

Molecular Defects in Mastocytosis

KIT and Beyond *KIT*

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KEYWORDS

- Systemic mastocytosis • *KIT* • Mutation • Signaling • *TET2* • *ASXL1* • Spliceosome
- Targeted therapy

KEY POINTS

- The *KIT* D816V mutation is found in almost all the adult patients presenting with different subvariants of systemic mastocytosis (SM).
- The clinical course and prognosis of the different subvariants vary greatly among patients with SM.
- Additional genetic lesions and aberrant overexpression of signaling pathways are found in aggressive SM and SM with associated hematologic non-mast cell-lineage disease.
- These additional genetic aberrations or overexpression of signaling pathways are associated with progression of the disease and worsen significantly the prognosis of patients with SM.

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INTRODUCTION

Mast cells (MCs) are multifunctional immune cells derived from hematopoietic stem cell (HSC) in the bone marrow (BM). Committed BM MC progenitors (MCPs) are released into the bloodstream, where they have been identified in humans as CD34+/KIT+/CD13+/FcεRI- cells.¹ Human MCPs migrate into the peripheral tissues, where they differentiate terminally into 2 major subtypes: MC_{tryptase} (MC_T) and MC_{tryptase-chymase} (MC_{TC}). MC_T are found preferentially in mucosal tissues, whereas MC_{TC} are prominent in serosal tissues.^{2,3} For both MC subtypes, the major growth and differentiation factor is stem cell factor (SCF), which binds KIT (CD117), a transmembrane receptor with intrinsic tyrosine kinase (TK) activity.⁴

Mastocytosis comprises a heterogeneous group of rare diseases characterized by abnormal accumulation of more or less atypical MCs in 1 or more organs.⁵ Although mastocytosis can affect either children or adults, the behavior of the disease appears different not only in children (disease frequently restricted to the skin and attenuating at puberty) versus adults (disease constantly systemic and chronic) but also between adults with systemic involvement.⁵ Adult patients may suffer from an indolent form of the disease or may show aggressive or even leukemic variants.⁶ However, in most adults, a recurrent abnormality in the *KIT* gene (mainly *KIT* D816V) is found in neoplastic MCs.⁷ This discrepancy between the recurrent genotype and the variable severity of the disease has led several teams to investigate for, then to find, the presence of associated non-*KIT* molecular defects, particularly in advanced systemic mastocytosis (advSM).^{8–10}

In the first part of this review, an overview of the implications of *KIT* in MC development and functions is provided. The mechanisms involved in the transforming potential of *KIT* mutants found in the various subcategories of mastocytosis and their consequences for treatment are then discussed. The additional genetic defects found in several cohorts of patients and their impact in terms of severity and progression of the disease are described, as well as their possible implications for future therapeutic considerations.

CRITICAL ROLES OF SCF AND *KIT* IN THE DEVELOPMENT AND BIOLOGY OF MCs

Mice with loss-of-function mutations affecting either synthesis of SCF (Sl/Sld mice) or *Kit* (W/Wv mice) are virtually devoid of MCs, showing the importance of the SCF/*Kit* axis in MCs.¹¹ In contrast, gain-of-function mutations in the *KIT* proto-oncogene are associated with enhanced survival and autonomous growth of MCs and their progenitors.¹² In addition, the injection of SCF increases the number of MCs by more than 100 times near the site of injection.¹³ MCs are the only terminally differentiated hematopoietic cells expressing KIT, and SCF promotes not only differentiation of MCs from their MCPs but also adhesion, migration, and survival, as well as mediator release from mature MCs.²

Structure of SCF

SCF is encoded by a gene on chromosome 12q22-12q24 in humans.¹⁴ The gene is alternatively spliced, leading to 2 SCF isoforms, which differ in the absence or presence of a particular proteolytic cleavage site. The isoform containing the cleavage site undergoes proteolysis and becomes soluble (sSCF of 18 kDa), as a 165 amino acid protein, with the first 141 residues necessary for its receptor-binding activity.¹⁵ The isoform lacking the cleavage site remains cell associated as a 31-kDa membrane-bound form (mSCF).¹⁵

Normal Structure of KIT

The proto-oncogene *KIT*, located on chromosome 4q12 in humans,¹⁶ contains 21 exons transcribed/translated into a transmembrane receptor TK (RTK) of 145 kDa and 976 amino acids.¹⁷ KIT structure is characterized by 5 immunoglobulin (Ig)-like subunits in the extracellular domain (ECD), which contains the binding site for SCF, and is linked to a cytoplasmic region by a single transmembrane helix (Fig. 1).¹⁸

The cytoplasmic region of KIT consists of an autoinhibitory juxtamembrane domain (JMD) and a kinase domain (KD) arranged in a proximal (N-) and a distal (C-) lobe linked by a hinge region (see Fig. 1). The C-lobe of KIT includes a large kinase insert domain of 77 residues.

Binding of SCF to the ECD induces KIT dimerization, initiating the transphosphorylation of specific tyrosine residues.¹⁸ The intracellular portion of KIT shows 21 tyrosine residues, from which at least 10 are phosphorylated after activation of the receptor.¹⁸ They serve as binding sites to recruit downstream signaling

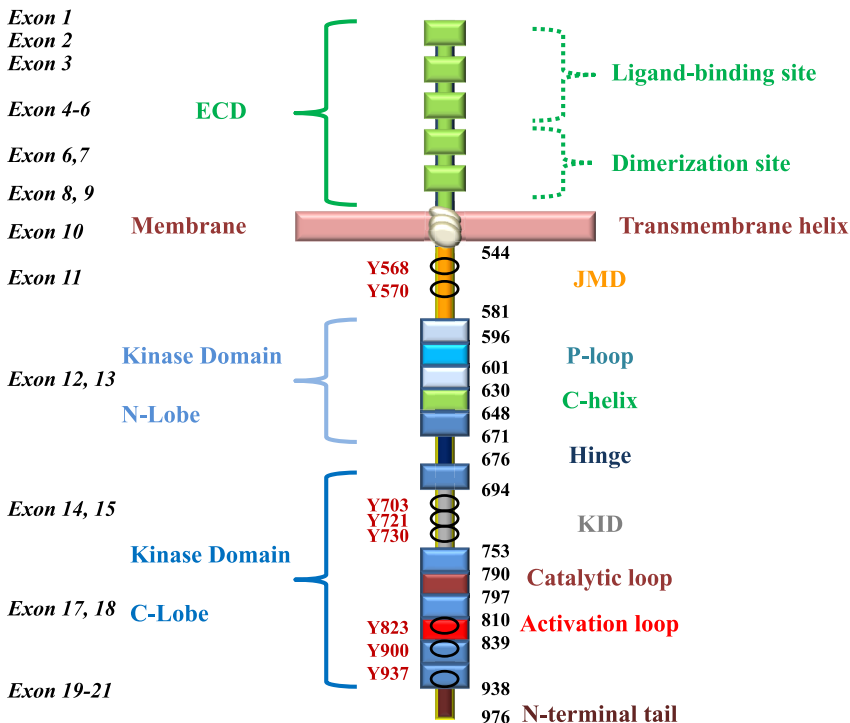


Fig. 1. Structure of normal KIT (KIT wild type). The *KIT* gene contains 21 exons transcribed/translated into a transmembrane RTK of 145 kDa and 976 amino acids. KIT structure is characterized by 5 Ig-like subunits in the extracellular domain (ECD), which contains the binding site for SCF and a dimerization site and is linked to a cytoplasmic region by a single transmembrane helix. The cytoplasmic region of KIT consists of an autoinhibitory juxtamembrane domain (JMD) of 35 residues and of a kinase domain (KD) arranged in a proximal (N-) and a distal (C-) lobe linked by a hinge region. The C-lobe includes a large kinase insert domain (KID) of 77 residues. In human KIT, the KID and the JMD contain 3 and 2 tyrosine residues, respectively. The receptor is presented under its monomeric form, and a dimer results from stem cell factor (SCF) ligation.

