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Leukemias

FLT3 Inhibition as Therapy in Acute Myeloid Leukemia: A Record of Trials and Tribulations

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Incorporate FLT3 mutational status into the initial diagnostic evaluation of AML to acquire prognostic information and guide the aggressiveness of consolidative therapy.
2. Select FLT3-mutant patients to participate in clinical trials of FLT3 inhibitors in order to help provide important insight into the future utility and promise of these compounds as adjuncts to therapy.

ABSTRACT

Acute myeloid leukemia (AML) is a hematologic malignancy with a poor prognosis. Approximately one quarter of the patients with AML also carry an internal tandem duplication (ITD) mutation in the gene encoding FMS-like tyrosine kinase 3 (*FLT3*), which has a significantly deleterious impact on prognosis. The ITD mutation renders FLT3 constitutively active and leads to uncontrolled proliferation of the leukemic blast. Over the course of the last decade, a variety of compounds have been developed in preclinical and clinical studies as potent inhibitors of FLT3. Many of the earlier agents under investigation, such as lestaurtinib, midostaurin, and sunitinib, were initially developed as inhibitors of other tyrosine kinases and as targeted therapies in a va-

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riety of malignancies. These compounds have been demonstrated to have some efficacy in clinical trials of AML, mainly manifesting as transient decreases in circulating blasts correlating with effective in vivo suppression of the FLT3 target. Nevertheless, the cumbersome pharmacokinetics of some compounds and the suboptimal specificity and potency of others have limited their therapeutic efficacy. In the last few years, newer, more potent and specific agents have been under investigation, with the leading example being AC220. This agent has shown significant promise in early phases of clinical investigation, and is currently in more advanced clinical trials. Hope remains that FLT3 inhibition will be become an effective therapeutic adjunct to our current treatment approach to AML. *The Oncologist* 2011;16: 1162–1174

Introduction

The internal tandem duplication (ITD) mutation in the gene encoding FMS-like tyrosine kinase 3 (*FLT3*) is found in approximately one quarter of the patients with acute myeloid leukemia (AML) and confers a markedly poor prognosis. Patients with this alteration often relapse following cytotoxic chemotherapy, and the majority die from their disease [1–4]. Recent studies have indicated 5-year overall survival (OS) and disease-free survival (DFS) rates as low as 15%–16% for patients with *FLT3*-ITD mutant disease, in contrast to the OS and DFS rates of ~40% in those with wild-type (WT) *FLT3* AML [1]. Inhibitors of the FLT3 tyrosine kinase have been extensively studied preclinically, and several have now been developed and investigated in clinical trials for the treatment of AML patients. Among these are a number of compounds initially developed to target other tyrosine kinases, but were later found to be potent inhibitors of FLT3. These include sorafenib, sunitinib (SU11428), lestaurtinib (CEP-701), semaxinib (SU5416), and midostaurin (PKC412) [5–8]. Midostaurin and lestaurtinib are currently the farthest along in clinical trials and have been associated with transient clinical responses. In recent years, attempts have been made to develop more specific and potent inhibitors of FLT3 for clinical investigation. One such agent, AC220, has shown great promise and dramatic responses in early-phase trials of patients with AML [9].

FLT3 as a Target

FLT3 was first cloned independently by two groups in the early 1990s [10, 11]. It resides on chromosome 13 and is comprised of 24 exons [12–14]. FLT3 is considered a type III receptor tyrosine kinase, a class that also includes KIT and platelet-derived growth factor receptor (PDGFR), proteins with very close homology to FLT3 [3, 15, 16]. FLT3 consists of an extracellular region with five immunoglobulin-like domains, a transmembrane region, a short intracellular juxtamembrane portion, followed by an intracellular tyrosine kinase domain (TKD). Upon binding of its ligand, FLT3 dimerizes, leading to eventual autophosphorylation on the inner leaflet of the membrane, with subsequent activation of the tyrosine kinase. phosphoinositide 3-kinase (PI3K), AKT, mitogen-activated protein kinase (MAPK), and signal transducer and activator of transcription (STAT)-5 are all significant mediators of downstream FLT3 signaling (Fig. 1) [17–25].

