Protocol for the Examination of Myeloid and Mixed / Ambiguous Lineage Neoplasms

Version: 1.0.0.0
Protocol Posting Date: September 2023
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.
This protocol applies to Myeloid and Mixed/Ambiguous Lineage Neoplasms involving bone marrow, blood, cutaneous, extranodal/mucosal, or any other anatomic site.

The following tumor types should be reported using this protocol:

Tumor Type
Myeloid precursor lesions
Myeloproliferative neoplasms
Mastocytosis
Myelodysplastic neoplasms with defining genetic abnormalities
Myelodysplastic neoplasms, morphologically defined
Myelodysplastic neoplasms of childhood
Myelodysplastic / myeloproliferative neoplasms
Acute myeloid leukemias with defining genetic abnormalities
Acute myeloid leukemias, defined by differentiation
Myeloid sarcoma
Myeloid / lymphoid neoplasms with eosinophilia and defining gene rearrangement
Acute leukemias of mixed or ambiguous lineage
Plasmacytoid dendritic cell neoplasms

The following tumor types should NOT be reported using this protocol:

Tumor Type
Precursor lymphoid malignancies including B-lymphoblastic leukemia/lymphoma and precursor T-cell neoplasms (use Precursor and Mature Lymphoid Malignancies Protocol)
Langerhans cells neoplasms
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Histiocytic neoplasms including Juvenile xanthogranuloma, Erdheim-Chester disease, Rosai-Dorfman disease, ALK-positive histiocytosis, Histiocytic sarcoma
Follicular dendritic cell neoplasms
Myofibroblastic tumor
Splenic vascular stromal tumors

Authors
Robert W. Allan, MD*; L. Jeffrey Medeiros, MD; Robert Seifert, MD; Claudio A. Mosse, MD, PhD; Samer Z. Al-Quran, MD; Joseph D. Khoury, MD.
With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
* Denotes primary author.

Accreditation Requirements
The use of this case summary is recommended for clinical care purposes but is not required for accreditation purposes. The core and conditional data elements are routinely reported. Non-core data elements are indicated with a plus sign (+) to allow for reporting information that may be of clinical value.

Replaced by version 1.1.0.0 on June 19, 2024, Obsolete as of March 2025 (8 months after newest release date)

Summary of Changes

v 1.0.0.0

- New protocol replacing retired Bone Marrow protocol

Reporting Template

Protocol Posting Date: September 2023
Select a single response unless otherwise indicated.

CASE SUMMARY: (MYELOID AND MIXED / AMBIGUOUS LINEAGE NEOPLASMS)

TUMOR (Note A)

Site of Tumor Involvement in Sample (select most involved site)
___ Bone marrow
Specify Percent Blasts / Blast Equivalents: _____ %
Specify Percent Marrow Cellularity: _____ %
___ Blood
Specify Percent Blasts / Blast Equivalents: _____ %
___ Anterior mediastinum
___ Lymph node
___ Cutaneous
___ Extranodal / mucosal site
___ Other (specify): _____

Final Integrated Diagnosis (Note B)
 # The Myeloid and Mixed / Ambiguous Lineage Neoplasms Cancer Case Summary is not required to be completed for Myeloid precursor lesions.
+___ Myeloid precursor lesions#
___ Clonal hematopoiesis of indeterminate potential (CHIP)
___ Clonal cytopenias of undetermined significance (CCUS)
___ Other myeloid precursor lesion (specify): _____
___ Myeloproliferative neoplasms
___ Chronic myeloid leukemia, chronic phase
___ Chronic myeloid leukemia, blast phase
___ Chronic neutrophilic leukemia
___ Chronic eosinophilic leukemia
___ Polycythemia vera
___ Essential thrombocythemia
___ Primary myelofibrosis, pre-fibrotic
___ Primary myelofibrosis, fibrotic
___ Juvenile myelomonocytic leukemia
___ Myeloproliferative neoplasm, NOS
___ Other myeloproliferative neoplasm (specify): _____
___ Mastocytosis
___ Bone marrow mastocytosis
___ Indolent systemic mastocytosis
___ Smoldering systemic mastocytosis
___ Aggressive systemic mastocytosis
___ Systemic mastocytosis with an associated hematologic neoplasm
___ Mast cell leukemia
___ Mast cell sarcoma
___ Other mastocytosis (specify): _____
___ Myelodysplastic neoplasms
Myelodysplastic neoplasms with defining genetic abnormalities

___ Myelodysplastic neoplasm with low blasts and 5q deletion
___ Myelodysplastic neoplasm with low blasts and SF3B1 mutation
___ Myelodysplastic neoplasm with biallelic TP53 inactivation
Myelodysplastic neoplasms, morphologically defined
___ Myelodysplastic neoplasm with low blasts
___ Myelodysplastic neoplasm, hypoplastic
___ Myelodysplastic neoplasm with increased blasts-1 (MDS-IB1)
___ Myelodysplastic neoplasm with increased blasts-2 (MDS-IB2)
___ Myelodysplastic neoplasm with increased blasts and fibrosis (MDS-F)
___ Other myelodysplastic neoplasm (specify): _____
Myelodysplastic neoplasms of childhood
___ Childhood myelodysplastic neoplasm with low blasts
___ Childhood myelodysplastic neoplasm with increased blasts
___ Other myelodysplastic neoplasm of childhood (specify): _____
Myelodysplastic / myeloproliferative neoplasms
___ Myelodysplastic chronic myelomonocytic leukemia (MD-CMML), CMML-1
___ Myelodysplastic chronic myelomonocytic leukemia (MD-CMML), CMML-2
___ Myeloproliferative chronic myelomonocytic leukemia (MP-CMML), CMML-1
___ Myeloproliferative chronic myelomonocytic leukemia (MP-CMML), CMML-2
___ Myelodysplastic neoplasm with neutrophilia
___ Myelodysplastic neoplasm with SF3B1 mutation and thrombocytosis
___ Myelodysplastic neoplasm, NOS
___ Other myelodysplastic / myeloproliferative neoplasm (specify): _____
___ Acute myeloid leukemias
Acute myeloid leukemias with defining genetic abnormalities
___ Acute promyelocytic leukemia with PML::RARA fusion
___ Acute myeloid leukemia with RUNX1::RUNX1T1 fusion
___ Acute myeloid leukemia with CBFB::MYH11 fusion
___ Acute myeloid leukemia with DEK::NUP214 fusion
___ Acute myeloid leukemia with RBM15::MRTFA fusion
___ Acute myeloid leukemia with BCR::ABL1 fusion
___ Acute myeloid leukemia with KMT2A rearrangement
___ Acute myeloid leukemia with MECOM rearrangement
___ Acute myeloid leukemia with NUP98 rearrangement
___ Acute myeloid leukemia with NPM1 mutation
___ Acute myeloid leukemia with CEBPA mutation
___ Acute myeloid leukemia, myelodysplasia-related
___ Acute myeloid leukemia with other defined genetic alterations (specify, if possible):
_____
Acute myeloid leukemias, defined by differentiation
___ Acute myeloid leukemia with minimal differentiation
___ Acute myeloid leukemia without maturation
___ Acute myeloid leukemia with maturation
___ Acute basophilic leukemia
___ Acute myelomonocytic leukemia
___ Acute monocytic leukemia
___ Pure erythroid leukemia
___ Acute megakaryoblastic leukemia
___ Other acute myeloid leukemia (specify): _____
Myeloid sarcoma
___ Myeloid sarcoma (specify myeloid neoplasm): _____

___ Myeloid neoplasms and proliferations associated with antecedent or predisposing conditions
___ Myeloid neoplasm post cytotoxic therapy (specify neoplasm and cytotoxic therapy, if possible): _____
___ Myeloid neoplasm associated with germline predisposition (specify neoplasm and germline predisposition, if possible): _____
___ Transient abnormal myelopoiesis associated with Down syndrome
___ Myeloid leukemia associated with Down syndrome
___ Other myeloid neoplasm and / or proliferation associated with antecedent or predisposing condition (specify): _____
___ Myeloid / lymphoid neoplasms
Myeloid / lymphoid neoplasms with eosinophilia and defining gene rearrangement
___ Myeloid / lymphoid neoplasm with PDGFRA rearrangement
___ Myeloid / lymphoid neoplasm with PDGFRB rearrangement
___ Myeloid / lymphoid neoplasm with FGFR1 rearrangement
___ Myeloid / lymphoid neoplasm with JAK2 rearrangement
___ Myeloid / lymphoid neoplasm with FLT3 rearrangement
___ Myeloid / lymphoid neoplasm with ETV6::ABL1 fusion
___ Myeloid / lymphoid neoplasms with other tyrosine kinase fusion genes
___ Other myeloid / lymphoid neoplasm (specify): _____
___ Acute leukemias of mixed or ambiguous lineage
___ Mixed-phenotype acute leukemia with BCR::ABL1 fusion
___ Mixed-phenotype acute leukemia with KMT2A rearrangement
___ Acute leukemia of ambiguous lineage with other defined genetic alterations
___ Mixed-phenotype acute leukemia, B / myeloid
___ Mixed-phenotype acute leukemia, T / myeloid
___ Mixed-phenotype acute leukemia, rare types
___ Acute leukemia of ambiguous lineage, NOS
___ Acute undifferentiated leukemia
___ Other acute leukemia of mixed or ambiguous lineage (specify): _____
___ Plasmacytoid dendritic cell neoplasms
___ Blastic plasmacytoid dendritic cell neoplasm (BPDCN)
___ Other plasmacytoid dendritic cell neoplasm (specify): _____

Dysplasia# (select all that apply)
 # Applicable only for myeloid malignancies
___ Not applicable
___ Absent
___ Erythroid
___ Granulocytic
___ Megakaryocytic
___ Cannot be determined (explain): _____

SPECIAL STUDIES (Note C)

Ring Sideroblasts / Iron Stain
___ Not applicable / not performed
___ Not evaluable
___ No ring sideroblasts seen on iron stain
___ Positive for ring sideroblasts on iron stain (less than 15%)
___ Positive for ring sideroblasts on iron stain (greater than or equal to 15%)

Bone Marrow Fibrosis Grade
___ Not applicable / not performed
___ MF-0
___ MF-1
___ MF-2
___ MF-3

Flow Cytometry
___ Not performed
___ No aberrancy detected at level of sensitivity of assay
___ Abnormal population (specify immunophenotype, if possible): _____
___ Pending

Conventional Cytogenetics
___ Not performed
___ Normal diploid karyotype
___ Abnormal karyotype (specify, if possible): _____
___ Pending

