Myeloid and Mixed or Ambiguous Lineage Neoplasms Histopathology Reporting Guide

Family/Last name

Given name(s)

Date of birth DD – MM – YYYY

Patient identifiers

Date of request DD – MM – YYYY

Accession/Laboratory number

Elements in **black text** are CORE. Elements in **grey text** are NON-CORE.

☐ indicates multi-select values ◯ indicates single select values

SCOPE OF THIS DATASET

CLINICAL INFORMATION (Note 1)

◯ Information not provided
◯ Information provided

History of previous myeloid or lymphoid neoplasms or clonal haematopoiesis

◯ Information not provided
◯ No
◯ Yes, *specify*

History of other relevant disease (e.g., solid organ cancers, immune deficiency)

◯ Information not provided
◯ No
◯ Yes, *specify*

Known germline predisposition

◯ Information not provided
◯ No
◯ Yes, *specify*

Previous therapy

◯ Information not provided
◯ No
◯ Yes (select all that apply)
- ☐ Cytotoxic therapy
- ☐ Radiation therapy
- ☐ Targeted therapy
- ☐ Other, *specify*

Other clinical information, *specify*

SAMPLING PROCEDURE (select all that apply) (Note 2)

◯ Not specified
☐ Bone marrow biopsy
☐ Bone marrow aspiration
☐ Fine needle aspiration
☐ Other, *specify*

SPECIMEN(S) SUBMITTED (select all that apply) (Note 3)

◯ Not specified
☐ Peripheral blood
☐ Bone marrow aspirate
- ◯ Adequate
- ◯ Inadequate due to marrow fibrosis
- ◯ Unsatisfactory, *specify*

☐ Bone marrow touch imprint
- ◯ Adequate
- ◯ Unsatisfactory, *specify*

☐ Bone marrow biopsy
- ◯ Adequate
- ◯ Unsatisfactory, *specify*

☐ Bone marrow clot
- ◯ Adequate
- ◯ Unsatisfactory, *specify*

☐ Other, *specify*

TUMOUR SITE (select all that apply) (Note 4)

- ◯ Not specified
- ☐ Peripheral blood
- ☐ Bone marrow
- ☐ Other, *specify*

BLOCK IDENTIFICATION KEY (Note 5)

(List overleaf or separately with an indication of the nature and origin of all tissue blocks)

FINAL INTEGRATED DIAGNOSIS (Note 6)

(Value list based on the World Health Organization Classification of Haematolymphoid Tumours, 5th Edition (2024))

- ◯ Myeloid precursor lesion
  - ◯ Clonal haematopoiesis
  - ◯ Clonal cytopenia of undetermined significance
- ◯ Myeloproliferative neoplasm
  - ◯ Chronic myeloid leukaemia
    - ◯ Chronic phase
    - ◯ Blast phase
  - ◯ Chronic neutrophilic leukaemia
  - ◯ Chronic eosinophilic leukaemia
  - ◯ Polycythaemia vera
    - ◯ Chronic phase
    - ◯ Accelerated phase
    - ◯ Blast phase
  - ◯ Essential thrombocythaemia
    - ◯ Chronic phase
    - ◯ Accelerated phase
    - ◯ Blast phase
  - ◯ Primary myelofibrosis
    - ◯ Chronic phase
    - ◯ Accelerated phase
    - ◯ Blast phase
  - ◯ Myeloproliferative neoplasm not otherwise specified (NOS) (unclassifiable)
- ◯ Mastocytosis
  - ◯ Cutaneous mastocytosis
  - ◯ Systemic mastocytosis
    - ◯ Bone marrow mastocytosis
    - ◯ Indolent systemic mastocytosis
    - ◯ Smouldering mastocytosis
    - ◯ Aggressive systemic mastocytosis
    - ◯ Systemic mastocytosis with an associated haematological neoplasm
    - ◯ Mast cell leukaemia
  - ◯ Mast cell sarcoma
- ◯ Myelodysplastic neoplasm (MDS)
  - ◯ MDS with defining genetic abnormalities
    - ◯ MDS with low blasts and 5q deletion
    - ◯ MDS with low blasts and *SF3B1* mutation
    - ◯ MDS with biallelic *TP53* inactivation
  - ◯ MDS defined morphologically
    - ◯ MDS with low blasts
    - ◯ MDS, hypoplastic
    - ◯ MDS with increased blasts-1
    - ◯ MDS with increased blasts-2
    - ◯ MDS with increased blasts and fibrosis
  - ◯ MDS of childhood
    - ◯ Childhood MDS with low blasts
    - ◯ Childhood MDS with increased blasts
- ◯ Myelodysplastic/myeloproliferative neoplasm (MDS/MPN)
  - ◯ Chronic myelomonocytic leukaemia-1
    - ◯ Myelodysplastic type
    - ◯ Myeloproliferative type
  - ◯ Chronic myelomonocytic leukaemia-2
    - ◯ Myelodysplastic type
    - ◯ Myeloproliferative type
  - ◯ MDS/MPN with neutrophilia
  - ◯ MDS/MPN with *SF3B1* mutation and thrombocytosis
  - ◯ MDS/MPN NOS (unclassifiable)
- ◯ Acute myeloid leukaemia (AML)
  - ◯ AML with defining genetic abnormalities
    - ◯ Acute promyelocytic leukaemia with *PML::RARA* fusion
    - ◯ AML with *RUNX1::RUNX1T1* fusion
    - ◯ AML with *CBFB::MYH11* fusion
    - ◯ AML with *DEK::NUP214* fusion
    - ◯ AML with *RBM15::MRTFA* fusion
    - ◯ AML with *BCR::ABL1* fusion
    - ◯ AML with *KMT2A* rearrangement
    - ◯ AML with *MECOM* rearrangement
    - ◯ AML with *NUP98* rearrangement
    - ◯ AML with *NPM1* mutation
    - ◯ AML with *CEBPA* mutation
    - ◯ AML, myelodysplasia-related
    - ◯ AML with other defined genetic alterations, *specify*
  - ◯ AML defined by differentiation
    - ◯ AML with minimal differentiation
    - ◯ AML without maturation
    - ◯ AML with maturation
    - ◯ Acute basophilic leukaemia
    - ◯ Acute myelomonocytic leukaemia
    - ◯ Acute monocytic leukaemia
    - ◯ Acute erythroid leukaemia
    - ◯ Acute megakaryoblastic leukaemia
- ◯ Myeloid sarcoma, *specify morphologic, immunophenotypic, cytogenetic, and molecular features*

