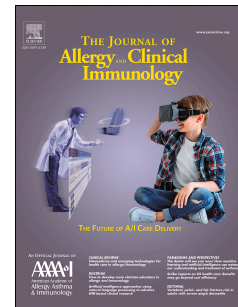


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Drug-Induced Mast Cell Eradication: A Novel Approach to Treat Mast Cell Activation Disorders?

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Abstract

Mast cell activation is a key event in allergic reactions, other inflammatory states, and mast cell activation syndromes. Mast cell-stabilizing agents, mediator-targeting drugs and drugs interfering with mediator effects are often prescribed in these patients. However, the clinical efficacy of these drugs varies, depending on the numbers of involved mast cells and the underlying pathology. One straightforward approach would be to eradicate the primary target cell. However, to date, no mast cell-eradicating treatment approach has been developed for patients suffering from mast cell activation disorders. Nevertheless, recent data suggest that long-term treatment with agents that effectively inhibit KIT-function results in the virtual eradication of tissue mast cells and a sustained decrease in serum tryptase levels. In many of these patients, mast cell depletion is associated with a substantial improvement in mediator-induced symptoms. In patients with an underlying KIT D816V+ mastocytosis, such mast cell eradication requires an effective inhibitor of KIT D816V, such as avapritinib. However, the use of KIT inhibitors must be balanced against potential side effects. We here discuss mast cell-eradicating strategies in various disease models, the feasibility of this approach, available clinical data, and future prospects for the use of KIT-targeting drugs in mast cell activation disorders.

Key Words: Mast cells • KIT • MCAS • TKI • Midostaurin • Avapritinib

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113 List of Abbreviations:

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115	ASM	Aggressive systemic mastocytosis
116	BM	Bone marrow
117	BMM	Bone marrow mastocytosis
118	CM	Cutaneous mastocytosis
119	FcεRI	Fc epsilon receptor type I
120	HαT	Hereditary alpha tryptasemia
121	HSCT	Hematopoietic stem cell transplantation
122	IgE	Immunoglobulin E
123	ISM	Indolent systemic mastocytosis
124	MC	Mast cells
125	MCAS	Mast cell activation syndrome
126	MCL	Mast cell leukemia
127	SM	Systemic mastocytosis
128	SM-AHN	Systemic mastocytosis with an associated hematologic neoplasm
129	SSM	Smoldering systemic mastocytosis
130	WHO	World Health Organization

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Introduction

Mast cells (MC) are key effector cells of allergic reactions and other inflammatory processes in various pathologic conditions.¹⁻⁴ These cells display high-affinity IgE receptors, also known as Fc epsilon receptor type I (FcεRI), as well as many other activation-linked surface antigens and receptors, such as C5aR or MRGPRX2, and store an array of vasoactive and inflammation-triggering mediators in their metachromatic granules.¹⁻⁴ During an anaphylactic reaction, allergen-induced cross-linking of MC FcεRI results in the release of preformed granule-stored mediators, including various proteases and histamine, and newly synthesized chemokines and cytokines.¹⁻⁴ In addition, activated MC release lipid membrane-derived arachidonic acid metabolites.¹⁻⁴ MC originate from multi-lineage hematopoietic stem and progenitor cells as well as MC-committed progenitors.⁴⁻⁹ These stem and progenitor cells reside in the bone marrow but are also detected in the peripheral blood and in extramedullary organs.⁴⁻¹¹ A complex network of cytokines and growth factor receptors is involved in the regulating the differentiation, maturation, migration, adhesion, homing and activation of MC in health and disease.⁴⁻¹² The key regulator of the development, survival and function of MC is stem cell factor (SCF) acting through its receptor, KIT. KIT itself is expressed on the surface of uncommitted stem cells, MC progenitor cells, and mature MC,⁴⁻¹² but is also expressed on a number of other cell types, including melanocytes, Cajal cells of the gastrointestinal tract, and germ cells.^{13,14} MC activation occurs in a number of different pathologic conditions and disorders, including allergic diseases, chronic inflammatory reactions, infectious diseases,

intolerance reactions, and toxic reactions.^{1-4,15-20} In patients with allergic (atopic) disorders as well as in mastocytosis, MC activation may be substantial and systemic, often resulting in overt anaphylaxis.^{4,15,16-19} When the symptoms are severe, episodic and recurrent, a MC activation syndrome (MCAS) may be diagnosed.^{15,18-21} In some of these cases, life-threatening anaphylaxis is seen, especially when one or more underlying conditions and contributing pathologies, such as a concomitant mastocytosis and/or allergy, are also present.^{15,18-21}

Based on mouse studies, symptoms typically recorded in an anaphylactic (allergen-induced and IgE-dependent) reaction are largely if not entirely dependent on the presence of MC.²²⁻²⁶ Basophils also can participate in anaphylactic reactions, but their contribution to the manifestations of severe anaphylaxis is assumed to be generally of less impact. Thus, MC are a critical target cell population in anaphylactic reactions in various disease models, including IgE-dependent allergies, mastocytosis, and MCAS.

