FLT3 Inhibitors for the Treatment of Acute Myeloid Leukemia

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Abstract: The fms-like receptor tyrosine kinase-3 (FLT3), which is important for the normal development of hematopoietic stem cells and cells of the immune system, is frequently mutated in patients with acute myeloid leukemia (AML). FLT3 is, therefore, a potential therapeutic target in AML. Recently, FLT3 inhibitors have shown therapeutic activity in AML patients with FLT3 mutations. Sorafenib and sunitinib were the first FLT3 inhibitors to be studied in the clinic and have the most clinically relevant data. Limited data are available for midostaurin (PKC412), lestaurtinib (CEP-701), tandutinib (MLN518), AC220, and KW-2449. It is likely that optimal application of these agents will involve combinations of inhibitors and combinations of inhibitors and chemotherapy, potentially with a mammalian target of rapamycin inhibitor such as everolimus or temsirolimus. This review discusses the theoretical rationale for the use of these agents and summarizes the relevant clinical data.

Introduction

The fms-like receptor tyrosine kinase-3 (FLT3) expressed by immature hematopoietic cells is important for the normal development and proliferation of hematopoietic stem cells and cells of the immune system.[1] Activating mutations are caused either by an internal tandem duplication (ITD) of between 3 and 33 (or more) amino acids in the juxtamembrane region or by a point mutation in the activation loop of the tyrosine kinase domain (TKD1) of FLT3. These mutations are present in up to 30% of acute myeloid leukemia (AML) patients, implicating FLT3 as a potential target for kinase inhibitor therapy.[2] FLT3 inhibitors have already shown promising activity in AML patients with FLT3 activating mutations.

FLT3-ITD mutations are the most common molecular abnormality associated with adult AML. FLT3-TKDs are associated with a favorable prognosis in AML in some studies,[3] but not all.[4] FLT3 mutations are unevenly distributed among French-American-British classification types and cytogenetic groups. They occur most frequently in acute promyelocytic leukemia (35–40%).[5] FLT3-ITD mutations negatively impact disease-free and overall survival in patients with intermediate-risk cytogenetics (normal karyotype), but not in patients with low-risk or poor-risk cytogenetics or patients with acute promyelocytic leukemia.

Newly diagnosed patients with FLT-ITD mutation (FLT3-ITD+) usually have heterozygous mutations (ie, a mutant and a wild-type allele), whereas relapsed patients are usually homozygous (with no wild-type allele). Various ratios of mutant to wild-type alleles have been observed. Wild-type allele signals may reduce the efficacy of kinase inhibitors in the treatment of AML in patients who have both wild-type and mutant alleles. This activity is a double-edged sword: wild-type signals may protect hematopoietic precursors from kinase inhibitor–induced marrow suppression, but they may reduce the therapeutic activity of such agents against leukemic blast cells.[6] Different FLT3 kinase inhibitors generate distinct, nonoverlapping resistance profiles, and therefore the resistance generated by one inhibitor does not interfere with the activity of another. This characteristic is in contrast to that of BCR/ABL inhibitors—such as imatinib (Gleevec, Novartis), nilotinib (Tasigna, Novartis), and dasatinib (Sprycel, Bristol-Myers Squibb)—which display overlapping resistance profiles. Therefore, combinations of FLT3 inhibitors may be useful to prevent FLT3 resistance mutations in the setting of FLT3-ITD+ AML.[7] Relapsed samples and samples with a high mutant allelic burden are more likely to be responsive to cytotoxicity from FLT3 inhibition than are samples obtained at diagnosis or samples with a low mutant allelic burden.[8]

Most FLT3 kinase inhibitors have mainly been evaluated in vitro against cell lines that express only mutant *FLT3* and not wild-type *FLT3*. Mori and colleagues analyzed how wild-type *FLT3* signals affect the inhibitory effect of FLT3 inhibitors on wild-type and mutant *FLT3*-expressing cells.[6] They demonstrated that *FLT3* wild-type signals reduced the inhibitory effects of FLT3 inhibitors. Potency against both wild-type and mutant FLT3 kinases may be required for sufficient efficacy against leukemia cells that express both.

