Supplementary Appendix to

Updated Diagnostic Criteria and Classification of Mast Cell Disorders: A Consensus Proposal

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Consensus Discussion and Development of Consensus Statements

The Year 2020 Working Conference on Mast Cell Disorders was organized in Vienna (Austria, Europe) from August 30 to September 1, 2020. The related consensus project with E-mail-based discussions and on-site in-depth discussions lasted from April 2020 to March 2021. The discussion phase was split into a pre-conference phase (E-mail-based and Web-based: April 2020 until August 2020), the conference, and a post-conference discussion phase (September 2020 to March 2021). Because of the corona pandemic, the Working Conference was organized as a hybrid (combined on-site and Web-based) meeting. The consensus discussion and the preparation of consensus statements were organized in accordance with available guidelines.[1]

In the final discussion round, the paper-draft was discussed and adjusted based on input provided by all faculty members as in our previous projects.[2] 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 were 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 faculty members who joined and actively contributed during the conference are included as co-authors on the final document.

Historical Overview: Criteria and Classification of Mastocytosis until 2020

In 1869, Nettleship and Tay described an atypical, pigmented form of an exanthema that exhibited urticarial wheals upon rubbing or scratching.[3,4] A few years later, the term urticaria pigmentosa (UP) was proposed. In 1887, Unna reported that the pigmented lesions of UP contain accumulations of mast cells (MC).[4] For many decades, mastocytosis was considered to be a disease limited to the skin. However, in 1949, Ellis reported a first patient with systemic mastocytosis (SM) involving internal organs.[5] Subsequent research and clinical observations provided evidence that there are two major forms of mastocytosis, namely cutaneous mastocytosis (CM) limited to

the skin, and SM where internal organs are always affected and the skin may or may not be involved.[4] Patients with CM were mostly classified as UP and found to have an excellent prognosis.[6] Later, a localized form of the disease in the skin (mastocytoma) was also described. Contrasting pure CM, patients with SM were found to have a less favorable prognosis, especially when the disease progresses and hematological problems occur.[4,7-9] In addition, a leukemic variant of SM was identified and called MC leukemia (MCL).[7-10] Moreover, patients with SM were found to develop associated hematologic (mostly myeloid) malignancies in a considerable number of cases.[7,8,11,12] Based on these observations, a first classification of mastocytosis was proposed by the Kiel group led by Karl Lennert in 1979.[7,8] Later, a first consensus classification of mastocytosis was proposed by Dean Metcalfe.[9]

After many years, the basic classification of mastocytosis is still valid. However, in the past 30 years, a number of markers and features with prognostic relevance have been identified.[13-21] Between 1990 and 2000, several of these parameters (with obvious diagnostic and/or prognostic impact) were tested in a series of multi-center studies to prepare and formulate robust diagnostic criteria.[13-22] These parameters and criteria were discussed in the Year 2000 Working Conference on Mastocytosis.[23] The resulting consensus criteria and the related (updated) classification proposed by our EU/US consensus group were adopted by the World Health Organization (WHO) in 2001.[23,24] This WHO classification and the related criteria were further up-dated and re-confirmed in 2008 and 2017.[4,25-27] Supplementary Table S1 shows the current WHO classification of mastocytosis and its prognostic impact.

To assist the WHO, the EU/US consensus group organized additional Working Conferences in 2005[28], 2010[2], 2012[29], 2015[4,27] and 2020 (Supplementary Table S2). Moreover, both in the EU and in the US, competence networks have been established. In 2002, the European Competence Network on Mastocytosis (ECNM) was inaugurated[30] and in 2019 the American Initiative in Mast Cell Disorders (AIM) was established.[31] Both networks are interconnected in various collaborations, joint efforts, and meeting series, and both have the aim to improve patient management, to foster research, and to support the development of diagnostic criteria and standards in MC disorders. In addition, both networks are closely collaborating with various patient

groups in the EU and in the US. In 2010, the EU/US consensus group also established criteria and a classification for MC activation syndromes (MCAS).[2] In addition, the consensus group proposed a global classification for all MC disorders.[2]

Involvement of Patients, Patient Groups, and their Representatives

In the Year 2010 Working Conference on Mast Cell Disorders, our consensus group invited patients and their representatives (patient organizations and self-support groups from the EU and the US) to support the consensus group by developing a priority-list of 10 most urgent and important open issues, needs, questions and suggestions (collectively termed top 10 issues herein) in the field of mastocytosis to the scientific community.[2] In the current project, we were following the same strategy and again involved patients, patient groups and their representatives. However, this time, a total of 12 countries and regions were invited to join (Supplementary Table S3). In addition, we asked for the top 10 issues to the scientific community in two fields, i) mastocytosis and ii) MCAS. The project was developed in a step-wise approach. In a first step, patient groups were invited to join and to prepare their top 10 points in mastocytosis and their top 10 points in MCAS (2 lists of top 10 issues) together with their patients and their local expert moderators. In a second step the patient representatives presented their 2 lists of top 10 issues in the Year 2020 Working Conference on Mastocytosis in Vienna in a separate Web-based session. In each country, the patient representatives were supported by one or two experts (expert moderators) who assisted the patients in the formulation and preparation of their 2 lists of top 10 issues. The patient representatives also had the opportunity to exchange their experiences, visions and thoughts with patient representatives from other countries. In a final step, the patient representatives revised and completed their 2 lists of top 10 issues and forwarded these points to the consensus group.

A summary of the top 10 issues raised by patients is provided later in this supplement. In addition, the patients extended their analyses and will publish a summary of all their issues, needs, questions, visions, and suggestions (not only the top 10) to the community in a separate joint publication. Our consensus group is thankful to all

patient group representatives and expert moderators who worked in this project and supported their patient groups in formulating their top priorities and top suggestions to the scientific community in this project.

Standards and Standardization of Markers, Assays and Evaluations

During the past 20 years, our EU/US consensus group has proposed diagnostic standards, assays, and algorithms for patients with suspected mastocytosis.[2,4,13-29,32-38] These standards and the WHO-based markers and criteria should be followed and applied in the daily practice of medicine whenever and wherever possible. During the current project, our consensus group reviewed and refined these standards, based on new developments in the field. These changes are presented and discussed below and in the main document of this consensus manuscript.

Evaluation of Skin Involvement: Recent Updates and Current Standards

When evaluating skin involvement, the general standards in diagnostic evaluations proposed by our consensus group and the WHO remain valid.[35,37,39] In particular, we are of the opinion that the Darier´s sign is an important diagnostic feature and thus a criterion of cutaneous involvement with mastocytosis even if, rarely, false-negative results may be obtained.[28,37,39] Our group also concluded that a standardized way (procedure) of performing the investigation would help to avoid a false-negative or false-positive Darier´s test results.

A skin biopsy is also regarded a standard of evaluation of skin involvement in mastocytosis, especially when the skin lesions are atypical.[28,37,39] Whereas an increase and accumulation of MC is often seen, the numbers of MC in lesional skin may also on occasion appear to be normal or only slightly elevated. Today, MC derived from lesional skin can also be examined for the presence of *KIT* mutations, including *KIT* D816V.[28,36,37] The presence of such a mutation confirms the diagnosis of mastocytosis in these patients, but does not support the diagnosis of SM. It is also standard to estimate or define the area of skin affected by mastocytosis (in percent of total skin

area) in each case.[28,37] Whereas the estimated percentage of involved skin provides valuable information, a machine-based (computer image-based) assessment would be preferable in the future.

The faculty also discussed updates in skin response evaluations in patients treated with cytoreductive agents, targeted drugs or with psoralen with ultraviolet light (PUVA). Whereas previously published standards[28,37,38] may still be helpful and valid, a more objective (computer-based) response evaluation would be preferable. The consensus group has the plan to publish updated evaluations of skin involvement in mastocytosis in a separate manuscript.

