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ADVANCES AND CONTROVERSIES IN THE DIAGNOSIS, PATHOGENESIS, AND TREATMENT OF SYSTEMIC MASTOCYTOSIS

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Abstract

The term systemic mastocytosis (SM) encompasses a group of hematopoietic malignancies characterized by excessive proliferation of neoplastic mast cells that accumulate in the bone marrow and visceral organs. Most patients with SM, particularly those presenting with aggressive clinical courses, carry somatic mutations of the *KIT* gene. *KIT* mutations are considered central events in the pathogenesis of SM and serve as diagnostic markers and putative therapeutic targets. The heterogeneity in the clinical course of patients with SM, and recent advances in the genetic and immunophenotypic characterization of neoplastic mast cells may help in improving current diagnostic, taxonomic, and therapeutic approaches in SM.

Introduction

Systemic mastocytosis (SM) encompass a group of heterogeneous myeloproliferative neoplasias (MPNs) characterized by excessive proliferation of neoplastic mast cells that accumulate in one or more organs, most frequently in hematopoietic tissues such as the bone marrow, spleen, and lymph nodes, as well as in the skeletal system and liver, among others.^{1, 2} The clinical course of patients with SM is highly variable and ranges from indolent, with normal life expectancy, to highly aggressive, associated with multisystem involvement and poor overall survival. SM has been frequently associated with the presence of somatic mutations in the *KIT* oncogene, which encodes a transmembrane receptor protein with kinase activity whose ligand is the stem cell factor (SCF). *KIT* mutations are believed to play a critical role in the pathogenesis of SM and have been postulated as diagnostic markers and potential therapeutic targets. Herein, we discuss controversial issues regarding diagnostic criteria and taxonomic aspects of SM and provide an account of recent therapeutic developments in the field.

Classification of SM

Mastocytosis can be classified into several subtypes according to the 2008 World Health Organization (WHO) classification system: 1) cutaneous mastocytosis (limited to the skin),

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2) extracutaneous mastocytosis (unifocal mast cell tumor with low-grade cellular atypia and non-destructive features), 3) mast cell sarcoma (unifocal mast cell tumor with destructive features and poorly differentiated mast cells), and 4) SM, which almost invariably involves the bone marrow and the most frequently diagnosed mast cell disorder diagnosed in adults.³ SM can be subdivided in four subcategories: 1) indolent systemic mastocytosis (ISM; no evidence of organ dysfunction), which includes two provisional indolent subvariants: isolated bone marrow mastocytosis (BMM) and smoldering SM (SSM), 2) SM associated with another clonal hematological non-mast cell lineage disease (SM-AHNMD), most commonly chronic myelomonocytic leukemia (CMML); 3) aggressive SM (ASM), characterized by life-threatening impaired organ function from mast cell infiltration, and 4) mast cell leukemia, a very aggressive form of SM.³ While the WHO classification segregates relatively discreet subtypes of SM, an outstanding question is whether such groups also represent distinct prognostic categories.

Validation and refinement of the WHO classification of SM

The prognostic significance of the WHO classification has been recently validated in 342 patients treated at the Mayo Clinic.⁴ In such series, the percentage of patients with SM classified as ISM, ASM, or SM-AHNMD was 46%, 12%, and 40%, respectively. The median survival in ISM (198 months) was better than in ASM (41 months), SM-AHNMD (24 months), or mast cell leukemia (2 months). Shorter survival was independently associated with advanced age, WHO subtype, weight loss, anemia, >5% bone marrow blasts, and low platelet or albumin levels.⁴ The 2008 WHO proposal recognizes two different variants of ISM, smoldering systemic mastocytosis (SSM), characterized by high mast cell burden, and isolated bone marrow mastocytosis (BMM), characterized by bone marrow involvement but no cutaneous involvement.³ A recent report identified SSM and BMM in 14% and 23% of 159 patients with ISM analyzed.⁵ The remainder 63% of patients were classified as ISM-other. Patients with SSM exhibited a higher incidence of constitutional symptoms, anemia, mast cell mediator levels, and a higher age at presentation. Of note, mast cell mediator symptoms were more frequent in patients with BMM. The median survival of patients with ISM-other, SSM, and BMM was 301, 120, and not reached ($p<0.01$), respectively.⁵ In another series, BMM was diagnosed in 46 of 99 consecutive patients with ISM, supporting the notion that this subcategory of SM has been largely underdiagnosed.⁶