FINAL INTEGRATED DIAGNOSIS (Note 6) **continued**

- ◯ Myeloid/lymphoid neoplasm with eosinophilia and tyrosine kinase gene fusions
  - ◯ 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* rearrangement
  - ◯ Myeloid/lymphoid neoplasm with other tyrosine kinase gene fusions, *specify*
- ◯ Acute leukaemia of mixed or 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, *specify*
  - ◯ Acute leukaemia of ambiguous lineage defined immunophenotypically
    - ◯ Mixed phenotype acute leukaemia, B/myeloid
    - ◯ Mixed phenotype acute leukaemia, T/myeloid
    - ◯ Mixed phenotype acute leukaemia, rare types
    - ◯ Acute leukaemia of ambiguous lineage NOS
    - ◯ Acute undifferentiated leukaemia
- ◯ Myeloid neoplasm and proliferation associated with antecedent or predisposing conditions
  - ◯ Myeloid neoplasm post cytotoxic therapy, *specify*
  - ◯ Myeloid neoplasm associated with germline predisposition, *specify*
  - ◯ Myeloid proliferation associated with Down syndrome, *specify*
- ◯ Secondary involvement of bone marrow: non-haematopoietic tumour
  - ◯ Accompanying bone marrow and peripheral blood pathology, *specify*
  - ◯ Other, *specify*
- ◯ Other, *specify*

Comments

COMPLETE BLOOD COUNT (Note 7)

White blood cells

◯ Information not provided

10^9/L

Haemoglobin

◯ Information not provided

g/dL OR mmol/L

MCV

◯ Information not provided

fL

Platelets

◯ Information not provided

10^9/L

Red blood cell distribution width (RDW)

◯ Information not provided

%

White blood cell differential

◯ Differential not provided or performed

◯ Differential provided or performed (select all that apply)

- ☐ Blasts %
- ☐ Neutrophils %
- ☐ Immature granulocytes %
- ☐ Lymphocytes %
- ☐ Monocytes %
- ☐ Eosinophils %
- ☐ Basophils %
- ☐ Other, *specify* %

BONE MARROW DIFFERENTIAL (Note 8)

- ◯ Differential not performed, *specify*
- ◯ Differential performed

Specimen type (select all that apply)

◯ Aspirate smears ◯ Touch imprint

- ☐ Blasts ____ %
- ☐ Granulocytic precursors ____ %
- ☐ Eosinophils ____ %
- ☐ Monocytes ____ %
- ☐ Lymphocytes ____ %
- ☐ Plasma cells ____ %
- ☐ Erythroid precursors ____ %
- ☐ Other cell populations (e.g., mast cells, abnormal basophils), *specify* ____ ____ %
- ☐ Myeloid-to-erythroid (M:E) ratio ____ :
- ☐ Total cells counted ____

BLASTS/BLAST EQUIVALENTS (select all that apply) (Note 9)

- ☐ Peripheral blood
  ____ % of total white blood cells
- ☐ Bone marrow aspirate or touch imprint
  ____ % of total nucleated cells by morphologic enumeration
- ☐ Bone marrow core biopsy or clot section
  ____ % of total nucleated cells by immunohistochemistry (e.g., CD34, CD117), *specify*
  ____
- ☐ Morphologic features of blasts, *specify*
  ____

MORPHOLOGIC ABNORMALITY AND DYSPLASIA (Note 10)

- ◯ Absent
- ◯ Present (select all that apply)
  - ☐ Peripheral blood
    - ☐ Neutrophilic, *specify*
      ____
    - ☐ Monocytic, *specify*
      ____
    - ☐ Red blood cells, *specify*
      ____
    - ☐ Platelets, *specify*
      ____
  - ☐ Bone marrow
    - ☐ Erythroid
      ____ % of erythroid precursors, *specify*
      ____
    - ☐ Myeloid
      ____ % of myeloid precursors
      Granulocytic, *specify*
      ____
      Monocytic, *specify*
      ____
    - ☐ Megakaryocytic
      ____ % of megakaryocytes, *specify*
      ____
    - ☐ M:E ratio ____ :
    - ☐ Histotopography, *specify*
      ____
  - ☐ Morphologic features of non-bone marrow specimen, *specify*
    ____
- ◯ Cannot be assessed, *specify*
  ____

BONE MARROW CELLULARITY (Note 11)

Specimen type (select all that apply)

☐ Bone marrow biopsy ☐ Bone marrow clot

____ % cellularity

- ◯ Normocellular for age
- ◯ Hypercellular for age
- ◯ Hypocellular for age

IRON STAIN (Note 12)

- ◯ Not performed
- ◯ Performed
  - **Specimen type** (select all that apply)
    - ☐ Aspirate smears
    - ☐ Touch imprint
    - ☐ Clot section
    - ☐ Core biopsy
  - ◯ Ring sideroblasts
    - _____ % of erythroid precursors
  - ◯ Storage iron
    - ◯ Absent
    - ◯ Increased
    - ◯ Normal
    - ◯ Decreased
  - ◯ No ring sideroblasts

BONE MARROW FIBROSIS (Note 13)

- ◯ Special stains not performed
- ◯ Special stains performed (select all that apply)
  - ☐ Reticulin
  - ☐ Gömöri trichrome
  - ☐ Masson trichrome

Myelofibrosis grade[a]

- ◯ MF-0
- ◯ MF-1
  - Reticulin fibrosis _____
  - Collagen deposition _____
  - Osteosclerosis _____
- ◯ MF-2
  - Reticulin fibrosis _____
  - Collagen deposition _____
  - Osteosclerosis _____
- ◯ MF-3
  - Reticulin fibrosis _____
  - Collagen deposition _____
  - Osteosclerosis _____

[a] *Refer to Table 2.*

IMMUNOPROFILING/PHENOTYPING STUDIES[b] (Note 14)

Immunohistochemistry (select all that apply)

- ◯ Not performed
- ◯ Performed (select all that apply)
  - ☐ Blasts
    - ☐ CD34
      - _____ % of marrow cellularity
    - ☐ Other, *specify* _____
      - _____ % of marrow cellularity
  - ☐ Other neoplastic population, *specify* _____
    - _____ % of marrow cellularity

Comments _____

Flow cytometry

- ◯ Not performed
- ◯ Performed (select all that apply)
  - ◯ Peripheral blood
  - ◯ Bone marrow

Specify panels _____

- ☐ No abnormal myeloid blast/blast equivalent population or maturing myeloid/monocytic population identified
- ☐ Abnormal myeloid blast/blast equivalent population identified
  - _____ % of total white blood cells
  - Specify immunophenotype _____
- ☐ Abnormal mast cell population identified
  - _____ % of total white blood cells
  - Specify immunophenotype _____
- ☐ Abnormal maturing myeloid/monocytic population identified
  - _____ % of total white blood cells
  - Specify immunophenotype _____
- ☐ Other findings, *specify* _____

Comments _____

[b] *Refer to Table 3.*

CYTOGENETIC AND MOLECULAR STUDIES

CYTOGENETIC STUDIES (Note 15)

Karyotyping

- ◯ Not performed
- ◯ Performed
  - ◯ Peripheral blood ◯ Bone marrow
  - Karyotype

FISH analysis

- ◯ Not performed
- ◯ Performed
  - ◯ Peripheral blood ◯ Bone marrow
  - Specify probe sets and results

