

Tyrosine Kinase Inhibition in Mastocytosis

KIT and Beyond *KIT*



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KEYWORDS

- Mast cells • Mastocytosis • *KIT* mutations • Advanced disease
- Tyrosine kinase inhibitors • Targeted drugs • Drug development

KEY POINTS

- Mastocytosis is a group of orphan diseases characterized by abnormal accumulation/proliferation of mast cells in one or several organs.
- In adults, mastocytosis is mostly systemic and chronic, affecting the bone marrow and other internal organs, with or without skin involvement.
- Indolent systemic mastocytosis (SM) is usually well controlled by symptomatic therapy, whereas, in advanced SM, cytoreductive drugs are needed.
- In most patients with SM, a recurrent activating *KIT* mutation (D816V) is found in the neoplastic mast cells.
- In advanced SM, patients may benefit from treatment by *KIT*-targeting tyrosine kinase inhibitors; but new targeted drugs or drug combinations are still needed to improve patients' outcome.

INTRODUCTION

Mast cells (MCs) are hematopoietic stem cells–derived tissue resident granulated cells found close to blood vessels, nerves, and mucosal surfaces, such as respiratory and gastrointestinal (GI) tracts.¹ The major growth and differentiation factor for the MC lineage is stem cell factor (SCF), which binds *KIT*, a transmembrane receptor with intrinsic tyrosine kinase (TK) activity (**Fig. 1**).²

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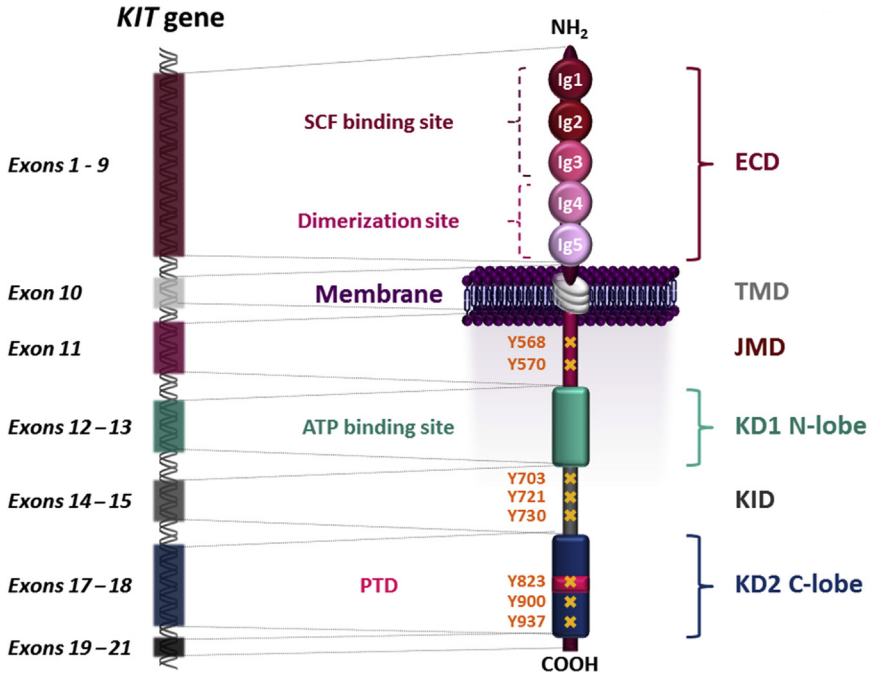


Fig. 1. Structure of the human normal *KIT* gene and of the corresponding KIT receptor. The *KIT* gene (left) contains 21 exons, encoding for a 145 kDa transmembrane protein, namely, the KIT receptor (right). KIT is represented in its monomeric form with its major structural and functional domains. The extracellular domain (ECD) is made by a longitudinal arrangement of 5 immunoglobulin-like (Ig-like) domains; the first 3 Ig-like domains of the ECD bind the SCF, whereas Ig-like domain 4 and 5 are involved in the dimerization process. The transmembrane domain (TMD) is composed of a hydrophobic α -helix allowing insertion of the receptor into the plasma membrane. The intracellular region contains the auto-inhibitory juxta-membrane domain (JMD) and a kinase domain (KD) divided by an insert region called kinase insert domain (KID) into a proximal N-lobe (KD1), which binds adenosine triphosphate (ATP) and a distal C-lobe, which contains the phosphotransferase domain (PTD). Yellow crosses indicate the position of the 8 tyrosine residues, which are phosphorylated upon activation of the receptor after ligation of SCF and dimerization.

Mastocytosis is a group of orphan diseases characterized by abnormal expansion of neoplastic MCs in at least one organ or tissue.³ The most frequently affected organs/tissues are skin, bone marrow (BM), and GI tract.³ Mastocytosis can affect both children and adults, with mostly pure cutaneous involvement in children, whereas systemic involvement is usually found in adults.³ Most pediatric cases tend to resolve at adolescence, although some cases persist into adulthood.³ By contrast, adult patients usually present with a chronic and indolent disease (indolent systemic mastocytosis [ISM]).³ However, in some adult patients, more advanced subtypes of systemic mastocytosis (SM) may be diagnosed, for example, aggressive SM (ASM), SM with an associated hematologic neoplasm (SM-AHN), and MC leukemia (MCL).^{3,4} Aggressive SM, SM-AHN, and MCL are collectively termed advanced SM (advSM). In ISM, clinical signs and symptoms are mostly related to increased mediator release by neoplastic MCs, whereas in advSM mediator-related symptoms are accompanied by organs damages following infiltration by neoplastic MCs.³

