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FLT3 Inhibitors in AML: Are We There Yet?

Akshay Sudhindra · Catherine Choy Smith

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Abstract FMS-like tyrosine kinase 3 (*FLT3*) is the most frequently mutated gene in AML. Thirty percent of patients with acute myeloid leukemia (AML) harbor activating mutations in FLT3, either internal tandem duplication mutations in the juxtamembrane domain (FLT3-ITD) or point mutations in the tyrosine kinase domain (FLT3 TKD). Small molecule FLT3 inhibitors have emerged as an attractive therapeutic option in patients with FLT3 mutations; however, the clinical activity of early inhibitors was limited by a lack of selectivity, potency and unfavorable pharmacokinetic properties. Newer agents such as quizartinib have improved potency and selectivity associated with much higher bone marrow response rates; however, response duration is limited by the development of secondary resistance. We will review here a number of FLT3 inhibitors that have been evaluated in clinical trials and discuss challenges facing the use of these agents in AML.

Keywords AML · FLT3-ITD · FLT3 mutation · FLT3 inhibitor · Midostaurin · Lestaurtinib · Sunitinib · Sorafenib · Quizartinib · PLX3397 · Ponatinib · Resistance

Introduction

FMS-like tyrosine kinase 3 (*FLT3*) is the most commonly mutated gene in acute myeloid leukemia (AML). About 30 % of AML patients harbor a *FLT3* mutation [1••], either in the form of an in-frame internal tandem duplication, usually in the juxtamembrane (JM) domain of FLT3 (*FLT3-ITD*), or as a point mutation in the tyrosine kinase domain (*FLT3* TKD). While the clinical significance of *FLT3* TKD mutations in AML remains controversial [2, 3], patients with *FLT3-ITD* mutant AML have aggressive disease characterized by early relapse and decreased survival [2, 4–6] compared to those with *FLT3* wild type (*FLT3* WT) AML. Studies suggest that use of allogeneic stem cell transplant in first remission can improve survival [7, 8, 9•, 10•, 11•]; however, a large number of patients are either not suitable candidates for this procedure or relapse prior to transplant. There has been significant interest in targeting the FLT3 receptor tyrosine kinase with small molecule inhibitors for many years. Unfortunately, first-generation FLT3 inhibitors were hindered by lack of selectivity and potency, resulting in unimpressive clinical activity as monotherapy. Preclinically, quizartinib (AC220), a second-generation FLT3 tyrosine kinase inhibitor (TKI), has been shown to be a highly selective inhibitor of FLT3 signaling for both FLT3-ITD mutant and wild type receptors [12]. Clinically, as a single agent it has achieved high response rates in patients with relapsed or refractory AML and has rekindled enthusiasm for FLT3 inhibition as a therapeutic approach in patients with *FLT3-ITD* mutant AML [13••, 14••, 15••], though response duration has been limited by the development of secondary resistance-conferring kinase domain (KD) mutations [16••]. While the early clinical success of quizartinib in AML is promising, the optimal strategy for the use of FLT3 inhibitors in AML remains unclear. Here we will review a number of FLT3 inhibitors that have been evaluated or are undergoing evaluation in clinical trials and discuss challenges underlying use of these agents (Tables 1, 2 and 3).

FLT3 Function and Signaling

FLT3 is a receptor tyrosine kinase that is normally expressed on immature hematopoietic cells and functions in the

A. Sudhindra · C. C. Smith (✉)
University of California, San Francisco, 505 Parnassus Ave, Box 1270, San Francisco, CA 94143, USA
e-mail: csmith@medicine.ucsf.edu

A. Sudhindra
e-mail: Akshay.Sudhindra@ucsf.edu

Table 1 Comparison of properties of FLT3 inhibitors tested in AML clinical trials

