12p) or 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)
- t(3;5)(q25.3;q35.1)

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Table 8. Cytogenetic and molecular abnormalities defining acute myeloid leukaemia, myelodysplasia-related.

Defining cytogenetic abnormalities
Complex karyotype (≥3 abnormalities)
5q deletion or loss of 5q due to unbalanced translocation
Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation
11q deletion
12p deletion or loss of 12p due to unbalanced translocation
Monosomy 13 or 13q deletion
17p deletion or loss of 17p due to unbalanced translocation
Isochromosome 17q
idic(X)(q13)
Defining somatic mutations
ASXL1
BCOR
EZH2
SF3B1
SRSF2
STAG2
U2AF1
ZRSR2

- The presence of one or more cytogenetic or molecular abnormalities listed in Table and/or history of MDS or MDS/MPN are required for diagnosing AML-MR.

Acute myeloid leukaemia, defined by differentiation

- Acute myeloid leukaemia with minimal differentiation
- Acute myeloid leukaemia without maturation
- Acute myeloid leukaemia with maturation
- Acute basophilic leukaemia
- Acute myelomonocytic leukaemia
- Acute monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia

Table 9. Differentiation markers and criteria for acute myeloid leukaemia (AML) types defined by differentiation.

Type	Diagnostic criteria*
AML with minimal differentiation	• Blasts are negative (<3%) for MPO and SBB by cytochemistry
	• Expression of two or more myeloid-associated antigens, such as CD13, CD33, and CD117
AML without maturation	• ≥3% blasts positive for MPO (by immunophenotyping or cytochemistry) or SBB and negative for NSE by cytochemistry
	• Maturing cells of the granulocytic lineage constitute <10% of the nucleated bone marrow cells
	• Expression of two or more myeloid-associated antigens, such as MPO, CD13, CD33, and CD117
AML with maturation	• ≥3% blasts positive for MPO (by immunophenotyping or cytochemistry) or SBB by cytochemistry
	• Maturing cells of the granulocytic lineage constitute ≥10% of the nucleated bone marrow cells
	• Monocyte lineage cells constitute < 20% of bone marrow cells
	• Expression of two or more myeloid-associated antigens, such as MPO, CD13, CD33, and CD117
Acute basophilic leukemia	• Blasts & immature/mature basophils with metachromasia on toluidine blue staining
	• Blasts are negative for cytochemical MPO, SBB, and NSE
	• No expression of strong CD117 equivalent (to exclude mast cell leukemia)
Acute myelomonocytic leukaemia	• ≥20% monocytes and their precursors
	• ≥20% maturing granulocytic cells
	• ≥3% of blasts positive for MPO (by immunophenotyping or cytochemistry)
Acute monocytic leukaemia	• ≥80% monocytes and/or their precursors (monoblasts and/or promonocytes)
	• <20% maturing granulocytic cells
	• Blasts and promonocytes expressing at least two monocytic markers including CD11c, CD14, CD36 and CD64, or NSE positivity on cytochemistry
Acute erythroid leukaemia	• ≥30% immature erythroid cells (proerythroblasts)
	• Bone marrow with erythroid predominance, usually ≥80% of cellularity
Acute megakaryoblastic leukaemia	• Blasts express at least one or more of the platelet glycoproteins: CD41 (glycoprotein IIb), CD61 (glycoprotein IIIa), or CD42b (glycoprotein Ib)[b]

*Shared diagnostic criteria include:
- ≥20% blasts in bone marrow and/or blood (except for acute erythroid leukaemia).
- Criteria for AML types with defined genetic alterations are not met.
- Criteria for mixed-phenotype acute leukaemia are not met (relevant for AML with minimal differentiation).
- Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy.
- No prior history of myeloproliferative neoplasm.

BM bone marrow, *MPO* myeloperoxidase, *NSE* nonspecific esterase, *PB* peripheral blood, *SBB* Sudan Black B.

ACUTE ERYTHROID LEUKEMIA

- Acute erythroid leukaemia (AEL) (previously pure erythroid leukaemia, an acceptable related term in this edition) is a distinct AML type characterized by neoplastic proliferation of erythroid cells with features of maturation arrest and high prevalence of biallelic TP53 alterations.
- Diagnostic criteria include erythroid predominance, usually ≥80% of bone marrow elements, of which ≥30% are proerythroblasts (or pronormoblasts).
- The occurrence of AEL cases in which nucleated erythroid cells constitute less than 80% of bone marrow cellularity is recognized; such cases share the same clinicopathologic features of other AEL
- The central role that biallelic TP53 mutations play in this aggressive AML type is underscored .The diagnosis of AEL supersedes AML-MR.