Chromosomal microarray

- ◯ Not performed
- ◯ Performed
  - ◯ Peripheral blood ◯ Bone marrow
  - Specify results

Other, *specify*

MOLECULAR STUDIES[c] (Note 16)

- ◯ Not performed
- ◯ Performed (select all that apply)
  - ◯ Peripheral blood ◯ Bone marrow aspirate ◯ Clot/particle preparation
    - ☐ Genetic abnormalities by PCR-based methods
      - ☐ *NPM1*
      - ☐ *FLT3* ITD
      - ☐ *FLT3* TKD
      - ☐ *CEBPA*
      - ☐ *JAK2*
      - ☐ *CALR*
      - ☐ *MPL*
      - ☐ *BCR::ABL1*
        - ◯ *p210*
        - ◯ *p190*
        - ◯ Other, *specify*
      - ☐ *PML::RARA*
      - ☐ *RUNX1::RUNX1T1*
      - ☐ *CBFB::MYH11*
      - ☐ *KIT* p.D816V
      - ☐ Other, *specify*
    - ☐ Gene mutations by next generation sequencing (NGS) myeloid gene panel
      - Specify gene panel and results
    - ☐ Gene fusions by RNA sequencing or other techniques
      - Specify gene panel and results
    - ☐ Structural variants by DNA sequencing
      - Specify gene panel and results
    - ☐ Other, *specify*

Comments

Representative blocks for ancillary studies, *specify those blocks best representing tumour and/or normal tissue for further study*

[c] *Refer to Table 4.*

Definitions

CORE elements

CORE elements are those which are essential for the clinical management, staging or prognosis of the cancer. These elements will either have evidentiary support at Level P2 or higher (based on the Hierarchy of Research Evidence for Tumour Pathology).[1] In rare circumstances, where level P2 evidence is not available an element may be made a CORE element where there is unanimous agreement in the Dataset Authoring Committee (DAC).

Molecular and immunophenotypic (by immunohistochemistry and flow cytometry) testing is a growing feature of cancer reporting. However, in many parts of the world this type of testing is limited by the available resources. In order to encourage the global adoption of ancillary tests for patient benefit, International Collaboration on Cancer Reporting (ICCR) includes the most relevant ancillary testing in ICCR Datasets as CORE elements, especially when they are necessary for the diagnosis. Where the technical capability does not yet exist, laboratories may consider temporarily using these data elements as NON-CORE items.

The summation of all CORE elements is considered to be the minimum reporting standard for a specific cancer.

NON-CORE elements

NON-CORE elements are those which are unanimously agreed should be included in the dataset but are not supported by level P2 evidence. These elements may be clinically important and recommended as good practice but are not yet validated or regularly used in patient management.

Key information other than that which is essential for clinical management, staging or prognosis of the cancer such as macroscopic observations and interpretation, which are fundamental to the histological diagnosis and conclusion e.g., macroscopic tumour details, may be included as either CORE or NON-CORE elements by consensus of the DAC.

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Scope

The dataset has been developed for the pathology reporting of myeloid and mixed or ambiguous lineage haematopoietic neoplasms and applies to specimens from peripheral blood, bone marrow, lymph node, spleen, skin, central nervous system, or other anatomic sites. Histologic, immunophenotypic, and genetic findings are integrated to formulate the final diagnosis.

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Note 1 – Clinical information (Core and Non-core)

Clinical data are essential for the accurate interpretation of morphologic, immunophenotypic, and genetic findings and are required for establishing a definitive diagnosis and classification. They also have significant prognostic implications and influence risk stratification and therapeutic decision making.[2-4]

The presence of clonal haematopoiesis (CH) is associated with a higher risk of myeloid and lymphoid neoplasms and increased all-cause mortality.[5,6] Other relevant clinical conditions include autoimmune disorders and inflammatory syndromes, such as VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome,[7,8] which demonstrate specific clinicopathologic associations that can impact disease classification and management. Knowledge of a history of prior cytotoxic or radiation therapy is critical for distinguishing myeloid neoplasms post cytotoxic therapy from de novo disease, as these entities have distinct pathogenetic mechanisms, prognostic implications, and therapeutic considerations.[9-14]

An increasing number of germline pathogenic or likely pathogenic variants have been identified that predispose individuals to myeloid or lymphoid neoplasms, and several germline variants are also associated with predisposition to CH.[15,16] Documentation of specific germline predisposition syndrome is important because myeloid neoplasms arising in certain predisposition syndromes, such as Fanconi anaemia,[17] confer a worse prognosis than sporadic cases, whereas those associated with other syndromes may have comparable outcomes. Recognition of an underlying germline predisposition informs treatment decisions and guides genetic counselling and family risk assessment.

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Note 2 – Sampling procedure (Core)

Accurate diagnosis and classification rely on adequate and representative sampling. The posterior iliac crest is the preferred bone marrow biopsy site, and specifying laterality (right or left) is recommended for clear documentation, correlation with imaging, and for comparison with prior or subsequent biopsies. For extramedullary manifestations or myeloid sarcoma, biopsy of the involved tissue (e.g., lymph node, skin, or soft tissue) and collection of involved body fluid (e.g., cerebrospinal fluid, pleural fluid) are recommended, as morphologic, immunophenotypic, and genetic features may differ from those in the concurrent peripheral blood or bone marrow.

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Note 3 – Specimen(s) submitted (Core)

Comprehensive evaluation of myeloid and mixed or ambiguous lineage haematopoietic neoplasms requires integration of findings from multiple specimen types, as each provides complementary diagnostic information. Peripheral blood is essential for assessing mature cell morphology, quantifying blast percentage, and identifying dysplastic features in circulating cells. It provides key quantitative information, including blood counts and the presence of cytosis or cytopenia, which guide interpretation of bone marrow findings and contribute to disease classification. A bone marrow aspirate and core biopsy are complementary and should be obtained whenever feasible. Aspirate smears or touch imprints are critical for detailed cytomorphologic evaluation, particularly to

identify dysplasia and perform a manual differential count of blasts and other haematopoietic lineages. Aspirate material also provides viable cells suitable for flow cytometric immunophenotyping, cytogenetic, and molecular analysis. Core biopsy and clot sections complement aspirate findings by allowing evaluation of overall cellularity, cellular components, architecture, and stromal or bone changes, including fibrosis or osteosclerosis. They also permit detection of focal infiltrates that may be missed or underrepresented on aspirate smears.

Adequacy of sampling should be assessed by correlating cellularity, the presence of spicules in the aspirate, and the size and integrity of the core biopsy. Haemodiluted or pauci-spicular aspirates may contain insufficient marrow elements for definitive morphologic assessment and can lead to underestimation of the blast concentration. Factors compromising aspirate smear adequacy include, but are not limited to, marrow fibrosis, paucity of spicules, thick or crushed smears, and suboptimal staining. Biopsy adequacy can be affected by insufficient core length, sampling artifact (such as subcortical sampling), aspiration artifact, crush artifact, and artifactual distortion. A repeat sample should be obtained when findings are non-diagnostic or inadequate for essential ancillary studies.

