Advanced systemic mastocytosis: from molecular and genetic progress to clinical practice

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Haematologica 2016
Volume 101(10):1133-1143

ABSTRACT

Systemic mastocytosis is a heterogeneous disease characterized by the accumulation of neoplastic mast cells in the bone marrow and other organ organs/tissues. Mutations in *KIT*, most frequently *KIT* D816V, are detected in over 80% of all systemic mastocytosis patients. While most systemic mastocytosis patients suffer from an indolent disease variant, some present with more aggressive variants, collectively called "advanced systemic mastocytosis", which include aggressive systemic mastocytosis, systemic mastocytosis with an associated hematologic, clonal non mast cell-lineage disease, and mast cell leukemia. Whereas patients with indolent systemic mastocytosis have a near normal life expectancy, patients with advanced systemic mastocytosis have a reduced life expectancy. Although cladribine and interferon-alpha are of benefit in a group of patients with advanced systemic mastocytosis, no curative therapy is available for these patients except possible allogeneic hematopoietic stem cell transplantation. Recent studies have also revealed additional somatic defects (apart from mutations in *KIT*) in a majority of patients with advanced systemic mastocytosis. These include *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *JAK2*, and/or *RAS* mutations, which may adversely impact prognosis and survival in particular systemic mastocytosis with an associated hematological neoplasm. In addition, several additional signaling molecules involved in the abnormal proliferation of mast cells in systemic mastocytosis have been identified. These advances have led to a better understanding of the biology of advanced systemic mastocytosis and to the development of new targeted treatment concepts. Herein, we review the biology and pathogenesis of advanced systemic mastocytosis, with a special focus on novel molecular findings as well as current and evolving therapeutic options.

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Received: March 31, 2016.
Accepted: May 25, 2016.
Pre-published: no prepublication.

doi:10.3324/haematol.2016.146563

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/101/10/1133

Introduction

Mastocytosis comprises a pathomorphologically and clinically heterogeneous spectrum of localized or systemic disorders characterized by an abnormal accumulation of mast cells (MCs) in one or more organs.[1] In children, the disease is mostly restricted to the skin (cutaneous mastocytosis: CM).[2,3] By contrast, adult patients usually present with systemic mastocytosis (SM). In patients with SM, neoplastic MCs are almost always detectable in the bone marrow (BM), and usually also in other internal organs.[1,4-7] The exact incidence of SM remains uncertain, but a prevalence of mastocytosis including all the subtypes is estimated to be approximately 1 in 10,000 people.[8] A recent study from Denmark showed the incidence rate for all SM, including CM, was 0.89 per 100,000/year.[9]

The World Health Organization (WHO) classification has defined major categories and variants of SM (*Online Supplementary Table S1*).[1,6,10] Most adult patients present with indolent SM (ISM), which is mainly characterized by mediator-related symptoms, frequent skin involvement, no organ dysfunction and a nearly normal life expectancy.[1] By contrast, in advanced variants of the disease (AdvSM), including SM with an associated clonal hematologic non-MC lineage disease (SM-AHNMD; recently updated to systemic mastocytosis with an associated hematological neoplasm (SM-AHN) by WHO),[11] aggressive SM (ASM), and mast cell leukemia (MCL), the malignant expansion and accumulation of neoplastic MCs can lead to organ damage ("C-findings", *Online Supplementary Table S2*).[6,7] No skin lesions are found in some patients.[12] Depending on the subtype, the survival of patients with AdvSM ranges from a few months to several years,[1,13,14] therefore cytoreductive therapy is indicated in most of these patients.[15]

Response criteria were developed (*Online Supplementary Table S2*),[16] and updated and detailed[17] for clinical trials by a consensus group.

Molecular defects found in advanced systemic mastocytosis KIT *mutations and their sensitivity to tyrosine kinase inhibitors*

KIT is a type III tyrosine kinase (TK) transmembrane receptor for stem cell factor (SCF), which is the major growth factor of MCs in humans (Figure 1).[18] Interestingly, in most cases of SM (overall >80%, in typical ISM >90%, and in AdvSM >70%), an acquired point mutation in the gene coding for *KIT* (CD117) is found. Although *KIT* D816V, an activation loop mutation, is the most common mutation found, more than 20 other mutations in *KIT* have been described in SM.[19,20] The exact percentages vary, depending on disease subtypes (e.g. ISM *vs.* ASM) and cell source [e.g. BM *vs.* peripheral blood (PB)].[18] The *KIT* D816V mutation is detected in AHN cells in the majority of cases, which reflects multilineage involvement.[21-23] There are, however, cases in which two independent (sub)clones exist and this might depend on the type of AHN.[24,25] *KIT* mutations often cause ligand-independent constitutive phosphorylation and activation of KIT, which transforms cell lines from factor-dependent growth to factor independence and tumorigenicity.[26-28] Longley *et al.* proposed to divide activating mutations of *KIT* into two types: "regulatory type" mutations affecting regulation of the kinase molecule, and "enzymatic pocket type" mutations, which change the amino acid sequence of the enzymatic site.[29] These latter mutations induce stabilization of the activation loop in an active conformation and/or structural alteration at the ATP-binding site of KIT, resulting in a decreased affinity for type I TK inhibitors (TKI), such as imatinib, that recognize the active conformation of a kinase. The MCL-like cell line HMC-1 has developed two sub-clones: HMC-1.1 which harbors a juxtamembrane domain (JMD) regulatory type mutation, *KITV560G*, and HMC-1.2 expressing both *KIT* D816V and *KITV*560G.[30] Imatinib inhibits only the regulatory type mutant affecting the juxtamembrane inhibitory helix, but does not significantly inhibit KIT D816V.[31] However, even some JMD-type *KIT* mutations (e.g. *KITV*559I) can cause imatinib resistance by leading to structural changes of the JMD of KIT, which affects the structure of the kinase domain.[32] Other TKIs, such as PKC412 (midostaurin) effectively suppress the activity of imatinib-resistant *KIT* mutants.[33-35] Of