Inhibitor	Type I or Type II	Relevant Kinase Targets	Potency (In Cell-Based Assays) * least potent *** most potent	FLT3 Kinase Domain Mutations Reported to Cause Resistance
Crenolanib	Type I	PDGFR, FLT3, CSF1R	**	F691L, D698N, Y693C [69•]
KW2449	Type I	Multikinase Inhibitor (including FLT3, ABL, Aurora A)	**	A627P, F691L, Y842C [75]
Lestaurtinib	Type I	Multikinase Inhibitor(including FLT3, TrkA/B/C, JAK2)	**	A627P [75]
Midostaurin	Type I	Multikinase Inhibitor (including FLT3, KIT)	**	N676K[§]/S/D, A627T/P, F691I/L, F691I, G697R/S [75, 76, 77]
PLX3397	Type II	FMS, KIT, FLT3	*	Multiple mutations in activation loop residues (D835[§], D839, N841, Y842, etc.) [69•]
Ponatinib	Type II	Multikinase Inhibitor (including FLT3, ABL)	**	D835V/Y/F/H, Y842C/H [68•]
Quizartinib	Type II	FLT3, RET, KIT, PDGFRA/B, CSF1R	***	D835V[§]/Y[§]/F[§], F691I/L[§], Y842C/H [16••, 68•]
Sorafenib	Type II	FLT3, RET, KIT, CSF1R, PDGFRA/B, VEGFR2	***	D835V/Y[§]/F/H[§], F691I/L[§], Y842C/H, A848P[§], A627P [16••, 65••, 68•, 75, 78]
Sunitinib	Type I	Multikinase Inhibitor (including KIT, VEGFR2, FLT3)	**	A848P[§], F691L, A627P, Y842C [75, 78]
Tandutinib	Undetermined	FLT3, PDGFRA/B, KIT, CSF1R	*	D835V/Y [79]

[§] identified in patients at time of relapse after initial response to inhibitor

development of both stem cells and the immune system [17]. Murine models have shown that the targeted disruption of *Flt3* results in adult mice with deficiencies in primitive B lymphoid progenitor cells. Additionally, stem cells lacking *Flt3* have a decreased ability to reconstitute T-cells and cells of the myeloid lineage [18]. The wild type FLT3 receptor is a monomeric transmembrane protein, which contains five extracellular immunoglobulin-like domains, a transmembrane domain, a JM domain and a split intracellular kinase domain. FLT3 is activated when its ligand, FLT3 ligand (FL) binds to the extracellular domain promoting dimerization [19]. Subsequent phosphorylation of the kinase activates downstream signaling pathways, including Ras/MAPK, PI3K/Akt and STAT5 signaling.

FLT3 is the most frequently mutated gene in AML, with an estimated 30 % of AML patients harboring *FLT3* mutations [1••, 20]. Approximately 20–25 % of AML patients harbor FLT3-ITD [21] mutations, which usually occur as in-frame duplications involving the FLT3 JM domain. Normally the JM domain exerts a negative regulatory function over the kinase domain [22]; however, with FLT3-ITD mutations this autoregulatory function is lost resulting in constitutive kinase activation. The added length conferred by the ITD mutation places tyrosines 589 and 591 in contact with aspartic acid 829 in the N-terminal of the activation loop, ultimately catalyzing phosphate transfer [23]. Recently, it was shown that about one-third of FLT3-ITD mutations are a result of an insertion outside of the JM domain, within the β1 sheet or the β2 sheet of the tyrosine kinase domain 1 (TKD1) [24, 25, 26•]. Another 5–7 % of AML patients have point mutations in the *FLT3* kinase domain (*FLT3* TKD) which result in single amino acid substitutions, usually within the activation loop of the FLT3 kinase domain at aspartate 835 [27, 28] and less commonly at tyrosine 842 or isoleucine 836 [28]. These FLT3 TKD mutations cause constitutive kinase activation by favoring the active kinase conformation. Recently, activating mutations at the TKD1 residue asparagine 676 have also been identified [29•].

Though both FLT3-ITD and FLT3 TKD mutations cause ligand-independent kinase activation, in vitro studies have identified differential autophosphorylation [30] and downstream signaling patterns for FLT3-ITD [31] compared to FLT3 TKD and native FLT3, particularly preferential activation of STAT5 [32] by FLT3-ITD, as well as increased proliferation and clonogenic growth potential [32]. These signaling differences may in part be the result of aberrant trafficking of FLT3-ITD mutant receptors resulting in prolonged retention in the endoplasmic reticulum and increased exposure to intracellular substrates such as STAT5 [33]. In a murine bone marrow transduction and transplantation model, FLT3 D835Y yields an oligoclonal lymphoid disorder with longer disease latency distinct from the myeloproliferative neoplasm (MPN) observed with FLT3-ITD [34]. Recently, Bailey et al. confirmed