The type of anticoagulant and fixative used directly affects the suitability of specimens for ancillary testing. Heparinised aspirate is preferred for conventional karyotyping and fluorescence in situ hybridisation (FISH); EDTA is recommended for flow cytometry and molecular studies.[15] Formalin or heavy metal based fixation (i.e., Bouin's solution or B-plus) and acid decalcification may compromise nucleic acid integrity in core biopsies,[18] whereas EDTA-based or other gentle decalcification methods better preserve genetic material for molecular testing.[19] When an aspirate specimen is unavailable, touch imprints of the core biopsy or non-decalcified clot sections can be an acceptable alternative source for molecular studies.

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Note 4 – Tumour site (Core)

Bone marrow and peripheral blood are the primary sites of involvement in most myeloid and mixed or ambiguous lineage haematopoietic neoplasms. Other extramedullary anatomic compartments may also be involved at initial presentation or during disease progression or relapse. Extramedullary lesions may display morphologic or immunophenotypic features distinct from those in the bone marrow or peripheral blood.[20] When the myeloid blasts, with or without maturation, efface tissue architecture of an extramedullary site, the term myeloid sarcoma is used, and correlation with bone marrow and peripheral blood findings is recommended to exclude or verify concurrent systemic disease. Documentation of all involved sites is essential for accurate diagnosis and clinicopathologic correlation and facilitates integrated reporting.

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Note 5 – Block identification key (Non-core)

The origin/designation of all slides and tissue blocks should be recorded. This information should ideally be documented in the laboratory records and the final pathology report of a case and is particularly important when further internal or external review arises. The reviewer needs to have an unequivocal description of the origin of each block in order to provide an informed expert opinion. It is highly encouraged to have a digital image (photograph) of the specimen and a record of the key to the tumour blocks.

Recording the origin/designation of tissue blocks also facilitates retrieval of blocks for further immunohistochemical or molecular analysis, research studies, or clinical trials.

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Note 6 – Final integrated diagnosis (Core and Non-core)

The final integrated diagnosis for myeloid and mixed or ambiguous lineage haematopoietic neoplasms follows the hierarchical diagnostic framework of the World Health Organization (WHO) Classification of Haematolymphoid Tumours, 5th edition, 2024 (WHO-HAEM5) (refer to Table 1).[21] This approach reflects updated diagnostic criteria from the revised 4th edition (WHO-HAEM4R), emphasising the combined interpretation of morphologic, immunophenotypic, cytogenetic, and molecular findings, in the context of relevant clinical and haematologic data.

Each diagnosis should be rendered according to the major categories defined in WHO-HAEM5,[21] which include myeloid precursor lesions, myeloproliferative neoplasms (MPN), mastocytosis, myelodysplastic neoplasms (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), acute myeloid leukaemias (AML), myeloid/lymphoid neoplasms with eosinophilia and defining tyrosine kinase gene fusions, acute leukaemias of mixed or ambiguous lineage, and myeloid neoplasms and proliferations associated with antecedent or predisposing conditions. Non-haematopoietic tumours involving peripheral blood and bone marrow are also recorded.

Whenever possible, the diagnosis should be assigned to the most specific entity within the category, based on the available evidence, and further defined by disease phase or subtype when indicated. Essential diagnostic criteria facilitate diagnostic practice in resource-limited settings. In laboratories with broad resources, investigational efforts should aim to apply both essential and desirable criteria to achieve optimal diagnostic accuracy. When ancillary testing is limited, classification should be made to the highest level supported by existing findings. If cytogenetic or molecular results are pending, a provisional diagnosis may be issued and amended with final integrative diagnosis when complete data are available.

Major updates introduced in WHO-HAM5 include:[21]

- Recognition of clonal haematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS) as myeloid precursor lesions, allowing for monitoring pre-neoplastic stage.
- Removal of chronic myeloid leukaemia (CML) accelerated phase as it no longer retains prognostic significance in the era of tyrosine kinase inhibitor (TKI) therapy.[22-24]
- Reclassification of MDS into groups defined by genetic abnormalities such as MDS with biallelic *TP53* alterations; inclusion of two new morphologic entities, hypoplastic MDS and MDS with increased blasts and fibrosis, and removal of MDS with single/multilineage dysplasia.
- Elimination of the 20% blast threshold for most AML with defining genetic abnormalities, with the exception of AML with *BCR::ABL1*, AML with *CEBPA* mutation, AML with other defining genetic alterations (e.g., AML with *KAT6A::CREBBP*), and AML, myelodysplasia-related which still require ≥20% blasts for diagnosis.
- Renaming or regrouping of several entities, including myeloid neoplasms post cytotoxic therapy, formerly known as therapy-related myeloid neoplasms, and MDS/MPN with neutrophilia, formerly known as atypical CML.

Clear and standardised designation of the final integrated diagnosis according to WHO-HAEM5[21] promotes consistency across laboratories and ensures accurate diagnostic communication and patient management.

Table 1: 5th edition of the World Health Organization Classification of myeloid and mixed or ambiguous lineage neoplasms.[21]

Descriptor	ICD-O codes[a]
Myeloproliferative neoplasms	
Chronic myeloid leukaemia	9875/3
Chronic neutrophilic leukaemia	9963/3
Chronic eosinophilic leukaemia	9964/3
Polycythaemia vera	9950/3
Essential thrombocythaemia	9962/3
Primary myelofibrosis	9961/3
Primary myelofibrosis, prefibrotic	9961/3
Primary myelofibrosis, fibrotic	9961/3
Myeloproliferative neoplasm, not otherwise specified (NOS) (unclassifiable)	9975/3
Mastocytosis	
Cutaneous mastocytosis	9740/1
Maculopapular cutaneous mastocytosis†	9740/1
Diffuse cutaneous mastocytosis	9740/1
Mastocytoma	9740/1
Systemic mastocytosis	9741/1
Indolent systemic mastocytosis	9741/1
Bone marrow mastocytosis†	9741/1
Smouldering systemic mastocytosis†	9741/1
Aggressive systemic mastocytosis	9741/3
Systemic mastocytosis with an associated haematological neoplasm	9741/3
Mast cell leukaemia	9742/3
Mast cell sarcoma	9740/3
Myelodysplastic neoplasms	
Myelodysplastic neoplasms with defining genetic abnormalities	
Myelodysplastic neoplasm with low blasts and 5q deletion†	9986/3
Myelodysplastic neoplasm with low blasts and *SF3B1* mutation†	9982/3
Myelodysplastic neoplasm with biallelic *TP53* inactivation†	9985/3
Myelodysplastic neoplasms defined morphologically	
Myelodysplastic neoplasm with low blasts†	9985/3