Most patients with mastocytosis (pediatric and adults) present *KIT* activating mutations in their neoplastic MCs.⁵ Of note, a recurrent *KIT* mutation (D816V) located in the phosphotransferase domain (PTD) of the receptor is detectable in most patients with SM, including those with advSM.⁵ Thus, it has been rapidly tempting to investigate the potential of KIT-targeted TK inhibitors (KIT-TKIs) for treatment. In this review, following a brief overview of the classification, physiopathology, and classic therapeutic options for SM, the authors focus on the present advances made to treat patients with advSM with KIT-TKIs. Because evidence suggest that such drugs might perhaps not be sufficient to cure patients with advSM, the authors finally discuss some future opportunities of alternative (combined) therapies.

CLASSIFICATION AND PHYSIOPATHOLOGY OF MASTOCYTOSIS

The World Health Organization (WHO) classifies mastocytosis into 3 broad categories: cutaneous mastocytosis (CM), SM, and MC sarcoma (MCS) (Table 1).⁶

Table 1 The 2016 updated World Health Organization's classification of mastocytosis, including criteria for diagnostic of systemic mastocytosis			
Categories	Subtypes	Diagnostic Criteria	Prognosis
CM	• MPCM = UP^a • DCM • Mastocytoma of skin	No systemic involvement (most patients are children)	+/- Good
SM ^b	ISM	• No B or C finding • Most patients are adults	Good
	SSM	• 2 or more B findings, no C findings	Good
	SM-AHN	• SM criteria and WHO diagnostic criteria for AHN are fulfilled • Frequently associated to myeloid AHNs (MPN, MDS, MPN/MDS), rarely to lymphoid AHNs	Depends on the type of SM and of the AHN
	ASM	• At least 1 C finding	Poor
	MCL	• BM smear >20% MCs • PB smear >10% MCs	Very poor
MCS		• Rare form of high-grade solid MC tumor • Very atypical MCs	Very poor

Abbreviations: DCM, diffuse CM; MDS, myelodysplastic syndrome; MPCM, maculopapular CM; MPN, myeloproliferative neoplasm; SSM, smoldering SM; UP, urticaria pigmentosa.

^a A recently published consensus report of the European Competence Network on Mastocytosis, the American Academy of Allergy, Asthma & Immunology, and the European Academy of Allergology and Clinical Immunology recommends that the typical maculopapular cutaneous lesions (urticaria pigmentosa) should be subdivided into 2 variants, namely, a monomorphic variant with small maculopapular lesions, typically seen in adult patients, and a polymorphic variant with larger lesions of variable size and shape, typically seen in pediatric patients. Clinical observations suggest that the monomorphic variant, if it develops in children, often persists into adulthood, whereas the polymorphic variant may resolve around puberty.⁷⁹

^b According to the WHO, SM diagnosis requires the presence of both the major criterion and one minor criterion or at least 3 minor criteria. The major criterion consists of the presence of multifocal, dense infiltrates of aggregated MCs (>15 MCs) detected in BM and/or other extracutaneous organs. Minor criteria are the following: (1) atypical morphology in greater than 25% of MCs in infiltrates; (2) presence of an activating *KIT* point mutation at codon 816 of in BM, blood, or an extracutaneous organ; (3) aberrant expression of CD2 and/or CD25 by neoplastic MCs; and (4) elevated serum tryptase level (>20 ng/mL).

Data from Refs.⁶⁻⁸

Pure CM is the most common form of mastocytosis, generally affecting children. Several variants of CM are described (see [Table 1](#)).⁷ Contrasting to CM, SM is detected mostly in adults. In SM, neoplastic MCs infiltrate internal organs, especially the BM, with or without skin involvement.³ Diagnostic criteria for SM have been established by the WHO (see [Table 1](#)).⁸

Once the diagnosis of SM is established, the next step is to classify the disease into 5 subtypes (see [Table 1](#)). This classification is based on the presence or absence of B (borderline benign) findings and/or C (consider cytorreduction) findings, reflecting, respectively, a high MC burden and end-organ damages.⁷ In the absence of B and C findings and of an AHN, patients are classified as having ISM. Such patients have a nearly normal life expectancy but may have MC mediator-related symptoms (pruritus, flush, gastrointestinal tract (GIT) symptoms, anaphylaxis, and so forth).⁹ Besides, in patients with SM, the presence of 2 or more B findings but no C findings reflects a smoldering SM, a subtype of slowly progressive SM with high MC burden and intermediate prognosis.¹⁰ By contrast, the presence of at least one C finding classifies the disease as ASM (MCs in BM smears <20% of nucleated cells) or MCL (MCs in BM smears >20% of nucleated cells), which share a poor prognosis.¹¹ Finally, SM-AHN is diagnosed when another hematological neoplasm is found associated with the different subtypes of SM.¹² In SM-AHN, the prognosis varies according to the aggressiveness of the SM component and to the one of the AHN.¹¹