proteins, crucial for the control of cellular activities such as proliferation or apoptosis.¹⁹

Two crucial regulatory segments, the activation loop (A-loop, residues 810–835) and the JMD (residues 547–581), undergo extensive conformational rearrangements during the activation/deactivation process. In the inactive autoinhibited state of the receptor, the A-loop is adjacent to the active site, and the highly conserved catalytic D810-F811-G812 (DFG) motif adopts an out conformation: the phenylalanine is flipped into the adenosine triphosphate (ATP)-binding site, impairing ATP and Mg²⁺ cofactor binding.²⁰ This conformation is stabilized by the JMD that inserts itself directly into the active site and prevents the A-loop from adopting an active conformation.²¹ On activation, the JMD moves from its autoinhibitory position to a completely solvent-exposed emplacement.²² This intermediate conformation presenting an inactive, but not autoinhibited, state of the KD is followed by a conformational swap of the A-loop from an inactive packed arrangement to an active extended conformation.²² This transition, together with a switch of the DFG motif to an “in” conformation, favors the stabilization of the active state, allowing ATP entrance and binding in the catalytic site.²²

Physiologic Signaling by Normal KIT in MCs

Binding of SCF to KIT leads to the recruitment of several downstream signaling pathways, which account for MC survival, proliferation, activation or migration. KIT wild type (WT) recruits the PI3-kinases (PI3-K)/AKT/mechanistic target of rapamycin (mTOR) pathway,^{23,24} the Janus Kinase 2 (JAK2)-Signal Transducer and Activator of Transcription (STAT)1/5 signaling,²⁵ the Src family kinases (SFK) pathway,^{26,27} the mitogen-activated protein kinase pathway,^{28–30} and phospholipases (particularly phospholipase C- γ).³¹ A more detailed survey on normal KIT signaling is provided in the article by Olivera and colleagues elsewhere in this issue.

CLASSIFICATION OF MASTOCYTOSIS

Mastocytosis is schematically divided into cutaneous mastocytosis (CM) and systemic mastocytosis (SM).⁶ Localized MC tumors (ie, mastocytomas and MC sarcoma [MCS]) are rare.³² CM is usually diagnosed in childhood.³³ However, in most adult patients, the disease is systemic (SM), although the skin is often also affected.³⁴

In patients with SM, the diagnosis is usually established by BM investigation using classic staining and specific tryptase stains.³⁵ Besides, apart from the BM, other organs, such as the liver or the gastrointestinal tract, may also be involved.³⁶ In addition, a high incidence of associated hematologic non-MC lineage diseases (AHNMD) is found in patients with SM.³⁷

The 2008 World Health Organization (WHO) classification defined the following categories of SM: indolent SM (ISM), SM-AHNMD, aggressive SM (ASM), and MC leukemia (MCL).³⁸ In addition, the WHO classification included CM and the rare, localized, MC tumors, namely mast cell sarcoma (MCS) and extracutaneous mastocytomas (Table 1).³⁸

The diagnosis of SM is based on 1 major and 4 minor criteria.³⁹ The major criterion of SM is the multifocal infiltration of MCs in the BM or other extracutaneous organs.⁴⁰ Minor SM criteria relate to the morphology of MCs by histology and on BM smears, aberrant expression of CD2 or CD25 by neoplastic MCs, an increased serum tryptase level (>20 ng/mL), and the presence of an activating *KIT* mutation at codon 816.⁴⁰ If at least 1 major and 1 minor criterion, or at least 3 minor criteria, are fulfilled, the final diagnosis is SM.

Table 1
WHO 2008 classification of mastocytosis

Category of Mastocytosis	Abbreviation	Subvariants
Cutaneous mastocytosis	CM	Urticaria pigmentosa (UP) Maculopapular CM (MPCM) Diffuse CM Mastocytoma of skin
Indolent systemic mastocytosis	ISM	Smoldering SM (SSM) Isolated bone marrow mastocytosis Well-differentiated SM
Systemic mastocytosis with an associated clonal hematologic non-mast cell disease	SM-AHNMD	SM-acute myeloid leukemia (AML) SM-myelodysplastic syndrome (MDS) SM-MPN SM-chronic myelomonocytic leukemia (CMML) SM-chronic eosinophilic leukemia (CEL) SM-non-Hodgkin lymphoma (NHL) SM-myeloma
Aggressive systemic mastocytosis	ASM	Lymphadenopathic SM with eosinophilia
Mast cell leukemia	MCL	Aleukemic MCL
Mast cell sarcoma	MCS	
Extracutaneous mastocytoma	ECM	

Adapted from Horny HP, Metcalfe DD, Bennett JM, et al. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, et al, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon (France): IARC Press; 2008. p. 54–63.

KIT D816V is detectable not only in ISM but also in advSM.⁴¹ From this observation, it must be concluded that, apart from *KIT* mutations, other factors might trigger the growth and survival of neoplastic MCs and that *KIT*-independent pathways may account for disease progression. Several of these additional *KIT*-independent oncogenic pathways may relate to the activation of LYN or BTK,⁴² or additional genetic defects.⁴³ There is also a special subvariant of SM, termed smoldering SM (SSM), which presents with high MC burden, B-findings (borderline benign), such as hypercellular BM, dysplasia, organomegaly without organ failure, and high serum tryptase levels, but has a clinical course stable over years. However, some of these patients progress to ASM, SM-AHNMD, or even MCL over time, whereas others remain in the smoldering state for decades.⁴⁴ Thus, SSM is by some aspects an advSM. In SSM, the *KIT* D816V mutation is usually found in MCs, as well as in non-MC-lineage cells, which often are dysplastic or even mimic a myeloproliferative or myelodysplastic disease.⁴⁵

INVOLVEMENT OF *KIT* DEFECTS IN MASTOCYTOSIS

Since SCF was identified as the major cytokine responsible for MC proliferation, various teams have investigated whether increased secretion of SCF could be involved in the pathophysiology of mastocytosis. No convincing data appeared, and investigations were thereafter conducted on its receptor *KIT*, which was reported to be responsible for autonomous growth of cell lines harboring *KIT* mutations, by constitutively activating downstream pathways in the absence of SCF.^{46,47} Neoplastic MCs of patients with SM⁴⁸ and then of children with CM⁴⁹ were more recently found to present similar recurrent anomalies of the *KIT* gene structure (mainly *KIT* D816V in adults). An additional proof of the involvement of *KIT* defects in the pathophysiology of SM

was provided by a model of animals transgenic for human *KIT* D816V, in which MC hyperplasia was observed, along with the expression of the transgene in MCs.⁵⁰

Although additional genetic defects apart from *KIT* mutations have recently been found in neoplastic cells of patients with advSM,^{8–10,43} *KIT* mutations are the only genetic alteration found in most mastocytosis cases (CM in children and ISM in adults) (Fig. 2). The following section emphasizes similarities and differences of the *KIT* defects found in mastocytosis variants as well as how the nature of the *KIT* mutant may affect the treatment of patients.