Table 2 Selected trials of type I FLT3 inhibitors

Inhibitor	Phase of Trial [reference]	Patient Population	Dosing	Response	Notes
Midostaurin	Ph II [42]	*n*=20; R/R AML or advanced MDS	PO, single agent, 75 mg 3× daily	PB Blasts <50 %: 70 % of patients BM Blasts <50 %: 30 % of patients	
	Ph IIb [43]	*n*=95; R/R AML or newly diagnosed unfit for intensive therapy; high risk MDS	PO, single agent, randomized to 50 or 100 mg, 2× daily	PB or BM blasts <50 %: 71 % in FLT3 mutated patients (*n*=35) 41 % in FLT3 WT patients (*n*=60)	
	Ph Ib [44]	*n*=69; Newly diagnosed AML age 18–60 years	PO, 50 or 100 mg 2× daily (concomitantly D1-7 and 15–21 or sequentially D 8–21) with chemotherapy	FLT WT (*n*=50): CR 74 %, 1 year OS 78 %, 2 year OS 52 %; FLT3 mutated (*n*=19): CR 92 %, 1 year OS 85 %, 2 year OS 62 %	Chemotherapy: Induction: Daunorubicin 60 mg/m^2 IV D1-3 and Cytarabine 200 mg/m^2 CIVI on D1-7; Consolidation: Cytarabine 3 g/m^2 IV q12 h (D1,3,5) for three cycles
Lestaurtinib	Ph I/II [45]	*n*=17; R/R AML age 18–74 years	PO single agent; 40 mg 2× daily q28 days with dose escalation to 80 mg 2× daily	6 % CRi; 24 % PB blast clearance	Response duration 2–3 weeks
	Ph III [39•]	*n*=224; FLT3 mutant AML in first relapse; age 20–81 years	Randomized with (*n*=112) or without (*n*=112) Lestaurtinib 80 mg 2× daily with chemotherapy	Chemotherapy + Lestaurtinib: CR 26 %; Chemotherapy alone: CR 21 %; no difference in OS	Chemotherapy: Mitoxantrone 8 mg/m^2, etoposide 100 mg/m^2, Cytarabine 1 g/m^2 D1-5 if CR1 <6 months; Cytarabine 1.5 g/m^2 D1-5 if CR1 6–24 months
Sunitinib	Ph I [50]	*n*=15; R/R AML unfit for chemotherapy	PO, single agent; 50–75 mg daily	PR or antileukemic effect seen in 100 % of FLT3 mutated patients (*n*=4) and 20 % of WT patients (*n*=10)	Response duration of 4–16 weeks
	Ph I/II [51]	*n*=22; newly diagnosed AML fit for intensive therapy, age 60–78 years	PO; 25 mg in combination with chemotherapy	CR 59 % (ITD: *n*=15; 53 %; TKD: *n*=7; 71 %) median OS: 18.8 months	Chemotherapy: Induction: Cytarabine 100 mg/m^2 D1-7; Daunorubicin 60 mg/m^2 D1-3; Consolidation: Cytarabine1 g/m^2 IV BID D1,3,5 three cycles

R/R relapsed/refractory, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *ITD* internal tandem duplication, *WT* wild type, *PO* per orem, *PB* peripheral blood, *BM* bone marrow, *RR* response rate, *CR* complete response, *CRi* complete response with incomplete count recovery, *PR* partial response, *OS* overall survival, *HSCT* hematopoetic stem cell transplant, *CIVI* continuous intravenous infusion

Table 3 Selected trials of type II FLT3 inhibitors

Inhibitor	Phase of Trial [reference]	Patient Population	Dosing	Response	Notes
Quizartinib	Ph I [60•]	*n*=76; R/R AML; age 23–86 years	PO, single agent, dose escalation; 12–450 mg/day	RR: 30 %; CR 13 %; PR 17 % FLT3-ITD mutant (*n*=17) RR 53 %; FLT3 WT (*n*=37) RR 14 %; FLT3 indeterminate (*n*=22) RR 41 %; median duration 13.3 months	MTD : 200 mg/day, DLT: grade 3 QT prolongation, responses at dose of 18 mg/day;
	Ph II [14••]	Cohort I, *n*=133, first relapse within 1 year or refractory to first line of therapy; ≥ 60 years	Oral solution, single agent, 135 mg daily for men; 90 mg daily for women	CRc: FLT3-ITD (*n*=100): 46 %; FLT3 WT (*n*=38) 32 %; median response duration: FLT3-ITD 12.1 weeks, FLT3 WT: 22.1 weeks	Secondary Kinase Domain mutations in activation loop D835 and gatekeeper F691 confer resistance [16••]
	Ph II [15••]	Cohort II: *n*=138, ≥ 18 years, relapsed after or refractory to second line of treatment or HSCT	Oral solution, single agent, 135 mg daily for men; 90 mg daily for women	CRc: FLT3-ITD (*n*=90): 54 %; FLT3 WT (*n*=42) 31 %; median response duration: FLT3-ITD 12.1 weeks, FLT3 WT: 7.0 weeks; 37 % of patients eventually taken to transplant	
Ponatinib	Ph I [67•]	*n*=12, R/R AML, age 30–72 years	PO, single agent, 45 mg daily	FLT3-ITD mutant patients (*n*=7): 29 % patients with clearance of BM blasts	Preclinical efficacy against quizartinib resistant FLT3 gatekeeper F691L mutations [68•]
Sorafenib	Ph I [52]	*n*=16; R/R AML, age 48–81 years	PO, single agent, dose escalation, 200–400 mg 2× daily	PB and BM decrease: 100 % ITD mutant patients (*n*=6) and 43 % WT patients (*n*=7)	No responses in patients with TKD mutations (*n*=3)
	Retrospective review [53••]	*n*=65; R/R AML; two cohorts: (a) *n*=29 after allogeneic HSCT; (b) *n*=36 after intensive chemotherapy	PO, single agent, 400 mg 2× daily	PB remission 37 %; CRi 8 %; CR 23 %, molecular remission 15 %	All patients with FLT3-ITD mutations
	Ph II [58••]	*n*=43 (37 evaluable for response), R/R AML, age 24–87 years; 40 patients with FLT3-ITD mutation	PO, Sorafenib 400 mg 2× daily, 5 Azacitadine 75 mg/m^2 IV daily D1-7	RR 46 %; CR 27 %; CRi 16 %; PR 3 %; median cycles to CR/CRi=2, median duration CR/CRi=2.3 months	64 % achieved 85 % inhibition of FLT3 during cycle 1, degree of FLT3 inhibition correlated with plasma sorafenib concentrations