Natural killer (NK) cells play an important role in immune surveillance in leukemia.[9] Cytokine production by NK cells is inhibited by some FLT3-ITD kinase inhibitors (sorafenib [Nexavar, Bayer Healthcare], midostaurin [PKC412, Novartis]) but not others (sunitinib [Sutent, Pfizer], TK1258 [Novartis]). Sorafenib, but not midostaurin, decreases phosphorylation of PI3K and extracellular signal-regulated kinase (ERK), which are important regulators of NK cells. Therefore, the selection of a kinase inhibitor may have important implications in therapy.[10]

Activating mutations of *FLT3* result in activation of mitogen-activated protein kinase (MEK) and ERK signaling pathways. Therefore, concomitant blockade of FLT3 and MEK signaling may be synergistic in causing apoptosis of leukemic cells that harbor *FLT3* activating mutations.[11]

Crosstalk between major downstream signaling pathways, such as PI3K/PTEN/Akt/mTOR, Ras/Raf/MEK/ERK, and JAK/STAT, might be exploited for therapeutic purposes through the targeting of key signaling molecules with selective inhibitors, such as mammalian target of rapamycin (mTOR) inhibitors, to overcome the rapid resistance to FLT3 inhibitors.[12]

Complete inhibition of FLT3 autophosphorylation does not always induce cell death, which implies that some FLT3/ITD+ AML is not truly addicted to FLT3 signaling.[13] This characteristic is observed only in patients at diagnosis. FLT3-ITD+ cells can be found in noncycling, dormant blast cells. These dormant cells may be insensitive to some FLT3 kinase inhibitors and may constitute an untargeted disease reservoir.[14]

Bone marrow–derived stromal cells inhibit chemotherapy-induced apoptosis in AML. Stromal-derived factor 1α (SDF-1α)/CXCR4 signaling plays a key role in leukemia cell-marrow microenvironment interactions. The CXCR4 inhibitor AMD3465 antagonizes SDF-1α–induced and stromal-induced chemotaxis and inhibits SDF-1α–induced activation of prosurvival signaling pathways in leukemic cells. CXCR4 inhibition also partially abrogates the protective effects of stromal cells on chemotherapy-induced apoptosis of AML cells. *FLT3* gene mutations activate CXCR4 signaling, and co-culture with stromal cells significantly diminishes the antileukemic effects of FT3 inhibitors in cells with activating mutations of *FLT3*. CXCR4 inhibition increases the sensitivity of *FLT3*-mutated cells to sorafenib. In vivo, AMD3465 mobilized AML cells into circulation and enhanced the antileukemic effects of chemotherapy and sorafenib, resulting in markedly reduced leukemic burden and prolonged survival in an animal model.[15]

Breitenbuecher and associates described a new mechanism of primary resistance to FLT3 tyrosine kinase inhibitors (TKIs) in AML: an FLT3 receptor that harbors a nonjuxtamembrane ITD atypically integrates into the ß-2 sheet of the first kinase domain (FLT3 ITD627E) and induces dramatic upregulation of the anti-apoptotic AML cell 1 protein (Mcl-1).[16] A similar study was conducted by Yoshimoto and coauthors.[17] Deregulated Mcl-1 protein expression was shown to play a major role in conferring the resistance phenotype of 32D ITD627E cells. This new mechanism of primary resistance to TKIs operates by reprogramming local and distant signal transduction events of the FLT3 kinase. These data suggest that particular ITDs of the FLT3 may be associated with rewired signaling and differential responsiveness to TKIs,[16] and that FLT3-ITD inhibitors may neutralize Mcl-1 and enhance p53-mediated apoptosis.[18]

FLT3 Inhibitors in the Treatment of AML

Sorafenib and sunitinib were the first FLT3 inhibitors to be studied in the clinic. There are, therefore, more

clinically relevant data on them than on the newer inhibitors. Limited clinical data are available for midostaurin, lestaurtinib (CEP-701, Cephalon), tandutinib (MLN518, Millennium Pharmaceuticals), AC220 (Ambit Biosciences), and KW-2449 (Kyowa Hakko Kirin).

Sorafenib

Sorafenib induces pronounced apoptosis in blast cells from AML patients in vitro, which is accompanied by ERK1/2 inactivation and caspase-independent downregulation of Mcl-1. Sorafenib mediates cell death in human AML cells, at least partly through downregulation of Mcl-1 via inhibition of translation.[19,20] Others have shown that sorafenib inhibits FLT3 enzymatic and signaling activities in leukemic cells with FLT3-ITD in vitro, which results in cell cycle arrest and apoptosis.[7] Sorafenib is an orally active multikinase inhibitor with potent activity against FLT3 and the Raf/ERK/MEK pathway.[21]