There are, however, other tenuous issues regarding the WHO classification of SM such as the lack of a clear recognition of cases of splenic, occult, and well-differentiated mastocytosis, or those categorized as mast cell activation syndrome with clonal mast cells,⁷ which resembles a *forme fruste* of SM. Immunophenotyping of neoplastic mast cells may prove an invaluable tool to further define these rarer forms of SM as well as to delineate subsets of SM with common pathogenetic features and similar outcomes within the categories currently recognized by the WHO classification. In addition to co-expression of CD25 and CD2, neoplastic mast cells have been recently found to frequently overexpress CD63, CD69, CD58, CD33, and several complement-associated molecules (e.g. CD11c, CD35).⁸ Three distinct immunophenotypic patterns have been identified in 123 patients with SM by flow cytometry analysis,⁸ which were associated with distinct markers of mast cell clonality and clinical outcomes. A more immature immunophenotype is present in ASM

(CD25+CD2-CD63+CD69+) and MCL (CD25+CD2-CD63-CD69-), whereas mature activated (ISM/clonal mast cell activation disorder) or resting (well differentiated SM, typically CD25-CD2-) bone marrow mast cell phenotypes, dependent on the presence or absence of the D816V *KIT* mutation, are associated with better prognostic categories of SM. The diagnosis of each specific subtype of SM (well differentiated vs ISM/clonal mast cell activation disorder vs ASM/MCL) could be made with a high sensitivity (67%, 86%, and 100%, respectively) and specificity (100%, 86%, and 88%, respectively).⁸ The use of immunophenotypic information may improve future taxonomic and diagnostic efforts in SM. A prognostically important subtype of ISM has been recently described.⁹ This group of patients is characterized by the presence of *KIT* mutations in all hematopoietic lineages, increased serum β -2 microglobulin, and a high risk of progression and early death.⁹ These results suggest the presence of different subgroup of patients with distinct clinical and prognostic characteristics.

Criteria for the diagnosis of SM

The diagnosis of SM is based on a set of diagnostic criteria set forth originally by the WHO in 2001. A diagnosis of SM requires the presence of one major and one minor criteria, or a combination of three minor diagnostic criteria (Table 1).¹⁰ Ultimately, the diagnosis of SM is underpinned by the identification of tissue mast cell infiltration. Bone marrow examination is imperative to establish a diagnosis of SM, as bone marrow involvement is easily identifiable in most adults with this malignancy. Approximately 20% of patients with SM are diagnosed after a bone marrow biopsy prompted by an abnormal blood count.¹¹ Bone marrow examination also assists in the diagnosis of SM-AHNMD.¹¹ This is important because the long-term prognosis of these patients relies heavily on the associated hematologic malignancy, with the worse prognosis being conferred by the presence of acute leukemia, followed by CMML, MDS, and MPN.¹² Neoplastic mast cells exhibit an atypical spindle-shape and form multifocal aggregates consisting of 15 or more cells,¹³ which frequently express the surface markers CD2 and/or CD25.¹⁴ A high prevalence of eosinophilia (34%) has been reported in patients with SM-AHNMD, particularly in those with SM-MPN but has no prognostic implications.¹² Serum tryptase level and urinary histamine levels (both released by mast cells) are typically elevated. Screening for the *KIT* D816V mutation confirms its presence in approximately 90% of patients with SM and should be considered when a diagnosis of SM is entertained. Similarly, the presence of eosinophilia should prompt testing for the *FIPIL1-PDGFR α* oncogene. However, most *FIPIL1-PDGFR α* carriers will not fulfill criteria for SM and are best classified as “myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFR α* , *PDGFR β* or *FGFR*” in accordance with the WHO 2008 classification.^{3, 15}

Once a diagnosis of SM is made, patients with this malignancy are further categorized according to the presence of “B and C findings”, which assess disease burden and disease aggressiveness, respectively (Table 2). Patients with SM with no findings are categorized as ISM, those with B findings are considered as having smoldering SM (SSM), a subtype of ISM with a more aggressive clinical course, and those with C findings are best classified as ASM and are candidates for immediate therapeutic intervention.

