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Midostaurin: a novel therapeutic agent for patients with FLT3-mutated acute myeloid leukemia and systemic mastocytosis

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***Abstract*:** The development of FLT3-targeted inhibitors represents an important paradigm shift in the management of patients with highly aggressive fms-like tyrosine kinase 3-mutated (FLT3-mut) acute myeloid leukemia (AML). Midostaurin is an orally administered type III tyrosine kinase inhibitor which in addition to FLT3 inhibits c-kit, platelet-derived growth factor receptors, src, and vascular endothelial growth factor receptor. Midostaurin is the first FLT3 inhibitor that has been shown to significantly improve survival in younger patients with FLT3-mut AML when given in combination with standard cytotoxic chemotherapy based on the recently completed RATIFY study. Its role for maintenance therapy after allogeneic transplantation and use in combination with hypomethylating agents for older patients with FLT3-mut has not yet been defined. Midostaurin also has recently been shown to have significant activity in systemic mastocytosis and related disorders due to its inhibitory effect on c-kit bearing a *D816V* mutation. Activation of downstream pathways in both of these myeloid malignancies likely plays an important role in the development of resistance, and strategies to inhibit these downstream targets may be synergistic. Incorporating patient factors and tumor characteristics, such as FLT3 mutant to wild-type allele ratios and resistance mutations, likely will be important in the optimization of midostaurin and other FLT3 inhibitors in the treatment of myeloid neoplasms.

Keywords: acute myeloid leukemia, allogeneic transplant, clinical trials, FLT3 mutation, midostaurin, systemic mastocytosis

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Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults, with over 20,000 new diagnoses each year in the United States.[1] Prognosis is highly dependent upon clinical and molecular characteristics, with complete response (CR) rates ranging from 50% to 80% depending upon patient characteristics, treatment tolerance, and genetic aberrations.[2–5] One of the most common mutations detected in AML occurs in the gene encoding fms-like tyrosine kinase 3 (FLT3), a type III receptor tyrosine kinase which regulates normal growth and differentiation of CD34+ hematopoietic cells *via* signaling through multiple pathways including PI3 kinase-Akt, MAPK, and STAT5a.[6,7] The overall incidence of FLT3 mutations in AML is about 30%; the majority are variable-length, in-frame insertion mutations within the juxtamembrane domain [termed internal tandem duplications (ITD), 23%], while the remainder (7%) are point mutations in the tyrosine kinase domain (TKD).[8–10] In patients under 60, the FLT3-ITD mutation represents an independent risk factor for higher relapse and induction death rates, as well as lower CR, disease-free survival (DFS), event-free survival (EFS), and overall survival (OS) rates,[10–15] particularly if there is a higher clonal burden of FLT3-ITD identified as the mutant to wild-type allelic ratio.[16,17] Based on these associations, the presence of the FLT3-ITD mutation is considered an unfavorable molecular risk marker in AML by the NCCN

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(National Comprehensive Cancer Network, Acute Myeloid Leukemia, version 1.2017, accessed 12April 2017). In contrast, the FLT3-TKD mutation is not an independent risk factor for poor outcomes; thus, many studies of FLT3 inhibitors have focused on patients with FLT3-ITD mutations.

Another myeloid disease is systemic mastocytosis (SM), a rare clonal myeloid neoplasm with an estimated prevalence of 10 per 100,000 in the United States.[18,19] SM is characterized by the accumulation of neoplastic mast cells in various organs such as the bone marrow and skin, and, in advanced cases, other visceral organs. Indolent or smoldering mastocytosis has a favorable prognosis and can be treated symptomatically, while patients suffering from advanced SM have a median survival of months to years.[20,21] Mutations in c-kit, a tyrosine kinase which signals through PI3 kinase, STAT5, mTOR, and FES, are nearly universal in SM.[22,23] The *D861V* mutation is found in over 80% of patients with SM, confers resistance to imatinib, and appears to play an important role in the pathogenesis by promoting mast cell differentiation and maturation, making it an attractive therapeutic target in the treatment armamentarium for this rare disease.[21]

This review focuses on the multikinase inhibitor midostaurin (*aka* N-benzoyl staurosporine, PKC412, CGP 41251), an inhibitor of FLT3, c-kit, and other kinases. Midostaurin is the only FLT3 inhibitor approved by the FDA for the treatment of newly diagnosed FLT3-mut AML in combination with systemic chemotherapy, and was also recently approved for the treatment of SM, mastocytosis with associated hematologic neoplasm, and mast cell leukemia (www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm555756.htm). We will review the mechanisms of action and pharmacologic properties of midostaurin, as well as clinical results of trials of midostaurin in myeloid malignancies including AML, myelodysplastic syndrome (MDS), and SM. The potential for synergy with other agents, mechanisms of resistance, and current trials of interest will also be addressed.

Midostaurin: mechanisms of action, pharmacokinetics, pharmacodynamics, and tolerability

Midostaurin is an alkaloid derived from the bacterium *Streptomyces staurosporeus*, and was initially characterized as an inhibitor of protein kinase C. Subsequent *in vitro* analyses identified inhibitory activities toward c-kit, platelet-derived growth factor receptors (PDGFRs) alpha and beta, cyclin-dependent kinase 1 (cdk1), the major vascular endothelial growth factor (VEGF) receptor KDR, src, Fgr, and spleen tyrosine kinase (Syk). Due to its broad kinase inhibition profile, and its ability to slow growth of diverse tumor xenografts in animal models, midostaurin was initially predicted to serve as a versatile anticancer agent with activity against diverse neoplasms.[24,25]

Midostaurin's FLT3-inhibitory activity was discovered in a screen for apoptosis-inducing compounds in Ba/F3 cells expressing FLT3-ITD.[26–28] Consistent with current understanding of FLT3 signaling, midostaurin treatment decreases FLT3 autophosphorylation and antagonizes downstream signaling through p38 MAPK and STAT5.[7,28–30] Subsequent studies of midostaurin in myeloid cells expressing FLT3-WT and various FLT3-TKDs (including *D835Y*) demonstrated comparable FLT3-inhibitory activity among FLT3 mutants, but tenfold lower inhibition of FLT3-WT.[31,32] Midostaurin's inhibitory properties towards FLT3 – particularly its mutant forms – make it a logical agent for investigation in FLT3-mut AML.

