

Kit Mutations

New Insights and Diagnostic Value



Lorenzo Falchi, MD^a, Srdan Verstovsek, MD, PhD^{b,*}

KEYWORDS

- KIT mutations • *KIT* D816V • Cutaneous mastocytosis • Systemic mastocytosis
- Imatinib • Midostaurin • Avapritinib

KEY POINTS

- KIT mutations are the molecular hallmark of mastocytosis and are present in the vast majority of patients, despite the clinical heterogeneity of the disease.
- *KIT* D816V is the most common KIT mutation, particularly in patients with more advanced forms, and it is believed to represent a driver lesion of the disease.
- Testing for *KIT* D816V is part of the diagnostic criteria for mastocytosis. The mutation can be detected through allele-specific-oligonucleotide quantitative polymerase chain reaction in peripheral blood in most patients.
- Mutated KIT inhibition with imatinib mesylate has represented the first advancement in the targeted therapy of systemic mastocytosis, but it is ineffective in patients with *KIT* D816V.
- Novel agents capable of overcoming *KIT* D816V-mediated tyrosine kinase inhibitor resistance include the multikinase inhibitor midostaurin, the selective *KIT* D816V inhibitor avapritinib, and the so-called switch-pocket inhibitors.

INTRODUCTION

Mastocytosis is a neoplastic disorder originating from the malignant transformation and clonal proliferation of mast cells (MCs), which accumulate in one or multiple organs. The true incidence of the disease is difficult to ascertain due to potential underdiagnosis. It is estimated, however, at 0.89 per 100,000 per year, according to a recent European study.¹ The prevalence of mastocytosis was hypothesized to be approximately 1 in 10,000 people.²

The clinical spectrum of mastocytosis ranges from isolated cutaneous forms (cutaneous mastocytosis [CM]) that is more frequent in younger patients, with tendency to

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^a Division of Hematology/Oncology, Columbia University Medical Center, 177 Fort Washington Avenue, MHB 6GN-435, New York, NY 10032, USA; ^b Leukemia Department, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 428, Houston, TX 77030, USA

* Corresponding author.

E-mail address: sverstov@mdanderson.org

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spontaneously resolve,³ to aggressive diffuse variants (systemic mastocytosis [SM]) typically seen in adult patients and associated with poorer outcome.⁴ In addition, because SM is a hematopoietic stem cell disorder, it can coexist with other World Health Organization (WHO)-defined myeloid (or, more rarely, lymphoid) neoplasms.⁵

Once comprised within the group of myeloproliferative neoplasms (MPNs), mastocytosis is now recognized as a distinct clinicopathologic entity in the 2016 revision of the WHO classification of myeloid tumors (**Box 1**).⁶

The symptoms of mastocytosis are largely due to the release of MC products. Histamine (acting through receptors H₁–H₄) mediates vasodilation, bronchoconstriction, gastrointestinal hypermotility, and increased gastric acid production, which account for rapid-onset symptoms like headache, hypotension, pruritus, urticaria, angioedema, cramping, diarrhea, and anaphylaxis.⁷ Chymase is responsible for cardiovascular symptoms like arrhythmias, myocardial ischemia, and hypotension. Other mediators of mastocytosis symptoms include platelet-activating factor, prostaglandin D₂,⁸ serotonin, and substance P.⁹ Rare manifestations of mastocytosis include tissue eosinophilia (due to interleukin [IL]-5 production) and bone remodeling (due to space-occupying MC burden, as well as to IL-1 β and IL-6 secreted by MCs).¹⁰

CM, the most common form of mastocytosis, can present as urticaria pigmentosa, particularly on the torso and extremities; diffuse CM; or solitary skin mastocytoma. SM is defined by MC involvement of end organs other than the skin (most commonly the bone marrow [BM]). Indolent SM primarily causes gastrointestinal, skin, and cognitive symptoms, but does not affect organ function (e.g., BM, although involved with mastocytosis, functions well and patients have normal blood cell counts). Smoldering SM is a subtype of indolent SM, with organ enlargement (B findings) but preserved organ function, and possibly with more variable course.¹¹ Although indolent SM is a rather benign condition, end-organ damage is the

Box 1

World Health Organization classification of mastocytosis (2016)

1. Cutaneous mastocytosis
 - a. Urticaria pigmentosa/maculopapular cutaneous mastocytosis
 - b. Diffuse cutaneous mastocytosis
 - c. Solitary mastocytoma of skin
2. SM
 - a. ISM: meets criteria for SM; no C findings; no evidence of associated clonal hematologic non-MC lineage disease^a
 - b. Smoldering SM: as above (ISM) but with 2 or more B findings and no C findings^a
 - c. SM-AHN^b: meets criteria for SM and criteria for an associated hematologic neoplasm as a distinct entity per the WHO classification
 - d. ASM: meets criteria for SM; 1 or more C findings; no evidence of MCL^a
 - e. MCL
3. MC sarcoma
 - a. Unifocal MC tumor; no evidence of SM; destructive growth pattern; high-grade cytology

^a These subtypes require information regarding B findings and C findings for complete diagnosis (see **Box 2**), all of which may not be available at the time of initial tissue diagnosis.

^b The terms, systemic mastocytosis with an associated clonal hematologic non-MC lineage disease (SM-AHNMD) and SM-AHN, can be used synonymously.

Adapted from Swerdlow SH, Campo E, Harris N, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th edition. Lyon (France): International Agency for Research on Cancer (IARC); 2017; with permission.

hallmark of aggressive SM (C findings). SM with associated hematologic neoplasms (SM-AHN) exhibits a yet more aggressive behavior and can associate with chronic myelomonocytic leukemia, myelodysplastic syndrome, B-cell lymphoma/leukemia, plasma cell neoplasms, MPNs, and acute myeloid leukemia.¹² MC leukemia (MCL) is a form of acute nonlymphoid leukemia and it is associated with dismal outcomes.

Characteristics of mastocytosis differ in children and adults. In the former, the disease is usually cutaneous and self-limited, anaphylaxis is rare, and the basal tryptase level is less than 20 $\mu\text{g/L}$ [70]. Tryptase levels greater than 20 $\mu\text{g/L}$ usually portend diffuse skin involvement and possible underlying systemic disease.¹³ Cutaneous manifestations of adulthood-onset mastocytosis exhibit a more chronic course, typically presenting with diffuse maculopapular lesions, anaphylaxis that is more common, and tryptase levels frequently greater than 20 $\mu\text{g/L}$.¹⁴ In general, serum tryptase levels tend to increase in more aggressive forms.⁴ This test, however, is nonspecific because tryptase can be increased in other conditions, such as acute myeloid leukemia, chronic myeloid leukemia, and myelodysplastic syndrome.¹⁵

In adults with suspected SM, a BM biopsy is always performed because it allows confirming the diagnosis and ruling out an associated hematologic neoplasm.⁵ BM histopathology reveals dense perivascular and/or paratrabecular aggregates of spindle-shaped MCs with hypogranulation or abnormal nuclei. Tryptase staining is extremely sensitive for the detection of MCs,¹⁶ but it is also found in normal MCs¹⁷ and other myeloid neoplasms.⁵ In contrast, expressions of CD2 and CD25⁶ are more specific markers.¹⁸ WHO criteria for the diagnosis of SM are outlined in **Box 2**. Additional descriptors of the severity of the disease, the so-called B finding and C findings, are also reported.

THE ROLE OF KIT IN THE PATHOBIOLOGY OF MASTOCYTOSIS

Over the past few years significant progress has been made in the understanding of the pathobiology of mastocytosis. This has led to the discovery, development, and, in some cases, regulatory approval of targeted therapies. Other agents specifically interfering with the pathogenetic mechanisms of mastocytosis are under preclinical or clinical development.