Caveats of the WHO diagnostic criteria

While the criteria issued by the WHO remain the gold standard for the diagnosis of SM, several criticisms can be made that challenge their currency. The detection of multifocal, dense aggregates of mast cells in bone marrow (confirmed by immunohistochemistry and/or flow cytometry), elevated serum tryptase levels, and KIT exon 17 mutations, is diagnostic in 90% of cases of SM. However, atypical mast cell morphology can be identified in all cases and an aberrant immunophenotype is present in 96% of cases of suspected SM, thus highlighting the sensitivity of these pathological features in the diagnosis of SM. However, such features are only categorized as “minor” by the WHO diagnostic system. It must be emphasized that tryptase immunostain on a bone marrow biopsy or clot specimens is not adequate to reliably assess mast cell morphology, the latter being best assessed on a bone marrow aspiration specimen.¹⁶ The presence of a substitution of Val for Asp at codon 816 (D816V) of the *KIT* gene is an important diagnostic criterion.¹⁷ However, in our experience, the presence of KIT D816V was detected in only 75% of patients with suspected SM, which is highly reliant on the sensitivity of the technique employed as well as on the type of sample used for testing.¹⁶ KIT mutations are typically assessed by direct sequencing techniques, which have a sensitivity of only 10%–15%, which renders an inferior sensitivity for the diagnosis of SM to that of the assessment of CD25 on the surface of aberrant mast cells.¹⁶ Interestingly, it has been shown that the sensitivity of detection of KIT D816V can be increased when testing is carried out in bone marrow mononuclear cells enriched for CD25+ cells, which contain the aberrant mast cell population.¹⁸ A contentious issue regarding KIT D816V detection in SM is the frequency of this mutation in patients with SM-HNMD. The prevailing view is that the majority of patients with SM-AHNMD will carry KIT D816V mutations. However, a recent series has shown, by using laser microdissection to separate the mast cell and the AHNMD components, that the frequency of this mutation varies significantly depending of the type of hematologic malignancy associated with SM.¹⁹ Patients with SM associated with CMML carry the KIT D816V in 89% of cases, while this frequency decreases to 20% and 30% for patients with SM associated with MDS or acute myeloid leukemia (AML), respectively and could not be detected in any case of lymphoproliferative AHNMD associated with SM.¹⁹

Widely considered a marker of mast cell clonality, the specificity of CD25 positivity in SM has been challenged as it has been shown that a fraction of patients with idiopathic anaphylaxis express CD25 on the surface of mast cells in addition to exhibiting at least one minor criteria of SM and other markers of clonality such as KIT D816V.¹⁸ In addition, as previously discussed, some cases of SM lack CD25 expression. The recent discovery that neoplastic mast cells from patients with advanced SM co-express CD30 suggests that the detection of this surface molecule may be used as a grading marker for diagnostic purposes as well as a therapeutic target.²⁰ The presence of a serum tryptase level persistently 20 ng/ml is another minor criterion for the diagnosis of SM. In our experience, this criterion is present in 85% of patients. However, the specificity of this parameter is limited by the fact that marked elevations in tryptase have been reported in other myeloid malignancies or in severe allergic reactions.²¹ Furthermore, the utility of this criterion is further limited by its unreliability in patients with SM-AHNMD, which precludes its use in that particular subtype

of SM. Perhaps the specificity of serum tryptase levels could be improved by raising the threshold in patients with SM-AHNMD, as most present with levels over 100 ng/ml. The above described limitations of the 2008 WHO diagnostic criteria result in a fraction of patients with clinically suspected SM in whom a diagnosis cannot be definitely made exclusively on the basis of such criteria. In those cases, clinical features of mast cell activation become particularly relevant. Improvements are clearly needed to increase the sensitivity and specificity of criteria for the diagnosis of SM.

