Proposed Diagnostic Criteria for Classical CMML, CMML Variants and Pre-CMML Conditions

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Abstract

Chronic myelomonocytic leukemia (CMML) is a myeloid neoplasm characterized by dysplasia, abnormal production and accumulation of monocytic cells and an elevated risk to transform into acute leukemia. Over the past two decades, our knowledge about the pathogenesis and molecular mechanisms in CMML has increased substantially. In parallel, better diagnostic criteria and therapeutic strategies have been developed. However, many questions remain regarding prognostication and optimal therapy. In addition, there is a need to define potential pre-phases of CMML and special CMML variants, and to separate these entities from each other and from conditions mimicking CMML. To address these unmet needs, an international consensus group met in a Working Conference in August 2018 and discussed open questions and issues around CMML, its variants, and pre-CMML conditions. The outcomes of this meeting are summarized herein and include diagnostic criteria and a proposed classification of pre-CMML conditions as well as refined minimal diagnostic criteria for classical CMML and special CMML variants, including oligomonocytic CMML and CMML associated with systemic mastocytosis. Moreover, we propose diagnostic standards and tools to delineate between ´normal´, pre-CMML and CMML entities. These criteria and standards should facilitate diagnostic and prognostic evaluations in daily practice and clinical studies in applied hematology.

Introduction

Chronic myelomonocytic leukemia (CMML) is a myeloid stem cell disease characterized by an abnormal production and accumulation of monocytic cells, often in association with other signs of myeloproliferation, substantial dysplasia in one or more hematopoietic cell lineages, and an increased risk of transformation into secondary acute myeloid leukemia (sAML).[1-5] As per definition, the Philadelphia chromosome (Ph) and the related *BCR-ABL1* fusion gene are absent in CMML. Other disease-related drivers, such as the *JAK2* mutation V617F or the *KIT* mutation D816V, may be detected and may indicate a special variant of CMML, such as CMML associated with systemic mastocytosis (SM-CMML).[6-8] However, most somatic mutations identified in CMML patients, such as mutations in *SRSF2*, *TET2*, or *RAS*, are not disease-specific, but are also detected in myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), or AML.[8-11]

For many years, CMML was listed as a separate variant amongst the MDS in the classification of the French-American-British (FAB) working group.[2,12] However, in 2001, the World Health Organization (WHO) reclassified CMML into a newly created MDS/MPN overlap group, defined by the presence of both, MDS-related and MPN-related morphologic and clinical features.[13] Depending on the leukocyte count, CMML can be divided into a ´dysplastic´ variant (leukocyte count $\leq 13x10^9$/L) and a ´proliferative´ variant (leukocyte count $>13x10^9$/L).[2] In 2001 and 2008, the WHO also proposed a split into CMML-1 and CMML-2, based on the percentage of blast cells in the blood and BM.[13,14] In the most recent update of the WHO 2016 classification,

CMML is again listed amongst the MDS/MPN overlap disorders.[15,16] Based on the percentage of blasts, CMML is now divided into CMML-0, CMML-1, and CMML-2.[15-19] Moreover, contrasting the 2008 WHO classification, the diagnosis of CMML now requires both, an absolute monocytosis ($\geq 1 \times 10^9$/L) and relative monocytosis (≥10% of leukocytes) in the peripheral blood (PB).[15,16,18,19] In the 2008 and 2016 update of the WHO classification, CMML can only be diagnosed per definition when rearrangements in *PDGFRA*, *PDGFRB* or *FGFR1* genes have been excluded, and in the 2016 update, the *PCM1-JAK2* fusion gene was added as an excluding criterion.[14-16,19] These molecular aberrations are commonly found in eosinophilia-associated neoplasms such as chronic eosinophilic leukemia.[20,21] However, CMML is also listed as an underlying variant in these molecular ´entities´ in the WHO classification system.[20,21]

Over the past two decades, our knowledge about molecular features and mechanisms in CMML has increased substantially.[4-11,22-26] Moreover, new diagnostic criteria, prognostic markers, and therapeutic concepts have been developed.[26-29] Nevertheless, a number of questions remain concerning basic diagnostic standards, prognostication, optimal management and therapeutic options. Furthermore, there is a need to define clinically relevant pre-phases of CMML and distinct CMML variants by clinical variables, histomorphologic features, flow cytometric phenotypes, molecular markers and cytogenetic findings. It is also important to separate CMML and pre-CMML conditions from diverse mimickers. To address these unmet needs, an international consensus group discussed open questions and issues around CMML, its variants and pre-CMML entities in a Working Conference held in August 2018. The outcomes of this meeting are summarized in this article and include proposed diagnostic criteria

and a classification of pre-CMML conditions as well as updated minimal diagnostic criteria for CMML and its variants. In addition, diagnostic standards and diagnostic algorithms are proposed. Details concerning the conference format, pre- and post-conference discussion and consensus-finding are described in the supplement.

Definition of CMML and Minimal Diagnostic Criteria

The diagnostic criteria of CMML, as defined by the WHO[15,16] are depicted in Supplementary Table S1. Our faculty is of the opinion that these criteria are valid in general for the classical form of CMML, but need adjustments for special variants of CMML. Based on consensus discussion, the following concept is proposed:

The classical form of CMML is defined by the following pre-requisite criteria: 1) persistent (at least 3 months) absolute PB monocytosis ($\geq 1\times10^9$/L) and relative monocytosis (≥10% of PB leukocytes), 2) exclusion of *BCR-ABL1*+ leukemia, classical MPN and all other hematologic neoplasms that may serve as primary source of monocytosis, and 3) a blast cell count of 0-19% in PB and/or BM smears and exclusion of all (other) histopathologic, morphologic, phenotypic, molecular and cytogenetic signs that qualify as evidence of AML. In addition, morphologic and/or histopathologic evidence for diagnostic dysplasia in one or more of the 3 major BM cell lineages (≥10% of megakaryocytes and/or erythroid precursor cells and/or neutrophilic cells) has to be present. If dysplasia is absent or not diagnostic (<10%), the presence of cytogenetic or molecular lesions (mutations) typically found in CMML and/or the presence of CMML-related flow cytometry abnormalities may be employed as co-criteria and may lead to the diagnosis of CMML, provided that the pre-requisite

criteria listed above are fulfilled. Pre-requisite criteria and co-criteria of the classical form of CMML are depicted in Table 1.

The exclusion of various reactive states producing monocytosis (and sometimes even dysplasia) was also discussed and regarded as being of great importance. However, these mimickers cannot ´a priori´ exclude the presence of a concomitant CMML, but may indeed occur in CMML patients in the context of certain infections. Furthermore, most of these mimickers do not produce persistent monocytosis. Proof of clonality by molecular and cytogenetic studies, and other disease-specific parameters, together with global and specific laboratory (e.g., microbial screen) tests should easily lead to the conclusion that the patient is suffering from reactive monocytosis but not from (or also from) CMML.