Physiology

KIT (or CD117) is a type III receptor tyrosine kinase found on various cell types, including hematopoietic progenitor cells, MCs, germ cells, melanocytes, and interstitial cells of Cajal. It plays an important role in organogenesis, development, and physiologic regulation of several organs/systems.¹⁹ In the hematopoietic system, KIT expression is lost in all mature cells, but it remains high in MCs. The 145-kDa KIT molecule is encoded by a 21-exon gene on chromosome 4q12 and is composed of 5 extracellular immunoglobulin-like domains (ECD), a juxtamembrane domain (JMD), and a tyrosine-kinase domain (TKD). The first 3 Ig-like domains bind to stem cell factor (SCF), the KIT ligand. Domains 4 and 5 initiate intracellular signaling in cooperation with the JMD. The TKD contains 2 subdomains: a phosphotransferase domain (PTD) and an ATP binding site.^{20–22}

SCF is expressed by fibroblasts and endothelial cells^{23,24} and subsequently cleaved to be released in soluble form.²⁵ Binding to KIT triggers conformational changes²² that cause activation of multiple downstream kinases, and signaling through the mitogen-activated protein kinase pathway, which is required for MC activation,²⁶ or JAK-STAT pathway, required for MC development, survival, and proliferation (**Fig. 1**).²⁷

Box 2**World Health Organization criteria for the diagnosis of systemic mastocytosis**

Major criterion

Multifocal, dense infiltrates of MCs (≥ 15 MCs in aggregates) detected in sections of BM and/or other extracutaneous organs

Minor criteria

- In biopsy sections of bone marrow or other extracutaneous organs, greater than 25% of the MCs in the infiltrate are spindle-shaped or have atypical morphology or, of all MCs in BM aspirate smears, greater than 25% are immature or atypical.
- Detection of an activating point mutation at codon 816 of *KIT* in BM, blood, or other extracutaneous organ.
- MCs in BM, blood, or other extracutaneous organ express CD2 and/or CD25 in addition to normal MC markers.
- Serum total tryptase persistently exceeds 20 ng/mL (unless there is an associated clonal myeloid disorder, in which case this parameter is not valid).

Diagnosis of SM requires major criterion + 1 minor criterion or greater than or equal to 3 minor criteria

B findings

- Greater than 30% BM infiltration by MCs in focal, dense aggregates and/or serum total tryptase level greater than 20 ng/mL
- Signs of dysplasia or myeloproliferation, in non-MC lineage(s), not meeting criteria for a hematopoietic neoplasm, with normal or slightly abnormal blood counts
- Hepatomegaly and/or splenomegaly without functional impairment, and/or lymphadenopathy

C findings

- One or more cytopenia(s) without obvious non-MC hematopoietic malignancy
- Palpable hepatomegaly with impairment of liver function, ascites, and/or portal hypertension
- Osteolytic lesions and/or pathologic fractures
- Palpable splenomegaly with hypersplenism
- Malabsorption with weight loss due to gastrointestinal MC infiltrates

Adapted from Swerdlow SH, Campo E, Harris N, et al. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edition. Lyon (France): International Agency for Research on Cancer (IARC); 2017; with permission.

Molecular Epidemiology of *KIT* Mutations

A point mutation resulting in the replacement of aspartate with valine in codon 816 of the *KIT* sequence (D816V) was originally identified in the peripheral blood (PB) of both patients with CM and SM.²⁸ Later it became clear that an activating mutation of *KIT*, primarily affecting the TKD, could be found in most SM patients across disease subtypes.⁴ At a cellular level, this point mutation results in enhanced survival and autonomous growth of neoplastic MCs.²⁹

In adult patients, D816V is the most common *KIT* mutation (>90% of cases). Other mutations, such as V560G,^{30,31} D815K,³² D816Y,^{32–35} insV1815–816,³³ D816F,^{32,35} D816H,³⁶ and D820G,³⁷ have been identified. In children, mastocytosis is also associated with activating *KIT* mutations, but the frequency of *KIT* D816V is lower (25%–36%)^{19,38–40} and the type and location of alterations in the remaining cases are varied (often in the ECD), potentially accounting for the different characteristics and clinical course of the disease in the two age groups. Mutations in exons 8 and 9 have been found in rare familial mastocytosis, alone or as part of clinical syndromes.^{41–44} A synopsis of the most common *KIT* mutations and their distribution is illustrated in **Fig. 2**.

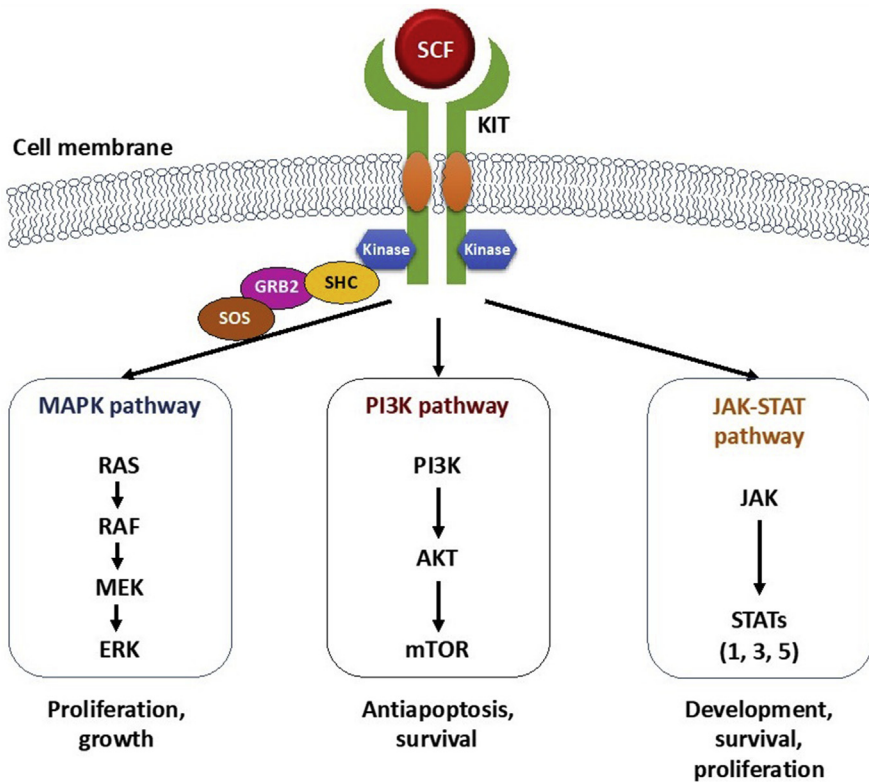


Fig. 1. Kit-mediated signaling pathways in normal MCs. SCF in its soluble form binds to KIT and promotes its dimerization and autophosphorylation. The latter causes activation of multiple downstream signaling pathways, including the mitogen-activated protein kinase pathway (through the recruitment of intermediary molecules, such as SHC and GRB2, required for MC activation), the PI3K pathways, important in MC survival, and the JAK-STAT pathway, required for MC development, survival, and proliferation). ERK, extracellular signal-regulated kinases; GRB2, growth-factor-receptor-bound protein 2; JAK-STAT, Janus kinase-signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; MEK, MAP/extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma SHC, SRC homology 2 (SH2)-domain-containing transforming protein C; SOS, son of sevenless homologue.