The diagnostic criteria used to diagnose and classify CM and CM sub-variants are discussed in the main document. Our faculty concludes that it is important to follow these criteria and to define the exact diagnosis in each case.[28,37] In children, it is important to be aware that no bone marrow biopsy is required to diagnose CM in most patients, and that the maculopapular form of CM (MPCM) in children can be divided into two distinct variants with distinct prognosis, namely the monomorphic form (with monomorphic small-sized lesions that usually persist into adulthood) and the polymorphic variant (with polymorphic smaller and larger lesions that usually disappear before adulthood) (Table 1 in main document).[37]

It is also important to know that most children are suffering from CM but not SM.[23-27] By contrast, in adulthood, most patients suffer from SM, whereas CM in adulthood is quite unusual.[4,23-27] However, when indeed diagnosed in adulthood, the prognosis of CM is excellent.

As per definition, CM in adults is diagnosed by excluding SM using staging investigations, including bone marrow studies and SM criteria (Table 1 in main document).[23-27] In adults with typical skin lesions who did not have a complete staging with bone marrow (BM) analyses, the provisional diagnosis is ´mastocytosis in the skin (MIS)´ (Supplementary Table S4).[2] In children, the diagnosis MIS does not apply unless i) serum tryptase levels exceed 100 ng/ml and/or ii) signs for a systemic hematologic disease are found in other investigations and iii) no BM studies were performed (Supplementary Table S4). Otherwise, the diagnosis in children is CM.

Pathology Report: Updated Standards

A detailed histologic, cytomorphologic, and immunophenotypic examination of a well-prepared BM trephine biopsy and BM aspirate remains an integral component of diagnostic evaluations in patients with known or suspected SM.[4,7,12,23-28,40,41] Good quality smears are stained with Wright-Giemsa, Giemsa or May Grunwald Giemsa (MGG), and are examined for the presence, morphology and numbers (percentage) of MC as well as other leukocytes.[21,23,28] The percentage of MC is determined in reasonable distance from any BM particles.[23,28] Morphologic assessment of a good quality BM smear is also mandatory in patients with (suspected) AHN. For example, it is of utmost importance to determine dysplastic features of BM cells and the percentage of blasts.[21,23,28]

For histologic and immune-histochemical assessments, a solid uncrushed BM cylinder of at least 1.5 cm in length is standard.[28,40,41] Recommended fixation and processing methods have been described elsewhere and should be followed.[12,19,40,41] Paraffin-embedded material is cut into thin sections and stained with standard stains, including a Giemsa stain (Wright-Giemsa or MGG) as well as antibodies against major MC-related and other hematologic determinants, including CD34, KIT, and tryptase as well as CD25 and other cell-specific antigens (Supplementary Table S5).[19,40-43]

In most patients, MC can be detected and enumerated in BM biopsy samples using tryptase and/or KIT as immunohistochemical markers.[19,40,41] Sometimes, some, or even most MC stain only weakly positive or are negative for tryptase. Therefore, KIT should always be added to the panel of MC-specific markers, especially when MC are immature as in advanced SM.[19,23,28,40] Since CD30 is now regarded as a novel SM criterion, we propose that the standard panel of markers to be used in (suspected) SM includes CD2 (optional), CD25 and CD30 (Supplementary Table S5).[4,44] In addition, other relevant cell types (numbers and expansion and morphologic features related to AHN) should also be examined by immunohistochemistry in each case.[40,41] The panel of markers applied should include CD34 (to detect and count blast cells), CD14 (monocytes), CD71 (erythroid cells), CD38 and/or CD138 (plasma cells), CD3 (T

cells), CD20 (B cells), and a megakaryocyte-related marker such as CD31, CD42b or CD61 (Supplementary Table S5).[28,40,41]

Our faculty also discussed the value of chymase (even more specific for MC than tryptase but not expressed on all MC types[45]) and of basophil-related markers, including 2D7 and BB1 (basogranulin). In most cases, there will be no need to apply these markers. However, in patients with suspected basophil-lineage involvement, chronic myeloid leukemia (CML), or basophilic leukemia, application of chymase and a basophil-related marker should be considered. Supplementary Table S5 shows a summary of immunohistochemical markers that can be applied in patients with known or suspected SM.

In extramedullary organs, such as the skin, gastrointestinal tract, spleen, lymph nodes or liver, immunohistochemical studies for MC and other cell types is also standard in patients with suspected SM (diagnostic screens) or known SM (staging and grading investigations).[28,23-26] In those with known SM, grading and staging investigations may lead to the conclusion that the patient is suffering from ASM or another form of advanced SM. In fact, the presence of a C-Finding is best confirmed by a thorough histologic and immunohistochemical investigation of the affected end organ.[28,23-26,33]

However, when applying immunohistochemistry, a few important aspects are to be considered. First, some of the markers may not be expressed in all neoplastic MC in a given organ section. For example, in the gastrointestinal (GI) tract, tryptase expression in MC is dim to negative in SM (contrasting KIT). Therefore, the application of both, tryptase and KIT in all biopsy sections and organs is essential. Another important aspect is that CD2 and CD25 (or even CD30) may not be easily applicable in these organs because of the presence of (activated) lymphocytes which may also display these antigens. Finally, depending on the organ system and tissue site, neoplastic MC usually express only trace amounts or no chymase.

With regard to AHN, most investigations have to be performed in the BM, but sometimes also the spleen and lymph nodes.[28,23-26] MC sarcoma or a MC sarcoma-like spread in advanced SM can often be detected in an extramedullary organ and should be documented in the pathology report. In the BM, the pathologist must also report on the presence and grade of fibrosis (reticulin stain), osteosclerosis, and thickening of

bony trabeculae as well as the myeloid-to-erythroid ratio and loss of fat cell content.[28,40,41] In addition, signs of myelodysplasia and/or myeloproliferation should be reported.[28,40,41] The final pathology report should also include a detailed description (or exclusion) of any type of AHN.[28] When detected, the AHN should be classified using WHO criteria. Finally, both the SM component and the AHN component of the disease can and should be sub-classified by the pathologist when sufficient clinical and lab-based information is available. When this is not the case, the pathology report should describe all relevant histopathological and immunohistochemical features and should relate findings to potential variants of the disease (e.g., ´… pattern would best fit with the diagnosis of ASM´). In this regard it should be emphasized that it is of critical importance that the pathologist receives adequate material from affected tissues, several unstained BM and blood smears, and all clinical and lab-based information required to define the final variant and diagnosis.[28]

Flow Cytometry: Novel Markers and Diagnostic Standards

A thorough flow cytometric evaluation of BM and blood leukocytes is an integral diagnostic approach and standard in patients with known or suspected mastocytosis.[20,46-49] In these patients, the diagnostic algorithms of the Spanish Network on Mastocytosis (REMA) should be followed.[46] These analyses comprise phenotypic studies of $KIT^+/CD34^-$ MC as well as examinations of other BM cells, including blast cells, neutrophils, and monocytes with the aim to exclude or define the presence and nature of an AHN, such as CMML or AML.[28,46-50] When performing flow cytometry on aspirated BM cells in patients with suspected (or known) SM, it is important that good quality samples with sufficient amounts of BM cells are collected, and blood cell contamination is avoided. Heparin or EDTA is usually recommended as anti-coagulant.[28,46] BM cells should be analyzed by flow cytometry within a reasonable time (<24 hours) and with reagents (antibodies) recommended for use in routine practice by the local institution or by (inter)national flow cytometry communities or societies.[46]