Fluorescence in situ Hybridization (select all that apply)
___ Not performed
___ Normal probes (specify loci tested): _____
___ Abnormal probes (specify loci tested): _____
___ Pending

Molecular Alterations Detected# (select all that apply)
 # Select all those with significant mutations
___ ASXL1 mutation (specify): _____
___ BCOR mutation (specify): _____
___ BCORL1 mutation (specify): _____
___ BCR::ABL1 p190 fusion transcript (specify): _____
___ BCR::ABL1 p210 fusion transcript (specify): _____
___ BCR::ABL1, unspecified transcript (specify): _____
___ BRAF mutation (specify): _____
___ CALR mutation (specify): _____
___ CBFB::MYH11 fusion (specify): _____
___ CEBPA (mono-allelic) mutation (specify): _____
___ CBL mutation (specify): _____
___ CTCF mutation (specify): _____
___ CSF3R mutation (specify): _____
___ DEK::NUP214 fusion (specify): _____
___ DNMT3A mutation (specify): _____
___ ETV6 mutation (specify): _____
___ ETV6::RUNX1 fusion (specify): _____
___ EZH2 mutation (specify): _____
___ FGFR1 rearrangement (specify): _____
___ FLT3 interim tandem duplication (ITD) (specify): _____
___ FLT3 p.D865 tyrosine kinase domain (specify): _____
___ GATA2 mutation (specify): _____

___ GNAS mutation (specify): _____
___ HRAS mutation (specify): _____
___ IDH1 mutation (specify): _____
___ IDH2 mutation (specify): _____
___ IGH::IL3 rearrangement (specify): _____
___ IKZF1 mutation (specify): _____
___ JAK2 mutation (specify): _____
___ JAK3 mutation (specify): _____
___ KIT mutation (specify): _____
___ KMT2A rearrangement (specify): _____
___ KRAS mutation (specify): _____
___ MECOM rearrangement (specify): _____
___ MPL mutation (specify): _____
___ MYD88 mutation (specify): _____
___ NF1 mutation (specify): _____
___ NOTCH1 mutation (specify): _____
___ NPM1 mutation (specify): _____
___ NRAS mutation (specify): _____
___ NUP98 rearrangement (specify): _____
___ PDGFRA rearrangement (specify): _____
___ PDGFRB rearrangement (specify): _____
___ PCM1::JAK2 fusion (specify): _____
___ PHF6 mutation (specify): _____
___ PML::RARA fusion (specify): _____
___ PTEN mutation (specify): _____
___ PTPN11 mutation (specify): _____
___ PRPF8 mutation (specify): _____
___ RB1 mutation (specify): _____
___ RBM15::MKL1 fusion (specify): _____
___ RUNX1 mutation (specify): _____
___ RUNX1::RUNX1T1 fusion (specify): _____
___ SETBP1 mutation (specify): _____
___ SH2B3 mutation (specify): _____
___ SF3B1 mutation (specify): _____
___ SRSF2 mutation (specify): _____
___ STAG2 mutation (specify): _____
___ TET2 mutation (specify): _____
___ TCF3::PBX1 rearrangement (specify): _____
___ TCF3::HLF fusion rearrangement (specify): _____
___ TP53 mutation (specify): _____
___ U2AF1 mutation (specify): _____
___ WT1 mutation (specify): _____
___ ZRSR2 mutation (specify): _____
___ Other alterations detected (specify): _____
___ Pending: _____

+Specify Molecular Alterations Assayed: _____

COMMENTS

Comment(s): _____

Explanatory Notes

A. Site of Involvement in Sample

Select the most significantly involved site of involvement in the sample as a single select choice. If both bone marrow and blood are involved, as is often the case, select bone marrow and report the bone marrow blast/blast equivalent percentage and the bone marrow cellularity. If the sample is blood or if the percentage of blasts is higher in the blood compared to bone marrow (i.e., more significant), select the option of blood and specify the blasts/blast equivalents.

For bone marrow samples, bone marrow aspirate smears stained with Wright-Giemsa or May-Grunwald-Giemsa should be used to perform a cell differential count to enumerate blasts and other nucleated cells in the bone marrow sample. While a 500-cell differential count is the traditional number used; studies indicate that 300-cell differential counts give similar results.[1] It is important to avoid hemodiluted areas that may not reflect bone marrow cellularity when performing the differential count. In those instances where the sample adequacy does not permit an adequate manual count, the best estimate of blasts can be given based on ancillary studies, typically immunohistochemistry for CD34 or other appropriate blast marker based on the known phenotype of the blast cells.

In addition, the overall bone marrow cellularity should be reported based on the area of hematopoietic bone marrow relative to the area of adipose tissue. This may be estimated on the bone marrow core biopsy specimen or intact areas of the bone marrow clot section. Bone marrow cellularity varies with age, but it is also important to note that cellularity may be lower in subcortical areas of bone marrow or may show significant heterogeneity and thus not be accurate in small samples.

References

1. Ahmed A Abdulrahman, MD, Kirtesh H Patel, MD, Tong Yang, MD, David D Koch, PhD, Sarah M Sivers, MLS (ASCP)CM, Geoffrey H Smith, MD, David L Jaye, MD, Is a 500-Cell Count Necessary for Bone Marrow Differentials? A Proposed Analytical Method for Validating a Lower Cutoff, *American Journal of Clinical Pathology*, Volume 150, Issue 1, July 2018, Pages 84–91, https://doi.org/10.1093/ajcp/aqy034.

B. Final Integrated Diagnosis

The final integrated diagnoses for the myeloid neoplasms are derived from the WHO 5th edition of Haematolymphoid Tumors.[1] This represents an update since the last revision of the WHO 4th edition in 2017 and reflects updates in the diagnostic criteria and increasing reliance of ancillary studies such as conventional karyotyping, fluorescence in-situ hybridization (FISH), and molecular genetic profiling- the latter increasingly utilizing large multigene interrogations utilizing next-generation sequencing with abilities to detect point mutations, insertions, deletions, copy number alterations, and gene rearrangements.
The final integrated diagnosis is categorized into the major subsections of the WHO 5th edition, including myeloid precursor lesions, myeloproliferative neoplasms, mastocytosis, myelodysplastic neoplasms, myelodysplastic/myeloproliferative neoplasms, acute myeloid leukemia, myeloid neoplasms, secondary, myeloid/lymphoid neoplasms, acute leukemias of mixed or ambiguous lineage and blastic plasmacytoid dendritic cell neoplasm. Leukemias of mixed lineage and ambiguous lineage are included in the myeloid neoplasm cancer case summary for ease of reporting, as these neoplasms will share more elements with myeloid neoplasms and more frequently contain a myeloid component in addition to a lymphoid component.

Histiocytic/dendritic cell neoplasms with the exception of blastic plasmacytoid dendritic cell neoplasm, are excluded from the protocol due to infrequent involvement of blood and bone marrow and distinct clinicopathologic features; these include neoplasms of Langerhans cells (Langerhans cell histiocytosis,

Langerhans cell sarcoma), indeterminate dendritic cell tumor, interdigitating dendritic cell sarcoma and histiocytic neoplasms (Juvenile xanthogranuloma, Erdheim-Chester disease, Rosai-Dorfman disease, ALK-positive histiocytosis, and histiocytic sarcoma).

Precursor myeloid lesions, clonal hematopoiesis of indeterminate potential (CHIP), and clonal cytopenia of undetermined significance (CCUS) are included as optional elements. These may have utility for some institutions for longitudinal tracking and documentation of pre-neoplastic states.

This cancer case summary contains certain common subtypes as the initial reporting option and includes staging/phase information as the primary diagnostic selection to emphasize its importance for these conditions and to ease reporting by minimizing additional deprecated selections. For example, chronic myeloid leukemia, chronic phase, and chronic myeloid leukemia, blast phase are listed as diagnostic choices instead of chronic myeloid leukemia with the option to further select chronic or blast phase (deprecated selections). Chronic myelomonocytic leukemia (CMML) includes both the subtype (myelodysplastic subtype vs. myeloproliferative subtype) and grading information (CMML-1 vs. CMML-2) as the primary choice (Myelodysplastic chronic myelomonocytic leukemia (MD-CMML), CMML-1).

This cancer case summary is designed to be used when a complete, integrated diagnosis can be rendered, including ancillary immunophenotypic, cytogenetic, and molecular results that are increasingly utilized to refine the diagnostic category. There may be some who wish to use this cancer summary to render a preliminary diagnosis prior to receipt of all pending ancillary studies; ideally, this approach would be used when a cancer case summary can be updated/addended/amended, or a new complete cancer protocol can be issued. To accommodate that use, one can use the “Other” category included as a diagnostic choice in each of the major sections of the cancer case summary. If using the “Other” option for cases pending further ancillary studies, it is strongly recommended to update the cancer case summary.

The full diagnostic criteria for the diagnostic categories in the WHO 5th edition are well summarized in the WHO monograph and beyond the scope of the explanatory notes. Essential diagnostic information is included in the explanatory notes to serve as a quick reference. Specific categories that may benefit from additional explanation, particularly regarding use of the cancer case summary, are discussed.

Myeloid precursor lesions

The WHO 5th edition introduces clonal hematopoiesis (CH)/clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenias of undetermined significance (CCUS) as putative myeloid precursor lesions. Clonal hematopoiesis (CH) refers to the clonal expansion of a mutated hematopoietic progenitor cells. Clonal hematopoiesis of indeterminate potential (CHIP) is defined as CH that harbors myeloid malignancy associated genes detected in blood or bone marrow at variant allele frequency (VAF) ≥2% (≥4% for X-linked gene mutations in males) in individuals without cytopenia or other diagnosed hematologic disorder. Clonal hematopoiesis of undetermined significance (CCUS) is diagnosed when CHIP criteria are accompanied by clinical cytopenia (Hgb <13g/dL males, <12g/dL females, absolute neutrophil count <1.8x10^9/L, platelet count <150x10^9/L) without evidence of diagnostic myeloid neoplasm. Reporting of CHIP or CCUS using the cancer case summary is optional.

Myeloid neoplasms, chronic

Myeloproliferative neoplasms

Myeloproliferative neoplasms represent a group of myeloid neoplasms characterized by the abnormal proliferation of one or more terminally differentiated myeloid cell lines, sometimes with increased bone marrow reticulin or collagen fibrosis. The WHO 5th edition retains the framework of the revised WHO 4th edition for diagnostic entities with minor changes. Chronic myeloid leukemia (CML) accelerated phase

(AP) has been removed as its relevance in the era of highly effective targeted tyrosine kinase inhibitors makes determination of ABL1 kinase resistance mutations, cytogenetic progression, and overt blast phase drive treatment decisions.[2]

The diagnostic criteria for chronic eosinophilic leukemia have been updated as follows: (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 blood or 5-19% in bone marrow) as an alternative to clonality.[1] Diagnostic criteria for chronic myeloproliferative neoplasms are summarized in Table 1.

