

REVIEW

Midostaurin: a magic bullet that blocks mast cell expansion and activation

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Clinically relevant features in patients with systemic mastocytosis (SM) include the cosmetic burden of lesional skin, mediator-related symptoms, and organ damage resulting from mast cell (MC) infiltration in advanced forms of SM. Regardless of the SM variant, expansion of neoplastic MC in the skin and other organs is triggered by mutant forms of KIT, the most prevalent being D816V. Activation of MC with subsequent release of chemical mediators is often caused by IgE-dependent mechanisms in these patients. Midostaurin, also known as PKC412, blocks the kinase activity of wild-type KIT and KIT D816V, counteracts KIT-dependent growth of neoplastic MC, and inhibits IgE-dependent mediator secretion. Based on this activity-profile, the drug has been used for treatment of patients with advanced SM. Indeed, encouraging results have been obtained with the drug in a recent multi-center phase II trial in patients with advanced SM, with an overall response rate of 60% and a substantial decrease in the burden of neoplastic MC in various organs. Moreover, midostaurin improved the overall survival and relapse-free survival in patients with advanced SM compared with historical controls. In addition, midostaurin was found to improve mediator-related symptoms and quality of life, suggesting that the drug may also be useful in patients with indolent SM suffering from mediator-related symptoms resistant to conventional therapies or those with MC activation syndromes. Ongoing and future studies will determine the actual value of midostaurin-induced MC depletion and MC deactivation in these additional indications.

Key words: mast cells, targeted drugs, IgE, mast cell activation, mast cell leukemia

Introduction

Systemic mastocytosis (SM) is a hematopoietic neoplasm characterized by expansion and accumulation of neoplastic mast cells (MC) in one or more internal organ systems, including the bone marrow (BM), liver, spleen, and gastrointestinal (GI) tract [1–3]. Based on organ involvement and clinical course, SM can essentially be divided into indolent, smoldering, and advanced disease variants [4–10]. Patients with indolent SM (ISM) may suffer from mediator-related symptoms, osteopathy, and/or from the

cosmetic consequences of the disease [4–7, 10–14]. Otherwise, ISM patients have a good prognosis with normal or near-normal life expectancy [10–16]. In contrast, patients with advanced SM, including aggressive SM (ASM), SM with an associated hematologic neoplasm (AHN), and MC leukemia (MCL) have a poor prognosis with reduced survival [5–9, 14–17]. In these patients, the ‘malignant’ expansion of neoplastic MC in the BM, liver, and other visceral organs leads to organ damage, also known as C-Findings [8–10]. Moreover, in advanced SM, neoplastic MC are often resistant to one or more cytoreductive drugs, including