Descriptor	ICD-O codes[a]
Myelodysplastic neoplasm with low blasts and single-lineage dysplasia†	9980/3
Myelodysplastic neoplasm with low blasts and multilineage dysplasia†	9985/3
Myelodysplastic neoplasm, hypoplastic†	9985/3
Myelodysplastic neoplasm with increased blasts†	9983/3
Myelodysplastic neoplasm with increased blasts-1†	9983/3
Myelodysplastic neoplasm with increased blasts-2†	9983/3
Myelodysplastic neoplasm with increased blasts and fibrosis†	9983/3
Myelodysplastic neoplasms of childhood	
Childhood myelodysplastic neoplasm with low blasts†	9985/3
Childhood myelodysplastic neoplasm with low blasts, hypocellular†	9985/3
Childhood myelodysplastic neoplasm with increased blasts†	9983/3
Myelodysplastic/myeloproliferative neoplasms	
Chronic myelomonocytic leukaemia	9945/3
Myelodysplastic chronic myelomonocytic leukaemia†	9945/3
Myeloproliferative chronic myelomonocytic leukaemia†	9945/3
Myelodysplastic/myeloproliferative neoplasm with neutrophilia†	9876/3
Myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis†	9982/3
Myelodysplastic/myeloproliferative neoplasm, NOS (unclassifiable)	9975/3
Acute myeloid leukaemia	
Acute myeloid leukaemia with defining genetic abnormalities	
Acute promyelocytic leukaemia with *PML::RARA* fusion	9866/3
Acute promyelocytic leukaemia with a variant *RARA* translocation†	9866/3
Acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion	9896/3
Acute myeloid leukaemia with *CBFB::MYH11* fusion	9871/3
Acute myeloid leukaemia with *DEK::NUP214* fusion	9865/3
Acute myeloid leukaemia with *RBM15::MRTFA* fusion	9911/3
Acute myeloid leukaemia with *BCR::ABL1* fusion	9912/3
Acute myeloid leukaemia with *KMT2A* rearrangement	9897/3
Acute myeloid leukaemia with *MECOM* rearrangement	9869/3
Acute myeloid leukaemia with *NUP98* rearrangement	9861/3
Acute myeloid leukaemia with *NPM1* mutation	9877/3
Acute myeloid leukaemia with *CEBPA* mutation	9878/3
Acute myeloid leukaemia, myelodysplasia-related	9895/3
Acute myeloid leukaemia with other defined genetic alterations†	9861/3
Acute myeloid leukaemia with *CBFA2T3::GLIS2* fusion	9861/3

Descriptor	ICD-O codes[a]
Acute myeloid leukaemia with *KAT6A::CREBBP* fusion	9861/3
Acute myeloid leukaemia with *FUS::ERG* fusion†	9861/3
Acute myeloid leukaemia with *MNX1::ETV6* fusion	9861/3
Acute myeloid leukaemia with *NPM1::MLF1* fusion	9861/3
Acute myeloid leukaemia defined by differentiation	
Acute myeloid leukaemia with minimal differentiation	9872/3
Acute myeloid leukaemia without maturation	9873/3
Acute myeloid leukaemia with maturation	9874/3
Acute basophilic leukaemia	9870/3
Acute myelomonocytic leukaemia	9867/3
Acute monocytic leukaemia	9891/3
Acute erythroid leukaemia	9840/3
Acute megakaryoblastic leukaemia	9910/3
Myeloid sarcoma	
Myeloid sarcoma	9930/3
Secondary myeloid neoplasms	
Myeloid neoplasms and proliferations associated with antecedent or predisposing conditions	
Myeloid neoplasm post cytotoxic therapy†	9920/3
Myelodysplastic neoplasm post cytotoxic therapy†	9987/3
Myelodysplastic/myeloproliferative neoplasm post cytotoxic therapy†	9920/3
Acute myeloid leukaemia post cytotoxic therapy†	9920/3
Myeloid neoplasms associated with germline predisposition (code as condition)	
Myeloid leukaemia associated with Down syndrome	9898/3
Transient abnormal myelopoiesis	9898/1
Myeloid/lymphoid neoplasms	
Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions	
Myeloid/lymphoid neoplasm with *PDGFRA* rearrangement	9965/3
Myeloid/lymphoid neoplasm with *PDGFRB* rearrangement	9966/3
Myeloid/lymphoid neoplasm with *FGFR1* rearrangement	9967/3
Myeloid/lymphoid neoplasm with *JAK2* rearrangement†	9968/3
Myeloid/lymphoid neoplasm with *FLT3* rearrangement†	9968/3
Myeloid/lymphoid neoplasm with *ETV6::ABL1* fusion†	9968/3
Myeloid/lymphoid neoplasms with other tyrosine kinase fusion genes†	9968/3
Acute leukaemias of mixed or ambiguous lineage	

Descriptor	ICD-O codes[a]
Acute leukaemia of ambiguous lineage with defining genetic abnormalities	
Mixed-phenotype acute leukaemia with *BCR::ABL1* fusion	9806/3
Mixed-phenotype acute leukaemia with *KMT2A* rearrangement†	9807/3
Acute leukaemia of ambiguous lineage with other defined genetic alterations†	9805/3
Mixed-phenotype acute leukaemia with *ZNF384* rearrangement†	9805/3
Acute leukaemia of ambiguous lineage with *BCL11B* rearrangement†	9805/3
Acute leukaemia of ambiguous lineage defined immunophenotypically	
Mixed-phenotype acute leukaemia, B/myeloid	9808/3
Mixed-phenotype acute leukaemia, T/myeloid	9809/3
Mixed-phenotype acute leukaemia, rare types	
Mixed-phenotype acute leukaemia, B/T†	9805/3
Mixed-phenotype acute leukaemia, B/T/myeloid†	9805/3
Mixed-phenotype acute leukaemia, T/megakaryocytic†	9805/3
Acute leukaemia of ambiguous lineage, NOS†	9805/3
Acute undifferentiated leukaemia	9801/3

[a] These morphology codes are from the International Classification of Diseases for Oncology, third edition, second revision (ICD-0-3.2).[25] Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour: /2 for carcinoma in situ and grade Ill intraepithelial neoplasia; /3 for malignant tumours, primary site; and /6 for malignant tumours, metastatic site. Behaviour code /6 is not generally used by cancer registries.

† Labels marked with a dagger have undergone a change in terminology of a previous code.

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Note 7 – Complete blood count (Core)

The complete blood count (CBC) provides essential quantitative data that complement bone marrow findings in the diagnosis and classification of myeloid and mixed or ambiguous lineage haematopoietic neoplasms. Differential cell percentages identify cytopenias or cytoses, quantify circulating blasts, and guide interpretation of marrow morphology and ancillary test results. Parameters such as haemoglobin, leukocyte, absolute neutrophil, and platelet counts, reticulocyte count, and red cell distribution have been reported to have significant impact on clinical outcomes in MDS and MPN.[2-4,26,27] CBC results should reflect the specimen obtained closest in time to the bone marrow examination. When available, the peripheral blood smear should be reviewed in conjunction with the CBC to verify automated results and evaluate morphologic features. Quantitative abnormalities also provide baseline data for monitoring disease course and therapeutic response.

