

Proceedings from the Inaugural American Initiative in Mast Cell Diseases (AIM) Investigator Conference

Jason Gotlib, MD, MS,^a Tracy I. George, MD,^b Melody C. Carter, MD,^c K. Frank Austen, MD,^d Bruce Bochner, MD,^e Daniel F. Dwyer, PhD,^d Jonathan J. Lyons, MD,^c Matthew J. Hamilton, MD,^f Joseph Butterfield, MD,^g Patrizia Bonadonna, MD,^h Catherine Weiler, MD, PhD,^g Stephen J. Galli, MD,^{i,j} Lawrence B. Schwartz, MD,^k Hanneke Oude Elberink, MD, PhD,^l Anne Maitland, MD, PhD,^m Theoharis Theoharides, MD, MS, MPhil, PhD,ⁿ Celalettin Ustun, MD,^o Hans-Peter Horny, MD,^p Alberto Orfao, MD, PhD,^q Michael Deininger, MD, PhD,^r Deepti Radia, MBBS, MRCPI, FRCPath, MSc Med Ed,^s Mohamad Jawhar, MD,^t Hanneke Kluin-Nelemans, MD, PhD,^u Dean D. Metcalfe, MD, PhD,^c Michel Arock, PharmD, PhD,^v Wolfgang R. Sperr, MD,^{w,x} Peter Valent, MD,^{w,x} Mariana Castells, MD, PhD,^{y,z} and Cem Akin, MD, PhD^{aa}

Stanford, Calif; Salt Lake City, Utah; Bethesda, Md; Boston, Mass; Chicago, Ill; Rochester, Minn; Verona, Italy; Richmond, Va; Groningen, The Netherlands; New York, NY; Munich and Mannheim, Germany; Salamanca, Spain; London, United Kingdom; Paris, France; Vienna, Austria; and Ann Arbor, Mich

From ^athe Division of Hematology, Stanford University School of Medicine/Stanford Cancer Institute, Stanford; ^bthe Department of Pathology, University of Utah School of Medicine, Salt Lake City; ^cthe Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; ^dthe Department of Medicine, Division of Rheumatology, Immunology and Allergy, Brigham & Women's Hospital, Boston; ^ethe Department of Medicine, Division of Allergy and Immunology, Northwestern University Feinberg School of Medicine, Chicago; ^fthe Division of Gastroenterology, Hepatology and Endoscopy, Brigham and Women's Hospital, Harvard Medical School, Boston; ^gMayo Clinic, Division of Allergic Diseases, Rochester; ^hthe Allergy Unit, Verona University Hospital, Verona; Departments of ⁱPathology and ^jMicrobiology and Immunology, Stanford University School of Medicine, Stanford; ^kthe Department of Internal Medicine, Division of Rheumatology, Allergy and Immunology, Virginia Commonwealth University, Richmond; ^lInternal Medicine, Section Allergology, University Medical Center Groningen, University of Groningen, Groningen; ^mthe Department of Medicine, Icahn School of Medicine at Mount Sinai, New York; ⁿthe Department of Immunology, Tufts University School of Medicine, Boston; ^othe Division of Hematology, Oncology and Cellular Therapy, Department of Medicine, Rush University, Chicago; ^pthe Institute of Pathology, Ludwig-Maximilians-University, Munich; ^qServicio Central de Citometría, Centro de Investigación del Cáncer (IBMCC; CSIC/USAL), IBSAL, CIBERONC and Department of Medicine, University of Salamanca, Salamanca; ^rthe Division of Hematology and Hematologic Malignancies, The University of Utah, and Huntsman Cancer Institute, Salt Lake City; ^sthe Department of Clinical Haematology, Guys and St Thomas' NHS Hospitals, London; ^tUniversity Hospital Mannheim, Heidelberg University, Mannheim; ^uthe Department of Haematology, University Medical Center Groningen, University of Groningen, Groningen; ^vthe Laboratory of Haematology, Pitié-Salpêtrière Hospital, Paris; ^wthe Department of Internal Medicine I, Division of Hematology and Hemostaseology, and ^xLudwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Vienna; ^yBrigham and Women's Hospital, Division of Allergy and Clinical Immunology, and ^zHarvard Medical School, Boston; and ^{aa}the Division of Allergy and Immunology, University of Michigan, Ann Arbor.

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Disclosure of potential conflict of interest: J. Gotlib has served as a Chair of the Study Steering Committee (SSC) for the global trial of midostaurin in advanced systemic mastocytosis (advSM) (Novartis), Chair of the Response Adjudication Committee (RAC) for studies of avapritinib in advSM (Blueprint Medicines), and SSC co-chair for the phase II trial of ripretinib in advSM (Deciphera Pharmaceuticals); has received