The minimal diagnostic panel to study the phenotype of $KIT^{+}/CD34^{-}$ MC in SM contexts includes CD2, CD25 and CD30.[20,46-50] In a first screening step, CD2 and CD30 can also be omitted. Other surface markers, such as CD35, CD59, CD63, CD64, CD69, CD88, or CD123 are also expressed abnormally or even aberrantly on MC in SM but are not included as minor SM criteria. As mentioned before, CD2 may be the most specific marker for MC in typical ISM but is not detected on MC in all patients (and usually not in advanced SM), whereas CD25 is the more sensitive marker.[28,46-49] It is of particular importance to know that MC in WDSM may lack both CD2 and CD25 but often display CD30.[49]

Another important point is that SM may or may not be accompanied by an AHN and that most AHN can be detected and graded using flow cytometry. Therefore, our group is of the opinion that flow cytometry should be applied with an expanded panel of markers in all patients with known or suspected SM in order to exclude or diagnose (and grade if possible) an AHN.[28,46] Depending on the type of AHN, the panels of markers to be used will vary according to the diagnostic algorithms used in each center as proposed for example by Euro Flow. A detailed description of all panels is beyond the scope of this article. We refer the reviewer to the available literature. However, we would like to point out that most AHN are myeloid neoplasms, whereas B cell neoplasms are very rare, and only a very few T cell lymphomas have been described in SM contexts.[23-27] Among the myeloid AHN in SM, chronic myelomonocytic leukemia (CMML) and AML are the most prevalent.[11,12,23-27] However, any other type of myeloid neoplasm may also develop, including myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). Because of this association, we also recommend that the antibody panels used for diagnostic evaluation and classification of myeloid neoplasms (e.g., AML, MDS and MDS/MPN) always include also the "CD117 and CD25 antibody combination" for exclusion or demonstration of the presence of SM-AHN.[46] In patients with SM-MPN, both *KIT* D816V and *JAK2* V617F may be detected. In each case, the percentage of blast cells should also be determined by morphologic studies and flow cytometry in order to define the prognosis and the variant of AHN.

Cytogenetic and Molecular Markers including *KIT* Mutations: Update 2021

In a substantial number of patients with advanced SM, cytogenetic abnormalities may be detected by conventional karyotyping and/or fluorescence in situ hybridization (FISH).[51-53] Especially in patients with an AHN, such chromosomal defects are often found. Usually, the AHN cells display these lesions and the cytogenetic anomalies detected reflect the type of AHN.[51-53] Overall, the presence of chromosomal defects in SM is of prognostic significance concerning survival and progression-free survival.[51-53] Therefore, we are of the opinion that karyotyping and FISH should be included in the panel of diagnostic markers to be applied in patients with SM.[4,27,28] Our faculty also discussed whether the presence of certain chromosomal anomalies can per se be diagnostic for an AHN. Whereas the chromosome defects alone may not be diagnostic, they can support the diagnosis of an AHN, depending on the number of involved metaphases (or interphases) and the type of anomaly. For example, when over 50% of all metaphases express an AML-related translocation such as t(8;21) and the patient has SM with an increase in blasts, the diagnosis is SM-AML. When neoplastic cells in a patient with SM and massive BM fibrosis express *JAK2* V617F, the diagnosis will be SM-AHN. In other patients with SM, the karyotype may not lead to the diagnosis of an AHN but may indicate the risk of a patient to develop SM-AHN. Thus, in all SM patients with obvious or suspected progression during follow-up, BM examinations should also include conventional karyotyping and FISH.[28]

Molecular studies in patients with SM include (high-sensitive) mutation analyses (PCR-based or sequencing-based) to detect or exclude *KIT* codon 816 mutations and mutations in other codons of *KIT* as well as next generation sequencing studies (NGS) covering most or all of the critical target genes (recommended screen: myeloid panel of the local referral laboratory) that may be affected in the context of SM and various types of AHN.[36,54-56] In both instances (PCR and NGS) the techniques applied need to have sufficient precision, specificity and sensitivity to be applied in daily practice. With regard to KIT D816V, PCR assays are commonly more sensitive than NGS. Highly sensitive PCR assays that work for detection of KIT D816V mRNA include digital droplet PCR (ddPCR) and allele-specific oligonucleotide-based PCR (ASO-

qPCR).[36,54-56] An important point is that contemporary NGS panels cover several or even most relevant *KIT* regions but do not reach the sensitivity of ASO-qPCR or digital PCR to detect or exclude the presence of *KIT* D816V at low variant allele frequency (VAF).

During the past few years, a number of novel *KIT* mutations (apart from D816V) have been detected in patients with CM and SM (Supplementary Table S6).[36] Some of these mutations are detected commonly in pediatric cases with CM, and some are expressed in germline configuration.[36] An interesting aspect is that some of these ´atypical´ *KIT* mutations are found in advanced SM rather than in ISM. These *KIT* mutations may play a similar (or even more important) role in disease evolution as *KIT* D816V (Supplementary Table S6). Therefore, these mutations should now also serve as minor SM criterion, provided that their impact as oncogenic driver has been documented. Reporting of molecular abnormalities in SM should always include the type of mutation as well as the VAF of the mutant. This is of utmost importance as the VAF may be indicative of a certain diagnosis. For example, a *KIT* D816V burden of ≥10% in BM or blood leukocytes now counts as B-Finding (Table 4 in main document).

Mutations in other driver or passenger genes, are mostly detected in patients with advanced SM, especially in those with SM-AHN.[36,54-58] In these patients, AHN cells often display mutations in one or more of the following genes: *TET2*, *SRSF2*, *ASXL1*, *CBL*, *RUNX1*, and *RAS*.[54-58] In addition, fusion driver genes of myeloproliferation, such as *JAK2* V617F, may be detected in neoplastic (AHN) cells in these patients.[36] As mentioned, such driver mutations may lead to the conclusion that the patient is suffering from SM-AHN. These mutations may be co-expressed with *KIT* D816V in the same cells or may be expressed in other myeloid cells but not MC, especially in SM-AHN. Based on colony-assay studies, acquisition of *KIT* D816V may be a late(r) event – at least in a subset of patients with SM-AHN.[58] Overall, the type and number of lesions (mutations) detectable in patients with multi-mutated SM correlates with the clinical course and prognosis.

In patients with true MC sarcoma (MCS) no *KIT* mutations are found whereas in those with MCS-like ASM, *KIT* D816V or other *KIT* mutations may be detected. Criteria to diagnose MCS are depicted in Supplementary Table S7.

Impact of Genetic Variables: Hereditary Alpha Tryptasemia (HαT) and Beyond

Several of the previously mentioned driver and passenger mutations may be detected in the germline of patients.[36] So far it remains unknown whether the presence of such germline mutations predispose to the development of CM, SM or an AHN. Previous studies have suggested that mastocytosis may also be associated with certain variants (polymorphisms) of genes coding for certain cytokines or cytokine receptors.[59] Again, however, the clinical impact of these gene variants remains unknown as most studies were only conducted on samples from a limited number of patients.