In patients with severe forms of MC activation and anaphylaxis, a number of drugs are employed in the hope of bringing MC activation under control.²⁷⁻³² In the acute phase of a MC activation-related event (anaphylactic shock), 'emergency drugs' such as epinephrine (first line immediate therapy) and corticosteroids, as well as antihistamines, are administered.²⁷⁻²⁹ In the symptom-free interval, MC-stabilizing agents, mediator-targeting drugs, and drugs interfering with the effects of these mediators on various target cells (e.g., histamine receptor blocker) are prescribed to help prevent or lessen an episode of recurrent anaphylaxis, should it occur.^{27,28,30-32}

In certain forms of MC activation, anaphylactic events may be life-threatening and resistant to conventional therapy.^{15-19,27-30} These include patients with an underlying MC neoplasm (mastocytosis), an underlying genetic predisposition, an atopic diathesis,

and/or multiple severe IgE-dependent allergies. When two or three of these conditions are present in the same patient, the risk for recurrent life-threatening events may be exceptionally high.¹⁵⁻²¹ In such cases a MCAS may be diagnosed.^{15,19-21} For patients with recurrent severe events, including those with MCAS not protected by conventional approaches, additional pharmacologic agents may be administered, including IgE-targeting antibodies (omalizumab), specific immunotherapy, and MC-stabilizing agents.³³⁻³⁸

An emerging novel strategy is to reduce the numbers of MC in the hope of decreasing or even eliminating the risk of occurrence of severe anaphylaxis. Especially in patients with both MCAS and systemic mastocytosis (SM), where the numbers of MC are excessively elevated, a reduction in, or eradication of, MC could be a highly effective therapeutic maneuver. In support of such an approach, several studies have shown that MC reduction induced by an effective inhibitor of KIT D816V, or other drugs with similar effects on MC, is followed by a decrease in the symptom burden and an improvement in quality of life.³⁹⁻⁴⁴ Indeed, KIT, a tyrosine kinase receptor for stem cell factor (SCF), is a well-known master regulator of MC and their progenitor cells^{10,11,13} and the KIT mutant form D816V has recently been identified as a primary target in MC-dependent disorders.³⁹ Currently, however, the approach of targeting KIT and thus suppressing MC development with a highly effective pharmacologic inhibitor, is offered almost exclusively to patients with advanced SM.³⁹⁻⁴⁴ On the other hand, there are a few studies in which the efficacy of KIT-targeting drugs in indolent SM has been explored with the goal of suppressing MC growth and MC activation.

Such experience prompts our discussion of the potential use of established and novel KIT-targeting drugs as MC-eradicating therapy in patients with anaphylaxis and MCAS,

particularly in the context of IgE-dependent allergies, atopic and other predisposing conditions, and SM. Moreover, we also review the available data and discuss future applications in clinical trials, as well as associated concerns related to safety, side effects and special indications.

Role of KIT in Expansion and Activation of MC

Human and mouse MC reportedly derive from transplantable hematopoietic stem and progenitor cells.⁴⁵⁻⁴⁷ The tyrosine kinase receptor KIT and its ligand, SCF, also called KIT-ligand, play a dominant role in the development and maturation of tissue MC.⁹⁻¹¹ In contrast to other myeloid cells, MC are long-lived. This is because the development of fully mature MC from their stem cells and pluripotent and committed MC progenitor cells may take several months.^{4-6,9,48-50} In addition, some mature tissue MC survive months or perhaps years when residing within local tissue microenvironments. Based on observations in patients with clonal MC disorders who have undergone hematopoietic stem cell transplantation, the *in vivo* development of human MC from donor stem cells takes at least 6 months.⁴⁷ Similarly, full differentiation of human MC from their stem and progenitor cells and full maturation *in vitro* takes at least 6-12 weeks.^{6,48-51} At maturation, MC reside in local tissue sites where SCF and other growth factors and other cytokines are expressed, supporting long-term survival as well as effector cell functions. It is important to note that the survival of mature MC in various tissues is dependent on SCF and KIT, and that after an effective blockade of the SCF-KIT-axis *in vivo*, it takes at least several weeks until (normal) mature tissue MC undergo apoptosis and disappear.^{10,11,47}

Other cytokines can also induce or promote MC differentiation. Whereas in the mouse and rat, several growth factors, including interleukin-3 (IL-3) and SCF may induce MC development^{2,9}, differentiation of human MC is promoted by only a few cytokines.⁴⁸⁻⁵⁶ In fact, in an early phase of MC development (stem and progenitor cell level), IL-3, IL-6, and IL-9 can promote proliferation and thus SCF-induced development of MC.^{48,51-53} IL-4, in turn, acts on a more mature stage of MC development. In particular, IL-4 promotes *in vitro* maturation, chymase expression and expression of FcεRI in maturing MC precursor cells cultured in the presence of SCF.⁵³⁻⁵⁶

Classes of KIT-Targeting Tyrosine Kinase Inhibitors (TKI), Drug-Target Interaction Profiles, and Pharmacology

KIT-targeting TKI can essentially be divided into i) drugs interacting with several different kinase targets, including wild type (wt) KIT but not with KIT D816V (example: imatinib), ii) drugs that interact primarily or selectively with wt KIT but not with KIT D816V or other kinase targets (example: masitinib), iii) drugs that recognize wt KIT, KIT D816V and several other oncogenic kinases (example: midostaurin), and iv) inhibitors of KIT D816V (and sometimes wt KIT) that interact with only a limited number of additional kinase targets (example: avapritinib). A compilation of such KIT-targeting drugs is provided in Table 1.

