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Incorporating FLT3 inhibitors into acute myeloid leukemia treatment regimens

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Abstract

FMS-Like-Tyrosine kinase-3 (FLT3) mutations are found in about 30% of cases of acute myeloid leukemia and confer an increased relapse rate and reduced overall survival. Targeting of this tyrosine kinase by direct inhibition is the focus of both preclinical and clinical research in AML. Several molecules in clinical development inhibit FLT3 with varying degrees of specificity. Preclinical models suggest that these compounds enhance the cytotoxicity of conventional chemotherapeutics against FLT3 mutant leukemia cells. The pharmacodynamic interactions between FLT3 inhibitors and chemotherapy appear to be sequence dependent. When the FLT3 inhibitor is used prior to chemotherapy, antagonism is displayed, while if FLT3 inhibition is instituted after to exposure to chemotherapy, synergistic cytotoxicity is seen. The combination of FLT3 inhibitors with chemotherapy is also complicated by potential pharmacokinetic obstacles, such as plasma protein binding and *p*-glycoprotein interactions. Ongoing and future studies are aimed at incorporating FLT3 inhibitors into conventional induction and consolidation therapy specifically for patients with FLT3 mutant AML.

Keywords

Pharmacotherapeutics; signal transduction; signalling therapies; myeloid leukemias and dysplasias

Introduction

Activating mutations of the receptor tyrosine kinase (RTK) FLT3 are one of the most common molecular abnormalities found in acute myeloid leukemia (AML). The presence of these mutations, in general, implies a poor prognosis, and for the last several years efforts have been underway throughout the world to develop a targeted therapy for this subtype of AML. More than 20 different small molecule inhibitors of FLT3 kinase activity have been introduced into the literature, several of which have advanced reasonably far in clinical trials. The general consensus in the field is that FLT3 inhibitors given as monotherapy will not measurably impact outcomes in AML patients harbouring FLT3 mutations, but that the addition of these drugs to chemotherapy holds great therapeutic promise. However, the best way of incorporating these compounds into standard treatment regimens for the disease remains unclear. The purpose of this review is to summarize the available data on combining FLT3 inhibitors with AML-directed chemotherapy, from *in vitro* models to animal systems to ongoing clinical trials, and to determine if these combinations show evidence of synergistic anti-leukemic effects.

FLT3

The human FLT3 (FMS-Like Tyrosine Kinase 3) gene was cloned from a stem cell-derived cDNA library over 15 years ago [1]. The protein contains 993 amino acids and is visualized as a doublet, consisting of a mature (glycosylated) form and an immature form, on electrophoretic gels [2]. FLT3 contains an extracellular ligand binding domain, a transmembrane domain, and, intracellularly, a juxtamembrane domain and tyrosine kinase domain. The kinase domain is interrupted by a short hydrophilic insert sequence, which allows FLT3 to be categorized with a group of RTKs sharing this structural feature: KIT, FMS, PDGF-R (α and β) and the VEGF receptors [3]. The homology shared within this “split-kinase domain” family of RTKs explains why small molecule inhibitors of FLT3 often have potent activity against these other receptors [4]. The juxtamembrane domain of FLT3, as with many other receptors, exerts a negative regulatory influence upon the tyrosine kinase activity [5,6]. Mutations within this juxtamembrane region can disrupt its negative regulatory functions, and this domain is the site of the most common and important of the FLT3 activating mutations, the internal tandem duplication (FLT3/ITD) mutations [4].

Upon binding FLT3 ligand (FL), FLT3 dimerizes, which in turn leads to a conformational change in its activation loop, allowing ATP access to the FLT3 active site. The dimerized receptor undergoes autophosphorylation, and subsequently transduces signals, via its kinase activity, to pathways that inhibit apoptosis and differentiation, and promote proliferation. Proteins within these pathways include Ras-GAP, PLC- β , STAT5, ERK1/2, Foxo proteins and Pim1 and Pim2 [7–16]. FLT3 has a fairly narrow range of cell expression, being localized primarily to hematopoietic and neural tissues, which presumably confines its functions to these cell types [2]. In bone marrow, FLT3 is expressed the CD34⁺ fraction of hematopoietic cells, and in a smaller fraction of CD34[−] cells destined to become dendritic cells [17]. In contrast, its ligand is expressed in virtually all cell types thus far examined [18,19]. FL acts in synergy with other cytokines to promote hematopoietic precursor expansion, and targeted disruption of either FLT3 or FL in mice leads to a reduction in hematopoietic precursors (although such disruption is non-lethal) [20–27].

