Published in final edited form as:

Best Pract Res Clin Haematol. 2010 December ; 23(4): 489–494. doi:10.1016/j.beha.2010.09.008.

Will newer tyrosine kinase inhibitors have an impact in AML?

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Abstract

FLT3 inhibition has been a goal of acute myeloid leukemia (AML) therapy since *FLT3* mutations were discovered to have a role in AML. Several FLT3 inhibitors have been developed in the last several years, beginning with less potent, less selective agents. The newer FLT3 inhibitors appear to be more potent in vivo and have shown more promise than the older agents in monotherapy trials.

Keywords

Acute myeloid leukemia; AML; FLT3; Inhibitor; Lestaurtinib; Midostaurin; Sorafenib; AC220; KW-2449

Introduction

The successful development of a FLT3 inhibitor that meaningfully improves outcomes for acute myeloid leukemia (AML) patients has been a holy grail of the field for several years now. As with that mythical chalice, is the object of the current quest ever receding, or has it at last drifted almost within our reach? In fact, on reviewing the progress over the last decade, it can be argued that the development of FLT3 inhibitors has actually followed an orderly progression, and that the first important phase in development is drawing to a conclusion.

From the original discovery of the internal tandem duplication (FLT3/ITD) mutations that cause constitutive activation of the FLT3 tyrosine kinase to the subsequent large studies out of the European and US cooperative groups that confirmed their strong negative prognostic impact, the efforts to identify and characterize candidate compounds has followed a rather predictable course [1–8]. The initial approach was to try “off-the-shelf” drugs that were already under investigation for other targets. In parallel with these efforts, pharmaceutical companies began from scratch, searching for compounds uniquely targeted to FLT3. The older drugs moved relatively rapidly into advance phase clinical trials, and while the results seemed disappointing, important information was nonetheless gleaned. The lessons learned from the earlier efforts cast a clearer light on the early results from the newer drugs, and we appear at last to be on the cusp of success. The real question here is just how much will an optimally designed FLT3 inhibitor benefit an AML patient with a FLT3 mutation. In other words, is the quest really worth the effort?

Conflict of interest statement

Mark J. Levis, MD, PhD, Consulting Fees: Ambit Biosciences Inc., Cephalon Inc.

The FLT3 tyrosine kinase

The receptor tyrosine kinase FLT3 was first cloned from the human genome in the early 1990s.[9] Constitutive activating mutations were identified and characterized primarily in studies taking place in Japan in the late 1990s,[1,2,10] and then followed a series of large studies of banked AML samples out of the European cooperative groups [3,5,6]. These reports established the profoundly negative effect of FLT3/ITD mutations on patient outcomes and provided significant impetus to develop FLT3 inhibitors.

Most kinase inhibitors are either adenosine triphosphate (ATP) analogues outright, or bear a structural resemblance to the intermediate between ATP and the residue being phosphorylated [11]. As such, kinase inhibitors are often rather promiscuous in their activity, a property that is often euphemistically termed “multitargeted.” With FLT3 now having established credentials as a potential therapeutic target, a wide variety of kinase inhibitors already under investigation for other malignancies were recognized as having activity against FLT3 [12]. These compounds represented the first generation of FLT3 inhibitors to be tested clinically. In parallel with this, efforts were launched to identify novel kinase inhibitors specifically for FLT3 inhibition. Over the past decade, therefore, the development of a FLT3 inhibitor has proceeded along two fronts. The first was to try the less selective multitargeted agents that were immediately available for clinical trials. The second was to start from scratch and proceed with preclinical and early phase clinical development of more selective and specific FLT3 inhibitors.

Is the targeted therapy hitting the target?

As a targeted therapy such as a kinase inhibitor moves into clinical trials, it is of paramount importance to establish whether or not the target in question is actually being inhibited. That is, while preclinical studies invariably demonstrate potent inhibition of tyrosine kinase activity, with resultant cytotoxic effects, it is technically much more challenging to prove that the same degree of inhibition is achieved *in vivo* in sustained fashion. In this regard, the development of a surrogate assay known as the plasma inhibitory activity (PIA) assay has been of some utility [13]. The PIA assay is carried out by isolating the plasma from blood collected from a patient receiving a FLT3 inhibitor, then using that plasma to assess inhibition of FLT3 in an established FLT3/ITD cell line. While not a direct measure of kinase activity in patient leukemia cells, this assay evaluates the FLT3 inhibitor’s ability to inhibit the target after any metabolism and plasma protein binding have occurred. This approach has been validated in studies of five inhibitors (lestaurtinib, midostaurin, sorafenib, KW-2449, and AC220) and can provide data that can complement conventional pharmacokinetics [13–18].