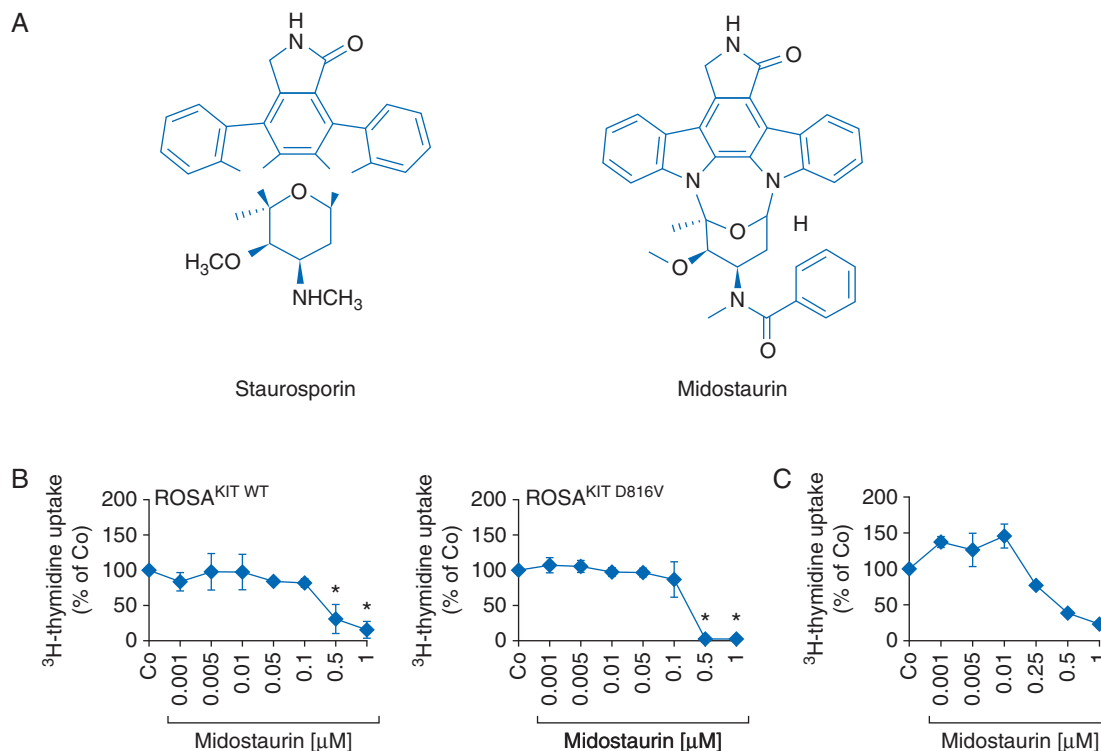


Figure 1. Chemical structure and anti-neoplastic effects of midostaurin. (A) Chemical structure of staurosporin and its derivative midostaurin. (B) The human mast cell lines ROSA^{KIT} WT (left panel) and ROSA^{KIT} D816V (right panel) were incubated in control medium (Co) or with increasing concentrations of midostaurin (PKC412) at 37 °C for 48 h. Results represent the percent of control and are expressed as mean ± SD of three independent experiments. *, $P < 0.05$. (C) Primary mast cells of a patient with aggressive systemic mastocytosis were cultured in the absence (Co) or presence of various concentrations of midostaurin at 37 °C for 48 h. After incubation, uptake of ³H-thymidine was measured. Results show the percent of control and are expressed as mean ± SD of triplicates.

interferon- α (IFN- α) and cladribine (2CdA) [18–20]. These patients are candidates for allogeneic hematopoietic stem cell transplantation (SCT) [21]. For those who are not eligible for SCT or refuse this therapy, investigational drugs are usually recommended [5, 9, 10, 22, 23]. In the past 10 years, several major efforts have been made to develop such drugs and to identify relevant therapeutic targets in neoplastic MC in advanced SM [24–27].

In over 80% of all patients with advanced SM, the *KIT* mutation D816V or other transforming *KIT* mutations are detectable in neoplastic cells [28–32]. These mutations cause ligand-independent activation of KIT and contribute to differentiation and expansion of MC in SM [25, 33]. Therefore, drugs interfering with the tyrosine kinase (TK) activity of KIT D816V have been proposed for patients with advanced SM [24–27, 34–36]. It is worth noting, however, that the D816V mutation of *KIT* confers resistance against several KIT kinase-inhibitors, including imatinib [25, 34–36].

Midostaurin was initially developed as a protein kinase C (PKC)-targeting drug [37]. However, it was soon discovered that midostaurin exerts strong inhibitory effects on a number of additional oncogenic kinases relevant in hematology [37]. In subsequent studies, midostaurin was found to suppress not only the kinase activity of wild-type (wt) KIT, but also mutated forms of the receptor, including D816V [25, 27, 35, 36]. Moreover, midostaurin was found to inhibit the growth of neoplastic MC exhibiting *KIT* D816V [25, 27]. Furthermore, midostaurin was found to

block IgE-dependent mediator release in human MC and basophils [38]. Based on these observations and first pilot cases, clinical trials were designed to explore anti-neoplastic effects of midostaurin in patients with advanced SM. Results from first clinical trials are now available and indeed suggest that a majority of patients with advanced SM respond to therapy with this agent [39, 40].

In the current article, we review the preclinical activities and the pharmacologic properties of midostaurin as well as the clinical efficacy of the drug in advanced SM. Moreover, we discuss additional indications of the drug in the field of SM and MC activation syndromes (MCAS) for which the drug may be useful. Finally, we discuss potential mechanisms of resistance against midostaurin and explore the potential value of drug combinations and other alternative therapies that may be useful and may overcome drug resistance.

Structure and target spectrum of midostaurin

Midostaurin, originally termed PKC412, is a semi-synthetic derivative of the alkaloid staurosporine, a well-known inhibitor of PKC (Figure 1A) [41]. Although initially considered a rather specific pharmacologic inhibitor of PKC, midostaurin interacts with a broad spectrum of clinically relevant kinase-targets, including KIT, FLT3, platelet-derived growth factor receptor α

(PDGFRA), PDGFRB, vascular endothelial growth factor receptor KDR, and FES (Table 1) [37, 42, 43]. Moreover, unlike other kinase inhibitors, midostaurin was found to inhibit not only the kinase activity of wt KIT, wt FLT3, and wt PDGFRA/B, but also the kinase activity of KIT D816V, other KIT mutants, FLT3 ITD, and various mutant forms of PDGFR, all of which are relevant clinically as they mediate resistance against other TK inhibitors (TKI) [25–27, 44–46]. Based on these observations, midostaurin is an emerging new anticancer agent in applied hematology. More recently, the kinase interaction profile of midostaurin was analyzed in the context of neoplastic MC [43]. In addition, the kinase interaction profile of midostaurin has been compared with that of two major metabolites, CGP62221 and CGP52421 [43]. Chemical proteomics profiling and drug-competition experiments revealed that midostaurin interacts with KIT and several additional oncogenic kinases, and also with other clinically relevant kinase-targets, such as SYK, in lysates prepared from primary MCL cells and HMC-1 cells [43]. SYK is an IgE-receptor downstream target that plays a major role in IgE-dependent MC activation [47, 48]. Interestingly, KIT and the key downstream-regulator FES were found to be recognized and dephosphorylated by midostaurin and CGP62221, but not by CGP52421, whereas SYK was recognized and blocked by both metabolites as well as by midostaurin [43]. These observations may be clinically relevant, in particular since CGP52421, following chronic dosing of midostaurin to patients, achieves pharmacologically relevant steady-state concentrations *in vivo* [49–52]. An overview of clinically relevant kinase targets recognized by midostaurin is shown in Table 1.