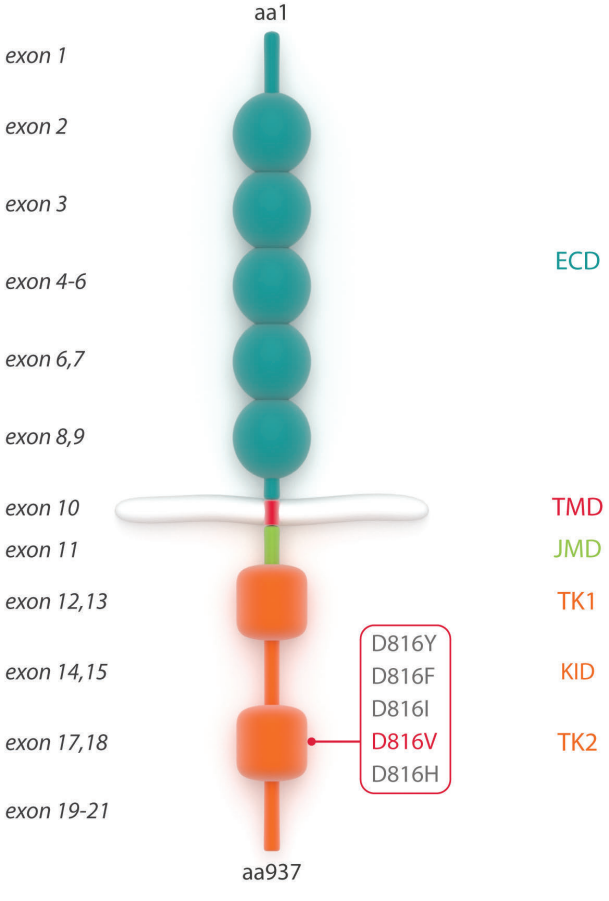

Figure 1. Structure of the KIT receptor and position of the major mutation (*KIT* D816V) found in systemic mastocytosis. The *KIT* gene, located on chromosome 4q12 in humans, contains 21 exons transcribed/translated into a transmembrane receptor tyrosine kinase (RTK) of 145 kDa and 976 amino acids. The Figure shows the receptor under its monomeric form, comprising 5 immunoglobulin (Ig)-like subunits in the extracellular domain (ECD) with a ligand binding site (SCF for KIT) and a dimerization site, and a cytoplasmic region with a transmembrane domain (TMD) made by a single helix. The cytoplasmic region of KIT contains an autoinhibitory juxtamembrane domain (JMD) and a kinase domain (in blue) arranged in a proximal (N-) and a distal (C-) lobe linked by a hinge region. The C-lobe of RTKs type III includes a large Kinase Insert Domain (KID) of ~ 60-100 residues. In adults, depending on the category of mastocytosis, the *KIT* D81V located in the phosphotransferase domain mutant (in red) is found in at least 80% of all patients, while other mutations at position 816 (in black) are less frequent by far. TK: tyrosine kinase.

note, the allele burden of the *KIT* mutant, determined by highly sensitive techniques, such as allele specific quantitative PCR (ASO-qPCR), correlates with the burden of neoplastic MCs, and with survival and prognosis.[18,36,37] Finally, although the *KIT* D816V mutant is recurrently found in SM patients, a recent report has pointed to the possibility that such patients may present with concurrent mutations in other codons of the *KIT* gene.[38] Indeed, out of 21 patients analyzed, the authors found 3 (15%) patients with *KIT* D816V and a concurrent mutation.[38] Overall, these data suggest an advantage for double mutations that might contribute to the aggressiveness of SM.

Tyrosine kinase inhibitors (TKI)

Midostaurin (*PKC412*): Midostaurin (PKC412) is an oral multi-kinase inhibitor with activity against protein kinase C (PKC), FMS-related tyrosine kinase 3 (FLT3), PDGFRA/B, vascular endothelial growth factor receptor 2 (VEGFR-2), and KIT. Midostaurin was evaluated in a centrally adjudicated, phase II multi-center international study in 116 patients with ASM, of which 89 were evaluable for efficacy.[39] Overall, 73 patients (82%) had ASM, 16 (18%) had MCL, and 63/89 patients (71%) had an AHN. Seventy-seven patients (87%) were positive for a codon 816 *KIT* mutation. After a median follow-up of 26 months (range 12-54 months), the overall response rate (ORR) was 60%. Most responses were major (75%), including decreases of >50% in serum tryptase and BM mast cell levels. These responses were durable: the median duration of response and median OS were 24.1 and 28.7 months, respectively. Median OS was 9.4 months in patients with MCL; however, responders in the MCL group did not reach a median OS. Midostaurin was tolerated fairly well with grade 1-2 gastrointestinal side effects being the most common adverse events (Table 1). Patient-reported outcomes, including symptoms and quality of life, measured by the Memorial Symptom Assessment Scale and the Short Form-12 Health Survey, respectively, significantly improved with midostaurin therapy. These results indicate that the drug has a favorable efficacy and safety pro-