Sorafenib upregulates proapoptotic Bim in patient-derived AML blasts in vitro, and silencing of Bim protein significantly abrogates sorafenib-induced apoptosis, which suggests that Bim plays a critical role in sorafenib-induced apoptosis in AML. Cytarabine was synergistic with sorafenib in these studies.[22] Other studies have shown that FLT3 inhibitors reduce antiapoptotic Mcl-1 levels and enhance p53-mediated mitochondrial apoptosis in FLT3-ITD+ blasts.[6,23] FLT3 inhibitors also induce proteasome-mediated degradation of Mcl-1, which results in the reduced ability of Mcl-1 to sequester pro-apoptotic Bim. Therefore, FLT3 inhibitors may promote p53 signaling toward mitochondrial apoptosis, which, in combination with FLT3 inhibition, might be a potent leukemia treatment strategy.[18] Sorafenib was 1,000-fold to 3,000-fold more effective in inducing growth arrest and apoptosis in AML cell lines that harbor *FLT3-ITD* mutations than in AML cell lines with wild-type *FLT3*, and it inhibited the phosphorylation of tyrosine residues in ITD mutant, but not wild-type, FLT3 protein.[23,24] Sorafenib also has potent activity against the Raf/ERK/MEK pathway.[21]

In a phase I clinical trial of 16 patients, sorafenib significantly reduced the number of leukemic blasts in the peripheral blood and marrow of 5 AML patients with FLT3-ITD, but not in patients without that mutation.[23,25] In another phase I trial, the maximally tolerated dose was 400 mg twice daily orally for 21 days per month.[26] Most common grade 3 or greater toxicities were fatigue (in 16% of patients) and hypokalemia (in 13% of patients). No complete responses (CRs) or partial responses (PRs) were observed, but 73% of patients had stable disease as the best response. Six patients had a reduction in marrow blasts after 1 course (3 had >50% reduction in marrow blasts). Sorafenib resulted in sustained complete inhibition of FLT3 and ERK in all 11 patients assessed. In both phase I trials, intermittent dosing was employed. Continuous dosing without interruption may have resulted in better clinical response rates.

Metzelder and coworkers collected data on 18 FLT-ITD+ AML patients treated with sorafenib.[27] Five patients were refractory to primary therapy, and 13 were treated in first relapse (n=11) or second relapse (n=2). Eight patients had relapsed after stem cell transplantation. The patients received oral sorafenib 200–800 mg/day for a median of 98 days (range, 16–425 days). All patients had a hematologic response. Among the 18 patients, the best responses were hematologic response in 9, marrow response in 4, CR in 1, and complete molecular response (negative for FLT3-ITD) in 4. After a median treatment duration of 180 days, 7 of the 18 patients (39%) developed clinical sorafenib resistance. Pancytopenia or thrombocytopenia of grades 3/4 were the most significant side effects (occurring in 13 patients).

Schroeder and coauthors presented data on 8 AML FLT3+ patients treated with sorafenib 800 mg/day for a median of 37 days before or after allogeneic stem cell transplantation.[28] Six patients had blasts with FLT3-ITD, and 2 had *FLT3-TKD* mutations. Seven patients were relapsed or refractory, and 1 patient was treated before initial induction therapy. Among the 4 patients who received sorafenib for refractory relapse after allogeneic stem cell transplant, 2 patients achieved CR, of which 1 response was at the molecular level. The molecular CR patient also had resolution of extramedullary granulocytic sarcomas. The CR patient had a second systemic relapse and died on day 164, and the molecular CR patient died of a central nervous system granulocytic sarcoma while still in bone marrow molecular CR on day 594. The other 2 patients treated for refractory disease had a hematologic response to sorafenib, but 1 died of progressive disease at day 188 and the other at day 329. Of the 4 patients treated prior to allogeneic stem cell transplant, 2 had relapsed after consolidation for a prior CR, 1 had refractory disease, and 1 was treated at diagnosis before induction therapy. Both refractory patients had a response to sorafenib that permitted transplantation (1 had a hematologic response and 1 had resolution of multiple lesions of leukemia cutis). Both patients were alive shortly after transplant, 1 in molecular CR (day +81) and 1 in clinical CR (day +16). Another primary refractory patient discontinued sorafenib after 13 days due to neurotoxicity. Except for that patient, the drug was well tolerated, with side effects limited to transient rashes in 2 patients.