FLT3 inhibitors in clinical development

These five FLT3 inhibitors—lestaurtinib, midostaurin, sorafenib, KW-2449, and AC220 are currently being evaluated in clinical trials. The first three of these (lestaurtinib, midostaurin, and sorafenib) are older multitargeted compounds that happened to also inhibit FLT3 [19–21]. These moved relatively quickly into clinical trials [14,22,23]. The latter two (KW-2449 and AC220) were more recently designed and more specifically developed as FLT3 inhibitors [24,25]. Using the PIA assay on plasma samples from patients treated with these five inhibitors reveals the striking differences between *in vitro* activity and *in vivo* activity. These differences are a reflection of the effects of both plasma protein binding and drug metabolism. The IC₅₀ values for inhibition of mutant FLT3 in culture medium and plasma, as well as an estimate of plasma half-life, are summarized in Table 1. While the IC₅₀ values for each of these drugs in cell culture medium are quite similar, the potencies in plasma differ by orders of magnitude. Here we see that the indolocarbazole inhibitors (midostaurin

and lestaurtinib) are 40-50-fold less potent in plasma than the newer compound AC220. Furthermore, the plasma half-life of these agents as determined from phase 1 pharmacokinetic studies are likewise highly variable [26,27]. Given that most cytotoxic effects of FLT3 inhibition are achieved in vitro with continued suppression of FLT3 over days, a compound with a short in vivo half-life is at a serious disadvantage.

Selectivity towards the target kinase is another parameter to be considered in this comparison of inhibitors. Four of the five FLT3 inhibitors listed in Table 1 have been evaluated for selectivity by a variety of in vitro kinase assays using panels of kinases. The conclusion that can be drawn from these studies is that lestaurtinib and midostaurin are highly promiscuous kinase inhibitors, while AC220 appears to be the most selective [13,28]. Sorafenib (and probably KW-2449) is somewhere in between. The general pattern that emerges, then, is that the early FLT3 inhibitors were less selective and less potent in vivo (but immediately available for study), while the newer generation compounds were more potent and more directly targeted at FLT3.

With this information about each of these inhibitors, the emerging results from clinical studies can be better understood.

Lestaurtinib (CEP-701)

This indolocarbazole derivative was initially developed as an inhibitor of TrkA, then subsequently characterized as a FLT3 inhibitor [19,29]. Two separate trials of lestaurtinib as a single agent yielded evidence of modest clinical activity, although no actual remissions were achieved in any FLT3 mutant patients [14,15]. In the company-sponsored trial Cephalon 204 and in the United Kingdom's Medical Research Council trial AML15, relapsed and newly diagnosed AML patients, respectively, were randomized to receive chemotherapy followed by lestaurtinib or chemotherapy alone [30,31]. All patients in these trials had *FLT3* ITD mutations. The newly diagnosed patients, those in AML15, had much more effective FLT3 inhibition than the relapsed patients, who were part of the 204 trial. Preliminary reports from both trials were encouraging, but the final results from the Cephalon 204 trial, reported at the American Society of Hematology meeting in December 2009, were disappointing. FLT3 inhibition in vivo correlated with remission rate, but treatment with lestaurtinib did not lead to any improvement in overall survival. Lestaurtinib's complex pharmacokinetics and overall lack of in vivo potency appear to be major obstacles to this drug's being of any utility for this disease.

Midostaurin (PKC412)

Midostaurin is the other major indolocarbazole derivative currently being investigated as a FLT3 inhibitor. Like lestaurtinib, this drug was originally developed for use against a different target (protein kinase C) and was found to have activity against FLT3 in vitro [20]. In vivo, as monotherapy, the drug was found to be reasonably potent at a dose of 75 mg administered three times a day [13,22]. In the ongoing RATIFY trial, however, in which the drug is being administered following chemotherapy, the dose is 50 mg twice a day [32]. It remains to be seen how effectively FLT3 will be inhibited in vivo in this context. As with lestaurtinib, complicated pharmacokinetics and off-target effects from its relative lack of selectivity may ultimately limit midostaurin's utility.

