Proposed diagnostic criteria for classical chronic myelomonocytic leukemia (CMML), CMML variants and pre-CMML conditions

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SUPPLEMENTAL APPENDIX TO:

Proposed diagnostic criteria for classical CMML, CMML variants and pre-CMML conditions

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Information on the Working Conference and the Consensus Discussion

The Working Conference on CMML and Pre-CMML conditions (official title: Standards and Standardization in Chronic Myelomonocytic Leukemia) was organized in Vienna in August 2018 (August 24-26, 2018) by the Medical University of Vienna in collaboration with the Vienna Cancer Stem Cell Club and the Ludwig Boltzmann Institute for Hematology and Oncology (LBI HO). The project included an in-depth discussion on CMML and Pre-CMML conditions, lasting from March 2018 until March 2019. The discussion phase was split into a pre-conference phase (via e-mails and smaller preparative meetings), the conference discussion (Working Conference: 3 days), and a post-conference discussion phase (September 2018 until March 2019). The consensus discussion and the consensus decision-making process were organized in accordance with recently published guidelines.[1]

In the final discussion round, the paper-draft was discussed and adjusted based on input provided by all faculty members and available information. Open discussion points were discussed in the faculty (consensus group = co-authors) until a clear-cut result (100% of faculty members agreed) was obtained or no consensus was reached. Only those statements, criteria, and definitions that are based on a 100% consensus among all faculty members were included in the final document.

The final document and its content were approved by all faculty members (all co-authors) before submission. All actively contributing (only those) faculty members are included as co-authors on the final document.

1. Graham R, Mancher M, Wolman DM, Greenfield S, Steinberg E, eds; Institute of Medicine; Board on Health Care Services; Committee on Standards for Developing Trustworthy Clinical Practice Guidelines. Clinical Practice Guidelines We Can Trust. Washington, DC: National Academies Press; 2011.

Supplementary Tables

Supplementary Table S1

Diagnostic WHO criteria of Chronic Myelomonocytic Leukemia (CMML) 2016

Persistent PB monocytosis ≥1x10^9/L, with monocytes accounting for ≥10% of the WBC count

Not meeting WHO criteria for BCR-ABL1+ CML, PMF, PV, or ET*

No evidence of PDGFRA, PDGFRB, or FGFR1 rearrangement or PCM1-JAK2 (should be specifically excluded in cases with eosinophilia)

<20% blasts in the blood and BM†

Dysplasia in 1 or more myeloid lineages:

If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are met and an acquired clonal cytogenetic or molecular genetic abnormality is present in hematopoietic cells‡

or

The monocytosis (as previously defined) has persisted for at least 3 month and all other causes of monocytosis have been excluded

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

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

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

Abbreviations: WHO, World Health Organization; PB, peripheral blood; WBC, white blood cell count; BM, bone marrow; CML, chronic myeloid leukemia; PMF, primary myelofibrosis; PV, polycythemia vera; ET, essential thrombocythemia.

Supplementary Table S2

Proposed Grading of Chronic Myelomonocytic Leukemia (CMML)*

Grading-based variants	Diagnostic features / criteria
CMML-0	<5% blasts in BM smears and <2% blasts in PB**
Dysplastic CMML-0	PB leukocytes $\leq 13x10^9$/L
Proliferative CMML-0	PB leukocytes $>13x10^9$/L
CMML-1	6-9% blasts in BM smears and 2-4% blasts in PB**
Dysplastic CMML-1	PB leukocytes $\leq 13x10^9$/L
Proliferative CMML-1	PB leukocytes $>13x10^9$/L
CMML-2***	10-19% blasts in BM smears and 5-19% in PB**
Dysplastic CMML-2	PB leukocytes $\leq 13x10^9$/L
Proliferative CMML-2	PB leukocytes $>13x10^9$/L***

*Initial grading of CMML is helpful to provide a first guess concerning prognosis. However, initial grading should not replace, but should rather be followed, by prognostic scoring using available scoring systems to predict the outcome in each individual patient with CMML.

**With regard to PB blast counts, grading should be based on at least 2 consecutive blood examinations and: in patients in whom results from BM and PB smears do not provide a definitive conclusion concerning the grade/variant of CMML (e.g., BM blasts 4% and PB blasts 6%) grading should always refer to the higher blast cell count.

***In patients who progress from CMML-1 to CMML-2, the CMML-status must be reconfirmed with all diagnostic approaches, including BM histology and immunohistochemistry, molecular studies and flow cytometry in order to exclude AML. This is of special importance in cases with proliferative CMML-2.