Evidence that midostaurin blocks proliferation, survival, and activation of MC

Earlier preclinical studies have shown that midostaurin inhibits the proliferation of Ba/F3 cells engineered to express either wt *KIT* or *KIT* D816V [25, 35]. Moreover, midostaurin suppressed the growth and survival of the human MCL-derived cell lines HMC-1.1 expressing the *KIT*V560G mutation (but not *KIT*D816V) and HMC-1.2 expressing both *KIT*V560G and *KIT*D816V [25, 27]. In both cell lines, relevant and comparable IC₅₀ values (50–250 nM) were obtained with midostaurin [25]. Similar effects were also seen when applying the drug in the recently established human MC lines ROSA^{KIT WT} and ROSA^{KIT D816V} [53]. In both cell lines, midostaurin exerts growth-inhibitory effects at submicromolar concentrations (Figure 1B). Furthermore, midostaurin was found to induce apoptosis and growth arrest in primary neoplastic MC obtained from patients with advanced SM (Figure 1C) [25, 27].

Midostaurin also produced synergistic growth-inhibitory effects in HMC-1 cells when combined with a number of targeted drugs, including other KIT inhibitors, cladribine (2CdA), BCL-2 family antagonists, or inhibitors of BRD4 [25, 27, 54–57]. In most studies, cooperative effects could be explained by the different target-interaction profiles these drugs exhibit [58]. For example, the synergistic effect of the drug combination ‘midostaurin + dasatinib’ was explained by inhibitory effects of dasatinib on BTK and LYN, since cooperative drug effects (midostaurin + dasatinib) were also obtained when applying the drug plus small interfering RNA (siRNA), namely ‘midostaurin + BTK-siRNA’ or ‘midostaurin + LYN-siRNA’ on neoplastic MC [58].

Table 1. Clinically relevant targets of midostaurin

Molecular target ^a	Major target cell populations and disorders
KIT wt	Mast cells, AML blasts, GIST cells
KIT D816V	Mast cells in SM, AML blasts, GIST cells
KIT V560G	Mast cells in SM, GIST cells
KIT K509I	Mast cells in CM
FES	Mast cells in KIT D816V+ SM (KIT D816V-downstream-signaling)
FLT3 wt	Myeloid (progenitor) cells
FLT3 ITD	AML blasts
FLT3 D835H	AML blasts
FLT3 D835Y	AML blasts
FLT3 N841I	AML blasts
PDGFRA wt	Fibroblasts, mesenchymal stem cells
FIP1L1-PDGFR (F/P)	Eosinophils, CEL, MPN-eo
F/P mutant forms	Eosinophils, CEL, MPN-eo
PDGFRB wt	Fibroblasts, mesenchymal stem cells
PDGFRB mutant forms	Eosinophils, CEL, MPN-eo
JAK2	Myeloid cells, MPN cells
JAK3	Various immune cells
AAK1	Notch adaptor in neoplastic cells
SYK	IgE-R+ cells, IgE-R-downstream signaling
AURKA	Mitosis regulator in various neoplastic cells
AURKB	Mitosis regulator in various neoplastic cells
MARK3	Oncogenic molecule in hepatocellular cancer
PKN1	Wnt/β-catenin repressor in cancer cells
FGFR3	B cells, multiple myeloma cells
FGFR3 TDII (K650E)	B cell lymphoma cells, multiple myeloma cells
TEL-FGFR3	T cell lymphoma cells, MPN, AML blasts

^aMolecular targets were identified in a cell-free screen [42] and in a chemical proteomics approach using primary neoplastic mast cells [43]. wt, wild-type; AML, acute myeloid leukemia; GIST, gastrointestinal stroma cell tumor; SM, systemic mastocytosis; CM, cutaneous mastocytosis; CEL, chronic eosinophilic leukemia; MPN, myeloproliferative neoplasm; IgE, immunoglobulin E; JAK, Janus kinase; AURK, Aurora kinase.

Another remarkable observation was that midostaurin almost completely blocks IgE-receptor mediated activation and mediator release in human MC and blood basophils (Figure 2A) [38, 43]. This effect was observed at pharmacologically relevant drug concentrations (0.5–2.0 μM) and may be explained by the strong effect of the drug on PKC, SYK, and other IgE receptor-downstream targets [43]. The effects of midostaurin on histamine release are dose-dependent, without a rebound effect or paradoxical stimulating effect at low concentrations, described for dasatinib in its effects on IgE-mediated histamine release [59]. More recently, midostaurin was also found to block IgE-dependent histamine release in *ex vivo* basophils obtained from SM patients receiving midostaurin [43]. It is also worth noting that midostaurin exerts little if any immunological side-effects *in vivo* (such as immunosuppression, lymphopenia, or hyperactivation of immune cells) at doses administered in clinical trials. Together, these data suggest that midostaurin is a promising agent that can