The ´a priori´ exclusion of AML as criterion should apply to both, the classical and the special variants of CMML, whereas the ´a priori´ exclusion of other indolent hematopoietic neoplasms should only apply to the classical variant of CMML and oligomonocytic CMML but not to other special CMML variants. This is because several previous and more recent studies have shown that CMML may be accompanied by (or may accompany) other myeloid or lymphoid neoplasms, such as systemic mastocytosis. In several of these patients, the CMML clone is dominant and the additional sub-clone is smaller in size and usually not relevant clinically, even if these smaller clones express certain driver mutations, such as *KIT* D816V or a rearranged *PDGFRA* or *PDGFRB*. Rarely, a Ph+ CML may develop as additional small-sized (sub)clone in a patient with CMML. Our faculty is of the opinion that the presence of additional (chronic) myeloid, mast cell, or lymphoid neoplasms does not exclude a diagnosis of CMML, provided that diagnostic WHO criteria for CMML are

fulfilled. Moreover, these concomitant neoplasms should not exclude a diagnosis of CMML even when the driver of the concomitant disease (e.g., *KIT* D816V) is detectable in CMML monocytes. Thus, whereas the occurrence of AML is always regarded as transformation of CMML, the occurrence of indolent myeloid, mast cell, or lymphoid neoplasms should be regarded as concomitant disorders. Co-existing myeloid neoplasms and CMML may be derived from the same original founder-clone. There are also patients in whom a certain driver of another BM neoplasm is present, such as a mutated *JAK2*, *PDGFRA/B,* or *FGFR1,* but only the diagnostic criteria for CMML (not that of the other BM neoplasm) are fulfilled. Our faculty concludes that these cases should also be regarded and diagnosed as special variants of CMML. This strategy is in line with the current WHO classification. In fact, whereas the primary molecular diagnosis is often based on a mutated form of *JAK2*, *PDGFRA/B* or other classical driver, the underlying or additional diagnosis may well be CMML.[20,21]

Grading of CMML

The grading system of CMML proposed by the WHO is regarded as the standard in clinical hematology. Our faculty recommends the use of this grading system as initial prognostic tool in classical CMML. In fact, classical CMML should be split into CMML-0, CMML-1 and CMML-2 based on the blast cell count (Supplementary Table S2).[15-19] In addition, CMML can be divided into a dysplastic variant and a proliferative variant based on leukocyte counts (threshold: $13x10^9$/L) (Supplementary Table S2). The resulting grading system defines 6 distinct CMML variants with variable clinical outcome.[17] However, grading may sometimes be challenging. For example, blast cell

counts obtained from BM smears may differ from those obtained in the PB so that the grade is in question. Our faculty recommends that in patients in whom results from BM and PB smears would not fit into one distinct grade of CMML (e.g., BM blasts 4% and PB blasts 6%) grading should be based on the higher blast cell percentage (Supplementary Table S2). It is worth noting that initial prognostication by grading does not include all essential prognostic parameters. Therefore, we recommend that in each case, deeper (full) prognostication should follow using multiparametric scoring systems (see later). It should be noted, however, that grading of CMML has only been validated in the classical form of CMML, but not in special CMML variants. Therefore, although grading is recommended also for special CMML entities, it is not regarded standard and the result must be interpreted with caution in these patients.

Special variants of CMML - Overview

As mentioned before, the classical form of CMML meets all pre-requisite criteria, and no signs (including molecular features) of an additional, concomitant BM neoplasm are detected. The special variants of CMML form a heterogeneous group of neoplasms comprising distinct clinical and biological entities. In one group of patients, the relative monocyte count (≥10%) is fulfilled without resulting in an absolute count equal or higher than 1×10^9/L, precluding the diagnosis of ´classical CMML´. Most of these patients are diagnosed as MDS or MPN/MDS-U by WHO criteria. In another group of patients, a molecular signature suggestive of a different type of myeloid neoplasm is detected but only the criteria for CMML (not that for the other neoplasm) are met. Such an example is CMML with *JAK2 V617F* (without definitive evidence of

a concomitant MPN). In a third group, CMML co-exists with another BM neoplasm, such as MPN or mastocytosis. In these patients, additional blood count abnormalities (e.g., eosinophilia), an elevated serum tryptase level and/or BM fibrosis, may be detected.

All variants of CMML (classical and special) can occur as a) primary CMML or as b) secondary CMML following a ´mutagenic´ event, such as chemotherapy (therapy-related CMML). In addition, our faculty is of the opinion, that the term secondary CMML may also be appropriate for those patients who develop CMML (months or years) after another indolent myeloid neoplasm, such as a MDS or systemic (indolent or aggressive) mastocytosis, had been diagnosed. In the following paragraphs, the clinical features and diagnostic criteria of special (atypical) variants of CMML are proposed and discussed. An overview of the special variants of CMML is provided in Table 2.

Oligomonocytic CMML

Over the past few years, more and more cases of cytopenic patients exhibiting relative monocytosis (≥10%) and moderately increased absolute blood monocytes not reaching the required threshold to diagnose classical CMML ($1.0x10^9$/L) have been described. These cases have recently been referred to as oligomonocytic CMML.[30] According to the WHO classification most of these patients would be classified as MDS (with monocytosis) or perhaps MPN/MDS-U. However, most of these patients exhibit typical features of CMML, including a typical morphology of PB and BM cells, splenomegaly, and CMML-related molecular features (e.g. mutations in *TET2* and

SRSF2).[30-32] Some of these patient have prominent BM monocytosis without diagnostic peripheral blood monocytosis at diagnosis.[32]

Whereas several of these cases remain stable without progression, the majority will develop ´overt´ CMML or eventually, secondary AML during follow-up. Therefore, oligomonocytic CMML may also be regarded as a potential pre-phase of classical CMML. Our faculty is of the opinion, that the term oligomonocytic CMML should be used in clinical practice. Diagnostic pre-requisite criteria for oligomonocytic CMML are: 1) persistent (at least 3 months lasting) absolute peripheral monocytosis of 0.5-0.9×10^9/L and relative blood monocytosis (≥10% of blood leukocytes) 2) exclusion of *BCR-ABL1*+ leukemia, classical MPN and all other myeloid neoplasms that can explain monocytosis, and 3) a blast cell count of 0-19% in PB and/or BM smears and exclusion of all histopathologic, morphologic, phenotypic, molecular and cytogenetic signs that count as proof of AML. Diagnostic dysplasia in one or more of the 3 major BM lineages (≥10%) must also be documented. If dysplasia is lacking or ´sub-diagnostic´ (<10%), the presence of cytogenetic or molecular lesions (mutations) typically found in CMML and/or the presence of CMML-related flow cytometry abnormalities, may also lead to the conclusion the patient has oligomonocytic CMML provided that the other diagnostic criteria described above are fulfilled and all other myeloid neoplasms have been excluded. The proposed criteria for oligomonocytic CMML are depicted in Table 3. Patients with oligomonocytic CMML should be managed and followed clinically in the same way as patients with classical CMML.

CMML associated with *KIT* D816V+ systemic mastocytosis (SM)

According to WHO criteria, SM can be divided into i) indolent SM (ISM) where life expectancy is normal, ii) smoldering SM (SSM) where signs of BM dysplasia, myeloproliferation and/or splenomegaly are found but survival and prognosis are still favorable, and iii) advanced SM defined by poor prognosis.[33-36] Advanced SM is further divided into aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN) and mast cell leukemia (MCL).[33-36] The most frequent AHN detected in patients with SM-AHN is CMML.[6-8,36] In these patients the SM component of the diseases may present as ISM, ASM or, rarely, as MCL. Our faculty concludes that diagnostic WHO criteria for SM and diagnostic criteria for classical CMML (except exclusion of SM) have to be fulfilled to diagnose SM-CMML.

Patients with SM may present with monocytosis resembling oligomonocytic CMML. However, the clinical features of SSM and advanced SM overlap largely with those found in patients with oligomonocytic CMML. Especially in SSM, myelo-proliferation, dysplasia and splenomegaly are diagnostic criteria.[33-35] Therefore our faculty is of the opinion that such patients should be classified as ISM, SSM or ASM with monocytosis rather than SM with oligomonocytic CMML.