Abbreviations: BM, bone marrow; PB, peripheral blood; AML, acute myeloid leukemia.

Supplementary Table S3

Diagnostic Criteria for Idiopathic Monocytosis of Undetermined Significance (IMUS) and Clonal Monocytosis of Undetermined Significance (CMUS)

IMUS:

- Persistent (≥3 months) absolute peripheral blood monocytosis ≥$0.5x10^9$/L and (plus) relative monocytosis of ≥10% of circulating peripheral blood leukoyctes

- Exclusion of diagnostic dysplasia and diagnostic myeloproliferation

- No signs and criteria of a myeloid or other hematopoietic neoplasm fulfilled

- No somatic mutation related to a myeloid, mast cell or lymphoid neoplasm is detected in leukocytes*

- No reactive condition/disease that could explain reactive monocytosis**

CMUS:

- Persistent (≥3 months) absolute peripheral blood monocytosis ≥$0.5x10^9$/L and (plus) relative monocytosis of ≥10% of circulating peripheral blood leukocytes

- Exclusion of diagnostic dysplasia and diagnostic myeloproliferation

- No signs and criteria of a myeloid or other hematopoietic neoplasm is fulfilled

- A CHIP-like somatic mutation, otherwise also found in myeloid neoplasms such as MDS or CMML, is detected in leukocytes***

- A reactive condition/disease that could explain reactive monocytosis may also be detected**

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*In IMUS, all somatic mutations indicative of a myeloid, mast cell or lymphoid neoplasm must be excluded because of the potential impact of such mutation. Example: in a healthy individual with IMUS who turns out to have BCR-ABL1 in a small-sized clone, the clinician´s primary attention and focus will be on BCR-ABL1.

**In IMUS all kinds of reactive causes have to be excluded by definition. By contrast, in CMUS, a reactive condition may well be diagnosed since in these patients, clonality of myeloid cells is the primary indicator of the risk to develop a myeloid neoplasm like CMML, irrespective of a co-existing inflammatory or other reactive disease. After successful treatment of the reactive disorder, monocytosis may disappear so that these cases are re-classified as CHIP (or CCUS when cytopenia is also present).

***In the CMUS context, only one CHIP-like somatic mutation (otherwise found in MDS or CMML) is detected. When two or more such mutations are found the patient is likely to suffer from oligomonocytic CMML.

Abbreviations: CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; CHIP, clonal hematopoiesis of indeterminate potential; CCUS, clonal cytopenia of undetermined significance.

Supplementary Table S4

Value and Impact of BM Histology and Immunohistochemistry (IHC) in CMML

Indication - Differential Diagnoses	Key (IHC) markers
Detection and enumeration of monocytes	CD14, CD68R
Detection and enumeration of blast cells	CD34, CD117/KIT**
Abnormal morphology / dysplasia of megakaryocytes*	CD31, CD42, CD62
Dysplastic versus proliferative CMML	Cellularity, CD14
CMML-0 vs CMML-1 vs CMML-2	CD34 (CD117/KIT**)
Separation from AML when smears are of poor quality or blood-contaminated	CD34 (CD117/KIT**)
Diagnosis of a concomitant (underlying) systemic mastocytosis (SM-CMML)	KIT, tryptase, CD25***
Detection of a concomitant lymphoproliferative disease	T cell / B cell markers Plasma cell antigens (CD38, CD138)
Multifocal accumulations of progenitor cells	CD34 (CD117/KIT)
Demonstration of bone marrow fibrosis	Reticulin stain (e.g., Gömöri)
Demonstration of increased angiogenesis	CD31, CD34

*In some patients with CMML, demonstration of megakaryocyte dysplasia is only possible by BM histology and immunohistochemistry. Note also that immature megakaryocytes (megakaryoblasts) may only be detectable by IHC.

**Evaluation of the numbers of $CD34^+$ cells by IHC can help exclude AML and assist in the delineation between CMML-0, CMML-1 and CMML-2 (when the smears are of poor quality or not available). When BM blasts lack CD34, CD117/KIT is used as alternative stain to label immature precursor cells and blast cells.

***Mast cells in systemic mastocytosis (SM) display CD25 in an aberrant manner.

Abbreviations: CMML, chronic myelomonocytic leukemia; BM, bone marrow; AML, acute myeloid leukemia; IHC, immunohistochemistry.