The type and frequency of *KIT* mutations vary to some degree in different subtypes of mastocytosis. For example, some patients with indolent SM (ISM) may carry a non-*KIT* D816V (or no *KIT* mutation), whereas those with smoldering SM, aggressive SM (ASM), and SM-AHN are nearly always *KIT* D816V-positive in MC^{17,37,45} and sometimes in non-MC populations.³³ A significant proportion of the latter also carries the mutation in the myeloid (virtually never lymphoid) AHN component,⁴ consistent with the hypothesis that transformation affects early progenitors capable of generating cells of different lineages that undergo further, distinct transformation events.¹² In patients with MCL, there is a relatively higher prevalence of non-D816V mutations or wild-type *KIT*. Finally, in MC sarcoma no *KIT* D816V mutations have been reported.⁴⁶

Several single-nucleotide polymorphisms have been described in the *KIT* gene although their clinical impact remains unclear.⁴⁷

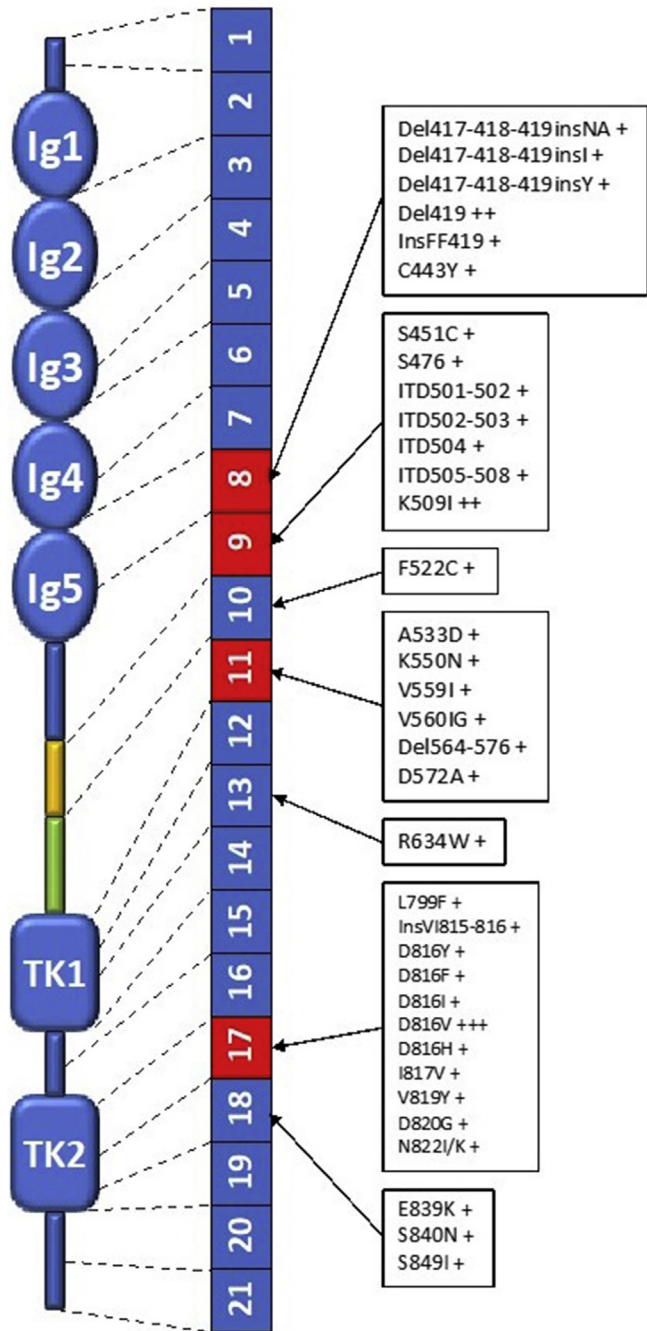


Fig. 2. Distribution of mutations of KIT (depicted in its monomeric form) in pediatric and adult patients with mastocytosis. In the former, *KIT* D816V PTD mutation (exon 17) is found in nearly 30% of the cases and in greater than 80% in the latter. A list of mutations described at each hotspot is represented. The approximate frequency of each mutation is also described: +, less than 10% of the patients; ++, 5% to 20%; +++ and approximately 30% (pediatric) or greater than 80% (adult). Del, deletion; Ig, immunoglobulin; Ins, insertion; ITD, internal tandem duplication. (Modified from Arock M, Sotlar K, Akin C, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 2015;29(6):1224–5; with permission.)

Oncogenicity

The *KIT* D816V is believed to induce conformational changes in the PTD of the receptor causing constitutive, ligand-independent activation.⁴⁸ Activating *KIT* mutations can affect regulation of the kinase domain or change the sequence of the enzymatic site itself, causing its stabilization in an activated state and/or a decreased affinity for type I tyrosine kinase inhibitors (TKIs), such as imatinib mesylate (IM), that recognize the active conformation of a kinase.⁴⁹ Rarely, more than 1 *KIT* mutation is present in the same patient.⁵⁰

In one set of experiments, transgenic mice expressed *KIT* D816V under the control of the chymase promoter. Upon sacrifice of the animals and a group of control mice, researchers noticed abnormal accumulation of MCs in spleen, heart, and lymph nodes (the chymase promoter would not be functional in hematopoietic progenitors, hence the lack of BM involvement). Only transgenic MCs could be maintained in continuous cultures. These, however, became growth factor-independent only after several months. Moreover, transgenic mice only developed clinical signs of mastocytosis after 12 months to 18 months and with a penetrance of only 30% (Fig. 3).⁵¹ These observations suggest that, despite the oncogenic potential of *KIT* D816V, additional genetic events are likely necessary for the development of overt mastocytosis in a majority of cases.

In another experience, transgenic mice expressing *KIT* D814V (the murine homolog of *KIT* D816V) under the *KIT* promoter, developed severe mastocytosis in 100% of cases early in life. In approximately 50% of them, an associated non-MC hematologic neoplasm was also found. Again, when the expression of *KIT* was restricted to more differentiated MCs, the phenotype was milder and developed later in life.⁵² Both experiments suggest that the severity of the disease might at least in part depend on the timing of acquisition of the *KIT* mutation along the differentiation chain of MCs.

Mutations of Genes other than *KIT*

The discrepancy between a unique defining mutation (*KIT* D816V) and a heterogeneous clinical course and prognosis may be explained in part by the presence of additional mutations in the MC clone. These additional alterations may also be responsible for the variable response to *KIT*-targeted therapy and are correlated with poorer outcomes.

For instance, a *TET2* mutation was detected in 20% to 29% of mastocytosis cases across subtypes, albeit more frequently in patients with aggressive forms, including SM-AHN.^{53–57} In half of these patients *KIT* D816V and *TET2* mutations coexisted. *ASXL1* is mutated in 12% to 20% of patients with mastocytosis and it seems to confer poorer prognosis.^{56–58} Other recurrent mutations involve genes of the spliceosome machinery (*SF3B1*, *SRSF2*, and *U2AF1*), *CBL*, *DNMT3A*, *KRAS*, *NRAS*, *JAK2*, *EZH2*, and *ETV6*.^{57,59,60} Some patients with SM, particularly in its later stage, harbor multiple mutations in the same MC clone,⁵⁷ often before acquiring the *KIT* D816V.⁵⁵ These individuals have a poorer prognosis. Although some mutations may contribute more strongly to the negative prognosis⁶¹ the precise contribution of each of these alterations and/or their order is presently unknown.