Table 1. SUMMARY OF DIAGNOSTIC FEATURES FOR CATEGORY OF CHRONIC MYELOPROLIFERATIVE NEOPLASMS[1]

Diagnosis	Essential Diagnostic Features	Post-fibrotic diagnostic features
Chronic myeloid leukemia (CML)	CML (chronic phase) criteria: 1. Blood leukocytosis 2. Detection of Ph chromosome and/or *BCR::ABL1* by cytogenetic and/or appropriate molecular genetic techniques 3. Do not meet criteria for blast phase CML (blast phase) criteria: 1. ≥20% blasts in the blood or bone marrow or 2. Presence of an extramedullary proliferation of blasts or 3. Presence of bona fide lymphoblasts in the blood or bone marrow (even if <10%)	

Chronic neutrophilic leukemia (CNL)	The diagnosis requires exclusion of reactive neutrophilia and other myeloproliferative and myelodysplastic/ myeloproliferative neoplasms: 1. Blood white blood cell count ≥25×10^9/L- Segmented neutrophils plus banded neutrophils constitute ≥ 80% of the white blood cells- Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) constitute <10% of the white blood cells- Myeloblasts rarely observed- Monocytes constitute <10% of blood leukocytes; absolute monocytosis not meeting criteria for CMML- No dysgranulopoiesis 2. Hypercellular bone marrow- Neutrophil granulocytes increased in percentage and number- Neutrophil maturation appears normal- Myeloblasts constitute < 5% of the nucleated cells 3. Not meeting WHO criteria for *BCR::ABL1*–positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, or primary myelofibrosis 4. No evidence of disease-defining gene rearrangements such as *PDGFRA*, *PDGFRB*, or *FGFR1*, and no *PCM1::JAK2* fusion 5. Presence of *CSF3R p.T618I* or another activating *CSF3R* mutation, OR Persistent neutrophilia (≥3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if a plasma cell neoplasm is present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies	
Chronic eosinophilic leukemia (CEL)	1. Hypereosinophilia, defined as blood eosinophilia >1.5x10^9/L on at least 2 occasions over an interval of at least 4 weeks 2. Evidence of clonality, excluding the possibility of clonal hematopoiesis of indeterminate potential (CHIP) 3. Abnormal bone marrow morphology typically hypercellularity with dysplastic megakaryocytes, increased eosinophils 4. WHO criteria for other myeloid or lymphoid neoplasms not met, including MPN, MDS/MPN, MDS, MLN-eo, mastocytosis, AML	
Polycythemia vera (PV)	Diagnostic criteria of polycythemia vera The diagnosis of polycythemia vera requires either all 3 major criteria or the first 2 major criteria plus the minor criterion. Major criteria: 1. Elevated hemoglobin concentration (>16.5 g/dL in men; >16.0 g/dL in women) or elevated hematocrit (>49% in men; >48% in women) 2. Bone marrow biopsy showing age-adjusted	Diagnostic criteria for post–polycythemia vera (PV) myelofibrosis Required criteria: Documentation of a previous diagnosis of WHO-defined PV Bone marrow fibrosis of grade 2-3 on a 0–3 scale

	hypercellularity with trilineage growth (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size) 3. Presence of *JAK2 V617F* or *JAK2* exon 12 mutation Minor criterion: 1. Subnormal serum erythropoietin level	Additional criteria (2 are required): 1. Anemia (i.e., below the reference range given age, sex, and altitude considerations) or sustained loss of requirement of either phlebotomy (in the absence of cytoreductive therapy) or cytoreductive treatment for erythrocytosis 2. Leukoerythroblastosis 3. Increasing splenomegaly, defined as either an increase in palpable splenomegaly of >5 cm from baseline (distance from the left costal margin) or the development of a newly palpable splenomegaly 4. Development of any 2 (or all 3) of the following constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5 °C)
Essential thrombocythemia	Diagnostic criteria for essential thrombocythemia The diagnosis of essential thrombocythemia requires that either all major criteria or the first 3 major criteria plus the minor criterion are met. Major criteria: 1. Platelet count ≥ 450×10^9/L 2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobated nuclei; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; very rarely a minor (grade 1) increase in reticulin fibers 3. WHO criteria for *BCR::ABL1*–positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, or other myeloid neoplasms are not met 4. *JAK2*, *CALR*, or *MPL* mutation Minor criterion: 1. Presence of a clonal marker or 2. Exclusion of reactive thrombocytosis	Diagnostic criteria for post–essential thrombocythemia (ET) myelofibrosis Required criteria: 1. Documentation of a previous diagnosis of WHO-defined ET 2. Bone marrow fibrosis of grade 2–3 on a 0–3 scale Additional criteria (2 are required): 1. Anemia (i.e., below the reference range given age, sex, and altitude considerations) and a >2g/dL decrease from baseline hemoglobin concentration 2. Leukoerythroblastosis 3. Increasing splenomegaly, defined as either an increase in palpable splenomegaly of >50 mm from baseline (distance from the left costal margin, or on imaging) or

		the development of a newly palpable splenomegaly 4. Elevated lactate dehydrogenase level (above the reference range) 5. Development of any 2 (or all 3) of the following constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C)
Primary myelofibrosis	Diagnostic criteria for primary myelofibrosis, prefibrotic The diagnosis of pre-fibrotic primary myelofibrosis requires that all 3 major criteria and at least 1 minor criterion are met. Major criteria: 1. Megakaryocytic proliferation and atypia, without reticulin fibrosis grade >1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and (often) decreased erythropoiesis 2. WHO criteria for *BCR-ABL1*–positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, myelodysplastic syndromes, or other myeloid neoplasms are not met 3. *JAK2, CALR*, or *MPL* mutation OR Presence of another clonal marker OR Absence of minor reactive bone marrow reticulin fibrosis Minor criteria: Presence of at least one of the following, confirmed in 2 consecutive determinations: 1. Anemia not attributed to a comorbid condition 2. Leukocytosis ≥11×10^9/L 3. Splenomegaly detected clinically and/or by imaging 4. Lactate dehydrogenase level above the upper limit of the institutional reference range 5. Leukoerythroblastic	Diagnostic criteria for primary myelofibrosis, fibrotic stage The diagnosis of overt primary myelofibrosis requires that all 3 major criteria and at least 1 minor criterion are met. Major criteria: 1. Megakaryocytic proliferation and atypia, accompanied by reticulin and/or collagen fibrosis grades 2 or 3 2. WHO criteria for essential thrombocythemia, polycythemia vera, *BCR-ABL1*–positive chronic, myeloid leukemia, myelodysplastic syndrome, or other myeloid neoplasms are not met 3. *JAK2*, *CALR*, or *MPL* mutation OR Presence of another clonal marker OR Absence of reactive myelofibrosis Minor criteria: Presence of at least one of the following, confirmed in 2 consecutive determinations: 1. Anemia not attributed to a comorbid condition 2. Leukocytosis ≥ 11×10^9/L 3. Splenomegaly detected clinically and/or by imaging 4. Lactate dehydrogenase

		level above the upper limit of the institutional reference range 5. Leukoerythroblastosis
Juvenile myelomonocytic leukemia (JMML)	Clinical, hematological, and laboratory criteria (all 5 criteria are required): 1. Blood monocyte count ≥1x10^9/L 2. Blast and promonocyte percentage in blood and bone marrow of <20% 3. Clinical evidence of organ infiltration, most commonly splenomegaly 4. No Philadelphia (Ph) chromosome or *BCR-ABL1* fusion 5. No *KMT2A* (*MLL1*) gene rearrangement Genetic criteria (any 1 criterion is sufficient): 1- Mutation in a component or a regulator of the canonical *RAS* pathway: • Clonal somatic mutation in *PTPN11*, *KRAS*, or *NRAS* • Clonal somatic or germline *NF1* mutation and loss of heterozygosity or compound heterozygosity of *NF1* • Clonal somatic or germline *CBL* mutation and loss of heterozygosity of *CBL* 2- Non-canonical clonal *RAS* pathway pathogenic variant or fusions causing activation of genes upstream of the *RAS* pathway, such as *ALK, PDGFR-B, ROS1*, among others Other criteria Cases that do not meet any of the genetic criteria listed above (or in conditions where genetic testing is not available) must meet the following criteria in addition to the aforementioned clinical, hematological, and laboratory criteria above: ≥2 of the following: • Increased hemoglobin F for age • Myeloid (promyelocytes, myelocytes, metamyelocytes) and erythroid precursors on blood smear • Thrombocytopenia with hypercellular marrow often showing decreased number of megakaryocytes. Dysplastic features may or may not be evident • Hypersensitivity of myeloid progenitors to GM-CSF as tested in clonogenic assays in methylcellulose or by measuring *STAT5* phosphorylation in the absence or with low dose of exogenous GM-CSF	
Myeloproliferative neoplasm, NOS	Most cases of MPN-NOS fall into one of these groups: • Early presentations where the characteristic	

	features of specific subtypes are not yet fully developed • A proportion of cases presenting with a portal or splanchnic vein thrombosis that fail to meet the diagnostic criteria for any of the specific MPN entities may also be considered to belong in this group • Advanced-stage MPN, in which pronounced myelofibrosis, osteosclerosis, or transformation to a more aggressive stage with increased blast counts and/or myelodysplastic changes obscures the underlying disorder • Cases with convincing evidence of an MPN in which a coexisting neoplastic or inflammatory disorder obscures some of the usual diagnostic clinical and/or morphological features	

Mastocytosis

Mastocytosis is an uncommon neoplasm with varying clinical presentation that is characterized by the abnormal accumulation of mast cells in various tissues, typically associated with mutations in *KIT* receptor that lead to constitutive activation. There have been updates to the minor diagnostic criteria of systemic mastocytosis in the WHO 5th edition including the expression of CD30 and any *KIT* mutation being a minor criterion. The subtype of systemic mastocytosis, bone marrow mastocytosis, has been added along with updates to the B- (burden of disease) criteria for subtyping systemic mastocytosis.