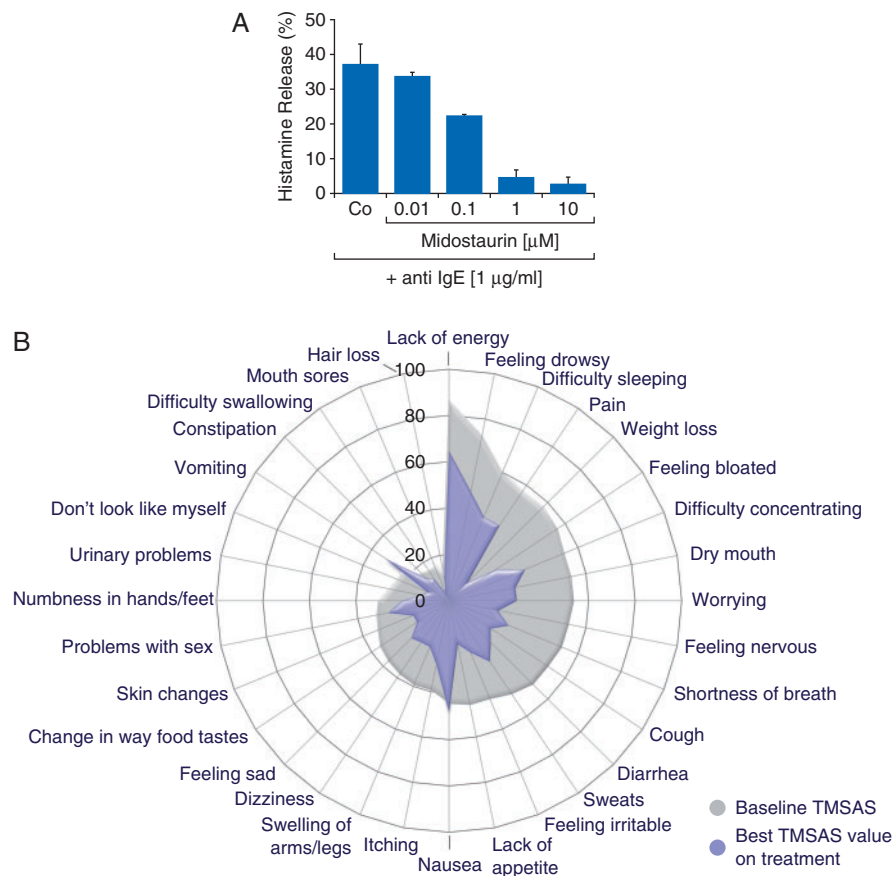


Figure 2. Effect of midostaurin on mediator release and mediator-induced symptoms. (A) Human basophils were incubated in control buffer or in the presence of various concentrations of midostaurin (as indicated). Histamine release was induced by incubating basophils with an anti-IgE antibody at 37 °C for 30 min. Histamine release was calculated as percent of total (cellular+extracellular) histamine and is expressed as mean $\pm$ SD of triplicates. (B) Spider plot delineation of symptom improvement in patients with advanced SM. Symptoms were recorded in 79 assessable patients in the global phase II trial examining the effects of midostaurin in advanced systemic mastocytosis [39]. Symptoms were assessed by memorial symptom assessment scale (MSAS) at baseline and during treatment with midostaurin. The spider plot shows the baseline MSAS values (gray shading) and the MSAS values at the time of best symptom improvement by total MSAS scoring during treatment (blue area). Reproduced from Gotlib et al. [39] (copyright: Massachusetts Medical Society) in slightly modified form with permission from the editor.

target neoplastic cell growth as well as IgE-dependent cell activation in patients with SM.

Results from a global phase II trial in patients with advanced SM

Between 2009 and 2012 a global multi-center, open-label, phase II (single-arm) trial enrolled patients with advanced SM to determine the safety and efficacy of single agent oral midostaurin. The drug was administered continuously at a dose of 100 mg twice daily in 4-week cycles [39]. A total of 116 patients with ASM, SM-AHN, or MCL were eligible for safety evaluation, and of these, 89 patients were eligible for the evaluation of midostaurin's efficacy based on the presence of organ damage attributable to SM (C-Findings). Treatment was continued until disease progression, death, or intolerable toxicity. The overall response rate was 60%, and thus the highest ever reported in this group of poor-risk patients [39]. More importantly, 45% of all patients achieved a major response defined by resolution of organ damage

(disappearance of at least 1 C-Finding) [39]. The response rates and the duration of responses were similar in ASM and SM-AHN. Successful treatment was also accompanied by a substantial decrease in the BM MC burden (median best decrease: -59%) and in the serum tryptase level (median best decrease: -58%). The median overall survival (OS) was 28.7 months, and the progression-free survival 14.1 months (Table 2) [39]. The primary cause of death was progression of ASM to MCL or to advanced AHN, often in form of secondary acute myeloid leukemia (AML). Among the 16 patients with MCL, 8 patients (50%) responded, including 7 with a major response. The median OS was 9.4 months for all MCL patients, but was not reached in responders. In a post hoc multivariate analysis, two outcome measures of midostaurin therapy, response (versus no response), and $\geq 50\%$ (versus $< 50\%$) reduction in BM MC burden were associated with longer survival. In 56% of the patients, the dose of midostaurin was transiently or permanently reduced because of toxicity. In 32% of these patients, midostaurin could later be re-escalated to the starting dose of 100 mg twice daily. The most frequently recorded side-effects were

Table 2. Clinical responses to midostaurin in patients with advanced SM: results from the global phase II trial

Response and clinical endpoint	Data recorded in a global phase II trial ^a	Improvement over historical controls
Overall response rate	60%	Yes (<50%)
Overall response rate in MCL	50%	Yes (<50%)
Major response rate	45%	Yes (<20%)
Median overall survival (OS)	28.7 months	Yes (<20 months)
Median OS of responders	44.4 months	Yes
Median P-F survival	14.1 months	Yes (<10 months)
Median OS in MCL	9.4 months	Yes (<6 months)
Decrease in BM MC burden by = 50%	57%	Yes
Decrease in serum tryptase by = 50%	60%	Yes
Decrease in spleen volume	77%	Yes

^aData were obtained from the recently published global midostaurin trial [39].
SM, systemic mastocytosis; MCL, mast cell leukemia; P-F, progression-free; BM MC, bone marrow mast cells.

low-grade nausea, vomiting, and diarrhea (Table 3) [39]. Grade 3/4 neutropenia, anemia, or thrombocytopenia developed in <50% of all patients, and in most of these patients, mild to moderate cytopenia had already been detected before therapy.