In addition, several antibody-based drugs against KIT or the KIT ligand SCF, and several CAR-T cell approaches employing KIT as a cellular target, are currently being developed and tested in preclinical studies and clinical trials. In one study, the anti-KIT antibody CDX-0159 produced profound and durable MC suppression in healthy

donors.⁵⁷ In addition, bi-specific antibodies targeting KIT and other key surface targets have been developed. However, in the current article, our focus is on small molecule-type KIT TKI, with more to follow as information accumulates on these other approaches targeting KIT-bearing cells.

Available KIT-targeting TKI vary in their efficacy against KIT and KIT variants, their half-life *in vivo*, their effects on SCF-dependent and IgE-mediated MC activation, and their toxicity profiles. For example, although dasatinib inhibits wt KIT and exerts effects on KIT D816V and IgE-dependent mediator secretion and MC activation *in vitro*⁵⁸⁻⁶⁰, the pharmacologic half-life of the drug (2-4 hours) is too limited to suppress KIT continuously and to block MC growth and activation *in vivo*. Another important consideration is that depending on their structure and chemical properties, some KIT-targeting drugs are degraded *in vivo* into more or less inactive metabolites. For example, midostaurin is degraded into two major metabolites.⁶¹ Whereas both midostaurin metabolites continue to block SYK and thus anti-IgE-induced histamine release, only one of these metabolites can sufficiently block KIT and KIT-downstream signaling, and thus SCF-induced or KIT D816V-mediated growth of MC.⁶²

When applying a KIT TKI in a routine setting or in clinical trials, a most important point to consider is that only a KIT TKI that can block KIT D816V (iii and iv) will be able to suppress the development and survival of neoplastic MC in patients with KIT D816V+ SM. Another important point is that highly specific KIT-targeting drugs lack activity against other oncogenic kinases and FcεRI-downstream signaling molecules. The consequence is that these agents may only be able to counteract MC activation and MCAS to a substantial degree when most or all MC are eliminated in a given patient.

It is also important to know that side effects correlate with the number and types of targets recognized and that most of these TKI have been developed to treat malignant diseases (cancer) but not MCAS or anaphylaxis. Finally, because expression of KIT is not limited to MC^{13,14}, consideration should also be given to the possibility of unknown adverse effects of administration of KIT TKI over longer periods of time.

Effects of KIT-Targeting TKI on Development of MC from their Progenitors

As mentioned, the development of normal and neoplastic MC is largely dependent on a functionally active KIT receptor. In normal MC, KIT activation is initiated by its ligand SCF.⁹⁻¹¹ By contrast, in neoplastic MC, specific point mutations in *KIT* lead to SCF-independent activation of KIT and thus to autonomous (SCF-independent) differentiation of MC from their stem and progenitor cells.^{63,64} In line with these observations, all 4 classes of KIT TKI can inhibit SCF-induced growth of stem cells and thus SCF-dependent *in vitro* development of MC from their normal stem- and progenitor cells.⁶⁵⁻⁶⁸ By contrast, only drugs targeting KIT D816V can suppress the growth and survival of MC and MC lines exhibiting KIT D816V, whereas these cells are resistant to imatinib, masitinib or other drugs lacking activity against KIT D816V (Table 1).⁶⁹⁻⁷³ Figure 1 shows the growth-inhibitory effects of avapritinib and midostaurin on neoplastic MC exhibiting *KIT* D816V.

The same efficacy profiles are also observed in patients *in vivo*. In particular, drugs targeting only wt KIT but not KIT D816V (imatinib, masitinib, and others) are unable to suppress growth of neoplastic MC in patients with KIT D816V+ advanced SM.⁷⁴⁻⁷⁹ By contrast, drugs targeting KIT D816V, such as midostaurin or avapritinib, suppress

MC expansion in patients with advanced SM.^{39-44,78-80} For these patients, avapritinib appears to be a more effective agent.^{43,44} An associated observation is that administration of avapritinib leads to a rapid decrease in the burden of neoplastic MC in these patients.^{43,44} This observation is best explained by the fact that this drug is a very effective inhibitor of KIT D816V, and that not only proliferating immature progenitor cells but also mature MC depend on KIT D816V for survival and thus represent a sensitive target.⁷²

Another remarkable phenomenon is that midostaurin induces rapid improvement of mediator-related symptoms in patients with advanced SM.⁴¹ This cannot be explained by a reduction of the MC burden alone, as MC numbers decrease only slowly (or not at all) with midostaurin.³⁹ Instead, this beneficial drug effect appears to be due to its impact on SYK activity in MC, and thus on IgE-dependent and -independent MC activation and mediator secretion.⁶² Indeed, even in patients without meaningful hematologic responses, midostaurin can alleviate mediator-induced symptoms and quality of life substantially.⁴¹ Moreover, in a phase II trial, midostaurin was able to improve refractory symptoms caused by MC mediator release and thus the quality of life in more than 70% of all patients with ISM.⁸¹ At high concentrations, avapritinib also exerts some inhibitory effects on MC and basophil activation and mediator release (Table 1).