The cytokine that binds FLT3, the FLT3 ligand (FL), is ubiquitous to most tissues but appears functionally important only in hematopoietic and neural tissue [26, 27]. In the hematopoietic environment, FLT3 expression exists predominantly in $CD34^+$ cells, although $CD34^-$ precursors of dendritic cells also express FLT3. FLT3 is a key mediator of early hematopoiesis and is involved with the reconstitution of early multilineage myeloid precursors [11, 28–30]. Disruption of FLT3 signaling in murine models is not lethal but does lead to a significant reduction in hematopoietic precursors. Specifically, when the *FL* gene was disrupted in mice, the numbers of myeloid and B lymphoid cells were markedly lower in the bone marrow. Interestingly, the numbers of dendritic and natural killer cells were also significantly lower in the spleen and thymus [31].

Overexpression of FLT3, or its constitutive activation, appears to play a major role in leukemias. Both FL and the FLT3 receptor have been demonstrated in the majority of human leukemia cell lines [32, 33]. FLT3 is expressed in higher amounts in AML blasts than in cells from normal bone marrow. Additionally, in this setting, its expression is no longer tightly associated with CD34 expression as it is in normal precursors. Indeed, the large majority of evaluated AML cell lines have amplified activity of FLT3 [34–36]. Some of these cells exhibited overexpression of WT *FLT3*, but others were found to have activating mutations that rendered FLT3 constitutively active [12, 37–40].

ITDs of nucleotide sequences in exon 14 were the first form of *FLT3* mutation discovered in AML, and are found in approximately 23% of patients with AML. These mutations localize to the juxtamembrane domain of the receptor tyrosine kinase, where they presumably offset the negative regulatory functions of this domain [41–44]. Another category of *FLT3* mutations consists of activating point mutations within the activation loop of the kinase domain, mostly localized at the aspartate 835 (D835) residue and found in an additional 7% of

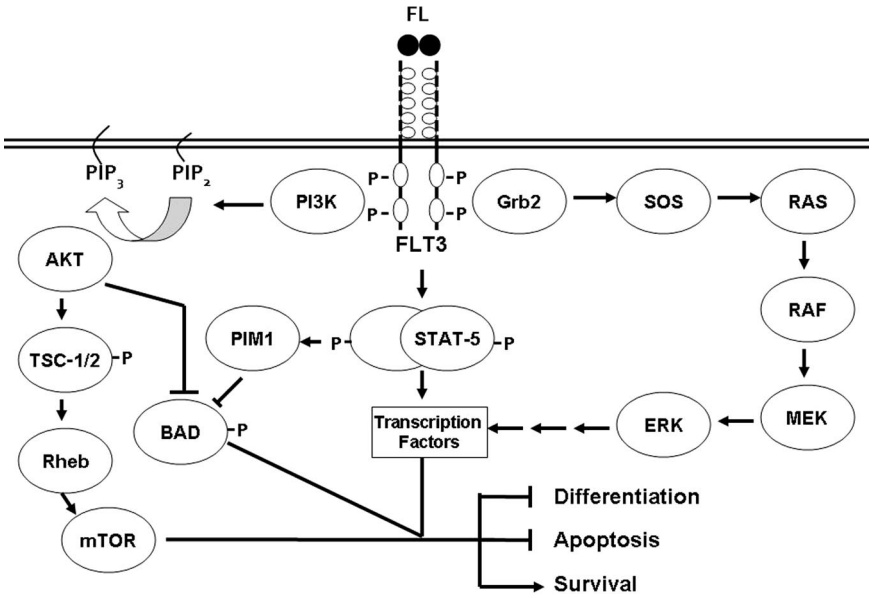


Figure 1. Simplified diagram of signaling cascades downstream of FLT3 that are thought to promote leukemogenesis.

Abbreviations: BAD, Bcl-2-associated death promoter; ERK, extracellular signal–related kinase; FL, FLT3 ligand; FLT3, FMS-like tyrosine kinase 3; Grb2, growth factor receptor-bound protein 2; MEK, mitogen-activated protein kinase/ERK kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PIM1, proto-oncogene serine/threonine-protein kinase 1; PIP_2, phosphatidylinositol-bisphosphate; PIP_3, phosphatidylinositol-trisphosphate; Rheb, Ras homolog enriched in brain; SOS, son of sevenless; STAT-5, signal transducer and activator of transcription 5; TSC, tuberous sclerosis protein.