Sorafenib rapidly lowered blood blast counts in some patients. Safaian and colleagues reported another case of molecular remission with sorafenib in a patient with AML FLT3-ITD+ who relapsed with extramedullary disease after an allogeneic stem cell transplant.[29] Metzelder and coworkers reported that sorafenib induced remissions

in 2 of 3 refractory FLT3-ITD+ patients and facilitated allogeneic transplantation for them that would otherwise not have been feasible.[30]

In a report by Lee and associates, sorafenib alone achieved complete resolution of extensive leukemia cutis in an elderly man with FLT3-ITD+ AML who was in marrow CR from standard chemotherapy.[31] The patient suffered no side effects from the treatment, but he had a marrow relapse several months later.

Ravandi-Kashani and coauthors reported a phase I/II study of idarubicin, high-dose cytarabine, and sorafenib in younger patients with relapsed AML.[32] In the phase I part of the study, treatment consisted of cytarabine 1.5 g/m^2 given over 24 hours for 4 days and idarubicin 12 mg/m^2 daily given for 3 days. Sorafenib was given daily, and the maximum tolerated dose was identified as 400 mg twice daily for 7 days during induction therapy. Patients who achieved CR received up to 5 courses of consolidation with idarubicin 8 mg/m^2 daily for 2 days and cytarabine 0.75 g/m^2 daily for 3 days, and sorafenib 400 mg twice daily for up to 28 days per cycle repeated every 4–6 weeks. Ten relapsed patients with a median age of 34 years were treated in the phase I part of the study, 7 of whom were FLT3-ITD+, and 4 of the 10 achieved CR. In the phase II part of the study, 45 patients with a median age of 53 years were treated. Twelve were FLT3-ITD+, 19 had normal karyotypes, and the others had poor-prognosis cytogenetics. Forty patients were evaluable for response, and 34 (85%) achieved a CR or PR, including 13 of 14 *FLT3*-mutated patients. The most frequent grade 3 or higher adverse events were hyperbilirubinemia in 5 patients, hand-foot syndrome in 3, elevated transaminase in 2, diarrhea in 2, and hypertension in 2. With a median follow-up of 5.4 months, the probability of survival at 6 months was 81.5%, and the median CR duration had not been reached. Of the 13 patients with *FLT3* mutations, 9 remained in CR at the time of the report. Mutant *FLT3* was suppressed in all 10 patients, with 5-fold greater suppression of mutant *FLT3* compared with wild-type *FLT3*. This study demonstrated that sorafenib can be safely administered with this chemotherapy regimen, and that potent inhibition of *FLT3* mutant signaling was achieved.

Sunitinib

Sunitinib is an oral multitargeted kinase inhibitor with selectivity for FLT3, PDGFα/β, VEGF receptor, and Kit receptor tyrosine kinases. Sunitinib was synergistic with cytarabine against AML cell lines with FL3-ITD, but not cell lines with wild-type FLT3.[33]

In a study by O'Farrell and colleagues, 29 AML patients each received a single dose of sunitinib, escalated from 50 mg to 350 mg, in increments of 50 mg and in cohorts of 3–6 patients.[34] FLT3 phosphorylation and plasma pharmacokinetics were evaluated at 7 time points over 48 hours after sunitinib administration, and the FLT3 genotype was identified. Toxicity was limited to grades 1/2 diarrhea and nausea in 31% of patients. Inhibition of FLT3 phosphorylation was observed in 50% of FLT3 wild-type patients and 100% of FLT3 mutated patients. Strong inhibition of FLT3 phosphorylation in more than 50% of patients was achieved in cohorts receiving sunitinib doses of 200 mg and higher. Downstream signaling pathways were also inhibited; signal transducer and activator of transcription 5 (STAT5) was reduced primarily in FLT3-ITD patients and at late time points in *FLT3* wild-type patients, whereas ERK activity was reduced in the majority of patients, independent of FLT3 inhibition.[34]

Fifteen patients with refractory AML were treated in another phase I study of sunitinib.[35] The most frequent grade 2 toxicities were edema, fatigue, and oral ulcerations occurring with a regimen of 50 mg/week for 4-week cycles followed by either a 2- or a 1-week rest period (13 patients). The 75-mg dose level was abandoned based on 1 case each of grade 4 fatigue, hypertension, and cardiac failure. All 4 patients with *FLT3* mutations had partial responses compared with 2 of 10 with wild-type *FLT3.* All responses were of short duration.