The pathophysiology of mastocytosis is governed by the presence of *KIT* activating mutations in neoplastic MCs.¹³ Various *KIT* mutations have been described, firstly in patients with SM¹⁴ and then in children with CM ([Table 2](#)).¹⁵ In adults patients with SM, *KIT* mutations affect primarily exon 17 encoding for PTD, usually D816V (>80% of all patients) (see [Table 2](#)).¹⁶ Other less frequent mutations affect exons 2, 8, and 9 encoding for the extracellular domain (ECD) or exons 13 and 14 encoding for Kinase Domain 1 (see [Fig. 1](#), [Table 2](#)).⁵ By contrast, in children, although a *KIT* mutation is found in 75% of skin biopsies, only 30% of the patients harbor the *KIT* D816V mutation.¹⁵ The other mutations affect the ECD (codon 8 and 9) (see [Table 2](#)).¹⁵ Finally, in the rare cases of familial mastocytosis, often represented by infant CM, most patients do not have *KIT* mutations or exhibit rare mutations, such as K509I, N835I, N835K, A533D, M835K, S841I, or K839E.⁵

The *KIT* D816V mutation induces *KIT* constitutive activation in the absence of the SCF ([Fig. 2](#)), the exact mechanism of which being not yet fully understood. The mutant receptor could either dimerize spontaneously with another mutant receptor or with a wild-type (WT) *KIT* receptor or could trigger a signaling cascade in its monomeric form.¹⁷ Anyway, structural changes induced by the mutation affect normal *KIT* signaling and induce resistance to TKIs, such as imatinib.¹⁸

The development of neoplastic MCs is principally governed by the PI3K/AKT (phosphoinositide 3-kinase[PI3K]) and Janus kinase/Signal transducer and activator of transcription 5 (JAK/STAT5) signaling pathways activated downstream of the *KIT* mutant receptor (see [Fig. 2](#)).^{19,20} AKT and STAT5 are constitutively activated in neoplastic MCs from patients with *KIT* D816V⁺ SM, and their specific inhibition induces growth arrest of such cells.^{19,21} Besides, other intracellular pathways and molecules, such as the Feline sarcoma oncoprotein (FES),²² or the mammalian target of rapamycin (mTOR) complex,²³ are also potential actors of oncogenesis linked to the *KIT* D816V mutant (see [Fig. 2](#)). In addition, the mutant can activate Extracellular signal-regulated kinase (ERK) independently of Sarcoma kinase (SRC), in contrast to *KIT* WT.²⁴ Finally, Lyn and Btk are found activated in neoplastic MCs, in a *KIT* D816V-independent manner.²⁵

In ISM, the *KIT* D816V mutant seems to be the unique genetic abnormality found, which might explain the low incidence of progression.^{26,27} By contrast, additional and recurrent somatic mutations of particular genes have been reported in advSM,

Table 2
Updated frequencies of the different *KIT* mutations found in mastocytosis

Mutation	KIT Domain	Frequency (Adults/Children) (%)
Y269C	ECD	<10
Del417–418–419insNA		<10
Del417–418–419insI		<10
Del417–418–419insY		<10
Del419		5–20 children
InsFF419		<10
C443Y		<10
S451C	TMD	<10
S476I		<10
ITD501–502		1–5 children
ITD502–503		1–5% children
Y503–504insAY		<10
ITD504		<10
ITD505–508		<10
K509I		5–20 children
F522C	JMD	<10
A533D		<10
K550N		<10
V559I		<10
V560G		<10
Del564–576		<10
D572A		<10
R633W	KD1	<10
K642E		<10
L799F	KD2	<10
InsVI815–816		<10
D816Y		Children < adults
D816F		<10
D816I		Children < adults
D816V		30 in children/90 in adults
D816H		<10
I817V		<10
V819Y		<10
D820G		<10
N822I/K		<10
E839K		<10
S840N		<10
S849I		<10

Abbreviations: Del, deletion; ECD, extracellular domain; ins, insertion; ITD, internal tandem duplication; JMD, juxta-membrane domain; KD, kinase domain; TMD, transmembrane domain.

Data from Arock M, Sotlar K, Akin C, et al. *KIT* mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 2015;29(6):1223–32.

particularly in SM-AHN, the genes most frequently affected are *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, *JAK2*, *N/KRAS*, and *CBL*.^{26–30} By contrast, defects in *EZH2*, *IDH2*, *ETV6*, *U2AF*, or *SF3B1* are less frequent.³¹ These defects usually contribute to aggressiveness of the disease, particularly in multi-mutated patients,^{26,28–30} although a recent study led on 70 patients with *KIT* D816V⁺ advSM has revealed that overall survival (OS) is influenced by *SRSF2*, *ASXL1*, and *RUNX1* (S/A/R) mutations but not by *TET2* or *JAK2* mutations.²⁷