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Note 8 – Bone marrow differential (Core)

A cell differential count on the Wright Giemsa or May Grünwald Giemsa-stained bone marrow aspirate or touch imprint is an important quantitative component of marrow evaluation. Differential counting should be performed on well-preserved, representative cellular areas adjacent to bone marrow particles. A 300-cell count provides comparable accuracy for most clinical purposes to a 500-cell differential as traditionally recommended[28] and should be adequate for bone marrow counts in most cases.

The relative percentages of major haematopoietic cell lineages provide important diagnostic clues, defining marrow composition and maturation pattern. Blast percentage is a critical diagnostic criterion for classification of myeloid neoplasms. Haemodilution can alter the relative proportion of cell types, particularly underestimating blast percentage, and distort lineage ratios. When aspiration yields inadequate material or smears are suboptimal for accurate enumeration, estimation of relative cell proportions may be performed in biopsy or clot sections using ancillary methods such as immunohistochemistry. The myeloid to erythroid (M:E) ratio provides a useful index of marrow cellular balance and must be interpreted in the context of peripheral blood counts, overall marrow cellularity, and clinical setting.

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Note 9 – Blasts/blast equivalents (Core)

Accurate enumeration of blasts/blast equivalents is essential for diagnosis, subclassification, prognosis, and disease monitoring in myeloid neoplasms.[3,29,30] Blast percentage thresholds define key diagnostic boundaries. For example, a diagnosis of AML without defining genetic abnormalities requires ≥20% blasts, and in MDS with increased blasts (MDS-IB), the blast percentage is used to subclassify cases into MDS-IB1 (≥5% and <10% in the bone marrow and/or ≥2% and <5% blasts in the peripheral blood) and MDS-IB2 (≥10% and <20% in the bone marrow and/or ≥5% and <20% blasts in the peripheral blood). The blast equivalent includes promyelocytes in acute promyelocytic leukaemia (APL), monoblasts, promonocytes, erythroblasts, and megakaryoblasts. The percentage of blasts/blast equivalents is primarily determined from peripheral blood and bone marrow aspirate smears or touch imprints by morphologic enumeration. Correlation with bone marrow biopsy findings using immunohistochemistry is advised to confirm concordance.

When aspirate smears are haemodilute, aspicular, represent dry taps due to fibrotic marrows, or in cases of CD56-positive myeloid neoplasms,[31] blasts/blast equivalents should be quantified on the bone marrow biopsy or clot sections using immunohistochemistry. For CD34-positive blasts, CD34 immunohistochemistry, preferably with the QBEND10 clone which has been extensively validated,[32-34] provides a reliable estimate of blast percentage. Interpretation of CD34 staining should take into account both the overall percentage of positive cells and their distribution pattern. The International Council for Standardization in Haematology (ICSH) recommends four major diagnostic cut-off points (1%, 5%, 10%, 20%) and/or ‘clusters’ (≥3 CD34+ blasts in direct contact; report when ≥2 clusters) and ‘hot-spots’ (≥1 400x high power field (HPF) and ≤50% of biopsy with CD34+ blasts percentage significantly higher than background).[34] The presence of ‘hot-spots’ or two or more clusters carries diagnostic and prognostic significance.

CD34 partially positive and CD34-negative blasts are common in blast equivalents, including promyelocytes in APL, monoblasts and promonocytes in AML with monocytic differentiation (e.g., AML with *NPM1* mutation, AML with *KMT2A* rearrangement), erythroblasts in acute erythroid leukaemia (AEL), and megakaryoblasts in acute

megakaryoblastic leukaemia. In such cases, other markers, such as CD56, CD61, CD71, CD117, CD123,[35] IRF8,[36] or mutated NPM1[37] can be applied to facilitate recognition of blast equivalents, though the supporting evidence is variably limited.

Morphologic features of blasts/blast equivalents should be documented, including nuclear details and cytoplasmic granularity or vacuolisation, as these may provide diagnostic clues to specific subtypes, for immunophenotypic and/or cytogenetic correlation and to guide subsequent ancillary testing.

In discrepant cases, the higher blast count obtained by any method should be considered of greater diagnostic weight.

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Note 10 – Morphologic abnormality and dysplasia (Core)

Assessment of morphologic dysplasia in the erythroid, myeloid, and megakaryocytic lineages is an integral component in the diagnosis and classification of myeloid neoplasms.[26,30,38,39] The distribution and morphology of megakaryocytes are specifically important in MPNs and in assessing treatment response in CML.[30] Dysplasia is considered significant when present in ≥10% of cells in a given lineage. The presence, extent, and lineage distribution of dysplasia are recorded systematically.

In peripheral blood and bone marrow aspirate, dysplasia of neutrophils and monocytes and atypical features of red blood cells and platelets are reported when evaluable and relevant. In bone marrow samples, evaluation of three major lineages and other cell types (i.e., basophils, eosinophils, and mast cells) is expected where possible. Common dysplastic features include nuclear budding, internuclear bridging, nuclear irregularity, multinuclearity, megaloblastic changes, karyorrhexis, ring sideroblasts, vacuolisation, and PAS positivity in erythroid precursors;[40] hyposegmentation (pseudo-Pelger-Huët), hypersegmentation, hypogranularity, pseudo-Chédiak-Higashi granules, small size, and Auer rods in granulocytes;[41] and micromegakaryocytes or hypolobation and multinucleation, or widely separated nuclear lobes in megakaryocytes.[42] If reliable assessment is not feasible due to suboptimal smears or hypocellularity, this should be documented as 'Cannot be assessed', with the reason specified. In disease entities where dysplasia is not applicable (e.g., myeloid sarcoma), it should be noted accordingly. Histotopography in bone marrow biopsy, including the spatial arrangement and clustering of haematopoietic elements and megakaryocytes, is also evaluated and reported to provide additional diagnostic clues. M:E ratio is relevant in the subclassification of chronic MPN[30] as well as in the classification of specific AML subtypes, such as AEL, where erythroid predominance informs diagnostic criteria.

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Note 11 – Bone marrow cellularity (Core)

Evaluation of bone marrow cellularity is a key parameter for assessing overall bone marrow composition and haematopoietic cell distribution. It contributes to accurate disease classification in distinguishing between entities with overlapping clinical and morphologic features and provides valuable information for prognosis and monitoring treatment response in MPN.[26,30,39]

Overall bone marrow cellularity reflects the proportion of haematopoietic bone marrow relative to adipose tissue. This is estimated from the bone marrow core biopsy specimen or intact areas of the clot section. Cellularity should be compared with the patient's age-related reference, with findings described as hypercellular, normocellular, or hypocellular relative to the expected range. It is also important to note that cellularity may be lower in subcortical areas of bone marrow or may show significant heterogeneity, which may limit accuracy in small or fragmented samples. Focal variation in cellularity should be interpreted in the context of biopsy adequacy, sampling site, and concurrent morphologic findings. In aspirate smears, the relative cellular composition is expected to correlate with the biopsy findings. Discrepancy between aspirate and core biopsy cellularity may reflect haemodilution, patchy involvement, or sampling artifact.

