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Investigational FMS-Like Tyrosine Kinase 3 (FLT3) Inhibitors in Treatment of Acute Myeloid Leukemia (AML)

Naveen Pemmaraju, **Hagop Kantarjian**, **Michael Andreeff**, **Jorge Cortes**, and **Farhad Ravandi**

Abstract

Introduction—Outcomes for the majority of patients with Acute Myeloid Leukemia (AML) remain poor. Over the past decade, significant progress has been made in the understanding of the cytogenetic and molecular determinants of AML pathogenesis. One such advance is the identification of recurring mutations in the FMS-like tyrosine kinase 3 gene(*FLT3*). Currently, this marker, which appears in approximately one third of all AML patients, signifies a poorer prognosis, but also identifies an important target for therapy. FLT3 kinase inhibitors have now undergone clinical evaluation in phase I, II and III clinical trials, as both single agents and in combination with chemotherapeutics. Unfortunately, to date, none of the FLT3 inhibitors have gained FDA approval for the treatment of patients with AML. Yet, there are several promising FLT3 inhibitors are being evaluated in all phases of drug development.

Areas covered—This review aims to highlight the agents furthest along in their development. It also focuses on those FLT3 inhibitors that are being evaluated in combination with other anti-leukemia agents.

Expert opinion—The authors believe that the field of research for FLT3 inhibitors remains promising, despite the historically poor prognosis of this subgroup of patients with AML. The most promising areas of research will likely be the elucidation of the mechanisms of resistance to FLT3 inhibitors, and development of potent FLT3 inhibitors alone, or in cominbation with hypomethylating agents, cytotoxic chemotherapy, or with other targeted agents.

Keywords

FLT3; FLT3 inhibitor; sorafenib; quizartinib; crenolanib

1. Introduction

Overall, the prognosis of patients with acute myeloid leukemia (AML) remains poor with dismal outcomes at 5 years for the majority of patients and especially in those older than 65 years[1,2]. Importantly, the biologic landscape of AML has changed over the past decade, with growing recognition and importance that both cytogenetic and molecular information about

Corresponding author: Farhad Ravandi, MD, Professor, Department of Leukemia, MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 428, Houston TX 77030, fravandi@mdanderson.org.

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a patient's disease are necessary for correct diagnosis, prognostic stratification, and treatment decision planning[3-5].

Among these cytogenetic and molecular abnormalities, one of the most important prognostic factors is the presence of *FLT3* gene mutations[6,7]. *FLT3* is mutated in approximately 30% of patients with AML, and with the *FLT3*-internal tandem duplication (ITD) mutations accounting for the majority; these have been clearly associated with an overall worse prognosis compared to patients with *FLT3*-wildtype AML[8]. Less clear, is the information with regards to the prognostic value of the other commonly occurring *FLT3* mutations, notably, the less frequent FLT3 point mutations which occur in the tyrosine kinase domain(TKD) (e.g. D835)[9].

As a result of the poor response of patients with *FLT3*-ITD mutations to standard cytotoxic agents, many groups worldwide have investigated a targeted approach involving kinase inhibitors directed against *FLT3* mutated AML cells. Several of these studies have been conducted in the salvage setting, where no standard of care therapies exist[10]. Currently, there are no FDA-approved FLT3 inhibitors in use in the clinic available for patients with AML. Therefore, most patients with *FLT3* mutations either receive an allogeneic stem cell transplant (in selected patients with available donors who are fit to undergo such procedures), or are considered for enrollment on clinical trials of FLT3 inhibitors[11]. Allogeneic transplant has been shown in some studies to benefit some patients with *FLT3*-ITD AML[12] and several groups have examined using FLT3 inhibitors as a post-transplant maintenance strategy in order to prevent relapse in this setting[13,14].

Several FLT3 inhibitors have undergone clinical evaluation. Some, such as sunitinib and lestaurtinib, have not demonstrated sufficient promise for further development and have been reviewed elsewhere[8,11,15,16]. It should be noted that most of the FLT3 inhibitors discussed are not inhibitors of FLT3 specifically; rather, these drugs often have multiple kinase targets (listed separately with each drug discussed). These non-*FLT3* targets may help to explain the clinical benefit of these drugs observed in even non *FLT3*-mutated AML patients.

In this review, we will focus on the more recently investigated FLT3 inhibitors that are currently in trials for patients with *FLT3* mutated AML, either alone or in combination with hypomethylating agents or chemotherapy. These include sorafenib, AC220 (quizartinib), PKC412 (midostaurin), and crenolanib.