Table 1. Treatment and outcomes in advanced SM

Author, (Reference#)	Therapy	Patient#	Study Type	Complications	Outcomes
Vega-Ruiz(40)	Imatinib	20 with ISM or AdvSM (n=9)	Prospective, Phase II	**Grade IV:** Thrombocytopenia 5% Neutropenia 5% Symptomatic improvement, 30% Median OS was NR	CR, 5%
Verstovsek(50)	Dasatinib	33 with ISM or AdvSM (n=15)	Prospective, Phase II	**No Grade IV** **Grade III:** Pleural effusion 21% Thrombocytopenia 18% Nausea, headache Fatigue, pain, dyspnea	ORR, 33% CR, 6.6% Median OS was NR
Gotlib(39)	Midostaurin	116 with AdvSM	Prospective, Phase II	**Grade III/IV:** Neutropenia 5% Leukopenia 4% Anemia 3% Febrile neutropenia 3% Thrombocytopenia 3% **Non-hematologic AEs:** Nausea 6%, increased lipase 4%, fatigue 4%	ORR, 60% MR, 75% IR, 36% PCR, 28% Unspecified, 11%. Good PR, 21% Minor PR, 4% **Median OS** All, 29 mos MCL 9.4 mos **Median duration of response 24 mos**
Kluin-Nelemans(119)	Cladribine	10 with ISM and AdvM (n=6)	Prospective	Cytopenia	All patients responded, no CR Median OS was NR
Barete(123)	Cladribine	68 with ISM and AdvSM (n=32)	Retrospective, registry study with a long follow-up (>10 years)	**Grade III/IV** Lymphopenia 82% Neutropenia 47% Infections 13%	ORR, 72% , No CR ORR in AdvSM 50% **Median duration of response 44 mos**
Ustun(125)	Allo-HCT	57 with Adv SM	Retrospective	TRM at 6 months: 11%	OS at 3 years: 57% SM-AHN: 74% ASM: 43% MCL: 17% DFS at 3 years: 51% SM-AHN: 63% ASM: 43% MCL: 17%

AHN: associated hematological neoplasm; AdvSM: advanced systemic mastocytosis; AE: adverse event; Allo-HCT: allogeneic hematopoietic cell transplantation; CR: complete remission; DFS: disease-free survival; IR: incomplete remission; ISM: indolent systemic mastocytosis; MCL: mast cell leukemia; Mos, months; MR: major response; NR, not reported; ORR: overall response rate; OS: overall survival; PCR, pure clinical response; PR: partial response; SM: systemic mastocytosis; TRM: transplant-related mortality.

file with activity in AdvSM regardless of *KIT* mutation status. Although midostaurin has not been approved by major drug authorities in either Europe or the USA, it is available for patients with AdvSM within a compassionate use program sponsored by the drug company.

Imatinib: After the remarkable success of TKIs in chronic myeloid leukemia (CML), significant enthusiasm for TKI in the treatment of SM emerged in the early 2000s.[19] However, imatinib is largely ineffective in patients with *KIT* D816V$^+$ SM.[40] On the other hand, some patients with SM may respond very well to imatinib, especially those with other *KIT* mutations such as K509I,[41] F522C[42] or *KIT* WT.[43] In patients with *FIP1L1-PDGFRA*-positive myeloid neoplasms with eosinophilia, small doses of imatinib (100 mg/d) will effect durable hematologic and cytogenetic/molecular remission in almost all cases.[44,45] Although some of these patients may exhibit scattered/interstitial distributions of increased abnormal CD25$^+$ MCs in the BM, these cases are not considered a subtype of SM by the WHO because typical dense infiltrates of spindle-shaped mast cells are missing. In SM patients with *KIT* WT, imatinib may even induce CR with the disappearance of skin lesions and return of elevated serum tryptase levels to the normal range (<15 ng/mL).[46] Imatinib (400 mg daily) is still the only TKI approved by the US Food and Drug Administration (FDA) for adult patients who have ASM either without the *KIT* D816V mutation or with unknown *KIT* mutational status.

Dasatinib: Dasatinib, a multikinase inhibitor (e.g. BCR-ABL1, KIT, and PDGFRα),[47,48] has proven to be effective *in vitro* against *KIT* D816V+ neoplastic MCs.[49] However, the half-life of the drug is very short, and no durable and meaningful clinical responses were observed in clinical studies in AdvSM (Table 1).[50]

Masitinib: Masitinib, which inhibits *KIT* WT and LYN,[51] is an effective drug for canine MC tumors.[52] However, in humans, the *KIT* D816V mutation introduces resistance against masitinib. In one study, masitinib was administered daily (3-6 mg orally) for 12 weeks in 25 patients diagnosed as having SM or CM with a related "handicap" (i.e. disabilities associated with flushes, depression, pruritus and quality of life).[53] ORR was 56% by AFIRMM response criteria.[54] Severe toxicities occurred in <10% of all patients. Currently, a larger study is being performed in patients with CM and ISM with a "handicap" (AdvSM was excluded).

Nilotinib: In a phase II trial of 61 patients with SM (37 with AdvSM), nilotinib (400 mg twice a day) induced overall responses of 21.6% (including a decrease in serum tryptase and BM mast cells) and of 21% in ASM.[55] All responders had the *KIT* D816V mutation. Nine AdvSM patients died during 34.7 months of follow-up. No active study is currently being performed with nilotinib.

Other targeted small-molecule inhibitors: Most of the data on these TKIs resulted from pre-clinical studies or case reports. Ponatinib, a multi-kinase blocker, inhibits the kinase activity of *KITV*560G and, less effectively, *KIT* D816V in HMC-1 cells.[35,56] Ponatinib induced dose-dependent growth inhibition and apoptosis in primary neoplastic MCs, HMC-1.1 cells, and HMC-1.2 cells.[56] Ponatinib and midostaurin were found to exert synergistic growth-inhibitory effects against neoplastic MCs harboring the *KIT* D816V mutant.[56] Other novel TKIs with potent TKI inhibiting properties (e.g. EXEL-0862)[57] and thiazole amine derivatives inhibiting β-catenin signaling (e.g. semaxinib (SU5416) and compound 126332).[58,59] BLU-285, a selective KIT D816V inhibitor with encouraging pre-clinical activity and a narrow target profile, is expected to enter clinical trial testing in AdvSM in the near future.[60]

Progress in somatic mutations other than KIT *in SM*

Recent studies have reported the presence of additional, recurrent somatic mutations (apart from *KIT* mutations) in AdvSM, especially in SM-AHN, including mutations in *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *JAK2*, and/or *RAS* (Figure 2).[61-65]

