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Bench to Bedside Targeting of FLT3 in Acute Leukemia

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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 this tyrosine kinase by direction inhibition is the focus of both preclinical and clinical research in AML. Several molecules in clinical development inhibit FLT3, but thus far clinical responses have been limited. Correlative studies from monotherapy trials have established that responses require sustained, effective FLT3 inhibition *in vivo*. Studies combining FLT3 inhibitors with chemotherapy have demonstrated increased remission rates to date but have yet to produce a survival advantage. Currently the only approved FLT3 inhibitor available for off-label use is sorafenib, which clearly has clinical activity but does not commonly lead to a complete response. Several FLT3 inhibitors are currently being tested as single agents and in combination with chemotherapy, and it seems likely that a clinically useful drug will eventually emerge.

Keywords

Acute myeloid leukemia; FLT3; Tyrosine kinase inhibitors

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. Thus far FLT3 inhibitors given as monotherapy have yet to measurably impact outcomes in AML patients harboring FLT3 mutations, but progress in the development of these agents with the addition to chemotherapy holds great therapeutic promise. The best way of incorporating these compounds into standard treatment regimens for the disease remains unclear. Here we will summarize the available clinical data on FLT3 inhibitors, including those studies combining these agents with AML-directed chemotherapy, and to provide some insight into their potential for improving survival in AML patients.

FLT3

The human FLT3 (FMS-Like Tyrosine Kinase 3) gene was cloned from a stem cell-derived cDNA library in 1991[1] and is found on chromosome 13q12 in humans [2]. The protein contains 993 amino acids and is visualized as a doublet, consisting of a mature

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(glycosylated) form and an immature form, on electrophoretic gels [3]. 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 (alpha and beta), and the VEGF receptors [4]. 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 [5]. The juxtamembrane domain of FLT3, as with many other receptors, exerts a negative regulatory influence upon the tyrosine kinase activity [6,7]. 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 which were discovered in 1996 [5]. Activating point mutations in the kinase domain were discovered in 2001 [8].

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 , ERK1/2, PI3K/AKT, Foxo proteins, and Pim1 and Pim2 [9–18]. 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 [3]. 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 [19]. In contrast, its ligand is expressed in virtually all cell types thus far examined [20,21]. 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) [22–29]. FLT3^{−/−} mice develop normally with only mild hematologic dyscrasias primarily effecting the B-cell lineage [22] suggesting specific pharmacologic targeting of FLT3 may have limited toxic effects.

Signaling aberrations associated with FLT3 ITD have been described and are somewhat different than those found in FLT3 tyrosine kinase domain mutants [30]. FLT3 ITD activation is associated with STAT5 activation and downstream repression of transcription factor CEBP α and Pu.1 while WT FLT3 or FLT3 TKD does not activate STAT5 [31–33]. There have been no significant differences in FLT3 ITD signaling through ERK1/2, AKT or Shc [30]. Signaling aberrancy is not just associated with mutation type but appears to also be related to intracellular location of FLT3 ITD [34].

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 [35–39]. In 1996, a polymerase chain reaction (PCR) screen of AML cases revealed a subset of patients whose leukemia cells harbored internal tandem duplication mutations within the FLT3 gene [40]. 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 [7,41,42]. 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 [8,43]. 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 pediatric AML samples have been published [44–56]. 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 [5]. 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 [57].

FLT3 INHIBITORS

More than 20 compounds have been reported to have inhibitory activity against FLT3, 28 of which are listed in Table 1. Several of these agents have now been tested in clinical trials [58–62]. 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 [63–65]. While most of them likely fit into the ATP binding pocket of FLT3, the exact mechanism probably varies from inhibitor to inhibitor [66]. FLT3 inhibitors have been found to have variable potency against different activating mutations [67]. 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 harbor 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 harboring 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 tumors (GIST).

All of the compounds in Table 1 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.