In patients with CMML, a concomitant SM is often overlooked especially when the disease does not present with cutaneous lesions. In other patients, CMML is diagnosed long before SM is detected by chance or after the *KIT* D816V is identified: even though it is tempting to call these conditions CMML-SM, our faculty agreed that the classical terminology should be SM-CMML which is also in line with the WHO classification[34,35] and that the subtype of SM and of CMML should be defined in the final diagnosis (e.g., ISM-CMML-1 or ASM-CMML-2) with recognition that in the SM-context, CMML is always a secondary neoplasm.[6,36] Furthermore our faculty is of

the opinion that it is standard to examine BM and blood leukocytes for the presence of *KIT* D816V in all patients with (suspected) CMML. In almost all patients with SM-CMML, neoplastic monocytes display *KIT* D816V.[7] In these monocytes, mutated KIT is not expressed on the cell surface but acts as a cytoplasmic driver lesion. In line with this hypothesis drugs targeting *KIT* D816V can sometimes induce a major decrease in monocyte counts in patients with ASM-CMML.[37]

Therapy of SM-CMML should be based on a bi-directional strategy: in fact the SM component of the disease should be treated as if no CMML was diagnosed and CMML should be treated as if no SM was found, with recognition of drug-drug interactions and the possibility of drug-induced anaphylaxis.[33-35] In many cases (ISM-CMML) the SM component of the disease is only treated symptomatically.[33-35]

CMML associated with mutated *JAK2*, rearranged *PDGFRA/B* or other drivers

Patients with CMML may present with the *JAK2* mutation V617F, a rearranged *PDGFRA* or *PDGFRB*, often in the context of hypereosinophilia, or other drivers related to distinct hematopoietic neoplasms as defined by the WHO.[5,9-11,38-43]

a) CMML with rearranged *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-JAK2*:

In these patients, persistent substantial monocytosis ($\geq 1.0 \times 10^9$/L) is detected and all other consensus criteria of classical CMML (see previous paragraphs) are also met, except the following specific exclusion criteria: CMML to be excluded in the presence of a well characterized diagnosis of myeloid/lymphoid neoplasm with rearranged *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-JAK2* (Table 2). Except for neglecting the above mentioned criteria, our proposal is otherwise fully in agreement with all of the

other tenets postulated by the WHO classification.[20,21] In relation to neoplasm with rearranged *PDGFRA/B*, *FGFR1* or *PCM1-JAK2*, their definition of ´myeloid/lymphoid neoplasms´ is too generic and there is a clinical need to know whether the underlying myeloid neoplasm is an aggressive disease, like AML, or a chronic neoplasm such as CMML or chronic eosinophilic leukemia (CEL). Our faculty is of the opinion that (unlike in previous times) the presence of one criteria-confirmed myeloid neoplasm should not ´a priori´ exclude the presence of another (second concomitant) myeloid or lymphoid neoplasm. Hence, when CMML is encountered in the context of another molecularly defined myeloid/lymphoid neoplasm (as a final diagnosis), it should be delineated as a specific subtype of the myeloid/lymphoid neoplasm with eosinophilia along with the specific associated gene rearrangement (*PDGFRA/B* or *FGFR1* or *PCM1-JAK2*).

a) CMML with *JAK2* V617F:

In these patients the situation is different. First, *JAK2* V617F itself may be considered as a criterion of myeloproliferation in MDS/MPN, e.g. in cases with MDS/MPN with ring sideroblasts and thrombocytosis. In the CMML-context, the *JAK2* mutation is also typically associated with other signs of myeloproliferation (including BM fibrosis) and with the ´myeloproliferative variant´ of CMML.[39,42,43] Therefore, our faculty concludes that *JAK2* V617F should also count as a molecular co-criterion of MDS/MPN and thus for CMML. Second, the presence of a *JAK2*-mutated MPN does not exclude the presence of a concomitant CMML if diagnostic criteria for both neoplasms are fulfilled. If this is not the case because the size of the MPN-like clone carrying *JAK2* V617F is too small and/or other MPN features are clearly missing, the final diagnosis will be CMML with *JAK2* V617F. On the other hand, in patients in whom the *JAK2*

allelic burden is high and clinical and laboratory features argue for an overt MPN rather than CMML (e.g., polycythemia and/or BM fibrosis without dysplasia and without molecular or flow cytometry-based signs of CMML) the final diagnosis will be *JAK2* V617F+ MPN with monocytosis.[43] In a third group of patients, diagnostic criteria for both, a distinct MPN and CMML, are fulfilled and the mutation status confirms the presence of an overt *JAK2*-mutated MPN (usually with high allelic burden). These patients are suffering from both, MPN and CMML or from a gray zone disease displaying hybrid features between MPN and CMML.[44,45] Our faculty concludes that it is therefore important to measure the *JAK2* V617F allele burden in all patients with CMML.[39,42,43] Other drivers, such as *BCR-ABL1*, are rarely found in patients with CMML. However, although in classical CMML, the presence of *BCR-ABL1* must be excluded, it may be detected in rare patients suggesting the existence of a special variant of CMML (defined by a co-existing CML). In some of these cases, the CML clone may be small-sized. In other patients, however, the CML may even mask CMML at initial diagnosis.[46]

Management and therapy of patients with special variants of CMML depends on the subtype of the disease and the molecular driver involved, like *FIP1L1/PDGFRA*, other gene abnormalities involving *PDGFRA* or *PDGFRB*, *KIT* D816V or *JAK2* V617F. Therefore, it is of crucial importance to screen (ask) for all these drivers in all patients with CMML. The type of therapy to consider in these patients depends on clinical features, the histopathological diagnosis, the size of the mutated clone(s) and the type of driver. The latter is of considerable importance since novel treatments directed against these drivers, are often extremely effective.[47-50] For example, imatinib can induce long-lasting molecular and hematologic complete remissions (CR) in patients

with *FIP1L1/PDGFRA*-rearranged myeloid neoplasms with features of CMML or MPN.[47-49] Even in patients who develop CMML and secondary AML in the context of *FIP1L1/PDGFRA*, the disease may respond to imatinib.[50] Therefore, it is important to diagnose all patients based on molecular markers and to define the major drivers and therapeutic targets expressed by malignant cells in order to provide optimal management and therapy.

CMML associated with lymphoid neoplasms

In a small group of patients with CMML, a co-existing lymphoproliferative neoplasm is diagnosed, such as a lymphocytic leukemia, non-Hodgkin lymphoma or multiple myeloma.[51-60] In most patients, the lymphoid neoplasm is detected first, and CMML is considered to develop as treatment-induced, secondary, leukemia.[51,57] In other patients, CMML is first diagnosed, and later, a lymphoid neoplasm is detected during follow-up.[52-56] It is worth noting that in patients with CMML, polyclonal hypergammaglobulinemia is often recorded which must be distinguished from the monoclonal gammopathy of concomitant myeloma, monoclonal gammopathies of undetermined significance (MGUS) and both low-count and high-count monoclonal B lymphocytosis (MBL) which represent pre-malignant conditions.

Management and treatment of lymphoid neoplasms presenting with concomitant (secondary) CMML is a clinical challenge. In non-transplantable cases, both diseases require separate treatment plans. Because of the high-risk regarding transformation to AML, allogeneic hematopoietic stem cell transplantation (allo-HSCT) should be

considered in young and fit patients, especially when it can be expected that the lymphoid neoplasm will also be eradicated by this approach.