Mutations in *TET2*, also detected in healthy individuals,[66] cause loss of function (i.e. regulating gene expression at the cellular level),[67] and are associated with increased self-renewal capacity of hematopoietic stem cells.[68] 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.[69] Patients with mutant *TET2*+ myeloid disorders show a decreased level of 5-hmC with hypomethylation or hypermethylation of DNA.[70] Altogether these data show that TET2 plays a role in various hematologic malignancies. In line with these recently published data, *TET2* mutations have been reported in 20-40% of *KIT* D816V-positive AdvSM patients.[25,61,62,64] The cooperation between *KIT* D816V and loss of function of *TET2* in MC results in transformation to a more aggressive disease phenotype in mice.[71] It has also been suggested that *TET2* mutations can occur before *KIT* D816V in ASM-AHN patients.[72] Thus, the acquisition of *KIT* D816V might act as a phenotype modifier of ASM in these cases.[72] Patients carrying a combination of *TET2* and *DNMT3A* (a DNA methyltransferase) mutations have a poor prognosis compared to those with wild-type genes.[61] *In vitro*, a combination of dasatinib and decitabine (a hypomethylating agent) was more effective at inducing apoptosis and cell death in HMC-1.2 cells harboring a *TET2* mutant compared to each compound alone.[71] This combination also had less effect in *TET2* wild-type cells due to a lower efficacy of decitabine. The impact of *TET2* mutations on overall survival remains uncertain.[61,62,73]

The spliceosome machinery includes SRSF2, U2AF1, and SF3B1 proteins, and is involved in the removal of introns from a transcribed pre-mRNA.[74] Mutations in the spliceosome machinery have recently been identified using whole exome/genome technologies in MDS and MPN.[75] A mutation in the hotspot region of *SRSF2* (codon P95) is found in approximately 1/3 of AdvSM patients[64,65] but is usually not detectable in patients with ISM or SSM.[25] It is more common in ASM-AHN[25,64,65] and precedes *KIT* D816V in these patients.[25] The frequency of *SF3B1* mutations in AdvSM is low, ranging from 0 to 5%.[64,65] *U2AF1* mutations are less frequently reported in SM.[64,65]

The gene *ASXL1* (additional sex combs–like 1) encodes for a protein of the polycomb group and trithorax complex family, which interacts with retinoic acid receptor and may be involved in chromatin remodeling.[76] The presence of *ASXL1* mutations has been reported in SM at various frequencies,[25,61,64,73] and alone or with other mutations seems to be a poor prognostic factor for OS in patients.[61,64,73] *RUNX1*, and less frequently, *JAK2* mutations,

are found in AdvSM, but not in ISM or SSM.[64] The frequency of *RAS* mutations (e.g. *NRAS*, *KRAS* or *HRAS*) in SM has been investigated, with *KRAS* and *NRAS* mutants being found in AdvSM at a relatively low frequency, and not usually detectable in patients with ISM.[63,64]

The presence of additional genetic defects in *KIT* D816V+ AdvSM patients may confer adverse prognosis as compared with patients without such abnormalities.[64,72] In a recent study, Jawhar *et al.* have analyzed the impact of several additional defects on 70 multi-mutated *KIT* D816V+ patients with an AHN.[77] In this study, the most frequently identified mutated genes were *TET2* (n=33 of 70 patients), *SRSF2* (n=30), *ASXL1* (n=20), *RUNX1* (n=16) and *JAK2* (n=11).[77] In multivariate analysis, *SRSF2* and *ASXL1* remained the most predictive adverse indicators concerning OS. Furthermore, the authors found that inferior OS and adverse clinical characteristics were significantly influenced by the number of mutated genes in the *SRSF2/ASXL1/RUNX1* (S/A/R) panel ($P<0.0001$).[77]

It appears that, based on these findings, the inclusion of molecular markers should be considered in upcoming prognostic scoring systems for patients with SM. This might be particularly important for patients with SM-AHN given that most of these studies were done in patients with SM-AHN.[73,78] Although it is arguable that these mutations could be detected due to the copresence of an AHN, there are recent studies in pure SM showing these mutations as well.[72,79] In addition, it has been described in many previous reports that *KIT* mutations are not restricted to the mast cell disease components in SM-AHN.[80] Although we are at an early stage in the understanding of the clinical and biological importance of these mutations in SM, most likely these mutations affect hematopoietic stem and progenitor cells, and the rate of multilineage involvement increases with the aggressiveness of SM.

In addition, recent investigations on mutational profiles of colonies grown from granulocyte-macrophage colony-forming progenitor cells (CFU-GM) and microdissected mature cells (tryptase or CD15 positive) revealed that these additional mutations develop prior to *KIT* D816V in almost all patients, indicating a multi-mutated stem cell disease with strong phenotype modification (i.e. the mastocytosis component) driven by *KIT* D816V.[25]

Critical intracellular pro-oncogenic pathways in neoplastic mast cells as novel potential therapeutic targets

Several studies have reported that the ability of wild-type and oncogenic mutant forms of KIT to induce signal transduction differs not only quantitatively but also qualitatively. These altered pathways, which are presented in Figure 3 together with potential targeted drugs, may have an effect on several properties of neoplastic MCs by reducing apoptosis and/or by inducing alterations in the cell cycle.