R/R relapsed/refractory, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *ITD* internal tandem duplication, *WT* wild type, *PO* = per orem, *PB* peripheral blood, *BM* bone marrow, *RR* response rate, *CR* complete response, *CRi* complete response with incomplete count recovery, *CRc* composite complete response, *PR* partial response, *OS* overall survival

in a knock-in mouse model where *Flt3* is expressed under control of the endogenous promoter that disease induced by FLT3 D835Y is phenotypically distinct from disease induced by FLT3-ITD. In contrast to FLT3-ITD mice, which exclusively develop MPN [35], FLT3 D835Y mice develop a MPN with longer latency and broader range of disease phenotypes, including extranodal B-cell masses and T-cell rich B-cell lymphoma, suggesting an increased permissiveness of FLT3 D835Y for lymphoid neoplasms.

FLT3 Mutations in AML: Clinical Features, Management and Outcomes with Standard Approaches

The presence of a *FLT3-ITD* mutation in young patients with normal cytogenetic AML confers a poor prognosis [5]. This effect is lost in older patients which may be because this group carries such a poor prognosis that it is difficult to detect any additional negative effect [36]. Despite clarity on the prognostic effect of *FLT3-ITD* mutations in young patients with normal cytogenetic AML, this is not the case in patients with *FLT3* TKD mutations. Patients with *FLT3-ITD* mutant AML often present with marked leukocytosis and high percentages of peripheral blood and bone marrow blasts [37]. A number of studies have shown that remission rates with standard induction regimens are similar for patients with *FLT3-ITD*+ AML and patients with unmutated FLT3 [2, 4–6], but remission duration is short, with a median time to relapse of only 6–7 months [37]. Furthermore, after relapse, the prognosis for patients with *FLT3-ITD*+ AML is worse than those with *FLT3* WT AML, with a median survival of less than 5 months [38, 39•]. As such, patients with *FLT3-ITD* mutant AML represent a particularly challenging population to treat. Given the aggressive nature of *FLT3-ITD* mutant AML, many studies have investigated the role of allogeneic stem cell transplant as consolidation therapy in first remission. While none of these studies are prospective in nature, most studies suggest that patients with *FLT3-ITD* mutant AML consolidated with allogeneic transplant in first remission have better outcomes than those patients consolidated with chemotherapy alone [7, 8, 9•, 10•, 11•, 40, 41•]. Most experts currently recommend consolidation with allogeneic transplant in first remission if possible based on donor availability and host suitability. Unfortunately, not all patients are transplant candidates and a large proportion of patients relapse prior to transplant.

