

Mastocytosis: Advances in Diagnosis and Treatment

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Mastocytosis is characterized by pathologic mast cell accumulation and activation in tissues. Establishment of objective histopathologic and molecular criteria for diagnosis of mastocytosis has allowed sensitive detection of mast cells with aberrant features in patients presenting with suspected mast cell activation symptoms. Frequent detection of the D816V *c-kit* tyrosine kinase mutation in mastocytosis has led to evaluation of small-molecular-weight tyrosine kinase inhibitors as mast cell cytoreductive agents. In vitro experiments, however, showed that mast cells carrying the D816V *c-kit* mutation were resistant to the prototypical tyrosine kinase inhibitor imatinib. Efficacy of newer generation tyrosine inhibitors in mast cell disease is currently being evaluated.

Introduction

Mastocytosis is a disorder characterized by pathologic accumulation and activation of aberrant mast cells in tissues [1•]. Excessive numbers of mast cells can be found in the skin, bone marrow, and internal organs such as the liver, spleen, lymph nodes, and gastrointestinal tract. Symptoms in patients with mastocytosis are most often due to induced or spontaneous activation of mast cells resulting in release of vasoactive mediators such as histamine, arachidonic acid metabolites (prostaglandin D₂ [PGD₂] and leukotriene C₄ [LTC₄]), proteases, and cytokines. Mastocytosis is therefore suspected in differential diagnosis of patients with recurrent unexplained flushing, anaphylaxis, and gastrointestinal symptoms (eg, cramping and diarrhea). Less frequently, in rare disease categories such as aggressive systemic mastocytosis and mast cell leukemia, infiltration of the mast cells in tissues causes end-organ dysfunction such as hepatomegaly,

ascites, malabsorption, pathologic bone fractures, and bone marrow suppression. Some patients may have a non-mast cell myeloid or lymphoid neoplasm associated with mastocytosis, and mastocytosis is diagnosed during the workup of the associated hematologic disorder [2].

The World Health Organization (WHO) classification recognizes seven disease categories based on tissue involvement, diagnostic findings, and prognosis (Table 1) [3,4]. Involvement of skin and/or bone marrow separates the disease into two broad categories: cutaneous mastocytosis limited to skin is most commonly diagnosed in children within the first year of life; and systemic mastocytosis with bone marrow involvement is commonly diagnosed in adults. Systemic mastocytosis (SM) is further divided into four categories: indolent systemic mastocytosis (ISM); mastocytosis with an associated clonal non-mast cell lineage disease (SM-AHNMD); aggressive systemic mastocytosis (ASM); and mast cell leukemia (MCL). Mast cell sarcoma and extracutaneous mastocytoma are rare mast cell tumors without diagnostic criteria of systemic mastocytosis [4].

Diagnosis of Cutaneous Mastocytosis

Cutaneous mastocytosis is a benign category of mastocytosis and includes urticaria pigmentosa, mastocytoma, diffuse cutaneous mastocytosis, and telangiectasia macularis eruptive perstans (TMEP) [5]. Most cases of isolated cutaneous mastocytosis occur in the pediatric population. Urticaria pigmentosa typically presents as a fixed, red-brown maculopapular rash distributed over the entire body, generally sparing sun-exposed areas. Darier's sign occurs as local wheal formation if the lesion is scratched, but may be absent if the patient is taking antihistamine medications. Skin lesions of urticaria pigmentosa tend to be larger and fewer in children than in adults. Childhood-onset cutaneous mastocytosis develops within the first year of life, and often resolves by puberty [6]. Children with cutaneous mastocytosis may also experience blistering within the first 3 years of life.

Although diagnosis can be established by an experienced physician based on appearance of lesions, skin biopsy is recommended in most instances to confirm the diagnosis. Biopsy of an urticaria pigmentosa lesion will

Table 1. Classification of mastocytosis

Cutaneous mastocytosis
Indolent systemic mastocytosis (without associated hematologic disease)
Systemic mastocytosis with an associated clonal hematologic non-mast cell lineage disease
Aggressive systemic mastocytosis
Mast cell leukemia
Mast cell sarcoma
Extracutaneous mastocytoma

(Adapted from Valent et al. [3] and Valent et al. [4].)

demonstrate increased mast cells (generally more than 20 mast cells per high-power field) that frequently involve the perivascular regions of the upper dermis, without a significant increase in other inflammatory cells [5]. In contrast to childhood-onset urticaria pigmentosa, adult-onset urticaria pigmentosa should always prompt an investigation for systemic (bone marrow) disease.