The importance of the activating c-kit mutation *D816V* in SM (see mastocytosis section, below) prompted further investigation into midostaurin's inhibitory activity toward wild-type and mutant c-kit. The c-kit mutation *D816V* is prevalent in SM, is considered a disease driver, and confers resistance to imatinib.[33] Midostaurin inhibits phosphorylation of c-kit *D816V* and its downstream targets STAT5 and STAT3 in Ba/F3 c-kit *D816V* cells, correlating with increased apoptosis in these cells and identifying midostaurin as a potential therapeutic agent for this rare neoplasm.[34]

Midostaurin is orally administered, with published dose ranges from 12.5 mg daily to 100 mg thrice daily; most studies report doses of 50–100 mg twice daily alone or in combination with other agents.[35] Midostaurin is generally well tolerated, with nausea and vomiting representing the most common adverse effects reported in single-agent trials, and cytopenias in combination trials.[35] Heart rate-corrected QT interval (QT_c) prolongation was not observed in healthy volunteers administered a dose of 75 mg PO twice daily for 3

days; nevertheless, concurrent use of agents with QT_c prolonging effects was avoided in many studies.[36–39] Midostaurin is not associated with significant renal or hepatic toxicities, but 8 of 63 patients in early phase trials died of pulmonary toxicity (primarily infection).[37,40,41] Adverse events documented in combination trials will be discussed in the next section.

Midostaurin is rapidly absorbed after oral administration, with C_{max} reached in 1–1.5 h.[36] In human plasma, midostaurin binds tightly to alpha-1 acidic glycoprotein (AAG), which interferes with its inhibitory activity *in vitro* and extends its terminal elimination half-life *in vivo*.[25] Midostaurin is metabolized in the liver by CYP3A4, and interactions with CYP3A4 inhibitors and inducers (e.g. ketoconazole and rifampicin) have been documented in healthy volunteers.[42] No specific dose adjustments are recommended by the manufacturer,[43] but an empiric 12.5% reduction in dose in patients taking strong CYP3A4 inhibitors was not associated with decreased efficacy in a phase II trial in which midostaurin was combined with induction chemotherapy and continued as maintenance after completion of chemotherapy or SCT (see section Role of midostaurin and other FLT3 inhibitors after allogeneic transplantation for AML).[44] In cultured CD4 cells, midostaurin reactivated latent HIV-1 expression through activation of the NFκB pathway; however, the potential for midostaurin to reactivate HIV-1 *in vivo* is not yet known.[45]

Midostaurin's two major metabolites are CGP 52421 (the 7-hydroxyl derivative) and CGP 62221 (the O-demethylated product), both of which exhibit pharmacologic activity *in vitro*. In Ba/F3 FLT3-ITD cells, CGP 52421 inhibits FLT3 signaling and induces apoptosis (activity of CGP 62221 not reported).[26] In mast cells, CGP 62221 blocks c-kit-dependent proliferation, while both midostaurin metabolites inhibit IgE-mediated histamine release, which is associated with SM symptom burden.[46] *In vivo*, CGP 62221 exhibits a markedly longer terminal elimination half-life than CGP 52421 or the parent compound (median terminal elimination half-life = 36 days), remaining detectable during treatment breaks and for days after cessation of treatment.[36] The longer $t_{1/2}$ of the GCP 62221 metabolite has been suggested to reflect AAG binding and/or CYP3A4 autoinduction, but other mechanisms are possible.[35] Ongoing studies of midostaurin's pharmacokinetics are investigating the feasibility of administering midostaurin in formulations to circumvent plasma protein binding (e.g. gold nanoparticles), and high-dose pulse dosing as an alternative to prolonged daily dosing.[47,48]

Clinical studies of midostaurin alone and in combination with chemotherapy

Single-agent studies of midostaurin have enrolled FLT3-WT and FLT3-mut patients with relapsed/refractory MDS, AML, and (in one study) MLL-rearranged ALL.[35,41,49,50] In a phase II study by Stone and colleagues, midostaurin was given at a dose of 75 mg three times daily to 20 patients with relapsed or refractory FLT3 mutated AML.[41] Transient reduction in peripheral blasts ⩾50% was observed in the majority of patients and three patients had a clearing of bone marrow blasts, one of whom achieved a 'near CR'. Two fatal treatment-related pulmonary events occurred of unclear pathophysiology. A >50% decrease in peripheral blasts was observed in 71% and 42% of FLT3-mut and wild-type AML/MDS, respectively, in a phase IIb study of midostaurin given at either 50 or 100 mg twice daily without any reported pulmonary toxicity.[49] A phase I/II study in pediatric patients observed dose-limiting grade 3 elevation in ALT at a midostaurin dose of 60 mg/m^2, but no dose-limiting toxicities at the 30 mg/m^2 dose.[50] In general, single-agent midostaurin treatment resulted in transient clearing of peripheral blasts which bridged a minority of patients to transplant, but did not produce consistent bone marrow responses. These modest responses may reflect insufficient target engagement (i.e. FLT3 inhibition), declining concentration of midostaurin with prolonged administration, a requirement for engagement of additional targets (i.e. combination therapy), or other mechanisms, such as stromal protection of leukemic cells within the bone marrow (see section Agents exhibiting additive or synergistic effects with midostaurin in FLT3-mut AML). Furthermore, the development of higher FLT3 mutant allelic ratio frequently observed at relapse may render these leukemias more resistant to multi-targeted FLT3 inhibitors such as midostaurin, and single-agent studies of more specific inhibitors are in progress.[51–53]

Phase I/II combination trials with intensive cytotoxic chemotherapy and demethylating agents in patients with both FLT3-WT and -mut AML are summarized in Table 1. A phase Ib study by

Table 1. Published phase I–II studies of midostaurin in combination with other agents in patients with FLT3-mut and FLT3-WT AML.