2. Mechanism of Action: Pre-Clinical Data and Rationale

The important role of personalized, molecularly-directed treatment in leukemia was most notably demonstrated by the development of the tyrosine kinase inhibitor (TKI) imatinib mesylate and the subsequent next generation TKIs for therapy in chronic myeloid leukemia (CML)[17-19]. Based on the impressive success of TKIs in CML, investigators began to explore this approach in AML, particularly in pediatric and adult patients with *FLT3*-mutated AML[20]. Based on the observation that constitutively activated FLT3 receptor in *FLT3*-mutated AML cell lines resulted in transformation to leukemia[21,22] and that this leukemic transformation via FLT3 needed a kinase domain activation[22], Levis et al

performed cytotoxic assays on AML primary blasts from patient samples using a tyrosine kinase inhibitor with FLT3 inhibitory activity (AG1295). This study found that the AG1295 directly inhibited *FLT3* and that apoptosis was induced in the patients samples carrying *FLT3*-ITD mutations, therefore indicating that this drug had specific activity against AML blasts that carried *FLT3*-ITD mutations[23]. Kelly et al demonstrated the activity of another FLT3 inhibitor (CT53518) in both human AML (*FLT3*-mutated) cell lines and in mouse models. In the human cell lines, this drug led to inhibition of phosphorylation of *FLT3*-ITD and to apoptosis[24]. Weisberg et al reported similar FLT3 inhibiting activity with another tyrosine kinase inhibitor,PKC412, or midostaurin. Addition of midostaurin led to apoptosis in *FLT3*-mutant cell lines (Ba/F3) and prolonged survival in *FLT3*-ITD murine models, again demonstrating the activity of small molecule/tyrosine kinase inhibitors (TKI) in *FLT3*-mutated AML[25].

The development of FLT3 inhibitors has thus far demonstrated that inhibitors of FLT3 signaling have varying levels of activity at the nanomolar level and have the ability to inhibit *FLT3*-mutated cell lines and murine models harboring *FLT3* mutations[26]. These TKIs have a unifying feature of acting as direct inhibitors of *FLT3* via competition with ATP for ATP-binding sites in the FLT3 receptor kinase domain[27]. The variations in conformational states (inactive versus active) of the kinase domains of *FLT3* have led to the different types of FLT3 inhibitors and likely in part the avidity of their efficacy and activity in *FLT3*-mutated AML[9,17,28]. While most FLT3 inhibitors available have been found to target the inactive conformation (so called Type II inhibitors), crenolanib has been found to target both the inactive and active conformational states (Type I inhibitor). This is notable, as not only has crenolanib been shown to inhibit classic *FLT3*-ITD cell lines and xenografts, it has also been demonstrated to have activity against point mutations, including the *FLT3*-D835 point mutation[9,28-30].

3. Sorafenib

Table 1 summarizes the key information for each of the FLT3 kinase inhibitors discussed in this article. Sorafenib is a multi-tyrosine kinase inhibitor that has served as an effective antineoplastic chemotherapeutic agent in a number of different malignancies. Among its multiple targets, sorafenib is known to inhibit *VEGF, c-kit, PDGFR, BRAF, and FLT3*[31]. Sorafenib is approved by the FDA for the treatment of hepatocellular cancer[32], renal cell carcinoma[33], and most recently thyroid cancer[34]. Importantly, in the context of AML, sorafenib has also been shown to be a potent inhibitor of the FLT3 kinase[35]. In vitro, cell line data suggested that sorafenib would potentially be an effective FLT3 inhibitor in patients with AML whose leukemic cells harbored the mutation. Therefore, based on induction of apoptosis in Ba/F3 AML cells harboring FLT3-ITD mutations, clinical trials of the agent in various settings have been conducted[36,37].

3.1 Sorafenib monotherapy in FLT3 mutated AML

Various groups have utilized sorafenib for its FLT3 inhibitor properties[38] for treating patients with AML. In a phase I study, Crump et al[39] evaluated sorafenib for treating patients with relapsed myelodysplastic syndrome (MDS) or AML older than 65. In various dosing regimens tested, among 42 patients, 1 CR was observed in a *FLT3*-ITD mutated

patient with AML. In another phase I clinical trial, by Borthakur et al[40], 50 AML patients were treated with two different dose schedules of sorafenib (once or twice daily for five days per week, weekly for 21 days for 1 cycle, or in the second schedule once or twice daily for 14 days every 21 days). CR or CRp were demonstrated in 5 patients (10%), all of whom had *FLT3*-ITD. Additionally, blast reduction (either bone marrow or blood) was noted in 17 more patients, which was durable in 11 patients, lasting for 2 cycles or more. The authors concluded that sorafenib was well tolerated, active in AML, especially in patients with *FLT3*-ITD.