A lack of selectivity would be expected to narrow the clinical therapeutic index of an inhibitor. However, such non-selectivity may offer an advantage. We recently screened six FLT3 inhibitors (lestaurinib, midostaurin, sorafenib, AC220, KW-2449, and sunitinib) for cytotoxic activity against a panel of primary FLT3/ITD leukemia samples [68]. First, we found that inhibition of FLT3 autophosphorylation in a FLT3/ITD specimen does not always induce cell death, implying that some FLT3/ITD AML is not truly addicted to FLT3 signaling. Additionally, we noted that at diagnosis, FLT3/ITD AML typically harbors a lower mutant allelic burden and is less sensitive to the highly selective FLT3 inhibitors such

as AC220 suggesting FLT3 oncogene addiction may not play as important role for initial clearance of leukemia. Conversely, FLT3/ITD samples obtained at relapse, (in which the mutant allelic burden typically increases), were generally more responsive to the more specific inhibitors. In other words, in a newly-diagnosed FLT3/ITD patient, the AML cells may not be fully addicted to mutant FLT3 signaling, and therefore the off-target effects of drugs such as lestaurtinib or midostaurin may offer a cytotoxic advantage.

Nine of the compounds listed in Table 1 have been tested in clinical trials specifically to assess their efficacy in AML patients harboring FLT3 mutations: Lestaurtinib (CEP-701), Midostaurin (PKC412A), Sunitinib (SU11248), Tandutinib (MLN518), SU5146, Sorafenib, KW2449, LS104, and AC 220. All drugs were demonstrated to inhibit FLT3 phosphorylation *in vivo* in significant numbers of patients. Each displayed a consistent, modest clinical activity, namely the clearance of peripheral blood leukemia cells. The two compounds with the greatest *in vivo* potency and longest half-life sorafenib and AC220 [69], have been associated with some complete remissions, suggesting that the disappointing results seen in early FLT3 inhibitor trials were due to a failure to effectively inhibit FLT3 *in vivo*. In general, 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) [70], so conclusions regarding their limitations as monotherapy may be somewhat premature. Conversely, our *in vitro* studies of relapsed disease would suggest an increased sensitivity to FLT3 targeting which was not apparent in these studies [68]. 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.

ASSESSMENT OF *IN VIVO* TARGET INHIBITION

One approach to determining the degree of target inhibition by a kinase inhibitor is to assay the target directly in the malignant cells. Even in leukemia patients, however, this represents a considerable technical challenge, as trial patients frequently have little or no circulating leukemia cells, or those that do have widely fluctuating levels of circulating blasts. Reliable correlation between the degree of inhibition of a target such as FLT3 and plasma drug levels is therefore difficult to achieve using conventional methods. We developed the plasma inhibitory assay (PIA) assay as a surrogate means of quantifying FLT3 inhibition over time in a consistent fashion for large numbers of patients [71]. The utility of this approach is the uniform data that can be obtained, and unlike pharmacokinetic values, PIA data takes into account protein binding, active metabolite levels, and cytokine levels which may influence target sensitivity to inhibition. While not a direct measure of kinase activity in patient leukemia cells, this assay evaluates the plasma of FLT3 inhibitor-treated patients for the ability to inhibit target under the most ideal of settings, thus setting a minimal threshold to achieve to assure the possibility of target inhibition *in vivo*. We have validated this approach in studies of five inhibitors (lestaurtinib, PKC-412, sorafenib, KW-2449, and AC220) [60,71–73], and believe this approach provides data more reflective of clinical conditions than conventional pharmacokinetics which only provide drug levels.

RESISTANCE TO FLT3 TARGETED THERAPY

Several potential mechanisms of resistance to FLT3 targeted therapy have been postulated but only a few have been described to occur clinically. Like resistant ABL kinase in CML, FLT3 has been found to develop kinase domain point mutations under selective pressure *in vitro* [74,75] and clinically [76]. Likewise, *in vitro* exposure to FLT3 targeted therapy is associated with up regulation of parallel signaling pathways such as PI3K and MAPK [77] as a mechanism of resistance. Other potential mechanisms to resistance to FLT3 targeted

therapy have involved atypical involvement of the ITD into a non-juxta-membrane domain of the receptor and this has been observed clinically in a primary refractory patient on a PKC412 trial [78].