Reference	Phase, regimen	Population	Key clinical results	Comments
Stone and colleagues[54]	Phase Ib Daunorubicin 60 mg/m^2 IV (days 1–3), cytarabine 200 mg/m^2 continuous (days 1–7) Midostaurin 50–100 mg PO twice daily concurrently or sequentially	Untreated patients aged 18–60 years with AML FLT3-mut and FLT3-WT ($n = 69$)	Concurrent midostaurin poorly tolerated at 100 mg PO twice daily; 50 mg PO twice daily sequentially best tolerated. CR rate 92% for FLT3-mut, 74% FLT3-WT and 1 year survival equivalent	Concurrent midostaurin may increase daunorubicin concentration
Walker and colleagues[55]	Phase I Midostaurin 50 mg PO twice daily + bortezomib 1–1.3 mg/m^2 (varying frequency) alone or following mitoxantrone 4–6 mg/m^2 (days 1–6), etoposide 40–80 mg/m^2 IV (days 1–6), cytarabine 1 g/m^2 IV (days 1–6)	Relapsed/refractory AML FLT3-mutant (9 FLT3-ITD, 1 FLT3-TKD), FLT3-WT (22), and unknown FLT3 mutation status (1) ($n = 34$)	No clinical response or DLTs without MEC 56.5% CR 82.5% ORR (66.7% ORR in FLT3-ITD patients, 88.2% ORR in non-FLT3-ITD patients) 12/19 patients who achieved CR/Cri proceeded to allo-SCT Neurotoxicity, GI toxicity, and cardiotoxicity dose limiting. DLT not defined	Dual anti-tyrosine kinase activity predicted based on inhibition of tyrosine kinase genes expression from disruption of Sp1/NF-κB transactivating complex by bortezomib and midostaurin High response rate, poorly tolerated
Ramsingh and colleagues[56]	Phase I CLAG (cladribine 5 mg/m^2 IV on days 2–6, Ara-C 2 g/m^2 IV on days 2–6, GCSF 300 mcg SC on days 1–6) followed by all-transretinoic acid (15 mg/m^2) PO daily in two divided doses days 7–20 and midostaurin at 25 or 50 mg PO twice daily days 7–20	Relapsed/refractory AML FLT3-mut (5 FLT3-ITD, 1 FLT3-TKD), FLT3-WT (4), and unknown (1) FLT3 mutation status ($n = 10$)	22% CR, 11% CRi No DLTs midostaurin at 50 mg recommended dose	No major pharmacokinetic interaction between ATRA and midostaurin. Azoles not allowed
Strati and colleagues[36]	Phase I–II 5-azaciditine days 1–7 (dosed per discretion of treating investigator) followed by midostaurin at 25 or 50 mg PO twice daily for 14 days (days 8–21, or 1–28). Cycles repeated every 28 days	MDS or AML, 76% previously treated, 43% received a prior hypomethylating agent 68% FLT3-ITD (median ITD ratio 0.35), 6% FLT3-ITD + FLT3-TKD (median D835 ratio 0.46), no isolated FLT3-TKD mut ($n = 54$)	ORR 26% (33% in FLT3-mut not previously exposed to FLT inhibitors) Prior FLT3 inhibitors and HCT associated with worse response. No DLTs midostaurin 50 mg for phase II	Midostaurin and metabolite CGP 62221 reached steady state by day 15; CGP52421 continued to rise. Mean reduction in phospho-FT3 was 49% on day 15
Cooper and colleagues[35]	Phase I 5-azacitidine 75 mg/m^2 IV (days 1–7) followed by midostaurin 25, 50, or 75 mg PO twice (daily days 8–21). Cycles repeated every 28 days	Untreated, unfit, AML patients ⩾70 Relapsed AML at any age Unfit patients at any age FLT3-WT (100%) ($n = 17$)	CR in 3/12 treatment-naïve patients of 7, 12, 12 mo No DLTs Three grade 3 GI toxicities after cycle 1	MTD 75 mg twice daily. Increased trough levels of midostaurin during cycle 2 Persistent and increased levels of CGP52421 during cycle 2
Williams and colleagues[38]	Phase I Decitabine 20 mg/m^2 (days 1–5) with midostaurin 25 or 50 mg PO twice daily (days 8–21) or midostaurin 50 mg PO twice daily (days 1–28)	Untreated >60-year-old AML patients (8/16), relapsed/refractory AML (any age), (8/16) 2/16 FLT3-ITD ($n = 16$)	4/16 patients achieved CR or CRi Grade 5 pulmonary toxicity in 2/3 patients treated with concurrent decitabine and midostaurin	No change in midostaurin pharmacokinetics between sequential and concurrent dosing

AML, acute myeloid leukemia; ATRA, all-trans retinoic acid; CLAG, cladribine, cytarabine, and filgrastim; CR, complete remission; Cri, complete remission, incomplete marrow response; DLT, dose-limiting toxicity; HCT, hematopoietic cell transplant; MEC, mitoxantrone, etoposide, cytarabine; myelodysplastic syndrome (MDS), mut, mutated; ORR, overall response rate; WT, wild-type.

Stone and colleagues evaluated the combination of midostaurin, at a dose of 50 or 100 mg twice daily, given either concurrently or sequentially with standard daunorubicin and cytarabine induction in previously untreated FLT3-mut or -WT AML.[54] Maintenance therapy with midostaurin for 14 days every 28 days was allowed after completing cytarabine consolidation, but was not a focus of this study. The 100 mg dose was not tolerated due to grade 3 nausea, vomiting, and diarrhea, and the 50 mg dose was slightly better tolerated when given sequentially rather than concurrently. Most notable was the impressive CR rate of 92% in the patients with FLT3-mut AML compared to 74% in patients with FLT3-WT AML at the 50 mg twice daily dose [5/6 (83%) *versus* 13/29 (45%) at the 100 mg twice daily dose]. In this small study, OS probability at 1 and 2 years was identical in the FLT3-mut and -WT groups at 0.86 and 0.78 and 0.62 and 0.52, respectively, suggesting that the addition of midostaurin may negate the adverse prognosis of FLT3 mutation. This study formed the basis of two large phase II and III studies discussed below.

Other mechanism-based phase I trials combined midostaurin with standard salvage regimens. Walker and colleagues administered midostaurin and bortezomib either as stand-alone salvage therapy or after salvage mitoxantrone, etoposide, and cytarabine; only patients who received the latter combination demonstrated clinical responses, which occurred at the expense of increased toxicity (Table 1).[55] A sequential salvage regimen of cladribine, cytarabine, and filgastim followed by all-trans retinoic acid (ATRA) and midostaurin was generally well tolerated, with two of 11 patients demonstrating CR (complete remission) and one with a CRi (complete remission with incomplete bone marrow recovery).[56]

Preclinical data suggest that combined treatment with midostaurin and hypomethylating agents inhibits growth of FLT3-ITD cell lines and blocks phosphorylation of the downstream targets STAT5 and AKT in a synergistic fashion.[40] Three phase I/II studies of hypomethylating agents in combination with midostaurin have been published.[38,35,36] In general, the combinations were well tolerated when given in a sequential fashion. Response rates of about 30% have been observed in patients with both FLT3-mut and -WT AML, many of whom had relapsed or were refractory to prior therapies. In addition to expected hematologic toxicities, gastrointestinal toxicities appear to be increased compared to demethylating agents alone.[35] In the study by Cooper and colleagues, three patients developed grade 3 gastrointestinal toxicities after cycle 1, which included one episode of ischemic bowel, a diverticular abscess, and a small-bowel obstruction.[37] Fatal dose-limiting pulmonary toxicity was observed in 2/3 patients who received midostaurin at a dose of 50 mg twice daily concurrently with decitabine, but was not seen when the combination was given sequentially.[40]