Finally, it must be noted that there are adult patients with SM in whom neoplastic MC do not exhibit KIT D816V but have other mutant forms of KIT or even wild type (wt) KIT.⁸²⁻⁸⁶ These include patients with well-differentiated SM, a subset of cases with MC leukemia (MCL) including MCL arising from mast cell sarcoma (MCS), and some with aggressive SM (ASM).⁸²⁻⁸⁶ In these patients, KIT is often sensitive not only to midostaurin but also to imatinib or other wt KIT-targeting drugs (Table 1).⁸²⁻⁸⁶

Therefore, imatinib may be considered as a MC-depleting therapy in such patients, especially those with a well-differentiated MC morphology. Indeed, major responses or even remissions have been reported during imatinib therapy in these patients.⁸²⁻⁸⁶ It should also be noted that imatinib is a FDA- and EMA-approved drug for this indication (KIT D816V-negative SM).

Will a Potent KIT-Targeting TKI Eradicate Normal Mast Cells?

To date, little is known about the ability of KIT-targeting drugs to reduce or even eradicate normal MC and their progenitors in patients. In one report, patients with chronic myeloid leukemia (CML) were treated with imatinib for several years. During this therapy, MC numbers in the bone marrow and serum tryptase levels were determined. The numbers of MC decreased after 12 months, and after 24 months, MC were almost completely absent in bone marrow sections.⁶⁸ Simultaneously, serum tryptase levels decreased to the point where they were low to undetectable.⁶⁸ These observations strongly suggest that a potent wt KIT inhibitor, like imatinib, can eradicate normal MC when administered for at least 24 months. By contrast, after 12 months MC decreased in number but were still detectable.⁶⁸ Whether this is due to the relatively weak effect of imatinib on non-dividing mature MC (having a lifetime of several months *in vivo*) and/or due to the fact that imatinib preferentially blocks the growth of (dividing) MC progenitors, remains at present unknown. In several of the CML patients examined, allergy-related symptoms also decreased or disappeared and patients were able to limit or stop anti-allergic drugs (P.V., unpublished observation).

Can a Potent KIT D816V-Targeting TKI induce MC Eradication in SM?

Several promising TKI targeting KIT D816V have recently been developed, and many more may be developed in the near future. Available TKI at the time of writing of this manuscript include midostaurin, ripretinib, avapritinib, BLU-263, bezucastinib, and nintedanib.^{39-44,70-73,87} Midostaurin and avapritinib, in particular, have rapidly been developed and translated into clinical applications.³⁹⁻⁴⁴ Their clinical efficacy is summarized in Table 2. Interestingly, these two drugs behave differentially in patients with KIT D816V+ advanced SM. Midostaurin blocks MC activation by interfering with KIT-dependent and IgE-dependent signaling pathways in neoplastic MC⁶² and exhibits some MC reducing ability in most patients with KIT D816V+ advanced SM, but usually midostaurin is unable to eliminate all neoplastic MC in patients.^{39,40} By contrast, avapritinib is often able to induce substantial MC cytoreduction and thus hematologic remission in a substantial subset of patients with advanced KIT D816V+ SM^{43,44}, and at higher concentrations, also shows some inhibitory effects on IgE-dependent MC activation (Table 1, Figure 2). Whether this additional effect of avapritinib can explain the rapid clinical improvement sometimes seen in patients with MC disorders⁸⁸, remains at present unknown.

In summary, both midostaurin and avapritinib are highly effective in reducing the symptoms of MC activation in patients with advanced SM, but the primary mechanisms of drug action appear to vary, and only avapritinib may qualify as a drug inducing MC eradication in a subset of patients with advanced SM.

Effects of KIT-Targeting TKI on SCF-dependent and IgE-Dependent Mediator Secretion in MC

The releasability of MC depends on a number of different factors, such as the type of allergen, numbers of IgE receptors, and cytokine exposure. For example, interleukin-4 (IL-4) can promote the expression of high-affinity IgE-binding sites on MC^{89,90}, and SCF can augment releasability in MC stimulated by IgE-dependent or IgE-independent stimuli.⁹¹⁻⁹⁴ At higher concentrations and with prolonged exposure, SCF itself is capable of inducing mediator secretion in MC through activation of KIT.⁹¹⁻⁹⁴ Therefore, potent KIT inhibitors or antibodies against KIT may be able to block SCF-induced MC activation and possibly SCF-augmented (priming) effects on IgE-dependent MC activation (Table 1). However, these effects of KIT TKI may be negligible in clinical (*in vivo*) contexts. Rather, MC activation induced by exogenous antigens (allergens) is usually not effectively blocked by pharmacologically meaningful concentrations of a specific inhibitor of wt KIT or KIT D816V.