There are significant differences between sorafenib and sunitinib that may be clinically relevant. For instance, sunitinib induces monocytic differentiation of AML cells that is enhanced by 1,25-dihydroxyvitamin D(3).[36] Sorafenib is substantially more bound in human plasma than is sunitinib,[37] yet there is more intracellular accumulation of sorafenib than sunitinib in AML cells.[41] Sorafenib inhibits FLT3-ITD more potently than FLT3-D835Y, and sunitinib is equally effective against both mutant forms of *FLT3*.[38]

The combination of sunitinib and oral everolimus (Afinitor, Novartis) may be at least additive in FLT3-ITD+ AML. Everolimus enhanced the ability of sunitinib to inhibit the proliferation of FLT3-ITD+ AML cells and to downregulate certain mTOR effectors in such cells.[39] Both sorafenib and sunitinib have no effect on AKT signaling.[37]

Midostaurin (PKC412)

Midostaurin is a multitargeted kinase inhibitor with demonstrated clinical activity in *FLT3*-mutant and *FLT3*-wild type AML, but it rarely produces complete remissions. Midostaurin inhibits growth and induces megakaryocytic differentiation in human leukemia cells. It enhances the surface expression of the megakaryocytic marker CD61 and upregulates the expression of the signaling of c-Mpl, a thrombopoietin receptor encoding gene in some cell lines.[40]

Stone and associates conducted a phase Ib study of midostaurin (100 mg or 50 mg twice daily on days

8–21 or on days 1–7 and 15–21) in combination with daunorubicin (60 mg/m^2 daily on days 1–3) and cytarabine (100 mg/m^2/day ×7 as a continuous infusion) in induction followed by high-dose cytarabine (3 g/m^2 every 12 hours on days 1, 3, and 5 for 3 cycles) consolidation.[41] Patients were younger than 61 years and had newly diagnosed AML. Overall survival of patients whose blasts had *FLT3* mutations was similar to that of patients with wild-type *FLT3*. The 100 mg midostaurin dose was poorly tolerated due to nausea and vomiting, but the 50-mg dose was well tolerated in both schedules. Median midostaurin exposure was 133 days for *FLT3*-mutant patients and 90 days for wild-type patients. Median age was 50 years for wild-type patients and 46 years for mutant patients. Normal cytogenetics were present in 77% of the mutant patients; 15% had adverse cytogenetics, and 8% had other intermediate cytogenetics. Among wild-type patients, 18.5% had normal cytogenetics, 26% had adverse cytogenetics, 26% had other intermediate cytogenetics, 18.5% had favorable karyotypes, and 11% had unknown karyotypes. CR occurred in 80% of all patients (74% of wild-type patients and 92% of mutant patients). For mutant patients, 1-year and 2-year overall survival rates were 85% and 62%, respectively. For wild-type patients, 1-year and 2-year overall survival rates were 81% and 59%, respectively. In this study, there were only 27 *FLT3* wild-type and 13 *FLT3*-mutant patients (9 with ITD), and they were not stratified for type of *FLT3* mutation (TKD, ITD, ITD length, location, or allelic ratio).

Based on the above study, an international study from the Cancer and Leukemia Group B (CALGB 10603) is ongoing in patients with newly diagnosed AML who are older than 60 years and who have FLT3 mutations. Study subjects will receive the addition of midostaurin 50 mg or placebo to induction and consolidation therapy (similar to the previous study), and they will be randomized to midostaurin or placebo as continuation therapy for up to 12 months. The results of this study will obviously be of significant interest.

Lestaurtinib (CEP-701)

In vitro study data show that lestaurtinib inhibits *JAK2* wild-type and mutant JAK2+ erythroid precursors in patients with myeloproliferative disorders but spares normal erythroid progenitors.[42] Investigators have reported potential antagonistic effects when dosing lestaurtinib prior to chemotherapy utilizing both in vitro cell lines[43] and primary patient samples.[44]

AML blasts with an *FLT3-TKD* mutation are less sensitive to lestaurtinib than blasts with an *FLT3-ITD* mutation. The blasts from 14 patients with *FLT3* wild-type mutation, 11 patients with *FLT3-ITD* mutation, and 11 patients with *FLT3-TKD* mutation were studied in vitro with cytarabine alone, lestaurtinib alone, or a combination of both agents. All 3 cell types showed similar sensitivity to the cytotoxicity of cytarabine alone, but the *FLT3-ITD* mutant level was inversely correlated with cytarabine cytotoxicity (P=.04), whereas the *FLT3-TKD* mutant level had no impact. FLT3-TKD cells and *FLT3* wild-type cells had a similar response to lestaurtinib, and FLT3-ITD cells were more sensitive (P=.004). The *FLT3-ITD* mutant level had no effect on sensitivity to lestaurtinib. Synergistic sensitivity of lestaurtinib plus cytarabine was demonstrated in all 3 groups of cells. The results suggest that all 3 groups would benefit from treatment with the combination.[3]