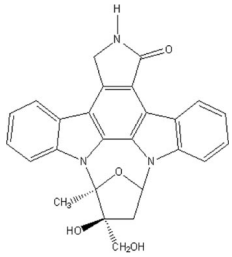
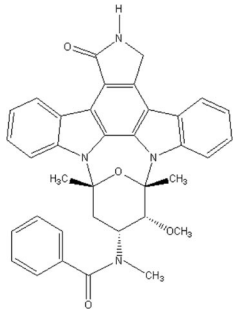
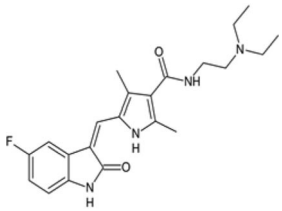
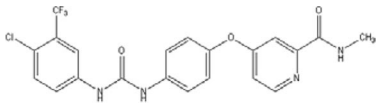
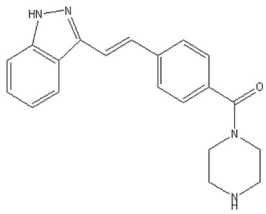
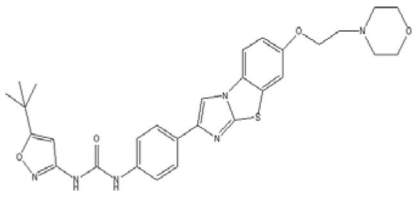
Figure derived from one obtained courtesy of Dr. Mark Levis, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins, Baltimore, MD.

patients [12, 45]. *FLT3* mutations result in constitutive activation, leading in turn to activation of STAT-5 as well as the MAPK and AKT signaling cascades. This results in suppression of apoptosis and dysregulated cell proliferation [38, 46, 47]. The ITD mutations, in particular, have been consistently found to have a negative prognostic impact. This was confirmed, in large part, by studies of banked AML samples from the European cooperative groups and multiple subsequent clinical studies, which have demonstrated that patients with *FLT3* ITD mutations often present with leukocytosis, experience significantly higher rates of relapse, and have shorter DFS and OS times. According to recent studies, patients with newly diagnosed FLT3-ITD AML have DFS and OS rates as low as 15% at 5 years, in comparison with the ~40% rates in those with WT *FLT3*. *FLT3* point mutations, on the other hand, do not appear to carry with them the same degree of negative prognostic impact as *FLT3* ITD mutations [1, 2, 4, 48–51]. These findings have brought forth a rationale and provided significant impetus to develop effective FLT3 inhibitors as therapy for AML patients.

In recent years, multiple compounds that inhibit the kinase function of FLT3 have undergone preclinical investigation (Table 1). Most are structural mimics of the purine component of ATP, and in this manner occupy the ATP-binding pocket of the tyrosine kinase [52, 53]. A number of assays have been employed to assess the in vitro potency and selectivity of FLT3 inhibitors, including in vitro kinase assays, cell-based receptor autophosphorylation assays, and cytotoxicity assays. Results from these studies have indicated that, in general, a specific FLT3 inhibitor induces preferential cytotoxicity, as demonstrated by a significantly lower 50% inhibitory concentration (IC_{50}), in FLT3-ITD AML cells. In addition, sustained and potent FLT3 inhibition (<10% FLT3 activity) is required to produce effective cell death of myeloblasts [5, 54].

The validation of FLT3 as a viable therapeutic target has also been supported by studies that have sought to suppress FLT3 by alternative mechanisms. One such manner of targeting FLT3 has been by RNA interference (RNAi) to induce downregulation of the tyrosine kinase. In those studies, downregulation of FLT3 led to a lower rate of phosphorylation of the FLT3 targets STAT-5, AKT, and MAPK. More importantly, the viability and growth of these cell lines were significantly lower following knockdown of *FLT3*. The investigators further demonstrated that the sensitivity of these cell lines to the FLT3 inhibitor tandutinib was enhanced by RNAi-induced downregulation of the FLT3 target [55]. Preclinical studies have therefore led to the conclusion that the activation of FLT3 results in activation of downstream signaling pathways and promotion of cell survival, and its downregulation can promote cytotoxicity, and therefore act as a potential therapeutic mechanism.

Table 1. IC_{50} and chemical structure of FLT3 inhibitors under current investigation for AML

FLT3 inhibitor	IC_{50}, in vitro FLT3 inhibition, media [38, 58, 98]	IC_{50}, in vitro FLT3 inhibition, plasma [58]	Chemical structure
Lestaurtinib	2–3 nM	700 nM	
Midostaurin	3–10 nM	1,700 nM	
Sunitinib	50 nM	Not determined	
Sorafenib	3 nM	484 nM	
KW-2449	10 nM	144 nM	
AC220	1 nM	18 nM	

IC_{50} indicates the concentration of drug that inhibits the autophosphorylation of FLT3 in vitro to 50% of its baseline value. IC_{50} values were calculated in cell-based assays using mutant *FLT3* cell lines in medium and primary patient leukemia samples in plasma [58].
Abbreviations: AML, acute myeloid leukemia; FLT3, FMS-like tyrosine kinase 3; IC_{50}, 50% inhibitory concentration.