Pathogenesis of SM

Genetic screens in samples of patients with MPNs have unveiled the presence of recurrent somatically acquired mutations, some of them encoding protein products susceptible to pharmacologic inhibition. Somatic gain-of-function point mutations in the kinase domain of the protein encoded by the *KIT* oncogene which encodes the receptor for SCF, are recurring genetic abnormalities present in the majority of patients with SM. *KIT* plays an important role in normal hematopoiesis and its expression declines in hematopoietic cells with maturation, except in mast cells. Mice genetically lacking *KIT* or SCF expression are devoid of mast cells.^{22, 23} The most frequently detected single-point mutation in neoplastic mast cells is *KIT* D816V, which maps to the *KIT* tyrosine kinase domain.^{24, 25} However, the *KIT* genotype has been shown to vary significantly with the age of mastocytosis onset.²⁶ Thus, mutations at *KIT* D816 are much more common in adults compared with SM pediatric patients (77% vs 42%; $p < 0.001$).²⁶ *KIT* D816V disrupts the hydrogen bond between residues D816 and N819, which destabilizes the inactive conformation of the kinase domain,²⁷ thus leading to constitutive *KIT* autophosphorylation and deregulated mast cell proliferation.²⁸ *KIT* D816V is present in 90% of patients with SM.²⁹ However, *KIT*-independent pathways have also been implicated in the pathogenesis of SM, such as the activation of *LYN* and *BTK*.³⁰ Patients with MPNs can also acquire loss of heterozygosity and somatic deletions at chromosome 4q24, which involves the *TET* oncogene family member 2 (*TET2*) that is frequently mutated in patients with myeloid malignancies such as MPNs and MDS.³¹ The presence of *TET2* mutations was investigated in 48 patients with SM, including 6 with the *FIPIL1-PDGFRa* abnormality, using high-throughput DNA sequence analysis.³² Twelve (29%) patients with SM but none of those with *FIPIL1-PDGFRa*, carried mutations in *TET2*. Six (50%) of the 12 patients with *TET2* mutations also carried *KIT* D816V mutations and one the *JAK2* V617V mutation. However, none of the 6 patients carried the *KIT* D816V mutation. The presence of *TET2* mutations appeared to be prognostically irrelevant.

A recent single nucleotide polymorphism array-based karyotyping analysis of 35 patients (9 with cutaneous mastocytosis, 14 with ISM, 9 with SM-AHNMD, 2 with ASM, 1 with mast cell sarcoma) with mastocytosis has unveiled 20 novel genetic lesions in 10 of them (1 cutaneous mastocytosis, 4 ISM, 4 SM-AHNMD, 1 ASM).³³ Uniparental disomy was only found in patients with SM-AHNMD and ASM and involved chromosomes 2p, 4p, 7p, and 13q. The presence of these lesions was not associated with a different overall survival. *KIT* D816V, *TET2*, *ASXL1*, and *CBL* mutations were found in 29%, 24%, 9%, and 3% of patients, respectively. Patients carrying *KIT* D816V and *TET2* mutations had shorter overall survival (10 months) compared to those carrying *KIT* D816V (not reached), *TET2* (32

months), or none (not reached) ($p < 0.0001$).³³ Activating mutations in N-ras have also been documented in patients with advanced KIT D816V SM but not in those with KIT D816V ISM. Interestingly, N-ras mutations but not KIT D816V were detected in the CD34+ compartment, suggesting that the former may precede the acquisition of KIT D816V in clonal development.³⁴ Whole genome gene expression profiling on peripheral blood samples of ISM confirmed the overexpression of 2303 genes (compared with healthy controls) implicated in pathways such as MAPK, ubiquitin-mediated proteolysis, and JAK-STAT.³⁵ The challenge ahead will be to precisely define the exact pathogenetic role and the role as therapeutic targets for these abnormalities.

CONVENTIONAL TREATMENT

SM remains incurable and therapy is largely palliative. Several support measures such as the use of oral H1 antihistamines or epinephrine, cromolyn sodium, short courses of prednisone, or PUVA (psoralen-ultraviolet A) have been used with different levels of success to attenuate symptoms caused by mast cell mediators such as urticaria, anaphylaxis, or ascites.³⁶ Omalizumab (a humanized murine monoclonal antibody that inhibits immunoglobulin E binding to mast cells and basophils) has been shown to benefit patients with syncopal episodes related to mastocytosis³⁷ and for SM cutaneous manifestations.³⁸ Cytoreductive agents such as interferon- α (IFN- α) and cladribine are typically reserved for patients with ASM, mast cell leukemia, and selected patients with ISM with high mast cell burden, poor quality of life, and/or severe osteoporosis.³⁶

IFN- α —Several groups have tested the activity of IFN- α in SM.^{39–41} In a retrospective analysis of 108 patients with SM treated with cytoreductive therapy, 47 patients (11 ISM, 14 ASM, and 22 SM-AHNMD) received IFN- α with ($n=20$) or without ($n=27$) prednisone (20–60 mg daily with a slow tapering).⁴² For 31 patients, IFN- α ($0.5\text{--}10 \times 10^6$ units three times per week) represented first line therapy. Complete, major, and partial remission rates were 3%, 15%, and 35%, respectively, which were frequently accompanied by marked reductions in serum and urine mast cell-derived metabolites. The overall response rate for patients with ISM, ASM, and SM-AHNMD were 60%, 60%, and 45%. Responses, which lasted for a median of 12 months, did not improve by the concomitant use of prednisone. The presence of mediator symptoms was associated with a favorable response to IFN- α .⁴²