In KIT D816V+ MC in patients with SM, the situation is similar. Here, SCF is no longer required to activate KIT as KIT is activated in an autonomous manner⁶³ and the affected cells appear to be chronically activated and possibly desensitized against KIT-dependent stimuli. Therefore, KIT inhibitors may only be capable of interfering with MC activation when these drugs are also directed against other critical targets relevant in IgE-dependent and/or KIT-dependent MC activation, such as SYK, LYN or BTK (Table 1).⁶² Indeed, midostaurin has been reported to suppress IgE-dependent activation and mediator secretion in MC and basophils.^{62,95} In addition, avapritinib can suppress IgE-dependent histamine release in patients with SM (P.V., unpublished observation). So

far, it remains unknown whether midostaurin and/or avapritinib can also suppress MC activation triggered through other surface antigens, such as C5aR or MRGPRX2.

Clinical Efficacy of KIT TKI in Patients with Mast Cell Activation: Do we have Evidence for a New Concept?

To date, little is known about the *in vivo* efficacy of KIT-specific or multi-targeted TKI in the treatment of patients with IgE-dependent allergies and MC activation disorders. This is of interest, as several studies have shown that KIT, the KIT ligand SCF, and MC, are involved in a number of atopic/allergic disease states^{23-26,96-99} and KIT-targeting drugs have been reported to interfere with, or even fully block, MC activation and mediator-induced symptoms in allergy-related animal models.¹⁰⁰⁻¹⁰³ However, very few studies have investigated the potential beneficial effects of KIT-targeting drugs in patients with severe allergic disorders where MC activation apparently plays a role.

In one study, masitinib (3-6 mg/kg/day orally) was administered in 44 patients with severe corticosteroid-dependent asthma.¹⁰⁴ After 16 weeks, oral corticosteroid therapy could be reduced to a greater degree and in more patients in the treatment arm compared to the placebo group.¹⁰⁴ However, lung function parameters did not change when comparing the treatment group with the placebo arm. Reported side effects during masitinib treatment were nausea (30.3%), a skin rash (30.3%), peripheral edema (18.2%), diarrhea (18.2%), vomiting (12.1%), fatigue (12.1%), and pruritus (12.1%).¹⁰⁴ No reports on patients treated with masitinib over 24 months (or longer) have been published so far.

In another study, 62 patients with poorly controlled or uncontrolled severe asthma who had airway hyperresponsiveness despite receiving maximal medical therapy were treated with imatinib (400 mg/day orally) for 24 weeks.¹⁰⁵ Therapy with imatinib was found to reduce airway hyperresponsiveness more effectively in these patients compared to the placebo control.¹⁰⁵ MC numbers measured in the airway fluids declined in both groups without a significant difference between imatinib and the placebo control. Clinically relevant side effects included muscle cramps and hypophosphatemia, both of which were more frequently recorded in the imatinib arm.¹⁰⁵ Interestingly, the authors also reported on a slight decrease in serum tryptase levels and tryptase levels in the bronchial alveolar fluids.¹⁰⁵ However, no studies of long-term-imatinib-treated patients with severe asthma have been published to date.

On the other hand, there are studies available where changes in allergy-related symptoms in patients with advanced SM under long-term treatment with midostaurin have been reported.^{39,40} In most of these patients, relevant mediator-related symptoms decreased and did not return during long-term therapy. However, as mentioned, midostaurin also exerts direct effects on MC activation, which makes it difficult to define MC-depleting effects of this drug in the context of allergic reactions and related symptoms. In patients treated with avapritinib, symptoms also disappeared rapidly, and in many of these cases, MC-depletion may indeed have been responsible (as an underlying drug action) for the observed improvement of their symptoms and their quality of life.^{43,44}

However, to date, long-term results are lacking. Such data might not only support a new pharmacologic concept (MC eradication to stop allergies and MC activation disorders) but would also provide additional proof that MC are indeed the most important and

relevant effector cells in human allergic disorders, thereby also reconfirming that the terms MC activation disorders and MCAS are justified.

There are, of course, additional remaining questions to consider. First, long-term treatment (over many years) with multi-targeted anti-cancer agents, like midostaurin or avapritinib, may cause unexpected long-term side effects. Therefore, one question is whether next-generation TKI targeting KIT even more specifically or KIT-targeting antibodies, will be developed and may be even more suitable to apply in patients with MC-dependent (otherwise drug resistant) allergic disorders or MC activation disorders. It is also worth noting that several different normal cell types, such as germ cells, hematopoietic stem cells, and melanoblasts, display KIT and that application of such drugs may be associated, for example, with mutagenic or teratogenic effects.