FLT3 in leukemia

The FLT3 receptor is expressed on the blasts in most cases of AML, but unlike hematopoietic precursors, FLT3 expression is no longer tightly coupled with CD34 expression [28–32]. In 1996, a polymerase chain reaction (PCR) screen of AML cases revealed a subset of patients whose leukemia cells harboured internal tandem duplication mutations within the FLT3 gene [33]. Subsequent work revealed that these FLT3/ITD mutations disrupted the negative regulatory function of the juxtamembrane domain of FLT3, leading to constitutive tyrosine kinase activation [6,34,35]. Following the discovery of the FLT3/ITD mutations, point mutations at amino acid residue D835 (in the activation loop of the kinase domain) were identified [36,37]. These mutations are analogous to the mutations occurring at residue D816 of KIT, and likewise constitutively activate FLT3. Following these initial observations, dozens of studies comprising the results of screening more than 5000 adult and paediatric AML samples have been published [38–50]. From these studies, FLT3/ITD mutations can be estimated to occur in 22.9% of *de novo* AML (i.e. AML not arising from pre-existing myelodysplasia) and their presence clearly confers a worse prognosis [4]. D835 mutations occur in roughly 7% of cases, with a less certain clinical impact. The typical AML patient with a FLT3/ITD mutation presents with pronounced leukocytosis, a hypercellular bone marrow and intermediate risk cytogenetics. The complete remission (CR) rate for these patients is generally reported to be similar to non-mutant AML patients, but the rate of relapse is much higher. Overall, FLT3 mutations now represent one of the most common molecular abnormalities in AML, and the large body of data regarding

the incidence and prognostic impact of FLT3 mutations has engendered tremendous interest in developing FLT3 inhibitors for therapeutic use in these patients [51].

FLT3 inhibitors

More than 20 compounds have been reported to have inhibitory activity against FLT3, 15 of which are listed in Table I. Several of these agents have now been tested in clinical trials [74–78]. The FLT3 inhibitors characterized to date are heterocyclic compounds that either act as ATP competitors, or structurally resemble the intermediary complex of a tyrosine covalently bound to ATP. Crystal structure data from other drug-receptor combinations, as well as from studies of the FLT3 receptor allow some speculation about the structure activity relationships of these inhibitors [79–81]. While most of them likely fit into the ATP binding pocket of FLT3, the exact mechanism probably varies from inhibitor to inhibitor. FLT3 inhibitors have been found to have variable potency against different activating mutations [82]. This is perhaps not a surprising finding, since FLT3 activating mutations all likely have direct influence over the ATP-binding pocket where the inhibitors bind.

FLT3 inhibitors are selectively cytotoxic to leukemia cells that harbour FLT3 activating mutations. This applies to model cell lines transfected with mutant FLT3 constructs so as to confer growth factor independence (such as the murine 32D or Ba/F3 lines), AML cell lines with naturally occurring FLT3 mutations such as MV4-11 and Molm-13, and primary AML cells harbouring FLT3 mutations. Most of the inhibitors, in contrast, have little or no effect on cells lacking activating FLT3 mutations. The activating mutation, then, serves as a marker of a cell that is relatively dependent (or “addicted”) on this oncogene for growth and survival. This phenomenon is similar to that seen with other kinase inhibitors targeted to different malignancies, such as EGF receptor inhibitors in lung cancer, or imatinib in gastrointestinal stromal tumours (GIST).