Supplementary Table S5

Recommended Immunohistochemical Markers in CMML

A: Minimal Panel

marker(s)	cell type(s)**
- CD14*	monocytes and macrophages
- CD34*	blast cells, progenitors, endothelial cells
- CD31, CD41, CD42, CD62	megakaryocytes
- Tryptase*	mast cells, immature/leukemic basophils
- CD117 (KIT)*	blast cells, progenitor cells, mast cells

B: Extended Panel – according to the cell lineage to be examined

marker(s)	cell type(s)**
- CD3	T cells
- CD71, E-Cadherin	erythroid cells
- CD15	neutrophils and monocytes
- CD20	B cells
- CD25	neoplastic mast cells, B cell subset
- CD38, CD138	plasma cells
- CD68, CD68R*	monocytes, macrophages, myeloid cells
- 2D7, BB1	basophils
- MBP, ECP	eosinophils

*In a very few patients with CMML, myeloblasts may be CD34-negative. In these patients, CD117 can be applied as an alternative stain, whereas tryptase usually is negative or shows only a weak reactivity with blast cells. CD34 and monocyte/macrophage markers may be helpful to discriminate between immature monocytic cells and blasts (CMML versus AML).
**The minimal panel includes markers directed against monocytes, mast cells (to exclude or reveal mastocytosis) and megakaryocytes (to define megakaryocyte dysplasia). In the extended panel, additional cell types are examined (depending on differential counts and other features), such as neoplastic mast cells (CD25), erythroid cells, basophils and eosinophils. These stains are of special importance in special variants of CMML.
Abbreviations: CMML, chronic myelomonocytic leukemia; MBP, major basic protein; ECP, eosinophil cationic protein.

Supplementary Table S6

Cytogenetic abnormalities detected in CMML

A: Most frequent cytogenetic abnormalities detected by chromosome banding

Abnormality	Prevalence (mean, range)*
Trisomy 8	6.5% (4-10%)
-7/del(7q)	5% (3-8.5%)
-Y	4.5% (3-6%)
Complex	4.1% (3-6%)
-20/del(20q)	2.8% (1-5%)
Trisomy 21	1.3% (0.5-2%)

B: Kryptic deletions in CMML detected by interphase FISH

Abnormality	Prevalence (mean, range)*
TET2-deletions	8.3% (6-10%)
NF1-deletions	5% (4-6%)
ETV6-deletions	3% (2-4%)

*Data refer to the available literature, the experience of our faculty (Haase et al., unpublished observations). Abbreviations: CMML, chronic myelomoncytic leukemia; FISH, fluorescence in situ hybridization.

Supplementary Table S7

Some Driver Genes Detectable in Patients with Special CMML Variants

Gene Name Abbreviation	Gene Class and function	Relative Frequency in CMML*	Clinical Impact
KIT D816V	Signaling and differentiation**	1-5%	SM-related drug target
JAK2 V617F	Signaling and differentiation**	5-10%	MPN-related drug target
PDGFRA-mutations***	Signaling and differentiation**	<5%	MPN-eo-related CEL-related**** drug target
PDGFRB-Mutations***	Signaling and differentiation**	<5%	MPN-eo-related CEL-related**** drug target

*The frequency relates to all patients with CMML (classical plus special variants). **Differentiation is dependent on the driver involved and includes mast cell differentiation (KIT D816V), myeloid, megakaryocyte and erythroid differentiation (JAK2 V617F), and eosinophil differentiation (PDGFRs). ***Most of these mutations are create distinct PDGFR fusion genes and are associated with specific gene rearrangements and certain karyotype abnormalities. ****In these patients, eosinophilia or even hyper-eosinophilia is commonly detected and the final WHO diagnosis may be MPN-eo, MDS/MPN-eo or CMML-eo. Chronic eosinophilic leukemia (CEL) may also be detected in these patients. However, in most of these CEL cases, the diagnostic criteria for CMML are not fulfilled, so that the final diagnosis remains CEL with monocytosis. Abbreviations: CMML, chronic myelomonocytic leukemia; SM, systemic mastocytosis; MPN, myeloproliferative neoplasm; MPN-eo, MPN with eosinophilia; CEL, chronic eosinophilic leukemia.