Two large seminal studies, which have been published in abstract form, have focused on fit, untreated FLT3-mut AML patients using standard induction followed by midostaurin at a dose of 50 mg by mouth twice daily on days 8–22 of a 28-day treatment cycle.[44,57] In both studies, midostaurin maintenance for a period of 1 year was planned after either standard cytarabine consolidation in combination with midostaurin or allogeneic transplantation. In the first study, AML SG 16-10 dose reduction was required if strong CYP3A4 inhibitors, such as posaconazole, were given. A 76% CR or CRi rate was observed after one or two inductions in both older ($\geqslant$60 years) and younger ($<$60 years) patients; there was no difference in cumulative incidence of relapse according to midostaurin dose adjustment. Induction deaths were 4% *versus* 10% in younger and older patients, respectively. In correlative laboratory studies, Theis and colleagues found that among patients with FLT3-ITD mutant to wild-type allelic ratio $\geqslant$0.5, reduction of phosphorylated FLT3 (pFLT3) to $<$20% was associated with a CR rate of 100% compared to 82% CR rate in patients who did not meet this target.[58] In contrast, in patients with a low mutant to wild-type ratio, clinical response was independent of pFLT3 reduction. They also observed that pFLT3 concentrations were lowest after induction and then increased, which is concerning for the development of midostaurin resistance after repeated cycles of chemotherapy.

The second trial was RATIFY, the first phase III placebo-controlled study of an FLT3 inhibitor (midostaurin) in untreated AML patients expressing either the FLT3-ITD or -TKD mutation. A total of 717 patients aged 18–60 years participated, and patients were allowed to proceed to allogeneic transplant in first remission. Serious adverse events, including gastrointestinal and

pulmonary toxicities, were similar between treatment arms with the exception of grade 3 dermatologic toxicity, which was more common in the midostaurin arm (13% *versus* 8%, $p = 0.02$). Although the CR rate was similar in both arms at 59% (midostaurin) *versus* 54% (placebo), 5-year OS and EFS were significantly better in the midostaurin arm at 50.8% *versus* 43.1% ($p = 0.0071$) and 26.7% and 19.2 % ($p = 0.004$), respectively, representing a 23% reduced rate of death (HR 0.77). Midostaurin appeared to benefit patients in all FLT3 groups, including those with high and low FLT3 mutant allelic ratios (<0.7 *versus* $\geqslant 0.7$) and patients with FLT3-TKD.[57] This seminal study confirms that midostaurin given sequentially after standard induction chemotherapy significantly improves survival in younger patient with FLT3-mut AML.

Role of midostaurin and other FLT3 inhibitors after allogeneic transplantation for AML

Although the prognosis of patients with FLT3-mut AML is improved by allogeneic transplantation, particularly in first CR, a significant percentage of patients relapse.[59] Therefore, maintenance strategies including FLT3 inhibitors after allogeneic transplantation to prevent relapse is an area of active research. Sorafenib, which until recently was the only available multi-targeted kinase and FLT3 inhibitor, has shown encouraging results when used as maintenance after allograft.[60,61]

Preliminary information regarding safety and efficacy of midostaurin after allogeneic transplantation has been published in three recent abstracts. A high incidence of grade 3–4 cytopenia (29%) was observed in patients receiving maintenance after allograft in all trials.[42,57,62] In the RATIFY study described above, 57% of the patients received an allogeneic transplant in first CR, of whom 100 were randomized to midostaurin and 79 were randomized to placebo. The group who received midostaurin as part of induction and later maintenance after allograft had a significantly better OS compared to patients receiving placebo (70% *versus* 60%, HR 0.61).[57] In the AML SG 16-10 study, Schlenk and colleagues reported on the first 86 patients who started maintenance therapy, 61 after allogeneic transplantation and 25 after high-dose cytarabine.[44] The cumulative incidence of relapse of 20% at 1-year follow up in both groups appears to be lower than expected, suggesting a potential beneficial role for midostaurin maintenance. Randomized trials of midostaurin in the post-transplant maintenance setting will be required to confirm this possible activity.

The Radius Trial was a prospective, phase II randomized comparison of standard care *versus* midostaurin maintenance for FLT3 positive patients who underwent allogeneic transplantation beginning 29–60 days after allograft.[62] For the first 56 patients enrolled, the planned dosing of midostaurin was 50 mg twice daily. Median dose received was 76.2 mg daily; 54% of patients required dose reduction, 46% due to GI toxicities (nausea/vomiting in 64% and diarrhea in 43%). Grade 3–4 toxicities in the midostaurin arm included diarrhea, elevated ALT, and cytopenias. Interestingly, 64% of patients in the midostaurin arm and 68% of patients in the standard of care arm stopped treatment for a variety of reasons. Notably, there was no increased incidence of GVHD in the midostaurin arm. These data suggest that midostaurin may have distinct safety and toxicity profiles in patients after allograft and lower doses or alternative schedules may need to be evaluated. Furthermore, it may be useful to incorporate standardized assays to detect minimal residual disease (MRD) in FLT3-mut AML to select which patients may benefit from maintenance treatment.[63]

Upcoming investigations include a phase II extension study of post-transplant midostaurin maintenance in patients receiving midostaurin and decitabine induction treatment [ClinicalTrials.gov identifier: NCT 02723435]. Other FLT3 inhibitors are also planned to be evaluated for maintenance after allograft, including gilteritinib (BMT CTN 1506, a placebo-controlled trial following allogeneic transplantation), quizartinib [ClinicalTrials.gov identifier: NCT 02668653], and crenolanib [ClinicalTrials.gov identifier: NCT02298166].

Tumor resistance to midostaurin and to other TKIs

Multiple mechanisms of resistance to FLT3 inhibitors have been identified, including secondary FLT3 mutations, upregulation of compensatory signaling pathways, overexpression of FLT3 ligand (FL), and secretion of pro-survival cytokines from the bone marrow stroma.[6,64] Single amino-acid substitutions are frequently associated with resistance to tyrosine kinase inhibitors.[65] Secondary

Table 2. Mechanisms of resistance to midostaurin in FLT3-mut AML patients and disease models.