Cutaneous mastocytomas in children are benign mast cell tumors and may be solitary or multiple, usually resolving as the child grows older. Diffuse cutaneous mastocytosis is a rare variant of the pediatric-onset type; it involves the entire surface of the skin, giving it a coarse appearance and a yellowish hue. Skin biopsy shows a high degree of mast cell infiltration. TMEP is a rare variant of cutaneous mastocytosis affecting adults. TMEP is characterized by generalized telangiectatic erythematous macules. If scratched, the macules may demonstrate Darier's sign. The biopsy of a TMEP lesion shows an increase in blood vessels surrounded by increased numbers of mast cells. The diagnosis of TMEP should be established only after considering the appearance of the skin lesion along with biopsy findings. Skin biopsy alone does not establish the diagnosis, because mast cells are generally found in close proximity to blood vessels in normal skin, and mild increases in numbers of mast cells may be observed in other skin lesions.

Diagnosis of Systemic Mastocytosis

In contrast to cutaneous mastocytosis, systemic mastocytosis is a persistent disease primarily affecting adults. Systemic mastocytosis is present when one or more organ systems other than skin are infiltrated with mast cells. The organ systems typically involved include bone marrow, lymph nodes, liver, spleen, and gastrointestinal tract.

The recommended diagnostic procedure when systemic mastocytosis is suspected is a bone marrow biopsy and aspirate [3,7]. This procedure provides information regarding the extent of the mast cell disease, determines whether other non-mast cell hematologic diseases are present, and allows analysis of cells for detection of *c-kit*

Table 2. Diagnostic criteria for systemic mastocytosis

Major

Characteristic multifocal dense infiltrates of mast cells (15 or more in clusters) in bone marrow or another extracutaneous tissue biopsy

Minor

Morphology of mast cells: spindle shaped

Detection of a codon 816 *c-kit* mutation

Flow cytometric co-expression of CD117, CD2, and CD25 by the bone marrow mast cell population

Serum tryptase > 20 ng/mL

mutations. All adult patients with urticaria pigmentosa are recommended to have a bone marrow biopsy due to the high incidence of systemic disease in this population. In contrast, a bone marrow biopsy is generally not required in children who present with skin lesions in the absence of lymphadenopathy, hepatomegaly, splenomegaly, or an abnormal complete blood count with differential. Biopsy of tissues other than bone marrow rarely provides reliable diagnostic information.

The WHO criteria for diagnosis of systemic mastocytosis include one major and four minor criteria (Table 2) [3]. The final diagnosis is made if one major and at least one minor criterion are met or if at least three minor criteria are present. The major diagnostic criterion requires demonstration of mast cells in multifocal compact aggregates of 15 or more cells in the bone marrow (or another extracutaneous tissue) via an appropriate mast cell stain (eg, tryptase or CD117 immunohistochemical staining). Mast cell clusters in bone marrow are typically found concentrated in perivascular or paratrabecular locations.

The recommended method of staining mast cells in tissues is immunohistochemical staining for tryptase [8]. Tryptase is a protease contained in mast cell granules and identifies mast cells in normal tissues in a fairly specific manner. Although basophils also contain small amounts of tryptase, the amount is not sufficient to highlight them with the stain. Early myeloid precursors may also stain positively with tryptase in certain myeloproliferative disorders such as acute myeloid leukemia [9].

Those patients who do not satisfy the major criterion must be carefully evaluated for minor diagnostic criteria before a diagnosis of systemic mastocytosis is ruled out. These criteria include atypical mast cell morphology, aberrant mast-cell surface immunophenotype of CD25 or CD2 expression, detection of a codon 816 *c-kit* mutation in the bone marrow, peripheral blood, or lesional tissue, and a serum or plasma tryptase level greater than 20 ng/mL. Demonstration of the first three of these minor criteria may require expert analysis in a mastocytosis research and referral center, and are discussed in more detail in the following paragraphs.

Pitfalls and Advances in Demonstration of Minor Criteria of Systemic Mastocytosis

Bone marrow aspirate smears from a patient with mastocytosis may show very few aberrant mast cells, and these may not resemble mature tissue mast cells. Their morphology is often elongated and hypogranulated, unlike the round and fully granular appearance of normal mast cells, and may escape attention.