KIT TESTING IN THE DIAGNOSIS OF MASTOCYTOSIS

The presence of *KIT* D816V represents a minor criterion for the diagnosis of mastocytosis according to the WHO.⁶ In general, in children with CM, *KIT* mutation is screened for only if the diagnosis is not unequivocal,³⁸ or an underlying ASM is suspected.⁶² The likelihood of detecting *KIT* mutations depends on the amount of MCs in the sample and

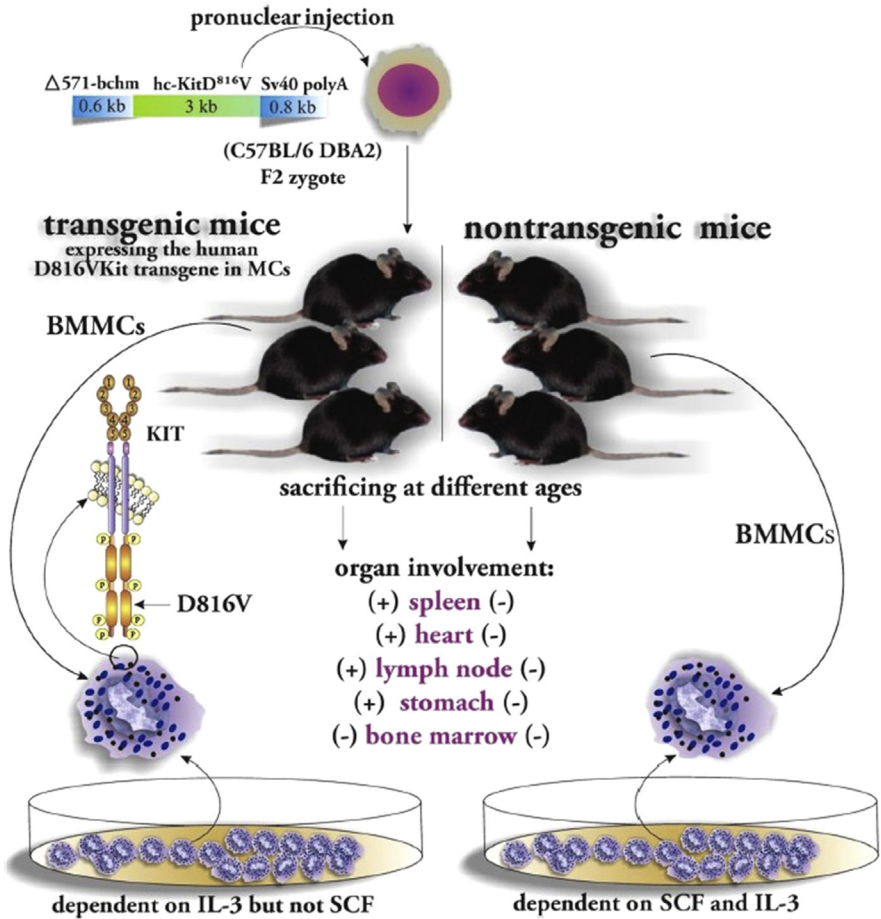


Fig. 3. Transgenic mice expressing the human D816V Kit transgene in MCs were assessed at different ages for developing SM symptoms and compared with normal nontransgenic mice. They were found to have organ involvement in the spleen, heart, lymph nodes, and stomach but not in the BM. Comparing BM-derived cultured MCs (BMMCs) of these 2 groups of mice revealed that BMMCs from the transgenic ones became independent to SCF for proliferation and activation. (From Komi DEA, Rambasek T, Wöhrl S. Mastocytosis: from a molecular point of view. *Clinic Rev Allerg Immunol* 2017; with permission.)

the sensitivity of the diagnostic technique.⁶³ In most SM patients, the *KIT* D816V allele burden is less than 1% by allele-specific oligonucleotide quantitative polymerase chain reaction (ASO-qPCR).^{64–66} Moreover, routine testing only detects *KIT* D816V. Therefore, for those SM patients who have *KIT* mutations other than D816V, dedicated assays, such as restriction fragment length polymorphism (RFLP) or high-performance liquid chromatography (HPLC) of PCR products, should be performed.^{64,67}

Historically, *KIT* testing has been performed by conventional sequencing of PCR products from BM-derived MCs.⁶⁸ Given the low diagnostic yield of this technique,⁶⁹ assays such as PCR plus RFLP,⁷⁰ peptide nucleic acid (PNA)-mediated PCR clamping,³² nested reverse transcriptase-PCR (RT-PCR) followed by denaturing HPLC (D-HPLC)⁶⁴ and ASO-qPCR assays^{64,71–73} were developed.

Several studies have consistently shown that PB ASO-qPCR allows detection of the *KIT* D816V in almost all adults with SM, regardless of the presence of MCs in the circulation.^{64–66,71} This test is currently recommended for the initial screening of individuals with suspected SM by the European Competence Network on Mastocytosis (ECNM).⁷⁴ ASO-qPCR presents other advantages. It can be performed both on fresh samples and stored material, such as on fresh-frozen paraffin-embedded tissue, including biopsy samples of the skin⁷⁵ and other affected organs.^{76,77} Moreover, because it is a quantitative method, it allows measurement of *KIT* D816V allele burden at the time of diagnosis and/or during the course of disease.^{65,71,73}

The technique has limitations. For instance, when the MC burden is very low, PB testing may be not sensitive enough and the BM by ASO-qPCR,^{64,71} or (less frequently) MC enrichment by laser microdissection or flow cytometry may need to be examined.^{33,78} In addition, ASO-qPCR cannot detect non-D816V *KIT* mutations. Therefore, in *KIT* D816V-negative patients with strong clinical suspicion for, but no formal diagnosis of, SM other techniques, such as PC plus RFLP or D-HPLC, should be considered, followed, if negative, by next-generation sequencing of the entire *KIT* coding region. This is especially important for patients with ASM or MCL who may have non-D816V *KIT* mutations sensitive to imatinib therapy. Finally, ASO-qPCR has not yet undergone rigorous standardization and harmonization among centers.

In this regard, digital droplet PCR (which allows a more accurate quantitative measurement of the target DNA without the need for calibration material)⁷⁹ was recently shown to be more sensitive than PNA-mediated PCR clamping and comparable to ASO-qPCR for the detection and quantitation of *KIT* D816V in PB and BM, with the appealing advantage of being suitable for readily interlaboratory standardization.⁷⁹ A comparison of standard assays for the detection of *KIT* mutations is outlined in **Table 1**.

Table 1 Advantages and disadvantages of standard techniques for the detection of <i>KIT</i> mutations		
Technique	Advantages	Disadvantages
RT-PCR and RFLP	• Easy to perform • Rapid • Reliable • Cost effective • Sensitivity: 0.05%	• Does not detect mutations other than in codon 816 • Not quantitative
Nested RT-PCR followed by D-HPLC of PCR products	• Detects all mutations in the amplified region	• Sensitivity: 0.5%–1% • Not quantitative • Time consuming • HPLC requires dedicated infrastructure
PNA-mediated PCR	• Detects other mutations in codon 816 or in adjacent codons • Method of choice for testing of formalin-fixed, paraffin-embedded tissue	• Not quantitative • Sensitivity: 0.1%
ASO-qPCR (performed on DNA or RNA-cDNA)	• Simple • Rapid • Inexpensive • Sensitivity: 0.01% • Quantitative	• Detects only <i>KIT</i> D816V mutation • Requires validation and interlaboratory standardization and harmonization

Abbreviations: cDNA, complementary DNA.