Supplementary Table S8

Recurrent Immunophenotypic Abnormalities detected in Patients with CMML

Monocytes
- relative increase in ´classical´ ($CD14^{bright}/CD16^{-}$) monocytes (MO1)
- relative decrease in ´intermediate´ ($CD14^{bright}/CD16^{+}$) monocytes (MO2)
- relative decrease in ´non-classical´ ($CD14^{dim}/CD16^{+}$) monocytes (MO3)
- abnormal granularity (sideward light scatter)
- abnormal distribution of immature and mature subsets
- abnormal (over)expression of CD2, CD5, CD10, CD23, or CD56
- lack or under-expression of CD13, CD14, CD33, CD36, CD38, CD45, or CD64
- abnormal expression of CD11b or HLA-DR

$CD34^{+}$ progenitor cells*
- increase in $CD34^{+}$ cells**
- absolute and relative (to all $CD34^{+}$) decrease in number of $CD34^{+}/CD10^{+}$ or $CD34^{+}/CD19^{+}$ cells
- abnormal expression of CD45, CD34 or CD117
- abnormal granularity (sideward light scatter)
- overexpression or lack of expression of CD13, CD33, or HLA-DR
- expression of ´lymphoid´ antigens: CD5, CD7, CD19, or CD56
- expression of CD11b and/or overexpression of CD15

Maturing Neutrophils*
- decreased granularity (sideward light scatter)
- abnormal distribution of immature and mature subsets
- lack of or abnormal expression of CD11b, CD13 or CD33
- delayed (or early) expression of CD16 or lack of CD10
- expression of CD56

Erythroid precursor cells*
- decreased or heterogeneous expression of CD36 and CD71
- abnormal frequency of $CD117^{+}$ erythroid precursors
- abnormal frequency of $CD105^{+}$ erythroid precursors
- abnormal CD105 fluorescence intensity

Mast cells (KIT^{+})***
- abnormal expression of CD25
- abnormal expression of CD2 or CD30

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*MDS-related phenotypic abnormalities that can be detected in patients with CMML. **An increase in $CD34^{+}$ precursors by flow cytometry can assist in the quantification of the blast cell compartment in CMML. In these cases, the percentage of $CD34^{+}$ cells usually correlates with the grading result. ***Aberrant expression of CD25 (or CD2 and/or CD30) on KIT^{+} mast cells is indicative of concomitant systemic mastocytosis (SM-CMML). Abbreviations: CMML, chronic myelomonocytic leukemia.

Supplementary Table S9

Reactive and Clonal Mimickers of CMML

Reactive:

- Subacute bacterial endocarditis/endo-myocarditis
- Tuberculosis
- Malaria infection
- EB virus infections
- Syphilis
- Typhoid fever
- Trypanosomiasis
- Drug-induced toxic reactions
- Corticosteroid therapy
- Treatment with GM-CSF
- Paraneoplastic (T cell lymphoma, Hodgkin disease, solid tumors)
- Chronic and acute autoimmune diseases
- Sarcoidosis
- Chronic hepatitis plus cirrhosis
- Collagen disease
- Asplenic state
- Pregnancy

Clonal:

- CMUS
- Low risk MDS with monocytosis
- High risk MDS with monocytosis
- Monoblastic AML
- MPN with monocytosis
- GATA2 deficiency with monocytosis
- RASopathies: CBL syndromes and others*
- JMML
- Histiocytosis

Abbreviations: CMML, chronic myelomonocytic leukemia; EB, Epstein Barr; GM-CSF, granulocyte-monocyte colony-stimulating factor; CMUS, clonal monocytosis of unknown clinical significance; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; JMML, juvenile myelomoncytic leukemia.

*RASopathies carry a high risk to develop into JMML

Supplementary Table S10

Examples for Therapy-Options for Non-Transplantable Patients with CMML*

Intensive Therapy

- Polychemotherapy (AML-like protocols)

Less Intensive Disease-Modifying Therapy

- 5-Azacytidine
- Decitabine

Experimental Therapy Options (many of them tested currently in clinical trials)

- Combinations of polychemotherapy and targeted drugs (e.g., plus GO)
- Combinations of 5-Azacytidine with targeted drugs:
  - BCL2 family member blocker: venetoxclax
  - JAK2 blocker: ruxolitinib
  - HDAC blocker: panobinostat
  - IMiDs: lenalidomide, thalidomide
  - Others: luspatercept, tipifarnib, SL401, lenziluzumab, etc.

Palliative Therapy

- Hydroxyurea
- Low dose ARA-C
- 5-Azacytidine or decitabine
- Others

Therapy for Special Variants of CMML

- Midostaurin and cladribine (KIT D816V+ SM-CMML)
- Ruxolitinib (JAK2 V617F+ CMML or MPN+CMML)
- Imatinib or other PDGFR-blocker (PDGFR-rearranged CMML)
- Lenalidomide (CMML with isolated 5q- abnormality)

Treatment recommendations and recommendations for determining responses to interventional drugs (treatment response criteria) have recently been proposed by an expert group representing the European Hematology Association (EHA) and the European Leukemia Net (ELN) (reference number #140 in main text).