KW-2449

KW-2449 is a novel compound with potent activity against FLT3 and, curiously, the T315I variant of BCR-ABL [24]. The compound was tested in a phase 1 trial in relapsed or refractory AML patients [16]. While the drug was confirmed to be a potent inhibitor of FLT3 in vivo, a different type of pharmacokinetic problem surfaced. KW-2449 proved to

have a very short half-life in vivo. The limited clinical activity of a compound that could only inhibit FLT3 for a few hours a day quickly became evident, and development of KW-2449 as a FLT3 inhibitor was discontinued. Nonetheless, KW-2449 serves as a useful illustration of the importance of sustained FLT3 inhibition for clinical benefit.

Sorafenib

Sorafenib was first developed as an inhibitor of raf kinase [21]. In clinical trials of solid tumor patients, significant activity was observed in renal cell carcinoma and hepatocellular carcinoma [33,34]. The exact target remains unclear, although inhibition of the vascular endothelial growth factor receptors (VEGFR) remains a distinct possibility. When administered as monotherapy, sorafenib seems to be much more effective than either lestaurtinib or midostaurin at inhibiting FLT3 in vivo [18]. When sorafenib is metabolized by the liver, an N-oxide metabolite of sorafenib is produced. This metabolite is a more potent FLT3 inhibitor than the parent compound; in plasma, the IC₅₀ of sorafenib is 308 nM, while the IC₅₀ of sorafenib N-oxide is 21 nM [17]. The combination of parent and metabolite brings the in vivo IC₅₀ below 300 nM. Furthermore, the drug has a relatively long half-life in vivo. This combination of in vivo potency and half-life makes it a more effective FLT3 inhibitor than either KW-2449 or the indolocarbazoles. Possibly in confirmation of this, monotherapy of FLT3/ITD AML with sorafenib can induce remissions, albeit in somewhat sporadic fashion [35,36]. When the agent was combined with chemotherapy, it was well-tolerated, but of unclear efficacy.[18] It has not yet been tested in a randomized trial, but several such trials are in the planning stages.

AC220

The newest FLT3 inhibitor to arrive on the scene is AC220. This drug is actually the first agent specifically designed with the intent of targeting FLT3 [25]. Preliminary in vitro studies suggest it is the most potent and selective FLT3 inhibitor identified to date, and the phase 1 trial, intriguingly, yielded a number of complete remissions. AC220 monotherapy, even at very low doses, is effective in completely inhibiting both mutant and wild-type FLT3 [37]. Furthermore, FLT3 inhibition continues for more than a day after AC220 is administered, suggesting that it has a half-life longer than a day [38]. A multicenter phase 2 trial of AC220 monotherapy in FLT3/ITD AML patients is currently accruing, and combination trials of chemotherapy and AC220 are in the planning stages.

Conclusion

The perception that the clinical development of a FLT3 inhibitor is proceeding slowly is, perhaps, a reflection of the impatience of physicians treating this terrible disease. On review of the work over the past 10 years, it seems we are actually making progress. Midostaurin and lestaurtinib are broad-spectrum kinase inhibitors with some activity against FLT3. As such, results from trials of these agents should be interpreted with extreme caution. It is still important to note that a great deal was learned from the early FLT3 inhibitor trials. We learned that the only AML patients likely to benefit from them were those harboring *FLT3* activating mutations. We have learned that in vivo FLT3 inhibition correlates with response. We are just on the cusp of seeing what sustained in vivo FLT3 inhibition can do with this disease, both as monotherapy and in combination with chemotherapy. It seems as if the grail is almost within our reach.

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Table 1

FLT3 inhibitors, with comparison of IC₅₀ estimates for inhibition of FLT3/ITD autophosphorylation in cell culture medium versus plasma.

	IC ₅₀ , culture medium	IC ₅₀ plasma	Plasma half-life
Lestaurtinib	2 nM ¹⁹	700 nM ¹³	8–12 hours ²⁷
Midostaurin	6 nM ¹³	1000 nM ¹³	~ 1 day ¹³
Sorafenib	3 nM ¹⁷	265 nM ¹⁷	~ 1 day ¹⁷
KW-2449	13 nM ¹⁶	144 nM ¹⁶	4 hours ¹⁶
AC220	1 nM ²⁵	18 nM ³⁸	>1 day ³⁷