MCL-1, a *BCL-2* family member with anti-apoptotic properties, is expressed in primary neoplastic MCs in SM as well as in the HMC-1.1 and HMC-1.2 cell lines.[81] The targeting of *MCL-1* by antisense oligonucleotides (ASOs) or *MCL-1*-specific siRNA resulted in reduced survival and increased apoptosis in these cell lines.[81] Moreover, *MCL-1* ASOs cooperated with various KIT-targeting TKIs in producing growth inhibition in neoplastic MC lines.[81]

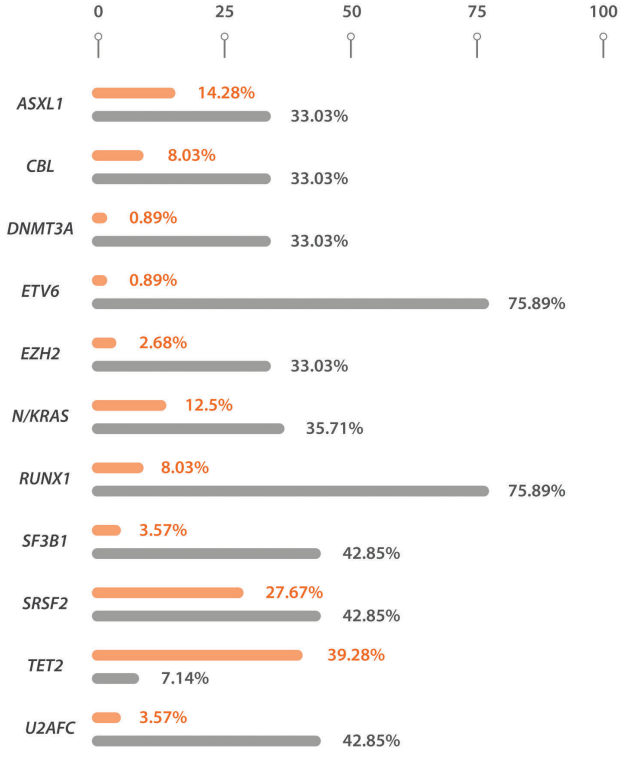

Figure 2. Synthesis of the frequency of the various molecular defects found in AdvSM, which sums up all the advanced (AdvSM) SM patients (n=122) reported in the studies by Tefferi *et al.*,[62] Wilson *et al.*,[63] Traina *et al.*,[61] Schwaab *et al.*[64] and Hanssens *et al.*[65] The frequency (%) of cases found positive for each genetic defect is represented in red, whereas the frequency of patients for whom the corresponding defect was not tested is represented in blue.

BIM, a pro-apoptotic member of the BCL-2 family, has been identified as a tumor suppressor in neoplastic MCs.[82] BIM is downregulated in neoplastic MCs by SCF as well as by KIT D816V.[82] Midostaurin, bortezomib (a proteasome inhibitor), and obatoclax (a pan-BCL-2 family blocker) reportedly upregulate BIM expression in HMC-1 cells and may thereby promote apoptosis.[82,83] Obatoclax also increased apoptosis in these cells.[83]

Activated LYN and BTK are expressed in neoplastic MCs in a KIT-independent manner in patients with ASM and MCL, and may thus contribute to malignant transformation.[49] LYN is a member of the SRC family involved in cellular signaling processes regulating growth, differentiation, and apoptosis. Activated LYN regulates BTK function and may influence the process of degranulation and cytokine production in MCs.[84,85] Dasatinib and bosutinib (SRC inhibitors) disrupt LYN and BTK activation and oncogenic signaling in neoplastic MCs.[49] Bosutinib inhibits the growth of neoplastic MCs *in vitro* at relatively high concentrations, with no effect on KIT.[49,86] Bosutinib acts synergistically with midostaurin on HMC-1 cell proliferation.[49] However, bosutinib is unable to induce any response in patients with AdvSM.[87]