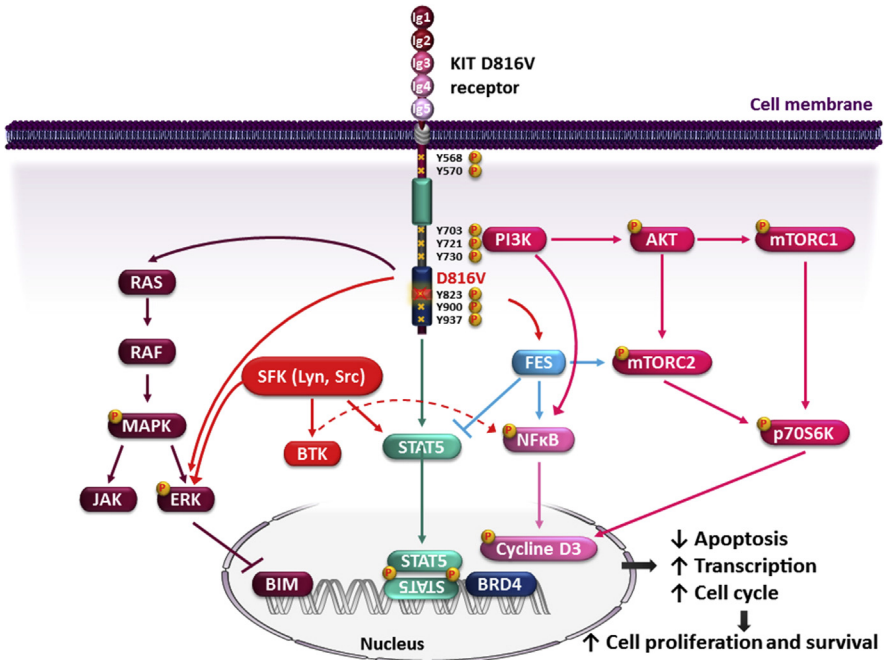


Fig. 2. Aberrant signaling pathways evoked by the KIT D816V mutant receptor. The D816V mutation activates constitutively the KIT receptor in the absence of SCF and alters the specificity of the protein, which shows substrate specificity that resembles that of SRC and ABL tyrosine kinases. The KIT mutant recruits downstream signaling pathways, though to be critical for the development of neoplastic MCs, such as PI3K/AKT and JAK/STAT5 signaling pathways. Indeed, AKT and STAT5 are constitutively activated in *KIT* D816V⁺ neoplastic MCs, suggesting that these molecules play a crucial role in the pathogenesis of SM. Besides, some other intracellular TKs, such as the Feline sarcoma oncoprotein (FES), or the mammalian target of rapamycin (mTOR) complex, are also potential actors of oncogenesis linked to the KIT D816V mutant. In addition, the mutant can activate ERK independently of SRC. Of note, in advanced SM, Lyn and Btk are activated in neoplastic MCs in a KIT-independent manner. All these KIT-dependent and KIT-independent activated pathways and molecules combine to increase cell proliferation and decrease apoptosis of neoplastic MCs. BRD4, Bromodomain-containing protein 4; BTK, Bruton tyrosine kinase; ERK, Extracellular signal-regulated kinase; JAK, Janus kinase; MAPK, Mitogen Activated Protein Kinase; NFκB, nuclear factor-κ B; PI3K, phosphoinositide 3-kinase; SFK, Src family kinase; STAT5, Signal transducer and activator of transcription 5. (Modified from Bibi S, Langenfeld F, Jeanningros S, et al. Molecular defects in mastocytosis: KIT and beyond KIT. *Immunol Allergy Clin North Am* 2014;34(2):248; with permission).

NON-TARGETED TREATMENTS OF MASTOCYTOSIS

To date, there is no curative treatment of SM and caring is adapted depending on the case, the symptoms, and disease manifestations. Overall, SM treatments aim:

1. At counteracting mediator release symptoms (symptomatic treatments) and
2. In advSM, at reducing organ damage due to neoplastic MC infiltration (non-targeted cytoreductive drugs)

However, KIT-TKIs have recently emerged as promising agents to treat patients with advSM.

Symptomatic Treatments

Patients with ISM usually require only symptomatic treatments. The main objective is to reduce the symptoms of MC activation.³² In case of pruritus or skin manifestations, antihistamines H1 (anti-H1) are used.^{3,32} For GI tract manifestations, anti-H2 are effective alone or combined with anti-H1, disodium cromoglycate, or leukotriene inhibitors.³² Besides, corticoids can suppress antihistamine recalcitrant symptoms.⁹ Epinephrine is indicated for hypotension, which may be spontaneous or observed after an insect bite.³ Indeed, some patients with SM may have a bee or wasp venom allergy.³³ In these patients, specific immunotherapy should be administered lifelong to ensure protection.³⁴ Finally, patients with SM with osteoporosis are treated with bisphosphonates together with supplementation of calcium and vitamin D.³⁵

Non-targeted Chemotherapy and Allogeneic Stem Cell Transplantation

In advSM, symptomatic treatment is often associated with cytoreductive therapy. Interferon- α may be effective in a subset of patients, despite numerous side effects.¹¹ Cladribine provides high response rates together with long-lasting responses in some patients.^{36,37} Besides, hydroxyurea (HU) is given in patients with SM-AHN, although HU is only active on the AHN component, but not on neoplastic MCs.³⁸ Of note, allogeneic stem cell transplantation (allo-SCT) is rarely used and reserved to fit patients with very aggressive, life-threatening advSM and a suitable donor.³⁹ Finally, allo-SCT provides more frequently good response rates and complete remissions in SM-AHN and ASM than in MCL.⁴⁰

TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF MASTOCYTOSIS

Recent approaches have concentrated on inhibitors targeting the KIT mutant receptor in patients with advSM. However, despite the fact that most patients with advSM harbor the imatinib-resistant *KIT* D816V mutant, it is of utmost importance to determine the *KIT* mutational status in each patient before applying KIT-TKIs, as some patients may present with *KIT* WT, or *KIT* mutant outside exon 17, potentially responsive to imatinib.