FLT3 Inhibitors: Early Agents and Limitations

For patients with *FLT3-ITD* mutant AML, small molecule FLT3 inhibitors have been an attractive investigational treatment alternative for a number of years. A number of small molecule TKIs with activity against FLT3, including midostaurin (PKC412), lestaurtinib (CEP-701), tandutinib (MLN-518), KW2449, sunitinib (SU11248), and sorafenib (BAY 43–9006) were initially evaluated in patients with *FLT3-ITD* mutant AML, both as single agents and in combination with intensive chemotherapy. Despite initial optimism, response rates and response duration were limited in early phase I and phase II trials in patients with relapsed or refractory AML, at least in part due to lack of sustained FLT3 inhibition in vivo. The cytotoxic activity of these agents appears to be dependent on significant and sustained FLT3 inhibition. Unfortunately, because these agents are multikinase inhibitors, a lack of potency meant that many early agents were limited by the inability to achieve effective FLT3 inhibition without dose-limiting toxicity. Additionally, clinical efficacy of these agents was limited by high degrees of protein binding and/or short plasma half-lives in patients. Moreover, it was unclear if rare clinical responses to these early inhibitors were achieved through inhibition of FLT3 signaling or through off-target effects.

FLT3 Inhibitors: The Agents

Midostaurin (PKC-412)

Midostaurin (PKC412) is a small molecule TKI with broad activity against cKIT and PDGFR in addition to FLT3. Unfortunately, single-agent trials with midostaurin in patients with relapsed or refractory AML were unimpressive, resulting largely in clearance of peripheral myeloid blasts and rare bone marrow responses [42, 43]. Despite, the lack of single-agent efficacy, a phase Ib study provided some optimism for the benefit of midostaurin combined with chemotherapy in previously untreated patients. In this study, midostaurin was combined with standard induction and consolidation chemotherapy in patients with newly diagnosed AML. Of the 69 patients in the trial, 19 harbored a FLT3 mutation, 13 of which were evaluable for a response. In the FLT3 mutated patients, the complete remission (CR) rate was 92 % with a 1-year overall survival (OS) of 85 % and a 2-year OS of 62 % [44]. Based on these results, a multi-center, randomized, phase III trial of midostaurin or placebo combined with induction chemotherapy using daunorubicin/cytarabine and consolidation chemotherapy using high-dose cytarabine in patients with newly diagnosed FLT3 mutated AML was initiated. Results of this trial are expected to be reported in 2014.

Lestaurtinib (CEP-701)

Like midostaurin, lestaurtinib is a small molecule TKI with broad activity against a number of receptor tyrosine kinases including JAK2, VEGFR and TrkA in addition to FLT3. In a phase I/II trial, lestaurtinib was evaluated as a single agent in

patients with relapsed or refractory *FLT3* mutant AML. Five of the 17 patients were noted to have clinical evidence of biological activity and measurable clinical response, which, similar to midostaurin, manifested mostly as short-lived reductions in peripheral and bone marrow blasts [45]. A randomized phase III trial of lestaurtinib in combination with chemotherapy in *FLT3* mutant AML patients in first relapse showed no benefit to the addition of lestaurtinib, though correlative studies suggested that only a small number of lestaurtinib-treated patients achieved target FLT3 inhibition [39•].

Tandutinib (MLN-518)

Tandutinib, is a small molecule TKI with activity against c-KIT and PDGFR in addition to FLT3. In a phase I trial in AML, single-agent tandutinib showed little clinical activity. Eight of 40 patients evaluated in this study had *FLT3-ITD* mutations. No CRs or PRs were observed at either of the dose levels tested [46]. A subsequent phase II trial showed similar results [47]. When combined with induction and consolidation chemotherapy in patients with previously untreated AML, the CR rate was 73 %, though only five of the 29 patients had a FLT3-ITD mutation [48].

KW2449

KW-2449 is a small molecule multikinase inhibitor with activity against FLT3, aurora kinase, FGFR-1, and ABL kinase, which was tested in a phase I trial in leukemia and myelodysplastic syndrome using a twice daily dosing schedule ranging from doses of 25 mg to 500 mg per day. In five of 11 patients with *FLT3-ITD* mutations, peripheral blast reductions of greater than 50 % were observed at day 14, without evidence of bone marrow responses. Careful correlative studies revealed that clinical response was limited by a high degree of plasma protein binding and short inhibitor half-life resulting in effective target inhibition in patients for only 4–6 h per dose [49].

Sunitinib (SU11248)

Sunitinib is a multikinase inhibitor which carries FDA indications for the treatment of a number of solid tumors including advanced clear cell renal cell carcinoma, gastrointestinal stromal tumors and advanced pancreatic neuroendocrine tumors. An early clinical trial assessing single-agent efficacy of sunitinib in patients with relapsed or refractory AML showed only modest activity with partial responses of short duration [50]. However, despite the lack of single-agent activity, a phase I/II study assessing the efficacy of sunitinib in combination with induction and consolidation chemotherapy in older patients with AML and *FLT3* activating mutations showed some promise. CR rates were 53 % (8/15) and 71 % (5/7) for patients with *FLT3-ITD* and *FLT3* TKD mutations, respectively. The 13 patients who achieved CR went on to be consolidated with high dose cytarabine and 7/13 received sunitinib maintenance. The median overall survival in this study was 18.8 months [51].