Results were recently reported for a phase II trial of oral lestaurtinib alone for 8 weeks. The study subjects were untreated older patients with AML who were unfit for standard therapy. They were included irrespective of FLT3 mutational status. The initial dose was 60 mg twice daily, escalating to 80 mg twice daily, which was well tolerated. Transient reductions in marrow and blood blasts and longer periods of transfusion independence were seen in 3 of 5 patients with mutated *FLT3* and in 5 of 22 evaluable patients with wild-type mutations.[45]

Levis and colleagues conducted a study of salvage chemotherapy followed by lestaurtinib for *FLT3* mutant AML patients in first relapse.[46] Patients were randomized to receive chemotherapy alone (mitoxantrone, etoposide, and cytarabine) or high-dose cytarabine, depending on the duration of the previous CR, or chemotherapy followed by 80 mg lestaurtinib orally twice daily for up to 112 days. Of the 224 patients who entered the study, 220 were evaluable. The median age was 53.5 years among patients in the control arm and 58.5 years among patients in the lestaurtinib arm. The duration of first CR was less than 6 months in 47% of patients in each arm. On the lestaurtinib arm, 107 patients received at least 1 week of lestaurtinib (mean, 60.1 days). Lestaurtinib was generally well tolerated following chemotherapy, with 104 grade 3/4 adverse events in the lestaurtinib arm and 99 in the chemotherapy-alone arm. In the intention-to-treat population, there were 29 CRs/PRs in the lestaurtinib arm and 23 in the control arm (26% vs 21%; P value was not significant), with no significant difference in overall survival between the arms (4.73 months in the lestaurtinib arm and 4.57 months in the control arm). FLT3 inhibition by lestaurtinib—achieved in 58% of patients—correlated with better CR rates (39%). A poor CR rate (9%) was observed in those patients for whom the drug did not reach the target level of FLT3 inhibition. Ligand levels rose from a baseline of 15.6 pg/mL to 1,148 pg/mL by day 15 in this study. In summary, this study failed to demonstrate efficacy for lestaurtinib at the dose and schedule employed.[46]

These disappointing results may be related to the half maximal inhibitory concentration (IC_{50}) for lestaurtinib inhibition of FLT3-ITD autophosphorylation. The IC_{50} for inhibition of FLT3-ITD autophosphorylation was as follows: lestaurtinib, 2 nM; AC220, 1 nM; KW-2449, 10 nM; sorafenib, 3 nM; and sunitinib, 1 nM. For patients with the wild-type mutation, these values were: lestaurtinib, 10 nM; AC220, 5 nM; KW-2449, 36 nM; sorafenib, 28 nM; and sunitinib 2 nM. When plasma protein binding was taken into account, AC220 was predicted to be the most potent of these compounds in vivo (IC_{50} in plasma, 18.4 nM), and lestaurtinib was predicted to be the least potent in vivo (IC_{50} in plasma, 700 nM).[13]

Another problem for lestaurtinib and other FLT3 inhibitors is the influence of the FLT3 ligand on their effectiveness. Sato and coauthors compared in vivo FLT3 inhibition in newly diagnosed AML patients and relapsed patients.[47] FLT3 inhibition by lestaurtinib was less effective in relapsed patients. FLT3 ligand levels increased dramatically following myelosuppressive chemotherapy. Ligand levels averaged 2.7 pg/mL in newly diagnosed patients; the average level was 15.6 pg/mL in patients at first relapse. Levels rose to a mean of 402 pg/mL on day 15 of induction therapy for newly diagnosed patients and to a mean of 1,174 pg/mL after induction therapy for relapsed patients. Ligand levels exerted a negative influence on FLT3 inhibition by lestaurtinib, AC220, KW-2449, PKC-412, and sorafenib in vitro by shifting upward the cytotoxic IC_{50} of FLT3 inhibitors 2-fold to 4-fold in patients with only the *FLT3-ITD* mutation. In patients with both wild-type and *ITD*-mutated *FLT3*, exogenous ligand in vitro increased the degree of FLT3 autophosphorylation and dramatically increased cell survival. Ligand is an important driver in *FLT3* mutant AML.