The cancer case summary lists the subtypes of systemic mastocytosis as the diagnostic choices. The approach to subtyping requires first the establishment of the diagnosis of systemic mastocytosis (see Table 2 for diagnostic criteria), followed by the application of the criteria (see Table 3 for B- and C-findings required for subtypes) for each of the subtypes shown in Table 4. A rare morphologic pattern that is worth noting, but not a defined subtype, is well-differentiated systemic mastocytosis (WDSM) which is characterized by round and well-granulated mast cells that are usually negative for *KIT* codon 816 mutation (15% positive, may possess *KIT* mutations outside TK2 domain) and negative for CD2 and CD25, but positive for CD30.[3]

Mast cell sarcoma is a very rare clinically aggressive form of mastocytosis characterized by the presence of atypical mast cells that results in a locally destructive lesion that typically express, similarly to systemic mastocytosis, CD2, CD25 and/or CD30. Unlike systemic mastocytosis, *KIT p. D816V* mutations are uncommon in de novo/classical mast cell sarcoma, suggesting a distinct origin from systemic mastocytosis. Mast cell sarcoma may exist as a transformation of a systemic mastocytosis.[4]

Cutaneous mastocytosis (CM), a form of mastocytosis primarily affecting the skin is not included in the cancer case summary due to typically indolent behavior and need for correlation with clinical and pathologic signs for systemic mastocytosis that are often not available at time of evaluation of skin biopsy samples.

TABLE 2. SUMMARY OF ESSENTIAL DIAGNOSTIC FEATURES FOR SYSTEMIC MASTOCYTOSIS[1]

Diagnosis	Essential Diagnostic Features
Systemic Mastocytosis (SM)	The diagnosis is SM if at least 1 major and 1 minor or 3 minor criteria are fulfilled. 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. >25% of all mast cells are atypical cells on bone marrow smears or are spindle-shaped in dense and diffuse mast cell infiltrates in sections of BM or other extracutaneous organ(s) 2. Activating *KIT* point mutation(s) at codon 816 or in other critical regions of *KIT* in bone marrow or another extracutaneous organ(s) 3. Mast cells in bone marrow, blood, or another extracutaneous organ(s) aberrantly express one or more of the following antigens (flow cytometry or immunohistochemistry): CD2, CD25, CD30 4. Baseline serum tryptase concentration >20ng/mL in the absence of a myeloid associated hematologic neoplasm (AHN). In the case of a known hereditary alpha-tryptasemia (HαT), the tryptase level could be adjusted[5]

TABLE 3. SYSTEMIC MASTOCYTOSIS B-FINDINGS (BURDEN OF DISEASE) AND C-FINDINGS (CYTOREDUCTION-REQUIRING)[1]

Diagnosis	Essential Diagnostic Features
B-findings	1. High MC burden: infiltration grade (MC) in BM ≥30% in histology (IHC) and/or serum tryptase ≥200 ng/mL and/or *KIT p.D816V* VAF ≥10% in BM or PB leukocytes 2. Signs of myeloproliferation and/or myelodysplasia: hypercellular BM with loss of fat cells and prominent myelopoiesis ± left shift and eosinophilia ± leukocytosis and eosinophilia and/or discrete signs of myelodysplasia (<10% neutrophils, erythrocytes, and megakaryocytes) 3. Organomegaly: Palpable (or documented by US, CT, or MRI) hepatomegaly without ascites or other signs of organ damage or/and palpable splenomegaly without hypersplenism and without weight loss or/and lymphadenopathy palpable or visceral LN-enlargement found in ULS or CT (>20 mm)
C-findings	1. Cytopenia/s (one or more found): • ANC <1x10^9/L • Hb <10 g/dL • PLT <1.0x10^9/L 2. Hepatopathy: ascites and elevated liver enzymes ± hepatomegaly or cirrhotic liver ± portal hypertension 3. Spleen: palpable splenomegaly with hypersplenism ± weight loss ± hypalbuminemia 4. GI tract: malabsorption with hypoalbuminemia ± weight loss 5. Bone: large-sized osteolysis (≥20 mm) ± pathologic fracture ± bone pain

TABLE 4. SUMMARY OF SUBTYPES OF SYSTEMIC MASTOCYTOSIS[1]

Subtype	Diagnostic criteria	Morphologic features
Bone marrow mastocytosis (BMM)	• SM criteria fulfilled • No skin lesions • No B-finding(s) • Basal serum tryptase <125ng/mL • No dense SM infiltrates in an extramedullary organ	• Low to very low MC burden in the BM • Compact MC infiltrates >70% of cases • Morphologic features otherwise as in ISM • WD morphology rarely observed

Indolent Systemic Mastocytosis (ISM)	• SM criteria fulfilled • Typical skin lesions • ≤1 B-finding • ISM without skin lesions: ≤1 B finding and/or basal serum tryptase ≥125 ng/mL and/or dense SM infiltrates in an extramedullary organ	• Low MC burden in the BM (usually <5-10% of section area) • Compact MC infiltrates >70% of cases, typically of mixed morphology, containing numerous eosinophils, lymphocytes, fibroblasts and histiocytes in addition to neoplastic MC • Usually >25% spindle-shaped MC • Hemopoiesis usually normal, mild reactive changes possible • Paratrabecular compact MC infiltrates often with prominent reticulin or collagen fibrosis and osteosclerotic changes in adjacent trabeculae • In BM smears atypical MC (usually type I) • WD morphology possible
Smoldering systemic mastocytosis (SSM)	• SM criteria fulfilled • ≥2 B-findings • No C-finding	• High MC burden in the BM (usually >30% of section area) • Diffuse-compact MC infiltration pattern • Usually spindle-shaped MC embedded in a dense fibrotic stroma with pronounced osteosclerosis • Hemopoiesis often with mild dysplastic changes (not fulfilling WHO criteria for AHN) • WD morphology rarely observed
Aggressive systemic mastocytosis (ASM)	• SM criteria fulfilled • ≥1 C-finding	• High to very high MC burden in the BM (up to 80% of the section area) • Diffuse-compact MC infiltration pattern • Usually spindle-shaped MC embedded in a dense fibrotic stroma with pronounced osteosclerosis • Pure ASM less frequent than ASM-AHN • MCs in BM smears between >5% and <20% indicating ASM in transformation (ASM-t) • Hemopoiesis often with mild dysplastic changes (not fulfilling WHO criteria for AHN) • WD morphology possible
Systemic mastocytosis with an associated hematologic neoplasm (SM-AHN)	• SM criteria fulfilled • Criteria for a WHO-defined hematologic neoplasm. Both disease compartments are classified according to WHO-definitions, e.g., BMM-ET, or MCL-CMML-1, etc	• Any subtype of SM and any type of WHO-defined myeloid and lymphoid neoplasm can occur; CMML or other MDS/MPN overlap neoplasms are most frequently found • In most cases, AHN obscures SM • In ASM or MCL, an AHN may be difficult to detect • In every case of suspected SM-AHN, not only immunostains for neoplastic MC (tryptase, CD117/KIT, CD25, CD30), but also for myeloid neoplasms (including CD14, CD34, CD42b, and CD71) should be applied • WD morphology possible

Mast cell leukemia (MCL)	• SM criteria fulfilled • ≥20% MCs in BM smears • In classic MCL ≥10% MCs, in aleukaemic MCL <10% MCs in blood smears • In acute MCL C-findings are detectable; chronic MCL (no C-findings with much better prognosis	• High MC burden in the BM (usually >50% of section area) • Diffuse-compact MC infiltration pattern • Predominantly round and hypogranulated MCs in most cases • Rarely highly pleomorphic MCs with hypogranulation and cytoplasmatic vacuolization • Reticulin content usually much lower than in SSM/ASM • Pure MCL is a diagnosis of exclusion of AHN • WD morphology rarely observed (chronic MCL)

Myelodysplastic Neoplasms

Myelodysplastic syndromes are now referred to as myelodysplastic neoplasms (MDS) in the WHO 5th edition to emphasize the clonal, neoplastic nature and to be consistent with diagnostic terminology for myeloproliferative neoplasms. The new classification groups MDS into two broad categories; those that are defined by genetic abnormalities and those that are morphologically defined. This approach is analogous to the groupings of acute myeloid leukemia and will enhance diagnostic consistency and highlight the importance of certain genetic alterations to MDS. The threshold for presence of dysplasia is set at 10% of cells of that lineage (erythroid, myeloid, megakaryocytic). Furthermore, diagnostic categories are appended with low blasts or increased blasts for additional clarity.

The major diagnostic criteria for MDS with defining genetic abnormalities are shown in Table 5. Myelodysplastic neoplasm with low blasts and 5q deletion (MDS-5q) may possess *SF3B1* mutations and non-biallelic *TP53* alterations (niTP53) if present. Myelodysplastic neoplasm with low blasts and *SF3B1* mutation (MDS-*SF3B1*) was introduced to capture the high association between the presence of *SF3B1* mutation and ring sideroblasts (>90% of cases of MDS with ≥5% ring sideroblasts). If ring sideroblasts are identified and *SF3B1* mutation is absent, after exclusion of secondary causes of ring sideroblasts, it is acceptable to diagnose these cases as MDS with low blasts and ring sideroblasts in the narrative report. These can be reported as MDS with low blasts as the diagnostic category and denote the presence of ring sideroblasts. Biallelic *TP53* (bi*TP53*) alterations are a poor prognosis category of MDS characterized by alterations in *TP53* that result in no functional/wild-type *TP53*.[6] The presence of one or more *TP53* mutations with copy number loss or evidence of copy neutral loss of heterozygosity (LOH) are considered sufficient for demonstration of biTP53/loss of wild type *TP53*. *TP53* with >49% variant allele frequency (VAF) may be regarded as presumptive evidence of allelic loss or copy neutral LOH if a constitutional *TP53* variant can be excluded.[7]

TABLE 5. SUMMARY OF SUBTYPES OF MYELODYSPLASTIC NEOPLASMS WITH DEFINING GENETIC ABNORMALITIES[1]

Subtype	Essential diagnostic criteria
Myelodysplastic neoplasm with low blasts and 5q deletion (MDS-5q)	• Anemia, with or without other cytopenias and/or thrombocytosis • Dysplasia involving megakaryocytes, often micromegakaryocytes with or without dysplasia involving other lineages • Blasts <5% in the bone marrow and <2% in the blood • Detection of 5q deletion, isolated or with one other cytogenetic aberration other than monosomy 7 or 7q deletion • Not fulfilling diagnostic criteria of AML, MDS with biallelic *TP53* inactivation, MDS with increased blasts, or MDS/MPN

Myelodysplastic neoplasm with low blasts and *SF3B1* mutation (MDS-*SF3B1*)	• Cytopenia involving one or more lineages, without thrombocytosis • Erythroid lineage dysplasia • Blasts <5% in the bone marrow and <2% in the blood • Detection of *SF3B1* mutation. If *SF3B1* mutation analysis is not available, demonstration of ring sideroblasts comprising ≥15% of erythroid precursors • Absence of 5q deletion, monosomy 7/7q deletion, or complex karyotype • Not fulfilling diagnostic criteria of AML, MDS with low blasts and 5q deletion, MDS with biallelic *TP53* inactivation, MDS with increased blasts or any MDS/MPN type
Myelodysplastic neoplasm with biallelic (or multi-hit) *TP53* alterations (MDS-bi*TP53*)	• Myeloid neoplasm fulfilling diagnostic criteria of MDS • Detection of one or more *TP53* mutations • In the presence of one *TP53* mutation, evidence of *TP53* copy loss or copy neutral LOH

Myelodysplastic neoplasms morphologically defined is a new category in the WHO 5th edition. The new diagnostic category of hypoplastic myelodysplastic neoplasm (MDS-h) is recognized to capture this distinctive type of MDS characterized by bone marrow hypocellularity, cytopenias, and dysplastic changes. It is important to exclude secondary causes of hypocellularity with dysplastic changes such as drug or toxin exposure and exclude aplastic anemia (AA) or paroxysmal nocturnal hemoglobinuria (PNH). In addition, exclusion of a genetic predisposition to bone marrow failure, particularly in younger patients, should be considered.