Sorafenib (BAY 43–9006)

Like sunitinib, sorafenib is a multikinase inhibitor that is FDA approved for the treatment of patients with advanced clear cell renal cell carcinoma, advanced hepatocellular carcinoma and radioactive iodine refractory thyroid cancer. The antileukemic effect of sorafenib was assessed in a phase I study of patients with relapsed or refractory AML. In this study, six of 16 patients had *FLT3-ITD* mutations; all *FLT3-ITD*+ patients achieved a reduction in peripheral and bone marrow myeloid blasts after sorafenib treatment. In patients who were *FLT3* WT there was limited activity, with only three of seven patients responding. No activity was observed in patients with *FLT3* TKD mutations [52]. Despite the relatively modest activity observed in this initial study, multiple studies have since reported the activity of sorafenib in *FLT3-ITD*+ AML. In a recent report of 65 patients with *FLT3-ITD* mutant AML, clinical efficacy of sorafenib monotherapy was seen in patients relapsed after both chemotherapy and allogeneic stem cell transplant [53••]. A number of additional studies have clinically assessed the antileukemic efficacy of sorafenib in a variety of settings [54–57], including a recent phase II study of sorafenib in combination with 5-azacitadine in relapsed/refractory *FLT3-ITD* mutant AML, which demonstrated a response rate of 46 %, mostly consisting of CR or CR with incomplete count recovery [58••]. In contrast, another recent randomized placebo-controlled study of sorafenib in combination with induction and consolidation chemotherapy in an elderly patient population showed no benefit and increased toxicity [59••]. In this trial, however, patients were not selected for *FLT3* mutation status and only 14/95 placebo-treated and 15/102 sorafenib-treated patients in this study were *FLT3-ITD*+. Given the observed anecdotal clinical efficacy of sorafenib monotherapy in *FLT3-ITD* mutant AML patients, especially in the post-allogeneic transplant setting, the use of sorafenib in this patient population has become increasingly common despite the fact that this agent is not FDA approved for the indication. Underlying reasons for the particular efficacy of FLT3 inhibitors in the post-allogeneic transplant setting remain unclear, though may be attributable to selection of a FLT3-mutant clone at relapse post-transplant or due to unknown effects of FLT3 inhibitors on the graft immune system.

Quizartinib (AC-220)

Quizartinib (AC220) is a second generation FLT3 inhibitor, which unlike earlier FLT3 inhibitors, has resulted in a high

rate of response in patients with relapsed or refractory AML. Studies have shown that quizartinib, in contrast to early FLT3 inhibitors, is both highly potent and selective for WT FLT3 and FLT3-ITD [12]. The safety and tolerability of quizartinib was evaluated in a phase I dose escalation trial of unselected patients with relapsed or refractory AML. The most commonly observed treatment-related adverse events were prolongation in QTc, gastrointestinal symptoms, and myelosuppression. The trial included 76 patients: 17 were *FLT3-ITD*+, 37 were *FLT3-ITD* negative and 22 were indeterminate for *FLT3* mutation status. Nine of 17 patients (53 %) who were *FLT3-ITD* mutant+ responded versus five of 37 (14 %) *FLT3-ITD* negative patients. The median response duration was 13.3 weeks, with responses noted at doses as low as 18 mg/day [60•]. The efficacy of single-agent quizartinib was subsequently assessed in a phase II study in patients with relapsed or refractory AML. This study consisted of two treatment cohorts, one consisting of older patients (>60 years of age) after one line of chemotherapy and one consisting of patients >18 years of age relapsed or refractory to second-line therapy or transplant. The primary end point was the composite CR rate (CRc), which was defined as combination of CR, CR with incomplete platelet recovery (CRp) and CR with incomplete hematologic recovery (CRi). Overall, the CRc rate (mostly CRi or CRp) in patient with *FLT3-ITD* mutant AML in both cohorts was an impressive 44–54 % [14••, 15••] with continuous daily dosing of 90 mg/day (females) or 135 mg/day (males); a notable ~30 % CRc rate was also observed in *FLT3-ITD* negative patients in both cohorts. It is also significant to note that patients who had relapsed after allogeneic stem cell HSCT had the highest remission rates and that a large proportion (35 %) of the *FLT3-ITD*+ patients in the younger cohort were bridged to transplant, suggesting a benefit of quizartinib in the peri-transplant setting. As was seen in the previous phase I study, the most common adverse events were gastrointestinal effects, reversible QTc prolongation and myelosuppression