More recently, duplications or higher replications of the *TPSAB1* gene encoding for alpha-tryptase have been described as a potential predisposing condition and trigger of severe hypersensitivity symptoms in the context of MC activation and allergic reactions.[60-63] Individuals found to have multiple copies of the alpha tryptase gene are designated as having Hereditary alpha Tryptasemia (HAT=HαT).[60-63] Carriers of this genetic constellation usually have elevated serum tryptase levels and a higher risk to develop mediator-related symptoms in the context of an allergy or mastocytosis or when they develop both mastocytosis and a concomitant allergic disease.[60-63] Moreover, the prevalence of HαT is higher in patients with SM (up to 20%) compared to the healthy population (around 5%).[63] It is also worth noting that many HαT carriers are asymptomatic even when they develop CM or SM. However, their risk to develop severe mediator-induced symptoms or even MCAS is obviously higher compared to CM or SM patients without HαT.[63]

Although the testing for *TPSAB1* copy numbers is not yet available in many centers, the HαT carrier status may have implications for the evaluation of SM criteria and B-Findings. In fact, the basal tryptase level may in part be influenced by HαT and may be substantially higher in those who have multiple extra copy numbers of the *TPSAB1* gene. Therefore, our faculty discussed how the basal serum tryptase levels obtained in patients with SM can be corrected for HαT. One suggested approach discussed in the conference was to correct for HαT by dividing the basal tryptase level by one plus the number of extra alpha tryptase gene-copies. For example, an individual with 3 beta

and 2 alpha tryptase alleles harbors one extra alpha allele in addition to canonical tryptase genotypes and is classified as HαT. If this individual with one extra copy of *TPSAB1* presents with 38 ng/ml basal tryptase, the minor SM criterion would not be fulfilled as the HαT-corrected basal tryptase level (38 divided by 2 = 19) is below 20 ng/ml. However, the question remains whether this approach may sometimes overcompensate tryptase levels in individual patients. Therefore, we believe that further validation studies are required to define the optimal correction-model for tryptase in carriers of HαT.

Diagnostic Algorithms in Daily Practice

In the past 2 decades, diagnostic algorithms for patients with suspected mastocytosis have been established by our consensus group.[2,4,22,27-29,32-35] These algorithms remain valid and should be followed in the daily practice of medicine. An important aspect is that the algorithms vary in different age groups and patients who have or do not have typical skin lesions. In almost all children, the diagnostic algorithm does not include BM investigations since a clinically relevant BM involvement is rarely found.[28] In fact, BM studies are only performed in childhood patients when signs and symptoms are indicative of the presence of advanced SM or another hematologic malignancy, or when the disease persists through adolescence and into adulthood.[28] By contrast, BM studies are included as an integral component of the diagnostic algorithm in adult patients with suspected or known mastocytosis. In fact, CM and SM can only be diagnosed in adults when cytological, histological, immunological, and molecular studies of BM cells have been performed.[2,23-28] When no BM studies are performed in an adult, the provisional diagnosis of mastocytosis in the skin (MIS) can be established.[2,23-28] The individual risk of a patient with MIS to have or to develop SM can be estimated using a recently established scoring system.[64] When no skin lesions are present in a patient with suspected SM, several laboratory screening tests, including the basal serum tryptase level and *KIT* D816V in peripheral blood leukocytes can be determined. In adults who have *KIT* D816V, a high tryptase level, or other signs of SM, a BM investigation should be performed.[2,23-28,65] When no BM

examination is performed, the risk of such a patient to have or develop SM can be estimated based on the REMA score.[65]

More recently the EU/US consensus group has also proposed diagnostic algorithms for patients with suspected mast cell activation syndromes (MCAS).[66] Since the value and robustness of these criteria have been well-documented in several validation studies, our faculty is of the opinion that this algorithm and the related consensus criteria should be applied in the evaluation of patients with suspected MCAS in daily practice.[66] There are also other diagnostic algorithms and criteria that have been discussed and proposed recently by others, but these criteria lack specificity and validation.[67] Therefore, in order to avoid misdiagnosis and delays in diagnoses, it is of importance to apply the consensus criteria of MCAS and to follow the diagnostic algorithm of the consensus group so that the correct final diagnosis is established in each case.

When applying diagnostic algorithms in daily practice, it is important to be aware of the fact that patients with CM or SM may also suffer from MCAS and/or may also be carriers of HαT. Thus, in some of the patients, several diagnostic algorithms have to be applied at the same time, with recognition that the clinical features may overlap in SM and MCAS.

Mast Cell Activation Syndromes and Related Disorders

As mentioned, the diagnostic criteria and classification of MCAS set by the EU/US consensus group should always be applied in each case in order to avoid misdiagnoses.[2,68-70] Supplementary Table S8 shows the diagnostic consensus criteria for MCAS and Supplementary Table S9 provides the classification of MCAS as proposed by the consensus group. In fact, MCAS is classified based on the presence of clonal mast cells or an underlying reactive disease such as an IgE-dependent allergy. In particular, MCAS is divided into i) primary (monoclonal) MCAS (=MMAS) where clonal MC (and usually SM or CM) are present, ii) secondary MCAS, where an allergic disease or another reactive condition is found, and iii) idiopathic MCAS where the criteria in Supplementary Table S8 are fulfilled but neither monoclonal MC nor

another underlying condition or disease is identified.[2,68-70] An important aspect is that MCAS can present as a mixed form where both, SM and an underlying IgE-dependent allergy have been diagnosed.[70] These patients are at high risk to develop fatal MCAS episodes.

Another important aspect is that in some patients a local mono-organ or chronic form of apparent MC activation may be detected. However, it is often difficult or impossible to demonstrate the impact of MC in such conditions, and in many instances, other cell types (but not MC) may be causative elicitors of clinical symptoms. In other words, the dilemma in these cases is that the terms "MC activation" or "MC involvement" are not really justified and may be misleading from a scientific point of view.[67-70]

Nevertheless, our group discussed where and when the term MC activation could still be appropriate and how these conditions and also the predisposing conditions could be incorporated in an updated global classification of MC pathologies and diseases. This updated global classification is shown in Supplementary Table S10. It includes MC hyperplasia, where increased numbers of MC are detected but neither an underlying clonal MC disease nor signs or symptoms of MC activation are found, mast cell neoplasms (including CM, SM and myelomastocytic leukemia), pathologies and conditions associated with MC activation, including various forms of MCAS, and conditions and pathologies predisposing to MCAS, such as IgE-dependent allergies, atopic diseases, and HαT. Our consensus group also related these conditions and pathologies to the recently established ICD codes (Supplementary Table S10). Finally, our group tried to define clinical features (criteria) specific for two ICD-based conditions, namely ´unspecified MC activation = MC activation, unspecified´ (D8940) and ´other MC activation disorders´ (D8949). The proposed features defining these conditions are shown in Supplementary Table S11. In both entities, criteria to diagnose MCAS are not met. ´Other MC activation disorders´ should exhibit features of a local or systemic MC activation, including biochemical evidence of MC involvement, but the full array of criteria qualifying for MCAS are not fulfilled. By contrast, in ´MC activation, unspecified´, the involvement of MC may not be demonstrable to relate to clinical findings with certainty. Thus, in these cases, some of the clinical or laboratory-based findings are indicative of the presence of MC activation, but the etiology

remains uncertain and some or most of the symptoms may be caused by mediators released by other cells, such as basophils or lymphocytes. These patients may be labeled with ´probably MC activation-related´ or ´non-confirmed MC activation´.
Based on the above-mentioned issues, our faculty is of the opinion that it is of utmost importance to exclude all other potential etiologies before establishing a (provisional) diagnosis of ´MC activation, unspecified´ or ´other MC activation disorder´ and before labeling a patient as suffering from ´MC activation´ or a MC activation disorder.[67-70]