Pediatric CM

Unlike in adult patients, the frequency and the role of *KIT* mutations in childhood-onset mastocytosis, and whether it is a clonal disease, have long remained unclear. Longley and colleagues⁴⁹ found that 6 pediatric patients lacked mutations in codon 816, but 3 had a dominant inactivating mutation, K839E. Similarly, Buttner and colleagues⁵¹ reported that none of 11 pediatric patients had codon 816 mutations, suggesting that childhood mastocytosis may be a reactive rather than a clonal disease. Conversely, Yanagihori and colleagues⁵² found mutations in codon 816 in 10 of 12 children with mastocytosis, and Verzijl and colleagues⁵³ found that 25% of children with urticaria pigmentosa had the D816V mutation.

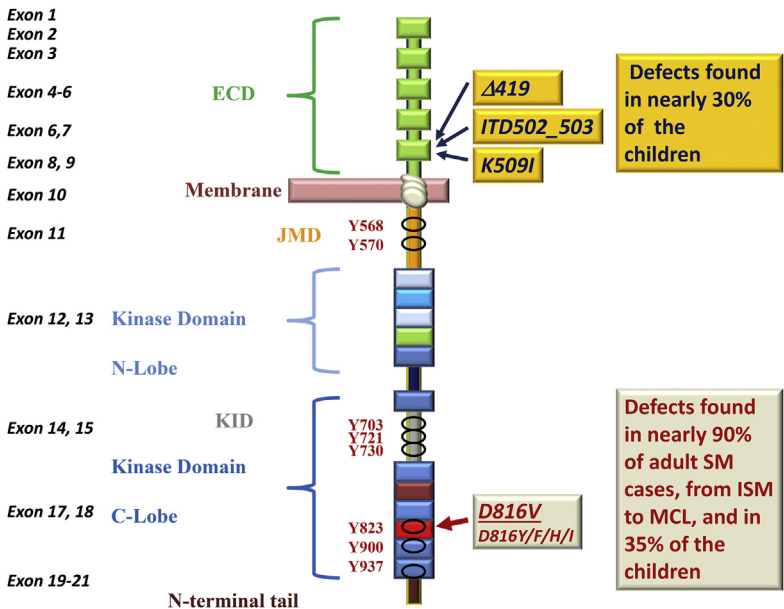


Fig. 2. KIT structure, showing localization of the more frequently observed mutations in the *KIT* sequence in pediatric or adult patients with mastocytosis. The receptor is presented under its monomeric form, whereas its wild type counterpart dimerizes on ligation with SCF before being activated in normal cells. The *KIT* D816V PTD mutant is found in up to 90% of the adult patients with SM, whereas ECD mutants are found in nearly 40% of affected children. Only the major mutations found in pediatric patients are represented here; see Table 2 for a complete list of *KIT* mutants retrieved in children. Δ, deletion; ITD, internal tandem duplication; JMD, juxtamembrane domain; KI, kinase insert; PTD, phosphotransferase domain; TMD, transmembrane domain.

In a recent study conducted on a large cohort of pediatric patients (50 patients younger than 16 years), we examined the presence of *KIT* mutations.⁵⁴ We found 18 children (36%) with the D816V point mutation and 3 additional patients with other mutations in codon 816: D816Y (n = 2) and D816I (n = 1). Other new *KIT* mutations were identified in our cohort, mainly located in exons 8 and 9 (Δ 417-418, D419Y, C443Y, S476I, ITD502-503, K509I) and exon 11 (D572A). All these new mutations were mutually exclusive of mutations in codon 816 and caused constitutive activation of *KIT*.⁵⁴ Further study of 60 additional pediatric cases provided similar results compared with our first set of patients (Table 2, Dubreuil and colleagues, unpublished data, 2014). From our studies, 76% of the pediatric patients have alterations in *KIT*, supporting the hypothesis that childhood mastocytosis is a clonal disease similar to SM in adults but with a less restricted spectrum of *KIT* mutations than in adults. However, how the pediatric form can spontaneously resolve remains to be elucidated.

ISM

KIT D816V mutant is the most frequent *KIT* abnormality, detected in virtually 100% of the ISM cases, when using a sensitive technique on purified BM MCs.^{55,56} In a small fraction of patients with ISM, the disease progresses into an aggressive variant and the presence of *KIT* D816V mutation in non-MC lineages seems to be a predictor for risk of progression to ASM.⁵⁵ However, in a recent study conducted on 25 patients with ISM, the *KIT* D816V mutation was detected in both BM and peripheral blood (PB) cells in all cases using a very sensitive quantitative polymerase chain reaction,

Table 2
Analysis of *KIT* mutations in an extended pediatric cohort of 110 patients

Affected Exon	<i>KIT</i> Mutation (aa)	Number of Patients Affected	Frequency (%)
8	Del417-418-419 InsAsn-Ala	1	0.91
8	Del417-418-419 InsIle	1	0.91
8	Del417-418-419 InsTyr	1	0.91
8	Del419Asp	19	17.27
8	419ins6	1	0.91
8	C443Y	1	0.91
9	S451C	1	0.91
9	S476I	1	0.91
9	Ins6 501-502	2	1.82
9	Ins6 502-503	6	5.45
9	Ins6 504	1	0.91
9	Ins12 505-508	1	0.91
9	K509I	6	5.45
11	D572A	1	0.91
11	Del564-576	1	0.91
17	D816I	2	1.82
17	D816V	34	30.91
17	D816Y	3	2.73
NA	WT	27	24.55

meaning that circulating *KIT* D816V+ non-MCs in PB can be considered characteristic of ISM.⁵⁷

Well-differentiated SM and SSM are subsets of ISM.⁴⁵ SSM has been described earlier. Well-differentiated SM is characterized by BM compact multifocal infiltrates, by round mature, CD25-negative MCs, and by a non-816 mutation of *KIT* or the absence of *KIT* mutation.⁵⁸

ASM

ASM is a subvariant of SM with a progressive evolution and a poor prognosis (median survival of 41 months) compared with ISM.⁵⁹ C-findings (clinical findings) are found, corresponding to organ(s) failure, such as impaired BM function, hepatic failure and ascites, splenomegaly with hypersplenism, severe osteolysis and fractures, or malabsorption and weight loss.⁶⁰ In addition, ASM can transform into MCL.⁶¹ Most patients with ASM present the *KIT* D816V mutation, but other *KIT* mutations (D820G or V559I) may also be found.^{62,63} Neoplastic MCs in ASM typically show an immature phenotype with clonal involvement of all myeloid lineages by the *KIT* D816V mutation.⁶⁴

Recently, reports, including ours, have proposed that additional genetic lesions apart from the *KIT* D816V mutation are present in ASM.^{9,10,43} This feature may explain the aggressiveness of the disease, as well as the resistance of many *KIT* D816V+ patients with ASM to midostaurin, a drug active *in vitro* on this mutant.⁶⁵