The study also examined potential effects of midostaurin on SM-related (including mediator-induced) symptoms and the quality of life (QOL) [39, 60]. Most commonly reported baseline symptoms, defined by Memorial Symptom Assessment Scale (MSAS), were lack of energy (86%), feeling drowsy (72%), and difficulty sleeping (60%). During treatment with midostaurin, most of the symptoms improved, and at the time of best response by MSAS, all 32 symptoms decreased, except for nausea and vomiting, the two known side-effects of midostaurin capsules (Figure 2B) [39, 60]. It is also noteworthy that IgE-dependent basophil histamine release rapidly decreased after intake of midostaurin [43]. A remarkable observation was that improvement in mediator-related symptoms and QOL was still observed even when no objective hematologic response was found and the disease relapsed. This is best explained by clinically relevant activity of midostaurin on MC activation, an effect that is separable from the impact of the drug on MC growth and survival, as also predicted from *in vitro* studies carried out with midostaurin and its metabolites [43]. In fact, both metabolites block IgE-dependent mediator secretion potently, but only one metabolite (CGP62221) blocks the *in vitro* growth of neoplastic MC whereas the other, accumulating, metabolite (CGP52421) is less effective [43].

Results from other clinical trials, cohorts, and case reports

Historically, the first patient with advanced SM (MCL) who was treated with midostaurin, achieved a major response with

Table 3. Side-effects observed with midostaurin in a global phase II trial in advanced SM^a

Reported event	Frequency (%)		Also known as a classical symptom in (advanced) SM
	All grades	Grade 3/4	
Hematologic			
Anemia ↑ ^b	63	41	Yes
Neutropenia ↑ ^b	48	24	Yes
Thrombocytopenia ↑ ^b	52	29	Yes
Non-hematologic			
Nausea	79	6	Yes ^c
Vomiting	66	6	Yes ^c
Diarrhea	54	8	Yes
Peripheral edema	34	4	No
Abdominal pain	28	3	Yes
Fatigue	28	9	Yes
Pyrexia	27	6	No
Constipation	24	1	No
Headache	23	2	Yes
Back pain	20	2	Yes
Pruritus	19	3	Yes
Arthralgia	17	2	No
Cough	16	1	No
Dyspnea	16	4	Yes
Musculoskeletal pain	16	4	Yes
Nasopharyngitis	15	0	No
Urinary tract infection	12	2	No
Dizziness	11	0	Yes
Epistaxis	11	3	No
Pleural effusion	11	3	No
QT-interval prolongation	10	1	No

^aData are derived from Gotlib et al. [39] with permission from the Editor.
^bWorsening (↑) of cytopenia was frequently observed (as sign of clone-eradication and poor stem cell reserve) and often followed by a good response.
^cNausea and vomiting are considered to be a result of drug (capsule) formulation rather than produced by midostaurin itself.
SM, systemic mastocytosis.

resolution of liver function abnormalities and almost complete disappearance of KIT D816V+ circulating neoplastic MC in the peripheral blood [61]. Based on this impressive response and the encouraging *in vitro* results obtained with the drug, clinical trials with midostaurin were started. The first study, an investigator-initiated trial of 26 patients with advanced SM, documented an organ response rate of 69%, including 50% major responses [62]. Notably, one patient with MCL achieved a complete remission, which included complete resolution of anemia and hypalbuminemia, normalization of a highly elevated tryptase (763 ng/ml), resolution of splenomegaly, and elimination of a huge infiltrate (70%–80%) of neoplastic MC in the BM [62]. This response is of particular interest from a molecular perspective since the patient carried tandem *KIT* D816V and *FLT3* ITD mutants, both therapeutic targets of midostaurin (Table 1). These data provided

proof of principle of the activity of midostaurin in advanced SM, and led to the aforementioned global phase II trial of midostaurin. With now more than 9 years of median follow-up, the median overall and progression free-survival of the pilot cohort examined are 40 and 41 months, respectively (J. Gotlib, personal communication).

In a more recent analysis, the response of 28 patients with advanced SM treated with midostaurin on a French compassionate use program was compared with a historical control group of patients with SM treated with other anti-neoplastic drugs, including cladribine [40]. After a median treatment-duration of 10.5 months and a median follow-up of 18.5 months, the overall response rate in the midostaurin group was 71%, including major responses seen in 57% of the cases. After a median treatment-duration of about 10 months, the OS rate was 42.7% in the midostaurin group and thus significantly higher than the OS rate in the control group (14.9%) [40]. Other studies suggest that midostaurin can also reduce the burden of neoplastic MC in the skin in patients with ISM (A. Reiter, H. C. Kluin-Nelemans, and J. Gotlib, unpublished observation).