Prognostication and Prognostic Scoring Systems

During the past few years, a number of new prognostic variables have been identified in patients with CM and SM and have been validated. Several of these validation studies have been performed using the data set of the registry of the European Competence Network on Mastocytosis (ECNM). In this data set, over 4000 patients with CM or SM were enrolled through 2020.[71] Novel markers predicting shorter overall and/or progression-free survival in SM include, among others, age, male gender, BM sclerosis, multi-lineage involvement with *KIT* D816V (determined by analyzing sorted BM or blood leukocyte fractions), organomegaly, and the presence of certain cytogenetic and/or molecular abnormalities.[51-58,72-76] Among molecular abnormalities, mutations in the S/A/R panel of target genes (*SRSF2*/*ASXL1*/*RUNX1*) and the *KIT* D816V allele burden in the BM appear to confer a particularly poor prognosis.[55-58] Based on the prognostic impact of individual prognostic factors, several new prognostic scoring systems have been established for patients with SM, including the Mayo Clinic score[72], the International Prognostic Scoring System for Mastocytosis (IPSM)[73], the Molecular Adjusted Risk Score for advanced mastocytosis (MARS)[74], the REMA score[75,76], and the Global Prognostic Score for mastocytosis (GPSM).[77]
All these scores may assist in prognostication of patients with SM, and we recommend their use in daily practice. However, our faculty also recommends that additional, individual factors with clear prognostic impact should also be considered in the management plan in each case, following the principles of personalized medicine. This is of particular importance in patients with certain co-morbidities and in those who are

exposed to certain drugs that can provoke side effects. Some of the markers may also be indicative of a response to certain drugs, such as KIT-targeting tyrosine kinase inhibitors (TKI). For example, in some patients with advanced SM, especially those with well-differentiated MC, neoplastic cells may display KIT mutant forms that are responsive to imatinib.[36]

Quality of Life (QOL) and QOL Assessment

In all variants of CM and SM, the quality of life (QOL) may be impaired, even substantially, by the underlying disease. This is due to the cosmetic, social and psychological consequences of the disease as well as to the impact of MC infiltration (B- and C-Findings) and the effects of various MC-derived mediators. Therefore, we believe that measurement of QOL before, during, and after therapy is an important component in the management plan in all patients.[38] Our faculty also concludes that standard assessment procedures, including symptom assessment forms (SAF) should be applied both in daily practice and in clinical trials. A number of these approaches have been developed and have been tested in patients with different forms of mastocytosis, such as the Mastocytosis Quality of Life Questionnaires (MC-QoL) in ISM, and the 12-Item Short-Form Health Survey (SF-12), the Memorial Symptom Assessment Scale (MSAS), and the Advanced SM SAF (AdvSM-SAF) in advanced SM.[78-81] These tools should be further developed and should be applied whenever and wherever possible in clinical trials and in daily practice. For example, during effective therapy, these scores can document the beneficial effects of therapy on QOL in patients with various categories of mastocytosis.[80]

Patients´ Perspectives and Views: Outcomes from the Patients´ Project

Patient groups and their representatives from 12 countries and regions worldwide prepared major issues, concerns, wishes, and recommendations (collectively termed issues in this document) to the scientific community. The patient representatives were supported by local expert moderators (experts in their countries) who assisted in the

formulation and development of these issues. The patients and patient representatives were also asked to prioritize their concerns and issues and to summarize the top 10 points in mastocytosis and the top 10 points in MCAS. During the Year 2020 Working Conference, the patient representatives presented these top 10 issues to the community and to other patient groups. Then, the patient representatives refined their 2 lists of top 10 issues and forwarded these summary lists to our consensus group. In a final step, we extracted the most important top 10 issues and concerns for mastocytosis and MCAS by selecting the most frequently mentioned and top listed issues from all countries. This summary-extract of top 10 concerns and issues is shown in Supplementary Table S11 (mastocytosis) and Supplementary Table S12 (MCAS).

Among the top issues in both mastocytosis and MCAS were ´better education, increased awareness, and better knowledge of physicians, better (easier) access to specialized centers and effective drugs, and development of new more effective agents against mastocytosis and/or MCAS.

One remarkable aspect in this study was that the concerns and issues raised were similar in general, but prioritization revealed some country/region-specific needs and concerns which may be of importance when considering national or regional efforts (versus global efforts) to address these concerns. A more detailed description of all these (not only top 10) concerns, issues, thoughts, and recommendations of patients to the scientific communities will be presented in a separate publication prepared by patient representatives and their advisors.

Therapeutic Options for Patients with CM and ISM

A comprehensive review of treatment options in CM and ISM is beyond the scope of this article. With regard to specific drugs, therapeutic algorithms, and new developments in the field we refer the reader to the available literature.[4,27,33,38,82-88] The same holds true for patients with MCAS and related disorders.[38,68-70] In patients with CM and ISM, prophylactic therapies usually consist of mediator-targeting drugs, including histamine receptor (HR) antagonists.[4,27,38,85] In addition, all patients are advised to avoid all potential and known triggers and elicitors of hypersensitivity

reactions. In addition, adult patients and severely affected pediatric patients (their parents) are advised to carry epinephrine auto-injectors for emergency situations.

In patients with mediator-related symptoms, HR blocker (combinations of HR1 and HR2 blocker) are also recommended in a first step.[4,27,38,85] In patients with marked or severe symptoms despite anti-HR therapy, additional drugs, such as proton pump inhibitors (GI tract symptoms), glucocorticosteroids, cromolyn sodium, ketotifen, or leukotriene antagonists, may be applied.[4,27,38,82-85] The type of drugs are selected based on the organ systems involved, age, severity of symptoms and the underlying etiology.[82-85] Sometimes, the use of aspirin is recommended, with recognition that dose-related side effects may be an issue. Some of the patients with SM are suffering from severe bee or wasp venom allergy. In these patients, specific immunotherapy should be administered life-long to ensure protection.[38] If immunotherapy is not effective or not possible, (additional) anti-IgE treatment with omalizumab or other experimental therapies should be considered.[86,87] More recently, clinical trials examining the efficacy of KIT-targeting TKI such as avapritinib, in ISM patients with mediator-related symptoms have been initiated.

Another clinical challenge in SM is osteoporosis. In all patients with SM in whom the T score arrives at -2, bisphosphonates should be initiated if possible.[38] In bisphosphonate-resistant cases, RANKL-inhibitors or IFN-alpha may be considered.[38]

Treatment Options in Patients with Advanced SM

Over the past 20 years, a number of treatment options for patients with advanced SM, including ASM, SM-AHN and MCL have been developed. The detailed treatment plan depends on the variant of disease, molecular markers and target expression profiles, and patient-related variables, including age, co-morbidities and fitness. A detailed review of all treatment options is beyond the scope of this article. We refer the interested reader to the available literature.[80,89-100] In previous decades, patients with advanced SM and slow progression were often treated with prednisolone, IFN-alpha, or hydroxyurea.[33,82,89-93] Later, cladribine was considered a standard of treatment for patients with advanced SM.[33,89,93] In rare cases in whom neoplastic cells were found to

display wild type KIT or imatinib-sensitive KIT-mutant forms, imatinib was often found to be efficacious.[33,95] In these patients, imatinib is still considered a reasonable treatment option. However, most patients with ASM, SM-AHN and MCL present with *KIT* D816V+ disease. In these patients, the current standards of therapy are KIT D816V-targeting drugs (such as midostaurin or avapritinib), chemotherapy, and allogeneic hematopoietic stem cell transplantation.[96-99]

One advantage of midostaurin is that this drug blocks not only the growth of *KIT* D816V+ MC but also IgE-dependent mediator secretion.[98] Therefore, midostaurin is also efficacious in patients suffering from mediator-related symptoms and is thus able to rapidly improve the QOL in these patients.[80,98] However, not all patients respond to midostaurin or they relapse after some time.[98,99] In these patients alternative KIT-targeting drugs such as avapritinib (recently approved by FDA for use in patients with advanced SM) or more intensive therapy must be considered. For patients with mulit-resistant advanced SM or rapidly progressing ASM/MCL, more intensive therapy may be required to keep the disease under control. One option is to offer poly-chemotherapy and hematopoietic stem cell transplantation (SCT).[4,27,33,96,99] The same holds true for patients with MC sarcoma (MCS) or MCS-like progression in ASM.