All of the compounds in Table I have been shown to induce apoptosis in FLT3-dependent cell lines. However, the cytotoxic effects in many cases are not necessarily exclusively due to FLT3 inhibition. In general, FLT3 inhibitors are only selective for FLT3, not specific. Each one inhibits other kinases (and potentially, a wide variety of cellular enzymes) with variable potency, and this degree of non-selectivity for FLT3 likely contributes to the cytotoxic effect against FLT3-expressing cell lines. The less selective the agent is for FLT3 (i.e. the more non-specific it is), the more generally cytotoxic it is to cell lines, irrespective of the FLT3 mutation status. While generalized cytotoxicity against leukemia cells may seem to be desirable, such a property would also be expected to narrow the therapeutic index of an inhibitor.

Four of the compounds listed in Table I have been tested in clinical trials specifically to assess their efficacy in AML patients harbouring FLT3 mutations: Lestaurtinib (CEP-701), Midostaurin (PKC412A), Sunitinib (SU11248) and Tandutinib (MLN518). All four drugs were demonstrated to inhibit FLT3 phosphorylation *in vivo* in significant numbers of patients. Each displayed essentially the same type of modest clinical activity, namely the clearance of peripheral blood leukemia cells. Only occasional effects on bone marrow were seen, and all responses were relatively transient, lasting weeks to months. Admittedly, the patients in most of these trials were heavily pre-treated and/or refractory (although one trial used a FLT3 inhibitor in untreated elderly patients) [83], so conclusions regarding their limitations as monotherapy may be somewhat premature. Nonetheless, it seems clear that while FLT3 inhibition is a biologically active and well tolerated therapy, these agents will have to be used in combination with other agents in order to achieve their maximum clinical benefit.

Combining FLT3 inhibitors with chemotherapy: Preclinical models

Ph⁺ ALL provides an interesting parallel to FLT3 mutant AML, in that monotherapy with a tyrosine kinase inhibitor, imatinib, leads to transient clinical responses in this disease. *In vitro* studies suggested that synergistic activity would result when imatinib was combined with chemotherapy [84,85], and subsequent clinical studies have borne this out [86–88]. Incorporation of imatinib is now a standard in the treatment of Ph⁺ ALL. Several FLT3 inhibitors have since been tested *in vitro* in combination with conventional DNA-damaging chemotherapeutic agents.

Lestaurtinib (CEP-701)

This indolocarbazole derivative was initially introduced as an inhibitor of TrkA for possible use in prostate cancer [89] but was recognized subsequently as a FLT3 inhibitor [52]. Lestaurtinib is quite potent against FLT3 *in vitro*, with a 2 nM IC₅₀ for inhibition of autophosphorylation using cells incubated in culture medium supplemented with 10% serum. In human plasma, however, the drug is highly bound to α -1-acid glycoprotein (AAG), which shifts to IC₅₀ 350-fold upwards, to 700 nM [90].

Lestaurtinib has been examined in combination with chemotherapy in a series of *in vitro* studies. In the first study [91], lestaurtinib in combination with cytarabine, daunorubicin, etoposide and mitoxantrone was tested in FLT3 mutant cell lines as well as in a primary AML sample harbouring a FLT3/ITD mutation. Three treatment sequences were examined: Pre-treatment of leukemia cells with lestaurtinib followed by one of the chemotherapeutic agents, simultaneous treatment with lestaurtinib and chemotherapy, and, finally, exposure of cells to chemotherapy followed by lestaurtinib. Pre-treatment with lestaurtinib antagonized the cytotoxic effects of the chemotherapy. FLT3 inhibition induced a cell cycle arrest (confirmed by propidium iodine staining and FACS analysis showing decreased cells in S phase), which presumably blunted the S-phase selective effects of agents such as cytarabine and etoposide. Simultaneous treatment with chemotherapy, or lestaurtinib exposure after chemotherapy induced synergistic cytotoxicity. However, a potential pharmacodynamic interaction between lestaurtinib and daunorubicin was identified – because both drugs are bound in plasma to AAG, free levels of the anthracycline could increase if they were to be administered simultaneously. Therefore, the conclusion drawn from this work was that chemotherapy treatment followed by the FLT3 inhibitor offered the most potential for safety and efficacy.