3.2 Sorafenib in combination with other agents

Several studies in both pediatric and adult patients with AML have been published combining sorafenib with either cytotoxic chemotherapy[41] or with hypomethylating/histone modifying agents[42,43]. These, in general, have continued to demonstrate the safety and efficacy of this agent for the treatment of patients with AML. Ravandi et al published the results of a phase I/II study of sorafenib in combination with idarubicin and cytarabine for patients younger than 65 years[44]. Of 51 patients (previously untreated for AML) treated in the phase II portion, 38 patients (75%) attained CR, which included 14/15 (93%) of the FLT3-mutated patients. The most common grade 3 or higher toxicities reported as possibly related to sorafenib during induction chemotherapy were elevated transaminases (n=5), bilirubin (n=4) and diarrhea (n=4). The authors concluded that sorafenib can be safely combined with intensive cytotoxic chemotherapy and produce high rates of CR, particularly among *FLT3*-mutated AML patients. In addition, the plasma inhibitory assay confirmed the on-target effect of sorafenib on the kinase activity of FLT3. Originally described by Levis et al, the plasma inhibitory activity assay was developed for the assessment of inhibiton of FLT3 in patients who are treated with FLT3 inhibiting drugs[45]. The first clinical application was for assessment in both midostaurin and lestaurtanib clinical trials. The assay, in conjunction with *in vitro* phosphorylation and cytoxocity assays, was able to identify the degree to which each FLT3 inhibitor was able to inhibit FLT3 activity in patient samples. Based on this early experience, the assay has since been validated in other FLT3 inhibitor trials and is being utilized in the context of sorafenib-based and other FLT3 inhibitor clinical trials[44,46-48].

Ravandi et al also reported their data from a study combining sorafenib with 5-azacytidine in patients with relapsed AML[49]. This novel combination was based on the observation that increased FLT3 ligand levels as a result of cytotoxic chemotherapy regimens accounted for a potential mechanism of resistance to tyrosine kinase inhibitors such as sorafenib[47,50]. The authors hypothesized that combination with hypomethylating agents, rather than cytotoxic intensive chemotherapy, would lead to decreased levels of FLT3 ligand and potentially less resistance. In this phase II single institution, single arm trial, 43 patients, mostly with multiply relapsed AML were treated with sorafenib 400mg orally twice daily continuously together with 5-azacytidine at 75mg/m^2 intravenously for 7 days. Forty patients (90%) had FLT3 mutations. Among 37 evaluable patients, 6 patients had received no prior therapy, 12 patients were primary refractory to treatment, and 19 patients had relapsed disease. The median number of prior therapies was 2 (range 0-7), with nine patients failing prior FLT3 inhibitor therapy. The overall response rate was reported as 46%, including 10 patients

(37%) with CRi, 6 with CR, and 1 PR. The most commonly noted side effect was fatigue in 47% of patients, usually grade 1 in degree. The most frequent grade 3 or higher toxicities were: thrombocytopenia, neutropenia, anemia, and neutropenic fever. Hepatic toxicity was observed (both elevated bilirubin and elevated transaminases) but most of these events were grade 1 or 2. Correlative studies demonstrated that, as hypothesized, FLT3 ligand levels did not increase to levels observed in prior cytotoxic combination trials. The authors concluded that sorafenib in combination with hypomethyaltor therapy is effective in treatment of patients with AML with *FLT3*-ITD.

4. Quizartinib (AC220)

4.1 Quizartinib monotherapy in AML

Preclinical studies conducted by Zarrinkar et al demonstrated that AC220, which has been named as quizartinib, had strong potency and selectivity against the FLT3 kinase[51]. Although highly selective, this second-generation FLT3 kinase inhibitor has also been noted to have activity against KIT, PDGFRA, PDGRB and RET kinases[52]. Quizartinib has also been reported to have other effects on AML cells including induction of cell-cycle arrest and differentiation as well as induction of apoptosis[53]. On the basis of these pre-clinical data demonstrating the activity and selectivity of FLT3 kinase, Cortes et al conducted the first-in-human study with quizartinib, a phase I clinical trial in patients with relapsed/refractory AML[48]. The median age of the cohort was 60 years (range 23-86 years), and the patients had a median of three previous treatment regimens (range 0-12). The drug was given as a single agent, orally, in a dose escalation manner. Of 76 patients evaluated, 23 (30%) had responses, including 2 CR, 3 CRp, and 5 CRi; additionally, 13 patients (17%) had PR. Among the 17 patients with *FLT3*-ITD, 9 (53%) had responses (1 CR, 1 CRp, 2CRi, 5 PR). Notably, 12% of patients developed prolonged QT interval, representing the 2nd most common adverse event; the dose-limiting toxicity was reported as grade 3 QT prolongation. The maximum tolerated dose for quizartinib was found to be 200mg per day. As in other previous FLT3 inhibitor trials, the plasma inhibitory assay demonstrated on-target inhibition of FLT3 phosphokinase.