Because of high rates of QTc prolongation and myelosuppression in the initial phase II study, a second randomized phase II study explored lower doses of quizartinib (30 or 60 mg) in a similar patient population. Again, an ~50 % rate of CRc was observed at both dose levels and was associated with a decreased rate of QTc prolongation; however, most remissions still occurred in the setting of incomplete neutrophil or platelet recovery [13••]. It is also notable that instead of the hypocellular response associated with chemotherapy, in some patients response to quizartinib appears to be associated with a syndrome of terminal myeloid differentiation resulting in marrow hypercellularity associated with a neutrophil surge and inflammatory tissue infiltrates [61••], further suggesting that remissions on FLT3 kinase inhibitor treatment may appear different from those achieved with standard chemotherapy. The lack of traditional CR with full neutrophil and/or platelet count recovery observed in these studies has sparked controversy as to whether the non-conventional endpoint of CRc is associated with true clinical benefit and/or prolongation of overall survival compared to standard chemotherapy. To answer this question, a randomized phase III clinical trial of quizartinib monotherapy versus salvage chemotherapy in *FLT3-ITD*+ AML patients in first relapse is expected to begin in 2014.

The single-agent clinical efficacy of quizartinib in patients with relapsed or refractory *FLT3-ITD* mutant AML has led to a number of studies assessing its activity in different clinical settings. Two recent phase I clinical trials assessed the safety and tolerability of quizartinib in combination with induction and consolidation chemotherapy in younger [62•] and older [63•] adult patients with newly diagnosed AML. Another trial assessed the safety of quizartinib in combination with etoposide and cytarabine in a pediatric population with relapsed AML [64•]. In all three trials, quizartinib was safely administered in combination with chemotherapy and further combination studies are being planned. Currently, a phase I trial assessing the efficacy of quizartinib as maintenance therapy after allogeneic stem cell transplant is also recruiting patients.

Even with the increased response rates observed with quizartinib, the lack of clinical activity of early FLT3 inhibitors called into question whether the activity of quizartinib could be definitively attributed to inhibition of FLT3 signaling. The development of drug-resistant KD mutations on the *FLT3-ITD* allele at the time of relapse in eight of eight patients who achieved remission on quizartinib monotherapy confirmed that these initial responses were achieved via FLT3 inhibition and that relapse was mediated by reactivation of FLT3 signaling [16••]. The most common resistance mutations occurred at the activation loop residue D835. Mutations at the FLT3 gatekeeper residue F691 were less commonly found. These mutations have also been associated with acquired clinical resistance to sorafenib [65••, 66•]. Taken together, these observations establish that inhibition of FLT3 signaling holds therapeutic promise, provided that sufficiently potent and prolonged inhibition can be achieved.

Novel FLT3 Inhibitors

The emergence of TKI-resistant *FLT3* KD mutations at the time of relapse on clinically active FLT3 inhibitors such as quizartinib has emphasized the need for additional potent and selective FLT3 inhibitors that are also invulnerable to resistance-causing FLT3 KD mutations. To this end, a number of FLT3 inhibitors that have demonstrated pre-clinical activity against quizartinib-resistant mutations have undergone or are currently undergoing evaluation in early phase clinical trials in *FLT3* mutant AML.

The less clinically common FLT3-ITD F691L "gatekeeper" mutation appears to cause quizartinib resistance by disrupting a π-π ring stacking interaction between the aromatic side chain of the phenylalanine residue and the benzo-imidazol-thiazol moiety of quizartinib [16••]. Mutations at this residue may be overcome by structurally diverse inhibitors less dependent on this interaction. The ABL/FLT3 inhibitor ponatinib, which is FDA approved for the treatment of TKI-resistant CML, achieved bone marrow remission in 2/7 TKI naïve *FLT3-ITD*+ AML patients treated on aphase I study [67•] and has demonstrated preclinical activity against the quizartinib-resistant F691L mutation [68•]. However, ponatinib's clinical activity in AML and its ability to suppress the F691L mutation needs to be assessed in larger clinical trial experience. It is unclear if the dose-limiting cardiovascular toxicity recently described in CML patients will impact the ability to administer ponatinib at clinically efficacious does in a FLT3 mutant AML population. PLX3397 is an inhibitor of KIT, FMS and FLT3 that has also demonstrated equipotent preclinical activity against the FLT3-ITD F691L mutation and is currently being evaluated in a phase I/II trial in FLT3-ITD+ AML. Results from this trial have not yet been reported.