In another study, two indolocarbazole FLT3 inhibitors – lestaurtinib and midostaurin – were compared for cytotoxic effect against primary AML cells, alone and in combination with cytarabine [83]. Lestaurtinib was found to be synergistic with cytarabine in inducing cytotoxicity in primary samples with mutant, but not wild type, FLT3.

The timing of drug delivery was examined in a study of lestaurtinib using primary blasts from MLL rearranged ALL cells [92]. In this study, leukemia cells were treated with the FLT3 inhibitor before, during and after exposure to standard induction agents (vincristine, daunorubicin, etoposide, cytarabine, dexamethasone and L-asparaginase). Lestaurtinib induced synergistic cytotoxicity when administered after cells were exposed to chemotherapeutic agents. The effects were additive when the agents were given at the same time, and, as with the AML study [91], there was antagonism when cells were treated with lestaurtinib prior to exposure to chemotherapeutic agents. These results reinforced the importance of timing and sequence in maximizing the efficacy of these combinations, and provide the basis for the rational design of clinical trials.

Sunitinib (SU11248)

An indolinone derivative, this agent inhibits FLT3, PDGF-R, VEGF-R 2 and KIT with similar potency [54,93]. Sunitinib was found to synergize with cytarabine- and daunorubicin-induced cytotoxicity in FLT3/ITD-transformed murine cells and MV4-11 cells [94]. Synergy was likewise seen with these combinations in primary AML cells harbouring FLT3/ITD mutations. The FLT3 inhibitor and chemotherapies were used simultaneously – no specific treatment sequences were studied.

Tandutinib (MLN518)

A piperazinyl quinazoline derivative, this agent inhibits FLT3, PDGF-B and KIT with similar IC₅₀ values [56,95]. There are no published reports examining the *in vitro* effects of combinations of tandutinib with chemotherapy for AML. However, in an important earlier study, tandutinib was administered to mice that had received myelosuppressive doses of cyclophosphamide and was shown to preferentially inhibit FLT3 ITD blast formation when compared with FLT3 negative controls [96]. Furthermore, the drug had only minimal effect on recovery of hematopoiesis following marrow transplantation in this mouse study.

Midostaurin (PKC412)

As described above, this indolocarbazole derivative was tested in combination with cytarabine against a series of primary AML samples, both wild type FLT3 and FLT3 mutant, for synergistic cytotoxicity. Interestingly, very little cytotoxicity was induced in either sample group by concentrations of midostaurin sufficient to inhibit FLT3. This result seemed to contrast with the results from the clinical trial in which a significant proportion of AML patients with FLT3 mutations responded to monotherapy with midostaurin. However, recent work has suggested that much of the clinical effect of this drug in humans is due to the effects of a metabolite (CGP52421) that inhibits FLT3, but is less selective (and more generally cytotoxic) than the parent compound [90]. Therefore, while no synergy was observed when midostaurin was combined with cytarabine in any of these AML samples, it is quite possible that synergy would have resulted from a combination of the more active metabolite and chemotherapy.

In recent work, Mollegard et al. examined sequential *in vitro* combinations of midostaurin with cytarabine and daunorubicin in MV4-11 cells (FLT3/ITD mutant), HL60 cells (non-FLT3-dependent), as well as in a series of primary patient samples [97]. While the FLT3 mutant cells tended to respond synergistically to these combinations more often than the FLT3 wild type cells, there was no discernible pattern of response with regard to the sequence of administration. However, the concentration of midostaurin used in the combinations studied was 200 nM, which is an order of magnitude in excess of the concentrations needed to inhibit FLT3 [53]. Thus, the interactions observed in this study may have had little to do with FLT3 inhibition. Furthermore, it is unlikely that the equivalent concentration could be achieved in patients, owing to the high degree of plasma protein binding seen with midostaurin (a point conceded by these investigators) [77,90,98].

Furukawa et al., examined simultaneous combinations of midostaurin with cytarabine, doxorubicin, idarubicin, mitoxantrone, etoposide, 4-HC, methotrexate and vincristine in three leukemia cell lines with FLT3 mutations and five without [99]. Midostaurin was synergistic with all agents except methotrexate in the FLT3 positive cell lines, but antagonistic in the FLT3 negative cell lines. Cell cycle arrest after exposure to midostaurin occurred at G1 phase in the FLT3 mutant lines, but not in the cell lines with wild type FLT3.