Cortes et al reported the initial results of a phase II open-label trial of quizartinib in relapsed/refractory patients with *FLT3*-ITD positive AML[54]. In a planned analysis of the first 62 patients on this study, and among the 53 patients (85%) evaluable for efficacy. the composite CR rate (which included CR, CRp, and Cri) was 45% (2 CRp and 22 CRi) with a PR rate of 24%. The most commonly reported adverse events related to quizartinib were nausea, QTc prolongation, and vomiting. Importantly, QTc prolongation was noted in 21 patients (34%), grade 3 in 11 patients (18%). The occurrence of QTc prolongation was reduced by decreasing the quizartinib starting dose from 200mg/day (35%) to 135mg/day (8.3%) (males) and 90mg/day (5.9%) in females. More recently, Levis at al[55] presented the final analysis of this study including 137 patients with relapsed or refractory AML, aged 18 years or older. The composite CR rate for patients with *FLT3*-ITD was 44% (40% CRi, 0 CRp, 4% CR). The median duration of response was 11.3 weeks and the median survival was 23.1 weeks. The three most commonly reported adverse events (treatment-related) were nausea (38%), anemia (29%) and QTc prolongation (26%). QTc prolongation occurred in 36

(26%) patients; it was grade 3 in 13 patients (10%) and no instances were reported as grade 4. To date, no definitive mechanisms have been identified to explain differences in QTc prolongation between men and women. It was noted that approximately one-third of the patients in this study were able to proceed to stem cell transplantation, including many patients who were refractory to prior therapy before responding to quizartinib.

4.2 Quizartinib in combination with other agents

Quizartinib has been safely combined with chemotherapy in two phase I clinical trials. Burnett et al reported their results from the AML 18 Pilot trial[56]. In this study, quizartinib was combined with either Ara-C, daunorubicin, etoposide or daunorubicin plus Ara-C alone. Fifty five newly diagnosed patients with AML (median age 69 years, range 62-87) were evaluated in dose-escalation cohorts. Patients with or without *FLT3*-ITD mutation were eligible for enrollment on the protocol. CR was noted in 33 out of 42 patients evaluated (79%), which included all 4 patients with *FLT3*-ITD. The authors concluded that quizartinib, at dose 40mg daily for 14 days, could be safely administered after intensive chemotherapy in older patients with newly diagnosed AML.

In another study, Altman et al studied quizartinib in combination with chemotherapy in younger patients with AML[57]. In this phase I dose-escalation study, patients with median age 43 years (range 22-60) with newly diagnosed AML received Ara-C and daunorubicin (in the 7+3 regimen) for induction chemotherapy and high dose Ara-C for consolidation. Following this, quizartinib was given daily for either 7 or 14 days. Sixteen of 18 patients enrolled (89%) had *FLT3*-ITD mutations. The maximum tolerated dose (MTD) from this study was determined to be 40mg daily for 14 days or 60mg daily for 7 days.

Several ongoing phase I and II trials combining quizartinib with either hypomethylating agents or chemotherapy in *FLT3*-mutated and wild type patients with AML will further determine the feasibility and efficacy of this strategy.

5. Midostaurin (PK412)

5.1 Midostaurin Single-agent activity

Originally tested in solid tumors, midostaurin was found to not have strong clinical activity in metastatic melanoma[58]. Midostaurin targets multiple kinases, including *VEGFR, PDGFR, FGFR, KIT*[59]. However, this multi-kinase inhibitor was also found to have inhibitory activity against the FLT3 kinase[25]. In this context, the drug was investigated as a candidate for testing in patients with AML patients. In a phase IIB trial, Fischer et al studied midostaurin in 95 patients with either MDS or AML, either wild-type *FLT3* or mutated *FLT3*. They reported 1 PR in a *FLT3*-mutated patient, and blast reduction of 50% or greater in 71% of patients with mutated *FLT3* and in 42% of patients with wild-type *FLT3*. The drug was well tolerated and the authors proposed that further studies were needed with this active agent for *FLT3*-mutated AML[60].

5.2 Midostaruin in combination with other agents

Williams et al report on both pre-clinical and phase I data in a small pilot study of combination of midostaurin with a hypomethylating agent, decitabine[61]. In the phase I portion, they enrolled 16 patients with AML; however, only 2/16 (13%) patients harbored *FLT3*-ITD mutations. The authors performed an intention-to-treat analysis and reported stable disease (SD) or better in 57% of the patients, with 25% having a complete hematologic response. The authors concluded that further studies were warranted with this combination; they also reported that sequential administration of decitabine followed by midostaurin was better tolerated than their concurrent administration.

Nazha et al[62] reported the results of a phase I/II clinical trial of midostaurin in combination with 5-azacytidine in 20 patients with relapsed/refractory MDS or AML. 5-azacytidine was administered either subcutaneously or intravenously at 75mg/m^2 for 7 days (days 1-7) for each cycle. Midostaurin was given in two dosing schedules (25mg or 50mg orally, twice daily, for 14 days (days 8-21)). Nine patients had *FLT3*-ITD mutations at time of enrollment with a response rate of 33% (3/9 patients). All reported side effects were grade 1 and 2, with no major differences between the two midostaurin dosing schedules.

