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FLT3 inhibitors: A Story of the Old and the New

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Abstract

Purpose of the Review—Ever since the recognition that FLT3 mutations exert a profound negative prognostic impact on the clinical outcome patients with acute myeloid leukemia (AML), researchers have sought to find effective small molecule inhibitors of this receptor tyrosine kinase. This review will attempt to provide a survey of the FLT3 inhibitors currently under investigation and provide a discussion on their current status in clinical trials.

Recent findings—Over the past 10 years, a number of different compounds have been studied *in vitro* and clinically as FLT3 inhibitors. The first inhibitors studied were hampered by cumbersome pharmacokinetics and a general lack of potency. However, some agents have shown promise in clinical trials with transient responses in AML. Newer compounds, such as AC220, have demonstrated profound selectivity and potency against the FLT3 target, and are currently being investigated in clinical trials.

Summary—Clinical trials have so far demonstrated that inhibitors of FLT3 do have clinical activity in patients with FLT3-mutant AML, although this activity is often transient and correlates with effective *in vivo* suppression of the FLT3 target. As newer, more potent agents are now entering advanced clinical trials, opportunities will emerge for real progress against this grim disease.

Keywords

FLT3; acute myeloid leukemia; tyrosine kinase inhibitors; internal tandem duplication

Introduction

Internal tandem duplication mutation of FLT3 (FLT3/ITD mutations) occur in roughly 23% of newly-diagnosed de novo AML cases.¹ Over the course of the last ten years, a number of tyrosine kinase inhibitors have been found to effectively inhibit FLT3 and induce cytotoxicity in FLT3-ITD AML blasts.² Many of these agents were initially developed to target a variety of other tyrosine kinases and have been studied in both hematologic and solid tumor malignancies. These compounds include semaxinib (SU5416), sunitinib (SU11248), lestaurtinib (CEP-701) and midostaurin (PKC412). Lestaurtinib and midostaurin are the most extensively studied FLT3 inhibitors and the most advanced in clinical trials of AML. However, the relative lack of selectivity or potency of these agents against FLT3 has limited their utility in the treatment of AML, and to date, these agents have only resulted in transient and partial clinical responses. In parallel with these efforts, however, others have

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worked to develop drugs more specifically targeting the FLT3 kinase. The lessons learned from the early work in this field have cast a clearer light on the results from the newer drugs, and we appear at last to be on the verge of developing a clinically effective FLT3 inhibitor.

FLT3 as a therapeutic target

The receptor tyrosine kinase FLT3 was first cloned from the human genome in the early 1990s.³ It is a member of the so-called type III receptor tyrosine kinases, which also include KIT and PDGFR.⁴ Constitutive activating mutations of FLT3 were identified in AML patients and found to have negative prognostic impact.^{5–6} Soon after this followed a series of large studies of banked AML samples out of the European cooperative groups which established the profoundly negative effect of FLT3/ITD mutations on patient outcomes and provided significant impetus to develop FLT3 inhibitors.^{7–10}

FLT3 inhibitors: conclusions from pre-clinical studies

Over the past 10 years, well over a dozen different compounds have been examined in vitro and reported on as potential FLT3 inhibitors.^{11–24} In general, a FLT3 inhibitor induces a cytotoxic effect only in FLT3 mutated AML cells, and for that cytotoxic effect to occur, the inhibition needs to be to less than 10% of baseline activity, and it needs to be sustained over days.^{13,25} Therefore, presumably, for any FLT3 inhibitor to be successful clinically it must be able to accomplish this molecular feat in vivo. As trials of the early, non-selective compounds used to target FLT3 proceeded, a theme that emerged was that of insufficient in vivo inhibition.

FLT3 inhibitors: older, multi-targeted compounds

Semaxinib (SU5416), and indolinone-derived compound, was found in early studies to effectively inhibit a variety of tyrosine kinases, include c-KIT and VEGFR.^{26–27} It was studied in phase 2 trials of refractory AML and MDS where a few partial and transient hematologic responses were noted.^{28–29} Patients enrolled on these trials were not screened for FLT3 mutations. In AML, the agent was thought to induce cytotoxicity mainly through its inhibition of c-KIT and VEGF. However, given the structural homology of c-KIT and FLT3, semaxinib was evaluated for and found to have significant inhibitory effect on the FLT3 receptor tyrosine kinase in leukemia cell lines.¹⁸ A subsequent study analyzed myeloblasts obtained from patients with AML, and noted modest in vivo inhibition of FLT3 phosphorylation by semaxinib in a little over half of samples from treated patients.³⁰ The duration of inhibition was not examined. The downstream effects of FLT3 inhibition were noted in a subsequent study, when levels of STAT5 and Akt were downregulated after administration of semaxinib.³¹ In retrospect, the lack of any meaningful responses with this agent was certainly predictable from its relatively poor activity as a FLT3 inhibitor in vivo.