Sorafenib (BAY43-9006)

Sorafenib is a bi-aryl urea that was originally developed as an inhibitor of B-RAF. Subsequent *in vitro* studies have shown it to be a multi-targeted tyrosine kinase inhibitor, with activity not only against RAF kinase, but also against the VEGF receptors, wild type and ITD-mutated FLT3, the PDGF receptors, and RET kinase [70]. The drug has been studied extensively *in vitro* against human leukemia cell lines in general [100,101] and against FLT3 mutant lines in particular [102–104]. There are no published *in vitro* studies in which sorafenib was combined with conventional leukemia chemotherapy. However, it has been studied in combination with bortezomib and vorinostat and was found to be synergistic in both cases [105,106].

ABT-869

ABT-869 is a multi-targeted kinase inhibitor with *in vitro* activity against FLT3, KIT, CSF-1R, VEGFR-1 and VEGFR-2 [107]. This compound was evaluated in combination with cytarabine and doxorubicin in MV4-11 and Molm-13 cells (both FLT3/ITD mutated), and in primary leukemia samples [108]. Again, pre-treatment of the cell lines with the FLT3 inhibitor antagonized the efficacy of cytarabine or doxorubicin, while treating the cells with chemotherapy followed by FLT3 inhibition led to synergistic cytotoxicity. Inhibition of the MAPK pathway appeared essential to this synergy.

Pharmacokinetic issues

The *in vitro* studies described above focus primarily on pharmacodynamic interactions between chemotherapy and FLT3 inhibitors. However, the concomitant administration of small molecule kinase inhibitors with chemotherapeutic agents carries the possibility of a number of pharmacokinetic interactions, some of which may be deleterious to the patient.

p-Glycoprotein

p-Glycoprotein (MDR1) is a 170 kDa efflux pump that has been found to modulate multi-drug resistance in a variety of cancer types. In leukemia it is thought to play a role in the rapid efflux of anthracyclines, vinca alkaloids, topoisomerase inhibitors and many other toxins, prior to toxin activity within the cell [109]. Strategies employed to raise intracellular levels of selected drugs include the direct inhibition of the pump with verapamil [110,111]. *In vitro* studies indicate some low level inhibition of *p*-glycoprotein by midostaurin [112], suggesting that anthracycline metabolism could be affected by concomitant use of midostaurin.

α -1-Acid glycoprotein

AAG is one of the major acute phase reactant proteins in mammalian plasma, and is an important drug binding protein in humans [113]. It is the principle binding protein for a number of tyrosine kinase inhibitors, including imatinib, lestaurtinib, gefitinib and midostaurin [90,114,115]. For any such organic compounds in plasma, equilibrium exists between molecules bound to AAG and those “free” to bind to cellular targets. AAG levels can fluctuate in different metabolic or inflammatory states, and different compounds can compete or displace each other from AAG binding sites. A drug such as erythromycin, for example, could displace imatinib from AAG sites, thereby increasing free levels of imatinib and potentially improve *in vivo* potency. More concerning, however, would be an inhibitor displacing a drug such as daunorubicin from AAG. Anthracyclines are well established to bind to AAG in plasma, and higher free levels of these drugs could well result in serious toxicity [116].

Conclusions from pre-clinical studies

From these studies it can be concluded that, in general, FLT3 inhibition will synergize with conventional chemotherapeutic agents in killing FLT3 mutant AML cells. However, a number of issues have been brought to light by this work. *In vitro* studies, performed primarily using rapidly dividing cell lines in culture medium, cannot precisely imitate the conditions encountered in a patient treated with infusional chemotherapy. For example, in contrast to cell lines in culture, only about 6% of AML cells in human marrow are in S-phase [117]. Cytarabine is typically administered over 3–7 days to allow a maximal fraction of leukemia cells to enter S-phase, when the drug will have maximum effect. If a FLT3 inhibitor is administered at the start of a cytarabine infusion, there exists the potential to arrest leukemia cells in G phase, thereby antagonizing the effects of the chemotherapeutic drug. This phenomenon was observed in more than one pre-clinical study, but it remains to be seen if it might occur in patients.