Analysis of mast cell expression of CD25 or CD2 can be accomplished by flow cytometry or immunohistochemistry. These aberrant markers are detectable only on mastocytosis but not on normal or reactive mast cells. Flow cytometric analysis of mast cells requires special gating techniques and acquisition of more events than routine leukemia/lymphoma phenotyping studies [10••,11]. A common mistake is to rule out aberrant mast cells based on examination of too few cells. Some patients may require collection of 500,000 or more events (cells) for a reliable analysis of the mast cell population. This may be especially problematic in patients with low mast cell burden and lower tryptase levels. Therefore, referral to a mast cell disease research center should be considered for patients who have normal or mildly elevated tryptase levels (ie, < 20 ng/mL). Recent availability of an anti-CD25 antibody that can be used in routinely processed paraffin tissue sections enabled analysis of mast cell CD25 expression in biopsy sections [12]. Using this method, mast cells can be identified in serial sections stained for CD25, tryptase, or CD117. Immunohistochemistry, however, may not be as reliable as flow cytometry in patients with low mast cell burden due to inherent difficulties in identifying single scattered cells in serial tissue sections.

CD2 expression in aberrant mast cells shows great individual variation and may be very low or absent in advanced disease categories such as mast cell leukemia and aggressive systemic mastocytosis. CD2 expression is best analyzed by flow cytometry.

Mutational analysis of the *c-kit* gene may present another pitfall when evaluating the patient for presence of minor criteria [13]. The D816V *c-kit* mutation is a somatic event that is expressed in hematopoietic tissue in neoplastic mast cells or their precursors. Consequently, examination of a peripheral blood sample may yield false-negative results because there are no circulating mast cells in peripheral blood in most patients with mastocytosis except in MCL and ASM, to a lesser extent. Thus, the mutation may be detectable in peripheral blood in patients with MCL, ASM, SM-AHNMD, and ISM with extensive marrow involvement if it occurs in a pluripotent hematopoietic progenitor. In other patients with ISM, enrichment of neoplastic mast cells either by microdissection, magnetic bead selection, or fluorescence-activated cell sorting has yielded *c-kit* mutation detection rates of greater than 90% [13]. These techniques, however, are considered investigational and are not routinely employed by clinical diagnostic laboratories. A recent workshop

on standardization of diagnostic techniques in mastocytosis recommended analysis of bone marrow buffy coat or mononuclear cells for mutational analysis in order to meet WHO diagnostic criteria [14••]. Techniques recommended for detection of the mutation included reverse transcription polymerase chain reaction (RT-PCR) followed by restriction length fragment polymorphism assay, peptide nucleic acid (PNA)-mediated PCR, and allele-specific PCR.

Measurement of baseline serum or plasma total tryptase levels should be performed in all suspected cases of systemic mastocytosis [15•]. In a healthy population, median total tryptase levels are approximately 5 ng/mL. The tryptase level in systemic mastocytosis is often found to be elevated with higher levels correlating with increased mast cell burden. A total tryptase level greater than 20 ng/mL in the absence of a concurrent hematologic disorder is a minor diagnostic criterion for systemic mastocytosis. Tryptase level for the purpose of diagnosing mastocytosis should not be based on a sample collected during or shortly after an episode of anaphylaxis, because it may be found elevated due to the recent degranulation of mast cells. In these patients, tryptase levels should be checked at least 2 days after a degranulation episode, allowing the levels to return to baseline to more accurately reflect the mast cell burden. An elevated tryptase level by itself is not diagnostic for systemic mastocytosis because other disorders such as hypereosinophilic syndromes, myelodysplastic syndromes, myeloid neoplasms [9,16,17], and renal failure [18] can lead to elevated tryptase levels. In addition, several patients displaying idiopathic elevations in tryptase levels without diagnostic evidence of mastocytosis have been observed in our practice. Whether these patients have a form of mast cell activation disorder that is yet to be characterized is not known [19].