Tandutinib (MLN518)

Tandutinib, an inhibitor of FLT3, PDGFRβ, and Kit, was studied in a phase I trial in 40 AML and high-risk myelodysplasia patients. It was given orally at 50–700 mg twice daily. The main dose-limiting toxicities were reversible generalized muscle weakness, fatigue, or both, occurring at doses of 525 mg and 700 mg twice daily. Pharmacokinetics showed slow elimination, with achievement of steady-state plasma concentrations requiring more than 1 week of dosing. Among the 5 patients with FLT3-ITD treated with tandutinib 525 mg and 700 mg twice daily, 2 patients (1 at the 525 mg twice daily dosage and 1 at the 700 mg twice daily dosage) had decreases in both blood and marrow blasts. Tandutinib at 525 mg twice daily should be studied further.[48]

Tandutinib was combined with standard 3+7 induction therapy in AML. In contrast to other structurally unrelated FLT3 inhibitors, tandutinib displayed application-sequence, independent, synergistic antileukemic effects in combination with cytarabine and daunorubicin. Strong synergistic antiproliferative and proapoptotic effects were seen in FLT3-ITD+ blasts.[49] A 90% CR rate was observed in a trial by Cheng and Paz.[50] Clearly, tandutinib is a potentially useful agent.

AC220

AC220 is a second-generation FLT3 inhibitor with low nanomolar potency in biochemical and cellular assays and exceptional kinase selectivity. In animal models, it is efficacious at doses as low as 1 mg/kg orally once daily.[51,52]

In a phase I trial, AC220 was administered once daily as an oral solution for 14 days followed by a 14-day rest period (1 cycle), with a starting dose of 12 mg.[53] In the first 52 patients, it was well tolerated, and the dose was escalated to 450 mg/day. The drug has a long effective half-life (2.5 days). An active metabolite (AC886) has been identified in patient urine and plasma, which has similar potency and kinase selectivity to the parent compound. The IC_{50} for both compounds in FLT3-ITD–expressing patient blast cells is in the low nanomolar range (1.7 nM for AC220 and 0.3 nM for AC886). Both drugs potently inhibit wild-type *FLT3* at slightly higher nanomolar concentrations. Plasma from AC220-treated AML patients completely suppresses FLT3 phosphorylation on day 1, even at the lowest daily dose of 12 mg. AC220 is one of the first kinase inhibitors to completely suppress FLT3 phosphorylation ex vivo at doses that are easily achievable and sustainable in the clinic.[52,53] AC220 increased survival in 2 mouse leukemia models with FLT3-ITD homozygous and heterozygous genotypes, and chronic administration was more efficacious than limited course therapy.[54]

Belli and colleagues studied the effect of combining AC220 with cytarabine, cladribine, etoposide, and daunorubicin against the homozygous FLT3-ITD cell line MV4-11 in vitro.[55] The results indicate that delivery of AC220 the day of or the day following treatment with the individual chemotherapeutic agents generally provided additive synergistic effects. Similar results were obtained in in vivo experiments with mice bearing MV4-11 leukemia. This agent is currently being studied in a phase II clinical trial.

KW-2449

KW-2449 is a multikinase inhibitor of FLT3, ABL, ABL-T315I, and aurora A kinase.[56] Aurora kinases play an important role in chromosome alignment, segregation, and cytokinesis during mitosis. Aurora kinase A and B are aberrantly expressed in many leukemia cell lines and blasts from AML patients compared with marrow mononuclear cells from healthy volunteers. ZM447439 is a novel selective aurora kinase inhibitor. It induced growth inhibition and apoptosis in a number of leukemia cell lines and was especially effective in lines with wild-type

p53. It had virtually no effect on stem cells harvested from healthy volunteers.[57]

KW-2449 has antiproliferative activity and enhances apoptosis in patients with FLT3-ITD+ AML as well as AML with wild-type *FLT3*.[58] KW-2449 showed potent growth inhibitory effects on leukemia cells with *FLT3* mutations by inhibition of the FLT3 kinase, resulting in the downregulation of phosphorylated FLT3/STAT5, G1 arrest, and apoptosis. In wild-type *FLT3* human leukemia, it caused G2 arrest and apoptosis. It also caused apoptosis in imatinib-resistant human leukemia cells.[58]