FLT3 INHIBITORS AS SINGLE AGENTS

Lestaurtinib as Monotherapy

A clinical-laboratory correlative phase 1/2 trial in relapsed or refractory AML patients with FLT3 mutations was completed in 2003 [60]. 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 [70]. 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.

Midostaurin as Monotherapy

Midostaurin was clinically evaluated in a phase II trial for relapsed or refractory AML patients harboring a FLT3 mutation [61]. Of 20 patients treated at a dose of 75 mg three times daily, 14 displayed at least hematologic improvement, with 1 complete remission. An indolocarbazole derivative like lestaurtinib, midostaurin is tightly bound to Alpha-1 Acid Glycoprotein (AAG). Furthermore, midostaurin is converted in the liver to two metabolites, CGP62221 and CGP52421 [61]. 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 an important component of the activity seen in patients [71]. Responses in this trial likewise correlated very well with the degree of FLT3 inhibition achieved as determined by the PIA assay [71].

Tandutinib and Sunitinib as Monotherapy

Both tandutinib and sunitinib have been studied as single agents in AML patients with relapsed and refractory AML. Both agents resulted in transient blast reductions in peripheral blood counts [62,79]. Neither has advanced further in clinical trials. Tandutinib was probably unsuccessful due to poor FLT3 inhibition at clinically achievable concentrations, while sunitinib appeared to have been poorly tolerated by AML patients at doses required for sustained FLT3 inhibition *in vivo* [79].

Sorafenib

Sorafenib is a multi-targeted tyrosine kinase inhibitor, with activity against RAF kinase, VEGF receptors, wild type and ITD-mutated FLT3, PDGF receptors, c-KIT, and RET kinase [80]. Sorafenib has shown significant clinical activity in phase I/II studies in numerous solid tumors, [81,82] and it was recently approved by the U.S. Food and Drug Administration for the treatment of advanced renal cell cancer [83] and inoperable hepatocellular carcinoma [84]. Preclinical studies of sorafenib in acute leukemia have demonstrated down-regulation of the MAPK pathway, sensitization of human leukemia cells to receptor-mediated apoptosis by the down-regulation of Myeloid cell leukemia-1 (Mcl-1) [85,86], and potent growth inhibition of AML cells with FLT3/ITD mutations with evidence of clinical activity in FLT3/ITD patients with suppression of circulating blasts [87].

Sorafenib has been studied in refractory AML as a single agent on an intermittent schedule [87]. A clinical response was observed in nine of sixteen patients (56%) and in patients with

FLT3/ITD as a solitary mutation, six of six (100%) demonstrated a clinical response. Among FLT3/ITD patients a more robust response was found in clearing peripheral blasts which on average dropped 50%. In the bone marrow, the average improvement in blasts was only 27%. In FLT3/WT patients there was no significant change in either peripheral blood or marrow blasts.

In a separate Phase I dose escalation trial of the sorafenib in relapsed/refractory acute leukemias fifteen patients with advanced leukemia (13=AML, 2=ALL) and a median age of 63 (range 37–85) years were enrolled and treated on a dose escalation trial [72]. Toxicities grade 3 were present in 55% of cycles and the maximum tolerated dose (MTD) was determined to be 400 mg BID $\times$ 21 days in a 28 day cycle. Plasma inhibitory assays of kinase targets ERK and FLT3/ITD demonstrated excellent target inhibition, with FLT3/ITD silencing occurring below the MTD. The N-oxide metabolite of sorafenib appeared to be a more potent and selective inhibitor of FLT3/ITD than the parent compound. Despite marked target inhibition, no patients met criteria for complete or partial response in this monotherapy study. Eleven of fifteen patients experienced stable disease as best response. Although sorafenib demonstrated only modest clinical activity as a single agent in this heavily treated population, robust inhibition of FLT3 and ERK suggest there may be a potential important role in combination therapies in particular for FLT3/ITD AML.