Sunitinib (SU11248) is a tyrosine kinase inhibitor with multiple targets, including PDGF, VEGFR, c-KIT, and FLT3.¹⁷ The agent has been extensively studied in clinical trials and developed for targeted therapy of multiple solid tumor malignancies.^{32–34} It is now approved for use in metastatic renal cell carcinoma and gastrointestinal stromal (GIST) tumors based on data from advanced clinical trials. This agent has also been studied as a FLT3 inhibitor in AML, with initial preclinical studies by O'Farrell et al demonstrating that sunitinib is a potent inhibitor of wild-type and mutant FLT3 in AML cell lines.¹⁷ A phase I trial assessed the activity of sunitinib in 29 patients with AML, and demonstrated that FLT3 inhibition was more pronounced in those with FLT3-ITD mutations and that toxicity (mainly gastrointestinal) affected a third of patients. Five patients in this study experienced decreases in peripheral blast counts.³⁵ Another phase I trial of 15 patients with refractory AML reported six transient partial responses after treatment with sunitinib.³⁶ A phase I/II

trial of sunitinib with induction chemotherapy in older patients with FLT3 mutant AML is currently enrolling patients (clinicaltrials.gov #NCT00783653). Although initially showing promise as a FLT3 inhibitor, due to poor tolerability, patients could not be treated with continuous dosing. This feature almost certainly precludes sunitinib from being a successful agent for the treatment of FLT3 mutant AML.

Lestaurtinib is a polyaromatic indolocarbazole compound which displays potent inhibitory activity against FLT3.¹³ However, this agent is also quite non-selective and effectively inhibits other tyrosine kinases, including RET, JAK2, and TRK (Camoratto Int J cancer 97, Strock Cancer res 03, Hexner Blood 08).^{37–39} Indeed, as with the compounds described above, this agent was initially studied in solid tumor malignancies, with a focus on its activity against trk tyrosine kinase. A phase I trial of Lestaurtinib in patients with advanced solid tumor malignancies demonstrated that the drug was well-tolerated, but that no objective tumor responses were noted.⁴⁰ Given the effective inhibition of FLT3 by lestaurtinib, studies in AML soon followed. Preclinical studies of lestaurtinib suggested that it is a potent inhibitor of FLT3 and promotes apoptosis in FLT3-ITD leukemic blasts.¹³

A phase I/II trial of 17 relapsed/refractory FLT3 mutant patients demonstrated that a sustained suppression of FLT3 phosphorylation, as measured by a plasma inhibitory assay (PIA), correlated with clinical response and transient decreases in peripheral blasts.^{41–42} A multi-center phase II trial followed, enrolling 29 previously untreated elderly patients and not restricted on the basis of FLT3 mutational status.⁴³ Five patients were found to have activating FLT3 mutations, three of which experienced transient hematologic responses. Five patients with wildtype FLT3 were noted to have bone marrow responses with decreases in bone marrow blasts. These results were attributed to possible over-expression of FLT3 in these patients. The phosphorylation of FLT3 from all eight responding patients was demonstrated to be effectively suppressed to below 15% of baseline activity. These findings suggested that effective and sustained inhibition of the FLT3 target is important for clinical responses.

The above studies demonstrated that lestaurtinib was reasonably well-tolerated in patients with AML, with the most common toxicity being nausea, and that FLT3 inhibition in vivo appears to correlate directly with modest clinical effects of short duration. However, at the doses used in these studies (60–80 mg twice daily), in vivo inhibition of FLT3 was by no means 100% effective.^{41,44} In vitro studies had demonstrated that lestaurtinib administration after chemotherapy leads to synergistic cytotoxicity,^{45–46} promoting the rationale of combining cytotoxic chemotherapy with this agent. A phase II multi-center trial of patients with relapsed AML randomized subjects to receive induction chemotherapy alone or followed by lestaurtinib was initiated. The type of chemotherapy varied depending on the duration of initial remission, with those whose remission was shorter than six months receiving MEC (an induction regimen of mitoxantrone, etoposide, and cytarabine), and those with longer remissions receiving high dose infusional cytarabine (HiDAc). Given encouraging preliminary findings, this study was expanded to a phase III trial, the results of which were presented at the 2009 meeting of the American Society of Hematology (ASH).⁴⁷ Blood samples from 79 of the 107 lestaurtinib-treated patients were analyzed for FLT3 inhibitory activity, and of these, only 46 achieved >85% FLT3 target inhibition by Day 15. This may have been partially explained by elevations in FLT3 ligand and AGP (alpha glycoprotein) levels during the same period, both of which may have reduced the degree of FLT3 inhibition. Although effective FLT3 inhibition did again appear to correlate with clinical response and an increase remission rate, given the above pharmacokinetic factors, the overall clinical response was limited and no benefit in overall survival was found.