- De novo AEL and cases that arise following MDS or MDS/MPN share distinctive morphologic features, with prominent proerythroblast proliferation. Proerythroblast have been shown to play an important role in treatment resistance and poor prognosis in AML patients

ACUTE MEGAKARYOBLASTIC LEUKEMIA

- Several molecular drivers can give rise to acute megakaryoblastic leukaemia (AMKL), which arises within three clinical groups: children with Down syndrome, children without Down syndrome, and adults.
- Immunophenotyping and detection of markers of megakaryocytic differentiation are required to make a diagnosis Of AMKL and detect the newly described "RAM immunophenotype",* which correlates with CBFA2T3::GLIS2, a subtype of AML with other defined genetic alterations.
- *RAM immunophenotypebright CD 56 expression, dim to negative expression of CD 45 and CD 38 and lack of HLA DR
- Initially discovered in a paediatric patient of AML ,phenotype was named after patient's initials RAM with documented informed consent

Secondary myeloid neoplasms

- A newly segregated category encompassing diseases that arise in the setting of certain known predisposing factors
- Myeloid neoplasms that arise secondary to exposure to cytotoxic therapy or germline predisposition are grouped in this category.
- AML transformation of MPN is retained in the MPN category, while AML transformation of MDS and MDS/MPN is kept under AML-MR
- An overarching principle in this context is the requirement to consider "post cytotoxic therapy" and "associated with germline [gene] variant" as disease attributes that should be added as qualifiers to relevant myeloid disease types whose criteria are fulfilled as defined elsewhere in the classification, e.g. *AML with KMT2A rearrangement post cytotoxic therapy* or *MDS with low blasts associated with germline RUNX1 variant.*

Myeloid neoplasms post cytotoxic therapy (MN-pCT)

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- introduction of more precise terminology
- the diagnosis of MN-pCT entails fulfilment of criteria for a myeloid neoplasm in addition to a documented history of chemotherapy treatment or large-field radiation therapy for an unrelated neoplasm
- This would exclude CCUS, which by definition lacks sufficient support for morphologic dysplasia
- Exposure to PARP1 inhibitors is added as a qualifying criterion for MN-pCT, and methotrexate has been excluded.

Similarities to WHO 4th Edition

- As in previous editions, this category includes AML, MDS, and MDS/MPN arising in patients exposed to cytotoxic (DNA-damaging) therapy for an unrelated condition
- Cases with a 'de novo molecular signature' such as NPM1 mutation and core binding factor leukaemias should still be assigned to this category since the "post cytotoxic therapy" designation is based on the medical history, and the indication of the most specific diagnosis in the pathology report is recommended when possible.

Myeloid neoplasms post cytotoxic therapy (MN-pCT)

- It is recommended that specification of the type of myeloid neoplasm is made when possible, with the appendix "post cytotoxic therapy" appended,e.g. CMML post cytotoxic therapy.
- The majority of AML-pCT and MDS-pCT are associated with TP53 mutations. The outcomes of such patients are generally worse with biallelic (multi-hit) TP53 alterations, manifesting as ≥2 TP53 mutations, or with concomitant 17p/TP53 deletion or copy neutral LOH.
- Less frequent mutations involve genes such as PPM1D and DNA-damage response genes that may require additional work-up for germline predisposition.

Myeloid neoplasms associated with germline predisposition

- Myeloid neoplasms associated with germline predisposition include AML, MDS, MPN, and MDS/MPN that arise in individuals with genetic conditions associated with increased risk of myeloid malignancies.
- Myeloid neoplasms arising in individuals with Fanconi anemia, Down syndrome, and RASopathies are discussed in separate dedicated sections. These diseases are now classified using a formulaic approach that couples the myeloid disease phenotype with the predisposing germline genotype, e.g., *AML with germline pathogenic variants in RUNX1.*
- Myeloid proliferations associated with Down syndrome, typically associated with somatic exon 2 or 3 GATA1 mutation, continue to encompass two clonal conditions that arise in children with constitutional trisomy 21: transient abnormal myelopoiesis (TAM), which is confined to the first 6 months of life and myeloid leukaemia of Down syndrome (ML-DS).