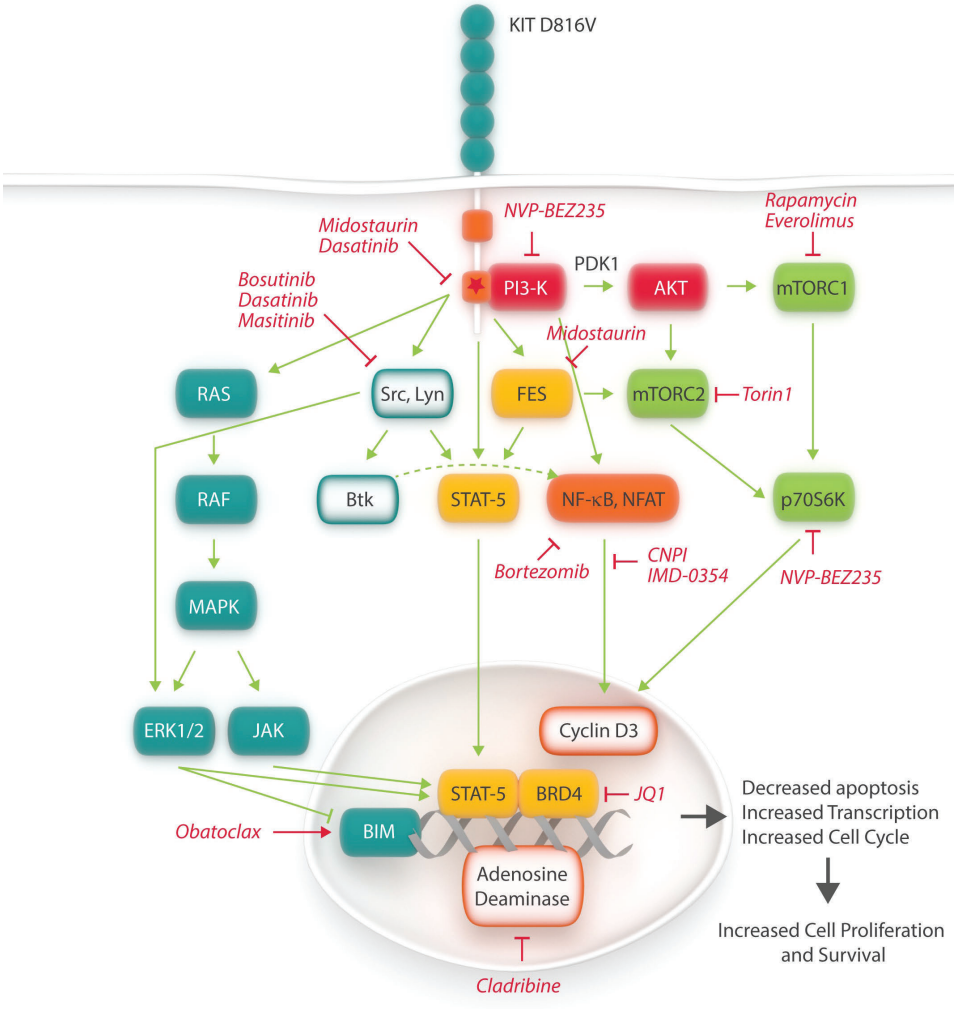

Figure 3. Intracellular pathways involved in the accumulation/proliferation of neoplastic mast cells in SM and agents which could be potentially used to target one or the other of these molecules. That KIT D816V dimerizes spontaneously with itself or with KIT WT, or is capable of transmitting oncogenic signals as a single molecule, remains largely unexplored. However, it has been postulated whether the *KIT* D816V protein could activate substrates under a monomeric form and could even be located in the cell cytoplasm. The KIT D816V oncogenic mutation alters the substrate specificity of the mutant protein, which shows a substrate specificity resembling 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 the 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 number of drugs (in red and in italics) can potentially selectively inhibit some of these critical pathways. Red arrows: inhibition; black arrows: induction of survival or functions; green arrows: activation of signaling pathways; dark blue arrow: induction of increased synthesis.

Phosphoinositide 3-kinase (PI3-K), a lipid kinase, is important for the function of intracellular signaling molecules, like BTK, AKT and PDK1, by inducing phosphatidylinositol 3,4,5-trisphosphate (PIP3) that provides membrane docking sites for these signaling molecules.[88] In both HMC-1 subclones (HMC-1.1 and HMC-1.2), mutated KIT leads to constitutive activation of PI3-K.[89] Once activated, the PI3-K subsequently activates AKT,[89] a key signaling molecule involved in KIT-dependent differentiation and growth of neoplastic MCs harboring oncogenic *KIT* mutants.[90] Indeed, AKT was found to be phosphorylated in neoplastic MCs in patients with *KIT* D816V+ SM and in the HMC-1.2 cell line.[90,91]

PI3-K and AKT are also important for the regulation of the mammalian target of rapamycin (mTOR), a serine/threonine kinase that interacts with 2 regulatory protein complexes called mTOR complex 1 (mTORC1) and complex 2 (mTORC2). PI3-K regulates the mTORC1 pathway *via* the activation of AKT which directly inactivates tuberin, the inhibitor of mTOR activation. Once activated, mTORC1 phosphorylates p70 ribosomal S6 kinase (p70S6K), resulting in increased gene transcription that regulates cell growth, survival, protein synthesis and metabolism. Smrz *et al.* showed that the expression and activation of mTORC1 and mTORC2 was increased in neoplastic human MC lines and in immature normal MCs, as compared with mature normal MCs.[92] Interestingly, the authors demonstrated that mTORC1 might contribute to MC survival, while mTORC2 might only fulfill critical functions in the context of proliferating (dividing) neoplastic and immature MCs.[92] Rapamycin, a specific inhibitor of mTORC1, has been shown to block FcεRI- and KIT-induced mTORC1-dependent p70S6K phosphorylation in normal MCs.[88] Furthermore, BEZ235, a dual PI3-K/mTOR blocker, exerted strong growth-inhibitory effects on neoplastic MCs *in vitro*.[93] Of note, BEZ235 was also found to reduce the engraftment and growth of HMC-1 cells in a xenotransplanted mouse model employing NMR1-Foxn1(nu) mice.[93] Everolimus, another mTOR-blocker, was ineffective in patients with SM.[94]

Neoplastic MCs express cytoplasmic and nuclear phospho-STAT5 (pSTAT5).[95] In an *in vitro* study,[90] knockdown of STAT5 was followed by growth inhibition of neoplastic MCs. Furthermore, it has been shown that KIT D816V directly promotes STAT5-activation, and that pSTAT5 contributes to the growth of neoplastic MCs.[95] This makes