To date, mutations at the FLT3 activation loop residue D835 have been the most frequently implicated in resistance to both quizartinib and sorafenib [16••, 65••]. D835 mutations appear to cause resistance to quizartinib by favoring an active kinase conformation, in which the phenylalanine at the base of the kinase activation loop is flipped "in" ("DFG-in") relative to its conformation in the inactive state ("DFG-out"). Quizartinib is a "type II" inhibitor, which binds to the kinase only in the inactive "DFG-out" conformation; therefore, D835 mutations induce a kinase conformation disadvantageous to quizartinib binding. By the same mechanism, D835 mutations and mutations at other activation loop residues such as Y842 and D839 cause similar resistance to other type II FLT3 inhibitors including sorafenib [16••], ponatinib [68•] and PLX3397 [69•], and pre-clinical mutagenesis studies have identified activation loop mutations as a common cause of resistance to these type II FLT3 inhibitors [16••, 68•, 69•]. The ideal FLT3 inhibitor which would be invulnerable to activation loop D835 mutations would likely be a "type I" inhibitor capable of binding the active, "DFG-in" kinase conformation. While earlier FLT3 inhibitors such as midostaurin and lestaurtinib are indeed type I inhibitors, lack of potency and selectivity limit their clinical efficacy. Crenolanib is a next-generation type I inhibitor that is highly selective for FLT3 and has demonstrated pre-clinical activity against FLT3 D835 mutations both in the presence and absence of an ITD [70•, 71•, 72•]. However, while phase II clinical trials in FLT3 mutant AML are ongoing, data on the clinical efficacy of crenolanib are not yet available.

FLT3 Inhibitors: How Should We Use Them?

While quizartinib has demonstrated high single-agent remission rates in FLT3-ITD+ patients, questions about the optimal use of FLT3 inhibitors in AML remain. Evidence suggests that AML is a polyclonal disease [73•]. While all patients with *FLT3-ITD* mutations are considered to have aggressive disease with a propensity for early relapse, significant evidence suggests that prognosis is related to the allelic burden of disease, with those patients with high mutant to wild type allele ratio having the worst prognosis [2, 74]. This phenomenon may be a reflection of the polyclonality of AML at the time of diagnosis, including disease cells with and without FLT3 mutations especially as FLT3 mutations are generally thought to occur later in leukemogenesis and not as "founder mutations" likely to exist in all leukemic clones. As the disease progresses, a dominant FLT3 addicted clone can emerge, which may account for the increased allelic burden of disease in many patients at the time of relapse [37]. This suggests that early in the disease course single-agent therapy with a FLT3 inhibitor would only affect a small subset of AML cells and may, therefore, have little clinical utility. While a dominant FLT3-addicted clone does seem to emerge in many patients at the time of relapse, the development of polyclonal drug-resistant FLT3 KD mutations may limit response duration to FLT3 inhibitors used as monotherapy [16••]. Together, the polyclonality of AML and the aggressive nature of relapsed *FLT3-ITD* mutant AML suggest that the optimal use of FLT3 inhibitors would be in combination with other agents, such as standard chemotherapy, hypomethylating agents or stem cell transplant, with a particular role for maintenance therapy for the suppression of a FLT3-addicted clone.

Based on clinical response rates, FLT3 inhibitors are most appropriately used in the patients with FLT3 mutations, particularly FLT3-ITD mutations. However, the ~30 % response rate observed with quizartinib in FLT3-ITD negative patients [14••, 15••] is intriguing and it is unclear if a subset of these response can be attributed to other FLT3 mutations not detected by clinical testing. The identification of other AML patient populations that might be ultimately responsive to potent FLT3 inhibitors will require careful molecular analysis of non-FLT3 mutant responders.

Conclusions

The clinical activity of early FLT3 inhibitors was limited by a lack of potency, selectivity and favorable pharmacokinetic properties. While quizartinib monotherapy has achieved a high rate of bone marrow remissions with incomplete recovery of blood counts in patients with relapsed and refractory *FLT3-ITD* mutant AML, the clinical meaningfulness of such responses is still in question. Furthermore, response duration

on quizartinib and other FLT3 inhibitors has been limited by the rapid development of secondary drug-resistant *FLT3* KD mutations that appear to be an Achilles heel for the majority of FLT3 inhibitors currently in clinical development. Future efforts will need to concentrate on elucidating the ideal strategy for the clinical use of FLT3 inhibitors, particularly in drug combinations or as remission maintenance, as well as identifying potent and selective FLT3 inhibitors with decreased vulnerability to resistance mutations.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Akshay Sudhindra declares no potential conflicts of interest relevant to this article.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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