Sorafenib possesses several qualities which may lend to favorable clinical responses in FLT3/ITD AML. It has a relatively long half-life in the plasma (~30 hours) allowing for levels to stabilize after 8 days [88]. Additionally, a major metabolite, sorafenib N-oxide likely significantly contributes to the specificity and potency of FLT3/ITD inhibition [72]. Our data suggests that doses below 400 mg twice a day is sufficient for FLT3/ITD silencing and may improve patient tolerability in long term treatment paradigms. Lastly, like many FLT3 inhibitors, Sorafenib preferentially inhibits FLT3/ITD over FLT3 WT which allows for more specific targeting of the malignant clone [87].

Clinically sorafenib is one of two known approved FLT3 inhibitors and several reports of compassionate use off protocol, with complete remissions, have been reported in the literature [89,90].

NEWER FLT3 INHIBITORS

KW-2449

KW-2449 is a small molecule tyrosine kinase inhibitor with known activity against FLT3, aurora kinase, FGFR-1 and Abl kinase [91]. Results from a phase I study of a KW-2449, which was specifically designed to establish in a quantitative fashion the degree of FLT3 inhibition achieved in patients at each dose level [73] again suggested that pharmacokinetic obstacles (such as a short drug half-life) may be responsible for the limited responses to FLT3 inhibitors in general [92]. Specifically, while transient inhibition of FLT3 autophosphorylation was readily achievable, this was insufficient both *in vitro* and *in vivo* for achieving significant cytotoxicity in leukemia cells. FLT3 inhibition needs to be sustained in order to effect killing of FLT3-dependent AML cells. The phase I trial of KW-2449 was halted and the dosing changed based on pharmacodynamic evaluations. Patients are currently accruing to the redesigned trial. This study highlighted the importance of using a phase 1 study of a kinase inhibitor to determine not just a safe and tolerable dose of a drug, but rather a kinase inhibitory dose that is safe, tolerable, and sustainable.

AC220

AC220 may well be the most potent and specific inhibitor of FLT3 currently in development [93]. A phase I study has recently completed studying activity in both FLT3/WT and ITD

relapsed and refractory AML [94]. A total of 76 patients were treated on two schedules: intermittent dosing (day 1–14) and continuous dosing (day 1–28). Pharmacokinetic studies revealed a prolonged plasma half life of ~36 hrs and excellent *ex vivo* target inhibition at dose levels above 12 mg per day. Additionally an active metabolite was found, which likely contributes significantly to the biologic activity of AC220. The dose limiting toxicity was QTc prolongation at 300 mg continuous dosing. Responses were documented in 30% of patients on study including 9 CR/CRi (12%). Interestingly at the MTD expansion dose of 200 mg daily 3/6 FLT3/ITD patients had a CR and one had a PR. Two of these patients were able to proceed to transplant in a remission. A Phase II study of AC220 is currently enrolling patients.

COMBINATIONAL TRIALS

Lestaurtinib Combined with Chemotherapy

Drawing on the results of the pre-clinical studies combining lestaurtinib with chemotherapy demonstrating sequential synergy [95], the Cephalon 204 trial began accruing patients in 2003. The trial design centers on three simple principles: 1) Only patients with FLT3 mutations are likely to benefit from treatment with a FLT3 inhibitor; 2) Because of the possibility of an antagonistic interaction if FLT3 inhibition occurs prior to chemotherapy, treatment with a FLT3 inhibitor should be initiated either simultaneously, or even after, chemotherapy; 3) FLT3 inhibition needs to be effective and sustained once treatment is initiated. AML patients were eligible for this trial if they were in first relapse and they harbored a FLT3 mutation. The trial was stratified according to the duration of first remission: Patients whose first remission lasted less than 6 months received MEC [96], while those whose first remission lasted greater than 6 months were treated with HiDac [97]. Patients were randomized to receive lestaurtinib at a dose of 80 mg twice per day beginning with the completion of chemotherapy and continuing for up to 16 weeks. The efficacy of target inhibition was determined through the use of the plasma inhibitory activity (PIA) assay for FLT3 [71]. The results, were presented in abstract form at the 2009 Annual Meeting of the American Society of Hematology [98]. In this trial, lestaurtinib plasma levels varied widely from patient to patient, and the degree of *in vivo* FLT3 inhibition was disappointing, with only 58% of patients on the lestaurtinib arm achieving a suppression of FLT3 activity to less than 15% of baseline. However, in patients achieving this degree of target inhibition, the CR/CRp rate was 39% *versus* only 9% for those not achieving target inhibition. By intention-to-treat analysis, there was no significant improvement in overall survival (4.73 *vs.* 4.57 mo) between the two arms. The complex pharmacokinetics of lestaurtinib appears to greatly limit its utility in the relapse setting. Nonetheless, the results of the trial seem to support the clinical benefit of FLT3 inhibition, if it can be achieved in sustained fashion.