Treatment-related CMML (t-CMML) and other secondary forms of CMML

Our faculty concludes that both the classical form of CMML and the special variants of CMML should be divided into primary (*de novo*) CMML and secondary CMML (sCMML). The latter group includes patients who i) received chemotherapy and/or radiation therapy in the past (therapy-related CMML) or ii) have a history of a preceding MDS, MPN or another indolent myeloid or mast cell neoplasm prior to the CMML diagnosis.[51,57,58,61-64] Recent data suggest that patients with therapy-related sCMML (t-CMML) may have shorter overall survival compared to patients with primary (*de novo*) CMML.[65] Although progression-free survival may not be different in these patients compared to *de novo* CMML, some of these patients progress rapidly to secondary AML. It is also worth noting that patients with t-CMML have a higher frequency of karyotypic abnormalities compared to *de novo* CMML.[66] Eligible patients in this group should be offered allo-HSCT.

Potential Pre-Phases of CMML

During the past few years evidence has accumulated suggesting that hematopoietic neoplasms, including MDS, MPN and MDS/MPN, develop in a step-wise manner. In the earliest phases of clonal development, patients present without overt signs or symptoms of a hematopoietic neoplasm but their leukocytes carry one or more somatic

mutations, usually (early, passenger-type) mutations otherwise also found in overt myeloid neoplasms (for example *TET2* mutations).[67-70] In the context of MDS and other myeloid neoplasms, these cases have been referred to as clonal hematopoiesis of indeterminate potential (CHIP), or, when accompanied by cytopenia, as clonal cytopenia of unknown significance (CCUS).[69-73] Since these mutations are frequently detected in older individuals, the condition is also called age-related clonal hematopoiesis (ARCH).[70,73] In a few healthy individuals, bona fide oncogeneic drivers (such as *BCR-ABL1*) are detected in a small subset of leukocytes. Because of the oncogenic potential of these drivers, these conditions are termed clonal hematopoiesis with oncogenic potential (CHOP).[71,73] CHIP, CCUS and CHOP may also be the earliest clonal conditions preceding CMML. For these cases, the definitions recently proposed for CHIP, CCUS and CHOP should apply.[69,71,73]

Apart from somatic mutations, other factors, such as epigenetic modifications, chronic inflammation or ageing-related processes, may also trigger the selection and expansion of pre-malignant neoplastic clones in myeloid neoplasms including CMML.[74-76] Some of these conditions may present with persistent monocytosis without signs of an overt myeloid neoplasm and may represent pre-phases of overt CMML. In other patients, however, no or another hematopoietic neoplasm develops during follow-up. Therefore, our faculty concluded that this pre-phase should be termed idiopathic monocytosis of unknown significance (IMUS), provided that the following criteria are met: i) persistent (at least 3 months) relative (≥10%) and absolute (>0.5x10^9/L) monocytosis, ii) no diagnostic dysplasia and no signs of myeloproliferation, iii) no signs and criteria of a myeloid or other hematopoietic neoplasm fulfilled, iv) no flow cytometric abnormalities or somatic mutations related to a myeloid, mast cell or lymphoid

neoplasm detected in leukocytes, and v) no reactive condition that would explain reactive monocytosis is detected (Table 4 and Supplementary Table S3). If in such patient CHIP-like mutations are found, but no hematopoietic neoplasm can be diagnosed using WHO criteria, the final diagnosis changes to clonal monocytosis of unknown significance (CMUS) (Supplementary Table S3). It is also worth noting that idiopathic cytopenias of unknown significance (ICUS) can precede CMML.[64,77-79] Especially in patients with idiopathic thrombocytopenia of unknown significance (ICUS-T), a CMML may be detected upon deeper investigations or during follow-up.[77-79] Finally, as mentioned before, oligomonocytic CMML, although proposed as a special variant of CMML, must also be regarded as a potential pre-phase of classical CMML. In this regard it is important to note that these patients should have a regular follow-up with repeated investigations of all disease-related parameters. A summary of non-clonal and clonal conditions potentially preceding CMML is shown in Table 4. With regard to criteria delineating non-clonal pre-diagnostic conditions, like ICUS from the clonal conditions described above (CHIP, CCUS, CHOP), we refer to the pertinent literature.[69,71,73]

PB and BM Smears: Proposed Standards and Recommendations

As in other myeloid neoplasms, a thorough examination of appropriately prepared and stained BM and PB smears is a crucial diagnostic approach in suspected CMML. It is standard to examine and count at least 100 leukocytes in the PB film and 200-500 nucleated cells in well-prepared thin BM films. BM cellularity, the erythroid-to-myeloid (E:M) ratio, and the percentage of blast cells (including monoblasts and

promonocytes), monocytes, mast cells, and other myeloid cells must be recorded (reported) in each case. Like in patients with MDS, at least 10% of cells in one of the major BM lineages (erythroid or/and neutrophil or/and megakaryocyte) need to be dysplastic to meet the dysplasia criterion of CMML.[13-18] It is also standard to study well-prepared and appropriately stained PB smears in CMML and to report the percentage of circulating monocytes, including normal (mature) and abnormal (immature) monocytes, blast cells, other immature myeloid cells, dysplastic (hypogranulated) neutrophils and other cell types in the PB. Overall, the same standards and recommendations that count for the evaluation of MDS by morphology (BM and PB stains)[12,80-83] also apply in cases with (suspected) CMML.[13-18] An important point is the classification of blast cells and monocytic cells in CMML (Table 5).[16,84] Blast cell types detectable in CMML include myeloblasts, monoblasts and also promonocytes (even if not named blast cells) (Table 5). Monocytes should be classified as normal (mature) or abnormal (immature).[16,84] The morphologic criteria used to delineate between these cell types are depicted in Table 5. Together with morphology, cytochemical staining for non-specific esterase can also assist in the morphologic delineation between monocytes, monoblasts and promononcytes.[16] An important aspect is that in many patients, megakaryocyte dysplasia is better documented and quantified in BM histology sections than in BM smears. Therefore, megakaryocyte dysplasia should only be recorded in BM smears when a sufficient number of these cells can be detected. Finally, the morphology of mast cells, when detected, should always be reported using established criteria and standards.[85]

BM Histology and Immunohistochemistry (IHC) in CMML

A thorough investigation of an appropriately processed and stained BM biopsy section by histology and IHC is standard in all cases with known or suspected CMML or a suspected pre-CMML condition.[14-16,30,86] Notably, BM histology and IHC are an essential approach to confirm the diagnosis of CMML and to exclude AML and other CMML-mimickers. Moreover, BM histology and IHC may provide important additional information, including BM fibrosis, focal accumulations of blast cells, increased angiogenesis, atypical (dysplastic) megakaryocytes, a hypocellular BM or concomitant mastocytosis (Supplementary Table S4).[33-35,86] The evaluation and enumeration of $CD14^+$ monocytes, $CD34^+$ progenitor cells and $CD117^+$/KIT^+ cells (progenitors and mast cells) by IHC in BM biopsy sections represent an integral part of the diagnostic assessment. This approach can also prevent diagnostic errors. For example, when the smear is of suboptimal quality, a preliminary diagnosis of CMML may change to AML based on BM histology and CD34 IHC.