Cladribine—In the largest series of patients with mastocytosis treated with cladribine to date ($n=44$: 3 cutaneous mastocytosis, 19 ISM, 3 SSM, 12 ASM, 6 SM-AHNMD, and 1 mast cell leukemia), major and partial responses were observed in 7 of 12 ASM, 3 of 3 SSM, 17 of 19 ISM, 2 of 3 cutaneous mastocytosis, but in none of those with ASM-AHNMD. Responses lasted for a median of 19.5 months.⁴³ In two other similar series, responses to cladribine, while occurring at similar rates, were only durable for 16⁴⁴ and 11 months,⁴² respectively. Myelosuppression is the main treatment-related toxicity.^{44, 45} Circulating immature myeloid cells and myeloproliferative features appear to predict for an inferior outcome with cladribine.⁴²

While available data support the use of IFN- α or cladribine as frontline therapy for patients with SM, they also highlight the urgent need to improve SM therapy as they only render responses in a limited number of patients, which are generally short-lived.

INVESTIGATIONAL THERAPIES

The mutant KIT D816V kinase can be detected in 90% of patients with SM.^{29, 46} Other *KIT* mutations mapping to the tyrosine kinase, juxtamembrane, or transmembrane domains have been described in sporadic cases of SM.⁴⁷ Several targeted agents with remarkable *in vitro* activity have been tested in clinical trials to counteract the constitutive kinase activity of KIT mutants. Until recently, results have been largely disappointing. However, preliminary results from a clinical study with midostaurin suggest activity of this agent in patients with SM.

1. Imatinib and Nilotinib—Imatinib is an effective inhibitor of ABL1 and KIT kinases,⁴⁸ but has negligible activity against KIT D816V.⁴⁹ Single-arm studies of imatinib⁵⁰ or imatinib in combination with prednisone⁵¹ have failed to show objective responses. Rare KIT mutations such as F522C, K509I, and del419 as well as the juxtamembrane mutations V560G and V559G (but not V559I) are sensitive to imatinib.^{47, 52} Imatinib 400mg/d was approved by the Food and Drug Administration (FDA) for adult patients with ASM without the KITD816V mutation, or with unknown KIT mutational status. Bone marrow eosinophilia is present in 19–33% of patients with SM and 56% of them have been shown to carry the *FIP1L1-PDGFR α* oncogene.⁵³ The majority of these patients respond to imatinib 100mg/d, while those without *FIP1L1-PDGFR α* fail to respond irrespective of KITD816V mutation status. Imatinib was also approved by the FDA for *FIP1L1-PDGFR α* -positive myeloid malignancies at 100mg/d with dose escalation to 400mg/d in patients with suboptimal response. In a recent phase II study, 186 patients with 40 different malignancies associated with imatinib-sensitive tyrosine kinases received imatinib at 400mg/d (hematologic malignancies) or 800mg/d (solid tumors).⁵⁴ Five patients with SM were treated (4 carrying KITD816V and 1 with KITD816T). The patient with KITD816T achieved a partial response, indicating that this mutation may be imatinib-sensitive, while of the 4 patients with KITD816V, one had stable disease.⁵⁴

Nilotinib is a TKI structurally related to imatinib and at 1 μ M potently inhibits KIT phosphorylation in human mast cell (HMC) 1.1 cells (carrying KIT V560G) but not in HMC 1.2 cells (carrying KIT D816V).⁵⁵ In a phase II study, 23 patients with SM were treated with nilotinib 400mg twice daily.⁵⁶ Three (13%) patients responded (2 partial and 1 minor response), based on serum tryptase, bone marrow mast cell infiltration and improvement of clinical symptoms. Overall, these data indicate that neither imatinib nor nilotinib are useful interventions for most patients with SM (carrying KIT D816V).