Reference	Resistance mechanism	Discovery	Comments
Cools and colleagues[66]	N676D, N676S, F691I, F691L, G697R, G697S	Ba/F3 cells transfected with a library of FLT3 point mutations	All mutations increased IC_{50} for midostaurin by 1.5–10-fold except G697R mutation, in which the IC_{50} could not be measured
Heidel and colleagues[67]	N676K	Clinical sample from midostaurin-resistant patient	Engineering N676K mutation into 32D FLT3-ITD cells increased IC_{50} for midostaurin and diminished midostaurin-dependent FLT3 autophosphorylation
von Bubnoff and colleagues[68]	N676D, N676I, N676S	Ba/F3 FLT3-ITD cells cultured in submicromolar concentrations of midostaurin and the mutagen N-ethyl-N-nitroso urea	Different FLT3 inhibitors resulted in distinct FLT3-mut profiles Cross-resistance between FLT3 inhibitors rare
Breitenbuecher and colleagues[70]	FLT3_ITD627E	Clinical sample from patient with primary refractoriness to midostaurin monotherapy	When integrated into 32D cells, mutation increases FLT3-GRB2 binding and upregulates MCL-1.
Stolzel and colleagues[71]	Upregulation of JAG-1, p53, MCL-1, c-kit, FL	Cytogenetic and microarray analysis of MV4-11r FLT3-ITD cells (midostaurin-resistant)	Resistant clones also exhibited alterations in chromosome 13q.
Sato and colleagues[72]	FLT3 ligand (FL)	Molm-14 cells	Micromolar concentrations of FL decrease the IC_{50} for midostaurin.
Williams and colleagues[69]	F691L, A267P	TF-1 FLT3-ITD cells cultured in the presence of FLT3 inhibitors	F691L increased IC_{50} for midostaurin fourfold and corresponded to increased phosphorylation of FLT3, STAT5, Akt, and MAPK compared to F691 WT cells A627P persistent phosphorylation of STAT5, Akt, and MAPK despite absent FLT3 phosphorylation, suggesting that the mutation either confers phosphorylation-independent activation of FLT3 or a FLT3-independent resistance mechanism

FLT3 point mutations that confer resistance to midostaurin have been identified in primary patient samples as well as in FLT3-ITD cells cultured in the presence of midostaurin (Table 2), among which point mutations in N676 are among the most frequently reported.[66–69] Asparagine 676 is located in the TKD, and although molecular modeling studies suggest that it does not contact FLT3 inhibitors directly, it may play a structural role stabilizing the hinge region of the receptor.[66] Interestingly, mutation at *N676* does not appear to be associated with exposure to other FLT3 inhibitors, and some *N676* mutations retain sensitivity to other TKIs.[68]

Additional FLT3 point mutations identified in *in vitro* screening experiments include *A627P, A627T, F691L, F691I, G697R*, and *G697S*, with variable effects on the IC_{50} for midostaurin but essentially consistent inhibition of downstream signaling pathways (Table 2). An atypical mutation (located in the beta-sheet rather than the juxtamembrane domain of the ITD) has been characterized in which the resistance mechanism was not related to maintenance of FLT3 phosphorylation. Rather, an increased binding of GRB-2 to the mutant receptor was observed, in turn upregulating the anti-apoptotic myeloid cell leukemia 1 protein (MCL-1). Other signaling pathways that may contribute to midostaurin resistance include JAG-1, p53, MCL-1, c-kit, and FL.[71] The latter observation is of particular clinical relevance, as increased levels of FL have been identified in refractory AML patients treated with the FLT3 inhibitor lestaurtinib, and FL diminishes the efficacy of midostaurin *in vitro*.[72]

In some cases, combinations of midostaurin with other agents (see synergy section, below) overcome resistance to midostaurin monotherapy. In

other cases, midostaurin monotherapy may 'rescue' cells which have developed resistance to other FLT3 inhibitors (Table 2); in fact, cross-resistance is not common among FLT3 inhibitors.[68] As FLT3 inhibitor use increases, understanding of resistance mechanisms will become increasingly important in the prevention and treatment of resistant disease. Although no current recommendations exist regarding FLT3 inhibitor-resistant disease, prevention strategies may include simultaneous treatment with multiple TKIs to maximize inhibition of compensatory pathways, and swift referral to transplant for eligible patients. In the future, FLT3 gene sequencing may become informative in treatment decisions in midostaurin-resistant patients. Given the variation in IC_{50} for midostaurin among FLT3 mutations, some patients could respond to increased doses of midostaurin, whereas others would require transition to an alternative agent.

Clinical activity of midostaurin in systemic mastocytosis

Given the generally favorable prognosis of indolent mastocytosis, most clinical studies have focused on patients with advanced SM which is defined by three main syndromes including aggressive SM (ASM), SM with associated hematologic neoplasm (ASM-AHN), and mast cell leukemia (MCL). Affected subjects have median survivals of 3.5 years, 2 years, and <6 months, respectively, and no standard treatment exists.[20,21]

As a single agent, midostaurin has shown significant activity in improving symptoms and survival in advanced mastocytosis conditions. Clinical response has been defined as improvement in mastocytosis- related symptoms in at least one organ.[73] In a small study, the French National Reference Center for mastocytosis (CEREMAST) reported a response rate of 71% within 3 months of initiating midostaurin in 28 advanced mastocytosis patients (4 ASM, 18 SM and ASM-AHN, 3 MCL). Median response duration was 17 (5–32) months and OS was 43% at a median follow up of 18.5 months.[74] Gotlib and colleagues recently published the results of an international study in a large cohort of 116 patients with advanced SM (89 evaluable for efficacy) in which midostaurin was given 100 mg orally twice daily. Median duration of treatment was 11 months, indicating that midostaurin was relatively well tolerated. However, grade 3–4 cytopenias were observed in about one-third of patients. Overall response rate was 60% in evaluable patients and was 75% among patients with ASM, 58% among patients with ASM-AHN, and 50% in MCL. Median duration of response was not reached in patients with ASM or MCL. Median survival was 28.7 months in the entire population, was not reached in ASM, and was 20.7 months in ASM-AHN and 9.4 months in MCL. Most impressive was that four of eight responding MCL patients had ongoing responses at 8, 19, 33, and 49 months. Although midostaurin is not curative in the vast majority of patients with advanced mastocytosis, these and other studies have established its role as a significant treatment advance.