Total serum or plasma tryptase levels reflect a combination of mature and protryptase levels encoded by alpha and beta tryptase genes. Alpha tryptase encodes an enzymatically inactive protryptase. Beta tryptase gene product is also protryptase, but gets processed to a mature enzyme by proteolytic cleavage and is stored in mast cell granules. In contrast, beta protryptase and alpha (pro-) tryptase are secreted out of the mast cell [20]. Therefore, baseline levels of total tryptase consist of a combination of alpha and beta protryptases and reflect total body mast cell burden, whereas mature (beta) tryptase is elevated after an anaphylactic episode [15•]. Fractionation of mature and protryptase levels in serum is available only in a few diagnostic laboratories and is generally not necessary for diagnosis of mastocytosis. It has been reported that approximately 25% of the population lacks the alpha tryptase gene [21]; however, deficiency of the alpha tryptase gene does not impact baseline tryptase levels or compromise the diagnostic value of tryptase measurements in patients with mastocytosis [22].

Recently, a group of patients with chronic eosinophilic leukemia associated with *FIP1L1-PDGFR*A rearrangement

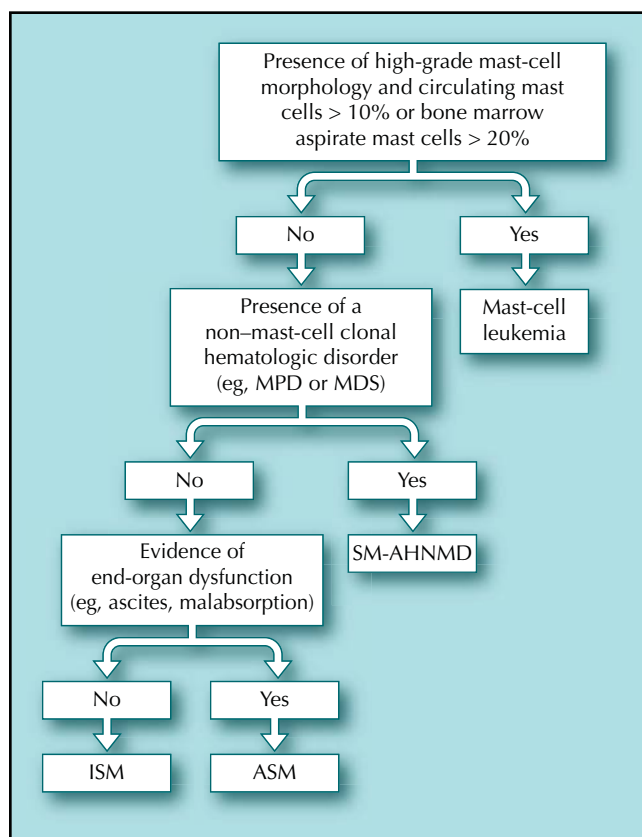


Figure 1. Suggested algorithm for classification of systemic mastocytosis. ASM—aggressive systemic mastocytosis; ISM—indolent systemic mastocytosis; MDS—myelodysplastic syndrome; MPD—myeloproliferative disorder; SM-AHNMD—systemic mastocytosis with associated clonal non-mast cell lineage disease. (From Akin and Metcalfe [1]; with permission.)

were described to have increased numbers of bone marrow mast cells as well as modest elevations of serum tryptase levels [16]. Some of these patients may meet the diagnostic criteria for systemic mastocytosis because their mast cells have atypical morphology and carry surface CD25 [23], although the clinical presentation is primarily due to eosinophil-mediated pathology or complications of progression of their myeloproliferative disease. Because this disease responds well to imatinib, it is recommended to check for the presence of *FIP1L1-PDGFR*A rearrangement in patients with chronic eosinophilia ($> 1.5 \times 10^9/L$) by RT-PCR or by cytogenetic detection of the deletion of the *CHIC2* locus intervening between the *FIP1L1* and *PDGFR*A loci in the long arm of the normal chromosome 4.

Once the diagnosis is established, the patient's disease must be classified into one of the four categories of systemic mastocytosis (Fig. 1) [1•]. The diagnosis of ASM and SM-AHNMD requires a high index of suspicion because these patients may not necessarily present with the skin lesions of urticaria pigmentosa. Most common non-mast cell hematologic disorders encountered in patients with SM-AHNMD are chronic myeloproliferative and myelodysplastic syndromes, although almost any hematologic

neoplasia can be associated with mastocytosis [2]. It is uncommon for a patient diagnosed with ISM to progress toward a more advanced disease category.