For patients with SM-AHN, separate treatment plans for the SM component and the AHN component have to be established.[4,27,33,38,82,99] The general recommendation is to treat the SM portion as if no AHN was diagnosed and the AHN as if no SM was found, with recognition that advanced AHN (for example AML) count as secondary AHN (e.g., sAML) and thus as a high-risk disease.[4,27] A future avenue of investigation may be to combine KIT TKI with AHN-targeting drugs. For example, in patients with ASM and concomitant high risk MDS, treatment with a KIT TKI (to treat ASM) and demethylating agents (for MDS) may an interesting and potentially useful approach.

Updated Response Criteria

In the past 20 years, response criteria for patients with CM, ISM and advanced SM have been developed and have been adjusted.[2,28,32] Whereas the initially proposed response criteria have been validated, with time it became clear that these criteria

require adaptations when used in clinical trials. These adaptations have been presented recently and are in general accepted as a global standard.[100] Still, however, there is an unmet need to further revise these criteria in order to offer appropriate response evaluations in all categories and sub-variants of mastocytosis and to delineate between responses of the SM and the AHN component of SM-AHN patients. These response criteria will be presented in a separate publication by the US/EU consensus group. There is also a need to update and revise the response criteria for patients with CM and ISM. In these updates, additional clinically relevant parameters, such as neurological, psychiatric, and mental aspects as well as the quality of life have to be addressed. In addition, a better evaluation of organ-specific measurements, such as a more accurate quantification of the involved skin surface area, need to be integrated in response evaluations. Our group will report on these new methods and the related computer and robot-based quantitative measurements in a separate position paper.

Supplementary Tables

Supplementary Table S1

WHO Classification of Mastocytosis and Prognostic Impact

Variant and Sub-Variant	Risk of Progression*	Risk of Anaphylaxis
Cutaneous Mastocytosis (CM)		
Maculopapular CM	Very Low**	Intermediate
Diffuse CM (DCM)	Very Low	High
Mastocytoma of Skin	Very Low	Low
Systemic Mastocytosis (SM)		
Bone Marrow Mastocytosis (BMM)***	Very Low	High
Indolent SM (ISM)	Low	Intermediate to High
Smoldering SM (SSM)	Intermediate	Intermediate
SM with an AHN (SM-AHN)	High	Low
Aggressive SM (ASM)	High	Low
Mast Cell Leukemia (MCL)	Intermediate	Low
Mast Cell Sarcoma (MCS)	Very High	Low

*Progression into a higher-grade mast cell neoplasm or from SM into SM-AHN.
**Although the risk of progression in CM is very low, a few patients may develop SM.
***In the latest update of the WHO classification, BMM is regarded as a provisional entity and subset of ISM. Abbreviations: WHO, World Health Organization; SM, systemic mastocytosis; AHN, associated hematologic neoplasm.

Supplementary Table S2

Overview of Working Conferences organized by the EU/US Consensus Group

Working Conference – Title	Location	Year	Consensus Report (Reference #)
Year 2000 Working Conference on Mastocytosis	Vienna	2000	23
Year 2005 Working Conference on Standards and Standardization in Mastocytosis	Vienna	2005	28
Year 2010 Working Conference on Mast Cell Disorders with Special Reference to Mast Cell Activation Syndromes (MCAS)	Vienna	2010	2
Mastocytosis Symposium and Consensus Meeting on the Classification and Diagnostic Criteria in Mastocytosis	Boston	2012	29
Paul Ehrlich Memorial Workshop on Mast Cells and Mastocytosis (Paul Ehrlich Meeting 2015)	Vienna	2015	4,27
Year 2020 Working Conference on Mast Cell Disorders and Related Conditions	Vienna	2020	-*

Consensus criteria and the consensus classification proposed by the working group were adopted by the World Health Organization (WHO) in 2001 and served as WHO criteria and WHO classification between 2001 and 2021. *Current consensus manuscript. Abbreviations: EU, Europe; US, United States of America.

Supplementary Table S3

Countries and Regions where Patients, Patient-Groups and their Representatives Prepared Top 10 Concerns and Issues in Mastocytosis and MCAS*

Participating Countries	Identifying number	Prepared Top 10 Issues in	
		Mastocytosis	MCAS
Australasia	i	+	+
Austria	ii	+	+
France	iii	+	+
Germany	iv	+	+
Italy	v	+	+
Mexico	vi	+	-**
Netherlands	vii	+	-**
Poland	viii	+	+
Romania	ix	+	+
Spain	x	+	+
UK	xi	+	+
USA	xii	+	+

*A total of 16 countries were invited and 12 of these invited countries joined in this project.
**In these countries, only the top 10 issues in mastocytosis were prepared for this publication.

Supplementary Table S4

Delineation between Mastocytosis in the Skin (MIS) and Cutaneous Mastocytosis (CM)

Variant	Discriminating Features / Criteria
Mastocytosis in the Skin in Adults (MIS)	Typical skin lesions Positive Darier´s sign* Positive skin histology (mastocytosis) (+/- *KIT* D816V in skin or peripheral blood) No bone marrow investigations yet performed No SM in other organs
Mastocytosis in the Skin in Children (MIS)	Typical skin lesions Positive Darier´s* sign or: Positive skin histology (mastocytosis) (+/- *KIT* mutations in skin or peripheral blood) Serum tryptase level ≥100 ng/ml or: Observable signs for systemic hematologic disease by other non-invasive examinations and: No bone marrow investigations yet performed
Cutaneous Mastocytosis in Adults (CM)	Typical skin lesions Positive Darier´s sign* or: Positive skin histology (mastocytosis) (+/- *KIT* D816V in skin or peripheral blood) Bone marrow studies exclude SM No SM in other organ biopsies
Cutaneous Mastocytosis in Children (CM)	Typical skin lesions Positive Darier´s sign* or: Positive skin histology (mastocytosis) (+/- *KIT* mutations in skin or peripheral blood) No signs of systemic hematologic disease by non-invasive examinations and: Serum tryptase level <100 ng/ml or: Bone marrow studies exclude SM

*The pathognomonic Darier´s sign is a key feature and criterion of cutaneous lesions in mastocytosis; it is defined by swelling and redness after stroking or rubbing of lesional skin in an affected individual. The sensitivity of the Darier´s sign is over 90%. Abbreviations: MIS, mastocytosis in the skin; CM, cutaneous mastocytosis; SM, systemic mastocytosis.

Supplementary Table S5

Immunohistochemical Markers recommended in Patients with SM

Marker	Cell Type(s)	Comments
Standard panel:		
Tryptase	Mast cells, basophils*	AML blasts* may also express some tryptase
Chymase	Mast cells	Not widely used, but mast cell-specific**
CD117 = KIT	Mast cells, myeloid and erythroid progenitors	Bright in mast cells; dim in progenitors and AML blasts
CD2***	Mast cells in SM	Also expressed in T cells and in a NK cell subset
CD25	Mast cells in SM	Also expressed in T and B cell subset
CD30	Mast cells in SM	Also expressed in a small subset of activated T cells
CD34	Blast cells, progenitors and endothelial cells	AML and ALL blasts usually express CD34
Extended panel:		
CD68, CD68R	Monocytes and mast cells	Mast cells in SM are also CD68-positive
Basogranulin (BB1)	Basophils, eosinophils****	Mast cells in SM may also react with antibodies directed against BB1 or 2D7
2D7	Basophils, eosinophils****	
CD14	Monocytes	
CD15	Neutrophils, eosinophils and monocytes	
CD3	T cells	
CD20	B cells	
CD38	Plasma cells	Progenitors may also display CD38
CD138	Plasma cells	
CD31	Megakaryocytes	Endothelial cells also express CD31
CD42b	Megakaryocytes	
CD61	Megakaryocytes	
CD71	Erythroid cells	
E-Cadherin	Erythroid precursor cells	

*Staining reactions in basophils and AML blasts are usually much weaker than staining reactions obtained with mast cells. **Mast cells in connective tissues in the skin and other organs also may display chymase. In SM, mast cells often lack chymase. ***CD2 is usually expressed weakly or is not detectable in mast cells in patients with SM. In advanced SM, mast cells usually stain negative for CD2. ****In the bone marrow, (reactive and neoplastic) eosinophils often react with antibodies against BB1 and 2D7. Abbreviations: SM, systemic mastocytosis; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia.