Genetic studies in systemic mastocytosis

Genetic aberrations are frequently seen in ASM, may have an adverse prognostic significance, and may predict response to midostaurin. Using next-generation sequencing (NGS), Schwaab and colleagues found additional molecular aberrations in 24/27 patients with advanced SM (5/5 with ASM-AHM and 19/22 with ASM/MCL).[75] The majority of these patients carried $\geqslant 3$ mutations of which the most commonly affected genes were *TET2, SRSF2, ASXL1, CBL*, and *RUNX1*. OS was significantly shortened in patients with additional mutational abnormalities compared to *KIT-D816V* alone. Jawhar and colleagues used the German Mastocytosis Registry to evaluate the impact of additional molecular markers and reduction of *KIT-D816V* allele burden on the outcome of 38 patients treated with midostaurin.[76] They found a significantly inferior response rate and survival in the patients with additional mutations in *SRSF2, ASXL1*, and *RUNX1* (S/A/R positive). Five of the six patients who progressed on midostaurin within the first 6 months were S/A/R positive. In multivariate analysis, S/A/R negative at time of diagnosis and $>25\%$ reduction in *KITD-816V* allele burden at 6 months were the most important factors associated with survival after midostaurin treatment. This same group evaluated 28 patients with primary or secondary MCL who had received midostaurin therapy during their disease course. They found a high prevalence of S/A/R mutations which, in multivariate analysis, were the most significant prognostic factors for predicting adverse survival.[77] These data suggest that NGS and monitoring of *KIT-D816V* allele burden may be useful in selecting treatment.

Table 3. Mechanisms of pharmacologic additivity and synergy with midostaurin in acute leukemia.

Reference	Combination agent(s)	Pathway(s) targeted	Comments
Nelson and colleagues[78]	Pimozide	STAT5	Combination poorly tolerated in animal model
Ahmad and colleagues[79]	CDDO-Me	STAT, NFκB	Phase I trial results pending
Mohi and colleagues[80]	UO126, rapamycin	MEK, mTOR	Phase I trial with everolimus complete[81]
Weisberg and colleagues[82]	Dasatinib, KIN112, KIN113	JAK	Synergy dependent upon presence of stroma-conditioned media (SCM)
Weisberg and colleagues[83]	Dasatinib, KIN112, KIN113, KIN001-102, MK2206, AT7867, GSK690693	Akt, JAK/STAT, Ras/Raf/MEK/ERK	Synergistic MAPK inhibition was less with SCM compared to stromal cell co-culture
Heidel and colleagues[84]	Bis (1H-indol-2-yl)methanones	STAT5, Akt, Ras/Raf/MEK/ERK	No reported clinical studies to date
Weisberg and colleagues[85]	NVP-AST487	STAT5	No reported clinical studies to date
Williams and colleagues[40]	Decitabine	Akt, STAT5	Phase I study complete, phase II study pending
Chi and colleagues[86]	All-trans retinoic acid (ATRA)	No specific pathway identified	Phase I trial of CLAG followed by ATRA and midostaurin at 25 and 50 mg twice daily published previously[56]
Edwards and colleagues[87]	CPX-351	No specific pathway identified	No reported clinical studies to date

Agents exhibiting additive or synergistic effects with midostaurin in FLT3-mut AML

Activated FLT3 signals through multiple pathways to promote survival of leukemic cells *in vitro*, including PI3K/Akt/mTOR, Ras/Raf/MEK/ERK, and STAT5a.[7,29,30] Strategies to boost midostaurin efficacy, minimize side effects, and prevent resistance have included combining this drug with agents that target common pathways with the goal of identifying synergistic relationships (Table 3; Figure 1).

Nelson and colleagues explored the synergistic relationship between midostaurin and pimozide, a STAT5 inhibitor that does not affect FLT3 activity.[78] They employed Ba/F3 FLT3-ITD and MV411 cells (all of which contain an endogenous FLT3-ITD mutation). Both agents decreased viability of each cell line independently, and the combination was synergistic across multiple drug concentrations, with combination index (CI) values ranging from 0.37 to 1.0. Although each agent exhibited anti-leukemic activity in mice injected intravenously with Ba/F3 FLT3-ITD cells, combination treatment was poorly tolerated. This study established the therapeutic potential for combining FLT3 and STAT5 inhibitors in FLT3-mut AML.[78]

Ahmad and colleagues investigated the synergistic potential of midostaurin in combination with the oleanolic acid derivative CDDO-Me, which has pleiotropic effects on myeloid cells including inhibition of STAT signaling and nuclear targeting of NFκB.[79] Midostaurin and CDDO-Me synergistically inhibited growth of Ba/F3 FLT3-ITD, Mv4-11, and MOLM-14 cells (all of which contain the FLT3-ITD mutation). They identified a CI of 0.56 at nanomolar concentrations of midostaurin and submicromolar concentrations of CDDO-Me. The experiments used primary AML cells isolated from three patients (one with FLT3-ITD mutation and the others FLT3-WT) and exhibited additive effects (only one concentration of each was reported so synergy could not be assessed).[79] A phase I trial of CDDO-Me in patients with advanced solid tumors or lymphoid malignancies has been completed but the results have not been reported [ClinicalTrials.gov identifier: NCT00529438].