OTHER USES OF KIT MUTATION TESTING

In retrospective series of patients with mastocytosis, the *KIT* D816V allele burden was found to gradually increase in advanced disease stages, from CM to ASM. Moreover, an increase in the allele burden over time correlated with disease progression. Conversely, a response to cytoreductive therapy was accompanied by a decrease of it.^{64,66} Based on these observations, the ECNM recommends measuring the *KIT* D816V allele burden in patients with previously stable ISM who display signs of disease progression and in patients with aggressive forms treated with cytoreductive therapies to monitor the response to treatment.⁸⁰

KIT testing can be helpful in certain diagnostically challenging situations. For instance, patients who do not meet criteria for a formal diagnosis of mastocytosis, but present with symptoms of MC activation and have clonal MCs by *KIT* mutation testing or CD25 staining, are assigned a provisional diagnosis of “monoclonal mast cell activation syndrome.” Its course and tendency to evolve into a more aggressive form are undefined.⁸¹ On the other hand, some patients with SM may have a relatively uninformative BM picture, but are found to have non-D816V *KIT* mutations and may be sensitive to IM therapy.^{33,43}

A recent prospective study confirmed the concordance of PB and BM *KIT* D816V mutation analysis (of 44 patients who tested positive in PB, 43 did so in BM as well). Moreover, among 13 patients with SM who had presented with Hymenoptera venom-induced anaphylaxis as the only manifestation of disease, 12 tested D816V-positive. Without molecular testing, an SM diagnosis could have been easily missed in these individuals. Among the 14 patients who tested negative, 5 were subsequently found to have SM, indicating that BM biopsy cannot be avoided in adults with strong suspicion for SM.⁸²

Finally, an important diagnostic distinction is made between *KIT*-mutated SM and Philadelphia chromosome-negative MPN with features of chronic eosinophilic leukemia associated with splenomegaly, marked elevation of vitamin B₁₂, elevation of tryptase, and increased BM MCs. In the latter, MCs are usually scattered rather than clustered and rearrangements of platelet derived growth factor A (*PDGFRA*) or *PDGFRB* are commonly found, conferring sensitivity to IM treatment.⁶

THERAPEUTIC RELEVANCE OF KIT MUTATIONS

In contrast to other hematologic malignancies, where the knowledge of specific genetic lesions has been critical to direct the therapeutic strategy, in SM, such knowledge has been less consequential, partly because of the limited efficacy of *KIT*-targeted approaches in this patient population and partly because of the significant genetic complexity of the disease.

Nonetheless, understanding the structural changes of *KIT* has helped explain how certain mutations determine sensitivity or resistance to targeted drugs.^{83,84}

Two drugs are currently approved by the US Food and Drug Administration (FDA) for the treatment of patients with SM. IM is approved for use in adult patients with ASM without the *KIT* D816V mutation as determined with an FDA-approved test or with *KIT* mutational status unknown. Midostaurin is approved for the treatment of patients with ASM, SM-AHN, or MCL, regardless of their *KIT* D816V mutational status.

Early small studies showed variable responses to IM in all-comer SM patients. Among those with *KIT* D816V responses were also variable (0%–36%).^{85–88} Because it is an ATP-competitive TKI, IM can only inhibit mutations that modulate the receptor's conformational change. Thus, patients with *KIT* mutations outside the PTD (ie, JMD or ECD) exhibit response to imatinib.^{42,89–91} In contrast, in *KIT* D816V mutants, the

receptor is found in a permanently active conformation that cannot be inhibited by imatinib.^{48,87,92} Other instances in which IM has shown remarkable efficacy include patients with transmembrane domain *KIT* mutations (F522C or K509I),^{42,43} individuals with familial syndromes associated with mastocytosis,⁴² and patients with wild-type *KIT*.⁹³

Midostaurin (PKC412) is a pleiotropic kinase inhibitor with activity against *KIT* mutants, including D816V.^{94,95} After encouraging preliminary results from small studies,⁹⁶ midostaurin was tested, at a dose of 100 mg twice daily, in a multinational phase 2 study that included 116 patients with advanced SM, 87% of whom were D816V-positive. Among 89 patients evaluable for efficacy, the overall response rate was 60%, consistently across ASM, SM-AHN, and MCL subtypes and regardless of the presence of *KIT* D816V. Remarkably, progression-free survival lasted approximately 1 year for patients in the latter 2 groups.⁹⁷ Based on these results, the FDA approved midostaurin for the treatment of aggressive SM on April 28, 2017.

In an effort to identify biomarkers of response, a recent study sought to evaluate the impact of mutations in *SRSF2*, *ASXL1*, and/or *RUNX1* [S/A/R] in 38 midostaurin-treated ASM patients. Responses and survival were significantly lower in S/A/R-positive compared with non-S/A/R patients. In multivariate analysis, however, only a reduction of *KIT* D816V allele burden greater than or equal to 25% was independently associated with improved survival. Patients with progression of disease had acquired additional mutations (*K/NRAS*, *RUNX1*, *IDH2*, or *NPM1*).⁹⁸

Other TKIs have been tested and/or are in development for SM. Dasatinib and nilotinib showed good preclinical activity against *KIT* mutants, including D816V, but modest clinical efficacy in patients with ASM.^{99–101} Ponatinib, another multikinase inhibitor, showed activity against *KIT* V560G and *KIT* D816V cell lines¹⁰² and may be synergistic with midostaurin.¹⁰³ Avapritinib (BLU-285) is a highly selective *KIT* D816V inhibitor, with activity in preclinical models, including those resistant to midostaurin.^{61,104} A phase 1 trial of this drug in patients with ASM, most with 1 or more additional mutations, was recently presented. Significant clinical benefit was seen in the majority of patients. Responses occurred rapidly and were durable. Treatment was well tolerated.¹⁰⁵ Masatinib mesilate (AB1010) is a *KIT* and *LYN* inhibitor¹⁰⁶ with weak activity against *KIT* D816V. This agent is being developed for patients with ISM, where it showed promising activity and good tolerability in pilot experiences^{107,108} and, more recently, proved superior to placebo in randomized phase 3 trial.¹⁰⁹ Finally, strategies have been developed to target the so-called switch-pocket region of *KIT*, which confers the receptor either an active or inactive conformation. DP-2976 and DP-4851 are non-ATP competitors that showed potent antineoplastic activity in primary malignant MCs.¹¹⁰ DCC-2618 is a type II switch-pocket control inhibitor, which potently inhibits exon 17 *KIT* mutations resistant to conventional TKI and is being tested in an ongoing phase 1 trial (NCT02571036).¹¹¹

SUMMARY

Mastocytosis is a rare WHO-defined distinct clonal disorder, according to the latest version of the WHO classification of myeloid tumors. Despite significant heterogeneity from clinical and prognostic points of view, at a molecular level mastocytosis is characterized by a mutation of the *KIT* gene sequence, most commonly the D816V, in almost all cases across disease subtypes. Thus, although *KIT* D816V can be considered a driver lesion based on experiments in animal models, other genetic events are likely responsible for the phenotypic diversity of the disease.

On the other hand, the discovery of *KIT* mutations as central to the pathobiology of mastocytosis, and the availability of *KIT* inhibitors, opened avenues for the development of targeted TKIs, including FDA-approved IM and midostaurin, as well as promising new agents (such as avapritinib).

Several other mutations involving genes other than *KIT*, such as *TET2*, *ASXL1*, and *DNMT3A*, among others, may preexist or accumulate in the neoplastic MC clone during the course of disease. These alterations likely contribute to the clinicopathologic diversity of mastocytosis, cause resistance to targeted (and conventional) therapy, and, ultimately, impart poorer prognosis. A deeper understanding of the complexity and dynamics of the genetic events occurring in mastocytosis is required to better identify patients who can most benefit from current therapies and to develop new agents capable of improving the outcome of patients with advanced SM.