Major KIT-Targeted Tyrosine Kinase Inhibitors

Imatinib (STI571, Glivec) and masitinib (AB1010)

Imatinib and masitinib are structurally similar type I TKI (Fig. 3) active on some tyrosine kinases, including KIT WT and KIT mutants outside the exon 17 (Table 3).^{41–43} In this respect, masitinib acts more potently than imatinib.⁴² Although imatinib is devoid of effects on MC degranulation, masitinib inhibits this phenomenon, as a result of its activity on Lyn and Fyn.⁴² Because imatinib has demonstrated clinical activity in single cases of patients with SM with *KIT* WT or *KIT* mutations outside exon 17 (reviewed in⁴⁴), it has been approved by the Food and Drug Administration (FDA) in 2006 to treat *KIT* D816V-negative ASM or ASM with an unknown *KIT* mutational status.

By contrast, masitinib has been used in clinical trials in patients with SM, whatever their *KIT* mutational status, in the aim to decrease mediator-related symptoms. Indeed, in a phase III clinical trial published recently, 135 severely symptomatic patients with ISM not controlled by optimal antimediator therapies received either masitinib (6 mg/kg/d) or placebo.⁴⁵ After 24 weeks of treatment, masitinib induced a 75% improvement in at least one of the following symptoms: pruritus, flushes, depression, and severe fatigue (18.7% for the masitinib arm compared with 7.4% for the placebo arm), whatever the *KIT* mutational status. The most frequent adverse events were diarrhea (11%), rash (6%), and asthenia (6%).⁴⁵ Interestingly, the study also reported slightly decreased serum tryptase levels and improvement of skin lesions in the

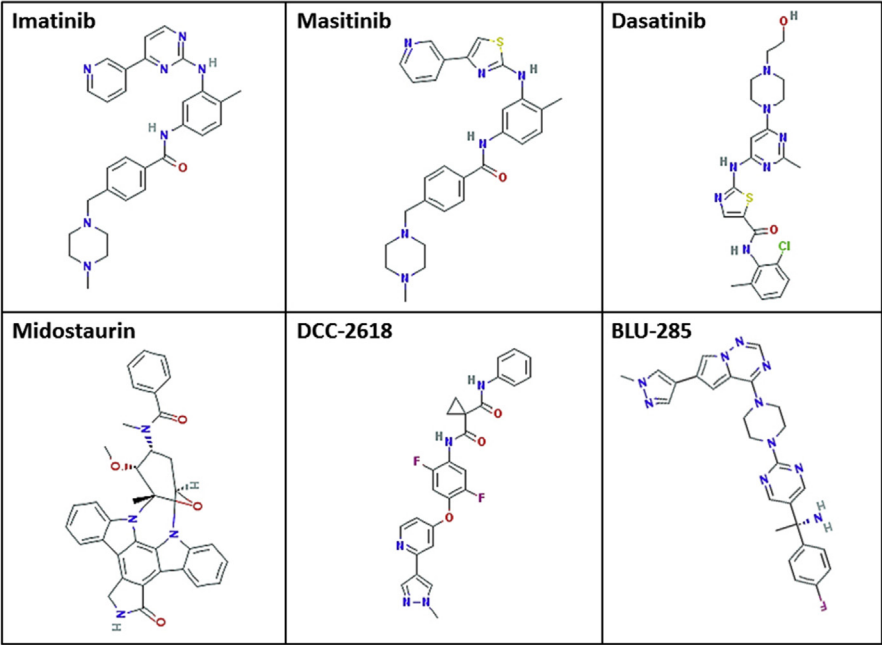


Fig. 3. Chemical structure of the major KIT-targeted TKIs. With the exception of compound DCC-2618, which targets the switch pocket of KIT, all the other KIT-targeted TKIs presented in this image are type I TKIs, which compete with adenosine triphosphate (ATP) for the ATP-binding site of the targeted kinase. Chemical formulas and molecular weights (MW) are as follows: imatinib: $C_{29}H_{31}N_7O$ (MW: 493.615); masitinib: $C_{28}H_{30}N_6OS$ (MW: 498.649); dasatinib: $C_{22}H_{26}ClN_7O_2S$ (MW: 488.007); midostaurin: $C_{35}H_{30}N_4O_4$ (MW: 570.649); DCC-2618: $C_{26}H_{21}F_2N_5O_3$ (MW: 489.483); BLU-285: $C_{26}H_{27}FN_{10}$ (MW: 498.57).

Table 3 Tyrosine kinase inhibitors of therapeutic interest in mastocytosis and their major molecular targets	
TKIs	Major Molecular Targets
Imatinib	ARG, ABL, KIT WT, ECD and JMD KIT mutants, PDGFR
Masitinib	KIT WT, ECD and JMD KIT mutants, FMS, Lyn, Fyn
Dasatinib	ARG, ABL, KIT WT, KIT D816V, SRC, LCK, PDGFR β
Midostaurin	PKC, KIT WT, KIT D816V, ECD and JMD KIT mutants, FLT3, PDGFR, FES, SYK, AURKA, AURKB, JAK2, JAK3, FGFR3, VEGFR2
DCC-2618	KIT WT, KIT D816V, ECD and JMD KIT mutants, PDGFR, KDR, FLT3, VEGFR2, TIE2, FMS
BLU-285	KIT WT, KIT D816V, KIT V560G, PDGFR α

Abbreviations: Abbreviations: AURK, aurora kinase; ECD; extracellular domain; FES, feline sarcoma; FGFR, fibroblast growth factor receptor; FLT3, Fms-like tyrosine kinase 3; FMS, M-CSF receptor; JAK, Janus kinase; JMD, juxta-membrane domain; KDR, Kinase insert domain receptor; LCK, lymphocyte-specific protein tyrosine kinase; PDGFR, platelet-derived growth factor receptor; SYK, Spleen tyrosine kinase; TIE2, tyrosine kinase with immunoglobulin and EGF homology domains; VEGFR, vascular endothelial growth factor receptor.

masitinib arm.⁴⁵ However, why masitinib is active only in a subset of patients independently of their *KIT* mutational status is still unclear and deserves further investigations.