Furthermore, due to its potent activity against wt KIT, midostaurin should also be able to induce near-complete MC-deficiency in the absence of SM, as has been recently described for imatinib [63]. However, due to the long-maturation time of MC-committed stem- and progenitor cells and the long survival of mature MC in the tissues [64], it may take about 2 years until a strong KIT-inhibitor is able to induce complete MC deficiency [63].

Pharmacologic aspects and impact of midostaurin metabolites

After multiple dosing, a non-linear pharmacokinetic of midostaurin was observed in patients with AML [49–51]. In fact, plasma concentrations of the drug increased during the first week of therapy, followed by a significant decrease, with a plateau being reached after 2–3 weeks [49–51]. It was also found, that during treatment, the following major drug metabolites can be measured in the plasma of these patients: CGP52421 and CGP62221 [49–51]. Whereas the pharmacokinetics of CGP62221 is similar to that of midostaurin, CGP52421 was found to accumulate over time and to reach steady-state concentrations after 1 month [49–52]. The pharmacologic basis of this phenomenon remains unclear, but may be related to cytochrome P450 enzyme induction in the liver. Recent data suggest that CGP62221 and midostaurin are equally effective in inhibiting the growth of neoplastic MC [43]. However, CGP52421 is less effective in inhibiting the growth of HMC-1 cells compared with midostaurin [43]. On the other hand, the metabolite still retains considerable activity against primary neoplastic MC *in vitro* in most patients with SM [43]. In addition, CGP52421 does not ‘antagonize’ the effects of midostaurin on MC when co-administered *in vitro* [43]. In addition, CGP52421 blocks IgE-dependent MC activation in the same way as midostaurin [43]. Furthermore, the metabolite cooperates with cladribine (2CdA) in the same way as midostaurin to block MC growth [43]. Finally, the metabolites can be washed out easily by discontinuing the drug for several days. Therefore, the metabolite issue, albeit recognized, may not be a major hurdle in the successful treatment of patients with advanced SM.

Mechanisms of resistance against midostaurin

Although midostaurin is regarded a breakthrough in the treatment of advanced SM, response-duration is variable, and in some patients, no clinical activity is observed. Although the mechanisms for primary or secondary resistance have not been formally elucidated [39, 61], a number of etiologies are discussed. These include: (i) secondary mutations in primary target genes, (ii) additional molecular lesions expressed in driver mutant-positive clones, (iii) selection and expansion of driver-negative sub-clones, (iv) intrinsic stem cell resistance, and (v) pharmacologic resistance.

Secondary mutations have been described in AML patients with *FLT3* ITD mutations [65] and patients with *PDGFR* mutations [66], but not in patients with advanced SM. This may be explained by the low number of patients examined so far, by a low mitotic rate and mutation-rate in *KIT*-mutated SM-clones or by the fact that the selection pressure of midostaurin is too weak to allow for outgrowth of sub-clones carrying such secondary mutations.

Additional molecular lesions and driver pathways are frequently detected in advanced SM [67–69]. Since KIT D816V may not be a primary defect in SM, such additional lesions and pathways are detectable in both, *KIT*-mutated sub-clones and ‘*KIT*-non-mutated’ sub-clones. In other words, midostaurin resistant patients may relapse with a *KIT* D816V-positive or with a *KIT* D816V-negative sub-clone or a mixture of such clones. As an example, the first MCL patient successfully treated with midostaurin relapsed a few months after a major initial response, and presented with an AML-like disease without *KIT* D816V [61]. A number of different mutations may be detected in advanced SM, mostly in the context of an AHN. These include, among others, known pathogenic variants of *TET2*, *SRSF2*, *ASXL1*, *CBL*, *RUNX1*, and *RAS* [67–69]. At least some of these mutations may contribute to resistance against midostaurin. In other SM-AHN patients, additional driver-lesions may be found, such as *JAK2* V617F or the AML-associated t(8;21) *RUNX1-RUNX1T1* fusion-gene.

Intrinsic resistance of leukemic stem cells (LSC) has been postulated in various malignancies and linked to certain molecules, including multidrug-resistance genes, such as MDR-1, immune-checkpoint receptors, such as PD-L1 (PD1 ligand) or CD47 (‘don’t eat me’ receptor), and interactions between LSC and the so-called stem cell niche. Such mechanisms may also apply in patients with advanced SM. For example, it has been described recently that neoplastic MC display PD-L1 [70]. Moreover, MCL-initiating cells (MCL LSC) have been described to express various niche-related receptors, such as CD44, KIT, and CD47 [71]. Pharmacologic resistance against midostaurin has also been discussed, especially in the context of midostaurin metabolites (see above). However, the clinical impact of pharmacologic resistance in the context of SM remains uncertain. Potential mechanisms of resistance against midostaurin are summarized in Table 4.