funding for the conduct of these trials; and has received honoraria and reimbursement of travel expenses from Novartis, Blueprint Medicines, and Deciphera Pharmaceuticals. T.I. George has served on the SSC for clinical trials in SM for Novartis and Blueprint Medicines and has received consulting fees and reimbursement of travel expenses from Novartis and Blueprint Medicines. B. Bochner receives remuneration for serving on the Scientific Advisory Board of Allakos, Inc; owns stock in Allakos; receives publication-related royalty payments from Elsevier and UpToDate; is a coinventor on existing Siglec-8-related patents and thus may be entitled to a share of royalties received by Johns Hopkins University during development and potential sales of such products; and is also a cofounder of Allakos, which makes him subject to certain restrictions under University policy. The terms of this arrangement are being managed by Johns Hopkins University and Northwestern University in accordance with their conflict-of-interest policies. M. J. Hamilton serves on a Scientific Advisory Board for Allakos. L. B. Schwartz is a consultant for Deciphera Pharmaceuticals, Blueprint Medicines, Allakos, and Genentech; participated in clinical trials sponsored by Deciphera Pharmaceuticals and Blueprint Medicines; and, as inventor of the commercial tryptase assay, receives funds from VCU collected as royalties from Thermo Fisher. C. Ustun has served as a consultant and received honoraria from Incyte, Inc, and Jazz Pharmaceuticals. H.-P. Horny serves on a scientific advisory board and SSC for Blueprint Medicines and Deciphera Pharmaceuticals; and has served as a consultant and received honoraria from Blueprint Medicines, Deciphera Pharmaceuticals, and Novartis. A. Orfao has received consultancy honoraria from Novartis. M. Deininger is a paid consultant and/or member of the Scientific Advisory Board for Fusion Pharma, Takeda, Novartis, Incyte, Sangama, SPARC, Pfizer, and Dispersol; and serves on the SSC for the Optic (Takeda), EXPLORER (Blueprint), PATHFINDER (Blueprint), and BFORE (Pfizer) clinical trials. D. Radia received funding for the conduct of the global trial of midostaurin in advSM (Novartis), and for studies of avapritinib in advSM (Blueprint Medicines); and has also served on the RAC for studies of avapritinib in advSM (Blueprint Medicines). M. Jawhar received consultancy honoraria from Novartis and Blueprint, and research support from Novartis. H. Kluin-Nelemans received institutional support from Novartis. M. Arock has served as a consultant and received honoraria from Blueprint Medicines and Novartis and has received a research grant from Deciphera Pharmaceuticals. W. R. Sperr received honoraria from Thermo Fisher, AbbVie, Novartis, Pfizer, Incyte, Deciphera Pharmaceuticals, Jazz Pharmaceuticals, Teva, and Celgene. P. Valent received consultancy honoraria from Novartis, Incyte, Blueprint, Deciphera Pharmaceuticals, and Thermo Fisher, and research support from Novartis, Blueprint, and Deciphera Pharmaceuticals. M. Castells is a consultant for Blueprint Medicines and one of the PIs of the PIONEER clinical trial for indolent SM. C. Akin has received research support from Blueprint Medicines and has served as a consultant for Blueprint Medicines and Novartis, including SSC for clinical trials in SM. The rest of the authors declare that they have no relevant conflicts of interest.

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Corresponding author: Jason Gotlib, MD, MS, Stanford Cancer Institute, 875 Blake Wilbur Dr, Rm 2324, Stanford, CA 94305-6555. E-mail: jason.gotlib@stanford.edu. 0091-6749/\$36.00

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The American Initiative in Mast Cell Diseases (AIM) held its inaugural investigator conference at Stanford University School of Medicine in May 2019. The overarching goal of this meeting was to establish a Pan-American organization of physicians and scientists with multidisciplinary expertise in mast cell disease. To serve this unmet need, AIM envisions a network where basic, translational, and clinical researchers could establish collaborations with both academia and biopharma to support the development of new diagnostic methods, enhanced understanding of the biology of mast cells in human health and disease, and the testing of novel therapies. In these AIM proceedings, we highlight selected topics relevant to mast cell biology and provide updates regarding the recently described hereditary alpha-tryptasemia. In addition, we discuss the evaluation and treatment of mast cell activation (syndromes), allergy and anaphylaxis in mast cell disorders, and the clinical and biologic heterogeneity of the more indolent forms of mastocytosis. Because mast cell disorders are relatively rare, AIM hopes to achieve a coordination of scientific efforts not only in the Americas but also in Europe by collaborating with the well-established European Competence Network on Mastocytosis. (*J Allergy Clin Immunol* 2021;■■■:■■■-■■■.)

Key words: Systemic mastocytosis, siglec-8, mast cell activation syndrome, hereditary alpha-tryptasemia, anaphylaxis, European Competence Network on Mastocytosis (ECNM), American Initiative in Mast Cell Diseases (AIM)

The evaluation and treatment of patients with mast cell (MC) diseases is undertaken by a cadre of multidisciplinary subspecialists. However, there is no organization in the Americas dedicated to the gathering of specialists to share research findings relevant to this patient population. With the inaugural American Initiative in Mast Cell Diseases (AIM) investigator conference, we sought to launch AIM's mission of advancing research, education, and treatment concerning mastocytosis and related MC diseases. In this report from the AIM proceedings, we focus on selected presentations related to MC biology, hereditary alpha-tryptasemia (H α T), MC activation syndromes (MCASs), allergy and anaphylaxis in MC disorders, and the heterogeneity of skin and gastrointestinal (GI) manifestations in mastocytosis. Lastly, we highlight a roadmap for future collaborations with the European Competence Network on Mastocytosis (ECNM).

SPECIAL RECOGNITION OF DR K. FRANK AUSTEN

A lifetime achievement award was bestowed upon Harvard Emeritus Professor K. Frank Austen, who delivered the keynote lecture on the extrinsic and intrinsic diversity of the major MC subclasses. This work is presented in the next section, titled: "Characterization of MC Subset Heterogeneity." Dr Austen is a legend in the field of allergy/immunology and the most prominent MC researcher of the last century. He has dedicated his professional life to understanding the physiological and pathological role of MCs and their impact in disease. His contributions, reflected in more than 1000 peer-reviewed publications since 1955, have translated into the development of therapies that have impacted patients with asthma, anaphylaxis, and mastocytosis. He has also been a pioneer in understanding the complement and kinin systems in human disease. One of his major research

Abbreviations used

AIM:	American Initiative in Mast Cell Diseases
BM:	Bone marrow
BST:	Basal serum tryptase
CM:	Cutaneous mastocytosis
ECNM:	European Competence Network on Mastocytosis
GI:	Gastrointestinal
H α T:	Hereditary alpha-tryptasemia
HVA:	Hymenoptera venom allergy
ISM:	Indolent SM
MC:	Mast cell
MCAS:	MC activation syndrome
PG:	Prostaglandin
SM:	Systemic mastocytosis

contributions has been elucidation of the pathways of arachidonic acid metabolism to cysteinyl leukotrienes and prostanoids, and the regulation of the action of each of these mediators in allergic inflammation. In 2016, his team described the first transcriptional signature of MCs, observing a unique identity within the mouse immune system with a greater than expected heterogeneity across tissues. As a clinician, he provided the first detailed clinicopathological characterization of human anaphylaxis, and since 1991, he has led international collaborations focused on the classification of clonal MC disorders.