MRC AML15/17 TRIAL

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, age 60 and under. Patients are randomized to receive lestaurtinib (80 mg twice daily) immediately following induction chemotherapy and continued until two 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, *personal communication*). Preliminary results suggest the combination is improving the remission rate, although overall survival data are not yet available. Consistent with other studies, there appears to be a high correlation between remission rate and FLT3 inhibition [99].

Midostaurin Combined with Chemotherapy

In a pilot trial, midostaurin was evaluated in combination with induction therapy using a conventional cytarabine and daunorubicin (“7+3”) regimen followed by high dose cytarabine consolidation. One arm gave midostaurin on day 1–7 & 15–21 and a second arm began midostaurin on day 8–21 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 occur [100]. This study was amended due to the high level of grade 3 nausea and vomiting and the results were presented at the 2009 Annual Meeting of the American Society of Hematology [101]. In the amended study, midostaurin, at an initial dose of 50 mg twice 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. Five patients received maintenance midostaurin on this protocol. Twenty patients in both arms were stratified by FLT3 status and those with a FLT3 ITD were found to have overall survival comparable to WT patients at both 1 and 2 years (85% and 62% for ITD respectively and 81% and 59% for WT patients respectively). This data supports the Phase III trial of midostaurin combined with chemotherapy for newly diagnosed FLT3 mutant AML patients under age 60 (RATIFY).

Sorafenib Combined with Chemotherapy

In a phase II single institution study, Sorafenib was administered with cytarabine and idarubicin in induction and consolidation, followed by a year of maintenance sorafenib [102]. Preliminary reports suggested the combination was tolerable, and the investigators reported a high CR rate in FLT3 mutated patients (13/14). With a median follow up of 10.6 months, median response duration has not been reached as of yet.

DISCUSSION

Clinical development of effective targeted therapies is proving to be a complex, multistage process, particularly in malignancies with multiple genetic, epigenetic, and genomic abnormalities such as those seen in AML. Once a potential target is identified, several steps need to be taken in order to successfully exploit the target as a weakness in a malignancy: 1) Discovery of a compound which affects the target *in vitro*; 2) Identification of patient subsets whose malignancies may be responsive to the targeted agent; 3) Demonstration of target modulation *in vivo*; 4) Assessment of dose limiting toxicities in the specific patient population; 5) Demonstration of clinical response correlated with target inhibition; 6) Incorporation of the targeted agent in combination with existing therapies. Several FLT3 inhibitors have advanced several steps along this pathway, but much work lies ahead refining the preliminary results discussed here.

Early development of targeted therapies too often fails to recognize issues that in hindsight reveal the inadequacies of the agent in development. For instance, many of these agents are highly protein bound, in some cases over 99%, and despite this knowledge many preclinical studies are performed in media consisting of only 10% plasma proteins. We have routinely begun screening agents using 100% inactivated normal human plasma as a way to address the protein binding issues and as a rule have found inhibitory concentrations 100–1000 fold different using conditions which more closely reflect *in vivo* tumor micro environment.

Correlative studies have also documented the importance of sustained complete or near complete inhibition of FLT-3 for maximal clinical benefit [60,71–73]. In the case of KW-2449, the preclinical PK data suggested a BID dosing design would be sufficient for

continuous target inhibition. This did not take into account both the shortened human half life of the agent, and the induced increased levels of enzymatic metabolism of the agent which was easy to demonstrate at day 14. Both sorafenib and AC220 have evidence of sustained FLT3 inhibition, and both of these agents have been associated with highest degree of clinical success.