BM biopsy specimens are usually taken from the iliac crest and should be of adequate length (≥2 cm). The specimen should be fixed in neutral formalin (or alternative standard fixation), decalcified in EDTA (for at least 8 hours) or by alternative standard decalcification, and embedded in paraffin-wax. Ideally 2-3 µm-thin sections should be prepared. Routine stains include hematoxylin-eosin, Giemsa, Prussian blue, AS-D chloroacetate esterase (CAE), Toluidine Blue and silver impregnation (Gömöri´s stain). BM cellularity should be measured and reported according to published standards.[87,88] For routine purposes, the pathologist should determine the cellularity as ´normocellular´, ´hypocellular´, or ´hypercellular´, based on an age-adapted estimate.[89] The presence of variable degrees of BM fibrosis (usually mild to moderate) has been

reported in CMML cases with several recent studies attempting to determine its prognostic value.[42,90,91] Indeed, although the data are not yet conclusive, the presence of marrow fibrosis in CMML seems to be of prognostic importance.[42,90,91]

The application of IHC markers is recommended in all patients with (suspected) CMML. The minimal IHC-panel includes CD14 (monocytes), CD34 (progenitors), CD117/KIT (progenitors and mast cells), tryptase (mast cells), and a megakaryocyte marker (CD41, CD42 or CD61) (Supplementary Table S5).[86,92,93] In unclear cases or when a co-existing (second) BM neoplasm is suspected, additional lineage-specific antibodies such as CD3, CD20, or CD25 (suspected mastocytosis) should be applied (Supplementary Table S4). When employing CD34 as a progenitor-related IHC marker, it is important to know that endothelial cells also express this antigen. Another important point is that blasts may sometimes be CD34-negative. In such cases, KIT/CD117 is applied as alternative marker (Supplementary Table S4). For the detection of monocytic cells, CD14 is a preferred IHC antigen.[71,86] Tryptase and CD117 are useful IHC markers to detect and quantify mast cells.[92,93] When spindle-shaped mast cells form compact clusters in the BM and express CD25, these cells usually also display *KIT* D816V – in these cases the final diagnosis is always SM-CMML.[93] In other cases, the pathologist will ask for *JAK2* V617F, based on an abnormal morphology and distribution of megakaryocytes. Like in MDS, megakaryocytes may also express CD34 in patients with CMML.

Karyotyping in CMML: Current Recommendations and Standards

Clonal cytogenetic abnormalities are detected in 20-30% of all patients with CMML. The most frequently identified aberrations are trisomy 8, abnormalities of chromosome 7 (especially monosomy 7 and deletion of 7q), and loss of the Y chromosome (-Y) (Supplementary Table 6).[94-97] Compared to MDS, isolated del(5q) and complex abnormal karyotypes are rarely detected in CMML. Our faculty is of the opinion that conventional karyotyping of BM cells should be performed in all patients with known or suspected CMML or a suspected pre-CMML condition. At least 20 metaphases should be examined.[98] In the case of a clear-cut result, even 10-20 metaphases may be sufficient to define the karyogram. Reporting of karyotypes should be performed using ISCN guidelines.[99] A clone is defined by 2 or more metaphases showing the same gain or structural rearrangement (deletion, inversion, translocation) of chromosomal material or at least 3 metaphases showing a monosomy of the same chromosome.[99] Several of the cytogenetic anomalies in CMML may be difficult to detect by conventional karyotyping. Therefore, we are of the opinion that fluorescence in situ-hybridization (FISH) should be performed in all patients with (suspected) CMML, at least in those where no karyotype anomaly was detected by conventional karyotyping. The FISH probes should cover all relevant regions, including 5q31, cep7, 7q31, 20q, cep8, cepY and p53. Special consideration should be directed to kryptic deletions of *TET2* (in 4q24), *NF1* (17q11), and *ETV6* (12p13) which can occur in up to 10% of CMML patients[10] and is only detectable by interphase FISH (Supplementary Table S6). It is worth noting that *NF1* deletions may occur during progression/katyotype evolution in CMML. The limitation of FISH is that is does not detect all karyotypic abnormalities. In some of the patients with CMML, clonal evolution is found. Subclones are defined by additional chromosomal defects (apart from the primary

chromosomal defect) in at least 2 cells (or 3 cells for monosomies) and absence of these additional chromosomal defects in the other clonal cells.[99] A complex karyotype is defined by at least 3 chromosome defects in one clone.[99] As in MDS, a complex karyotype in CMML is indicative of a poor prognosis. Overall, cytogenetic studies are of prognostic significance in CMML and have been used to optimize prognostic scoring systems.[97,100-102] In some patients with CMML, clonal evolution is observed over time and may then also be an adverse prognostic sign. Therefore, we recommend that chromosome analyses are performed each time when a BM investigation is done in the follow-up in order to detect (or exclude) clonal evolution.

Mutation Profiles in CMML: Current Standards and Limitations

In the vast majority of patients with CMML, somatic mutations are detectable.[8,11,103-106] The clonal architecture, clone-sizes and clonal evolution patterns vary from patient to patient.[106-108] In some cases, initially small-sized clones expand over time. Therefore, it is standard to apply next-generation sequencing (NGS) assays with sufficient sensitivity to identify bona fide somatic mutations associated with CMML. The most frequently detected somatic mutations in CMML are mutations in *TET2* (60%), *SRSF2* (50%), and *ASXL1* (40%) (Table 6).[31,103-110] The presence of a *SRSF2* mutation, particularly in combination with mutated *TET2*, correlates strongly with a CMML phenotype.[31,109,110] It is also worth noting that two of these mutations (*TET2*, *ASXL1*) are also known as CHIP/ARCH-related mutations. However, only mutated *ASXL1* has been associated with a poor prognosis in CMML.[104,109] An overview of somatic mutations recurrently detected in CMML is provided in Table 6. Somatic

mutations with independent prognostic impact include several RAS-pathway mutations as well as mutations in *ASXL1*, *RUNX1* and *SETBP1* (Table 6).[31,103-111] RAS-pathway mutations are triggering cell signaling and proliferation and have been associated with cytokine-independent growth of CMML progenitor cells, the proliferative variant of CMML, AML transformation and poor survival.[10,22,23,112-116] Other driver mutations involved in cell signaling, such as *JAK2* V617F or *KIT* D816V, are also (in addition) major triggers of cellular differentiation (Supplementary Table S7). These drivers alone cannot induce transformation, but they may act together with other (e.g., ´RAS pathway´) mutations to cause disease progression. Whereas *JAK2* V617F is a strong indicator of MPN-like differentiation, the presence of *KIT* D816V is almost always associated with concomitant mast cell differentiation and mastocytosis (SM-CMML).[6-8,32-36,39,42,43] The other mutations found in CMML act as modulators of epigenetic events and transcription (like *ASXL1*) or DNA methylation (like *TET2*), as regulators of the spliceosome machinery (like *SRSF2*), or as modulators of the DNA damage response, such as *TP53* (Table 6). During progression of CMML to sAML and especially during therapy, the mutational landscape(s) and clonal architecture(s) may change.[109-113] For example initially small-sized clones may expand and may be selected because of resistance-mediating molecular features. It is worth noting that several mutated gene products also serve as potential targets of therapy (Table 6).

Our faculty recommends that NGS studies should be regarded as a standard approach in all patients with suspected or known CMML as well as in patients with idiopathic monocytosis of unknown significance (IMUS) and in those with persistent reactive monocytosis (in order to exclude an additional clonal component). When a CMML-related mutation is found in an individual with IMUS or reactive monocytosis, the

diagnosis may change to CMUS or oligomonocytic CMML (O-CMML) depending on additional findings.