MC BIOLOGY

Characterization of MC subset heterogeneity

MCs can be classified into 2 distinct groups: innate and induced. Recognized MC progenitors in the mouse, for both innate and induced MC subclasses, express the integrin β 7, which is essential for their transendothelial migration from the blood into peripheral tissues. Innate MCs of adult mice arise in neonatal life from progenitors released in sequence from the extraembryonic yolk sac and then from the hemogenic endothelium, resulting in profound intrinsic regulation of peripheral phenotypes with minimal heterogeneity.^{1,2} A third wave of hemogenic endothelium-derived progenitors initially seeds the mucosa,² whereas adult bone marrow (BM) maintains mucosal populations and provides additional progenitors during a local organ-based T_H2 response.³ Adoptive transfer of a mouse BM-derived v-abl-immortalized MC line with limited protease expression showed expansion with immunodetection of 5 proteases for v-abl⁺ MCs in the liver, and shut down of all proteases in v-abl⁺ MCs in the small intestine.⁴ These mouse data suggest that BM-derived MCs have profound tissue-directed secretory protease heterogeneity. A subsequent study of the expansion of mouse BM-derived MCs in type 2 inflammation in the mouse gut with *Trichinella spiralis* infection and in the lung with ovalbumin sensitization and challenge allowed comparison with innate MCs. Five proteases were detected in innate MCs for both tissues, whereas the induced BM-derived MCs in the gut had only mMCP1, those in large bronchi mMCP1, mMCP6, and mMCP7, and those in the trachea additionally demonstrated mMCP4 and CPA.^{5,6} More recently, expression profiling of submucosal MCs from 5 mouse tissues and the peritoneal cavity revealed an MC transcriptional signature with minimal heterogeneity distinct from all other immune pathway cells,

including basophils.⁷ Thus, innate and induced mouse MCs are diverse with regard to their derivation from separate progenitors and their tissue-directed heterogeneity—which is minimal for innate and profound for induced. Innate are sentinel, whereas induced are “after the fact.” The key questions to resolve include (a) what are the functions of induced BM-derived MCs and (b) are amplified submucosal MCs in mouse models of inflammatory diseases BM-derived rather than an expansion of the innate? The implications for both questions relate to human target tissue MC expansion in bronchial asthma and rheumatoid synovitis.

As in mice, anatomically distinct compartments in human peripheral tissue contain MCs with distinct protease expression profiles, although humans have a greatly reduced protease profile relative to mice. MCs coexpressing tryptase and chymase in conjunction with CPA3 and Cathepsin G (MC_{TC}), thought to be analogous to innate murine MCs, can be found in the stromal compartment of most peripheral tissues. Supporting this concept, transcriptomics analysis identified considerable overlap between the resting transcriptome of murine constitutive skin MCs and human skin MC_{TC}.⁷ MCs that express tryptase with little to no chymase (MC_T), likely analogous to murine-induced MCs, reside in the epithelium of mucosal tissues. A BM-derived, circulating committed human MC progenitor has also been identified.⁸ Like its murine counterpart, this human MC progenitor expresses integrin b7 and is agranular.

Both MC_T and MC_{TC} expand during mucosal T_H2 inflammation, including asthma and nasal polyposis. However, the transcriptional programs underlying the heterogeneous MC subsets found in barrier tissues, the mechanisms underlying their expansion, and their relationship to the MC_T and MC_{TC} observed under homeostatic conditions are poorly understood. To assess this heterogeneity, Dwyer et al⁷ flow-sorted MCs on the basis of FcεR1α and CD117 expression from nasal polyps and profiled them using parallel single-cell RNA sequencing. They identified histochemically distinct stromal and intraepithelial MC subsets representing 2 distinctly polarized ends of a transcriptional gradient, with differential expression of proteases, cytokines, chemokines, and cell surface receptors. Through differential expression analysis and flow cytometric validation, they found that chymase-negative intraepithelial MCs are characterized by robust CD38 expression and low CD117 expression, whereas chymase-positive stromal MCs have high CD117 expression, lack CD38, and are highly transcriptionally distinct from MC_{TC} in the skin. A third subset was also identified, which coexpressed CD38 and CD117, and likely gives rise to both polarized MC subsets and contains a population of proliferating MCs. These findings thus suggest a common origin for the expanded human MC_T and MC_{TC} found within human mucosal tissue during T_H2 inflammation. Although murine studies suggest that the expanded MC populations observed during T_H2 responses arise from adult BM-derived progenitors,⁸ human MC progenitors have limited proliferative capacity. Thus, additional studies will be required to characterize the progenitor pool driving MC hyperplasia in nasal polyposis.

Siglec-8: MC biology and clinical targeting

Sialic acid-binding, immunoglobulin-like lectins (Siglecs) are single-pass transmembrane cell surface receptors found primarily on leukocytes that recognize different forms and conformations of sialic acid. Two natural ligands for Siglec-8 are known. Both involve the terminal sugar 6'-sulfated sialyl Lewis X on keratan

sulfate.⁹ In the lower airway, the keratan sulfate is displayed on the glycoprotein aggrecan; on the upper airway, it is displayed on a different glycoprotein, namely DMBT1. So far, the physiologic meaning of these natural ligands remains unknown.