Sustained and effective inhibition of FLT3 appears necessary for clinical responses in trials, and therefore, presumably, for any FLT3 inhibitor to be successful clinically it must be able to accomplish this molecular feat in vivo. This is sup-

ported by results from correlative studies of clinical trials of FLT3 inhibitors, in which effective suppression of FLT3 phosphorylation strongly correlated with clinical response [54, 56]. The earlier, more multitargeted, generation of FLT3 inhibitors has been generally associated with transient, though often dramatic, decreases in peripheral blast counts. The suboptimal performance of these agents in clinical trials has at times been linked to their pharmacokinetic parameters, and in other cases to a lack of effective and sustained FLT3 inhibition. In the last few years, however, FLT3 inhibitors with more potent and constant suppression of their target have been associated with more impressive clinical outcomes, albeit in earlier phases of clinical investigation.

INHIBITORS OF FLT3

As mentioned above, the majority of the characterized FLT3 inhibitors are heterocyclic compounds with a component resembling a purine ring. They can thus act as competitors of ATP for the FLT3 binding site. The exact mechanism of binding for all FLT3 inhibitors is not completely clear, although some, such as the staurosporine derivatives (e.g., lestaurtinib), bind via an induced fit mechanism, whereas others interact in a lock and key manner [53, 57]. The clinical effectiveness of FLT3 inhibitors currently under study is quite variable, and in many ways dependent on the pharmacodynamic and pharmacokinetic properties of the compounds. Like other tyrosine kinase inhibitors (TKIs), the degree and duration of inhibition is determined by the potency of the inhibitor, its susceptibility to metabolism and protein binding, its pharmacokinetic properties such as half-life and rate of elimination, as well as the related factor of dosing frequency. Perhaps, most importantly, clinical efficacy depends on the dependence of leukemic cells on the FLT3 pathway. Very recent studies have indeed demonstrated that relapsed leukemia and other samples with a high mutant *FLT3* allelic burden are more likely to be responsive to cytotoxicity from FLT3 inhibition [58].

We henceforth review the results from clinical evaluation of FLT3 inhibitors. The structure and in vitro potency of a number of the evaluated FLT3 inhibitors are demonstrated in Table 1. The results of key clinical trials are summarized in Table 2.

EARLY FLT3 INHIBITORS

Semaxinib

Semaxinib is an indolinone-derived TKI. This compound was not initially described as an inhibitor of FLT3 and entered clinical trials based on its suppression of other targets often upregulated in myeloblasts, such as c-KIT and vascular endothelial growth factor receptor (VEGFR). Semaxinib has been studied in clinical trials of refractory AML and myelodysplastic syndrome (MDS) patients. In one study of 55 patients, four instances of a transient hematologic response were noted, and in another phase II trial of 42 patients, one morphologic complete response (CR) and seven partial responses in the peripheral blood and marrow were found [59, 60]. Given the structural homology of c-KIT and FLT3, it was not surprising to find that semaxinib also effectively suppressed the latter target in leukemia cells [8]. Downstream effects of FLT3 inhibition by semaxinib included a lower rate of phosphorylation of the FLT3 targets STAT-5 and AKT. However, given the only modest clinical effects of this agent in patients with AML, no further advanced-phase studies have ensued.

Sunitinib

Like semaxinib, sunitinib can effectively inhibit multiple tyrosine kinases, including PDGFR, VEGFR, c-KIT, as well as FLT3 [61]. Sunitinib has been extensively studied in clinical trials of solid tumor malignancies [62–64] and is approved for use in metastatic renal cell carcinoma and gastrointestinal stromal tumors. O'Farrell et al. [61] demonstrated that sunitinib is a potent inhibitor of mutant *FLT3* in AML cell lines, with an IC_{50} of 50 nM and single doses achieving potent inhibition for up to 16 hours in preclinical in vitro models. Sunitinib also inhibits the phosphorylation of WT *FLT3* at an IC_{50} of 150 nM. A single-dose design, phase I trial of sunitinib followed in order to assess the in vivo effects of the compound in patients with AML. Clinical activity (decreases in peripheral blast counts) was noted in five of 29 patients, including subjects with both mutant *FLT3* and WT *FLT3* disease. Toxicity was mainly gastrointestinal and affected a third of patients [7]. A more traditional phase I trial of 15 patients with refractory AML again reported a small number of transient partial responses. These responses occurred in all four patients with mutant *FLT3* AML and two patients with WT *FLT3* disease. However, three patients experienced grade 3–4 toxicity, and six deaths were reported by the investigators (four cases of bleeding and two of cardiac dysfunction) [65].