Supplementary Table S6

Mutations (Variants) of *KIT* Described in Patients with Mastocytosis

KIT Variant	Frequency in Adulthood Mastocytosis (mostly SM)	Frequency in Childhood Mastocytosis (mostly CM)
Y269C	< 3%	< 3%
E414D	< 3%	< 3%
Del417-419insF	**< 3%**	**< 3%**
Del417-419insI	**< 3%**	**< 3%**
Del417-419insNA	**< 3%**	**< 3%**
Del417-419insY	**< 3%**	**< 3%**
Del419	**< 3%**	**15-20%***
InsFF419	**< 3%**	**< 3%**
C443Y	< 3%	< 3%
S451C	< 3%	< 3%*
S476I	< 3%	< 3%
ITD501-502	**< 3%**	**< 3%**
501_502InsAF	**< 3%**	**< 3%**
ITD502-503	**< 3%**	**3-7%**
503_504insAY	**< 3%**	**< 3%**
ITD504	**< 3%**	**< 3%**
ITD505-508	**< 3%**	**< 3%**
K509I	**< 3%**	**< 3%***
Q515H	< 3%	< 3%
F522C	**< 3%**	**< 3%***
A533D	< 3%	< 3%*
V540L	< 3%	< 3%
K550N	< 3%	< 3%
W557R	**< 3%**	**< 3%**
V559A	**< 3%**	**< 3%***
V559I	**< 3%**	**< 3%**
Del559-560	**< 3%**	**< 3%***
V560G	**< 3%**	**< 3%**
Del564-576	< 3%	< 3%
D572A	< 3%	< 3%
L576P	< 3%	< 3%*
R634W	< 3%	< 3%*
K642E	**< 3%**	**< 3%**
V654A	**< 3%**	**< 3%**
L799F	**< 3%**	**< 3%**
InsV815-816	< 3%	< 3%
D816A	**< 3%**	**< 3%**
D816F	**< 3%**	**< 3%**
D816H	**< 3%**	**< 3%**
D816I	**< 3%**	**< 3%**
D816V	**> 80% (>90% for ISM)**	**20-30%**
D816Y	**< 3%**	**< 3%**
D816T	**<1%**	**<1%**
I817V	< 3%	< 3%

N819Y	< 3%	< 3%
D820G	**< 3%**	**< 3%**
N822I	**< 3%**	**< 3%***
N822K	**< 3%**	**< 3%**
N822Y	< 3%	< 3%*
M835K	< 3%	< 3%*
E839K	< 3%	< 3%
S840N	< 3%	< 3%
S849I	< 3%	< 3%*
E885D	< 3%	< 3%

All mutations (variants) of *KIT* described in the literature in the context of mastocytosis (identified in PubMed) are listed. Mutants highlighted **in bold** reportedly activate KIT in a ligand-independent manner and thus qualify as oncogenic variants and a minor SM criterion.
*These gene variants denote *KIT* mutations that have (also) been detected in germline configuration in familial cases, mostly in the context of CM. Abbreviations: CM, cutaneous mastocytosis; Dup, duplication; Ins, insertion; ISM, indolent systemic mastocytosis; ITD, internal tandem duplication; SM, systemic mastocytosis.

Supplementary Table S7

Definition and Criteria for Mast Cell Sarcoma (MCS), MCS-like Progression in Systemic Mastocytosis (SM), and Extracutaneous Mastocytoma

Variant	Abbreviation	Discriminating Features / Criteria
Mast Cell Sarcoma	MCS	Local mast cell tumor with immature atypical mast cells and aggressive (invasive) growth pattern CM and SM criteria not fulfilled (CM and SM/MCL excluded) High rate of recurrence/relapse Resistance to therapy
MCS-like Progression (in patients with SM)	-	Local mast cell tumor with immature atypical mast cells and aggressive (invasive) growth pattern SM criteria fulfilled - often: Prior diagnosis ASM or MCL established High rate of recurrence/relapse Resistance to therapy
Extracutaneous Mastocytoma	-	Local mast cell tumor with mature atypical or round mast cells Benign growth behavior CM and SM criteria not fulfilled (CM and SM excluded) Disease stable – no therapy required Progression or relapse very unusual Most have been detected in the lung

Abbreviations: CM, cutaneous mastocytosis; SM, systemic mastocytosis; MCL, mast cell leukemia; ASM, aggressive SM.

Supplementary Table S8

Consensus Criteria for Mast Cell Activation Syndrome (MCAS)*

A) Typical clinical signs of severe, recurrent (episodic) systemic mast cell activation are present (often in the form of anaphylaxis) (definition of systemic: involving at least two organ systems)

B) Involvement of MC is documented by biochemical studies: preferred marker: increase in serum tryptase level from the individual´s baseline to plus 20% + 2 ng/ml**

C) Response of symptoms to therapy with MC-stabilizing agents, drugs directed against MC mediator production or drugs blocking mediator release or effects of MC-derived mediators***

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*The consensus criteria for MCAS were first published in (27). All three MCAS criteria (A+B+C) must be fulfilled to call a condition MCAS.
**Other MC-derived markers of MC activation (histamine and histamine metabolites, PGD2 metabolites, LTC4 metabolites) have also been proposed but are less specific compared to tryptase.
***Example: histamine receptor blockers.
Abbreviations: MC, mast cells; PGD2, prostaglandin D2; LTC4, leukotriene C4.

Supplementary Table S9

Classification of Mast Cell Activation Syndromes (MCAS)

Variant of MCAS*	Main Diagnostic Features
Primary MCAS (Clonal MCAS)**	the *KIT* D816V mutation is detected*** and mast cells aberrantly display CD25 in most cases a) with confirmed mastocytosis (CM or SM)**** b) with only two minor SM criteria****
Secondary MCAS	an IgE-mediated allergy, another hypersensitivity reaction or another immunologic disease that can induce MCA and thus MCAS, is diagnosed, but no neoplastic MC or *KIT* D816V is found*****
Idiopathic MCAS	criteria to diagnose MCAS are met (see Supplemental Table S8) but no related reactive disease, no IgE-dependent allergy, and no neoplastic/clonal MC are found*****

*Proposing a hereditary variant of MCAS defined by the HαT carrier status was also discussed by the faculty. This variant would be defined by MCAS criteria, documented HαT, and absence of both an IgE-dependent allergy and clonal *KIT*-mutated MC. Some experts also proposed that familial clustering of symptoms (i.e., more than one family member affected by MCAS) would be another criterion for such a familial form of MCAS. An ´idiopathic´ form of MCAS would in turn require that HαT be excluded (or is not known) in such extended classification. However, our faculty concluded that more data and confirmatory results from patients suffering from MCAS and HαT are required to propose a hereditary variant of MCAS as an official entity at this time

**The terms clonal MCAS and monoclonal MCAS (=MMAS) can be used synonymously with the term primary MCAS.

***Rarely, other *KIT* mutations in exon 17 or other KIT-activating mutations are detected.

****Most of the patients suffer from CM or SM. However, in some cases, only two minor SM criteria are detected and criteria for SM and CM are not fulfilled.