Human MCs express several Siglecs, including CD22 (low levels), CD33, Siglec-5 (low levels), Siglec-6, Siglec-7, Siglec-8, and Siglec-10 (low levels) (Fig 1).^{10,11} Among these, Siglec-8, first discovered in 2000, is expressed not only on MCs but also on eosinophils. Surface expression of Siglec-8 is maintained on cells within tissues, including BM MCs in systemic mastocytosis (SM).¹² It is now known that Siglec-8 engagement, either by specific antibodies (via multivalent, specific α2,3-linked sialylated, sulfated artificial, or endogenous glycan ligands) or by sulphonamide sialoside analogues,^{13,14} can result in a number of effects *in vitro*. These include reduced eosinophil survival and attenuated MC secretion responses. The latter responses have been shown to be a result of downstream signaling events mediated via Siglec-8's intracellular immunoreceptor tyrosine-based inhibitory motif domain to reduce FcεRI-mediated degranulation and calcium flux responses.¹⁵ Siglec-8 also is internalized following ligation, which allows it to function as an endocytic receptor that can be exploited to selectively deliver therapeutic payloads into these cells.¹⁶

Because Siglec-8 is expressed only on human and primate cells and not in lower species, novel knock-in strains of mice have been generated in which Siglec-8 is transgenically expressed on eosinophils and/or MCs.^{17–20} Together with “humanized” mice, these preclinical models have enabled studies of Siglec-8 function on MC responses *in vivo*, revealing that anti-Siglec-8 antibody administration induces both protection from anaphylaxis²¹ and reductions in eosinophils and MCs in a model of eosinophilic gastroenteritis.¹⁹ A humanized nonfucosylated IgG₁ mAb, AK002, has entered clinical trials for the treatment of various diseases involving MCs and/or eosinophils including eosinophilic gastritis and/or gastroenteritis, antihistamine-resistant chronic urticaria, severe allergic conjunctivitis, and indolent SM (ISM).¹⁰

HEREDITARY α-TRYPTASEMIA

An elevated basal serum tryptase (BST) has been traditionally defined as a level of greater than 11.4 ng/mL, and it is seen in approximately 5% of people from Western populations.^{22,23} This clinical finding can be associated with mastocytosis and other myeloid neoplasms, such as acute myeloid leukemia (about 30%), chronic myeloid leukemia (CML: about 20%), and myeloproliferative neoplasms (especially those associated with eosinophilia related to fusion tyrosine kinases genes involving *PDGFRA*, *PDGFRB*, *FGFR1*, or *JAK2*).^{24–27} Elevated BST is now recognized to be more commonly caused by a genetic trait called HαT. HαT is so-named as it results from increased *TPSAB1* copy number encoding wild-type alpha-tryptase. The increased copy number of *TPSAB1* leads to its overexpression and elevated levels of protryptases—ostensibly composed predominantly of α-tryptase—in the peripheral blood.²⁸ In the small number of studies to date characterizing individuals with HαT, an association has been observed between increased BST and skin symptoms such as pruritus and urticaria, as well as with a constellation of comorbidities, which may variably include certain connective tissue phenotypes such as joint hypermobility, functional GI complaints including irritable bowel syndrome-like complaints and dyspepsia, and symptoms suggestive of

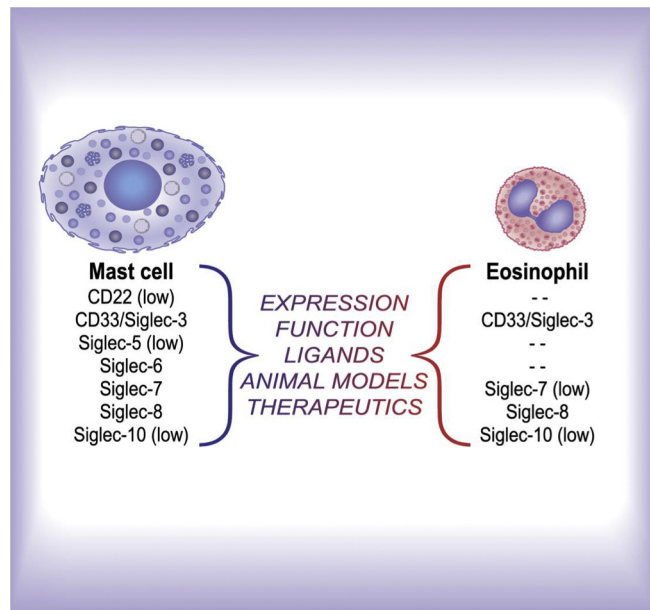


FIG 1. Siglecs on human MCs and eosinophils. Artwork by Jacqueline Schaffer. This online graphical abstract (<https://jlb.onlinelibrary.wiley.com/doi/10.1111/jlb.19383673/0/0>), which accompanies a recent publication,¹⁰ was reproduced with permission.

autonomic dysfunction.²⁹⁻³¹ Although these associations require further validation, several studies have demonstrated a similar link between elevated BST and some of these clinical phenotypes.^{22,32-38} More recently, the prevalence of H α T was found to be uniquely increased in patients with mastocytosis (especially ISM), and was associated with an increased risk of severe mediator symptoms/anaphylaxis in these individuals. It is unknown whether the number of germline copies of *TPSAB1* may confer a genetic predisposition for the later development of mastocytosis or whether alpha-tryptase expression level modulates MC homeostasis and neoplastic MC growth.^{39,40}

Variable expression of these clinical findings has been observed in H α T, with some individuals reporting few or none of the described symptoms. However, all individuals identified with H α T to date have been reported with BST of greater than or equal to 8 ng/mL (median BST in the general population is ~5 ng/mL), making this genetic trait fully penetrant. In the absence of severe acute systemic symptoms such as anaphylaxis, serum tryptase is composed of enzymatically inactive monomeric protryptases, irrespective of whether clonal MC disorders, myeloid disease, or H α T is the culprit.⁴¹ Unlike mature enzymatically active tryptases, which are released following MC degranulation, protryptases have no currently known biological function(s).⁴² Mature tryptases from subjects with H α T, as well as individuals producing *TPSAB1*-encoded α -tryptase, have recently been shown to include heterotetrameric $\alpha\beta$ -tryptases that increase with greater relative gene composition of α -tryptase.⁴³ Heterotetrameric tryptases, in contrast to α - or β -homotetramers, are able to cleave and directly activate protease-activated receptor-2, as well as augment activation of vibration-dependent EGF-like module-containing mucin-like hormone receptor-like 2.⁴³ These pathways have been associated with gut permeability *in vitro*, and an increased response to vibratory challenge *in vivo*, respectively. Thus, such heterotetramers may contribute to some of the symptoms reported

with H α T and is distinct from a severe form of familial vibratory urticaria caused by a gain-of-function variant in *Adhesion G protein-coupled receptor E2* encoding EGF-like module-containing mucin-like hormone receptor-like 2.⁴⁴ Whether a particular tryptase genotype may modify clonal or nonclonal MC-associated disorders and related clinical phenotypes is an area of ongoing investigation.