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Note 12 – Iron stain (Core)

Assessment of iron stores and the detection of ring sideroblasts by iron stain are essential for the classification of specific myeloid neoplasms.[43] Optimal evaluation is performed on bone marrow aspirate smears or touch imprints as they provide well preserved cytologic detail necessary for reliable identification of ring sideroblasts. Although iron stain may be attempted on core biopsy or clot sections when aspirate smears or touch imprints are not available, decalcification can reduce stainable iron and this preparation limits the visualisation of the ring sideroblasts. For non-marrow specimens (e.g., peripheral blood, lymph node, soft tissue), iron stain is not applicable, given the lack of relevance and technical limitations in those contexts.

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Note 13 – Bone marrow fibrosis (Core)

Grading of bone marrow fibrosis contributes significantly to the diagnostic and prognostic workup of myeloid neoplasms, particularly MPN, MDS, and MDS/MPN and informs disease progression and treatment monitoring (refer to Table 2).[2,4,21,30,44-48] Fibrosis may be primary, as in primary myelofibrosis, or secondary to other processes such as metastatic carcinoma or post-therapy changes. Reticulin and trichrome (Gömöri or Masson) stains are best performed on a core biopsy of sufficient length to determine the type and extent of fibrosis. Trichrome stain is recommended for cases with moderate to marked reticulin fibrosis to assess collagen deposition. Myelofibrosis is graded semi-quantitatively in areas of preserved haematopoiesis, primarily based on the patterns of reticulin fibrosis, collagen, and osteosclerosis. Areas distorted by crush artifact, haemorrhage, or decalcification should be avoided. The grade should reflect the highest level present in ≥30% of the evaluable marrow space.

Table 2: The World Health Organization grading system for myelofibrosis.[21,45]

Grade	Reticulin pattern[a]	Collagen pattern[b]	Osteosclerosis[c]
MF-0	Scattered linear reticulin with no intersections (crossovers), corresponding to normal bone marrow	Perivascular collagen only (normal)	Regular bone trabeculae (distinct paratrabecular borders)
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas	Focal paratrabecular or central collagen deposition with no connecting meshwork	Focal budding, hooks, spikes, or paratrabecular apposition of new bone
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibres mostly consistent with collagen and/or focal osteosclerosis	Paratrabecular or central deposition of collagen with focally connecting meshwork or generalized paratrabecular apposition of collagen	Diffuse paratrabecular formation of new bone with thickening of trabeculae, occasionally with focal interconnections
MF-3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibres consistent with collagen, usually associated with osteosclerosis	Diffuse (complete) connecting meshwork of collagen in >30% of marrow spaces	Extensive interconnecting meshwork of new bone with overall effacement of marrow spaces

[a] Reticulin and collagen fibre density should be assessed only in haematopoietic areas; if the pattern of reticulin fibrosis, collagen deposition, and/or osteosclerosis is heterogeneous, the final grade is determined by the highest grade present in >30% of the marrow area.

[b] Using trichrome staining (Masson trichrome or Martius Scarlet Blue); the stain is recommended for grades MF-2 and MF-3.

[c] Best performed on a bone marrow core biopsy of sufficient length, taken at a right angle from the cortical bone without significant fragmentation.

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Note 14 – Immunoprofiling/phenotyping studies (Core)

Immunoprofiling of haematopoietic cells is performed in addition to morphologic assessment to provide phenotypic information for disease diagnosis and classification.[49,50] Depending on the disease category, antibodies or antibody panels targeting markers of immaturity, erythroid, myeloid, monocytic, and megakaryocytic lineages, and other relevant populations[51] are selected to identify and characterise haematopoietic lineages, determine blast lineage, assess maturation patterns, and detect aberrant antigen expression that support diagnoses of myeloid neoplasms and acute leukaemias of mixed or ambiguous lineage (refer to Table 3).

Immunophenotyping may be performed by flow cytometry, immunohistochemistry, or other validated platforms, depending on sample type, laboratory capability, and test availability. Flow cytometry requires viable cells and offers rapid, semi-quantitative, and multiparametric evaluation of cell surface, cytoplasmic, and nuclear antigens on individual cells. Reporting of flow cytometry results should specify the antibody panels used and describe the immunophenotype of abnormal populations, including the percent of the population of interest, the presence or absence of antigen expression and the level/intensity of expression, for classification and subsequent monitoring of measurable residual disease (MRD). Immunohistochemistry can be performed on formalin-fixed, paraffin-embedded tissue to evaluate the immunophenotype of neoplastic populations and it is particularly useful in cases of dry tap or to assess cellular distribution in the context of tissue architecture. For blast quantification, immunohistochemistry for CD34 and/or CD117 generally provides more accurate blast percentage than flow cytometry, as flow cytometric enumeration can be affected by haemodilution, red cell lysis methodology (particularly for bone marrow aspirate), and may overestimate the blast count when using leukocytes rather than total nucleated cells as the denominator. Immunophenotypic results should be interpreted in conjunction with morphologic, cytogenetic, and molecular findings and clinical correlation.

Table 3: Recommended antibody panels[51]

Population of interest	Recommended antibody panels
Blasts/precursors	CD34, CD117, mutational specific NPM1
Lineage markers in MPAL	B lineage: CD19, CD10,* CD22, CD79a T lineage: cytoplasmic or surface CD3 Myeloid lineage: MPO, or monocytic differentiation (NSE, CD11c, CD14, CD64, lysozyme)
Erythroid cells	CD36, CD71, CD117, E-cadherin, glycophorin A
Myeloid cells	CD11b, CD13, CD15, CD33, CD64, CD65, CD117, CD177, MPO
Megakaryocytes	CD36, CD41, CD42b, CD61, vWF
Monocytic cells	CD4, CD11c, CD14, CD64, CD68, CD117, CD163, IRF8, lysozyme, NSE
Plasmacytoid dendritic cells	CD4, CD56, CD123, CD303, CD304, SOX4, TCL1, TCF4, IRF8
Mast cells	CD2, CD25, CD30, CD117, mast cell tryptase

*CD10 cannot be used in diagnosing B/T MPAL and B/T/myeloid MPAL.