SM-AHNMD

SM-AHNMD is a complex subtype of advSM. The SM compartment can be ISM, ASM, or MCL (MCL is rarely associated with AHNMD),⁶⁶ whereas the AHNMD is of myeloid origin in most cases (see **Table 1**).⁶⁷ However, AHNMDs cover all major subtypes of hematologic malignancies, including lymphomas (see **Table 1**).⁶⁸ These various AHNMDs may present with their own recurrent genetic defects, such as t(8;21) in acute myeloid leukemia (AML) cells or *JAK2* V617F mutation in *BCR-ABL1*-negative myeloproliferative neoplasm (MPN).³⁷ However, the existence of a clonal relationship between the 2 disease components in SM-AHNMD has been largely unexplored. In a recent study, 48 patients with SM-AHNMD were tested for the presence of mutant *KIT* in the SM and AHNMD components of the disease.⁶⁹ The *KIT* D816V mutant was retrieved in the SM compartment of almost all the patients excepted those with an SM-chronic eosinophilic leukemia⁶⁹ and in AHNMD cells of most patients with SM-chronic myelomonocytic leukemia (CMML), suggesting that the *KIT* D816V mutant appears in a common MC/monocytic precursor, unlike patients with SM-MPN (20%) or SM-AML (30%), in whom *KIT* D816V was less frequently detectable in the non-MC malignant compartment. None of the patients in whom AHNMDs were lymphoproliferations showed the *KIT* 816 mutation.⁶⁹

MCL

In a recent review compiling all the published cases (n = 51) of MCL, *KIT* D816V mutation was detected only in 13 of 28 patients with MCL analyzed (46%).⁶¹ In 2 patients without *KIT* D816V, *KIT* was WT, whereas in 6 other cases, mutations were found in exon 9 (n = 3), exon 10 (n = 1), exon 11 (n = 1), or exon 13 (n = 1).⁶¹

MCS

MCS is an extremely rare, aggressive neoplasm composed of atypical malignant MCs presenting as a solitary mass.³² *KIT* mutational status has been investigated in a few cases and showed either the absence of mutations or mutations in non-816 locations, such as *KIT* Δ419 or *KIT* N822K.⁷⁰

Familial Forms of Mastocytosis

Familial occurrence of mastocytosis has been reported in more than 50 families since the mid-1880s. Most of the cases were pediatric CM without *KIT* mutations or with uncommon mutations (ie, K509I, A533D, N822I, M835K, S849I or deletion of amino acids 419 or 559–560).^{71–73} Most of these *KIT* defects have been found activating and some were sensitive to imatinib, at least *in vitro*. In most cases, the *KIT* mutation found was of a germline nature, but we recently reported a family (mother and son) with adult-onset SM associated with a somatic *KIT* D816V mutation.⁷⁴

ABNORMAL SIGNALING EVOKED BY THE *KIT* D816V ONCOGENIC MUTANT

This section focuses on the signaling pathways evoked by the *KIT* D816V mutant receptor (**Fig. 3**), which is the most prominently *KIT* mutant found in all the variants of SM.

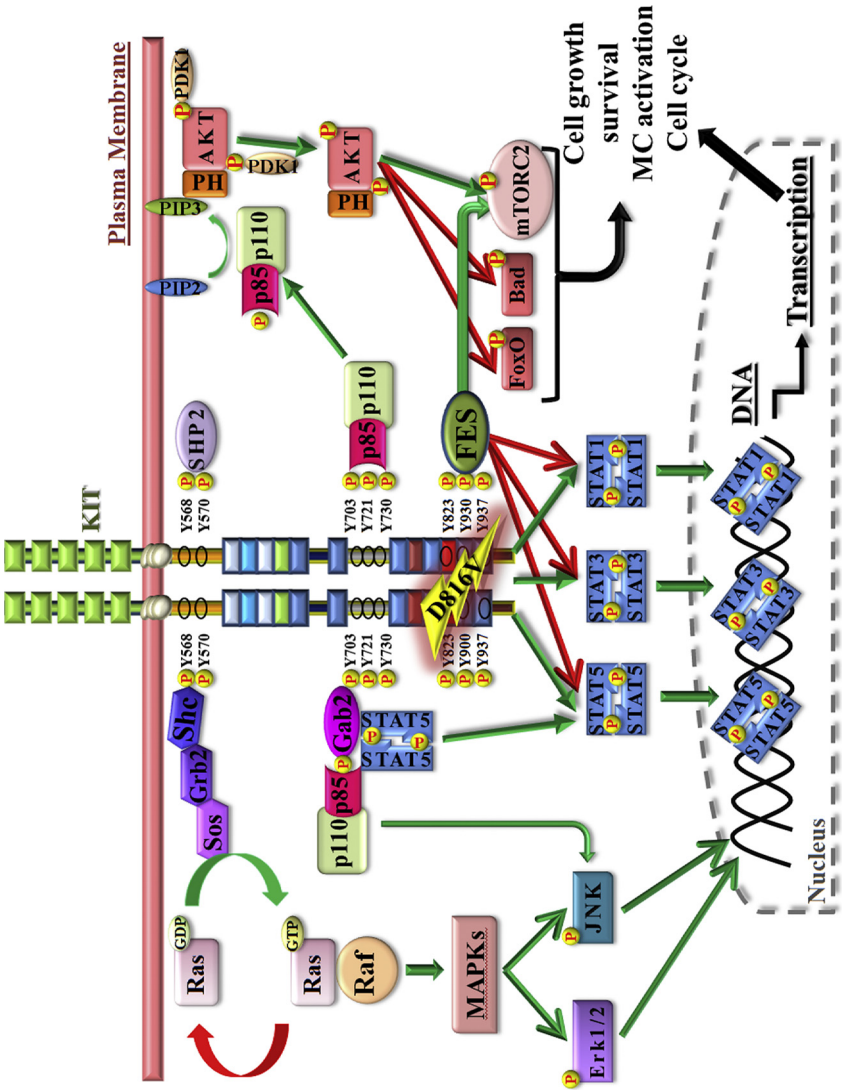
Although the mechanism behind the constitutive activation of the *KIT* D816V mutant receptor is still not fully understood, 1 possibility is that mutation within the phosphotransferase domain (PTD) results in a structural change that relieves autoinhibitory mechanisms. This hypothesis remains speculative, because no crystal structure of the KD of *KIT* with an activating mutation has been yet published.

Recently, an *in silico* analysis has suggested that D816 mutations cause a structural change in the A-loop but also weaken the binding of the JMD to the KD.²² Consequently, the inhibitory effect of the JMD on kinase activity is suppressed.²² In addition, it was proposed that the extended JMD could make contact with another *KIT* receptor, thereby promoting dimerization in the absence of ligand.²² In support of this hypothesis, recombinant KD with the D816Y mutation, but not the WT KD, was found to form dimers in solution.⁷⁵

Several groups have compared the ability of WT and oncogenic mutants of *KIT* to induce signal transduction and found that they differ not only quantitatively but also qualitatively. This finding is potentially important, because it suggests that there might be ways to selectively target oncogenic signaling with less impact on the normal situation. The reason for the different signaling abilities can stem from changes in intracellular localization of the mutant *KIT*, altered substrate specificity, or a combination of both.