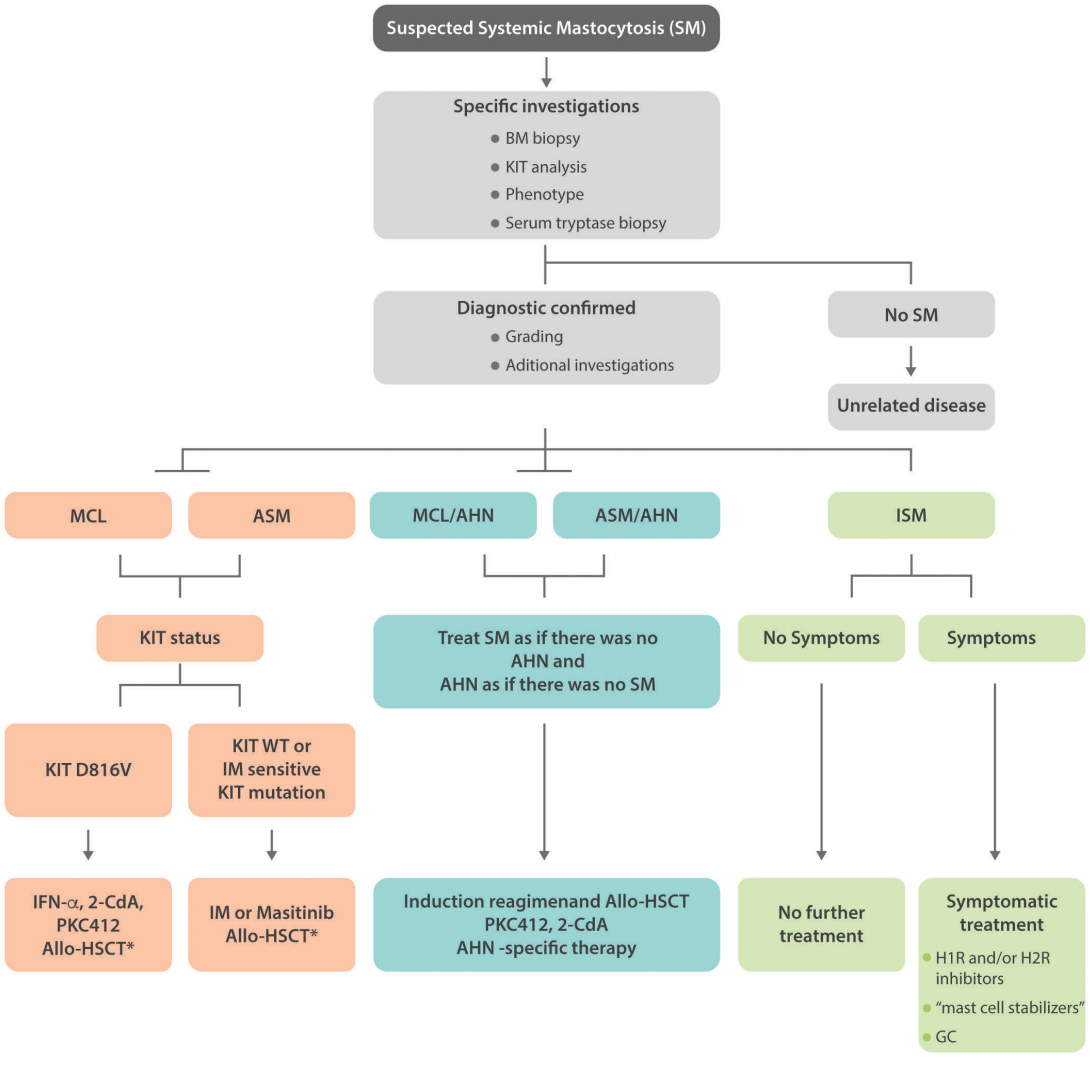

Figure 4. Proposed algorithm for the diagnosis and classification of the different categories of SM patients and for the preferable therapeutic options adapted to each category of the disease. AHN: associated hematological neoplasm; Allo-HSCT: allogeneic hematopoietic stem cell transplantation; ASM: aggressive systemic mastocytosis; BM: bone marrow; 2-CdA: cladribine; GC: glucocorticoids; HR: histamine receptors; IFN-α: interferon-alpha; IM: imatinib mesylate; ISM: indolent systemic mastocytosis; MCL: mast cell leukemia; PKC412: midostaurin; SM: systemic mastocytosis; WT: wild-type.

STAT5 an attractive target for therapy in AdvSM. However, until now, most drugs targeting STAT5 exert anti-neoplastic effects only at high, non-pharmacological concentrations *in vitro*. The inhibition of the JAK-STAT signaling pathway *in vitro* decreased KIT D816V-mediated cell growth.[96] Ruxolitinib, a JAK1/2 inhibitor, has shown clinical benefit in patients with MPN regardless of JAK2 V617F-mutation.[97] Ruxolitinib decreased spleen size and improved blood counts in a *KIT*-mutated but not *JAK2*-mutated patient with SM-MPN primary myelofibrosis.[98] Therefore, JAK1/2 blockers can be considered in studies of patients with SM-MPN.

NF-κB, a dimeric transcription factor of the REL family, was found to be spontaneously activated in HMC-1 cells.[99] IMD-0354 inhibited translocation of NF-κB to the nucleus, and thus led to decreased cyclin D3 expression and increased cell cycle arrest in HMC-1 cells *in vitro*.[99] Another transcription factor of the REL family, nuclear factor of activated T cells (NFAT), has also been found constitutively activated in *KIT*-mutated neoplastic MCs.[100] The combination of a KIT inhibitor and of a calcineurin phosphatase inhibitor (a NFAT regulator) exhibited a synergistic inhibitory effect on cell viability and survival in *KIT*-mutated MC lines.[100]

One promising class of targets within chromatin regulatory molecules and related antigens are the bromodomain (BRD)-containing proteins.[101-103] Indeed, inhibition of the epigenetic reader bromodomain-containing protein-4 (BRD4) by exposure to RNA interference or treatment with JQ1, a drug blocking the specific interactions between BRD4 and acetylated histones, resulted in major antileukemic effects in murine and human AML cells.[102] More recently, BRD4 has been identified as a novel drug target in AdvSM.[104] The authors showed that neoplastic MCs expressed substantial amounts of BRD4 in ASM and MCL, as assessed by immunohistochemistry and PCR.[104]

They also reported that the human MCL lines HMC-1 and ROSA also expressed BRD4, and that a BRD4-specific short hairpin RNA or the BRD4-targeting drug JQ1 induced dose-dependent growth inhibition and apoptosis in HMC-1 and ROSA cells, regardless of the presence or absence of the *KIT* D816V mutant.[104] Moreover, the authors demonstrated that JQ1 suppressed the proliferation of primary neoplastic MCs obtained from patients with ASM or MCL. Finally, in drug combination experiments, midostaurin (PKC412) and all-*trans* retinoic acids were found by the authors to cooperate with JQ1 in producing synergistic effects on survival in HMC-1 and ROSA cells.[104] Taken together, these data identified BRD4 as a promising drug target in advanced SM. However, whether JQ1 or other BET bromodomain inhibitors are effective *in vivo* in patients with AdvSM remains to be elucidated.