The distinction of single- and multilineage dysplasia in the classification of MDS has been removed to reflect the absence of a well-defined category and fluidity of lineage dysplasia over the disease course of MDS. MDS with increased blasts is now the preferred terminology to capture those cases with 5-19% bone marrow or 2-19% blood blasts. For the cancer case summary, select the appropriate subtype of MDS-IB- either MDS-IB-1, MDS-IB-2, or MDS-F based on the criteria shown in Table 6.

Table 6. SUMMARY OF MYELODYSPLASTIC NEOPLASMS, MORPHOLOGICALLY DEFINED WITH SUBTYPES OF MDS-IB[1]

Subtype	Essential diagnostic criteria
Myelodysplastic neoplasm with low blasts (MDS-LB)	• Cytopenia involving one or more lineages • Dysplastic changes in one or more lineages involving at least 10% of cells • <5% bone marrow blasts and <2% blood blasts • Exclusion of folate and vitamin B12 deficiency • No fulfilling diagnostic criteria of MDS with defining genetic alterations or hypoplastic MDS
Hypoplastic MDS (h-MDS)	• Cytopenia involving one or more lineages • Hypocellular bone marrow (assessed on a trephine core biopsy, adjusted for age of the patient) not explained by drug/toxin exposure or pertinent nutritional deficiency • Dysplasia involving myeloid and/or megakaryocytic lineages • <5% blasts in bone marrow and <2% blasts in blood • Not meeting criteria for MDS with defining genetic abnormalities or MDS with increased blasts
Myelodysplastic neoplasm with increased blasts (MDS-IB)	• Cytopenia involving one or more lineages • Dysplastic changes in one or more lineages, involving at least 10% of cells • ≥5% blasts in the bone marrow and/or ≥2% blasts in blood • No fulfilling diagnostic criteria of MDS with biallelic *TP53* inactivation or AML Subtypes of MDS-IB

	• MDS with increased blasts-1 (MDS-IB1): 5-9% blasts in the bone marrow and/or 2-4% blasts in the blood, without significant reticulin fibrosis • MDS with increased blasts-2 (MDS-IB2):10-19% blasts in the bone marrow and/or 5-19% blasts in the blood, without significant reticulin fibrosis; or, with presence of Auer rods • MDS with increased blasts and fibrosis (MDS-F): 5-19% blasts in the bone marrow and/or 2-19% blasts in the blood, with significant fibrosis (defined as grade 2 or 3)

Myelodysplastic neoplasms of childhood

Myelodysplastic neoplasms of childhood are distinct clinicopathologic and biologic syndromes characterized by ineffective hematopoiesis, resultant cytopenias, and increased risk of developing acute myeloid leukemia. Like adults, the WHO 5th edition divides these into the morphologically defined categories of cMDS with low blasts (cMDS-LB) and cMDS with increased blasts (cMDS-IB). cMDS-LB replaces refractory cytopenia of childhood (RCC) from the revised WHO 4th edition. Bone marrow hypocellularity is particularly common in cMDS-LB, and it is important to exclude secondary causes of hypocellularity (infection, toxin, nutritional deficiency, severe aplastic anemia, PNH).[8] The diagnostic criteria for cMDS are summarized in Table 7.

TABLE 7. SUMMARY OF SUBTYPES OF MYELODYSPLASTIC NEOPLASMS OF CHILDHOOD[1]

Subtype	Essential diagnostic criteria
Childhood myelodysplastic neoplasm with low blasts (cMDS-LB)	• Cytopenia involving one or more lineages • Dysplastic changes in one or more lineages, involving at least 10% of cells • <5% bone marrow blasts and <2% blood blasts • Meeting at least one of the following criteria: 1. Detection of clonal cytogenetic and/or molecular abnormality 2. Exclusion of other causes of cytopenia (non-neoplastic and some germline mutations)
Childhood myelodysplastic neoplasm with increased blasts (cMDS-IB)	• Cytopenia involving one or more lineages • Dysplastic changes in one or more lineages, involving at least 10% of cells • 5-19% bone marrow blasts and/or 2-19% blood blasts • Exclusion of Down syndrome, juvenile myelomonocytic leukemia, and AML with defining genetic abnormalities

Myelodysplastic/myeloproliferative neoplasms

The criteria for the diagnosis of chronic myelomonocytic leukemia (CMML) were revised in the WHO 5th edition to include prerequisite and supporting criteria. The initial prerequisite criteria include persistent absolute (≥0.5x10^9/L) and relative monocytosis (≥10%) in the blood. Of note, the absolute monocyte count in the blood was lowered from the 1.0x10^9/L cutoff in the revised WHO 4th edition. Another new criterion introduced was the abnormal partitioning of monocytes (>94%) into classical monocytes CD14+, CD16- compared to those with more variable levels of intensity of CD14 and CD16.[9] In addition, the presence of a *NPM1* mutation supersedes the diagnosis of CMML. In the current WHO 5th edition, these cases meet the criteria for AML with *NPM1* mutation.[10]

Supporting criteria are required after meeting the prerequisite criteria, with a higher absolute monocyte count (>1x10^9/L) requiring one additional supporting, and lower absolute monocyte count (>0.5x10^9/L) requiring 2 supporting criteria. CMML is further subtyped into myelodysplastic CMML (WBC <13x10^9/L) and myeloproliferative CMML (WBC ≥13x10^9/L) based on the white blood cell count (WBC). Furthermore, it is subgrouped based on the percentage of blasts and promonocytes in the blood and bone marrow (CMML-1: < 5% blasts and promonocytes in blood and < 10% in bone marrow; CMML-2: 6-19% blasts and promonocytes in blood and 10-19% in bone marrow). The cancer case summary removes choice

deprecation and allows for reporting of the four options for the final subtype to make reporting easier and more straightforward.

Atypical chronic myeloid leukemia was renamed MDS/MPN with neutrophilia in the WHO 5th edition to avoid potential confusion with chronic myeloid leukemia (CML); diagnostic criteria are unchanged. A summary of the diagnostic features for myelodysplastic/myeloproliferative neoplasms and subtypes/subgroups of CMML are shown in Table 8.

TABLE 8. SUMMARY OF MYELODYSPLASTIC/ MYELOPROLIFERATIVE NEOPLASMS INCLUDING SUBTYPES AND SUBGROUPS OF CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML)[1]

Subtype	Essential diagnostic criteria	Desirable diagnostic criteria/Notes
Chronic myelomonocytic leukemia (CMML)	Prerequisite criteria: 1. Persistent absolute (≥0.5 × 10^9/L) and relative (≥10%) blood monocytosis 2. Blasts and equivalents* constitute <20% of the cells in the blood and bone marrow. (*= myeloblasts, monoblasts, and promonocytes) 3. Not meeting diagnostic criteria of chronic myeloid leukemia or other myeloproliferative neoplasms 4. Not meeting diagnostic criteria of myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangements (e.g., *PDGFRA*, *PDGFRB*, FGFR1, or *JAK2*) 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 <1 × 10^9/L: supporting criteria 1 and 2 must be met	Supporting criteria: 1. Dysplasia involving ≥1 myeloid lineage (morphologic dysplasia ≥10% of cells in lineage) 2. Acquired clonal cytogenetic or molecular abnormality 3. Abnormal partitioning of blood monocyte subsets (Based on detection of increased classical monocytes (>94%) in the absence of known active autoimmune diseases and/or systemic inflammatory syndromes) Subtyping criteria: • Myelodysplastic CMML (MD-CMML): WBC count <13 × 10^9/L • Myeloproliferative CMML (MP-CMML): WBC count ≥13 × 10^9/L Subgrouping criteria: • CMML-1: < 5% blasts and promonocytes in blood and < 10% in bone marrow • CMML-2: 6-19% blasts and promonocytes in blood and 10-19% in bone marrow
Myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N)	• Blood leukocytosis ≥13 × 10^9/L, with neutrophilia and ≥10% circulating immature myeloid cells (promyelocytes, myelocytes and metamyelocytes), as well as	• Detection of *SETBP1* and/or *ETNK1* mutations • Absence of mutations in *JAK2*, *CALR, MPL*,

	neutrophilic dysplasia • Hypercellular bone marrow with granulocytic predominance and granulocytic dysplasia, with or without dysplasia in the megakaryocytic and erythroid lineages • <20% blasts in blood and bone marrow • Not meeting diagnostic criteria for myeloproliferative neoplasms (specifically, exclusion of *BCR::ABL1* fusion), myeloid neoplasms with eosinophilia and defining gene rearrangement, chronic myelomonocytic leukemia, or myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis	and *CSF3R* • Previously referred to as “Atypical CML”
Myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis (MDS/MPN-*SF3B1*-T)	• Anemia associated with dysplastic erythropoiesis and ≥15% ring sideroblasts, with or without dysplasia in the megakaryocytic and erythroid lineages • Persistent thrombocytosis, with platelet count ≥450 × 10^9/L • *SF3B1* mutation and concurrent *JAK2 p.V617F*, or, in the absence of these mutations, concurrent biologically similar mutations involving spliceosome factors and cell signaling (e.g., MPL or CBL) • Not meeting diagnostic criteria for myelodysplastic neoplasms, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia with *MECOM* rearrangement, or myeloid/lymphoid neoplasms with eosinophilia	
Myelodysplastic/myeloproliferative neoplasm, NOS (MDS/MPN-NOS)	• Blood with combination of cytopenia(s) and proliferative features • Bone marrow with both dysplasia and proliferative features • Molecular alterations seen in proliferative and dysplastic myeloid malignancies • Exclusion of: Therapy-related myeloid neoplasms, disease-defining gene fusions, exclusion of other specific MDS/MPN such as CMML, MDS/MPN with neutrophilia (MDS/MPN-N), MDS/MPN with	

	SF3B1 mutation and thrombocytosis	

Acute myeloid leukemia

The classification of acute myeloid leukemia (AML) was reorganized and made into two major categories; those with defining genetic abnormalities and those defined by differentiation. A major change was the elimination of the 20% blast requirement for all AML types with defining genetic abnormalities except for acute myeloid leukemia (AML) with *BCR::ABL1* fusion and acute myeloid leukemia (AML) with *CEBPA* mutation.