Dasatinib (Sprycel)

Dasatinib (see Fig. 3) is a second-generation multi-kinase inhibitor acting on several targets (see Table 3).⁴⁶ It has in vitro efficacy against various *KIT* mutants, including *KIT* D816V.⁴⁷ By contrast, in a clinical study led with dasatinib given to 33 patients with SM,⁴⁸ an overall response rate (ORR) of 33% was achieved but short-lasting complete responses were only observed in 2 patients with *KIT* D816V-negative SM-AHN.⁴⁸ In addition, grade 3 toxicities were recorded in 58% of patients, with edema and pleural effusions.⁴⁸ In another trial, led on 4 patients with *KIT* D816V⁺, 2 patients had a major response with decreased BM MC burden but with severe cutaneous side effects.⁴⁹ Thus, because of numerous side effects and modest efficacy, dasatinib is not recommended in the treatment of patients with AdvSM.

Midostaurin (Rydapt)

Midostaurin (see Fig. 3), originally termed PKC412, is a potent TKI initially developed as a protein kinase C inhibitor.⁵⁰ In fact, midostaurin is a multi-kinase inhibitor (see Table 3). Unlike most TKIs, midostaurin inhibits not only the WT form of its targets but also their mutant forms. Particularly, midostaurin suppresses not only the kinase activity of WT *KIT* but also that of *KIT* mutants, including the D816V mutant.^{47,51,52}

Indeed, midostaurin has in vitro growth-inhibitory activity on various cell lines expressing *KIT* WT, *KIT* V560G, or *KIT* D816V mutants, with half maximal inhibitory concentration (IC₅₀) ranging from 50 nM to 250 nM.^{43,52} Of note, these growth-inhibitory effects are accompanied by inhibition of *KIT* phosphorylation and apoptosis.^{43,52} Furthermore, midostaurin is also active on primary neoplastic MCs from patients with advSM.⁵² Moreover, midostaurin produces synergistic growth-inhibitory effects when combined with dasatinib,⁴⁷ cladribine,⁵² or JQ1 (a bromodomain 4 [BRD4] inhibitor).⁵³ Overall, these results have promptly encouraged the clinical use of midostaurin in patients with advSM.

Midostaurin was first used to treat a patient with *KIT* D816V⁺ MCL, resulting in a partial remission with a significant decrease in the percentage of circulating MCs, associated with decreased histamine level and *KIT* D816V mutation frequency in peripheral blood.⁵¹ More recently, an international phase II clinical trial has been performed on 116 patients with advSM (all patients receiving 100 mg twice daily in 4-week continuous cycles).⁵⁴ Based on the presence of C findings, 89 patients (16 ASM, 57 SM-AHN, 16 MCL) were eligible for evaluation. Among them, 77 patients (87%) were positive for *KIT* D816V/Y/L mutation. The ORR for the whole cohort was 60% (53 of 89 patients), whereas the ORR for patients with MCL was 50% (8 of 16 patients).⁵⁴ The treatment significantly decreased the BM MC burden, serum tryptase levels, and spleen volume in most patients.⁵⁴ In addition, the median OS of responders was 44.4 months, whereas in MCL, OS was 9.4 months.⁵⁴ All grades' side effects mostly seen were anemia, neutropenia, thrombocytopenia, digestive toxicities, peripheral edema, fatigue, pyrexia, headache, back pain, and pruritus; but grade 3/4 toxicities were essentially hematologic.⁵⁴ The main conclusions of this study were that midostaurin induced frequently durable responses in patients with advSM, with an acceptable safety profile.⁵⁴ This favorable profile of activity was confirmed in another clinical study whereby 28 patients with advSM received midostaurin at a dosage of 100 mg twice daily.⁵⁵ Recently, the long-term potential therapeutic benefit of midostaurin given as a single agent to patients with advSM was confirmed by a 10-year median follow-up of a phase II trial conducted on 26 patients (3 ASM, 17 SM-AHN, and

6 MCL).⁵⁶ In this study, midostaurin produced a 50% or greater reduction in the BM MC burden and serum tryptase level in 68% and 46% of the patients, respectively. Of note, the median OS for the entire cohort was 40 months and 18.5 months for patients with MCL.⁵⁶ Based on these positive data, midostaurin has been approved in monotherapy to treat adult patients with advSM by the FDA (April 2017) and by the European Commission (September 2017) and is considered a major component of the first-line treatment in advSM.

DCC-2618 compound

Deciphera Compound-2618 (DCC-2618) (see Fig. 3) is a switch control type II inhibitor, which arrests KIT in an inactive state, regardless of the type of activating mutations, by targeting the switch pocket of the receptor that regulates its catalytic conformation.^{57,58} Besides KIT, DCC-2618 recognizes several other oncogenic kinases (see Table 3).⁵⁸ DCC-2618 inhibits the proliferation and survival of human neoplastic MC lines expressing various *KIT* mutants, including *KIT* D816V, with IC₅₀ less than 0.5 μM.⁵⁹ In addition, DCC-2618 suppressed endothelial cell proliferation, suggesting additional drug effects on disease-related angiogenesis.⁵⁹ Besides, DCC-2618 blocked immunoglobulin E-mediated histamine release by basophils and tryptase release by MCs.⁵⁹ Altogether, these data suggest that DCC-2618 might represent a promising drug to treat patients with advSM. Thus, a first clinical trial has been initiated with DCC-2618 in patients with advanced kinase-driven malignancies, including advSM (NCT02571036).