*****No *KIT* mutation in codon 816 and no other KIT-activating *KIT* mutations is detected, and flow cytometry (if performed) will not detect a clonal population of CD25-positive MC.

Abbreviations: MC, mast cells; MCA, MC activation; CM, cutaneous mastocytosis; SM, systemic mastocytosis.

Supplementary Table S10

Mast Cell Disorders, Related Syndromes and Predisposing Conditions: ICD-10 Codes

Disorder/condition	Abbreviations	Related ICD-10 Code
Mast cell hyperplasia	-	-
Mastocytosis:		
Cutaneous mastocytosis	CM	D47.01
Childhood onset cutaneous mastocytosis	CM	Q82.20
Bone marrow mastocytosis	BMM	D47.02
Indolent systemic mastocytosis	ISM	D47.02
Smoldering systemic mastocytosis	SSM	D47.02
Aggressive systemic mastocytosis	ASM	C96.21
Systemic mastocytosis with an associated hematologic neoplasm	SM-AHN	D47.02 + code for AHN
Mast cell leukemia	MCL	C94.30
Mast cell sarcoma	MCS	C96.22
Mastocytoma NOS	-	D47.09
Mast cell activation-related disorders:		
Mast cell activation syndrome	MCAS	D89.40
Mast cell activation, unspecified*	MCA-NOS*	D89.40*
Monoclonal MCAS	MCAS-m	D89.41
Idiopathic MCAS	MCAS-i	D89.42
Secondary/reactive MCAS	MCAS-s/r	D89.43
Other mast cell activation disorder(s)*	-	D89.49*
Myelomastocytic leukemia	MML	-
Conditions predisposing to MCA:		
Hereditary alpha tryptasemia	HαT	D89.44
Atopic diseases	varia	varia
Hypersensitivity disorders (allergies)	varia	varia
Intolerance syndromes	varia	varia
Toxin exposure	varia	varia

*For these conditions, no validated criteria are available to date; an initial attempt and proposal to define features and criteria in these conditions is shown in Supplementary Table S11. However, it should be mentioned that these criteria should not be use in a global manner to replace MCAS as a diagnosis when MCAS criteria are not fulfilled. Rather in such individuals alternative diagnoses and etiologies must be considered.

Abbreviations: ICD, International Statistical Classification of Diseases and Related Health Problems; MCA, mast cell activation; NOS, not otherwise specified.

Supplementary Table S11

Features and Criteria of Mast Cell Activation-Related Conditions

Condition	ICD code	Proposed diagnostic features*
Mast cell activation, Unspecified*	03628 D89.40	1) Clinical and lab-based signs and symptoms of mast cell activation in one or more organs (e.g. histologic/flow evidence of mast cell activation and/or local or systemic elevation of a mast cell-derived mediator)** 2) Patients may or may not respond to drugs targeting mast cells or mast cell mediators 3) MCAS criteria are not fulfilled
Other mast cell activation disorder(s)*	03632 D89.49	1) Typical clinical symptoms (MCAS-like) affecting one or more end organ systems (with or without signs of anaphylaxis) 2) Event-related increase in a mast cell-specific mediator (tryptase, PGD2-met, histamine-met) in biological fluids – but below the MCAS thresholds* 3) Response of symptoms to drugs targeting mast cell activation, mast cell mediators, or mediator-effects (mediator-receptors) 4) Criteria to diagnose MCAS are not fulfilled

*No robust validated diagnostic criteria are available for these conditions to date. Unspecified should mean that the signs and symptoms of mast cell activation could not be confirmed as causal with certainty and that the possibility of involvement of other cells, such as basophils (an alternative potential source of tryptase, PGD2 and histamine, although typically in lower amounts than can be produced by mast cells), and lymphocytes, could not be eliminated.

**Indications of mast cell activation may be detected in histologic examinations, functional assays (CD63 or CD203c test utilizing mast cells), or biochemical assays (MCAS-like event-related increase in tryptase or other mast cell mediators), but also in clinical examinations (like in MCAS).

Abbreviations: ICD, International Statistical Classification of Diseases and Related Health Problems; MCAS, mast cell activation syndrome; PGD2, prostaglandin D2; PGD2-met, PGD2 and its metabolites; histamine-met, histamine metabolites.

Supplementary Table S12

Top 10 Concerns/Issues Raised in Mastocytosis Extracted from Reports of All Countries

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1. **Better education, increased awareness and better knowledge** about mastocytosis among general practitioners, specialists in various fields of medicine, and other health care providers

2. **More specialized centers** for mastocytosis patients, more knowledgeable doctors and other health care providers and improved collaboration between these health care providers and specialists

3. **Better and easier access to vital drugs**, new drugs, expensive drugs, and alternative medicines for all patients – and better access to clinical trials and compassionate use programs

4. Better **awareness and medical care of psychologic and neurologic symptoms** in mastocytosis

5. **Improved emergency algorithms and improved emergency care** for patients with mastocytosis

6. More formal and **better integration of mastocytosis in general health care systems**, including diagnosis-codes, recognition by insurances and approval by health care organizations

7. **Increased research efforts** to develop individualized targeted therapies and curative therapies for patients with **advanced mastocytosis**, including development of new targeted drugs

8. **More effective treatments** for patients with **mediator-related symptoms**, **constitutional symptoms**, and **organ-specific symptoms**

9. Improved **personalized treatment concepts** for patients with mastocytosis with special focus on possibilities to **increase the quality of life** in all patients

10. Development and validation of parameters to predict progression of mastocytosis in children and adults – **improved prognostication** in mastocytosis

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After having collected the top 10 concerns/wishes/issues/recommendations from patients in various countries, overall priorities were compared and listed to create a resulting overall top 10 master-list of top concerns/wishes/issues/recommendations from all countries. The top 10 concerns/wishes/ issues/recommendations from each individual country will be published in a separate manuscript.

Supplementary Table S13

Top 10 Concerns/Issues raised in MCAS Extracted from all Countries

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1. **Improved knowledge** about MCAS **of doctors** of various specialties and scientists

2. **More specialists** well trained to diagnose and treat MCAS – reducing the time between first symptoms and a correct diagnosis

3. Update and **improve diagnostic criteria** and the **classification** of MCAS and related disorders

4. More research to develop **new and better diagnostic tests for MCAS** patients and development of better diagnostic algorithms

5. **Better access to** existing **effective therapies** and development of more and better therapeutics to control MCAS – **more research** to develop anti-MCAS therapies

6. **Improved emergency care** and treatment of MCAS, and knowledge of (emergency) doctors about atypical forms of anaphylaxis and MCAS

7. Better access to **mental health support and psychological therapy** when/where needed

8. **Recognition of MCAS as a distinct disease** requiring more research and more clinical trials to improve outcomes

8. Generally accepted **guidelines for use of anti-mediator therapies** in patients with MCAS

9. **Therapeutic algorithms** for treatment of MCAS, including **generally accepted guidelines** for use of **anti-mediator therapies**

10. Establishing **more centers specialized on MCAS** and related disorders

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After having collected the top 10 concerns/wishes/issues/recommendations from patients in various countries, overall priorities were compared and listed to create a resulting overall top 10 master-list of top concerns/wishes/issues/recommendations from all countries. The top 10 concerns/wishes/ issues/recommendations from each individual country will be published in a separate manuscript.

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