Potential strategies aimed at overcoming resistance against midostaurin

A key question in advanced SM is how to avoid and to overcome resistance against midostaurin. One strategy may be to apply

Table 4. Potential mechanisms and strategies to overcome resistance against midostaurin

Potential mechanism	Example(s)	Proposed potential strategies to overcome drug resistance
(A) Molecular (acquired) resistance		
Secondary mutations in critical target/driver genes	FLT3 N676 in AML	Switch to other drug, CT+SCT, drug combinations
Expansion of driver target-	KIT D816V–	Switch to other drug, CT+SCT
Negative subclone(s) +/- additional oncogenic drivers trigger growth of neoplastic cells	Negative relapse	Drug combinations employing additional targeted drugs directed against other oncogenic drivers
	pAXL in AML	
	pBTK in MCL	
(B) Intrinsic LSC resistance		
Niche-related resistance	CXCR4-SDF-1	CXCR4 blockers
Checkpoint-related resistance	PD-L1 in MC and AML LSC	Checkpoint inhibitors
Drug-transporter activity	MDR-1 on LSC	Transporter-targeting drugs
(C) Pharmacologic resistance		
Metabolite accumulation	Accumulation of CGP52421 in AML patients	Cycle-based therapy with in-between wash out periods

AML, acute myeloid leukemia; CT, chemotherapy; SCT, stem cell transplantation; MCL, mast cell leukemia; MC, mast cells; LSC, leukemic stem cells.

combinations of drugs that exert clear synergistic growth-inhibitory effects on neoplastic MC [25, 27, 55–57, 72]. Probably the most encouraging drug combination to be considered is midostaurin + cladribine [25]. Based on *in vitro* data and clinical responses seen with both agents, this combination should be tested in forthcoming clinical trials in advanced SM.

Another potential strategy to overcome resistance may be to apply poly-chemotherapy (CT) and allogeneic SCT. Indeed, it has been shown that SCT is a potentially curative approach in advanced SM, particularly in patients with SM-AHN [21, 73]. One strategy for patients with drug-resistant SM may be to use midostaurin with or without chemotherapy (CT) such as cladribine or multi-agent induction regimens (especially for patients with MCL) as a bridge to SCT. Such debulking strategies may be particularly relevant for rapidly proliferating forms of ASM and MCL. Currently available safety and efficacy data suggest that high-risk hematologic neoplasms such as FLT3-positive AML can be managed effectively with midostaurin in combination with chemotherapy [74]. Another approach would be to use midostaurin after allogeneic SCT as maintenance therapy in order to reduce the risk of relapse. Observations in single case reports suggest that such an approach is feasible and associated with disease-free survival (Figure 3). CT and SCT may also be an optimal way to eradicate neoplastic stem cells in ASM and MCL. However, not all patients are transplantable, mainly because of age and comorbidities. For such elderly and/or comorbid patients, alternative stem-cell eradicating therapies have to be developed. One problem is that these cells are often quiescent and indistinguishable (by phenotype and target-profiles) from normal stem cells. One interesting approach to kill neoplastic stem cells (preferentially) may be to apply targeted antibodies directed against surface structures expressed on disease-initiating and propagating cells in ASM and MCL. Promising surface targets that are expressed not only on neoplastic MC, but also on disease-initiating and -propagating stem cells in ASM and MCL, include CD33, CD44, and CD52 [71, 75, 76]. Table 4 provides a

summary of strategies that may be useful for overcoming resistance of neoplastic MC against midostaurin. Another question may be how to address pharmacologic resistance and the accumulation of inactive midostaurin metabolites. One possible way may be to apply the drug in 14- or 21-day cycles, followed by a short wash-out period. Another method to address this form of resistance may be to apply drug combinations. In fact, it has been described that both midostaurin metabolites cooperate with cladribine in inducing growth inhibition of neoplastic MC [43].

Indications for midostaurin: status 2017 and outlook toward the future

Based on its beneficial effects observed in recent clinical trials [39, 40, 62], midostaurin is regarded as an emerging first-line standard therapy for patients with advanced SM. In addition, midostaurin can be considered standard salvage therapy for SM patients in whom lack or loss of response to IFN-A, cladribine, or other cytoreductive is seen. Additional indications may be based on the effects of the drug on MC activation. For example, patients with ISM or smoldering SM (SSM) with severe mediator-related symptoms unresponsive to conventional antimediation therapy may benefit from midostaurin [77]. In addition, patients with advanced SM who have undergone high-dose CT and/or SCT may benefit from treatment with midostaurin as maintenance therapy (Figure 3). However, thus far, no data from controlled clinical trials are available to support these indications. It also remains unknown whether and what combination treatments involving midostaurin are useful, and what subgroup of patients will optimally benefit. A reasonable approach may be to combine midostaurin with cladribine in patients who develop resistance against cladribine. Other unresolved questions are whether midostaurin can eradicate all neoplastic MC in patients with ISM and whether skin lesions will disappear in all patients. If this is the case, the benefits of midostaurin will need to be weighed against