BLU-285 compound

Blueprint-285 compound (avapritinib) (see Fig. 3) is a novel TKI that potently inhibits selectively *KIT* exon 17 mutants, including *KIT* D816V (see Table 3).⁶⁰ BLU-285 also inhibits STAT3 and AKT phosphorylation in vitro in the Human MC line (HMC-1.2 cell line) and decreases tumor progression in vivo in mice grafted with murine mastocytoma cells P815.⁶⁰ Recently, a phase 1 clinical trial has been initiated to assess the safety, pharmacokinetics (PK), and preliminary clinical activity of avapritinib in patients with advSM (NCT02561988). Preliminary data analyzing the effects of BLU-285 on 30 patients (15 ASM; 9 SM-AHN; 3 MCL; 3 other D816V-mutant positive hematologic neoplasms) treated with BLU-285 in 7 cohorts at dosages of 30 to 400 mg daily have been recently reported.⁶¹ Among these 30 patients, 24 had *KIT* D816V mutation, 2 had *KIT* D816Y mutation, 1 had *KIT* polymorphism M541L and 3 were *KIT* WT.⁶¹ Twenty-four patients had 1 or more co-occurring mutations in BM, most frequently *TET2* (17), *DNMT3A* (9), *ASXL1* (7), *SRSF2* (6), and *GATA2* (6). Interestingly, in these patients, BLU-285 seemed well tolerated and demonstrated significant clinical (significant decrease in spleen volume) and biological (significant decrease in serum tryptase levels, BM MC and *KIT* D816V allele burden) activities in all AdvSM subtypes, including in those who have failed previous midostaurin treatment.⁶¹ All in all, these are encouraging data that deserve long-term follow-up.

Other KIT-Targeted Tyrosine Kinase Inhibitors

Several TKIs have demonstrated in vitro efficacy on imatinib-resistant *KIT* mutants. Some of these drugs have been discontinued for safety issues, and others are already approved for the treatment of non-*KIT*-related malignancies, whereas the last drugs are still experimental. For instance, discontinued drugs comprise compound MLN518 (tandutinib),⁶² purine inhibitors AP23464 and AP23848,⁶³ compound EXEL-0862,⁶⁴ and semaxanib (SU5416).⁶⁵ Besides, ponatinib (Iclusig) a TKI FDA approved to treat patients with imatinib-resistant CML, inhibited the kinase activity of various *KIT* mutant receptors, such as *KIT* Y823D,⁶⁶ *KIT* V560G, and *KIT* D816V.⁶⁷ In addition to its effects on the kinase activity of mutant *KIT* proteins,

ponatinib inhibited in vitro the proliferation of *KIT* D816V⁺-malignant MCs by inducing apoptosis and that, synergistically with midostaurin.⁶⁷ Nevertheless, ponatinib has still not been used clinically. More recently, nilotinib, a strong inhibitor of BCR-ABL1 approved as a first-line treatment in CML but that has some marginal activities in vitro on *KIT* mutants, has been administered to 61 patients with SM (including 37 with advSM) in a clinical trial, with low response rates not being in favor of its use in advSM.⁶⁸ Other compounds that are experimental are 2 thiazole amine derivatives: compound 126332, which inhibits the *KIT* D816V mutant in vitro in neoplastic MCs and in vivo in solid tumors generated by subcutaneous injection of cells of the *KIT* D816V⁺ human mast cell line (HMC-1.2),⁶⁹ and compound BPR1J373, which suppresses the *KIT* D816V phosphorylation.⁷⁰ Finally, about 40 new *KIT* D816V inhibitors have been developed by the Korea Advanced Institute of Science and Technology, 7 of which having significant in vitro activity on HMC-1.2 cells (IC₅₀ <1.0 μ M).⁷¹ However, to date, no clinical trials have been initiated with these experimental drugs.

Non-KIT-Targeted Kinase Inhibitors

As described earlier, the PI3K/AKT/mTOR and JAK/STAT pathways, and to a lesser extent the mitogen-activated protein kinase (MAPK) pathway, seem crucial for the proliferation of *KIT* D816V⁺ malignant MCs.²⁰ These pathways might be alternative targets for specific inhibitors, used alone or in combination with *KIT*-TKIs. For instance, the pharmacologic inhibition of PI3K in vitro by NVP-BE235 induced a dose-dependent inhibition of HMC-1.2 cell proliferation, which was synergistically increased in combination with the MAPK inhibitor UO126.⁷² In addition, treatment of *KIT* D816V⁺ malignant MCs with AKT inhibitors reduced cell growth in vitro.⁴³ Besides, ruxolitinib, an inhibitor of JAK1 and JAK2, has been recently administered to 2 patients with SM, in 2 single case reports.^{73,74} In both cases, ruxolitinib reduced symptoms and increased quality of life.^{73,74} However, despite long-term treatment, no decrease in MC burden was recorded.^{73,74} Of note, Lyn and Btk, are phosphorylated independently of *KIT* in neoplastic MCs in SM (**Fig. 4**).⁴⁷ Interestingly, bosutinib, an ABL-targeting TKI approved for the treatment of CML, inhibits also the phosphorylation of Lyn and Btk and exerts antiproliferative effects in vitro on *KIT* D816V⁺ neoplastic MCs.⁴⁷ In addition, a combination with midostaurin provided synergistic antiproliferative and proapoptotic effects in vitro.⁴⁷ However, despite these promising data, bosutinib has been administered to one patient with advSM, without any clinical effects.⁷⁵