The entity designated AML with myelodysplasia-related changes in the revised WHO 4th edition classification has been renamed AML, myelodysplasia-related, and new diagnostic criteria have been implemented. In the WHO 5th edition, in addition to the morphologic requirements, both defining cytogenetic criteria and mutational-based criteria have been introduced. See Table 9 for a summary of the diagnostic criteria for AML with defining genetic abnormalities.

TABLE 9. SUMMARY OF ACUTE MYELOID LEUKEMIA WITH DEFINING GENETIC ABNORMALITIES[1]

Subtype	Essential diagnostic criteria	Desirable diagnostic criteria/ Notes
Acute promyelocytic leukemia (APL) with *PML::RARA* fusion	• Myeloid neoplasm with increased blood and/or bone marrow atypical promyelocytes showing characteristic abnormal hypergranular promyelocytes or microgranular blasts (may be <20%)	• Detection of t(15;17)(q24;q21)
Acute myeloid leukemia (AML) with *RUNX1::RUNX1T1* fusion	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%) • Detection of *RUNX1::RUNX1T1* • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	• Detection of t(8;21)(q22;q22.1)
Acute myeloid leukemia with *CBFB::MYH11* fusion	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%) • Detection of *CBFB::MYH11* • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	• Detection of inv(16)(p13.1q22.1) or t(16;16)(p13.1;q22.1)
Acute myeloid leukemia with *DEK::NUP214* fusion	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%) • Presence of *DEK::NUP214* fusion • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	• Detection of t(6;9)(p22.3;q34.1)
Acute myeloid leukemia (AML) with *RBM15::MRTFA* fusion	• Detection of *RBM15::MRTFA* fusion by fluorescence in situ hybridization and/or RT-PCR or similar molecular technique • Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%)	• Detection of t(1;22)(p13.3;q13.1) by karyotype analysis • Demonstration of megakaryocytic differentiation
Acute myeloid leukemia (AML) with *BCR::ABL1* fusion	• Myeloid neoplasm with >20% blasts expressing a myeloid immunophenotype in the bone marrow and/or blood	• Presence of t(9;22)(q34.1;q11.2) on conventional karyotyping

	• Detection of *BCR::ABL1* at initial diagnosis • Lack of features of CML prior to or at diagnosis or after therapy	• Determination of the *BCR::ABL1* transcript subtype and establishing a baseline level of *BCR::ABL1* transcript for monitoring treatment response
Acute myeloid leukemia with *KMT2A* rearrangement	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%), or presence of a myeloid sarcoma • Blasts express myeloid immunophenotype, not fulfilling immunophenotypic criteria for mixed-phenotype acute leukemia • Presence of a *KMT2A* rearrangement. • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	• Identification of the *KMT2A* fusion partner
Acute myeloid leukemia with *MECOM* rearrangement	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%) • Detection of *MECOM* rearrangement • No history of myeloproliferative neoplasm • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	• Detection of inv(3)(q21.3q26.2), t(3;3)(q21;q26), t(3;21)(q26.2;q22) or t(3;12)(q26.2;p13)
Acute myeloid leukemia (AML) with *NUP98* rearrangement	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%) • Detection of the *NUP98* rearrangement and/or specific fusion products such as *NUP98*::*NSD1*	• Identification of the *NUP98* fusion partner at diagnosis is desirable to enable PCR-based disease monitoring
Acute myeloid leukemia with *NPM1* mutation	• Myeloid neoplasm with increased blood and/or bone marrow blasts (may be <20%) • Detection of *NPM1* mutation • No history of exposure to cytotoxic therapy	
Acute myeloid leukemia (AML) with *CEBPA* mutation	• ≥20% blasts with myeloid immunophenotype in bone marrow or blood • Presence of biallelic mutations in *CEBPA*, or a single mutation located in the bZIP region • Absence of criteria allowing for classification into other AML with defining genetic abnormalities • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	
Acute myeloid leukemia, myelodysplasia-related (AML-MR)	• ≥20% blasts with myeloid immunophenotype in bone marrow or blood • History of MDS or MDS/MPN and/or detection of one or more chromosomal or molecular aberrations • Criteria for other AML types with defined genetic alterations are not met. • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy	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*
Acute myeloid leukemia (AML) with other defined genetic alterations	• ≥20% blasts with myeloid immunophenotype in bone marrow and/or blood • Detection of one or more cytogenetic or molecular aberrations listed in adjacent column • Not fulfilling diagnostic criteria for AML with defining genetic abnormalities, AML-MR, AML-pCT or MPAL	Other defined genetic alterations: • *CBFA2T3::GLIS2* • *KAT6A::CREBBP* • *FUS::ERG* • *MNX1::ETV6* • *NPM1::MLF1*

The WHO 5th edition classification maintains the traditional categories of AML defined by differentiation that lack defining genetic abnormalities. As advances in our understanding of AML and improvements in molecular genetic techniques occur, it is likely that the number of cases falling into this category will diminish. Acute erythroid leukemia (AEL) is distinctive in that it requires >30% pronormoblasts (proerythroblasts), typically with marked background erythroid predominance (≥80%) and maturation arrest in the erythroid lineage. Biallelic *TP53* alterations are present in most cases.[11] AEL would supersede the diagnoses of MDS with biallelic *TP53* alterations due to lake of blasts (pronormoblasts and myeloid blasts) and AML, myelodysplasia related due to the lack of sufficient myeloid blasts. Criteria for acute myeloid leukemia (AML), defined by differentiation is shown in Table 10.

Myeloid sarcoma describes the presence of a tumor mass arising at a site other than bone marrow (extramedullary) that effaces the tissue architecture and is composed of myeloid blasts or blast equivalents with or without accompanying myeloid maturation. Myeloid sarcoma may occur prior to, coincident with, or following treatment for bone marrow myeloid leukemia. Non-effacing or non-tumor forming extramedullary blastic proliferations can occur in AML, MPN, MDS, or MDS/MPN and are not to be diagnosed as myeloid sarcoma. When selecting myeloid sarcoma on the cancer case summary, specify the associated myeloid neoplasm that it is associated with, if possible. For example:

Myeloid sarcoma
_X_ Myeloid sarcoma, specify myeloid neoplasm: Acute myeloid leukemia (AML) with *RUNX1::RUNX1T1* fusion

Table 10. SUMMARY OF DIAGNOSTIC FEATURES OF ACUTE MYELOID LEUKEMIA, DEFINED BY DIFFERENTIATION[1]

Subtype	Diagnostic criteria
Acute myeloid leukemia (AML) with minimal differentiation	• ≥20% blasts in bone marrow and/or blood lacking morphological and cytochemical evidence of myeloid differentiation • Positive for ≥2 myeloid-associated markers (e.g., CD13, CD33, CD117) • Criteria for AML types with defined genetic alterations are not met • Criteria for mixed-phenotype acute leukemia are not met • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy
Acute myeloid leukemia (AML) without maturation	• ≥20% blasts in bone marrow and/or blood with cytochemical evidence of myeloid differentiation and limited (<10%) morphologic features of granulocytic maturation • Positive for ≥2 myeloid-associated markers (e.g., myeloperoxidase, CD13, CD33, CD117) • Criteria for AML types with defined genetic alterations are not met. • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy
Acute myeloid leukemia (AML) with maturation	• ≥20% blasts in bone marrow and/or blood with cytochemical evidence of myeloid differentiation and morphologic features of granulocytic maturation in ≥10% of bone marrow cells • Positive for ≥2 myeloid-associated markers (e.g., myeloperoxidase, CD13, CD33, CD117) • Monocyte lineage cells constitute <20% of bone marrow cells • Criteria for AML types with defined genetic alterations are not met • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy
Acute basophilic leukemia (ABL)	• ≥20% of blasts with increased immature and mature basophils • Blasts/basophils with metachromasia on toluidine blue staining and negative for myeloperoxidase, or SBB, and NSE • Blasts positive for ≥2 myeloid-associated markers (e.g., myeloperoxidase, CD13, CD33, CD117) • Criteria for AML types with defined genetic alterations are not met • Not fulfilling diagnostic criteria for myeloid neoplasm post cytotoxic therapy Desirable diagnostic criteria: Blasts positive for CD123, CD11b, CD9, and/or CD203c, and negative for HLA-DR.
Acute myelomonocytic leukemia	• ≥20% blasts and blast equivalents (promonocytes) in bone marrow and/or blood • Positive for myeloid-associated markers (e.g., myeloperoxidase, CD13, CD33, CD117) • Maturing granulocytes constitute ≥20% of bone marrow cells • Monocyte lineage cells constitute ≥20% of bone marrow cells • Criteria for AML types with defined genetic alterations are not met • No fulfillment of diagnostic criteria for myeloid neoplasm post cytotoxic therapy
Acute monocytic leukemia	• ≥20% blasts and blast equivalents (promonocytes) in bone marrow and/or blood • ≥80% of the leukemic cells are monocytes and their precursors, including monoblasts and promonocytes • <20% maturing granulocytic cells • Criteria for AML types with defining genetic abnormalities are not met • No fulfillment of diagnostic criteria for myeloid neoplasm post cytotoxic therapy
Acute erythroid leukemia (AEL)	• Erythroid predominance, usually ≥80% of bone marrow elements, of which ≥30% are erythroblasts. Desirable diagnostic criteria: Evidence of *TP53* mutation
Acute megakaryoblastic	• ≥20% blasts with megakaryocytic differentiation in bone marrow and/or blood. • Blasts express at least one or more of the platelet glycoproteins: CD41, CD61, or

leukemia (AMKL)	CD42b • Does not meet criteria for other defined AML types • No history of myeloproliferative neoplasm • Recommend evaluation for possible Down syndrome

Myeloid neoplasms and proliferations associated with antecedent or predisposing conditions

Myeloid neoplasms post cytotoxic therapy (MN-pCT) includes AML, MDS, and MDS/MPN that arises in patients with antecedent exposure to cytotoxic/DNA-damaging chemotherapy or large field radiation therapy for an unrelated neoplasm. Recognition of prior cytotoxic therapy is important as the prognosis of matched therapy-related compared to primary/de-novo myeloid neoplasms is worse[12]; lists of causative cytotoxic chemotherapy is provided in the WHO 5th edition Classification of Tumors of Hematopoietic and Lymphoid Tissue.[1] The reporting of therapy-related, and predisposing conditions are required to be added as qualifiers to the AML, MDS, and MDS/MPN diagnosis rendered. In the cancer case summary, this is accomplished by selecting the diagnostic category of Myeloid neoplasm, post cytotoxic therapy, or Myeloid neoplasm and further specifying the neoplasm and therapy or germline predisposition. Examples are shown below.