There are currently other studies of lestaurtinib in combination with cytotoxic chemotherapy. In 2007, lestaurtinib was incorporated into induction and consolidation chemotherapy for patients with FLT3 mutations in the MRC AML15 trial in the UK, and continued in the AML17 trial. Although the final results of this trial are not yet available, initial findings suggest effective inhibition (>85% inhibitory activity) of FLT3 in the large majority (82%) of evaluated patients.⁴⁸ In addition, it was reported that 77 of 83 evaluable patients (93%) achieved a complete remission, and that the most significant independent predictor of eventual relapse was >95% FLT3 PIA at one or more time points following initiation of lestaurtinib.

The staurosporine derivative, midostaurin (PKC412), initially described as an inhibitor of protein kinase C, was later found to be able to also inhibit VEGF-R, PDGF-R, and c-KIT. Its anti-angiogenic and antiproliferative activities in vitro potential promised a broad applicability to a variety of solid and hematologic tumors.⁴⁹ A phase I trial of the agent in solid tumors revealed that the primary toxicities were gastrointestinal, that the agent was generally well-tolerated, but that there was significant protein binding of the compound.⁵⁰ Following this trial, published data revealed that PKC412 is also a potent inhibitor of the FLT3 tyrosine kinase and that it is preferentially cytotoxic to FLT3-ITD cell lines, prompting further investigation of agent in myeloid malignancies.¹⁵

A phase I trial of PKC412 in 20 patients with relapsed/refractory AML again found that the agent was fairly well-tolerated, with seven patients experiencing transient decreases in peripheral blast counts and five experiencing significant decreases in bone marrow blasts.⁵¹ Another trial in Europe yielded similar results.⁵² A phase Ib trial of midostaurin in combination with cytarabine- and daunorubicin-based induction chemotherapy in newly diagnosed patients followed. Promising data, based on a small number of patients, was presented at the annual ASH meeting in 2009, with FLT3-mutant patients experiencing similar 2 year overall survival (62%) as compared to those patients with FLT3-WT AML.⁵³ The above results have prompted an ongoing randomized multi-center CALGB study of midostaurin with induction and consolidation chemotherapy, followed by midostaurin maintenance in newly diagnosed patients (clinicaltrials.gov #NCT00651261).

Sorafenib, a multi-kinase inhibitor, was initially developed to inhibit the Raf-1 kinase pathway. Sorafenib has since been approved for use in advanced renal cell and hepatocellular carcinomas, after improving survival parameters in phase III clinical trials.^{54–55} Sorafenib has also been demonstrated to be a potent inhibitor of multiple other receptor tyrosine kinases, including FLT3.⁵⁶ Many of the enzymes targeted by sorafenib, including FLT3, c-KIT, NRAS, and Raf kinase, are frequently mutated in AML and appear to arrest differentiation and drive proliferation in myeloblasts.⁵⁷ Preclinical studies of FLT3-mutant leukemic cell lines and patient samples demonstrated that sorafenib suppresses FLT3 phosphorylation and downstream signaling, and promotes apoptosis.^{21,58} Importantly, unlike the previous multi-targeted agents, sorafenib induced far more effective, sustained FLT3 inhibition in vivo. This may be the result of an N-oxide metabolite that is generated following administration of sorafenib. The metabolite was reported to be a more potent FLT3 inhibitor than the parent compound.⁵⁹

Emerging clinical data now suggest that sorafenib is well tolerated as a single agent in AML, and that it can promote transient, but significant, decreases in bone marrow blasts, particularly in patients with FLT3-ITD mutations.^{59–60} Investigators have reported dramatic cases of complete patient response to single agent sorafenib (Safaian Leuk Res 2009; Lee et al Am J Hematol 2009). Others have also described the use of sorafenib in the transplant setting. In one report, it was used, on a compassionate use-basis, in a small number of patients with refractory FLT3-mutant AML before and after allogeneic stem cell

transplantation.⁶¹ Two of three patients given sorafenib were able to proceed to transplant after remissions. Prolonged complete molecular remissions were also reported in a few patients post-transplant in this study. More recently, a phase I/II trial of 61 patients with newly diagnosed AML found that sorafenib, combined with cytarabine- and idarubicin-based induction, led to high rates of complete remission (88%) in the evaluable patients.⁶² Of note, eight patients who experienced a CR had FLT3-ITD mutations, and the drug was reported to effectively suppress FLT3-phosphorylation in correlative studies. There are now multiple ongoing trials evaluating the safety and efficacy of sorafenib in combination with induction regimens and multiple other cytotoxic agents (clinicaltrials.gov, NCT00516828, NCT00908167, NCT 00373373, NCT00893373, NCT00875745, NCT00943943).