MC ACTIVATION, ALLERGY, AND ANAPHYLAXIS IN MC DISEASES

MC activation syndrome

Localized MC activation is common and is presumed to be necessary for normal homeostasis.⁴⁵ Pathologic MC activation can occur in the context of mastocytosis (clonal), with IgE and non-IgE-mediated triggers (secondary) or idiopathically (eg, idiopathic anaphylaxis). MCAS is a severe systemic manifestation of pathologic MC activation. Based on an initial proposal published in 2010,⁴⁶ the first international consensus diagnostic criteria for MCAS were developed in 2012⁴⁷ and updated in 2019.^{48,49} All 3 criteria (Table I) must be met to establish a diagnosis of MCAS, which is divided into 3 subtypes (Table II).

Serum tryptase, when drawn within 4 hours of symptoms, is the most specific mediator clinically available for testing to confirm MC activation. A formula of 20% of BST plus 2 ng/mL is suggested as a minimal increase to confirm MC activation in a patient with compatible symptoms.⁵⁰ In the absence of tryptase, urinary metabolites of MC activation products may also be measured (see below). The benefit or harm caused by measuring mediators that are not validated, such as serum heparin and chromogranin,⁵¹ also needs to be addressed. The literature regarding MCAS includes a multitude of conditions that do not fulfill the outlined criteria, which in turn leads to confusion and frustration among patients and practitioners because of the lack of a definitive diagnosis.^{49,52}

Utility of urinary MC mediators in the evaluation of SM and MCAS

Currently, selected clinical laboratories in the United States can determine urinary levels of the metabolites of 3 MC mediators: histamine, leukotriene (LT) C₄, and prostaglandin (PG) D₂. The metabolites measured are, respectively, N-methylhistamine, LTE₄, and 2,3 dinor-11 β PGF₂ α .⁵³ A urinary sample for the 3 metabolites can be collected by patients. Because results are expressed “per gram or milligram creatinine,” 24-hour urinary collections are no longer necessary. “Spot” urinary samples for these markers are now available at selected centers. Samples mailed to the laboratory by overnight express are stable for at least 7 days, if kept refrigerated.

Significant elevations in urinary 11 β -prostaglandin F₂ α and N-methylhistamine correlate with BM findings in MC disorders⁵⁴; the results can be used (1) to exclude MC-induced symptoms from syndromes with features that symptomatically can overlap (pheochromocytoma, VIPoma, and carcinoid syndromes); (2) to verify which mediators are chronically or acutely elevated and thereby allow targeting of treatment; (3) to confirm a diagnosis of suspected MCAS in a patient with low tryptase levels; and (4) to distinguish an MC “event” from a basophil “event” because, although both MC and basophils produce histamine, PGD₂ is a product of MCs and *not* basophils. The finding of an

TABLE I. Consensus criteria for MCAS*

Criterion A: Typical clinical signs of severe, recurrent (episodic) systemic MC activation are present (often in form of anaphylaxis) (definition of systemic: involving at least 2 organ systems)
Criterion B: Involvement of MCs is documented by biochemical studies: preferred marker: increase in serum tryptase level from the individual's baseline to plus 20% + 2 ng/mL†
Criterion 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‡

*The consensus criteria for MCAS were first published in Valent et al.⁴⁷ All 3 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, and heparin) have also been proposed, but are less specific compared with tryptase.

‡Example: histamine receptor blockers.

TABLE II. Recognized variants of MCAS and diagnostic features

Variant	Feature
Primary MCAS (clonal MCAS)*	The <i>KIT</i> D816V mutation is detected and MCs aberrantly display CD25 in most cases (a) with confirmed mastocytosis (CM or SM)† (b) with only 2 minor SM criteria
Secondary MCAS	An IgE-mediated allergy, another hypersensitivity reaction, or another immunologic disease that can induce MC activation, and thus MCAS, is diagnosed, but no neoplastic MC or <i>KIT</i> D816V is found‡
Idiopathic MCAS	Criteria to diagnose MCAS are met, but no related reactive disease, no IgE-dependent allergy, and no neoplastic/clonal MCs are found‡

From Valent et al.⁴⁹

*The terms clonal MCAS and monoclonal MCAS can be used synonymously with the term primary MCAS. Whether to add HsT as a form of primary, nonclonal MCAS is under consideration.

†Most of the patients suffer from CM or SM. However, in some cases, only 2 minor SM criteria are detected and criteria for SM and CM are not fulfilled.

‡No *KIT* mutation at codon 816 is detected, and flow cytometry (if performed) will not detect a clonal population of CD25⁺ MCs.

elevation of the urinary metabolite of PGD₂ supports the diagnosis of MC activation; the specificity of a mildly elevated PGD₂ metabolite as the only marker for MCAS has not been studied.