Example of post cytotoxic therapy:
Myeloid neoplasms and proliferations associated with antecedent or predisposing conditions
_X_ Myeloid neoplasm post cytotoxic therapy
specify neoplasm: AML with *KMT2A* rearrangement, post cytotoxic therapy

Example of post-germline predisposition:
Myeloid neoplasms and proliferations associated with antecedent or predisposing conditions
_X_ Myeloid neoplasms associated with germline predisposition
specify neoplasm: MDS-LB
specify germline predisposition: germline *RUNX1* variant

Table 11. SUMMARY OF MYELOID NEOPLASMS AND PROLIFERATIONS ASSOCIATED WITH ANTECEDENT OR PREDISPOSING CONDITIONS[1]

Subtype	Essential diagnostic criteria	Desirable diagnostic criteria/ Notes
Myeloid neoplasms post cytotoxic therapy (MN-pCT)	• Myeloid neoplasm meeting diagnostic criteria of any myelodysplastic neoplasm, myelodysplastic/myeloproliferative neoplasm, or acute myeloid leukemia • History of prior exposure to cytotoxic therapy and/or large-field radiation therapy for an unrelated disorder • Not meeting diagnostic criteria of myeloproliferative neoplasms	
Myeloid neoplasms associated with germline predisposition	• Detection of germline mutation • Changes consistent with myeloid neoplasm with features of MDS, or with ≥20% blasts in blood and/or bone marrow	• Clonal molecular and/or cytogenetic abnormalities in addition to the germline mutation • Positive family history as determined by formal genetic counseling
Myeloid proliferations	Transient abnormal myelopoiesis associated with Down	• Mutation profiling and

associated with Down syndrome (DS)	syndrome: • Confirmation of constitutional trisomy 21 • Blood leukocytosis with increased blasts • Detection of exon 2/3 *GATA1* mutation (*GATA1* exon 2/3 sequencing should be performed in all cases with blood blasts >10%) Myeloid leukemia associated with Down syndrome: • Confirmation of constitutional trisomy 21 • Myeloid neoplasm with persistent increased blood and/or bone marrow blasts (may be <20%) • Detection of exon 2/3 *GATA1* mutation	detection of mutations in other genes, e.g., cohesin complex, *EZH2, KANSL1,* and/or *JAK3*

Myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangements

Myeloid/lymphoid neoplasms with eosinophilia and defining gene rearrangements are myeloid or lymphoid neoplasms that are driven by gene fusions resulting in constitutively active tyrosine kinase domains and resultant cell proliferation and survival. Clinical presentation is similar to myeloproliferative neoplasms with elevated white blood cells counts, bone marrow hypercellularity, and extramedullary involvement being common.[13] Unlike many of the AML with defining genetic alterations above, these neoplasms are classified as blast phase/acute myeloid leukemia only when blasts are ≥20% in blood or bone marrow. A summary table of salient diagnostic features is shown in Table 12. Several diagnostic points are worth emphasizing. First, eosinophilia is common in these disorders but not invariably present, so a high index of suspicion is warranted when some, but not all features are present. This is important as the *FIP1L1::PDGFRA* is not detected on routine karyotype and requires FISH or molecular studies to demonstrate the rearrangement. Second, many of the neoplasms in myeloid/lymphoid with eosinophilia and defining gene rearrangements may present with atypical mast cell infiltrates both morphologically (spindled, clustered) and by immunophenotype (CD25+), and therefore these should be excluded when considering the diagnosis of mast cell neoplasms.[14,15]

TABLE 12. SUMMARY OF MYELOID/ LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND DEFINING GENE REARRANGEMENT[1]

Subtype	Essential diagnostic criteria	Desirable diagnostic criteria/ Notes
Myeloid/lymphoid neoplasm with *PDGFRA* rearrangement	• A myeloid (more frequent) or lymphoid neoplasm, usually with prominent and/or tissue eosinophilia • Presence of a *PDGFRA* fusion gene, usually with *FIP1L1*	• In the absence of molecular demonstration of the fusion gene, the diagnosis should be suspected if there is a *BCR::ABL1*-negative myeloproliferative neoplasm with prominent eosinophilia associated with splenomegaly. • Marked elevation of serum vitamin B12, increased serum tryptase, and increased bone marrow mast cells

Myeloid/lymphoid neoplasm with *PDGFRB* rearrangement	• A myeloid or lymphoid neoplasm, often with prominent eosinophilia with varying degrees of neutrophilia or monocytosis associated with the formation of a *PDGFRB* fusion gene • Cases of *BCR::ABL1*-like B-ALL without evidence of an associated myeloid neoplasm are excluded from this category	• Cytogenetic and molecular identification of the partner gene, e.g., t(5;12)(q32;p13.2) with *ETV6::PDGFRB* or other partner genes
Myeloid/lymphoid neoplasm with *FGFR1* rearrangement	• Demonstration of t(8;13)(p11.2;q12.1) or a different translocation leading to formation of an *FGFR1* fusion gene is required • Phenotypically the disease may present as a myeloproliferative or myelodysplastic/myeloproliferative neoplasm with prominent eosinophilia, +/- neutrophilia or monocytosis or with increased blasts of myeloid, T-cell or B-cell lineage, or mixed phenotype, usually with eosinophilia	• Molecular identification of the partner gene of *FGFR1*
Myeloid/lymphoid neoplasm with *JAK2* rearrangement	• A myeloid or lymphoid neoplasm, often with prominent eosinophilia and the presence of a *JAK2* fusion gene • Cases of *BCR::ABL1*-like B-ALL without evidence of an associated myeloid neoplasm are excluded from this category	• Cytogenetic identification of the translocation. Molecular identification of the fusion gene, e.g., *PCM1::JAK2* • May have prominent erythroblastic islands in bone marrow and extramedullary locations
Myeloid/lymphoid neoplasms with *FLT3* rearrangement	• A myeloid or lymphoid neoplasm, with or without associated eosinophilia with chromosomal rearrangements leading to the formation of a *FLT3* fusion gene	
Myeloid/lymphoid neoplasm with *ETV6::ABL1* fusion	• A hematopoietic (myeloid or lymphoid) neoplasm in chronic phase associated with *ETV6::ABL1*	• Cytogenetics: t(9;12)(q34;p13) or complex aberrations involving other chromosomes
Myeloid/lymphoid neoplasms with other tyrosine kinase gene fusions	• A myeloid and/or lymphoid neoplasm • Detection of a tyrosine kinase fusion gene, other than those specifically defined as distinct entities (i.e., *PDGFRA, PDGFRB, FGFR1, JAK2, FLT3, ETV6::ABL1,* etc.)	• Eosinophilia • Cytogenetic identification of a translocation, suggesting the involvement of a tyrosine kinase gene and prompting the selection of appropriate break apart FISH probes or other molecular investigation • Reported alterations include but are not limited to: *ETV6::FGFR2; ETV6::LYN; ETV6::NTRK3;*

		RANBP2::ALK; BCR::RET; FGFR1OP::RET

Acute leukemias of mixed or ambiguous lineage

Acute leukemias of ambiguous lineage are neoplasms composed of ≥20% blasts in the blood or bone marrow that do not show differentiation along a single lineage (mixed phenotype acute leukemia/MPAL) or fail to demonstrate lineage differentiation (acute undifferentiated leukemia/AUL). MPAL may consist of a single, well-defined blast population that expresses lineage-defining antigens (see Table 13) of two or more lineages (termed biphenotypic), multiple distinct blast populations that each express lineage-defining antigens (bilineal/ bilineage) or a combination of these two. To clarify, in cases of bilineal/bilineage acute leukemia, the aggregate count of the two distinct abnormal blasts populations is used to define the total blast count and meet criteria for ≥20% blasts.

The assessment of antigen expression to determine lineage assignment is best performed by flow cytometry due to its ability to identify discrete populations based on multiparametric analysis and ability to quantify antigen expression. The criteria for the assignment of B-, T-, and myeloid lineage is shown in Table 13. Briefly, B-lineage is defined using CD19 expression coupled with additional antigens based on the intensity of CD19. T-lineage is defined by cytoplasmic or surface CD3 expression. Myeloid lineage by myeloperoxidase expression or expression of more than one marker of monocytic differentiation. Leukemias that have a well-defined single lineage assignment that express antigens associated with another lineage but not lineage-defining should be considered acute leukemia with aberrant antigen expression and not defined as MPAL.

Leukemias that can more accurately be assigned to another well-defined entity but meeting the criteria for MPAL or AUL should be assigned to that category; common examples include acute myeloid leukemia (AML) with *RUNX1::RUNX1T1* fusion, acute myeloid/lymphoid neoplasms with eosinophilia and blast phase chronic myeloid leukemia. A summary of the diagnostic features of leukemias with ambiguous lineage with defining genetic abnormalities and acute leukemias with ambiguous lineage, immunophenotypically defined as shown in Tables 14 and 15, respectively.