SUMMARY AND FUTURE CONSIDERATIONS

Current therapeutic options for patients with SM are based on symptomatic treatment and, in advSM, on the use of cytoreductive agents and TKIs. However, in advSM, with the exception of allo-SCT applicable only to a minority of patients, none of these treatments, including midostaurin, has demonstrated true curative potential; the prognosis of these patients is still poor. Besides, knowledge on the pathophysiology of SM has increased dramatically these last years, highlighting the complexity of the processes leading to abnormal accumulation/proliferation of neoplastic MCs in SM, particularly in advSM. Indeed, in these patients, malignant MC proliferation is driven not only by *KIT* mutations but also by altered signaling pathways and additional genetic defects. Thus, new targets and alternative therapies are needed to achieve lifelong lasting remission. Alternative targets could be, for instance, aberrantly activated *KIT*-dependent and *KIT*-independent signal transduction pathways described earlier and members of the Bcl-2 family overexpressed in neoplastic MCs (see **Fig. 4**).⁷⁶ Besides,

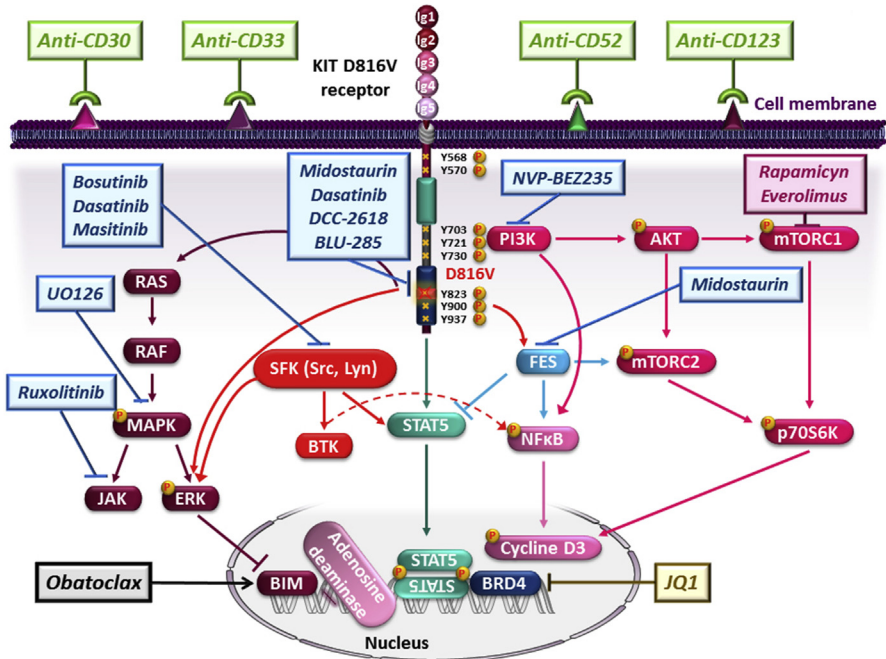


Fig. 4. Present and potential therapeutic targets in advanced systemic mastocytosis: *KIT* and beyond *KIT*. Several drugs can selectively inhibit the kinase activity of the *KIT* D816V mutant or some of the critical pathways aberrantly activated in a *KIT*-dependent or *KIT*-independent manner downstream of the mutant receptor, such as PI3-kinase/AKT/mTOR, MAPK, JAK/STAT and SFK pathways, the FES kinase, and the NFκB pathway. In addition, anti-apoptotic molecules, such as Mcl-1, Bcl-2, and Bcl-xL, may constitute potential targets since they are upregulated in *KIT* D816V⁺ neoplastic MCs⁷⁶ and because pan-Bcl-2 blockers can induce their apoptosis.⁷⁸ Besides, one promising class of targets within chromatin regulatory molecules are the BRD-containing proteins, such as BRD4, which is overexpressed in *KIT* D816V⁺ MCs in advanced SM and for which a specific inhibitor (JQ1) has proven efficacy on neoplastic MCs in vitro.⁵³ Finally, neoplastic MCs may express aberrantly some cell surface antigens, such as CD30, CD33, CD52, or CD123, which could be targeted by antibody-based toxin conjugates or other ligands. Kinase inhibitors are represented in blue, specific antibodies in green, Bcl-2 antagonist in black, inhibitor of BRD-containing proteins in brown, and mTOR inhibitors in purple. (Modified from Ustun C, Arock M, Kluin-Nelemans HC, et al. Advanced systemic mastocytosis: from molecular and genetic progress to clinical practice. *Haematologica* 2016;101(10):1137; with permission.)

other targets of potential interest should be cell surface antigens expressed by neoplastic MCs.⁷⁷ Some of these antigens are of particular interest (see Fig. 4), such as CD30, CD33, CD52, and CD123, for which targeted antibodies are already in clinical trials. All in all, these potential targets might serve as a basis for the development of new targeted therapies, which will be certainly used in combination with *KIT*-TKIs to improve the health outcome of patients with advSM.

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