FLT3 inhibitors: newer agents

The agents reviewed above are quite non-selective for FLT3, and indeed, were initially developed against other targets. This non-selectivity may enhance efficacy in some settings, such as newly diagnosed AML, since multiple pathways in addition to FLT3 may drive the proliferation of the tumor. However, non-selectivity also portends a broader range of toxicity, and in general, lower overall potency, properties which likely limit the therapeutic utility of these agents. In addition, FLT3-mutant AML in the relapsed setting often exhibits a high FLT3-mutant allele burden, and is addicted to and driven primarily by the constitutively active FLT3 receptor.⁶³ In such a scenario, more selective and potent targeted agents would theoretically be more active. In addition to the compounds already described, several have been developed to specifically target FLT3. These include tandutinib (MLN518/CT53518), KW-2449, and AC220.^{14,23–24}

Tandutinib (MLN518, previously referred to as CT53518) is quite selective for the FLT3 tyrosine kinase, but also displays inhibitory effects on c-KIT and PDGFR, both of which share significant structural homology to FLT3.¹⁴ In vitro studies found that tandutinib was preferentially cytotoxic against FLT3-ITD cell lines and inhibited cell signaling through the MAP kinase and PI-3 kinase pathways downstream of the FLT3 receptor.^{14,64} A phase I trial of tandutinib in patients with relapsed/refractory AML or high risk MDS suggested that tandutinib was fairly well-tolerated, though a significant degree of nausea and vomiting were reported as well as a dose-limiting toxicity of generalized muscle weakness.⁶⁵ The pharmacokinetics of the agent was not ideal with slow elimination and resultant elevated plasma concentrations in some patients. Furthermore, while it was very selective for type III receptor tyrosine kinases, it was not particularly potent. However, a fraction of the patients experienced partial transient responses with decreases in peripheral and bone marrow blasts. Tandutinib was also studied in combination with traditional induction agents, cytarabine and daunorubicin, and reported to display strong synergistic activity, specifically in FLT3-ITD samples.⁶⁶ Results of a recently concluded phase I trial of tandutinib in combination with induction therapy are not yet available (clinicaltrials.gov #NCT00274248).

KW-2449 is a tyrosine kinase inhibitor that effectively suppresses FLT3 phosphorylation, along with that of the Abl and aurora kinases.²³ This agent inhibited growth of FLT3-mutant leukemia cell lines and suppressed phosphorylation of FLT3 and its downstream target, STAT5. A phase I trial of KW-2449 demonstrated modest single agent clinical activity in 8 of 31 AML patients (26%), the majority of whom harbored FLT3 mutations.⁶⁷ As with other FLT3 inhibitors, the clinical responses consisted of transient decreases in blast count, which correlated with in vivo FLT3 inhibition. Correlative studies from this trial suggest that while FLT3 inhibition in vivo was effective, it was also quite transient, owing to the very short plasma half-life of the drug.²⁵ The experience with this agent underscores the impression that sustained FLT3 inhibition will be necessary if meaningful clinical responses are to be achieved.

AC220 is the most recent kinase inhibitor of FLT3 under clinical investigation. It has been demonstrated to have very selective in vitro and in vivo activity against FLT3.^{24,68} In comparison with other FLT3 inhibitors, AC220 appears to be 1–2 orders of magnitude more potent in vivo. In addition, it has ideal pharmacokinetics in that it has a very long plasma half-life. A phase I study in relapsed or refractory AML reported very promising preliminary results, with 11 of 45 patients (24%) experiencing transient clinical responses, and 4 patients achieving CR. Of note, three of the responders were patients with FLT3 mutations, but the rest exhibited wild-type FLT3.⁶⁹ Based on this promising phase I data, a phase II trial of AC220 in relapsed/refractory patients with FLT3-ITD activating mutations is currently enrolling (clinicaltrials.gov #NCT00651261).

Conclusion

FLT3-mutant AML has an especially poor prognosis, and is associated with significant leukocytosis and early relapse. A series of tyrosine kinase inhibitors have been identified as inhibitors of FLT3, and have been studied in clinical studies. The first generation of these agents has shown some promise, but these drugs have been mainly limited by suboptimal pharmacokinetics and specificity. Newer, more selective compounds, such as AC220, appear to offer more promise. Preclinical studies of this agent have demonstrated significant potency and specificity, and an early phase trial has reported a number of complete remissions.

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