Pharmacokinetic interaction between FLT3 inhibitors and chemotherapy is also an important concern. A number of inhibitors are highly protein bound (midostaurin, lestaurtinib and sorafenib [90,98,118]), and could compete with anthracyclines for plasma protein binding sites, with the theoretical potential of increasing free levels of anthracyclines. In addition, the indolocarbazoles (lestaurtinib and midostaurin) have activity as inhibitors of *p*-glycoprotein. This property could lead to *in vivo* interactions between a number of chemotherapy drugs and the inhibitors. An interaction between lestaurtinib and daunorubicin was observed *in vitro* in one study [90], and midostaurin (and by inference, lestaurtinib) is an inhibitor of *p*-glycoprotein [112]. Because of these potential pharmacokinetic interactions, caution should be employed when considering the combination of some of these agents with anthracyclines.

A third issue addressed in these studies was that of the effect of combination treatment on the bone marrow. A number of FLT3 inhibitors also inhibit KIT, a receptor important in early myelopoiesis. While transgenic mice with a FLT3 knockout survive with only mild abnormalities in hematopoiesis, lack of a functional KIT gene in mice generally leads to lethal hematopoietic defects [119]. There remains a concern, therefore, that recovery from chemotherapy induced marrow aplasia may be significantly hindered by a combination of FLT3 and KIT inhibition. To test for this possibility, investigators treated mice with cyclophosphamide to induce myelosuppression, and then followed this treatment with tandutinib, an equipotent inhibitor of FLT3 and KIT [96]. The tandutinib-treated mice were noted to have relatively normal recovery of hematopoiesis compared with the control group, suggesting that, at least in mice, combined inhibition of FLT3 and KIT may be tolerable in the setting of chemotherapy-induced aplasia.

Clinical studies

Lestaurtinib as monotherapy

A clinical-laboratory correlative phase 1/2 trial in relapsed or refractory AML patients with FLT3 mutations was completed in 2003 [77]. The correlative assays from this trial revealed that if a patient had leukemic blasts that died when exposed to CEP-701 *in vitro*, and if that patient achieved a level of CEP-701 in plasma sufficient to significantly inhibit FLT3 autophosphorylation in sustained fashion, then a clinical response was observed. In a second phase II study, elderly patients with AML not fit for conventional chemotherapy were treated with lestaurtinib as monotherapy [83]. The results showed partial response in 8 of 27 patients. The response rate among FLT3 mutants was 3 out of 5 patients. All 8 of the responders had plasma levels of drug sufficient to inhibit FLT3 phosphorylation to levels below 15% of baseline activity.

Lestaurtinib combined with chemotherapy

Drawing on the results of the pre-clinical studies combining lestaurtinib with chemotherapy, the Cephalon 204 trial began accruing patients in 2003. The trial design centres on three simple principles: (1) Only patients with FLT3 mutations are likely to benefit from treatment with a FLT3 inhibitor; (2) Treatment with a FLT3 inhibitor should be initiated towards the end of, or even after, chemotherapy infusion is complete, in order to avoid the hypothetical antagonistic effects of such a sequence; (3) FLT3 inhibition needs to be effective and sustained once treatment with lestaurtinib is initiated. AML patients are eligible for this trial if they are in first relapse and they harbour a FLT3 mutation. The trial is stratified according to the duration of first remission: Patients whose first remission lasted less than 6 months receive MEC [120], while those whose first remission lasted greater than 6 months are treated with HiDAc [121]. Patients are randomized to receive lestaurtinib at a dose of 80 mg twice per day beginning after chemotherapy is complete and continuing for up to 16 weeks. The efficacy of target inhibition is being determined through the application of a surrogate assay, the plasma inhibitory activity (PIA) assay for FLT3 [90]. The preliminary results from the first 44 patients enrolled on this trial, presented in abstract form at the 2005 Annual Meeting of the American Society of Hematology, were encouraging in that there were 12 remissions in the lestaurtinib arm versus 6 remissions in the control arm (55% versus 27%) [122]. Based on these encouraging findings, the Cephalon 204 trial has been expanded to a pivotal trial, with a target accrual of 220 patients.