Recently, clonal mast cells have been identified in bone marrow aspirates of some patients with recurrent anaphylactic episodes [24,25]. These patients may not have lesions of urticaria pigmentosa, or mast cell clusters in bone marrow biopsy, and may have normal or only mildly elevated tryptase levels. Examination of bone marrow mast cells reveals aberrant mast cells carrying one or two minor diagnostic criteria for systemic mastocytosis. This patient population with recurrent mast cell degranulation episodes and only one or two minor diagnostic criteria has been tentatively termed to have a monoclonal mast cell activation syndrome [25].

Treatment

The management of mastocytosis involves controlling the symptoms related to mediator release from mast cells in all categories and curtailing end-organ damage caused by infiltrating mast cells by using cytoreductive treatments in selected patients with ASM, SM-AHNMD, MCL, and MCS [26]. To date, there is no curative measure for mastocytosis that has been shown to be uniformly effective; thus, symptom control often becomes the main focus of treatment. Medications that are used to manage mast cell mediator symptoms include histamine₁ (H_1) and histamine₂ (H_2) receptor blockers, leukotriene antagonists, cromolyn sodium, topical and systemic glucocorticoids, and epinephrine.

Avoidance of known triggers for mast cell degranulation is an important part of the management strategy. Triggers show great individual variation between patients and may include exercise, heat, temperature changes, spicy foods, alcohol, physical and emotional stress, insect venoms, and drugs such as nonsteroidal anti-inflammatories, opioid analgesics, amphotericin B, quinine, general anesthetics, muscle relaxants, and radiocontrast material. Pretreatment regimens, including both H_1 and H_2 antihistamines with or without glucocorticoids, are generally considered before anesthesia [27]. Furthermore, using medications that were tolerated by the patient in previous procedures is suggested when possible.

Methoxypsoralen plus ultraviolet A (PUVA) radiation has provided temporary relief of itching and improvement of urticaria pigmentosa in some patients [28]. The increased risk of skin cancer in the long term and the temporary nature of relief provided by PUVA often outweigh the potential benefits of this treatment modality, except in some patients who continue to have cutaneous symptoms despite optimized medical therapy. There is no treatment modality to influence the natural course of urticaria pigmentosa or cause its regression permanently.

Cytoreductive therapies used in aggressive variants of systemic mastocytosis have included interferon alpha

(IFN- α), cladribine (2-CDA), polychemotherapy, bone marrow transplantation, and new small-molecular-weight tyrosine kinase inhibitors. Most experience has been reported with IFN- α [29]. It appears that IFN- α may be particularly useful in the subset of patients with severe osteoporosis and pathologic bone fractions. Studies using this drug have shown equivocal results [30–32]. Some patients have reported a reduction in symptoms related to their mast cell disease (eg, flushing, pruritus, abdominal pain, and diarrhea), which was generally not accompanied by improvement of the bone marrow pathology. The myelo-suppressive effects of the drug, as well as its severe flu-like side effects, limit its usefulness in treating mastocytosis.

The nucleoside analogue 2-chlorodeoxyadenosine has been reported to be effective in treatment of systemic mastocytosis [33]; however, the responses are generally transient and incomplete [34,35]. Severe immunosuppression and myelosuppression may be encountered as a result of therapy. Chemotherapeutic agents have been used to treat patients with poor prognostic categories of mastocytosis [36], but generally do not alter the course of the disease. Due to the rarity of the aggressive disease variants requiring chemotherapy, there have been no large clinical trials to look at the effectiveness of these agents.

Bone marrow transplantation has been used in treatment of patients with advanced disease and poor life expectancy. Two case reports describe patients with SM-AHNMD who received bone marrow transplants, which resulted in remission of the associated non-mast cell hematologic disorder but did not affect the mastocytosis [37,38]. A recent study of three patients with advanced systemic mastocytosis who underwent non-myeloablative stem cell transplantation similarly reported no clinically significant effect on mast cell disease despite engraftment of the donor cells [39]. In contrast to these studies, one patient who underwent a non-T-cell-depleted peripheral blood transplant was reported to have a complete remission of mast cell disease, having no detectable mast cells in the bone marrow at 2-year follow-up [40]. Another case report describes a patient with mast cell leukemia who was successfully treated with allogeneic bone marrow transplant and donor lymphocyte infusions, in whom the disease was converted to an indolent form [41].