Stone et al conducted a phase Ib clinical trial combining midostaurin with cytotoxic chemotherapy (daunorubicin and cytarabine)[63] in newly diagnosed, younger AML patients. Two dosing schedules were tested (50 mg twice daily versus 100mg twice daily). On the 50 mg twice daily arm, the CR rate was 80% including 12/13 (92%) patients with mutated *FLT3*. The authors concluded that further testing should be performed leading to an ongoing, multi-center Phase III clinical trial with midostaurin 50mg po twice daily for 14 days in combination with cytarabine plus anthracycline-based chemotherapy; the results of this trial are being eagerly awaited at this time[64].

6. Crenolanib

Initially evaluated in gastrointestinal stromal tumor (GIST) cell lines, crenolanib has been shown to have activity against a wide variety of kinases, including PDGFRA and FLT3[79]. Importantly, crenolanib has subsequently been shown to have pre-clinical activity against *FLT3*-mutated AML cell lines[28]. Furthermore, crenolanib appears to have selectivity against the *FLT3* point mutation, D835, a common mechanism of resistance in many patients with *FLT3*-ITD mutation[9,30]. An ongoing phase II clinical trial with crenolanib as single agent, for patients with relapsed/refractory AML patients with *FLT3* mutations, is currently enrolling patients (Clinicaltrials.gov NCT01522469, Arog Pharmaceuticals).

7. Ponatinib

A common etiology for acquired resistance to FLT3 inhibitor therapy, including with sorafenib and quizartinib, is the development of secondary mutations, usually point mutations of *FLT3* gene at the tyrosine kinase domain (TKD); novel strategies are therefore needed to overcome this resistance[80]. Ponatinib, is a multi-kinase inhibitor which is currently approved by the FDA for the treatment of patients with chronic myeloid leukemia (CML) with the T315I mutation, patients with Philadelphia positive acute lymphoblastic

leukemia (Ph+ ALL) with T315I mutation, or patients with CML or Ph+ALL in whom no other tyrosine kinase inhibitor therapy is indicated[81]. The pathway targets of ponatinib include PDGFRA, FGFR, KIT, FLT3 as well as the ABL kinase[82]. Preclinical data has demonstrated potent activity of ponatinib against *FLT3*-mutated AML cell lines as well as in the MV4-11 xenograft (mouse) model system[82]. Further reports have shown that ponatinib may overcome resistance due to a number of *FLT3* mutants including F691 in cell lines[80]. The authors suggested that treatment with ponatinib for *FLT3*-mutant AML may decrease the secondary resistance rates due to *FLT3*-TKD mutations[83]. Shah et al analyzed the results of 12 patients with AML who were treated in the phase I trial of ponatinib at the dose of 45 mg po daily[84]. The median age of the patients was 49 years (range, 30-72) and they were all heavily pre-treated (median number of prior therapies 3, range 1-7). *FLT3*-ITD was present in 7/12 patients (58%). Nine patients had one or more treatment-related adverse event with the most commonly noted toxicity being pancreatitis (3 patients, all grade 2). The overall response rate was 25% (3/12 patients), with 2 CRi and 1 PR. All 3 were noted to have *FLT3*-ITD mutations[84]. The authors concluded that ponatinib should be considered for further testing as a FLT3 kinase inhibitor in AML.

8. FLT3 Inhibitors and Post-Transplant Setting

The presence of *FLT3*-ITD mutation in patients appears to be a poor prognostic marker even in those patients who are able to go for allogeneic stem cell transplant, leading to interest in post-stem cell transplant maintenancestrategies[14]. The most studied FLT3 inhibitor in the post-transplant setting is sorafenib. Safaian et al reported a case in which a patient with *FLT3*-ITD mutated AML had extramedullary AML relapse after allogeneic stem cell transplant and achieved molecular remission with administration of sorafenib. [85]. Metzelder et al reported 6 patients with *FLT3*-ITD mutated AML who received sorafenib around the time of allogeneic stem cell transplant (2 patients before, 3 patients after, and 1 patient both before and after). Sorafenib led to 2 patients being transitioned to stem cell transplant in the pre-transplant setting and 2 patients who received sorafenib post-transplant had durable molecular remissions. This suggested that monotherapy with sorafenib in the peri-transplant period may be beneficial for patients with *FLT3* mutations.More recently, Metzelder et al evaluated 65 relapsed/refractory patients with *FLT3*-ITD mutated AML who received sorafenib in a multi-center setting[86]. In this study, 29 patients (45%) had prior allogeneic stem cell transplant. They reported that patients who received sorafenib after relapse following an allogeneic stem cell transplant, developed sorafenib resistance less commonly and significantly later in their disease course. Additionally, durable remissions occurred mostly in patients who had undergone a prior allogeneic stem cell transplant. The authors concluded that sorafenib results in durable remissions in some *FLT3*-ITD mutated patients who have relapsed post-transplant, and that there may be synergy between sorafenib and the immunological effects of an allogeneic stem cell transplant.[86].