Another unresolved question is whether all atopic/allergic disorders are strictly MC-dependent. In fact, some of these disorders may also be triggered by basophils, which also express IgE receptors and contain mediators of allergic reactions. It is worth noting that neither midostaurin nor avapritinib (nor any other KIT-targeting TKI) are known to eradicate blood basophils although some of these TKI do appear to decrease IgE- and antigen-dependent basophil activation.^{58,62,95} This is particularly relevant, as basophils may also participate in anaphylaxis reactions.^{24,95,106}

Finally, MC are thought to have important functions, as for example within the immune system (including contributing to resistance against certain parasite infections and mediating acute resistance to some venoms) and the reproductive systems, and long-term depletion of MC may not always be beneficial.^{2-4,9,107} In summary, more research and more clinical studies are required to define the exact value of TKI-induced MC-

depletion therapies as a new pharmacologic concept and to translate such concepts into clinical practice in patients with MC activation disorders.

Concluding Remarks and Future Perspectives

MC activation occurs in a number of different disease states and pathologies. The resulting clinical symptoms represent an emerging challenge in daily practice. In severe forms, life-threatening anaphylaxis may occur, leading to the diagnosis of a MC activation syndrome. Recent data suggest that therapy with KIT-targeting drugs may lead to a partial or even complete eradication of the MC lineage in patients with various MC diseases. In addition, some of these TKI can also suppress IgE-dependent activation of MC and basophils. Whereas several different KIT-targeting drugs are available that may suppress growth and activation of normal MC, suppression or even eradication of neoplastic MC exhibiting KIT D816V can only be achieved with novel TKI directed against this KIT mutant form. The most effective and specific KIT D816V-targeting drug available to date appears to be avapritinib. By contrast, whereas midostaurin is less effective in blocking the activity of KIT D816V, this drug also blocks SYK activity and thus IgE-dependent MC activation. Both drugs suppress MC expansion in patients with KIT D816V+ SM. Whether MC eradication induced by continuous treatment with an effective KIT TKI is safe and effective in patients with IgE-dependent allergies, and can help to avoid or mitigate the clinical symptoms of MCA and anaphylaxis in these patients, remains to be determined in forthcoming clinical studies.

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Legends to Figures

Figure 1

Effects of avapritinib and midostaurin on proliferation of neoplastic MC

HMC-1.2 cells expressing *KIT* D816V (upper panels) and primary neoplastic MC obtained from a patients with MCL (lower panels) were incubated in control medium (Co) or medium containing various concentrations of avapritinib (left panels) or midostaurin (right panels) at 37°C for 48 hours. Then, ³H-thymidine uptake was measured. Results are expressed as percent of control and represent the mean±S.D. from three independents (HMC-1.2) or triplicates (MCL cells). The data are in line with the available literature^{68,69,71,77} and suggest that the effective range and IC₅₀ values obtained with these cell lines are within the plasma concentrations (nM-range) measured in patients with mastocytosis receiving these drugs. Asterisk (*): p<0.05 compared to control. MCL, mast cell leukemia.

Figure 2

Target organs primarily involved in mastocytosis and MCAS

Several different organ systems may be involved in patients with mast cell activation syndromes (MCAS), non-advanced systemic mastocytosis (SM), and advanced SM. Whereas in patients with MCAS and non-advanced SM, mast cell-derived mediators usually affect organ function (examples: anaphylaxis, skin rash, ulcerative disease in the gastrointestinal tract), patients with advanced SM often suffer from organ damage induced by the local mast cell infiltrates (typical examples: severe cytopenia, hepatosplenomegaly with ascites and weight loss, or focal osteolysis).

Tables

Table 1

Molecular Targets and Activity Profiles of KIT-Targeting Tyrosine Kinase Inhibitors

Drug/name	KIT-blocking profile	Additional major targets	Blocks <i>in vitro</i> growth of		Blocks MC activation**
			normal MC: <i>KIT</i> -mutated MC:		
Imatinib	wt KIT, a few mutant forms	ABL1, ABL2, PDGFRs, many more	+	-	+/-
Masitinib	wt KIT, a few mutant forms	LYN	n.k.	-	+/-
Dasatinib	wt KIT, a few mutant forms (KIT D816V)*	ABL1, ABL2, BTK, PDGFRs, many more	++	+/-*	+
Nilotinib	wt KIT, a few mutant forms (KIT D816V)	ABL1, ABL2,	+	+/-	+/-
Midostaurin	wt KIT, most mutant forms, KIT D816V	FES, FGR, SYK, LYN, PDGFRs, many more	+	+	+
Avapritinib	wt KIT, most mutant forms, KIT D816V	PDGFRs	n.k.	+	+/-
Ripretinib	wt KIT, most mutant forms, KIT D816V	PDGFRs, VEGFR2	n.k.	+	+/-
Nintedanib	wt KIT, most mutant forms, KIT D816V	PDGFRs, VEGFR2, FGFR	n.k.	+	+/-
Bezuclastinib	KIT D816V and other KIT mutant forms	unknown	n.k.	n.k.	n.k.