Myeloid neoplasms with germline predisposition

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Table 7.01 Classification of myeloid neoplasms with germline predisposition

Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction

Acute myeloid leukaemia with germline *CEBPA* mutation
Myeloid neoplasms with germline *DDX41* mutation[a]

Myeloid neoplasms with germline predisposition and pre-existing platelet disorders

Myeloid neoplasms with germline *RUNX1* mutation[a]
Myeloid neoplasms with germline *ANKRD26* mutation[a]
Myeloid neoplasms with germline *ETV6* mutation[a]

Myeloid neoplasms with germline predisposition and other organ dysfunction

Myeloid neoplasms with germline *GATA2* mutation
Myeloid neoplasms associated with bone marrow failure syndromes[b]
Myeloid neoplasms associated with telomere biology disorders[b]
Juvenile myelomonocytic leukaemia associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome–like disorders[c]
Myeloid neoplasms associated with Down syndrome[a,d]

[a] Lymphoid neoplasms have also been reported.
[b] See Table 7.03 (p. 127) for specific genes.
[c] See *Juvenile myelomonocytic leukaemia*, p. 89.
[d] See *Myeloid proliferations associated with Down syndrome*, p. 169.

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Table 10. Subtypes of myeloid neoplasms associated with germline predisposition.

Myeloid neoplasms with germline predisposition without a pre-existing platelet disorder or organ dysfunction
• Germline *CEBPA* P/LP variant (CEBPA-associated familial AML)
• Germline *DDX41* P/LP variant[a]
• Germline *TP53* P/LP variant[a] (Li-Fraumeni syndrome)
Myeloid neoplasms with germline predisposition and pre-existing platelet disorder
• Germline *RUNX1* P/LP variant[a] (familial platelet disorder with associated myeloid malignancy, FPD-MM)
• Germline *ANKRD26* P/LP variant[a] (Thrombocytopenia 2)
• Germline *ETV6* P/LP variant[a] (Thrombocytopenia 5)
Myeloid neoplasms with germline predisposition and potential organ dysfunction
• Germline *GATA2* P/LP variant (GATA2-deficiency)
• Bone marrow failure syndromes
◦ Severe congenital neutropenia (SCN)
◦ Shwachman-Diamond syndrome (SDS)
◦ Fanconi anaemia (FA)
• Telomere biology disorders
• RASopathies (Neurofibromatosis type 1, CBL syndrome, Noonan syndrome or Noonan syndrome-like disorders[a,b])
• Down syndrome[a,b]
• Germline *SAMD9* P/LP variant (MIRAGE Syndrome)
• Germline *SAMD9L* P/LP variant (SAMD9L-related Ataxia Pancytopenia Syndrome)[c]
• Biallelic germline *BLM* P/LP variant (Bloom syndrome)

[a]Lymphoid neoplasms can also occur.
[b]See respective sections.
[c]Ataxia is not always present.
P pathogenic, *LP* likely pathogenic.

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND TYROSINE KINASE GENE FUSIONS

- Summary Box:
- ● Family renamed myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK).
- ● Recognition of novel types with JAK2 rearrangements, FLT3 rearrangements, and ETV6::ABL1 fusion.
- ● New scalable genetic framework introduced under MLN-TK with other defined tyrosine kinase fusions

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND TYROSINE KINASE GENE FUSIONS

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- Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangements
- Myeloid/lymphoid neoplasms with PDGFRA rearrangement
- Myeloid/lymphoid neoplasms with PDGFRB rearrangement
- Myeloid/lymphoid neoplasms with FGFR1 reaarangement
- Myeloid/lymphoid neoplasms with PCM1-JAK2 (provisional entity)

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- Table 11. Genetic abnormalities defining myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions.
- PDGFRA rearrangement
- PDGFRB rearrangement
- FGFR1 rearrangement
- JAK2 rearrangement
- FLT3 rearrangement
- ETV6::ABL1 fusion
- Other defined tyrosine kinase fusions: ETV6::FGFR2; ETV6::LYN; ETV6::NTRK3; RANBP2::ALK; BCR::RET; FGFR1OP::RET

MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND TYROSINE KINASE GENE FUSIONS

- From a diagnostic hierarchy standpoint, the diagnosis of MLN-TK supersedes other myeloid and lymphoid types, as well as SM.
- MLN-TK must be excluded before a diagnosis of CEL is rendered.
- The majority of MLN-TK cases associated with PDGFRA rearrangements have cytogenetically cryptic deletion of 4q12 resulting in FIP1L1::PDGFRA, but PDGFRA fusions involving other partners are also identified.