These assays have also shown that elevations of PGD₂ are much more common than elevations of histamine in MCAS.⁵⁵ This may explain the failure of antihistamines in a subset of these patients, whereas treatment with aspirin, an inhibitor of PG synthesis, may be effective in such cases. Available studies confirm increased excretion of leukotriene E₄ in SM when compared with a control population, especially in those patients with SM with active clinical symptoms.⁵⁶ The finding of increased excretion of all 3 urinary metabolites correlates with a diagnostic sensitivity for SM of 97% and specificity of 61%.⁵⁷ Although not formally included in the criteria for SM and MCAS, urinary MC mediator assays can improve diagnosis and target treatment in these disorders.⁵³

Antimediator therapies in clonal and nonclonal MC activation disorders

Symptoms of MC activation are common in clonal and nonclonal MC activation disorders and are derived from the effects of MC mediators systemically and in targeted tissues. Measurements of tryptase in serum, and urinary histamine, leukotrienes, and PGD₂ metabolites, have helped elucidate the clinical expression of MC activation disorders and response to antimediator therapies. The Brigham and Women's Hospital Mastocytosis Center database has captured more than 1500 patients, including more than 350 patients with nonclonal MC activation disorders, including MCAS.⁵⁸ Pediatric patients account for 109 patients, of which 17% presented with MCAS.

The association of nonclonal MCAS with postural orthostatic tachycardia syndrome, dysautonomia, or Ehlers-Danlos syndrome, findings also reported in patients with HsT,²⁸⁻³⁰ was found in 20% of MCAS cases, with most patients being female (82.5%), and close to 20% are younger than 30 years. MC mediator-related symptoms in such patients affect predominantly the skin (95%) and GI (90%), respiratory (60%), and skeletal (50%) systems, and can be constitutional (50%), neuropsychiatric (>30%), or systemic/anaphylactic (30%).⁵⁸

Avoidance of triggers is critical to reduce MC activation events. These triggers may include specific foods, medications ((nonsteroidal anti-inflammatory drugs in selected patients, vancomycin, quinolones), environmental allergens, and general triggers (temperature changes, lack of sleep, stress). Presence of such triggers provoking symptoms should be evaluated in each patient through a careful history and, when indicated, by food/drug testing and challenges.⁴⁵ Some events may be caused by IgE antibody-independent responsiveness of MCs to cationic substances, referred to as basic secretagogues. Binding of these molecules to the MRGPRX2 receptor on MCs can result in systemic pseudoallergic, or anaphylactoid reactions.⁵⁹

Premedications are generally recommended for radiological, endoscopic procedures and for invasive procedures with contrast dyes, surgery, dental work, and, in sensitive patients, before vaccinations. Antihistamine H₁ and H₂ blockers, leukotriene receptor antagonists, and in some cases, low-dose glucocorticoids are recommended on the basis of individual symptom profiles. Skin symptoms are targeted with antihistamine H₁ and H₂ blockers, ketotifen, leukotriene receptor antagonists, aspirin, and topical cromolyn sodium, which may have anecdotal benefit in cutaneous mastocytosis (CM) based on some of the authors' experience. GI symptoms are targeted with antihistamine H₂

blockers, proton pump inhibitors, sodium cromolyn, and ketotifen. Respiratory symptoms are treated with bronchodilators, nebulized cromolyn sodium, with and without glucocorticoids.

Severe hypotension and shock during systemic/anaphylactic events and which can occur in patients with MCAS are treated with injected epinephrine and patients are recommended to carry at least 2 epinephrine autoinjectors. Recurrent hypotensive events can be treated prophylactically with glucocorticoids with variable success. More recently, anecdotal evidence has shown that omalizumab may be effective in patients with MCAS alone or in mastocytosis with severe MCAS (associated with an IgE-dependent allergy).⁶⁰⁻⁶⁴ Neuropsychiatric symptoms have been treated with antihistamine H1 blockers, aspirin, anxiolytics, and cromolyn sodium; however, there are limited data regarding their efficacy for these symptoms, and the activity of these agents may reflect their ability to cross the blood-brain barrier and achieve pharmacologically active concentrations. Bone symptoms, including osteopenia, osteoporosis, and bone fractures, are treated with calcium, vitamin D, bisphosphonates, denosumab, and, if needed, IFN- α .^{65,66} Tyrosine kinase inhibitors currently are reserved for clonal MC activation syndromes, because there is no clinical evidence that activated TKs, including KIT, are involved in nonclonal MCAS. However, *in vitro* such inhibitors do attenuate MC activation. Future dissection of the biology of MC activation disorders, as well as the newly described H α T, will help identify disease-relevant therapeutics, such as antitrypsinase antibodies, which may have utility particularly in the latter condition.⁶⁷

Allergy and anaphylaxis in MC diseases

Patients with SM have an increased risk of anaphylaxis compared with the general population. Anaphylaxis occurs more often in patients with SM lacking cutaneous involvement and in those with atopic predisposition.⁶⁸ There is a strong correlation between mastocytosis and Hymenoptera allergy, and the most common IgE-mediated triggers for anaphylaxis in subjects with mastocytosis are Hymenoptera venoms from stings. Idiopathic anaphylaxis is second in frequency followed by pharmacologic agents and foods as triggers.⁶⁹⁻⁷¹ The prevalence of systemic reactions after Hymenoptera stings in patients with mastocytosis is significantly higher (20%-30%) compared with that in the general population (0.3%-8.9%); the prevalence of SM in patients with Hymenoptera venom allergy (HVA) (8%) is also higher than in the general population.⁷² Currently, there are no guidelines to support skin testing for Hymenoptera anaphylaxis in MCAS or mastocytosis. Patients with reactions are candidates for skin testing life-long immunotherapy. A subset of patients with Hymenoptera anaphylaxis has been found to have clonal MC disorders, either SM or monoclonal MCAS, but there is currently no evidence for an increased incidence of Hymenoptera anaphylaxis in patients with MCAS.

The initial association between HVA and SM was described in patients with urticaria pigmentosa, but more recently HVA has been described with increased frequency in patients with SM without skin involvement. Anaphylactic reactions in patients with SM typically present without angioedema or hives and instead with cardiovascular symptoms, such as hypotension and collapse. Patients may not report MC activation symptoms between acute episodes, and severe reactions to Hymenoptera venom may be the sole manifestation and presenting symptom of mastocytosis.