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Note 15 – Cytogenetic studies (Core)

Cytogenetic analysis is indispensable for diagnosis, risk stratification, and disease monitoring in myeloid neoplasms and mixed or ambiguous acute leukaemias.[52,53] Conventional karyotyping, FISH, and chromosomal microarray (CMA) are used to detect recurrent structural or numerical abnormalities with diagnostic and prognostic significance. Karyotyping remains the standard approach for comprehensive evaluation of chromosomal abnormalities and clonal evolution. A normal karyotype is established by analysis of at least 20 metaphase cells[54] and may be performed on bone marrow or peripheral blood containing circulating abnormal cells. FISH analysis can identify specific rearrangements and copy number variants. Screening for gene rearrangements by FISH is recommended when rapid information is required to guide therapy, when chromosome morphology is suboptimal, or when characteristic morphology is present, but the expected cytogenetic abnormality is not detected. Results of FISH studies should list the probes tested, indicating those with normal findings and those demonstrating abnormal signal patterns. CMA allows accurate characterisation of chromosomal copy number alterations and copy-neutral loss of heterozygosity with diagnostic, prognostic, and therapeutic significance in myeloid neoplasms,[55] especially when conventional cytogenetics are inconclusive.

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Note 16 – Molecular studies (Core and Non-core)

Somatic mutations and gene fusions drive the pathogenesis of myeloid neoplasms and mixed or ambiguous acute leukaemias and are fundamental to their diagnostic classification, prognostication, therapeutic management, and disease monitoring (refer to Table 4).[56-60] These genetic aberrations can be detected by currently validated molecular methods, including PCR-based assays, targeted myeloid gene panel by next-generation sequencing (NGS), targeted RNA sequencing, and other genomic technologies, applied according to laboratory capability and clinical context. Screening for gene mutations continues to evolve, with single-gene assays increasingly replaced by multigene panel testing that provides comprehensive molecular profiling.[51,61,62] Reporting of gene panel results should include all detected pathogenic/likely pathogenic variants and variants of uncertain significance (VUS), with relevant details for each affected gene such as the specific alteration and variant allele frequency (VAF).

Molecular results should be interpreted within the clinical and morphologic context, as certain mutations define specific entities (e.g., AML with *NPM1* mutation, MDS with biallelic *TP53* inactivation), while others provide prognostic or therapeutic guidance. Germline variants associated with hereditary predisposition to myeloid neoplasms may also be detected and require clinical correlation and genetic counselling when appropriate. Molecular studies are additionally used for MRD assessment, monitoring of treatment response, and detection of clonal evolution during disease progression or relapse.

Table 4: Recommended molecular studies

Disease category	Recommended panels for gene mutations (core/non-core)	Recommended panels for gene fusions (core/non-core)
CH and CCUS	Proposed gene panel* (core and non-core)	None
CML	*ABL1* (kinase domain mutation) (core) Proposed gene panel* (core and non-core)	*BCR::ABL1* (isoform) (core)
PV, ET, PMF	*JAK2, CALR, MPL* (core) Proposed gene panel* (core and non-core)	Exclude *BCR::ABL1* (CML) (core)
CNL	*CSF3R, JAK2, CALR, MPL* (core) Proposed gene panel* (core and non-core)	Exclude *BCR::ABL1* (CML), *PDGFRA*-r, *PDGFRB*-r, *FGFR1*-r, *JAK2*-r, *FLT3*-r, *ETV6::ABL1* (M/LN-Eo and TK) (core)
CEL	Proposed gene panel* to exclude SM, MPN, MDS/MPN, and MDS (core and non-core)	Exclude *BCR::ABL1* (CML), *CBFB::MYH11* (AML), *IGH::IL3* (B-ALL), *PDGFRA*-r, *PDGFRB*-r, *FGFR1*-r, *JAK2*-r, *FLT3*-r, *ETV6::ABL1* (M/LN-Eo and TK) (core)
SM	*KIT* (core) Proposed gene panel* to exclude MPN, MDS/MPN, and MDS (core and non-core)	
MDS/MPN	Gene panel: *ASXL1, BCOR, CBL, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, NF1, NPM1, NRAS, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2* (core as per 5th edition WHO for CMML[21]) Proposed gene panel* (core and non-core)	Exclude *BCR::ABL1* (CML), *PDGFRA*-r, *PDGFRB*-r, *FGFR1*-r, *JAK2*-r, *FLT3*-r, *ETV6::ABL1* (M/LN-Eo and TK) (core)
MDS	*SF3B1, TP53* (core) Proposed gene panel* (core and non-core)	*ASXL1, BCOR, EZH2, FLT3, IDH1, IDH2, NPM1, SETBP1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*
AML	*NPM1, CEBPA, FLT3* (core) Gene panel: *ASXL1, BCOR, CEBPA, EZH2, FLT3, IDH1, IDH2, NPM1, SETBP1, SF3B1, SRSF2, STAG2, TP53, U2AF1, ZRSR2* (core) Proposed gene panel* (core and non-core)	*PML::RARA, RUNX1::RUNX1T1, CBFB::MYH11, DEK::NUP214, RBM15::MRTFA, BCR::ABL1, KMT2A-r, MECOM-r, NUP98-r, CBFA2T3::GLIS2, KAT6A::CREBBP, FUS::ERG, MNX1::ETV6, NPM1::MLF1* (core and non-core)
ALAL	Gene panel: *ASXL1, BCOR, CEBPA, ETV6, EZH2, FLT3, IDH1, IDH2, IKZF1, NOTCH1, NPM1, PAX5, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TP53, U2AF1, WT1, ZRSR2* (core) Proposed gene panel* (core and non-core)	*BCR::ABL1, KMT2A*-r, *ZNF384*-r, *BCL11B*-r (core) Other AML and ALL related (core and non-core)

M/LN-Eo and TK	Proposed gene panel* (core and non-core)	*PDGFRA*-r, *PDGFRB*-r, *FGFR1*-r, *JAK2*-r, *FLT3*-r, *ETV6::ABL1* (core)

*Proposed gene panel:[61-65]

ASXL1, BCOR, BCORL1, CBL, CEBPA, CALR, CSF3R, DDX41, DNMT3A, ETV6, ETNK1, EZH2, FLT3, GATA2, GNB1, IDH1, IDH2, IKZF1, JAK2, KIT, KRAS, KMT2A-PTD, MPL, NF1, NOTCH1, NPM1, NRAS, PAX5, PHF6, PPM1D, PRPF8, PTPN11, RAD21, RUNX1, SAMD9, SAMD9L, SETBP1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, UBA1, UBTF, WT1, ZRSR2.

Abbreviations: ALAL, Acute leukaemias of mixed or ambiguous lineage; AML, acute myeloid leukaemia; CCUS, clonal cytopenia of undetermined significance; CEL, chronic eosinophilic leukaemia; CH, clonal haematopoiesis; CML, chronic myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; CNL, chronic neutrophilic leukaemia; ET, essential thrombocythaemia; MDS, myelodysplastic neoplasms; MDS/MPN, myelodysplastic/myeloproliferative neoplasms; M/LN-Eo and TK, myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions; MPN, myeloproliferative neoplasms; PMF, primary myelofibrosis; PV, polycythaemia vera; r, rearrangement; SM, systemic mastocytosis.

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