Importantly in this trial, the clinical outcomes correlated tightly with the degree of FLT3 inhibition observed in the patients. The percentage of patients achieving adequate FLT3 inhibition, as determined from the PIA assay, was a somewhat disappointing 60%. The primary pharmacokinetic impediment appears to be fluctuating levels of AAG. Because AAG is the principle plasma protein binding lestaurtinib, its levels can influence the concentration of free drug able to penetrate cells and inhibit FLT3 (*Mark Levis MD PhD Baltimore MD, unpublished data*). AAG is an acute phase reactant whose levels rise following chemotherapy. Thus, in the context of this trial, the pharmacodynamic advantage of treating with lestaurtinib immediately after chemotherapy is partially offset by the pharmacokinetic disadvantage posed by AAG levels.

Lestaurtinib is also currently under evaluation in FLT3 positive patients as part of AML15/17 trial in the United Kingdom (UK). In contrast to the Cephalon 204 trial, the UK study is enrolling newly diagnosed patients. Patients are randomized to receive lestaurtinib (80 mg twice daily) immediately following induction chemotherapy and continued until 2 days prior to the next cycle of chemotherapy. The AML15 version of this trial has completed accrual, but the lestaurtinib treatment will continue to be evaluated in the AML17 trial (Alan Burnett MD, Cardiff UK, *personal communication*).

Midostaurin as monotherapy

Midostaurin was clinically evaluated in a phase II trial for relapsed or refractory AML patients harbouring a FLT3 mutation [77]. Of 20 patients treated at a dose of 75 mg three times daily, 14 displayed at least hematologic improvement, with 1 CR. An indolocarbazole derivative like lestaurtinib, midostaurin is tightly bound to AAG. Furthermore, midostaurin is converted in the liver to two metabolites, CGP62221 and CGP52421 [77]. CGP52421, by virtue of its being less selective (hence more “multi-targeted”), less bound to AAG than either the parent drug or the other metabolite, and present at much higher levels in plasma, is likely the active compound in patients [90]. Responses in this trial likewise correlated very well with the degree of FLT3 inhibition achieved as determined by the PIA assay [90].

Midostaurin combined with chemotherapy

Midostaurin was next evaluated in combination with induction therapy using a conventional cytarabine and daunorubicin (“7+3”) regimen followed by high dose cytarabine consolidation. One arm started midostaurin on day one and a second arm began midostaurin on day 8 of chemotherapy. In general, midostaurin doses that were well-tolerated when used as monotherapy (100 mg orally twice daily) were intolerable (due to nausea) when given concomitantly or following chemotherapy. The metabolism of daunorubicin was reportedly delayed, suggesting that the predicted interaction between the indolocarbazole and anthracycline did in fact occur [123].

This study was amended due to the high level of grade 3 nausea and vomiting and the results were presented at the 2005 Annual Meeting of the American Society of Hematology [124]. In the amended study, midostaurin, at an initial dose of 75 mg three times daily, was given on Days 8–21 of induction in one arm, while in the other arm, it was given on Days 1–7 and Days 15–21. Toxicities included nausea, vomiting, transaminitis, diarrhoea and rash. In general, midostaurin was poorly tolerated in this context, and the dose was subsequently dropped to 50 mg twice daily dose on the same schedule. Nineteen patients were evaluated, and 6 out of 6 patients with a FLT3 mutation achieved a CR. By comparison, of the 13 patients with wild type FLT3, only 8 achieved CR. Based on the results of this pilot trial, a pivotal trial of midostaurin, at a dose of 50 mg twice daily following induction chemotherapy, is currently planned for newly diagnosed AML patients (Richard Stone MD, Boston, MA, *personal communication*).

Tandutinib as monotherapy

Tandutinib has been studied as a single agent in AML patients with relapsed and refractory AML. As a single agent it demonstrated >50% decreases in bone marrow and peripheral blast counts in 12/25 patients. Maximum tolerated dose in this study was reached at 525 mg twice a day and limiting symptoms were generalized muscle weakness and fatigue. In 5 patients with FLT3 ITD that were evaluable 2 of the 5 had evidence of anti-leukemic effect with lowered peripheral blood and bone marrow blast counts [78].