Changes in Intracellular Localization

KIT with gain-of-function mutations in the KD shows reduced cell surface expression, and inhibition of kinase activity in *KIT* KD mutations restored cell surface expression of the mutant receptor.⁷⁶ Another study reported that *KIT* D816V was primarily localized to and could transmit oncogenic signals from the Golgi apparatus, whereas *KIT* trapped in the endoplasmic reticulum (ER) could not.⁷⁷ In an attempt to elucidate the role of localization on signaling, cells with activating mutations of *KIT* were treated with brefeldin A, which causes protein accumulation in the ER, and then various signaling pathways were analyzed. Certain proteins such as AKT and Extracellular signal-Regulated Kinases (ERK)1/2 were not activated in brefeldin A-treated cells, whereas other proteins (eg, STAT5) were phosphorylated, suggesting that signals induced from different compartments can be qualitatively different.⁷⁸ Although *KIT* D816V has an increased intracellular localization, some is still surface exposed, and this has functional consequences. For instance, cells expressing *KIT* D816V still migrate toward soluble SCF.⁷⁹ Furthermore, even in cells expressing *KIT* D816V, SCF is still required to promote activation of AKT and ERK1/2.⁸⁰ In contrast, other pathways such as c-Jun N-terminal Kinases (JNKs), Casitas B-lineage Lymphoma (c-CBL), and Src Homology 2 domain-Containing (SHC) are constitutively activated in cells



expressing *KIT* D816V. The reason for the dependence of intracellular localization for signaling is unclear but may relate to which downstream substrates are available at different locations. Alternatively, it could be the result of modifications in the receptors (eg, phosphorylation or selectively dephosphorylation) by other enzymes that are located only in certain compartments.

Altered Substrate Specificity

A recent study reported that *KIT* D816V showed a substrate specificity that resembled that of SRC and Abelson murine leukemia viral oncogene homolog (ABL) TKs.⁸¹ Consequently, the mutant *KIT* could activate ERK1/2 independently of SRC, whereas *KIT* WT required SRC activity for these processes.⁸¹ Several studies have tried to identify signaling pathways critical for the transforming abilities of mutant *KIT*. Particularly, we showed that the Feline Sarcoma Oncogene (FES) TK was important for negative regulation of STAT and positive regulation of mTOR phosphorylation downstream of *KIT* D816V.⁸² More recently, the mTOR complex, composed by 2 molecules, mTORC1 and mTORC2, has been shown upregulated in neoplastic and developing immature MCs compared with their mature normal counterparts.⁸³ In addition, the use of selective inhibitors showed that, whereas mTORC1 may contribute to MC survival, mTORC2 was critical only for homeostasis of neoplastic and dividing immature MCs.⁸³ Because mTORC2 plays little role in the homeostasis of differentiated, nonproliferating, mature MCs, the investigators hypothesized that mTORC2 inhibition *via* a targeted approach might reduce the proliferation in MC disorders and leave normal differentiated MCs largely unaffected.⁸³

The importance of the PI3-K/AKT pathway in mastocytosis has been reported recently. AKT activation has been identified as a key signaling molecule involved in *KIT*-dependent differentiation and growth of neoplastic MCs harboring oncogenic *KIT* mutants.⁸⁴ AKT was found phosphorylated in neoplastic MCs from patients suffering from *KIT* D816V+ SM and in the HMC-1.2 cell line, raising the hypothesis that AKT activation plays a critical role in the pathogenesis of mastocytosis.^{74,84,85}

Because STAT5 is involved in growth and survival of normal MCs,²⁵ several teams studied the implication of STAT5 in neoplastic MC growth, survival, and transformation, and light was shed on its implication downstream of *KIT* D816V. A first study



Fig. 3. Aberrant signaling evoked by the *KIT* D816V mutant receptor. Although the receptor is presented under a dimeric form as a transmembrane protein, it has been postulated that the *KIT* D816V protein could activate substrates and be located in the cell cytoplasm. That *KIT* D816V dimerizes spontaneously with itself or with *KIT* WT, or is capable of transmitting oncogenic signals as a single molecule, remains largely unexplored. The *KIT* D816V oncogenic mutation alters the substrate specificity of the mutant protein, which shows a substrate specificity that resembled that of SRC and ABL TKs. In addition, FES TK is activated by mutant *KIT* protein and negatively regulates the STAT pathway, although it induced phosphorylation of mTOR. Furthermore, AKT activation has been identified as a key signaling molecule involved in *KIT* D816V-dependent differentiation and growth of neoplastic MCs. Also, STAT5 is believed to play a pivotal role in growth of *KIT* D816V+ neoplastic MCs and is constitutively phosphorylated in such cells, probably because *KIT* D816V can promote direct STAT5 activation, thus diverting the canonical JAK-STAT pathway. A noncanonical pSTAT5 cytoplasmic retention system is found in neoplastic MCs, namely pSTAT5 retention by docking to the p85 regulatory subunit of PI3K *via* the GAB2 scaffold protein that controls the AKT signaling. This cytoplasmic retention system might block nuclear accumulation of pSTAT5, keeping STAT5-mediated transcription at bay. Green arrows, activation; red arrows, inhibition; black arrows, induction of survival or functions.

showed that knockdown of STAT5 led to growth inhibition of neoplastic MC.⁸⁴ A second study has shown that neoplastic MCs express cytoplasmic and nuclear phospho-STAT5 (p-STAT5).⁸⁶ Furthermore, the same team showed that KIT D816V promotes direct STAT5-activation, and that p-STAT5 contributes to growth of neoplastic MCs.⁸⁶

CONSEQUENCES OF THE VARIOUS DEFECTS IN *KIT* FOR TARGETED THERAPY

There are strong changes in the sensitivity of KIT mutants to TK inhibitors (TKIs) type II (eg, imatinib or masitinib) compared with KIT WT.⁸⁷ Thus, before using targeted therapies aiming to inhibit KIT TK activity for the treatment of patients with SM, it is mandatory to know exactly the *KIT* mutational status of each patient. However, even with adequate targeting of the KIT mutant, the results could be deceiving, and no complete remission should be obtained with SM, particularly in advSM. For this reason, combined therapies, or therapies targeting signaling downstream KIT could be a promising approach for the future.

PTD Mutants

The A-loop mutations D816V/H/Y/N induce high KIT resistance to imatinib *in vitro*, as well as *ex vivo* on patients' cells.⁸⁷ Given this resistance of *KIT* D816V toward imatinib, several other more or less targeted TKIs have been tested *in vitro* and in clinical studies for patients with advSM.

Dasatinib is also a potent inhibitor of SFKs, which are important mediators of KIT actions, and thus, this inhibitor potentially has dual targets of KIT kinase activity *per se* and SFK-mediated responses.⁸⁸ However, although dasatinib strongly inhibits KIT D816 mutants in cell lines, as well as in cells of patients with SM, only a few patients with ASM have been treated with dasatinib, with disappointing results.⁸⁹

PKC412 (midostaurin) is an *N*-benzoylstaurosporine with potent inhibitory activity against protein kinase C, vascular endothelial growth factor receptor 2 (VEGFR-2), Alpha-type platelet-derived growth factor receptor (PDGFR α), Fms-related tyrosine kinase 3 (FLT3), kinase insert domain receptor (KDR), and KIT.⁹⁰ PKC412 decreased KIT phosphorylation in both HMC-1.1 cells and HMC-1.2 cells as well as in patients' cells at pharmacologic concentrations *in vitro*.⁹¹ Several clinical trials are ongoing to examine the potential effects of this multikinase inhibitor in patients with SM.⁶⁵ In patients with ASM, midostaurin was shown to be well tolerated and there was a high rate of durable responses, accompanied by significant reductions in MC burden, including in MCL.⁶⁵

JMD, Transmembrane Domain, and Extracellular Membrane Domain Mutants

By contrast to the PTD mutation D816V, the JMD mutation V560D/G increases the drug sensitivity of the receptor.⁹² Imatinib inhibits at low concentrations the proliferation of the HMC-1.1 subclone with only *KIT* V560G mutation.⁹² However, this mutant is rarely seen in SM, and thus, only limited data are available on the efficacy of imatinib in such patients. In addition, not all JMD mutations may be sensitive to imatinib (eg, V559I).⁹³