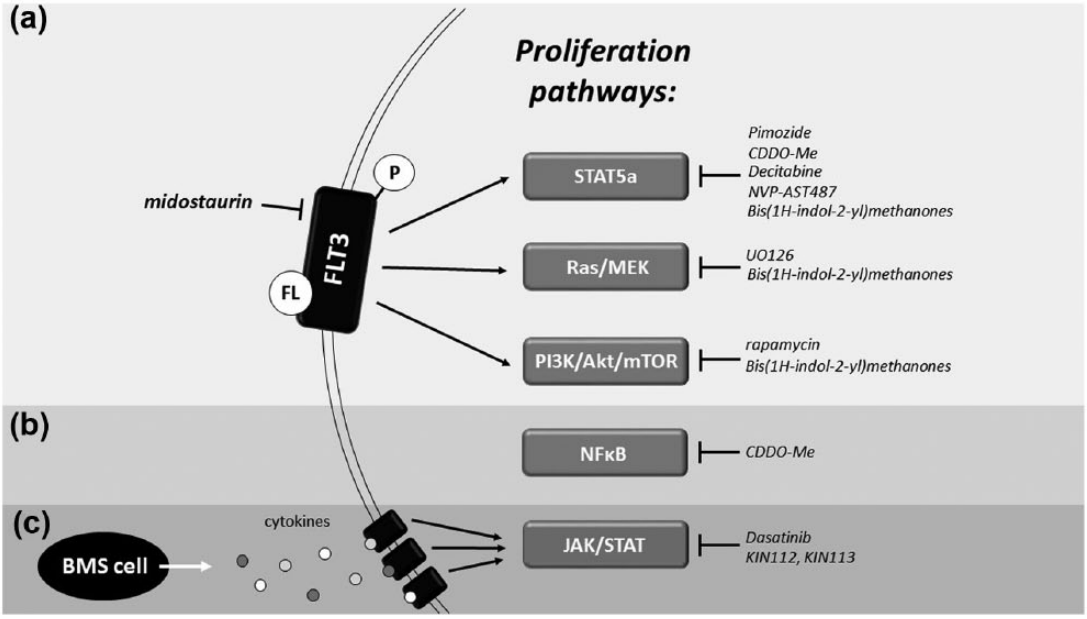

Figure 1. Agents with potential synergistic activity with midostaurin in AML. Agents with additive and/or synergistic potential when combined with midostaurin are illustrated alongside their respective target pathways. (a) STAT5a, Ras/MEK, and PI3K/Akt/mTOR are established FLT3-dependent signaling pathways, and may be inhibited further by the agents listed. (b) CDDO-Me targets multiple pathways, including NFκB which is not an established FLT3-dependent signaling pathway. (c) Activation of the JAK/STAT pathway by cytokines released from bone marrow stromal cells is hypothesized to overcome FLT3 inhibition within the bone marrow environment. Individual agents are discussed in detail in the section Agents exhibiting additive or synergistic effects with midostaurin in FLT3-mut AML.
FLT3-mut (FLT3-ITD and FLT3-TKD) are constitutively active; FLT3-WT requires FL stimulation.
FL, FLT3 ligand; BMS, bone marrow stromal.

The combinatorial effects of midostaurin with the MEK inhibitor UO126 and rapamycin (an inhibitor of mTOR, a downstream target of Akt) were investigated in Ba/F3 cell lines expressing FLT3-ITD. The combination of nanomolar concentrations of midostaurin with micromolar concentrations UO126 resulted in an additive inhibition of cell proliferation, which was further enhanced with the addition of rapamycin. The two-drug combination of midostaurin and rapamycin exhibited synergistic inhibition of proliferation at nanomolar concentrations, and overcame resistance to midostaurin monotherapy in the presence of the FLT3-ITD *F691I* mutation.[80] A phase I study of midostaurin and the mTOR inhibitor everolimus was conducted in patients with relapsed/refractory AML, newly diagnosed AML who were not candidates for cytotoxic chemotherapy, and CMML; disease biology included FLT3-WT, -ITD, and -TKD.[81] Only 1 of 29 patients achieved CR and this subject went on to receive a hematopoietic cell transplant (HCT); 3 patients achieved a significant reduction in blast count, 8 had stable disease, but 17 exhibited disease progression. Pharmacodynamic correlatives have not yet been reported.

Weisberg and colleagues investigated the role of bone marrow stromal cells in modulating the sensitivity of leukemic cells to midostaurin, and identified combination treatment strategies to maximize FLT3 inhibition in the setting of stromal cell-secreted cytokines.[82,83] Increasing evidence points to a cytoprotective role of bone marrow stromal cells toward leukemic cells, which is thought to be mediated by stromal-secreted cytokines that signal through pathways common to FLT3, including STAT, Akt, and MAPK. This group studied 3 JAK/STAT inhibitory agents (dasatinib, KIN112, and KIN113) to screen for compounds which could synergize with midostaurin. They studied growth inhibition of MOLM-13 cells cultured in stromal-cell conditioned medium (SCM) and noted greater inhibition of phosphorylated Akt and STAT5 than either midostaurin or TKI alone. Combination

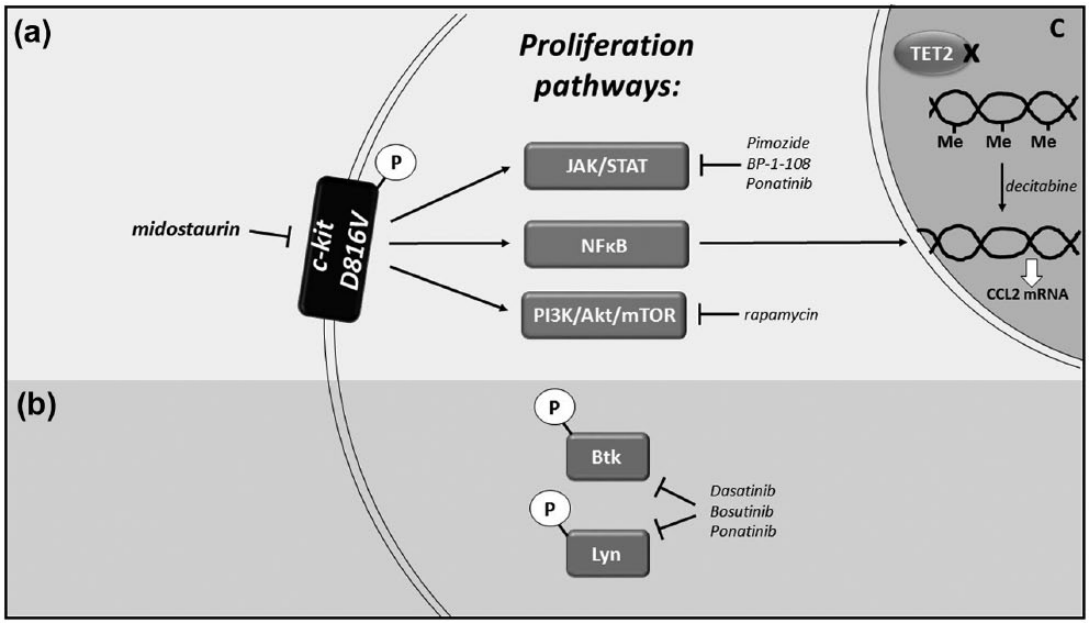

Figure 2. Agents with potential synergistic activity with midostaurin in systemic mastocytosis. Agents with additive and/or synergistic potential when combined with midostaurin are illustrated alongside their respective target pathways. (a) JAK/STAT and PI3K/Akt/mTOR are established c-kit-dependent signaling pathways, with the potential for inhibition by the agents listed. NFκB activation promotes transcription of CCL2, which promotes vascularization and fibrosis of the tumor microenvironment. (b) Phosphorylated Lyn and Btk (P = phosphorylated) are activated independently of c-kit in advanced systemic mastocytosis, and are inhibited by the agents illustrated. (c) Independent mutation of TET2 (mutation indicated with an X) leads to DNA hypermethylation (Me = methyl), which may be reversed with decitabine. Individual agents are discussed in detail in the section entitled Agents exhibiting additive or synergistic effects with midostaurin in advanced mastocytosis.

therapy also was additive to synergistic in samples from relapsed/refractory AML patients cultured in SCM. Importantly, synergy between midostaurin and these TKIs was dependent upon the presence of SCM, suggesting that inhibition of cytokine-triggered signals within leukemic cells contributes to the efficacy of cytotoxic therapy in FLT3-mut AML.[82]

Selective inhibitors of Akt and p38 MAPK also synergized with midostaurin in FLT3-ITD leukemic cells co-cultured with stromal cells or SCM.[83] These observations may reflect the proposed roles for Akt- and MAPK-mediated signaling in stromal cytoprotection, or can be explained by compensatory upregulation of Akt and MAPK pathways in the setting of FLT3 inhibition. Midostaurin's synergy with MAPK inhibitors was lessened in the presence of SCM compared to stromal-cell co-culture, suggesting that it may not be as potent in the presence of high concentrations of cytokines; Akt may be a preferable target for combination treatment *in vivo*.