TABLE 13. LINEAGE ASSIGNMENT CRITERIA FOR MIXED PHENOTYPE ACUTE LEUKEMIA- B-LINEAGE, T-LINEAGE AND MYELOID LINEAGE[1]

Lineage	Criterion	Notes
B lineage	• CD19 strong and • 1 or more also strongly expressed: CD10, CD22, or CD79a	• CD19 strong= intensity in part exceeds 50% of normal B cell progenitor by flow cytometry • CD19 weak= intensity does not exceed 50% of normal B cell progenitor by flow cytometry • If mixed T lineage is under consideration, CD79a cannot be used for B lineage assignment
	• CD19 weak and • 2 or more also strongly expressed: CD10, CD22, or CD79a	

T lineage	• Cytoplasmic CD3 or surface • Cytoplasmic CD3 or surface expression flow cytometry (CD3 epsilon chain antibodies); intensity exceeds 50% of mature T cells by flow cytometry • Cytoplasmic CD3 by immunohistochemistry using non-zeta chain antibody	• Surface CD3 expression rare in mixed phenotype/ ambiguous acute leukemia
Myeloid lineage	• Myeloperoxidase (MPO) • Intensity in population of interest exceeds 50% of mature neutrophil level	
	• Monocytic differentiation • 2 or more expressed: CD11c, CD14, CD36, CD64, diffuse non-specific esterase, lysozyme	

TABLE 14. SUMMARY OF ACUTE LEUKEMIA OF AMBIGOUS LINEAGE WITH DEFINING GENETIC ABNORMALITIES[1]

Subtype	Essential diagnostic criteria	Desirable diagnostic criteria/ Notes
Mixed-phenotype acute leukemia with *BCR::ABL1* fusion	• ≥20% blasts in bone marrow and/or blood with an immunophenotype that meets the diagnostic criteria for MPAL • *BCR::ABL1* and/or t(9;22)(q34;q11.2) detected at initial diagnosis • No prior or subsequent evidence of chronic myeloid leukemia • No history of exposure to cytotoxic therapy	Determination of the *BCR::ABL1* transcript subtype and establishment of a quantitative baseline for monitoring treatment response
Mixed-phenotype acute leukemia (MPAL) with *KMT2A* rearrangement	• ≥20% blasts in bone marrow and/or blood with an immunophenotype that meets the diagnostic criteria for MPAL • Presence of a *KMT2A* rearrangement • No history of exposure to cytotoxic therapy	Identification of the *KMT2A* fusion partner
Acute leukemia of ambiguous lineage with other defined genetic alterations	• ≥20% blasts in bone marrow and/or blood expressing mixed-lineage or ambiguous immunophenotype. • Detection of *ZNF386* or *BCL11B* rearrangement	

TABLE 15. SUMMARY OF ACUTE LEUKEMIA WITH AMBIGUOUS LINEAGE, IMMUNOPHENOTYPICALLY DEFINED[1]

Subtype	Essential diagnostic criteria	Desirable diagnostic criteria/ Notes
Mixed phenotype acute leukemia, B/Myeloid (MPAL-B/M)	• ≥20% blasts in bone marrow and/or blood expressing B lineage and myeloid lineage antigens • Not fulfilling diagnostic criteria of MPAL with defined genetic alterations • No history of exposure to cytotoxic therapy	
Mixed phenotype acute leukemia, T/Myeloid (MPAL-T/M)	• ≥20% blasts in bone marrow and/or blood expressing T lineage and myeloid lineage antigens • Not fulfilling diagnostic criteria of MPAL with defined genetic alterations	

	• No history of exposure to cytotoxic therapy	
Mixed-phenotype acute leukemia (MPAL), rare types	• ≥20% blasts in bone marrow and/or blood expressing combinations of B, T, myeloid and megakaryocytic (Mk) lineage markers • Not fulfilling diagnostic criteria of MPAL with defined genetic alterations, MPAL B/Myeloid, or MPAL T/Myeloid • No history of exposure to cytotoxic therapy • No history of myeloid neoplasms	Subtypes: • Mixed-phenotype acute leukemia, B/T (MPAL-B/T) • Mixed-phenotype acute leukemia, B/T/Myeloid (MPAL-B/T/M) • Mixed-phenotype acute leukemia, T/Megakaryocytic (MPAL-T/Mk)
Acute leukemia of ambiguous lineage, not otherwise specified (ALAL-NOS)	• ≥20% blasts in bone marrow and/or blood expressing combinations of immunophenotypic lineage markers that do not permit definitive lineage assignments • Not fulfilling diagnostic criteria of MPAL with defined genetic alterations	

Blastic plasmacytoid dendritic cell neoplasm

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a hematological neoplasm consisting of immature cells with plasmacytoid dendritic cell differentiation. While plasmacytoid dendritic cells can be derived from both myeloid and lymphoid cells, the predominant cell of origin is myeloid.[16] In addition, the frequent leukemic presentation and possible blastic transformation event of underlying myeloid neoplasm, typically MDS or MDS/MPN in 20-30% of cases make inclusion in this cancer case summary more apt. Diagnostic characteristics include immature cells with blast/blastoid features and immunophenotypic evidence of plasmacytoid dendritic cell differentiation including the expected expression of CD123, CD4, CD56, TCF4, TCL1, CD303, CD304 with typical absence of expression of other antigens such as CD3, CD14, CD19, CD34, Lysozyme, Myeloperoxidase.[17]

Dysplasia

The presence or absence of significant dysplasia (defined as 10% or greater of cells in each lineage) should be noted for each of the major cell lineages present in the sample- erythroid, myeloid, and megakaryocytes. If dysplasia cannot reliably be assessed due to the nature of the sample select- Cannot be determined and specify the reason. For blood, report dysplasia that you can evaluate- typically granulocytic. If dysplasia is not applicable, for example in myeloid sarcoma or blastic plasmacytoid dendritic cell neoplasm, report as not applicable.

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C. Special Studies

Ring sideroblasts/iron stain

An iron stain (Prussian blue) is necessary to evaluate for the presence of stainable iron and to identify ring sideroblasts.[1] Stainable iron is best evaluated on bone marrow aspirate smears or touch preparations of the core biopsy. Evaluation of the bone marrow core biopsy or clot section may hinder the visualization of the ring sideroblasts and decalcification diminishes stainable iron. For specimens other than bone marrow, select not applicable/not performed/not evaluated.

Bone marrow fibrosis grade

Bone marrow fibrosis is assessed using a semi-quantitative grade (MF-0 to MF-3) based on reticulin stains and, depending on the degree of fibrosis (MF-2 or MF-3), trichrome stain. The fiber density should only be assessed in areas of hematopoiesis; if there is variability the grade should be assigned based on the highest grade that is present in at least 30% of the bone marrow. The bone marrow fibrosis grading system is shown in Table 1.[2,3]

Table 1. Semi-quantitative grading of bone marrow fibrosis

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

Flow cytometry

Flow cytometry is a quantitative method for rapid, multiparametric evaluation of the expression of cell surface and cytoplasmic antigens of a large number of cells. If flow cytometry is performed, report if there was no aberrancy detected in the sample at the level of sensitivity of the assay or specify what specific alterations were detected. As there is substantial variability in the reporting of results the cancer case summary does not require a specific method. It is recommended to report the result on the tumor cell population in a semi-quantitative method that would allow those reviewing the report to determine if there is heterogenous expression (not all tumor cells are positive) and the level of expression on the population. Examples of this reporting include: CD34 dim+ (low-level expression of CD34), CD34+ (moderate level of

expression of CD34), CD34++ (bright expression of CD34), CD34-/+ het (variable heterogeneous expression of CD34).

Cytogenetics
Report if conventional/karyotype cytogenetic analysis was performed on the sample, and if performed, the result. For those samples with abnormal karyotypes, specify the result.

Fluorescence in-situ hybridization
Report the results of any fluorescence in-situ hybridization performed by listing probes that were tested with normal results and those that showed an abnormal signal pattern.

Molecular alterations detected
With the advent of increasingly sophisticated molecular genetic techniques such as next-generation sequencing (NGS), chromosomal microarrays, and large fluorescence in-situ hybridization panels reporting all of these results in a synoptic format is a challenge. As many of these alterations are diagnostically, prognostically, or therapeutically significant- reporting in a succinct manner is necessary. To make reporting easier for the pathologist and highlight what is most important for the treating clinician, this cancer case summary requires the reporting of any positive/abnormal alterations that were detected followed by an optional listing of all the alterations that were tested. The cancer case summary lists some of the most common genetic alterations that occur in myeloid neoplasms, but it is in no way comprehensive. The option exists to provide the molecular alterations not specifically listed under “Other alterations listed (specify),” where the user would enter what molecular alteration was detected.

After each alteration, there is a specific field where users can delineate, if desired, specific alterations, methodology (NGS, for example), or additional information (variant allele frequency VAF) that would be useful for that institution. While preferable to include specific information, this could also reference a separate report with this information (separate molecular pathology report). An example of how to use this section is as follows:

Molecular alterations detected (select all those with significant mutations)

_X_ *DNMT3A* mutation, (specify): *DNMT3A R882H*, VAF 47%, NGS
_X_ *FLT3* interim tandem duplication (ITD), (specify): (PCR)/Capillary Electrophoresis, signal ratio 9.5
_X_ *NPM1* mutation, (specify): c.578A>G→p.K193R, VAF 40%, NGS

Molecular alterations assayed:
(List): *ABL1, ANKRD26, APC, ARAF, ASXL1, ATM, ATRX, BCOR, BCORL1, BLM, BRAF, BRCA1, BRCA2, BRIP1, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CHEK2, CSF3R, CTC1, CUX1, CXCR4, DDX41, DKC1, DNMT3A, ELANE, EPCAM, ERCC4, ETNK1, ETV6, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FBXW7, FLT3, G6PC3, GATA1, GATA2, GFI1, GNAS, GNB1, HAX1, HRAS, IDH1, IDH2, IKZF1, IKZF3, ITPKB, JAK2, JAK3, KDM6A, KIT, KMT2A, KRAS, MAP2K1, MET, MLH1, MPL, MSH2, MSH6, MYD88, NF1, NHP2, NOP10, NOTCH1, NPM1, NRAS, PALB2, PDGFRA, PHF6, PIGA, PML, PMS2, PPM1D, PTEN, PTPN11, RAD21, RAD51C, RB1, RPL11, RPL35A, RPL5, RPS10, RPS17, RPS26, RPS7, RTEL1, RUNX1, SAMD9, SAMD9L, SBDS, SETBP1, SETD2, SF3B1, SH2B3, SLX4, SMC1A, SMC3, SRP72, SRSF2, STAG2, STAT3, STAT5B, SUZ12, TERC, TERT, TET2, TINF2, TP53, U2AF1, VHL, WAS, WRAP53, WT1, ZRSR2*

This method can simplify the reporting of large numbers of genes to highlight only those with alterations identified. It also provides a means of listing what was assayed- such lists can be typically obtained from

the laboratory performing the molecular studies. This is less labor-intensive than listing all the negative results.

References

1. Della Porta MG, Travaglino E, Boveri E, et al. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. *Leukemia* 2015;29: 66–75.
2. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica*. 2005 Aug;90(8):1128-32. PMID: 16079113.
3. Kvasnicka HM, Beham-Schmid C, Bob R, Dirnhofer S, Hussein K, Kreipe H, Kremer M, Schmitt-Graeff A, Schwarz S, Thiele J, Werner M, Stein H. Problems and pitfalls in grading of bone marrow fibrosis, collagen deposition and osteosclerosis - a consensus-based study. *Histopathology.* 2016 May;68(6):905-15. doi: 10.1111/his.12871. Epub 2015 Nov 20. PMID: 26402166.