Recent Advances in Treatment of Mastocytosis

Low-molecular-weight tyrosine kinase inhibitors targeting KIT have generated considerable interest as novel cytoreductive options in treatment of mastocytosis in recent years because the D816V *c-kit* mutation associated with the disease causes a constitutive activation of the intrinsic tyrosine kinase activity of KIT independent of binding of its ligand (stem cell factor) [42]. Imatinib was the first such drug to be approved by the US Food and Drug Administration (FDA) for treatment

of *bcr-abl*-positive chronic myeloid leukemia (CML). In addition to *abl* kinase, imatinib has also been shown to inhibit KIT and platelet-derived growth factor receptor (PDGFR) [43]. In vitro experiments have shown that the drug is a potent inducer of apoptosis in mast cells carrying wild-type KIT as well as juxtamembrane or transmembrane KIT mutations, but failed to show significant cytotoxicity in mast cells with the common D816V mutation. This is theorized to be due to the inability of imatinib to bind the activation loop of KIT, the structure of which is altered by the D816V mutation. These in vitro observations have been verified in clinical trials in which imatinib has effectively caused disease remission in patients with mastocytosis and chronic eosinophilic leukemia carrying *FIP1L1-PDGFR* rearrangement, and in a rare patient with a transmembrane *c-kit* mutation, but not in those with the more common D816V mutation [23,44]. A recent study reported symptomatic improvement and mild reductions in baseline tryptase levels in some patients with D816V *c-kit* mutation who were treated with imatinib and glucocorticoids [45]. These effects may be due to the cytotoxicity of imatinib in mast cells carrying the wild type *c-kit*, as one study showed mast cells carrying both wild type and D816V mutant KIT in different ratios in patients with mastocytosis [46]. The FDA has recently approved imatinib for treatment of aggressive systemic mastocytosis with unknown or negative *c-kit* codon 816 mutation. Imatinib therapy is not recommended for patients with indolent systemic mastocytosis or cutaneous mastocytosis.

Newer “second generation” tyrosine kinase inhibitors have been developed for use in imatinib-resistant diseases such as CML with *abl* point mutations or acute myeloid leukemia associated with *flt3* mutations. Some of these inhibitors, such as dasatinib and PKC412, have also been shown to be effective for in vitro killing of mast cells carrying D816V KIT [47,48,49]. Early clinical trial data using these inhibitors were presented at the 2006 annual meeting of the American Society of Hematology, in which the drugs showed disappointingly low efficacy in causing mast cell cytoreduction. This is presumed to be due to inability of the drugs to reach therapeutic tissue levels to sustain inhibitory levels without causing significant toxicity, or existence of other survival pathways in neoplastic mast cells independent of KIT. These observations highlighted the importance of efforts to identify new therapeutic targets in mast cell disease. In line with this concept, a recent article demonstrating the importance of anti-apoptotic protein mcl-1 for mast cell survival showed that combination of PKC412 with an antisense oligonucleotide targeting mcl-1 was more effective in killing neoplastic mast cells than either agent alone [50]. Other studies showed combination of two tyrosine kinase inhibitors with different structures or a tyrosine kinase inhibitor with 2-CDA has allowed lower concentrations of each drug to achieve mast cell cytotoxicity [49]. The

concept of combination therapy in treatment of systemic mastocytosis, therefore, appears to be a promising future approach to mast cell cyto-reductive therapy.

Conclusions

Establishment of objective diagnostic criteria for systemic mastocytosis and a recent conference report on standardization of diagnostic techniques and evaluation of patients with systemic mastocytosis should provide helpful guidance to physicians caring for patients with mast cell disease. Patients suspected of having mastocytosis who have normal or mildly elevated tryptase levels, as well as those with extensive mast cell burden requiring cyto-reductive therapies, may benefit from evaluation in a mastocytosis research and referral center. The current mainstay of management is directed toward symptomatic relief. To date, no treatment approach has been shown to be uniformly effective in achieving mast cell cyto-reduction. Combination therapies incorporating tyrosine kinase inhibitors should be evaluated in future clinical trials. Meanwhile, research efforts should continue in order to identify new survival pathways for neoplastic mast cells, better define the clinical spectrum and disease pathology, and more effectively counteract symptoms caused by pathologic mast cell activation.

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