REFERENCES

1. Cohen SS, Skovbo S, Vestergaard H, et al. Epidemiology of systemic mastocytosis in Denmark. *Br J Haematol* 2014;166(4):521–8.
2. Brockow K. Epidemiology, prognosis, and risk factors in mastocytosis. *Immunol Allergy Clin North Am* 2014;34(2):283–95.
3. Uzzaman A, Maric I, Noel P, et al. Pediatric-onset mastocytosis: a long term clinical follow-up and correlation with bone marrow histopathology. *Pediatr Blood Cancer* 2009;53(4):629–34.
4. Lim KH, Tefferi A, Lasho TL, et al. Systemic mastocytosis in 342 consecutive adults: survival studies and prognostic factors. *Blood* 2009;113(23):5727–36.
5. Horny HP, Sotlar K, Sperr WR, et al. Systemic mastocytosis with associated clonal haematological non-mast cell lineage diseases: a histopathological challenge. *J Clin Pathol* 2004;57(6):604–8.
6. Swerdlow SH, Campo E, Harris N, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th edition. Lyon (France): International Agency for research on Cancer (IARC); 2017.
7. Carter MC, Metcalfe DD, Komarow HD. Mastocytosis. *Immunol Allergy Clin North Am* 2014;34(1):181–96.
8. Picard M, Giavina-Bianchi P, Mezzano V, et al. Expanding spectrum of mast cell activation disorders: monoclonal and idiopathic mast cell activation syndromes. *Clin Ther* 2013;35(5):548–62.
9. Hannah-Shmouni F, Stratakis CA, Koch CA. Flushing in (neuro) endocrinology. *Rev Endocr Metab Disord* 2016;17(3):373–80.
10. Castells M, Austen KF. Mastocytosis: mediator-related signs and symptoms. *Int Arch Allergy Immunol* 2002;127(2):147–52.
11. Valent P, Akin C, Sperr WR, et al. Smouldering mastocytosis: a novel subtype of systemic mastocytosis with slow progression. *Int Arch Allergy Immunol* 2002;127(2):137–9.
12. Wang SA, Hutchinson L, Tang G, et al. Systemic mastocytosis with associated clonal hematological non-mast cell lineage disease: clinical significance and comparison of chromosomal abnormalities in SM and AHNMD components. *Am J Hematol* 2013;88(3):219–24.
13. Alvarez-Twose I, Vano-Galvan S, Sanchez-Munoz L, et al. Increased serum baseline tryptase levels and extensive skin involvement are predictors for the severity of mast cell activation episodes in children with mastocytosis. *Allergy* 2012;67(6):813–21.

14. Hartmann K, Escribano L, Grattan C, et al. Cutaneous manifestations in patients with mastocytosis: consensus report of the European competence network on mastocytosis; the American academy of allergy, asthma & immunology; and the European academy of allergology and clinical immunology. *J Allergy Clin Immunol* 2016;137(1):35–45.
15. Sperr WR, El-Samahi A, Kundi M, et al. Elevated tryptase levels selectively cluster in myeloid neoplasms: a novel diagnostic approach and screen marker in clinical haematology. *Eur J Clin Invest* 2009;39(10):914–23.
16. Horny HP, Sillaber C, Menke D, et al. Diagnostic value of immunostaining for tryptase in patients with mastocytosis. *Am J Surg Pathol* 1998;22(9):1132–40.
17. Jordan JH, Walchshofer S, Jurecka W, et al. Immunohistochemical properties of bone marrow mast cells in systemic mastocytosis: evidence for expression of CD2, CD117/Kit, and bcl-x(L). *Hum Pathol* 2001;32(5):545–52.
18. Sotlar K, Horny HP, Simonitsch I, et al. CD25 indicates the neoplastic phenotype of mast cells: a novel immunohistochemical marker for the diagnosis of systemic mastocytosis (SM) in routinely processed bone marrow biopsy specimens. *Am J Surg Pathol* 2004;28(10):1319–25.
19. Yanagihori H, Oyama N, Nakamura K, et al. c-kit Mutations in patients with childhood-onset mastocytosis and genotype-phenotype correlation. *J Mol Diagn* 2005;7(2):252–7.
20. Giebel LB, Strunk KM, Holmes SA, et al. Organization and nucleotide sequence of the human KIT (mast/stem cell growth factor receptor) proto-oncogene. *Oncogene* 1992;7(11):2207–17.
21. Chabot B, Stephenson DA, Chapman VM, et al. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature* 1988;335(6185):88–9.
22. Lennartsson J, Ronnstrand L. Stem cell factor receptor/c-Kit: from basic science to clinical implications. *Physiol Rev* 2012;92(4):1619–49.
23. Bai CG, Hou XW, Wang F, et al. Stem cell factor-mediated wild-type KIT receptor activation is critical for gastrointestinal stromal tumor cell growth. *World J Gastroenterol* 2012;18(23):2929–37.
24. Cruse G, Metcalfe DD, Olivera A. Functional deregulation of KIT: link to mast cell proliferative diseases and other neoplasms. *Immunol Allergy Clin North Am* 2014;34(2):219–37.
25. Duttlinger R, Manova K, Chu TY, et al. W-sash affects positive and negative elements controlling c-kit expression: ectopic c-kit expression at sites of kit-ligand expression affects melanogenesis. *Development* 1993;118(3):705–17.
26. Metcalfe DD. Mast cells and mastocytosis. *Blood* 2008;112(4):946–56.
27. Morales JK, Falanga YT, Depcrynski A, et al. Mast cell homeostasis and the JAK-STAT pathway. *Genes Immun* 2010;11(8):599–608.
28. Akin C, Kirshenbaum AS, Semere T, et al. Analysis of the surface expression of c-kit and occurrence of the c-kit Asp816Val activating mutation in T cells, B cells, and myelomonocytic cells in patients with mastocytosis. *Exp Hematol* 2000;28(2):140–7.
29. Piao X, Bernstein A. A point mutation in the catalytic domain of c-kit induces growth factor independence, tumorigenicity, and differentiation of mast cells. *Blood* 1996;87(8):3117–23.
30. Buttner C, Henz BM, Welker P, et al. Identification of activating c-kit mutations in adult-, but not in childhood-onset indolent mastocytosis: a possible explanation for divergent clinical behavior. *J Invest Dermatol* 1998;111(6):1227–31.

31. Furitsu T, Tsujimura T, Tono T, et al. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J Clin Invest* 1993; 92(4):1736–44.
32. Sotlar K, Escribano L, Landt O, et al. One-step detection of c-kit point mutations using peptide nucleic acid-mediated polymerase chain reaction clamping and hybridization probes. *Am J Pathol* 2003;162(3):737–46.
33. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood* 2006;108(7):2366–72.
34. Beghini A, Cairoli R, Morra E, et al. In vivo differentiation of mast cells from acute myeloid leukemia blasts carrying a novel activating ligand-independent C-kit mutation. *Blood Cells Mol Dis* 1998;24(2):262–70.
35. Longley BJ Jr, Metcalfe DD, Tharp M, et al. Activating and dominant inactivating c-KIT catalytic domain mutations in distinct clinical forms of human mastocytosis. *Proc Natl Acad Sci U S A* 1999;96(4):1609–14.
36. Pullarkat VA, Pullarkat ST, Calverley DC, et al. Mast cell disease associated with acute myeloid leukemia: detection of a new c-kit mutation Asp816His. *Am J Hematol* 2000;65(4):307–9.
37. Pignon JM, Giraudier S, Duquesnoy P, et al. A new c-kit mutation in a case of aggressive mast cell disease. *Br J Haematol* 1997;96(2):374–6.
38. Bodemer C, Hermine O, Palmerini F, et al. Pediatric mastocytosis is a clonal disease associated with D816V and other activating c-KIT mutations. *J Invest Dermatol* 2010;130(3):804–15.
39. Verzijl A, Heide R, Oranje AP, et al. C-kit Asp-816-Val mutation analysis in patients with mastocytosis. *Dermatology* 2007;214(1):15–20.
40. Bibi S, Langenfeld F, Jeanningros S, et al. Molecular defects in mastocytosis: KIT and beyond KIT. *Immunol Allergy Clin North Am* 2014;34(2):239–62.
41. Hartmann K, Wardelmann E, Ma Y, et al. Novel germline mutation of KIT associated with familial gastrointestinal stromal tumors and mastocytosis. *Gastroenterology* 2005;129(3):1042–6.
42. Zhang LY, Smith ML, Schultheis B, et al. A novel K509I mutation of KIT identified in familial mastocytosis-in vitro and in vivo responsiveness to imatinib therapy. *Leuk Res* 2006;30(4):373–8.
43. Akin C, Fumo G, Yavuz AS, et al. A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood* 2004;103(8):3222–5.
44. Tang X, Boxer M, Drummond A, et al. A germline mutation in KIT in familial diffuse cutaneous mastocytosis. *J Med Genet* 2004;41(6):e88.
45. Sotlar K, Colak S, Bache A, et al. Variable presence of KITD816V in clonal haematological non-mast cell lineage diseases associated with systemic mastocytosis (SM-AHNMD). *J Pathol* 2010;220(5):586–95.
46. Georgin-Lavialle S, Aguilar C, Guieze R, et al. Mast cell sarcoma: a rare and aggressive entity—report of two cases and review of the literature. *J Clin Oncol* 2013;31(6):e90–7.
47. Nagata H, Worobec AS, Metcalfe DD. Identification of a polymorphism in the transmembrane domain of the protooncogene c-kit in healthy subjects. *Exp Clin Immunogenet* 1996;13(3–4):210–4.
48. Laine E, Chauvot de Beauchene I, Perahia D, et al. Mutation D816V alters the internal structure and dynamics of c-KIT receptor cytoplasmic region:

- implications for dimerization and activation mechanisms. *PLoS Comput Biol* 2011;7(6):e1002068.
49. Longley BJ, Reguera MJ, Ma Y. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk Res* 2001;25(7):571–6.
 50. Lasho T, Finke C, Zblewski D, et al. Concurrent activating KIT mutations in systemic mastocytosis. *Br J Haematol* 2016;173(1):153–6.
 51. Zappulla JP, Dubreuil P, Desbois S, et al. Mastocytosis in mice expressing human Kit receptor with the activating Asp816Val mutation. *J Exp Med* 2005; 202(12):1635–41.
 52. Gerbaulet A, Wickenhauser C, Scholten J, et al. Mast cell hyperplasia, B-cell malignancy, and intestinal inflammation in mice with conditional expression of a constitutively active kit. *Blood* 2011;117(6):2012–21.
 53. Tefferi A, Levine RL, Lim KH, et al. Frequent TET2 mutations in systemic mastocytosis: clinical, KITD816V and FIP1L1-PDGFRA correlates. *Leukemia* 2009; 23(5):900–4.
 54. Soucie E, Hanssens K, Mercher T, et al. In aggressive forms of mastocytosis, TET2 loss cooperates with c-KITD816V to transform mast cells. *Blood* 2012; 120(24):4846–9.
 55. Jawhar M, Schwaab J, Schnittger S, et al. Molecular profiling of myeloid progenitor cells in multi-mutated advanced systemic mastocytosis identifies KIT D816V as a distinct and late event. *Leukemia* 2015;29(5):1115–22.
 56. Traina F, Visconte V, Jankowska AM, et al. Single nucleotide polymorphism array lesions, TET2, DNMT3A, ASXL1 and CBL mutations are present in systemic mastocytosis. *PLoS One* 2012;7(8):e43090.
 57. Schwaab J, Schnittger S, Sotlar K, et al. Comprehensive mutational profiling in advanced systemic mastocytosis. *Blood* 2013;122(14):2460–6.
 58. Damaj G, Joris M, Chandesris O, et al. ASXL1 but not TET2 mutations adversely impact overall survival of patients suffering systemic mastocytosis with associated clonal hematologic non-mast-cell diseases. *PLoS One* 2014;9(1):e85362.
 59. Wilson TM, Maric I, Simakova O, et al. Clonal analysis of NRAS activating mutations in KIT-D816V systemic mastocytosis. *Haematologica* 2011;96(3):459–63.
 60. Hanssens K, Brenet F, Agopian J, et al. SRSF2-p95 hotspot mutation is highly associated with advanced forms of mastocytosis and mutations in epigenetic regulator genes. *Haematologica* 2014;99(5):830–5.
 61. Jawhar M, Schwaab J, Schnittger S, et al. Additional mutations in SRSF2, ASXL1 and/or RUNX1 identify a high-risk group of patients with KIT D816V(+) advanced systemic mastocytosis. *Leukemia* 2016;30(1):136–43.
 62. Valent P, Akin C, Escribano L, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest* 2007;37(6):435–53.
 63. Akin C. Molecular diagnosis of mast cell disorders: a paper from the 2005 William Beaumont Hospital Symposium on Molecular Pathology. *J Mol Diagn* 2006; 8(4):412–9.
 64. Erben P, Schwaab J, Metzgeroth G, et al. The KIT D816V expressed allele burden for diagnosis and disease monitoring of systemic mastocytosis. *Ann Hematol* 2014;93(1):81–8.
 65. Kristensen T, Broesby-Olsen S, Vestergaard H, et al. Mastocytosis Centre Odense University Hospital (MastOUH). Circulating KIT D816V mutation-positive non-mast cells in peripheral blood are characteristic of indolent systemic mastocytosis. *Eur J Haematol* 2012;89(1):42–6.

66. Hoermann G, Gleixner KV, Dinu GE, et al. The KIT D816V allele burden predicts survival in patients with mastocytosis and correlates with the WHO type of the disease. *Allergy* 2014;69(6):810–3.
67. Valent P, Akin C, Sperr WR, et al. Mastocytosis: pathology, genetics, and current options for therapy. *Leuk Lymphoma* 2005;46(1):35–48.
68. Sotlar K. c-kit mutational analysis in paraffin material. *Methods Mol Biol* 2013; 999:59–78.
69. Corless CL, Harrell P, Lacouture M, et al. Allele-specific polymerase chain reaction for the imatinib-resistant KIT D816V and D816F mutations in mastocytosis and acute myelogenous leukemia. *J Mol Diagn* 2006;8(5):604–12.
70. Tan A, Westerman D, McArthur GA, et al. Sensitive detection of KIT D816V in patients with mastocytosis. *Clin Chem* 2006;52(12):2250–7.
71. Kristensen T, Vestergaard H, Bindslev-Jensen C, et al. Mastocytosis Centre OUH. Sensitive KIT D816V mutation analysis of blood as a diagnostic test in mastocytosis. *Am J Hematol* 2014;89(5):493–8.
72. Schumacher JA, Elenitoba-Johnson KS, Lim MS. Detection of the c-kit D816V mutation in systemic mastocytosis by allele-specific PCR. *J Clin Pathol* 2008; 61(1):109–14.
73. Kristensen T, Vestergaard H, Moller MB. Improved detection of the KIT D816V mutation in patients with systemic mastocytosis using a quantitative and highly sensitive real-time qPCR assay. *J Mol Diagn* 2011;13(2):180–8.
74. Valent P, Escribano L, Broesby-Olsen S, et al. Proposed diagnostic algorithm for patients with suspected mastocytosis: a proposal of the European Competence Network on Mastocytosis. *Allergy* 2014;69(10):1267–74.
75. Kristensen T, Broesby-Olsen S, Vestergaard H, et al. KIT D816V mutation-positive cell fractions in lesional skin biopsies from adults with systemic mastocytosis. *Dermatology* 2013;226(3):233–7.
76. Jensen RT. Gastrointestinal abnormalities and involvement in systemic mastocytosis. *Hematol Oncol Clin North Am* 2000;14(3):579–623.
77. Wimazal F, Schwarzmeier J, Sotlar K, et al. Splenic mastocytosis: report of two cases and detection of the transforming somatic C-KIT mutation D816V. *Leuk Lymphoma* 2004;45(4):723–9.
78. Sotlar K, Marafioti T, Griesser H, et al. Detection of c-kit mutation Asp 816 to Val in microdissected bone marrow infiltrates in a case of systemic mastocytosis associated with chronic myelomonocytic leukaemia. *Mol Pathol* 2000;53(4): 188–93.
79. Greiner G, Gurbisz M, Ratzinger F, et al. Digital PCR: a sensitive and precise method for KIT D816V quantification in mastocytosis. *Clin Chem* 2017;64(3): 547–55.
80. Arock M, Sotlar K, Akin C, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 2015;29(6):1223–32.
81. Valent P, Akin C, Arock M, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012;157(3):215–25.
82. Kristensen T, Vestergaard H, Bindslev-Jensen C, et al. Prospective evaluation of the diagnostic value of sensitive KIT D816V mutation analysis of blood in adults with suspected systemic mastocytosis. *Allergy* 2017;72(11):1737–43.
83. Frost MJ, Ferrao PT, Hughes TP, et al. Juxtamembrane mutant V560GKit is more sensitive to Imatinib (STI571) compared with wild-type c-kit whereas the kinase domain mutant D816VKit is resistant. *Mol Cancer Ther* 2002;1(12):1115–24.