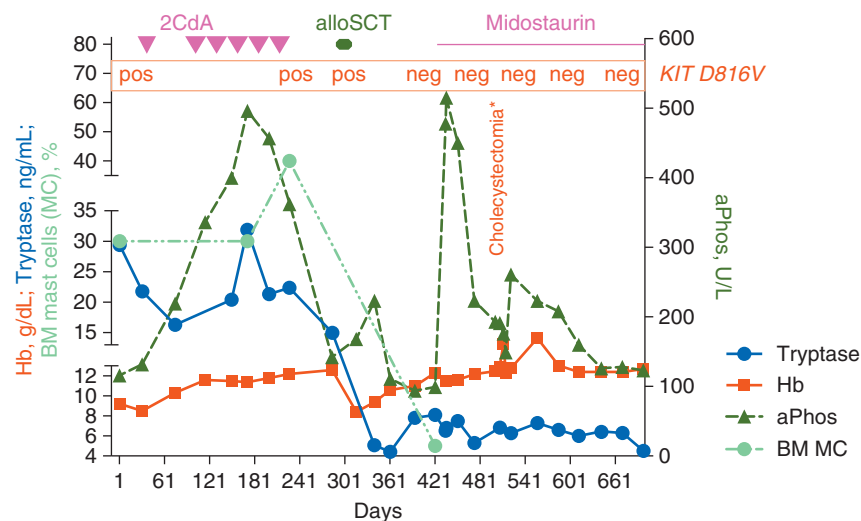


Figure 3. Clinical course in a patient with aggressive systemic mastocytosis (ASM). The patient was diagnosed with ASM with rapid clinical deterioration based on the local destructive growth of mast cells as well as on severe mediator-related symptoms. Initially, the patient’s condition did not allow for any intensive therapy. The patient was then treated with cladribine and glucocorticosteroids. After sufficient debulking the general condition improved dramatically, and the patient subsequently underwent allogeneic stem cell transplantation (SCT). After SCT, the patient had a transient increase in tryptase. Treatment with midostaurin was initiated and resulted in a decrease in tryptase and a complete disappearance of *KIT* D816V. At the same time, the patient had also developed cholecystitis and a huge (transient) increase in alkaline phosphatase. However, after cholecystectomy, clinical symptoms improved, and the alkaline phosphatase concentration (otherwise also known as a disease-indicator in ASM) decreased to normal levels. After 2 years, the patient has still a major clinical response and is in complete hematologic and molecular remission.

potential side-effects, including long-term toxicities that may not be fully appreciated at this time.

Because of midostaurin’s capacity to block IgE-mediated activation of MC and its ability to improve mediator-related symptoms in SM patients [39, 60], an additional indication worthy of future investigation may be secondary (IgE-dependent) MCAS and IgE-dependent allergies unresponsive to classical antimediator therapy. An important consideration in this regard is that it can take many months until the numbers of (normal) MC decrease substantially in patients treated with a potent KIT inhibitor [63]. The obvious advantage of midostaurin over other KIT inhibitors such as imatinib or masitinib would be that the drug immediately blocks IgE-dependent release of histamine [38, 43]. However, the side-effect profile of the drug should always be carefully considered against potential symptomatic improvements in non-neoplastic conditions. Whether midostaurin indeed works in patients with severe anaphylaxis or secondary MCAS remains to be elucidated in forthcoming clinical trials. A summary of potential future indications of midostaurin is shown in Table 5.

Discussion

Concluding remarks

The growth of normal and neoplastic MC is largely dependent on the kinase activity of KIT. The oncogenic *KIT* variant D816V is detected in most patients with SM and has been implicated as a major driver of MC survival and oncogenesis. As a result, KIT is an obvious target of therapy in advanced SM. Midostaurin is a multi-targeted drug that blocks not only the oncogenic variants

Table 5. Potential indications of midostaurin in applied hematology and immunology

Potential indication	Positive results from clinical trials available	Approval from health authorities
FLT3-mutated AML	+	+ ^a
KIT-mutated AML	–	–
Advanced SM including MCL	+	+ ^a
Smoldering SM (skin lesions, MCAS)	+/–	–
Indolent SM (skin lesions, MCAS)	+/–	–
Primary MCAS	–	–
Secondary (IgE-dependent) MCAS	–	–
IgE-dependent atopic diseases	–	–
Eosinophilic leukemias	–	–
PDGFR-rearranged MPN-eo	–	–
Other forms of MPN-eo	–	–

^aFor these indications midostaurin received approval from the FDA on April 28, 2017.

AML, acute myeloid leukemia; SM, systemic mastocytosis; MCL, mast cell leukemia; MCAS, mast cell activation syndrome; IgE, immunoglobulin E; PDGFR, platelet-derived growth factor receptor; MPN, myeloproliferative neoplasm; MPN-eo, MPN with hypereosinophilia.

of KIT and various KIT-downstream kinase targets, but also IgE-dependent secretion of histamine from MC and basophils. In first clinical trials, these effects have been confirmed and major clinical responses were seen with midostaurin in advanced SM. The next steps will be to explore the clinical efficacy of drug combinations in advanced SM and the potential value of midostaurin as monotherapy in patients with indolent or smoldering SM suffering from severe mediator-related symptoms (MCAS). Based on its broad target spectrum, its documented efficacy in advanced SM and *FLT3*-mutated AML, and its favorable toxicity profile, midostaurin is expected to become a broadly used agent in clinical hematology once it has been adopted in clinical practice. In the USA, midostaurin (Rydapt®; Novartis Pharmaceuticals Corp.) was approved for the treatment of advanced SM and *FLT3*-positive AML by the FDA on April 28, 2017.

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Disclosure

PWM is employed by Novartis, Switzerland; PV, KH, TIG, KS, HCK-N, H-PH, AR, and JG served as Scientific Advisory Board Members and as Consultants in a global phase II Novartis trial exploring the effects of midostaurin in advanced SM. PV, KH, TIG, KS, WRS, OH, HCK-N, H-PH, AR, and JG received honoraria from Novartis. The authors have declared no other conflicts of interest in this project.

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