2. Dasatinib—Dasatinib is a multikinase inhibitor 300-fold more potent than imatinib against BCR-ABL1 kinase and highly active against KIT (IC₅₀ 5 nM) and PDGFR β (IC₅₀ 28 nM).⁵⁷ Notably, dasatinib inhibits the kinase activity of KIT D816V with comparable efficacy to that of wild-type KIT (IC₅₀ of 37 and 79 nM, respectively) and at a concentration of 0.1 μ M displayed differential cytotoxicity against neoplastic mast cells in bone marrow

mononuclear cell cultures from patients with SM.⁵⁸ KIT D816Y was 10-fold more sensitive to dasatinib than KIT D816V/F, suggesting that the conformational changes of the KIT activation loop caused by different mutants greatly influence the inhibitory activity of dasatinib *in vitro*.⁵⁹ Disappointingly, in a phase II trial of dasatinib, only 2 out of 33 patients with SM (18 ISH, 9 ASH, and 6 SM-AHNMD) achieved CR that lasted for 8 and 18 months, respectively.⁶⁰ The marginal activity of dasatinib in SM has been corroborated in other small series.^{61, 62}

3. Midostaurin—Midostaurin is a potent multikinase inhibitor active against protein kinase C (PKC), FLT3, PDGFRA, VEGFR-2 and KIT.⁶³ Midostaurin 1 μ M abrogates the phosphorylation of KIT kinase in HMC-1.1 and in HMC-1.2 cells in a time- and dose-dependent fashion (IC_{50} of 50–250 nM). Midostaurin suppressed with similar efficacy the growth of human mast cells bearing either KIT D816V or wild-type KIT and synergized with nilotinib against both HMC-1 subclones, with cladribine against HMC-1.1 cells,⁵⁵ and with dasatinib.⁶⁴ In a phase II trial, midostaurin was administered orally at 100mg twice daily to 26 patients (4 ASM, 14 SM-CMML, 3 SM-MDS, 1 SM-MDS/MPN-U, and 4 mast cell leukemia).⁶⁵ KIT D816V was detected in 18 of 26 (69%) patients. Eighteen (69%) patients responded, including 10 (38%) with a major response (6 incomplete remissions and 4 pure clinical responses), 5 (19%) with a good partial response, and 3 with a minor partial response. Four patients had stable disease and four progressed. A positive correlation was observed between KIT D816V and major response ($p=0.0095$). High quality responses included normalization of albumin and liver function tests, improvement in platelet count and hemoglobin level, complete resolution of ascites and pleural effusion, normalization of weight, reductions of hepatosplenomegaly, improvement of cutaneous lesions, mediator symptoms and performance status.⁶⁵ These results with midostaurin suggest a role of this agent in the future management of patients with SM. However, more rigorous response criteria are warranted to properly assess the clinical benefit of midostaurin.⁶⁶

4. Masitinib—Masitinib (AB1010) is a TKI active against KIT, PDGFR, and LYN kinases.⁶⁷ Masitinib inhibits SCF-induced proliferation and KIT tyrosine phosphorylation with an IC_{50} of 150 ± 80 nM in Ba/F3 cells expressing human or mouse wild-type KIT. However, the activity of masitinib against KIT D816V was 1000-fold lower compared to that exhibited against cells carrying mutations in the juxtamembrane domain of KIT. A study of masitinib in 202 dogs with mast cell tumors showed proved this TKI to be safe and significantly active when compared to placebo.⁶⁸ It must be noted, however, that the effect of masitinib in dogs was observed regardless of KIT mutational status.⁶⁸ This may indicate that also some other mechanism is active than KIT inhibition. In a phase II study, 25 patients (1 smouldering mastocytosis, 17 ISM, and 7 cutaneous mastocytosis) were randomized to receive masitinib at either 3 mg/kg/day ($n=13$) or 6 mg/kg/day ($n=12$) for 12 weeks.⁶⁹ Symptomatic improvement was observed in 14 of 25 (56%) patients. However, only 1 of 15 assessable patients who experience reduction in bone marrow mast cell burden, and 7 of 14 patients had partial improvements in skin lesions. A dose of 6 mg/kg/day provided the best benefit:risk ratio and was recommended for future studies. However, only 24% of patients in this series carried the KIT D816V mutation and no cases of ASM or mast cell leukemia were included.

5. Other targeted small-molecule inhibitors—Many other small-molecule multikinase inhibitors have been identified as active preclinically against mutant KIT isoforms (Table 3). The latter include OSI-930, also a potent inhibitor of KDR (kinase insert domain receptor) and PDGFR β ,⁷⁰ tandutinib, a FLT3 inhibitor,⁷¹ PD180970 and PD173955, with activity against ABL1, and SRC,^{72–74} AP23464 and AP23848,⁷⁵ the indolinone-based compounds SU11652, SU11654, and SU11655,⁷⁶ AGL2043, which has also been found to inhibit FLT3 and PDGFR,⁷⁷ and EXEL-0862.⁷⁸ None of these agents has been tested in clinical trials yet.