- Cases with PDGFRB rearrangement result most commonly from t(5;12)(q32;p13.2) leading to ETV6:: PDGFRB; however, more than 30 other partners have been identified.
- Cases with FGFR1 rearrangement may manifest as chronic myeloid neoplasms or blast-phase disease of B-cell, T-cell, myeloid or mixed-phenotype origin, typically with associated eosinophilia. The characteristic cytogenetic feature is an aberration of chromosome 8p11.
- Detection of JAK2 rearrangements leading to fusion products with genes other than PCM1 have been recognized, supporting MLN-TK with JAK2 rearrangement as a distinct type .
- Cases with FLT3 fusion genes are particularly rare and result from rearrangements involving chromosome 13q12.2. They manifest as myeloid sarcoma with MPN feature in the bone marrow or T-ALL with associated eosinophilia, but disease features and phenotypic presentation may be variable and diverse.
- MLN-TK with ETV6::ABL1 should be separated from B-ALL with ETV6::ABL1
- The natural history of MLN-TK with PDGFRA or PDGFRB has been dramatically altered by TKI therapy, particularly imatinib.
- In contrast, patients with FGFR1, JAK2 and FLT3 fusions and ETV6::ABL1 have more variable sensitivity to available newer generation TKIs ; in most cases, long-term disease-free survival may only be achievable with allogeneic haematopoietic stem cell transplantation.

ACUTE LEUKAEMIAS OF MIXED OR AMBIGUOUS LINEAGE

Table 12. Acute leukaemias of ambiguous lineage.

Acute leukaemia of ambiguous lineage with defining genetic abnormalities
Mixed-phenotype acute leukaemia with *BCR*::*ABL1* fusion
Mixed-phenotype acute leukaemia with *KMT2A* rearrangement
Acute leukaemia of ambiguous lineage with other defined genetic alterations
Mixed-phenotype acute leukaemia with *ZNF384* rearrangement
Acute leukaemia of ambiguous lineage with *BCL11B* rearrangement
Acute leukaemia of ambiguous lineage, immunophenotypically defined
Mixed-phenotype acute leukaemia, B/myeloid
Mixed-phenotype acute leukaemia, T/myeloid
Mixed-phenotype acute leukaemia, rare types
Acute leukaemia of ambiguous lineage, not otherwise specified
Acute undifferentiated leukaemia

ACUTE LEUKAEMIAS OF MIXED OR AMBIGUOUS LINEAGE

- Two new subtypes of ALAL with defining genetic alterations are added.
- The first subtype is MPAL with ZNF384 rearrangement, which commonly has a B/myeloid immunophenotype and is identified in ~50% of pediatric B/myeloid MPAL with fusion partners including TCF3, EP300, TAF15, and CREBBP.
- ZNF384-rearranged B/myeloid MPAL and B-ALL have similar transcriptional profile, suggesting a biological continuum .
- The other subtype is ALAL with BCL11B rearrangement, which has a more heterogenous immunophenotype - identified in acute undifferentiated leukaemia (AUL) and ~20-30% of T/myeloid MPAL.

Table 13. Lineage assignment criteria for mixed-phenotype acute leukaemia.

	Criterion
B lineage	
CD19 strong[a]	1 or more also strongly expressed: CD10, CD22, or CD79a[c]
or,	
CD19 weak[b]	2 or more also strongly expressed: CD10, CD22, or CD79a[c]
T lineage	
CD3 (cytoplasmic or surface)[d]	Intensity in part exceeds 50% of mature T-cells level by flow cytometry or, Immunocytochemistry positive with non-zeta chain reagent
Myeloid lineage	
Myeloperoxidase	Intensity in part exceeds 50% of mature neutrophil level
or,	
Monocytic differentiation	2 or more expressed: Non-specific esterase, CD11c, CD14, CD64 or lysozyme

[a]CD19 intensity in part exceeds 50% of normal B cell progenitor by flow cytometry.
[b]CD19 intensity does not exceed 50% of normal B cell progenitor by flow cytometry.
[c]Provided T lineage not under consideration, otherwise cannot use CD79a.
[d]Using anti-CD3 epsilon chain antibody.