In contrast, another study evaluated 16 patients with *FLT3*-ITD mutated AML that relapsed after stem cell transplant. Half (8 patients) were treated with sorafenib alone and the other half with sorafenib and cytotoxic chemotherapy. A total of 3 patients achieved partial remissions and 2 of the patients were able to be transitioned to second allogeneic transplants (however, both patients relapsed within 3 months after second transplant). The authors

suggested that a better approach could involve sorafenib or other FLT3 inhibitor as a preventative strategy in high risk patients[13]. It will be important to assess other FLT3 inhibitors with different profiles compared to sorafenib (e.g. quizartinib, crenolanib) in the post-stem cell transplant setting, both as a maintenance strategy as well as for relapse.

9. Other Agents

Several other recently reported FLT3 kinase inhibitors are in earlier stages of development but have demonstrated potential in the preclinical setting. Table 2 summarizes the pre-clinical data of some of the newer FLT3 inhibitors in early development. BPR1J-340, a novel, potent FLT3 inhibitor, was studied in combination with a histone deacetylase inhibitor (HDAC), vorinostat and demonstrated synergistic induction of apoptosis in AML cell lines[87]. Initial reports of another novel FLT3 kinase inhibitor, TTT-3002, demonstrated high potency in FLT3-ITD mutated leukemia cell lines[88]. Futhermore, TTT-3002 was shown to have potent activity against the FLT3-D835 point mutation.

10. Novel *FLT3* Mutations and Resistance to FLT3 Inhibitors

Many patients with *FLT3*-mutated AML do not gain substantial benefit from FLT3 inhibitors, despite receiving several inhibitors sequentially[10]. Development of mutations in the kinase domain, has been described as an acquired form of drug resistance, responsible for this phenomenon [89]. This has been well-described with sorafenib with subsequent development of the D835 mutation[77]. In this study, mouse models infused with leukemia cells from AML patients before and after sorafenib administration demonstrated that the expansion of D835 mutant cells during therapy with sorafenib led to clinical resistance. Further studies have revealed similar developments with other FLT3 inhibitors such as quizartinib. Smith et al demonstrated the occurrence of novel point mutations at three residues in the *FLT3*-ITD kinase domain leading to resistance to quizartinib.[90] Further understanding of the mechanisms responsible for the development of these novel *FLT3* mutations occurring de novo and following drug exposure, remains an area of active research. Detailed characterization of these mechanisms may lead to improved therapeutic targeting or combination of drugs to target or prevent development of resistance.

11. Response Criteria

The issue of response criteria in patients with *FLT3*-mutated AML is an active area of research. While there are standard response criteria established for AML in general[65], these rely solely on conventional definitions based upon morphologic and cytogenetic responses, with no standard definition for molecular responses. A growing area of discussion is how best to incorporate molecular responses into remission criteria, and to note that increased overall survival, in *FLT3*-mutated AML, may be potentially achieved without achievement of morphological complete remission. The incorporation of FLT3 inhibitors into AML therapy, especially in the salvage setting, has been shown to lead to overall improvement in long term outcomes for patients with *FLT3*-mutated AML, a strategy especially important for elderly patients are those unfit for standard intensive chemotherapy[10].

12. Conclusion

The overall prognosis and outcome for patients with AML and *FLT3*-ITD mutations remains poor. Approximately one-third of all patients with AML have these mutations; as such, the development of FLT3 inhibitors has become an active area of research. Several inhibitors have been and are being evaluated in clinical trials, both as monotherapy, and in combination with either cytotoxic chemotherapy or hypomethylating agents. Importantly, due to the impressive activity of these agents in the relapsed setting, several ongoing studies are evaluating them in the frontline setting (in combination with other agents). As a myriad of FLT3 inhibitors are entering the clinic, resistance in patients who have previously achieved remission is being observed, with a number of potential mechanisms, most notably with acquisition of *FLT3* point mutations. Strategies for the optimum utilization of FLT3 inhibitors including use in the frontline setting, combinations with other agents with potential synergistic action, and sequencing before and after stem cell transplantation remain active areas of research. Much remains to be learned about this novel group of targeted agents in *FLT3*-mutated AML; future research is likely to provide us with much needed effective agents for combating this disease.