Agents exhibiting additive or synergistic effects with midostaurin in advanced mastocytosis

Synergistic combinations with midostaurin have also been investigated in models of mast cell neoplasms (Figure 2). Gleixner and associates investigated the potential of midostaurin to synergize with other TKIs to target Lyn and Btk, two *KIT-D816V*-independent signaling molecules which contribute to malignant growth in mast cells and are activated in patients with advanced mastocytosis.[88] In combination with midostaurin, these tyrosine kinase synergistically contribute to apoptosis in HMC-1 cells expressing c-kit with *V560G* ± *D816V* mutation, as well as in primary neoplastic MC cells. In addition, increased activation of c-kit-dependent pathways after exposure to midostaurin likely contributes to TKI resistance; thus, drugs that target these pathways may delay or prevent as well as demonstrate synergy. A list of other molecular and stromal factors which are potential targets in advanced mast cell neoplasms are shown in Table 4. Understanding overlapping

Table 4. Signaling pathways important in mast cell neoplasms with potential for synergy with midostaurin.

Target	Mechanism of resistance	Synergistic drug combination
KIT-dependent pathways[21,89] JAK2/STAT 1/5 PI3K/AKT/mTOR RAS/RAF/MEK	AKT and STAT5 become activated during disease progression, possibly driving oncogenesis	*STAT5 inhibitors*: Pimozide BP-1-108 Ponatinib *AKT inhibitors*: Various in development *mTOR pathway inhibitors*: Rapamycin
TET2[90]	Coexistence of *TET2* mutation with *KIT-D816V* associated with more aggressive disease	Combination of decitabine and midostaurin was effective in mast cell lines with *KIT-D816V* and *TET2* mutations
KIT-D816V-independent oncogenic signaling[88,91,92] Lyn Btk	Expressed in activated form in neoplastic mast cells	Dasatinib blocks KIT, Lyn and Btk activation; Bosutinib deactivates Lyn and BTk without blocking KIT in mast cells; Ponatinib causes dephosphorylation of KIT, Lyn and STAT5 in HMC-1 cells All above tyrosine kinases synergistic with midostaurin *in vitro*
Stromal factor: CCL2[93]	CCL2 is a *KIT-D816V*-dependent modulator of the BM microenvironment, regulates vascular cell migration and angiogenesis. *KIT-D816V* promotes expression of CCL2 through activation of NFκB in mast cells	Midostaurin reduces expression of CCL2 Possible mechanism of cross-talk with NFκB pathways in mast cell neoplasms

BM, bone marrow.

and distinct pathways downstream of *KIT-D816V* may provide insight to development of drug combinations with synergistic and potentially curative potential.

In summary, synergistic relationships have been reported between midostaurin and agents which target shared (e.g. Akt, MEK, STAT) and distinct signaling pathways in both leukemia and mastocytosis. In light of the broad specificity of midostaurin, it is possible that some synergistic relationships may be related to activities distinct from FLT3 inhibition. Testing this hypothesis, however, was beyond the scope of the studies reviewed. The role of combination therapy outside of induction chemotherapy for AML (see RATIFY trial, Combination trial section) has not yet been established, but may have particular importance in the prevention or treatment of disease which has acquired resistance to midostaurin monotherapy. To date, the combination regimen most amenable to further clinical investigation in the setting of relapsed/refractory AML may be midostaurin plus a JAK/STAT inhibitor, given the agents' availability, generally good patient tolerance, and rigorous supporting preclinical data.

Conclusion

Midostaurin, as well as other FLT3 inhibitors currently under development,[51–53] represent a major therapeutic advance for the management of patients who harbor the highly aggressive subtype of FLT3-ITD AML. Based on results of the RATIFY trial, midostaurin became the first FLT3 inhibitor to receive breakthrough therapy designation from the FDA for induction therapy for newly diagnosed FLT3-mut AML, and has since been approved in combination with induction chemotherapy for this indication (www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm555756.htm). Furthermore, preliminary data

suggest that maintenance treatment with midostaurin after either standard dose consolidation or allogeneic HCT could be considered to prevent disease recurrence.[42,57] The results of clinical studies of midostaurin in combination with demethylating agents for unfit, previously untreated FLT3-mut AML patients are eagerly awaited.

The activity of midostaurin in combination with induction chemotherapy in newly diagnosed FLT3-mut AML may reflect inhibition not only of FLT3, but also of other kinases important in AML, such as PDGFRs, c-kit, VEGF receptor, and protein kinase C.[24] As in the case of imatinib for CML, more specific, second-generation FLT3-targeted agents may become important additions to the therapeutic armamentarium for this aggressive disease, so that treatment may eventually become tailored based on prior exposure to FLT3 agents, FLT3 mutant to wild-type allelic ratio, patient and toxicity profiles, and secondary tyrosine kinase resistance mutations. Standardization of methods used to detect FLT3 mutations (both primary and secondary), measure MRD, and monitor FLT3 mutant allelic ratio will no doubt facilitate such efforts. In upcoming studies of novel FLT3 inhibitors in combination with cytotoxic chemotherapy, it may be prudent to include midostaurin rather than a placebo as the comparator arm. Moreover, pharmacodynamic monitoring of FLT3 inhibition during treatment may be predictive of response and allow opportunity for early treatment modification to optimize efficacy.[58,94]

Due to its broad inhibition profile of oncogenic kinases, midostaurin has potential activity in a variety of myeloid neoplasms, including advanced mastocytosis, a disease driven by the activating c-kit mutation *D816V*. Based upon its promising efficacy in recent trials, midostaurin has recently been approved by the FDA for this condition and will no doubt remain an important therapeutic advance for this malignancy, particularly if safe and effective synergistic combinations can be developed. We await with interest clinical studies in other hematologic malignancies based on midostaurin's ability to target diverse oncogenic kinases with good tolerability and compatibility with multiple other therapeutic agents.[95,96]

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Conflict of interest statement

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