Among those presenting in this manner, BST is lower than median levels in SM generally, and can be normal. This is likely related to a lower MC burden, because these patients have fewer aggregates in the BM.

The prevalence of mastocytosis among patients with drug hypersensitivity is lower than in patients with HVA, but the severity of reactions, including anaphylaxis, is similar. Patients with SM with HVA and documented by skin testing or serum IgE testing should be treated with life-long venom immunotherapy, which confers increased protection in most (86%) resting patients.⁷³ Patients afflicted with SM who also have severe systemic reactions and anaphylaxis should carry at least 2 epinephrine autoinjectors. Omalizumab has been found to be safe and effective in preventing recurrent unprovoked anaphylaxis.^{60-63,74}

CLINICAL HETEROGENEITY OF MASTOCYTOSIS

Skin disease in pediatric and adult SM

Cutaneous lesions in mastocytosis are highly heterogeneous with localized and disseminated forms. CM is divided into 3 variants: (1) maculopapular CM, also known as urticaria pigmentosa; (2) diffuse CM; and (3) mastocytoma of the skin. The classification of CM has been based on macroscopic features of skin lesions, their distribution, or the onset of the disease.⁷⁵⁻⁸⁰ The typical maculopapular cutaneous lesions (urticaria pigmentosa) should be subdivided into 2 variants, namely a monomorphic variant with small maculopapular lesions, typically seen in adult patients, and a polymorphic variant with large irregularly shaped lesions, typically observed in pediatric patients (Table III).⁷⁵ Clinical observations suggest that the monomorphic variant, if it develops in children, often persists into adulthood, whereas the polymorphic variant usually resolves around adolescence. Overall, more than 80% of all patients with mastocytosis exhibit characteristic brown or red skin lesions.⁸⁰ The Darier's sign, defined by swelling and reddening upon mechanical stroking or rubbing of the lesions, is usually demonstrable.⁸¹

Mastocytomas and diffuse CM occur almost exclusively in the pediatric population. In adult-onset mastocytosis, cutaneous lesions are usually associated with SM, most often ISM.⁸²⁻⁸⁴ In contrast, in most pediatric patients, CM is found without histologically evident involvement of other organs.⁸⁵⁻⁸⁸ Patients with well-differentiated SM mainly have a pediatric-onset disease,⁸⁹ with persistent skin manifestations (polymorphic maculopapular CM and diffuse CM) typically seen in pediatric patients. These patients have increased MCs in the BM that lack the characteristic markers (CD2, CD25) of the clonal MCs frequently seen in ISM. The course of mastocytosis in adults is usually chronic, whereas children often show a self-limited course with spontaneous resolution around puberty.⁹⁰⁻⁹² Studies have shown that adult and pediatric patients differ with respect to mutations in the *KIT* gene.⁹³⁻⁹⁶ More than 80% of adult patients with mastocytosis carry the *KIT* D816V missense variant in exon 17, whereas patients with childhood-onset mastocytosis may not have detectable *KIT* mutations or express other *KIT* variants affecting exons 8, 9, 11, or 17.⁹⁵

Large polymorphic lesions in children generally have a favorable prognosis for disease resolution.⁹⁷ A recent report documented the similarities of CM in patients of different racial and ethnic groups. Although the lesions in these patients may demonstrate darker pigmentation, they are not so dissimilar that they would lead to a misdiagnosis.⁹⁸

TABLE III. Characteristics of skin lesions in adult vs childhood-onset mastocytosis⁷⁵

Characteristic	Adulthood-onset mastocytosis	Childhood-onset mastocytosis
Most frequent category of mastocytosis	ISM	CM
Typical course of the disease	Chronic	Temporary
Frequency of anaphylaxis (%)	50	<10
Typical tryptase level (μg/L)	>20	<20
Typical location of <i>KIT</i> mutation	Exon 17, most frequently <i>KIT</i> D816V	Exon 8, 9, 11, or 17 or absent
Most frequent type of cutaneous lesions	Maculopapular	Maculopapular
Typical morphology of maculopapular lesions	Monomorphic	Polymorphic
Typical size of maculopapular lesions	Small	Large
Typical distribution of maculopapular lesions	Thigh, trunk	Trunk, head, extremities

From Hartmann et al.⁷⁵

Therapy is targeted to avoid known triggers, such as physical and environmental insults, as well as patient-specific triggers, including foods, drugs, or venom. Additional management includes using local skin care with emollients, glucocorticoids, and antibiotic ointments when indicated, and psoralens and ultraviolet light on a limited basis in adults.

GI involvement in MC disease

The GI tract is a major compartment where MCs reside and are thought to carry out various functions such as homeostasis and pathogen defense.⁹⁹ However, MCs may become inappropriately activated in the setting of allergy, recent infection, inflammation, and as part of a primary MC disorder, sometimes with signs of MCAS. At the mucosal surface, MCs may affect the epithelial barrier and secretion, which may result in heartburn, diarrhea, and abdominal bloat and cramps. On the serosal side, MCs may interact with the autonomic nervous system and may contribute to abdominal pain and cramping, as well as alterations in GI motility.

The study of patients with MCAS may serve as a model to determine how MCs interact with other immune and stromal cells within the GI compartment in certain pathogenic states, such as in postinfectious irritable bowel syndrome. When MCs in patients with MCAS have been activated, the aforementioned GI symptoms may be associated with MC mediator-related symptoms in other organs, which may be worsened by predictable triggers. These symptoms are typically treatable with a combination of medications that block MC activation and/or MC mediators, dietary changes (eg, avoidance of dyes, chemicals, preservatives, and alcohol), and stress reduction.¹⁰⁰ Diagnostic studies, such as endoscopy and radiology examinations, are primarily used to exclude other GI disorders, because most patients with MCAS will have normal-appearing GI tracts and normal numbers and appearance of MCs as seen on histology. With some exceptions, the MCs in MCAS are dispersed throughout the lamina propria of the mucosal layer without clustering or reaching significantly elevated numbers. Indeed, the numbers of MCs observed in the small intestine and colon are not used to diagnose MCAS per current diagnostic guidelines.