A transmembrane domain mutation in *KIT* is a rare event in SM. Only 1 well-documented case has been reported. Akin and colleagues⁹⁴ described the case of a 25-year-old patient with SM with a germline *KIT* F522C mutation and a history of CM during childhood. Therapy with imatinib resulted in a dramatic improvement in MC burden and clinical symptoms in this patient.⁹⁴

Most of the *KIT* mutants found in the ECD of the receptor, particularly those described in pediatric patients, were found highly sensitive to imatinib, at least

in vitro.⁷⁴ Imatinib was even shown to be effective in a case of MCL with a defect in the ECD of KIT (p.A502_Y503dup).⁹⁵

However, in rare cases of SM (or even MCL) with no defect in KIT (*KIT* WT), imatinib or masitinib have shown a significant activity *in vivo* in isolated cases.^{96,97}

OTHER (ADDITIONAL) MOLECULAR DEFECTS FOUND IN VARIOUS FORMS OF MASTOCYTOSIS: BEYOND *KIT*

TET2 Mutations

TET2 functions and impact in hematologic malignancies

TET2 is a member of a family of 3 proteins (*TET1*, *TET2*, and *TET3*) that catalyze the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) in the DNA.⁹⁸ 5-hmC may initiate DNA demethylation by actively excluding DNA methyltransferases from CpG islands. Thus, the *TET* family could be implied in epigenetic regulation *via* the induction of 5-hmC.

The *TET2* gene is located on chromosome 4q24, which is a breakpoint in various AML-associated translocations.⁹⁹ Mice knockout or heterozygous for *TET2* have abnormal hematopoiesis with, first, HSC expansion, then, the development of myeloid malignancies resembling CMML or myelodysplasia.¹⁰⁰ Recently, several investigators have identified *TET2* mutations, scattered across several of its 12 exons in 1 or both *TET2* alleles, as an early event during the development of various malignancies, including MPN, CMML, myelodysplastic syndrome (MDS), leukemia, and B- and T-cell lymphomas.¹⁰¹ Patients with mutant *TET2*+ myeloid disorders show a decreased level of 5-hmC with hypomethylation or hypermethylation of DNA.¹⁰² Altogether these data show that *TET2* plays a role in various hematologic malignancies.

TET2 and mastocytosis

The first report of an association between mastocytosis and alterations in *TET2* came from Tefferi and colleagues in 2009.⁴³ On a cohort of 48 patients with SM (comprising 6 patients with *FIP1L1-PDGFRFA* fusion gene), these investigators found at the BM level that 29% of patients with SM, but no *FIP1L1-PDGFRFA*+ patients, had *TET2* mutations. The presence of *TET2* defects was documented in 15% of 13 patients with ISM, 40% of 5 patients with ASM, and 35% of 23 patients with SM-AHNMD.⁴³ In addition, the investigators reported a significant association between monocytosis and the presence of *TET2* mutations. Furthermore, they showed a correlation between *TET2* lesions and presence of *KIT* D816V mutant. *KIT* D816V was found in 50% of patients with the *TET2* mutation, but in only 20% of patients without alteration in the *TET2* gene.⁴³ From these data, the investigators concluded that *TET2* mutations are frequent in SM, segregate with *KIT* D816V, and influence phenotype, without necessarily altering prognosis.

The presence of *TET2* mutations in mastocytosis was further confirmed by Traina and colleagues⁸ on 26 patients (15 ISM, 8 SM-AHNMD, 2 ASM, and 1 MCS); these investigators found *TET2* mutations in 6 patients (23%), mostly in patients with SM-AHNMD (5 of the 6 patients with *TET2* mutations), whereas only 1 patient with ISM was found positive. Although the number of affected cases was low, the investigators claimed that the presence of *TET2* mutations conferred a poor prognosis regarding overall survival (OS).⁸

In a recent report, Schwaab and colleagues¹⁰ have analyzed the complete coding regions of *TET2*, as well as of several other genes of interest, in a cohort of 39 patients with advSM. Regarding *TET2* defects, the investigators found 29 mutations identified in 15 patients. These 15 patients had an SM-AHNMD, with the SM compartment being

indolent, aggressive, or even leukemic, whereas the AHNMD was frequently an MDS or a CMML.¹⁰ In addition, the investigators compared the OS of the patients with *KIT* D816V mutation alone with one of the *KIT* D816V+ patients bearing *TET2* mutations and found a shorter OS in the second group of patients.¹⁰

We have recently analyzed a cohort of 74 patients with mastocytosis, categorized according to the WHO classification into aggressive or nonaggressive disease.⁹ Consistent with previous studies, most of our patients (82.5%) were positive for *KIT* mutation (78.4% *KIT* D816V+). *TET2* mutations were detected in 20.3% of the patients and were distributed along the gene in a pattern similar to that reported in AML and CMML.⁹ Two distinct mutations in the *TET2* gene were detected in some patients, and in all cases, *TET2* mutation occurred in conjunction with *KIT* mutation. *TET2* mutation correlated significantly with aggressive forms of mastocytosis.⁹ Both *TET2* and *KIT* mutations were found in primary MCs sorted from patients, and clonal analysis from BM biopsies of 2 patients was also consistent with a model in which *TET2* mutation is an early event preceding clonal disease onset of aggressive disease.⁹

To understand the mechanism underlying the impact of *TET2* on the severity of the disease, we derived BM-derived MCs (BMMCs) from *Tet2*^{-/-} mice and we transduced these BMMCs with *KIT* D816V, together with BMMCs obtained from *Tet2* WT mice. We observed a significant and competitive growth advantage for *KIT* D816V+ *Tet2*^{-/-} BMMCs compared with *KIT* D816V+ *Tet2* WT cells.⁹

Taken together, these data provide evidence for the existence of an oncogenic cooperation between *KIT* and *TET2* mutations in MCs and show *TET2* mutation as a potential marker to diagnose and predict severe forms of mastocytosis. However, this observation does not apply to all the subtypes of advSM. In a recent study focused on 62 patients with SM-AHNMD, 27% of the patients were found to have a *TET2* mutation.¹⁰³ However, in our hands, in univariate analysis as well as in multivariate analysis, the presence of *TET2* mutation was not associated with an affected OS.¹⁰³

Mutations in the Spliceosome Machinery

In humans, most protein-coding transcripts contain introns that are removed by messenger RNA splicing carried out by spliceosomes. Mutations in the spliceosome machinery, which include the splicing factor 3 subunit b1 (*SF3b1*), the U2 small nuclear RNA auxiliary factor 1 (*U2AF1*) and the serine arginine rich splicing factor 2 (*SRSF2*) have recently been identified using whole exome/genome technologies in MDS, CMML, and primary fibrosis.¹⁰⁴

There is only 1 published report, by Schwaab and colleagues,¹⁰ in which the structure of *SF3B1* has been analyzed. Although the cohort comprised several patients with SM-CMML or SM-MDS, none of the 39 patients tested was found positive for hotspot mutations lying in the exon 15 of *SF3B1*.¹⁰ More recently, we have analyzed the structure of *SF3B1* on a cohort of 72 patients and found mutations in only 4 patients (5.6%), with 2 patients having mutations in codon 14 and 2 patients in codon 15.¹⁰⁵ We also analyzed the structure of *U2AF1* and found only 2 patients with a mutation in codon 2. Mutations in *SF3B1*, *U2AF1*, and *SRSF2* were found mutually exclusive in our cohort of patients.¹⁰⁵

Schwaab and colleagues¹⁰ also analyzed the occurrence of *SRSF2* mutations in their cohort of 39 patients. These investigators found 14 patients (35%) presenting a mutation in the hotspot region of *SRSF2*.