Our faculty also recommends that the NGS assay should have sufficient sensitivity (to detect 2-5% clonal cells) and should cover all relevant lesions depicted in Table 6. In the context of CHIP/ARCH, a cutoff of 2% variant allele frequency (VAF) is considered diagnostic[69], whereas in the CMML context, we propose a 10% VAF as a diagnostic cut off and thus marker to count as a co-criterion of CMML when, for example, no diagnostic morphologic dysplasia could be documented (Tables 1 and 3) similar to the definition in MDS.[71,73] Determining the VAF is also useful to document the clinical impact of certain driver lesions in special CMML variants (e.g., with *JAK2* V617F or *KIT* D816V) and clone-expansion during follow-up. Therefore, our faculty recommends that molecular studies in CMML should report VAFs with sufficient precision and sufficient sensitivity – in the same way as in MDS.[71,73] Finally, our faculty recommends that molecular markers should increasingly be used to optimize prognostic scoring systems in CMML.[117-120]

Flow Cytometry in CMML: Standards and Limitations

Flow cytometry studies are an essential diagnostic tool in patients with (suspected) classical CMML, pre-CMML conditions and special CMML variants.[121-132] Therefore, our faculty is of the opinion that it is standard to perform multi-color flow cytometry (MFC) in the PB and BM in all cases with suspected or known CMML or a suspected pre-CMML condition. MFC studies are helpful to confirm the monocyte and blast cell counts in these patients and to exclude AML. In addition, MFC is useful to confirm the

presence of distinct monocyte populations. Monocytes are defined as CD14+ cells in these analyses. Based on expression of CD14 and CD16, monocytes are further divided into classical (MO1) monocytes ($CD14^{bright}/CD16^{-}$), intermediate (MO2) monocytes ($CD14^{bright}/CD16^{+}$) and non-classical (MO3) monocytes ($CD14^{dim}/CD16^{+}$) (Table 7).[127,128,132] Compared to age-matched healthy donors[133] and patients with reactive monocytosis, but also myeloid neoplasms other than CMML (even MDS), the percentages of MO1 monocytes in the peripheral blood are higher and the percentage of MO3 monocytes is lower in patients with CMML.[127,131,132] When the absolute monocyte count is increased in the PB, a cutoff value of >94% MO1 monocytes, based on their immunophenotype, can identify CMML with a sensitivity of >90% and a specificity of >95%.[127,129,131] Moreover, during successful therapy, the distribution of MO1, MO2, and MO3 monocytes changes back to near normal or normal.[128] Therefore, our faculty recommends that the percentages of MO1 monocytes are quantified in the peripheral blood by MFC in all cases with suspected or known CMML at diagnosis and during follow-up.

In many cases with CMML, neoplastic monocytes aberrantly display CD2, CD5, CD10, CD23, and/or CD56.[121-124] Of all aberrantly expressed surface markers, CD56 is most commonly detected on CMML monocytes.[121-124] CD5 is only (very) weakly expressed on neoplastic monocytes in most cases with CMML. The most frequently underexpressed antigens may be CD14 and CD15. Overall, however, the use of decreased expression of these markers as a diagnostic test in CMML is limited by a relatively low sensitivity. An abnormal immunophenotype of monocytes is also seen in other myeloid neoplasms, including MDS. On the other hand, phenotypically aberrant monocytes (as described above) are typically neoplastic cells (unless the patient has

been treated with growth factors). Therefore, our faculty recommends that MFC studies in patients with (suspected) CMML employ antibodies directed against aberrantly expressed surface markers, including CD2 and CD56. Additionally several surface markers are ´under-expressed´ on CMML monocytes when comparing to normal blood monocytes. These antigens include, among others, CD13, CD14, CD33, CD36, CD38, CD45, and CD64.[121-124,129,131]

Other cell types may also express aberrant markers by MFC in CMML. For example, myeloid progenitor cells may express CD56 in CMML and often exhibit the same phenotypic abnormalities like in MDS; this holds also true for neutrophils and erythroid cells (Supplementary Table S8). Other cell types that may show aberrant phenotypes are dentritic cells and mast cells. Especially mast cells are of considerable importance as these cells may be indicative of the presence of a concomitant mastocytosis (SM-CMML). In these cases, mast cells almost invariably express CD25 in MFC analyses (Supplementary Table S8).[134] Overall, our faculty is of the opinion that MFC studies should be performed on monocyte subsets, myeloid progenitors, neutrophils, erythroid cells and mast cells. An overview of immunophenotypic aberrancies detectable in CMML is shown in Supplementary Table S8.

Differential Diagnoses of CMML: Reactive and Clonal Mimickers

A number of conditions can mimick CMML and have to be taken into account when patients with unexplained monocytosis are evaluated. Reactive disorders mimicking CMML include, among others, certain chronic bacterial infections (examples: tuberculosis or subacute endomyocarditis), fungal infections, chronic auto-immune

processes and non-hematologic neoplasms. There are also hematologic malignancies which may present as a CMML-like disease. For example, Ph^+ CML is usually presenting with (absolute) monocytosis and can also show signs of dysplasia. Particularly high monocyte counts are recorded in CML cases expressing *BCR-ABL1*$_{p190}$. When cryptic variants of *BCR-ABL1* are expressed by leukemic cells, it can be difficult to exclude CMML. Myeloid neoplasms (MDS or MPN) in progression and myelomonocytic or monocytic AML may also resemble CMML. The reactive and clonal mimickers of CMML are listed in Supplementary Table S9.

Scoring Systems in CMML: Recommended Standards

Although several prognostic variables have been identified in CMML regarding survival and AML evolution, accurate prediction of the clinical course and survival remains a clinical challenge. A first step in prognostication is grading into CMML-0, CMML-1 and CMML-2. To delineate the prognosis in CMML more accurately, a number of scoring systems have been developed in the past.[29,117-121,135-138] Until 2012, the international prognostic scoring system (IPSS) served as a golden standard of prognostication in MDS and (dysplastic) CMML.[135]

However, a number of more specific scoring systemic taking CMML-related features into account have also been proposed.[117-120,136-138] During the past few years, researchers have successfully started to integrate cytogenetic and molecular variables into these scoring models.[117-121] Our faculty concludes that these novel approaches should be followed and developed into clinical application.

Management Strategies and Therapeutic Options in CMML

Several new treatment strategies for CMML have been developed during the past 15 years. A detailed description of therapeutic options is beyond the scope of this article. The reader is referred to a series of excellent published review articles.[139-146] A disappointing fact is that all drug therapies are still non-curative. The only curative therapy in CMML remains allo-HSCT.[147,148] For most young and eligible patients with acceptable transplant-related risk, allo-HSCT is therefore recommended. All other forms of treatment are cytoreductive, experimental or palliative in nature. Some of these drugs, like the hypomethylating agents (5-azacytidine, decitabine) may induce long-term disease control in a subset of patients with classical CMML.[139-145] In general, cytoreductive and palliative drugs should be applied according to available recommendations provided by major societies.[145,148] Similarly, treatment response assessment should be performed in line with available (accepted) guidelines.[146,150]

Specific therapy may work in those patients who suffer from a special variant of CMML. For example, in CMML patients with a transforming *PDGFRA/B* mutation, treatment with imatinib or other similar TKI usually induces major responses or even long-lasting remissions.[47-49,151] In patients with SM-CMML, midostaurin may result in disease control, especially when the CMML-portion of the disease exhibits *KIT* D816V. However, in many cases, relapses occur. Treatment options in CMML and its variants are summarized in Supplementary Table S10.