Patients with ISM may present with similar GI symptoms with or without signs of MCAS, but endoscopy and biopsy may be used to detect the clonal MCs and diagnostic features may be identified, such as clustering or sheets of more than 15 MCs/hpf in the mucosa, typically just below the surface epithelium. Clonal MCs will usually stain positive for CD25.¹⁰¹ Patients with

smoldering SM or aggressive SM may experience more persistent diarrhea, associated with infiltration of clonal MCs beneath the epithelial surface, leading to malabsorption in cases of severe advanced SM. Oral budesonide or systemic glucocorticoids have been used to decrease the MC burden and to relieve diarrhea in this setting, and may also correct malabsorption; however, further cyto-reduction is usually required for GI-related organ damage in advanced SM. Further studies are needed to phenotype the populations of MCs in the GI tract of patients with various MC disorders to personalize treatments.

LESSONS LEARNED FROM THE ECNM EXPERIENCE: A ROADMAP FOR AIM

The ECNM was established as a multidisciplinary cooperative initiative in 2002.^{102,103} During the last 18 years, the ECNM contributed to the development of new markers, definitions, and standards in the field of mastocytosis, and has supported the World Health Organization and the international community in establishing classifications of MC disorders.¹⁰⁴⁻¹⁰⁸ Members of the ECNM organized annual meetings, as well as several working conferences and workshops.¹⁰³ In addition, the ECNM supported the development and conduct of interventional and observational cohort studies. The ECNM structure consists of reference centers, which typically have a specific unitary focus on MC disorders (eg, pathology or allergy/immunology), and centers of excellence, which have multidisciplinary expertise in MC disorders. The major strategic goals of the ECNM are aligned with, and provide a roadmap for AIM. These are (1) to provide new essential information about the disease to patients and physicians and (2) to encourage academic and biopharma collaborations to develop and implement tools for prognostication and treatment of various MC disorders.^{102,103}

A series of multicenter studies on diagnostic criteria for mastocytosis published between 1990 and 2000 served as the basis for the 2001 World Health Organization classification.^{104,108} The prognostic significance of the diagnostic criteria and of the World Health Organization classification was confirmed in several different validation studies.^{83,106} In addition, a number of new potential prognostic markers were identified.¹⁰¹ However, because of the rarity of advanced SM subtypes, the study cohorts were too small to reach definitive conclusions. To address open issues in these rare and complex diseases, the ECNM initiated a multicenter registry, with the aim to (1) create a web-based collection of data from patients with mastocytosis that is regularly updated by the participants; (2) perform prospective evaluations of

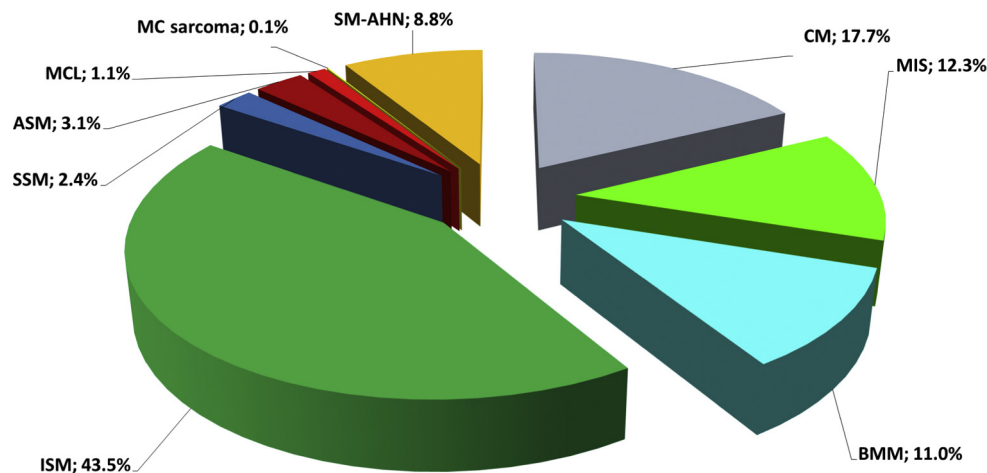


FIG 2. Distribution of mastocytosis subtypes from the ECNM Registry. The total number of patients included in the graph is 2361 from 23 centers. The date of data cutoff is November 2017. Permission from Dr Wolfgang Sperr and members of the ECNM who contribute to the registry. *ASM*, Aggressive SM; *BMM*, BM mastocytosis; *MIS*, mastocytosis in the skin; *MCL*, MC leukemia; *SM-AHN*, SM with an associated hematologic neoplasm; *SSM*, smoldering SM.

prognostic factors in patients with mastocytosis; (3) analyze the course of disease and treatment responses in different patient cohorts; and (4) establish multiparametric scores in mastocytosis.

ECNM registry projects are approved by local ethics committees of participating centers. The first patients were enrolled in 2011, and by 2019, 32 centers from 15 European countries and 1 US center (Stanford, Calif) had joined the registry and accrued 3830 patients with more than 8000 follow-up examinations. Moreover, data on symptomatic and cytoreductive therapies have been captured. Since 2015, 29 ECNM registry projects have been initiated in various centers. Four of these projects have been published.¹⁰⁹⁻¹¹² Fig 2 highlights the distribution of mastocytosis subtypes comprising the registry.

To address new developments in the field, several patient- and disease-related parameters, including comorbidities and genetic data, are included in the registry. Moreover, the ECNM consortium has a plan to link the ECNM registry data with a robust biobank system. Overall, the analyses and projects arising on the basis of the ECNM registry should improve prognostication and individualized management of patients with mastocytosis. A major goal will be to merge ECNM and AIM efforts to establish and maintain a common ECNM/AIM registry. In turn, observations gleaned from these combined efforts will further inform opportunities for interorganizational collaborations as well as for productive engagement with patient-centered groups.

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