Biologic agents for the treatment of SM

Thalidomide, an immunomodulatory drug (ImiD) was given at 100–300 mg daily to 2 patients with advanced SM.⁷⁹ After a follow-up of 12 months, thalidomide rendered a partial remission in both patients, with significant improvement of symptoms, peripheral blood counts, marrow mast cell burden, and reductions in hepatosplenomegaly. By contrast, lenalidomide, a more potent ImiD, did not show any activity when given to 2 patients with SM associated with multiple myeloma.⁸⁰ Neoplastic mast cells express the low-affinity interleukin-2 receptor (IL-2R) CD25,⁸¹ which lends itself as a therapeutic target. A potential approach to target CD25 is that of using denileukin diftotox (DAB₃₈₉IL-2, OntakTM), a DNA-derived cytotoxic protein composed of the amino acid sequences of diphtheria toxin fragments A and B followed by the sequences for IL-2. On binding to CD25, denileukin diftotox is internalized and cleaved, thus liberating the diphtheria toxin, which induces cell death. In a pilot study, denileukin diftotox was given to 8 symptomatic patients with SM (despite optimal supportive care) for a median of 6 cycles, but none of the patients responded.⁸²

Daclizumab is a monoclonal humanized antibody that targets CD25, the IL-2R alpha subunit, aberrantly expressed on the surface of neoplastic mast cells. Four patients with symptomatic *KIT D816V*-positive ASM were treated with daclizumab at 1mg/kg intravenously over 30 minutes on days 1, 4, 8, 15, and 22 of each cycle.⁸³ One of the patients, who had previously failed therapy with imatinib and denileukin diftotox experienced massive fragmentation of mast cells in the bone marrow after the first cycle of treatment, suggesting extensive daclizumab-induced mast cell destruction with remarkable improvement of SM-associated symptoms.⁸³

Novel experimental therapies

Although mutant KIT constitutively activates the cell growth regulator mammalian target of rapamycin (mTOR), results with the mTOR inhibitor everolimus in 10 patients enrolled in a phase II trial have been disappointing.⁸⁴ Similarly, JAK2 kinase has been proposed as an integral part of the SCF/KIT signaling pathway.⁸⁵ Treatment of HMC-1.1 or HMC-1.2 cells with the selective JAK2 inhibitor TG101348 resulted in marked inhibition of cell proliferation (IC₅₀ 740 and 407 nM, respectively).⁸⁶ Importantly, the combination of TG101348 with dasatinib at sub-IC₅₀ concentrations resulted in synergistic inhibition of HMC-1.2 cell proliferation.⁸⁶

Recently, the proapoptotic Bcl-2 family member Bim has been found to be a tumor suppressor in neoplastic mast cells,⁸⁷ which is downregulated by SCF stimulation and by the oncogenic KITD816V kinase.^{87, 88} Accordingly, the KIT inhibitor midostaurin but also the proteasome inhibitor bortezomib, have been shown to upregulate Bim in HMC-1.1 and HMC-1.2 cells. More importantly, both agents cooperated with the BH3-mimetic obatoclax to re-express Bim and abrogate the growth of neoplastic mast cells,⁸⁷ potentially opening a new avenue for the treatment of SM.

Inhibition of KIT mRNAs with short hairpin RNAs (shRNA) decreases the proliferation of both HMC-1.1 and HMC-1.2 cells followed by a recovery period of 5 days.⁸⁹ Anti-KIT shRNA combined with α -tocopherol sensitizes cells to a caspase-dependent apoptosis that results in a higher percentage of cell death compared with anti-KIT shRNA therapy alone.⁹⁰ These results were replicated in a nude mice injected with HMC-1.1 cells.⁹⁰ Triptolide has also been shown to inhibit the growth of human mast cells harboring D816V and D816Y KIT mutations via inhibition of KIT mRNA levels, induction of apoptosis through downregulation of Mcl-1 and XIAP, and reduction of phosphorylated and total levels of the downstream KIT targets STAT3, Akt, and Erk1/2.⁹¹ Similar activity has been described for the inhibitor of Mcl-1 and protein synthesis inhibitor homoharringtonine. Treatment with this agent induced marked inhibition of the growth of mast cells carrying both V560G and D816V or D814Y KIT isoforms, which was associated with inhibition of KIT-dependent phosphorylation of STAT3/5 and Akt.⁹²