84. Lasota J. Not all c-kit mutations can be corrected by imatinib. *Lab Invest* 2007; 87(4):317.
85. Lim KH, Pardanani A, Butterfield JH, et al. Cytoreductive therapy in 108 adults with systemic mastocytosis: outcome analysis and response prediction during treatment with interferon-alpha, hydroxyurea, imatinib mesylate or 2-chlorodeoxyadenosine. *Am J Hematol* 2009;84(12):790–4.
86. Droogendijk HJ, Kluin-Nelemans HJ, van Doormaal JJ, et al. Imatinib mesylate in the treatment of systemic mastocytosis: a phase II trial. *Cancer* 2006;107(2): 345–51.
87. Vega-Ruiz A, Cortes JE, Sever M, et al. Phase II study of imatinib mesylate as therapy for patients with systemic mastocytosis. *Leuk Res* 2009;33(11):1481–4.
88. Pagano L, Valentini CG, Caira M, et al. Advanced mast cell disease: an Italian Hematological Multicenter experience. *Int J Hematol* 2008;88(5):483–8.
89. Hoffmann KM, Moser A, Lohse P, et al. Successful treatment of progressive cutaneous mastocytosis with imatinib in a 2-year-old boy carrying a somatic KIT mutation. *Blood* 2008;112(5):1655–7.
90. Alvarez-Twose I, Gonzalez P, Morgado JM, et al. Complete response after imatinib mesylate therapy in a patient with well-differentiated systemic mastocytosis. *J Clin Oncol* 2012;30(12):e126–9.
91. Mital A, Piskorz A, Lewandowski K, et al. A case of mast cell leukaemia with exon 9 KIT mutation and good response to imatinib. *Eur J Haematol* 2011; 86(6):531–5.
92. Foster R, Griffith R, Ferrao P, et al. Molecular basis of the constitutive activity and STI571 resistance of Asp816Val mutant KIT receptor tyrosine kinase. *J Mol Graph Model* 2004;23(2):139–52.
93. Georgin-Lavialle S, Lhermitte L, Suarez F, et al. Mast cell leukemia: identification of a new c-Kit mutation, dup(501-502), and response to masitinib, a c-Kit tyrosine kinase inhibitor. *Eur J Haematol* 2012;89(1):47–52.
94. Fabbro D, Ruetz S, Bodis S, et al. PKC412—a protein kinase inhibitor with a broad therapeutic potential. *Anticancer Drug Des* 2000;15(1):17–28.
95. Gowney JD, Clark JJ, Adelsperger J, et al. Activation mutations of human c-KIT resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. *Blood* 2005;106(2):721–4.
96. Gallogly MM, Lazarus HM. Midostaurin: an emerging treatment for acute myeloid leukemia patients. *J Blood Med* 2016;7:73–83.
97. Gotlib J, Kluin-Nelemans HC, George TI, et al. Efficacy and safety of midostaurin in advanced systemic mastocytosis. *N Engl J Med* 2016;374(26):2530–41.
98. Jawhar M, Schwaab J, Naumann N, et al. Response and progression on midostaurin in advanced systemic mastocytosis: KIT D816V and other molecular markers. *Blood* 2017;130(2):137–45.
99. Verstovsek S, Akin C, Manshouri T, et al. Effects of AMN107, a novel aminopyrimidine tyrosine kinase inhibitor, on human mast cells bearing wild-type or mutated codon 816 c-kit. *Leuk Res* 2006;30(11):1365–70.
100. Shah NP, Lee FY, Luo R, et al. Dasatinib (BMS-354825) inhibits KITD816V, an imatinib-resistant activating mutation that triggers neoplastic growth in most patients with systemic mastocytosis. *Blood* 2006;108(1):286–91.
101. Hochhaus A, Baccarani M, Giles FJ, et al. Nilotinib in patients with systemic mastocytosis: analysis of the phase 2, open-label, single-arm nilotinib registration study. *J Cancer Res Clin Oncol* 2015;141(11):2047–60.

102. Jin B, Ding K, Pan J. Ponatinib induces apoptosis in imatinib-resistant human mast cells by dephosphorylating mutant D816V KIT and silencing beta-catenin signaling. *Mol Cancer Ther* 2014;13(5):1217–30.
103. Gleixner KV, Peter B, Blatt K, et al. Synergistic growth-inhibitory effects of ponatinib and midostaurin (PKC412) on neoplastic mast cells carrying KIT D816V. *Haematologica* 2013;98(9):1450–7.
104. Evans EK, Gardino AK, Kim JL, et al. A precision therapy against cancers driven by KIT/PDGFRA mutations. *Sci Transl Med* 2017;9(414) [pii:eaao1690].
105. DeAngelo DJ, Quiery AT, Radia D, et al. Clinical activity in a phase 1 study of Blu-285, a potent, highly-selective inhibitor of KIT D816V in advanced systemic mastocytosis (AdvSM). *Blood* 2017;130:2.
106. Dubreuil P, Letard S, Ciufolini M, et al. Masitinib (AB1010), a potent and selective tyrosine kinase inhibitor targeting KIT. *PLoS One* 2009;4(9):e7258.
107. Paul C, Sans B, Suarez F, et al. Masitinib for the treatment of systemic and cutaneous mastocytosis with handicap: a phase 2a study. *Am J Hematol* 2010;85(12):921–5.
108. Hermine O, Lortholary O, Leventhal PS, et al. Case-control cohort study of patients' perceptions of disability in mastocytosis. *PLoS One* 2008;3(5):e2266.
109. Lortholary O, Chandesris MO, Bulai Livideanu C, et al. Masitinib for treatment of severely symptomatic indolent systemic mastocytosis: a randomised, placebo-controlled, phase 3 study. *Lancet* 2017;389(10069):612–20.
110. Bai Y, Bandara G, Ching Chan E, et al. Targeting the KIT activating switch control pocket: a novel mechanism to inhibit neoplastic mast cell proliferation and mast cell activation. *Leukemia* 2013;27(2):278–85.
111. Schneeweiss MA, Peter B, Blatt K, et al. The multi-kinase inhibitor DCC-2618 inhibits proliferation and survival of neoplastic mast cells and other cell types involved in systemic mastocytosis. *Blood* 2016;128:1965.