We also tested a cohort of 72 patients for *SRSF2*-P95 mutation and found that after *KIT* mutations (81%), the *SRSF2*-P95 hotspot mutation was the most frequent defect found in our patients: 17 of 72 (23.6%) patients, whereas *TET2* mutants were found in 21% of the patients.¹⁰⁵ We found that *SRSF2*-P95 hotspot mutation was highly

correlated with the presence of an AHNMD (17 patients of 17 positive had an AHNMD). Nevertheless, we were able to show at the single-cell level that, in these cases of *SRSF2*-P95+ SM-AHNMD, the mutant was found in MCs as well as in monocytes, supporting a role for *SRSF2*-P95 mutation in MC transformation. In addition, we found *TET2* and *SRSF2*-P95 mutations were statistically highly associated, suggesting a mechanistic link between these 2 factors. However, the presence of the *SRSF2*-P95 mutation in patients did not induce a shortening in OS.¹⁰⁵

Regarding *U2AF1* mutations in mastocytosis, Schwaab and colleagues¹⁰ found only 2 patients of 39 with this defect. One of the 2 patients had an ISM and presented only with the *U2AF1* mutation, whereas the other patient had an SM-MDS/MPN and presented several additional genetic defects.¹⁰ Confirming this low frequency, we also analyzed the structure of *U2AF1* in our cohort of 72 patients and found also only 2 patients affected by a mutation of this gene.¹⁰⁵

***ASXL1* Mutations**

The gene *ASXL1* (additional sex combs-like 1) is located in the chromosomal region 20q11 and encodes for a protein of the polycomb group and trithorax complex family *ASXL1*.¹⁰⁶ *ASXL1* can interact with retinoic acid receptor and seems to be involved in chromatin remodeling, but its function remains unknown.¹⁰⁷ *ASXL1* is expressed in most hematopoietic cell types, and a knockout mouse model showed mild defects in myelopoiesis but did not develop MDS or other hematologic malignancy.¹⁰⁸ Several studies have shown that *ASXL1* mutations (particularly frequent in exon 12 and often heterozygous) occur frequently in MDS, AML, chronic myelogenous leukemia (CML), CMML, and MPN and worsen the prognosis.^{109,110}

The first description of occurrence of *ASXL1* defects in mastocytosis came in 2012 from the group of Traina and colleagues,⁸ who sequenced *ASXL1* gene in 26 patients with SM (15 ISM, 8 SM-AHNMD, 2 ASM, and 1 MCS) and found 3 different *ASXL1* heterozygous mutations in 3 of the 26 (12%) patients with SM. *ASXL1* mutations were found in 1 of 15 of the patients with ISM and 2 of 8 of the patients with SM-AHNMD.⁸ An *ASXL1* defect was the only genetic alteration (apart from a *KIT* D816V mutation) found in the patient with ISM, whereas the 2 patients with SM-AHNMD found positive for *ASXL1* mutation were also positive for *TET2* defects.

More recently, Schwaab and colleagues¹⁰ found *ASXL1* defects in 8 patients of 39. All 8 patients were found to have an SM-AHNMD, with the SM compartment being either indolent, aggressive, or even leukemic, whereas the AHNMD was found frequently to be CMML.¹⁰ Four of 8 presented with an associated *TET2* mutation, and 7 of 8 presented with 1 or several additional defects.¹⁰ In addition, the investigators compared the OS of the patients with *KIT* D816V mutation alone with OS of the *KIT* D816V+ patients bearing at least 1 additional genetic defect. In all cases, including for *ASXL1* mutations, OS was significantly shorter in the second group of patients.¹⁰

In our cohort of 62 French patients with SM-AHNMD (AHNMD being mostly myeloid, and comprising MDS, CMML, or MPN), we have analyzed the structure of *KIT*, *TET2*, *ASXL1*, and *CBL* genes (see earlier discussion). We found defects in *ASXL1* in 14% of the patients.¹⁰³ In univariate analysis, we found that the presence of C-findings, the AHNMD subtypes (SM-MDS/CMML/AML vs SM-MPN/hypereosinophilia), neutropenia, high monocyte levels, and the presence of *ASXL1* mutation had statistically significant detrimental effects on OS.¹⁰³ In our hands, with the use of multivariate analysis and a penalized Cox model, only the presence of the *ASXL1* mutation remained an independent prognostic factor, which had a significant and negative effect on OS.¹⁰³

The literature tends to show that *ASXL1* mutations are found at a frequency ranging between 12% and 20% of patients with SM, preferably in advSM, and in most cases in SM-AHNMD. In addition, it seems that the presence of *ASXL1* mutants could be detrimental for patients' outcome.

RAS Mutations

Acquired mutations in the *RAS* family are frequently implicated in malignant transformation and tumor maintenance.¹¹¹ *RAS* and proliferation of MCs were linked a long time ago by studying an interleukin 3–dependent mouse MC line (BP-3c) immortalized after introduction of the *v-HRas* oncogene producing mastocytoma.¹¹² More recently, another study confirmed that *NRASV12* expression induces the development of an ASM or even an MCL-like disease in mice.¹¹³ Using an improved mouse BM transduction and transplantation model, a third study showed that an oncogenic form of *NRAS* induced rapidly and efficiently a CMML-like or AML-like disease and also an SM-like disease *in vivo*.¹¹⁴ These last 2 studies indicated that oncogenic *NRAS* could be of importance in the pathogenesis of mastocytosis *in vivo*.

Wilson and colleagues¹¹⁵ evaluated the coexistence of *NRAS*, *KRAS*, *HRAS*, or *MRAS* mutations on 44 *KIT* D816V+ patients (27 with ISM, 9 with SSM, 4 with SM-AHNMD, and 4 with ASM). These investigators found activating *NRAS* mutations in 2 patients (4.5%), one with ASM and the other with SM-CMML. In these 2 patients, BM CD34+ were positive for *NRAS* mutation but negative for *KIT* D816V mutant, whereas clonal MCs and circulating myeloid and lymphoid mature cells harbored both mutations, suggesting that *NRAS* mutations may have the potential to precede *KIT* D816V in clonal development.¹¹⁵

More recently, the Reiter group analyzed 39 *KIT* D816V+ patients with various subtype of ASM for the presence of *KRAS* and *NRAS* mutations.¹⁰ These investigators found 4 patients (10.2%) with *KRAS* mutations (3 with an ASM and 1 MCL) and 2 other patients with ASM (5.1%) with *NRAS* mutant forms. No mutations in *RAS* were found in the 10 patients with ISM included in the cohort.¹⁰

We have recently analyzed the presence of *NRAS* mutations in our cohort of 72 patients with different subtypes of mastocytosis and found only 2 patients classified in the ASM-AHNMD group with mutant *NRAS* (2.8%).¹⁰⁵

CBL, DNMT3A, ETV6, EZH2, RUNX1, and SETBP1 Mutations

These mutations are usually found at lower frequencies than *TET2*, *ASXL1*, or *SRSF2* mutations and are more frequently found in patients with SM-AHNMD. Because few data are available, it is difficult to determine whether they target both the MC and the non-MC components of the disease or only the non-MC part of the malignant cells, or to characterize their impact on prognosis, response to treatment, or OS of the patients. Here, we propose a rapid survey of their frequency, analyzing the few cohorts of patients published.