*Although dasatinib blocks the activity of wild type KIT and KIT D816V and is capable of suppressing the *in vitro* growth and differentiation of human MC, dasatinib is usually unable to block the expansion of KIT D816V+ neoplastic MC in patients with mastocytosis, which is primarily due to the low *in vivo* half-life time (a few hours) of this drug.

**All KIT-targeting drugs can suppress stem cell factor-mediated (KIT-dependent) MC activation and SCF-induced mediator secretion, whereas only two of these drugs, midostaurin and dasatinib, both of which recognize IgE receptor-downstream targets, also substantially block IgE-dependent mediator secretion in MC. +, substantial suppression/blockage; +/-, some suppressing effects under certain conditions; -, no suppression (even with high drug concentrations) seen.

Abbreviations: MC, mast cells; PDGFR, platelet-derived growth factor receptor; wt, wild type; BTK, Bruton's tyrosine kinase; VEGFR, vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; n.k., not known.

Table 2

Clinical Efficacy Profiles of KIT-Targeting Tyrosine Kinase Inhibitors (TKI) in SM

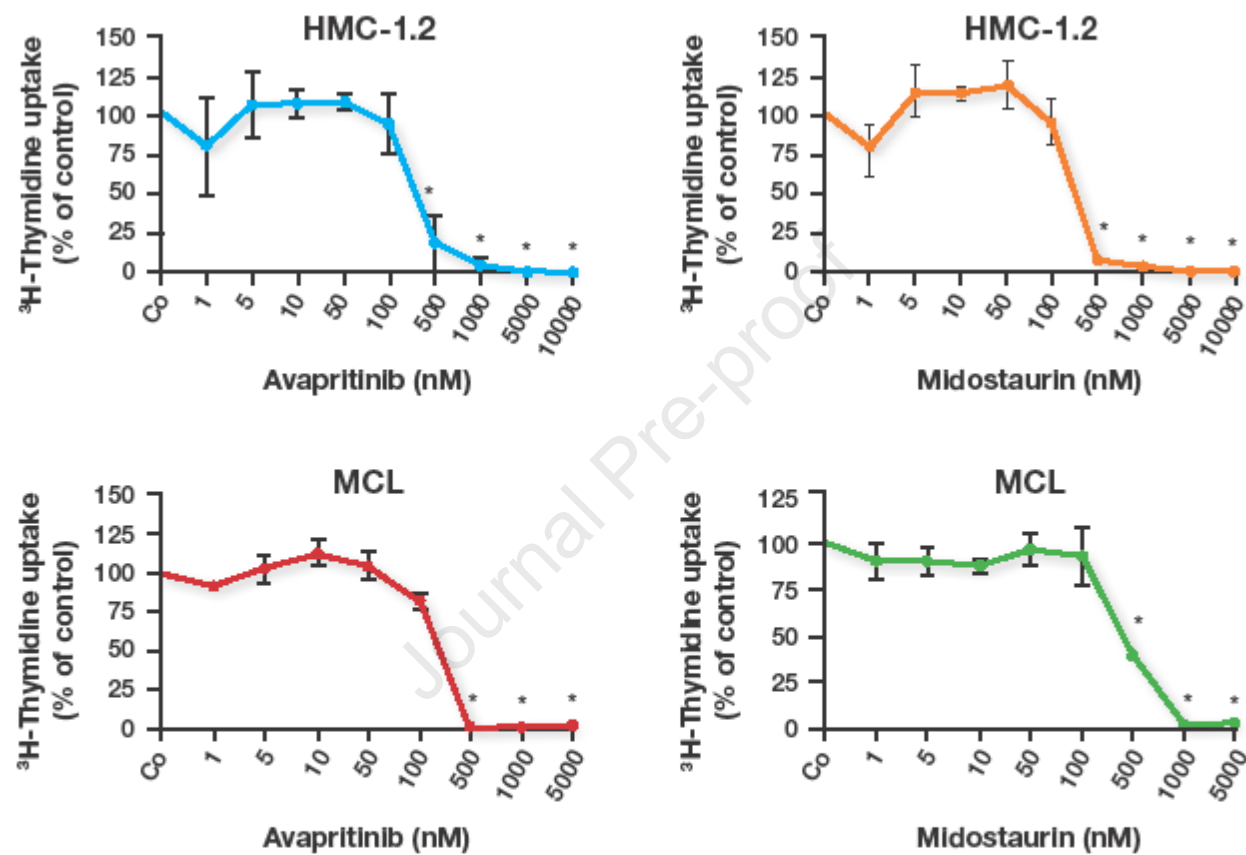
Name of TKI	Approved by FDA for	Complete mast cell eradication	Effects on mediator-induced symptoms	Recurrent and clinically relevant adverse events
Imatinib	SM without <i>KIT</i> D816V or unknown <i>KIT</i> mutation status	Seen in few cases with wt <i>KIT</i> , or <i>KIT</i> K509I, or <i>KIT</i> F522C, or other <i>KIT</i> variants	Limited or no effects	Facial edema, skin depigmentation, obstipation, renal function impairment*, diarrhea
Midostaurin	Advanced SM	Seen in few cases with <i>KIT</i> D816V	Major effects on mediator-related symptoms in most patients with advanced SM	Nausea, vomiting, diarrhea
Avapritinib	Advanced SM	Seen in 30-40% of patients with <i>KIT</i> D816V+ advanced SM**	Major effects on mediator-related symptoms in most patients with advanced SM	Cytopenia, facial edema, skin depigmentation, diarrhea, nausea, intracranial bleeding***

*After several years of treatment, less than 5% of all patients with chronic myeloid leukemia develop an impairment in kidney function – in many of these patients, a renal disease is also detected.