KW-2449 induces cytotoxicity in MOLM-14 cells that harbor the *FLT3-ITD* mutation. Transient reductions in peripheral blast counts were observed in a phase I trial of 37 patients with refractory/relapsed AML, acute lymphoblastic lymphoma, or myelodysplastic syndromes, or with resistant/intolerant CML. FLT3 was inhibited, but only transiently.[58] A dose of 12.5–250 mg twice daily for 14 or 28 days with a recovery period of 7–28 days between cycles was initially used, but the 28-day schedule was terminated during the study. The mean duration of therapy was 2 cycles in AML and 4 cycles in CML. The half-life of the drug ranged from 2.4 hours to 4.9 hours. The near-complete downregulation of phosphorylated FLT3 was at a level of 400 mg/day 2-hours postdose and was generally absent 12 hours postdose. Adverse events were as follows: nausea in 70.3%, vomiting in 48.6%, fatigue in 45.9%, diarrhea in 32.4%, dyspnea in 29.7%, febrile neutropenia in 29.7%, pain in extremities in 29.7%, and arthralgia in 27.0% of patients. The most frequent grade 3/4 adverse events were febrile neutropenia in 24.3%, pneumonia in 10.8%, and thrombocytopenia in 10.8% of patients. Atrial fibrillation and pleural effusion may have been drug-related. The reduction in blood and/or marrow blasts at the end of cycle 1 was less than 50% in 26% of AML patients (5 patients with FLT3 mutation, 3 without) and in 1 of 5 CML patients. Only 1 AML patient had a reduction in blasts (500 mg/day) exceeding 50%. One CML patient with T315I+ BCR-ABL lost the mutant clone while in the study. Sustained inhibition of phosphorylated FLT3 was not achieved. Dosing every 6–8 hours should be evaluated, given the short half-life of the drug.[59,60]

General Considerations

At M.D. Anderson Cancer Center, Pemmaraju and associates retrospectively reviewed the outcome of patients with AML or myelodysplastic syndromes who received FLT3 inhibitors as part of their therapy.[61] Among the 128 patients, 51 were FLT3 wild-type, 56 were FLT3-ITD+, 11 were FLT3-D835+, and 10 were both FLT3-ITD+ and FLT3-D835+. The overall median age was 62 years, and 50% of the patients were women. Twenty-three patients received FLT3 inhibitors with induction chemotherapy, and 22 patients received FLT3 inhibitors as first salvage therapy. Nine patients (all with FLT3 mutations) achieved CR or PR with FLT3 inhibitors for a median of 8 months (range, 3 to ≥12 months). The overall median survival for the 128 patients was 3.8 months, with 3.1 months for the 51 patients with *FLT3* wild-type and 4.2 months for the 77 patients with FLT3 mutations (P=.03). The researchers concluded that FLT3 inhibitors have the potential to improve outcome in AML patients with FLT3 mutations.[61]

Certain FLT3-ITD+ murine and human leukemia cell lines are resistant to cytarabine due to downregulation of the equilibrative nucleoside transporter 1, a transporter responsible for the cellular uptake of cytarabine. This effect may in part be responsible for the poor prognosis of FLT3-ITD+ AML patients.[62] It can be prevented by pretreating the cells with an FLT3 inhibitor.

FLT3 ligand plasma levels rise following chemotherapy and may interfere with the activity of FLT3 kinase inhibitors by increasing the degree of FLT3 autophosphorylation and increasing cell survival.[47] In 4 of 5 patients studied, an *FLT3-ITD* mutation was present in noncycling, dormant cells. At least 25% of noncycling cells in patients with the *FLT3-ITD* mutation harbored the mutation. The majority of noncycling AML cells that harbor FLT3-ITD are unaffected by FLT3 inhibitors, and may constitute an untargeted disease reservoir.[19]

Conclusions

FLT3 inhibitor studies have offered both promise and disappointment. In most studies, FLT3-ITD inhibition has been accomplished only transiently, and resistance has become evident after relatively short periods of treatment. Mechanisms of resistance are only now becoming clear. Multikinase inhibitor resistance may be due to inadequate dosing; ligand interference; poorly understood relationships between mutation and wild-type signals; the presence of residual dormant, noncycling cells that harbor *FLT3-ITD* mutations; and/or other as-yet unknown mechanisms. It is likely that optimal application of these agents will involve combinations of inhibitors. For example, the combination of an FLT3-ITD inhibitor with an mTOR inhibitor such as everolimus or temsirolimus (Torisel, Wyeth) seems worthy of evaluation, based on study data. Finally, the most effective combination of multikinase inhibitors with chemotherapy still needs to be elucidated. At present, the most promising FL3-ITD inhibitor appears to be AC220, which appears to completely suppress FLT3-ITD autophosphorylation in some studies. However, it is too early to completely discard any of the agents discussed. They all have different pharmacokinetic properties and affect different pathways in addition to *FLT3* mutation signals.

These agents provide us with an entirely new approach to the treatment of AML, and their importance will only increase as we learn more about them and the biologic pathways with which they interact.

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