An alternative experimental approach is that of using heat shock protein 90 (HSP90) inhibitors. HSP90 is a cellular chaperone responsible for a number of client proteins such as KIT, p53, and AKT. Treatment of wild-type and mutant KIT canine bone marrow-derived cultured mast cells with the novel HSP90 inhibitor STA-9090 results in growth inhibition, caspase-3/7-dependent apoptosis, and decreased phospho-KIT and -AKT, but not ERK both *in vitro* and in a canine mastocytoma xenograft model.⁹³

Conclusion and future directions

Treatment of SM is indicated in symptomatic cases. In that case, both IFN and cladribine are reasonable options. Despite the availability of several agents with activity against KIT D816V, the therapeutic paradigm of KIT inhibition in patients with SM has not translated into tangible clinical benefits in patients with SM. A prime example supporting this contention is the poor clinical activity reported with the use of potent *in vitro* inhibitors of mutant KIT such as dasatinib. These data suggest that in spite of the importance of KIT for the homeostasis of mast cells, the constitutive kinase activity of mutant KIT may be, at least in part, dispensable for the maintenance of the malignant SM clone. It is conceivable that approaches using combinations of KIT D816V inhibitors with agents that abrogate the activity of signaling molecules downstream from KIT kinase (e.g. STAT5, JAK2) or other pathways involved in mast cell biology (e.g. bcl-2) may render better results.

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Table 1

World Health Organization diagnostic criteria for systemic mastocytosis

- (A) *Major criteria*
Multifocal dense mast cell infiltrates (≥ 15 mast cells in clusters) in bone marrow biopsy sections and/or in other extracutaneous organ(s).
- (B) *Minor criteria*
- 1 More than 25% mast cells in bone marrow or other extracutaneous organ(s) showing an atypical (spindle-shaped) morphology.
 - 2 *KIT* mutation at codon 816 is present in extracutaneous tissues, bone marrow, or blood.
 - 3 Mast cells in bone marrow co-expressing CD117 and either CD2, CD25, or both, as assessed by flow cytometry.
 - 4 Serum tryptase persistently ≥ 20 ng/ml (not applicable to patients with an associated clonal hematological non-mast cell disorder).

A diagnosis of SM requires the fulfillment of either one major and one minor criterion or 3 minor criteria.

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Table 2

B findings and C findings in systemic mastocytosis

B findings: Indication of high mast cell burden and expansion of the genetic defect into various myeloid lineages	
1	Infiltration grade of mast cells in bone marrow >30% on histology and serum total tryptase levels >200 ng/mL
2	Hypercellular bone marrow with loss of fat cells, discrete signs of dysmyelopoiesis without substantial cytopenias, or World Health Organization criteria for myelodysplastic syndrome or myeloproliferative disorder
3	Organomegaly: palpable hepatomegaly, splenomegaly, or lymphadenopathy (>2 cm on computed tomography or ultrasound) without impaired organ function
C findings: Indication of impaired organ function because of mast cell infiltration (confirmed by biopsy in most patients)	
1	Cytopenia(s): absolute neutrophil count <1000/ μ L, or hemoglobin <10 g/dL, or platelets <100,000/ μ L
2	Hepatomegaly with ascites and impaired liver function
3	Palpable splenomegaly with hypersplenism
4	Malabsorption with hypoalbuminemia and weight loss
5	Skeletal lesions: large osteolyses or/and severe osteoporosis causing pathologic fractures

Table 3

Preclinical activity of novel KIT tyrosine kinase inhibitors

Study	Compound	KIT mutation tested	Cell proliferation inhibition (IC ₅₀)
Dubreuil <i>et al</i>⁶⁷	Masitinib	Wild-type D816V Del 547–555	150±80 nM 5.0±2.0 μM 5.0±0.3 nM
Garton <i>et al</i>⁷⁰	OSI-930	Wild-type V560G	<100 nM <100 nM
Corbin <i>et al</i>⁹⁴	Tandutinib	V560G D816V D814V	40 nM 250 nM 600 nM
Corbin <i>et al</i>⁹⁴	PD180970	V560G D816V	40 nM insensitive
Corbin <i>et al</i>⁷⁵	AP23464	V560G D816Y D816V D816F D814V	100 nM 3 nM 11 nM 4 nM 20 nM
Liao <i>et al</i>⁹⁵	SU11652, SU11654, & SU11655	Wild-type Juxtamembrane domain Catalytic domain	10 nM 10–100 nM 250–500 nM
Pan <i>et al</i>⁷⁸	EXEL-0862	V560G D816V	510 nM 350 nM