13. Expert Opinion

Development of drug resistance to FLT3 kinase inhibitors remains a potentially significant limitation of treatment with these agents. Several potential mechanisms of resistance to the current FLT3 inhibitors have been proposed. The tumor microenvironment has been demonstrated to play a key role in resistance to kinase inhibitors in AML[70]. Because the SDF-1 alpha/CXCR4 signaling pathway is a critical component of the interaction between leukemia cells and the bone marrow microenvironment, inhibition of CXCR4 has been postulated as a novel strategy of targeting leukemic cells, especially in *FLT3*-ITD positive patients with AML[70,71]. Building on this strategy, Andreeff et al are conducting a phase I study of combination of sorafenib with plerixafor and G-CSF[72]. Initial results are encouraging, and the authors demonstrated that the mobilization of AML cells was successful in removing them from their protective bone marrow environments. Another area of investigation is the hypoxic bone marrow niche, as demonstrated by Konopleva et al[73,74]. Multiple studies have suggested that dual inhibition of both *PI3K* and *FLT3* may serve as a means of overcoming resistance of the bone marrow stroma as a protective environment for leukemic cells, and should be further investigated as a novel therapeutic approach in patients with *FLT3*-mutated AML[75,76]. Another potential mechanism of acquired resistance to FLT3 kinase inhibitors is the development of point mutations in the kinase domain, notably the *FLT3* D835 point mutation[77]. The acquisition of point mutations in patients previously exposed to FLT3 kinase inhibitors has been well described as a predictor of relapse and resistance to clinically available FLT3 inhibitors, such as sorafenib[78] and has led to the investigation of several newer agents reported to overcome this mechanism

Another approach is the application of additive and/or syngerstic combinations of FLT3 inhibitors with other targeted inhibitors, or dual-targeted therapy. One example is with STAT5 pathway; *FLT3*-ITD activates *STAT5* in a *JAK*-independent fashion. It has been reported that the psychotropic drug pimozide inhibits *STAT5* and synergizes with

midostaurin in order to induce apoptsois [91]. Another novel combination strategy involves dual inhibition of the *FLT3* and *SYK* pathways. A recent in vitro study of quizartinib in combination with a SYK inhibitor [P505-15 (PRT062607)], demonstrated that *SYK* may be an important regulator of *FLT3* signaling in AML.[92] Weisberg et al, suggested that combinations of FLT3 inhibitors with JAK/STAT inhibitors such as pacritinib (a dual JAK2/FLT3 inhibitor[93]) or with multi-kinase inhibitors such as dasatinib may be beneficial[94]. Furthermore, the PI3K and *mTOR* pathways appear to play key roles in AML cell growth and survival, including mutated AML. Reikvam et al have demonstrated the essential role of cytokine-activated *PI3K/AKT/mTOR* pathway as a common component in AML[95,96]. Chen et al demonstrated that mTOR signaling is downstream of FLT3 kinase and is important for leukemia cell survival, suggesting *that* combinations of mTOR and FLT3 inhibtiors may have potential for clinical activity.[97] Greater understanding of the pathobiology of the underlying mechanisms of resistance to FLT3 inhibitors will lead to the development of more potent inhibitors and combinations strategies that can overcome or even prevent the occurrence of mutations responsible for resistance.

We believe that the field of research for FLT3 inhibitors remains promising, as it represents an important aspect of drug development for patients with AML. Historically, patients with *FLT3*-ITD mutation have done extremely poorly with standard therapies, and the current recommendation is for chemotherapy aimed at achieving complete remission followed by an allogeneic stem cell transplant in first remission, if possible. However, as many patients are unable to undergo stem cell transplant due to more advanced age, presence of comorbidity, or lack of an appropriate donor, incorporation of targeted therapy with FLT3 inhibitors represents a strategy with high likelihood of significant impact. This concept is particularly important in older AML patients, and those not suited for intensive chemotherapy, as treatment with FLT3 inhibitors may provide lower toxicity and better tolerated options in *FLT3*-mutated patients compared to standard chemotherapy. The presence or absence of *FLT3* mutations should be established in all patients with newly diagnosed AML. Furthermore, as some patients may acquire the mutation later on in the course of their disease, there is reason for this testing to be repeated at relapse. This not only allows for better determination of the patient's prognosis allowing consideration for an allogeneic stem cell transplant early in the course of therapy, but also helps select a personalized targeted therapy approach by enrolling patients into clinical trials evaluating FLT3 inhibitors. The most eagerly awaited results in 2014 are those of the ongoing phase III trial of combination of midostaurin with cytarabine plus anthracycline-based chemotherapy, which represents the FLT3 inhibitor strategy furthest along in development, as well as the continued development of quizartinib as it enters into phase II and III testing both as a single agent and in combination with chemotherapy and with hypomethylating agents. We remain hopeful that FLT3 inhibitors will demonstrate proven efficacy, and will represent one of many novel approaches as a step forward for some of the AML patients with the poorest prognosis, those with *FLT3*-ITD mutations.