Concluding Remarks and Future Perspectives

CMML is a unique and rare hematopoietic neoplasm with a complex biology and pathology. In the past 10 years, several different pre-CMML conditions and sub-variants of CMML have been defined. In the current article, we propose minimal diagnostic criteria for classical CMML and for special CMML variants. These criteria should help in the diagnosis of pre-CMML conditions, classical CMML, special CMML variants, and conditions that mimick CMML. In addition, we propose standards and tools for the diagnosis, prognostication and management of CMML. Contemporary assays define all major histopathologic, molecular, cytogenetic and flow cytometry-based features of neoplastic cells, and thereby cover all CMML variants, including oligomonocytic CMML and CMML associated with certain drivers or a concomitant myeloid neoplasm, such as mastocytosis. Different aberration profiles may also be found, resulting in a quite heterogeneous clinical picture and a variable clinical course. Although the course is often unpredictable, initial grading and consecutive application of CMML-directed prognostic scores are standard tools that support the prognostication of patients with CMML concerning survival and AML evolution. The application of criteria, tools and standards proposed herein should assist in the diagnosis, prognostication and management of patients with CMML.

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Contributions

All co-authors contributed by establishing concepts and recommendations, by participating in the pre-conference and post-conference discussion-phases, by actively participating in the Working Conference, by formulating consensus statements, by writing parts of the manuscript, and by correcting the draft and approving the final version of the document. Consensus statements were based on a 100% agreement (all faculty members agreed) and only those statements were included in this article.

Conflict of Interest Statements

The authors declare that they have no conflict of interest in this study and paper. Conflicts of interest (past 3 years) outside the study are as follows:

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A.Ora.: no conflict of interest

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Tables

Table 1

Minimal Diagnostic Criteria of Classical CMML*

A. Prerequisite Criteria (all must be fulfilled)

- Persistent (3 months) peripheral blood monocytosis ≥1x10^9/L and (plus) relative monocytosis of ≥10% of circulating peripheral blood leukoyctes

- Exclusion of *BCR-ABL1*+ leukemia, classical MPN and all other bone marrow neoplasms that could serve as a primary source of chronic persistent monocytosis

- Blast cell count of <20% in peripheral blood and bone marrow smears and (plus) exclusion of all other histopathological, morphologic, molecular and cytogenetic features that count as evidence for the presence of acute myeloid leukemie (AML)**

B. Morphologic criterion = Dysplasia

- Dysplasia in at least 10% of all cells in one of the following lineages in the bone marrow smear: erythroid; neutrophilic; megakaryocytic

C. Co-Criteria (for patients fulfilling A but not B, and otherwise show typical clinical features of CMML such as splenomegaly)

- Typical chromosome abnormalities by conventional karyotyping or FISH***

- Abnormal findings in histologic and/or immunohistochemical studies of bone marrow biopsy sections supporting the diagnosis of CMML****

- Abnormal immunophenotype of bone marrow and blood cells by flow cytometry, with multiple CMML-associated phenotypic aberrancies indicating the presence of an abnormal/dysplastic population of monocytic and other myeloid cells*****

- Evidence of a clonal population of myeloid cells determined by molecular (sequencing) studies revealing CMML-related mutations******

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*The diagnosis of classical CMML can be established when all prerequisite criteria (´A´) and either morphologic dysplasia (´B´) or one or more of the co-criteria (´C´) are fulfilled.

**Examples: Auer rods, overt AML by histology and immunohistochemistry; presence of AML-specific diagnostic cytogenetic and/or molecular markers (e.g., inv16).

***Typical cytogenetic abnormalities found in CMML (Supplementary Table S6).

****Leukemic infiltration of $CD14^+$ monocytes and exclusion of AML.

*****Utilizing a cutoff value of >94% MO1 monocytes, phenotyping can identify CMML cases with a sensitivity of >90% and a specificity of >95%, and the decrease in MO3 monocytes is even as diagnostic as the increase in circulating MO1 cells.[122,124,126]

******Genes that are often mutated in the CMML/MDS context include, among other, *TET2*, *SRSF2*, *ASXL1* and *SETBP1*. Minimal allele burden proposed to count as co-criterion: ≥10%.

Abbreviations: CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasm(s); MDS, myelodysplastic syndrome(s); FISH, fluorescence in situ hybridization.

Table 2

Overview of Special Variants of CMML

Special variant	Key diagnostic features that discriminate the variant from classical CMML
Oligomonocytic CMML	Absolute PB monocyte count $<1x10^9$/L
Systemic mastocytosis (SM) with concomitant CMML = SM-CMML	WHO criteria for SM fulfilled; in most patients CMML monocytes exhibit *KIT* D816V
CMML with a concomitant myeloid neoplasm* expressing a classical MPN-driver, such as *JAK2* V617F, *BCR-ABL1* or rearranged *PDGFRA/B*** or *FGFR1*.	WHO criteria for a classical MPN, such as CML**, PMF, or a myeloid neoplasm with rearranged *PDGFRA/B* are fulfilled in addition to the criteria of CMML.
CMML with expression of a molecular MPN-driver – examples: CMML with *JAK2* V617F or CMML with a rearranged *PDGFRA/B* or CMML with rearranged *FGFR1*.	Molecular drivers of classical MPN, such as JAK2 V617F**** or rearranged *PDGFRA/B**** are found but diagnostic criteria for such classical MPN are not fulfilled (only criteria for CMML are met)
CMML with a concomitant lymphoid/ lymphoproliferative neoplasm	WHO criteria for a lymphoid neoplasm are fulfilled

*These conditions have to be separated from MPN with concomitant monocytosis that does not fulfil the diagnostic criteria of CMML.

**Unlike in SM-CMML where monocytes display *KIT* D816V or CMML with rearranged *PDGFRA*, the CMML monocytes must not express *BCR-ABL1* in patients with CML plus CMML.

***Several different translocations and fusion genes involving *PDGFRA* or *PDGFRB* may be dected, such as the t(5;12) associated with the *TEL-PDGFRB* fusion gene.

****_JAK2_ V617F itself counts as a feature of MPN; therefore, detection of *JAK2* V617F can confirm the diagnosis CMML (as MPN/MDS overlap disease) when other signs of myeloproliferation are absent (e.g., no splenomegaly and no leukocytosis).

Abbreviations: CMML, chronic myelomonocytic leukemia; PB, peripheral blood; WHO, World health organization; CML chronic myeloid leukemia.

Table 3

Proposed Minimal Diagnostic Criteria for Oligomonocytic CMML*

A. Prerequisite Criteria (all must be fulfilled)

- Persistent (3 months) peripheral blood monocytosis 0.5-0.9x10^9/L and (plus) relative monocytosis of ≥10% of circulating peripheral blood leukoyctes

- Exclusion of *BCR-ABL1*+ leukemia, classical MPN and all other bone marrow neoplasms that could serve as a primary source of chronic persistent monocytosis

- Blast cell count <20% in peripheral blood and bone marrow smears and (plus) exclusion of all other histopathological, morphologic, molecular and cytogenetic features that count as evidence for the presence of acute myeloid leukemie (AML)**

B. Morphologic criterion = Dysplasia

- Dysplasia in at least 10% of all cells in one of the following lineages in the bone marrow smear: erythroid; neutrophilic; megakaryocytic

C. Co-Criteria (for patients fulfilling A but not B, and otherwise show typical clinical features of CMML such as splenomegaly)

- Typical chromosome abnormalities by conventional karyotyping or FISH***

- Abnormal findings in histologic and/or immunohistochemical studies of bone marrow biopsy sections supporting the diagnosis of CMML****

- Abnormal immunophenotype of bone marrow and blood cells by flow cytometry, with multiple CMML-associated phenotypic aberrancies indicating the presence of an abnormal/dysplastic population of monocytic (and other myeloid) cells*****

- Evidence of a clonal population of myeloid cells determined by molecular (sequencing) studies revealing CMML-related mutations******

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*The diagnosis of classical CMML can be established when all prerequisite criteria (´A´) and either morphologic dysplasia (´B´) or one or more of the co-criteria (´C´) are fulfilled.