Antibody-mediated therapeutic approach to target neoplastic mast cells and stem cells

Based on recent knowledge on the phenotype of malignant MCs and their neoplastic progenitors, a number of cell surface antigens might be aberrantly expressed, including CD13, CD25, CD30, CD33, CD44, CD52, CD87, and CD117, and therefore might be considered also as potential targets of therapy in AdvSM.[105-110] For example, neoplastic MCs and their progenitors have been shown to respond *in vitro* to gemtuzumab ozogamicin (a monoclonal antibody targeting CD33 combined to a cytostatic agent).[111] The CD52-targeting antibody alemtuzumab induces cell death in neoplastic MCs *in vitro* and in mice xenotransplanted with HMC-1 cells.[108] CD30 is expressed on the surface of neoplastic MCs in a proportion of patients with AdvSM, but not on normal/reactive MCs, making this antigen an attractive target of specific therapy in these patients.[107,112,113] A single-arm, open-label clinical trial applying brentuximab vedotin (SGN-35) to patients with CD30-positive AdvSM (*clinicaltrials.gov identifier: 01807598*) is ongoing in the US. Neoplastic (leukemic) stem cells (LSCs) have recently been identified in AdvSM. These cells reside within a $CD34^+$ cell fraction and co-express aminopeptidase N (CD13), leukosialin (CD43), Pgp-1 (CD44), the IL-3R α-chain (CD123), AC133 (CD133), CXCR4 (CD184), CD33, CD52 and CD117.[114,115] As observed in chronic myeloid leukemia, a part of these LSCs might be non-cycling and therefore probably resistant to treatment with TKIs. Thus, a combination of a TKI that targets KIT on neoplastic MCs and a mAb targeting a surface antigen, such as CD52 for instance, expressed on non-cycling LSCs, may help to achieve a minimal residual disease negative state in AdvSM.

Conventional therapies with anti-neoplastic drugs and allogeneic hematopoietic cell transplantation

Cytarabine, fludarabine, hydroxyurea (a drug of choice in palliative care)[15] and interferon-alpha (IFN–α),[116-118] have been frequently used for cytoreduction in the treatment of AdvSM. Hydroxyurea is useful to control leukocyte counts in AdvSM, especially in SM-AHN (palliative therapy) and in patients with comorbidity. Cladribine (2-CDA) is the most effective and frequently used drug. Kluin-Nelemans *et al.*, used 2-CdA in 10 patients with SM, most of them suffering from AdvSM (Table 1).[119] All patients responded concerning clinical symptoms and MC burden as reflected in declining serum tryptase values and urinary histamine metabolite excretion. Although no patient achieved a complete remission (CR), clinically meaningful and some durable responses were seen, suggesting that 2-CdA may be a potentially effective treatment option for some patients with severe SM.[119] These results have been supported by more recent studies.[120-123] For instance, in a study on 44 SM patients, the median duration of response was 20 months; however, none of the patients with SM-AHN responded.[122] However, 2-CdA usually does not control the disease for prolonged periods of time in rapidly progressing ASM and MCL. For these patients, more intensive therapy, such as AML-like multi-agent chemotherapy, including fludarabine and cytarabine[124] should be considered in induction therapy and then for allogeneic hematopoietic cell transplantation (HCT) for consolidation therapy.[125-127] Allogeneic HCT remains the only potentially curative treatment option for patients with AdvSM. We have recently reported data on the effect of allo-HCT in patients with AdvSM (Table 1).[125] Most patients (the median age was 46) received a graft from HLA-identical siblings (n=34) or unrelated donors (URD) (n=17). Overall survival (OS) and SM progression-free survival (PFS) at 3 years for all patients were 57% and 51%, respectively. They were significantly affected, however, by the type of advanced SM: 74% and 63%, respectively, for SM-AHN; 43% and 43%, respectively, for ASM; and 17% and 17%, respectively, for MCL. Although the data presented are very encouraging, future prospective studies, perhaps per recommended consensus opinion to homogenously collect data,[128] are required to confirm the safety[129] and efficacy of this treatment approach in AdvSM.

Miscellaneous aspects of management in AdvSM

Patients with SM-AHN should be treated according to generally accepted guidelines: the SM component of the disease is treated as if no AHN was diagnosed, and the AHN component of the disease is treated as if no SM was diagnosed, with the recognition of potential drug interactions[10,15] deciding whether the SM or AHN component is primarily contributing to organ damage or other related clinical, laboratory concerns. However, admittedly it is often not possible to clearly delineate whether one or the other component is responsible for the clinical issues/organ damage.

As a supportive therapy, H1-receptor antagonists, such as the classical antihistamine hydroxyzine, or non-sedating antihistamines, such as loratadine or fexofenadine, can be administered for the alleviation of symptoms caused by the release of the mediators (e.g. pruritus and flushing).[130-132]

Conclusion and perspectives

Advanced variants of SM share two major characteristics: i) the prognosis of the disease remains poor, and ii) other than allogeneic HCT no curative therapy is available. Only a few drugs have shown beneficial effects in AdvSM (2-CdA, interferon-alpha, and midostaurin). We propose a treatment algorithm with current therapy options (Figure 4). However, this is a subject to change in the future due to remarkable progress in the biology of AdvSM. Neoplastic cells in SM are usually driven by a canonical KIT-downstream pathway as well as by addi-

tional somatic mutations and KIT-independent pathways and molecules, including *TET2*, the spliceosome machinery, *ASXL1*, or *RAS*. We may better prognosticate AdvSM using these additional genetic defects. The PI3-kinase, AKT, STAT-5, BTK, FES, mTORC2, and BCL-2 family members as well as certain surface molecules and disease initiating (quiescent) neoplastic stem cells can be a target for therapies in the future. Potentially, studies will combine the most effective targeted drugs with one another and/or with conventional chemotherapy options to improve patient survival.

Acknowledgments

The authors thank Sabrina Porter for her assistance in the manuscript preparation. M. Arock is supported by Fondation de France. J. Gotlib is supported by the Charles and Ann Johnson Foundation. O. Hermine is supported by Agence nationale pour la recherche (ANR) and by Fondation pour la recherche médicale (FRM). P. Valent is supported by Austrian Science Funds (FWF) Projects SFB F4611 and SFB F4704-B20. A. Reiter is supported by research grants from the 'Deutsche José Carreras Leukämie-Stiftung e.V. (H 11/03 and R 13/05)). D. D. Metcalfe is supported by the Division of Intramural Research, NIAID/NIH.

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