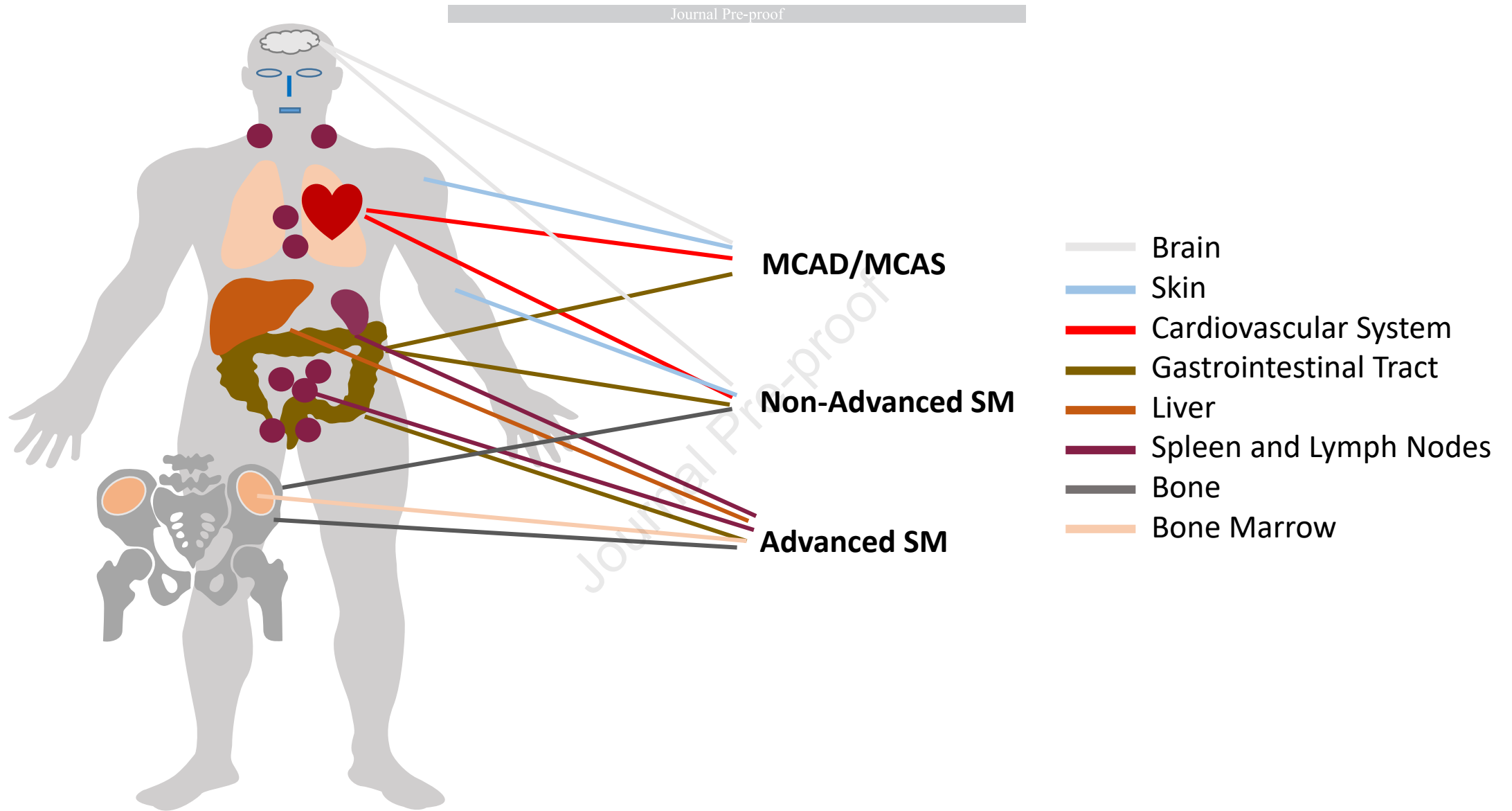
**In about 30% of these patients, avapritinib also induced molecular remission defined as no evidence of *KIT* D816V using droplet digital PCR (ddPCR).

Abbreviations: FDA, Food and Drug Administration; SM; systemic mastocytosis.

***Intracranial bleeding mostly has been reported in patients with severe thrombocytopenia.



Valent et al, Figure 1



Valent et al, Figure 2