**Examples: Auer rods, overt AML by histology and immunohistochemistry; presence of AML-specific diagnostic cytogenetic and/or molecular markers (e.g., inv16).

***Typical cytogenetic abnormalities found in CMML (Supplementary Table S6).

****Leukemic infiltration of $CD14^+$ monocytes and exclusion of AML.

*****Utilizing a cutoff value of >94% MO1 monocytes, phenotyping can identify CMML cases with a sensitivity of >90% and a specificity of >95%, and the decrease in MO3 monocytes is even as diagnostic as the increase in circulating MO1 cells.[122,124,126]

******Genes that are often mutated in the CMML/MDS context include, among other, *TET2*, *SRSF2*, *ASXL1* and *SETBP1*. Minimal allele burden proposed to count as co-criterion: ≥10%.

Abbreviations: CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasm(s); MDS, myelodysplastic syndrome(s); FISH, fluorescence in situ hybridization.

Table 4

Overview of Non-Clonal and Clonal Conditions that may precede CMML

	Pre-CMML conditions and comparison to classical CMML						
Feature	IMUS	ICUS	CCUS	CHIP/CHOP	CMUS	O-CMML	CMML
Absolute Monocytosis ($\geq 0.5x10^9$/L)	+	+/-	+/-	+/-	+	+	+
Substantial Monocytosis ($\geq 1x10^9$/L)	+/-	-	-	-	+/-	-	+
Relative Monocytosis (>10% of leukocytes)	+	-	-	-	+	+	+
Dysplasia*	-	-	-	-	-	+	+
Cytopenia(s)**	-	+	+	-	-	+/-	+/-
BM blasts	<5%	<5%	<5%	<5%	<5%	<20%	<20%
Flow abnormalities	-	-	+/-	+/-	-	++	++
Cytogenetic Abnormalit(y)ies	-***	-***	+/-	+/-	-***	++	++
Molecular Aberration/s****	-	-	+	+	+****	++	++

*At least 10% of all cells in a given lineage (erythroid, neutrophil, or platelet) are dysplastic.
**Persistent cytopenia(s) recorded over a time-period of at least 4 months.
***In a subset of cases, a small-sized clone is detectable by FISH.
****A molecular aberration is defined by CMML/MDS-related mutations and an allele burden of ≥2%. The working definition for pre-CMML conditions is also ≥2% allele burden, whereas the minimal allele burden to count as a co-criterion of CMML is 10%. In most patients with overt CMML, multiple gene mutations/aberrations are found.
Abbreviations: CMML, chronic myelomonocytic leukemia; IMUS, idiopathic monocytosis of unknown (undetermined) significance; ICUS, idiopathic cytopenia of undetermined significance; CCUS, clonal cytopenia of undetermined significance; CHIP, clonal hematopiesis of indeterminate potential; CCUS, clonal cytopenia of undetermined significance; CMUS, clonal monocytosis of unknown (undetermined) significance; O-CMML, oligomonocytic CMML; MDS, myelodysplastic syndrome; BM, bone marrow; FISH, fluorescence in situ hybridization.
****Here a CHIP-like mutation is detected – if more than one CHIP-like mutations are found the question is whether the final diagnoses changes to O-CMML.

Table 5

Classification of Blast Cells and Monocytes in Patients with CMML

Cell Type	Nuclear shape	Chromatin	Cytoplasm	Size relative to mature monocytes
Blast cells:				
Myeloblast	Round/oval	Fine with nucleoli	Basophilic, rare or no granules	Smaller
Monoblast	Round/oval	Delicate / lace-like, nucleoli	Basophilic, rare azurophilic granules	Large (20-30 µM)
Promonocyte	Convoluted/ indented*	Delicate / lace-like, nucleoli	Variably basophilic, variable azurophilic granules	Large
Monocytes:				
Abnormal/immature monocyte	Convoluted/ indented	More condensed, rare nucleoli	Intermediate basophilic**	Smaller
Mature monocyte	Lobulated/ indented	Condensed, no nucleoli	Grey or pinkish with occasional azuroophilic granules and vacuoles	=

*The most important delineating feature discriminating promonocytes from monoblasts.
**Less basophilic than promonocytes and more basophilic than mature monocytes.

Table 6

Commonly Mutated Genes Detectable in Patients with Classical CMML

Gene Name Abbreviation	Gene Class and function	Relative Frequency in CMML	Clinical Impact
ASXL1	Epigenetic regulation Histone modification	40%*	poor prognosis** CHIP/ARCH***
EZH2	Epigenetic regulation Histone modification	5%	
TET2	Epigenetic regulation DNA methylation	60%*	CHIP/ARCH***
DNMT3A	Epigenetic regulation DNA methylation	5%	poor prognosis** CHIP/ARCH***
IDH1	Epigenetic regulation	1%	drug target
IDH2	Epigenetic regulation	5-10%	drug target
CBL	Signaling	15%	RAS pathway
NRAS	Signaling	15%	poor prognosis** RAS pathway
KRAS	Signaling	10%	RAS pathway
PTPN11	Signaling	5%	RAS pathway
FLT3	Signaling	<5%	AML-related drug target
SRSF2	Pre-mRNA splicing	50%*	
SF3B1	Pre-mRNA splicing	5-10%	
U2AF1	Pre-mRNA splicing	5-10%	
ZRSR2	Pre-mRNA splicing	5%	
RUNX1	Gene transcription	15%	poor prognosis** AML-related
SETBP1	Gene transcription	15%	poor prognosis**
TP53	DNA damage	1%	poor prognosis**
PHF6	Chromatin adaptor	5%	

*These mutations can be regarded as CMML-related mutations, but only *SRSF2* mutations do not, in addition, also count as classical CHIP/ARCH mutations. **Mutations in these genes are independent adverse prognostic factors concerning survival in CMML. ***These genes are frequently detected in individuals with clonal hematopoiesis of interminate potential (CHIP) also known as age-related clonal hematopoiesis (ARCH). Therefore, the diagnostic impact of these mutations may be regared as somehow lower compared to other (CMML-related and other) mutations. Abbreviations: CMML, chronic myelomonocytic leukemia; AML, acute myeloid leukemia.

Table 7

Phenotypic Classification of Monocytes and Distribution of Monocyte-Subsets in CMML and Controls*

Monocyte -Subset	Defining Phenotype	Typical Relative Frequency in* CMML	MDS or MPN	Reactive BM
Classical (MO1)	$CD14^{bright}/CD16^{-}$	≥94%	70-97%	<94%
Intermediate (MO2)	$CD14^{bright}/CD16^{+}$	<20%	5-20%	5-15%
Non-classical (MO3)	$CD14^{dim}/CD16^{+}$	<5%	5-10%	5-20%

*Data refer to published results presented in references #118 through #123.
Abbreviations: CMML, chronic myelomonocytic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; BM, bone marrow.