Tandutinib combined with chemotherapy

Tandutinib was tested in combination with induction chemotherapy (“7+3”) for newly diagnosed AML patients in a phase I/II trial [125]. Initially, the drug was given at 200 mg twice daily throughout induction and consolidation beginning on day 1 and continuing 6 months after consolidation. Because of gastrointestinal intolerance, however, the protocol was amended and tandutinib was administered only on Days 1–14 of each cycle. In preliminary results CRs were reported in 5 out of 7 patients treated with the continuous tandutinib (i.e. prior to the amendment) and in 6 of 8 patients treated on the Day 1–14 regimen. Reported side effects included nausea, vomiting, diarrhoea, fatigue and generalized muscle weakness.

Sunitinib

In a single dose study in AML patients, sunitinib was found to significantly inhibit FLT3 phosphorylation in all FLT3-mutant patients tested and in 50% of patients with wild type FLT3 [75]. In this study, inhibition of phosphorylated STAT5 correlated with FLT3 inhibition in all patients with FLT3 mutations. Interestingly, phosphorylation of STAT5 was also inhibited in the late time point samples from wild type patients, suggesting that a second target was being inhibited by sunitinib. In a phase I study of sunitinib in AML, 4 of 15 enrolled patients harboured FLT3 mutations, and all 4 displayed partial or morphologic

responses, albeit transient [126]. There have been no published studies in which sunitinib has been combined with chemotherapy for the treatment of AML.

Sorafenib

Sorafenib has been studied in single agent phase I clinical trial in AML [127]. This two arm study looked at 5 day per week dosing versus 14 days out of 21. Preliminary results published at ASCO in 2007 showed responses in 6 out of 10 patients receiving a full cycle of therapy. Laboratory correlates in patients showing a response indicated inhibition of FLT3 phosphorylation in 3/3 patients studied. There have been no reported studies of sorafenib combined with chemotherapy for leukemia patients. However, a phase I trial in which AML patients are treated with cytarabine, an anthracycline and sorafenib, has recently begun accruing patients at the MD Andersen Cancer Center (Michael Andreef MD PhD, Houston TX, *personal communication*).

Conclusion

FLT3 represents a tantalizing target for oncologists seeking new avenues of therapy for AML. While the disappointingly modest clinical activity of FLT3 inhibitors can in part be attributed to pharmacokinetic limitations, it seems clear that FLT3 inhibition is much more likely to impact AML cure rates when incorporated into existing chemotherapy regimens. The pre-clinical studies published to date suggest that FLT3 inhibition synergizes with chemotherapy in killing AML cells, but this effect is for the most part sequence dependent. FLT3 inhibition should be applied during or immediately after, but not before, chemotherapy in order to achieve maximum cell kill. Preliminary clinical studies of combination regimens seem to confirm the pre-clinical pharmacodynamic predictions, but they have also high-lighted the importance of pharmacokinetic interactions between small molecule kinase inhibitors and potent drugs such as anthracyclines. The administration of any oral agent in the wake of the mucositis-inducing agents that constitute current AML chemotherapy will be a challenge, but the use of imatinib in ALL therapy stands as proof that it can be done.

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Table I

FLT3 inhibitors in publication.

CEP-701(Lestaurtinib) [52]
PKC412(Midostaurin) [53]
SU11248(Sunitinib) [54]
SU5416(Semaxinib) [55]
MLN518(Tandutinib) [56]
AG1295 [57]
AG1296 [58]
SU14813 [59]
ABT869 [60]
KRN383 [61]
KP372-1 [62]
SU5614 [55]
SU11657 [63]
CHIR-258 [64]
Ki23819 [65]
AGL-2043 [66]
AGL-2033 [66]
GTP-14564 [67]
D64406 [68]
R406 [69]
BAY43-9006(Sorafenib) [70]
AS602868 [71]
FI-700 [72]
KW-2449 [73]