With several of the agents in development, the metabolism of the parent drug can yield an active agent, which in some cases is the primary FLT3 modulating compound. Preclinical studies of PKC412 failed to reveal the importance of the less selective but more cytotoxic metabolite CGP52421 [71]. Likewise we have demonstrated evidence for active metabolites in patients on KW-2449 [73], AC220 [94] and Sorafenib [72], which to varying degrees improve the effectiveness of the agents once metabolized *in vivo*. The knowledge of the biologic activity aids the *in vitro* development of these agents by again more closely mimicking *in vivo* conditions.

One modulating issue again only seen *in vivo* is fluctuating cytokine levels in patients receiving multi-agent chemotherapy. It has been observed previously that humoral factors induced by chemotherapy influence sensitivities of leukemia clones to treatment [103]. These same humoral factors are likely to influence the effects of targeted therapies. The assumption that using a dosing regimen derived from single agent studies will lead to target inhibition in the setting of multi-agent chemotherapy is probably naïve. Correlative studies confirming target modulation should be just as vigorous in the advanced clinical setting as in the early phase trials.

CONCLUSION

The data to date suggests that successfully inhibiting FLT3 *in vivo* in AML patients harboring FLT3 mutations can be clinically beneficial to patients. The benefits include reducing blood or marrow blast counts, inducing the occasional complete remission as monotherapy, and, when inhibitors are combined with chemotherapy, improving the remission rate. The benefits to overall survival are not known.

What should the properties of an ideal FLT3 inhibitor be? It should be extremely potent *in vivo*—not just *in vitro*. It should have a pharmacokinetic profile that allows for sustained inhibition. In this regard, AC220 appears to be the clear winner. However, the selectivity of the inhibitor may ultimately be important. The highly selective inhibitors seem to be less efficacious (at least *in vitro*) against FLT3 mutant AML at diagnosis, especially if the mutant allelic burden is low. The less selective indolocarbazoles, lestaurtinib and midostaurin, thus offer better cytotoxicity, but at the cost of unfavorable pharmacokinetics.

When to incorporate FLT3 inhibition into AML therapy is still very much unclear. There is always the temptation to introduce therapy up-front, but these agents have not been studied as monotherapy in newly-diagnosed AML. The trials combining inhibitors with chemotherapy are making a number of assumptions about the potential benefit of these drugs that may not be justified. Remission rate is an important clinical parameter, but it should not be used as a surrogate for overall survival. We must await the results of the ongoing phase III trials for a definitive answer.

What to do with a FLT3 mutant patient currently? Most often, this question is asked in the setting of refractory disease. Enrollment in a clinical trial is always the best answer, but unfortunately, is often not an option. While we believe that a FLT3 inhibitor will eventually gain approval, for the time being, if there are no other options, off-label use of sorafenib, at a dose of 400 mg twice daily, can be considered.

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Table 1**FLT3 Inhibitors in Publication**

Compound name	Clinical trial phase
Lestaurtanib(CEP-701) [104]	Phase III
Midostaurin(PKC412) [105]	Phase II
Sunitinib (SU11248) [106]	Phase I
SU5416 [107]	Phase II
Tandutinib (MLN518) [108]	Phase II
AG1295 [109]	
AG1296 [110]	
SU14813 [111]	
ABT869 [112]	
KRN383 [113]	
KP372-1 [114]	
SU5614 [107]	
SU11657 [115]	
CHIR-258 [116]	
Ki23819 [117]	
AGL-2043 [118]	
AGL-2033 [118]	
GTP-14564 [119]	
D64406 [120]	
R406 [121]	
Sorafenib(Bay 43-9006) [80]	Phase II
AS602868 [122]	
FI-700 [123]	
KW-2449 [73]	Phase I
AC220 [93]	Phase II
LS104 [124]	Phase I
NVP-AST487 [125]	
JNJ-28312141 [126]	