CBL mutants were found at a frequency ranging from 3.8% (1 of 26 patients)⁸ to 20.5% (8 of 39)¹⁰ patients in mastocytosis. In both reports, the mutants were exclusively found in patients with SM-AHNMD, except for 1 mutation in a patient with ISM.

The presence of *DNMT3A* mutations in mastocytosis has been analyzed by Traina and colleagues,⁸ and 3 patients of 26 (11.5%) were found to have mutations in *DNMT3*: 2 patients with *KIT* D816V+ ISM and 1 patient with *KIT* WT SM-CMML.

The complete coding regions of the *ETV6* gene were analyzed in the cohort of 39 patients by Schwaab and colleagues¹⁰ and 1 mutation was found in 1 patient with Unclassifiable myelodysplastic/myeloproliferative neoplasm (MDS/MPNu), who also presented mutations in *ASXL1*, *CBL*, and *U2AF1* genes.

The *EZH2* gene was analyzed by Traina and colleagues,⁸ who found no alteration of the gene in a cohort of 26 patients, whereas the Reiter group found 2 of 39 patients with advSM (5.1%) with alterations in the structure of the gene, consisting of a missense mutation and 2 splice sites.¹⁰ One patient was categorized as having an SM-MDS/MPNu, with associated defects in *TET2*, *ASXL1*, and *CBL*, whereas the other patient, categorized as ASM-MDS/MPNu, presented associated defects in *TET2* and *CBL*.¹⁰

In the same study, Schwaab and colleagues¹⁰ analyzed additional genes known to be involved in hematologic malignancies, *SETBP1* and *RUNX1*. *SETBP1* gene codes for a nuclear localizing protein with mutations (mostly heterozygous missenses) that have been recently described in atypical CML, AML, MDS, and CMML with a low frequency.¹¹⁶ Only 1 patient (2.5%) with *KIT* D816V+ ISM was found positive for *SETBP1* mutation. Runt-related transcription factor 1 (*RUNX1*), also known as AML1 protein or core-binding factor subunit α_2 (*CBFA2*) is a transcription factor regulating HSC differentiation. Mutations in *RUNX1* are implicated in MDS and AML and associated with a poor prognosis.¹¹⁷ Such mutations (9 missense mutations, 1 frameshift) were also reported in 9 of the 39 *KIT* D816V+ patients with mastocytosis (23%) analyzed by the Reiter group.¹⁰ All patients who tested positive for *RUNX1* mutations were patients with ASM, with or without AHNMD. Seven of these 9 patients presented with 1 or more additional genetic defects targeting *TET2*, *SRSF2*, or less frequently, *ASXL1*, and 3 of the 4 patients found positive for *KRAS* mutations in the whole cohort were also positive for *RUNX1* mutations.¹⁰

SUMMARY AND FUTURE CONSIDERATIONS

In SM, the *KIT* D816V mutant is detected in most (>90%) patients.⁴⁵ Although TKIs specific for *KIT* D816V have shown excellent activity toward *KIT* D816V *in vitro*,^{91,118} these compounds have only modest activity *in vivo*. One possible explanation could be that the molecular pathogenesis of SM, particularly in advSM is more complex than the presence of a single gene defect in *KIT* and that additional genetic defects could

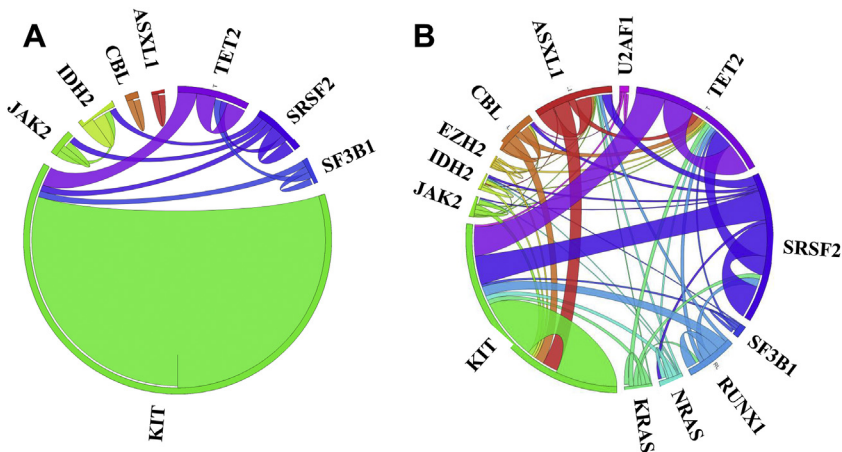


Fig. 4. Circos (circular genome data visualization) diagrams depicting the relative frequency and associations of the major mutations found in SM in nonaggressive (NA) forms (A) and in aggressive (A) forms (B) based on data from our work on 73 patients with SM (31 A, 42 NA)¹⁰⁵ and from the Reiter group study of 47 patients with SM (38 A, 9 NA).¹⁰ (NA forms: SSM ($\pm$ AHNMD), ISM ($\pm$ AHNMD), CM, SM, MCL); (A forms: ASM ($\pm$ AHNMD), MCS, MCL).

also influence the severity and progression of SM. This theory might explain the poor response to TKIs targeting KIT defects.

Data summarized here confirm that such genetic complexity exists in SM. Thus, in advSM (but not in ISM), there is increasing evidence that most patients present with 1 or several additional genetic defects, which might explain the aggressive evolution. Although some of these additional defects seem to be a rare event, encountered in only a few percent of patients with advSM tested, others are common and found in many of these patients. This finding is particularly the case for *TET2* mutants, which are found in up to 30% of patients with advSM.^{9,10} Experiments conducted by our team showed that *KIT* D816V and *TET2* mutations cooperate *in vitro* and *in vivo* to induce a more aggressive cell phenotype when present together.⁹ Also, mutations in *SRSF2* and in *ASXL1* are present in many of the patients tested and are related to a more aggressive disease.¹⁰ In addition, a significant part of the patients may have 2 to more than 5 additional mutations, as shown by studies in which *KIT* D816V+ patients were found simultaneously positive for *TET2*, *SRSF2*, *ASXL1*, *CBL*, and *RUNX1* defects.¹⁰ In line with the complexity of the associations of genetic defects found in SM by different teams, we present in **Fig. 4** Circos (circular genome data visualization) diagrams depicting the relative frequencies and associations of the major mutations in systemic SM in nonaggressive forms and in aggressive forms based on data from our work on 73 patients with SM (31 aggressive, 42 nonaggressive)¹⁰⁵ and from the Reiter group study on 47 patients with SM (38 aggressive, 9 nonaggressive).¹⁰

Data presented in this article suggest that ISM might be mainly a disease related to the sole presence of the *KIT* D816V mutant, whereas additional genetic defects (preexisting or acquired during disease evolution) might be responsible for progression to advanced phases of SM. However, in SM-AHNMD, these additional genetic defects could be related to the coexistence of 2 different diseases. This theory means that analysis of purified cell populations obtained by cell sorting or of single-cell derived clusters is mandatory to identify in which cell compartment one or the other of the mutations could be found. If these additional genetic defects are of obvious prognostic significance, their interest for future therapeutic in advSM remains to be explored.

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