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Article Highlights Box

- *FLT3* mutations are common in AML, occurring in approximately 30% of patients with AML and indicate a poor prognosis and higher risk for relapse
- In this poor prognosis group of patients, no definitive standard of care exists as, to date, there is no medication approved specifically for treatment of *FLT3*-mutated AML
- Many clinical trials testing FLT3 inhibitors alone or in combination with other agents are ongoing
- Among the FLT3 inhibitors in clinical trials, sorafenib, quizartinib, midostaurin, crenolanib, and ponatinib are the furthest along in clinical evaluation
- I Innovative combinations of FLT3 inhibitors with hypomethylating agents, cytotoxic chemotherapy, or with other targeted agents are being evaluated in both the pre- and post-stem cell transplant settings, and to overcome resistance to FLT3 kinase inhibitors

Table 1

Characteristics of Selected FLT3 inhibitors and Summary of Major Results from Clinical Trials

Drug	Current FDA approvals in oncology	Potential Drug Targets in AML	Key Single agent trial in FLT3-mutated AML	Key Combination trials in FLT3-mutated AML
Sorafenib	Hepatocellular cancer Renal cell carcinoma Thyroid cancer	*PDGFR, c-KIT, VEGF, BRAF, FLT3*	Phase I, relapsed;N=50; CR/CRp in 5 patients, all had FLT3-ITD; also seen: blast reduction in 17 additional patients (Borthakur, Haematologica 2010)[40]	Phase I/II;IA+sorafenib;n=51 phase II, untreated: 75% achieved CR, including 14/15 FLT3-mutated patients (Ravandi, JCO 2010)[44] Phase II, all but 6 patients had relapsed at start; n=37 evaluable; ORR 46% (10 CRi, 6 CR, 1 PR) (Ravandi Blood 2013)[49]
Quizartinib	None	*PDGFRA/B, RET, c-KIT, FLT3*	Ph II, relapsed, n=137; composite CR rate 44% (40% Cri, 0 CRp, 4% CR) (Levis ASH 2012)[55]	Phase I, quizartinib +chemotherapy, n=42 evaluable; untreated; CR in 79%, including all 4 FLT3-ITD patients (Burnett ASH 2013)[56]
Midostaurin	None	*PDGFR, FGFR, c-KIT, VEGFR*	Ph IIB, relapsed; n=95 MDS or AML; 1 PR in a FLT3-mutated patient, blast reduction seen in 71% of FLT3-mutated patients (Fischer JCO 2010)[60]	Phase III; frontline study, randomized, chemotherapy +/- midostaurin; multicenter; ongoing evaluation, results pending at time of publication
Crenolanib	None	*PDGFRA, FLT3*	Phase II; single-agent, ongoing, relapsed/refractory AML with FLT3 mutations	N/A
Ponatinib	CML w/T315I mutation or in whom no other TKI is indicated Philadephia-positive ALL w/T315I mutation	*PDGFRA, FGFR, c-KIT, ABL, FLT3*	Phase I; relapsed; n=12; ORR 25% (2 CRi, 1 PR, all 3 responses had FLT3-ITD mutations) (Shah Br J H 2013)[84]	N/A

Table 2

Pre-clinical characteristics of selected novel FLT3 inhibitors

Drug	Includes Inhibition of FLT3-D835?	Other potential drug targets	IC_{50} for FLT3 inhibition/ kinase assay	Proposed mechanisms of action	Potential for combination with other agents
TTT-3002(Hayley, Blood 2014)[88]	Yes	*AKT, MAPK*	100-250pM	Demonstrated potent inhibition of FLT3 phosphorylation, including activating FLT3 D835 mutations and STAT5	N/A
BPR1J-340 (Lin PLOS one, 2014)[87]	Yes	*VEGFR2, VEGFR3, TRKA*	25 nM	Inhibits phosphorylation of FLT3 and STAT5, led to apoptosis in FLT3-ITD mutated AML cells	Synergistic apoptosis in combination w/vorinostat (HDAC inhibitor) in MOLM-13 AML cell line
AMG925 (Keegan, Molecular Cancer Therapeutics 2014)[98]	Yes	*FLT3/CDK4 dual kinase inhibitor; CDK6*	0.002 uM	Inhibition of CDK4, STAT5, and specific inhibition of FLT3 in MOLM-13 cell line	A dual FLT3 and CDK4 inhibitor
Pacritinib (SB1518) (Hart, Blood Cancer 2011)[99]		*FLT3/JAK2 dual inhibitor; MAPK, PI3K*	22 nM	Inhibition of FLT3 phosphorylation and STAT, MAPK, PI3K	A dual FLT3 and JAK2 inhibitor