Those studies demonstrated that sunitinib does lead to clinical responses in a fraction of treated patients with AML. Its associated significant toxicity, however, which has affected a number of patients studied, has limited the ability to maintain continuous dosing of the drug in patients with AML. A phase I/II trial of sunitinib administered concurrently with induction 7+3 chemotherapy is currently enrolling older patients with mutant *FLT3* AML. Interim results were presented at the recent annual meeting of the American Society of Hematology (ASH). Twelve patients have been enrolled, eight of whom harbored *FLT3* ITD mutations, and four of whom had *FLT3* TKD mutations. Although seven of 10 evaluable patients achieved a CR or

Table 2. The status of FLT3 inhibitors investigated in clinical trials of AML

Compound	Clinical trial stage	Significant findings
Semaxinib (SU5416)	Phase II [60]	Minimal activity in MDS/AML, with few transient hematologic responses
Sunitinib (SU11248)	Phase I [7]	Reductions in peripheral blasts, but with approximately one third of patients experiencing drug-related toxicities
	Phase I/II [66]	Can be administered with chemotherapy with ~70% CR rate, but with greater nonhematologic toxicity
Sorafenib (BAY 43–9006)	Phase I [73, 74]	Fairly well tolerated as a single agent, with transient reductions in peripheral and marrow blasts
	Phase I/II [80, 81]	Can be safely combined with induction and consolidation chemotherapy, but does not seem to result in better outcomes in combination
Tandutinib (MLN518)	Phase I [84]	Poor pharmacokinetics, but produced transient reductions in peripheral and marrow blasts
	Phase I/II [86]	Tandutinib can be administered safely with cytotoxic chemotherapy
Lestaurtinib (CEP-701)	Phase I/II [54, 92]	Sustained FLT3 inhibition by lestaurtinib is associated with transient reductions in peripheral and marrow blasts
	Phase III [94]	• A U.S. trial demonstrated that lestaurtinib can be safely combined with chemotherapy, but that this did not improve overall survival in patients with advanced disease • British MRC trials are also currently investigating the combination of lestaurtinib with induction and consolidation chemotherapy
Midostaurin (PKC412)	Phase IIB [102]	The majority of patients with mutant *FLT3* AML and some with wild-type *FLT3* disease experienced reductions in peripheral and bone marrow blasts
	Phase IB [101]	Midostaurin can be administered safely in combination with cytotoxic chemotherapy
	Phase III (ongoing)	Randomized multinational trial, currently accruing patients, comparing traditional cytotoxic therapy with or without midostaurin
KW-2449	Phase I [56, 104] (ongoing)	Reductions in peripheral and marrow blasts
AC220	Phase I [107] (ongoing)	Single-agent AC220 leads to reductions in peripheral and marrow blasts in the majority of patients, as well as multiple instances of CR
	Phase II (ongoing)	Phase II study of AC220 currently enrolling patients

Abbreviations: AML, acute myeloid leukemia; CR, complete response; FLT3, FMS-like tyrosine kinase 3; MDS, myelodysplastic syndrome; MRC, Medical Research Council.

complete remission with insufficient blood count recovery, a high proportion of patients (>40%) experienced neutropenic fever, infection, and colitis [66], and long-term follow-up is not yet available.

Sorafenib

Sorafenib is now approved by the U.S. Food and Drug Administration for use in advanced renal cell and hepatocellular carcinoma patients [67, 68]. Sorafenib is a potent inhibitor of multiple receptor tyrosine kinases, including FLT3, c-KIT, NRAS, and RAF kinase, all of which can be upregulated in AML and appear to promote leukemogenesis and drive proliferation in myeloblasts [69, 70]. Indeed, sorafenib has been shown to suppress FLT3 phosphorylation and downstream signaling, leading to apoptosis of leukemia cells [71, 72]. In comparison with some other multitargeted FLT3 inhibitors, sorafenib is more effective in inducing sustained FLT3 inhibition in experimental models and in patients. This finding may be related to an N-oxide metabolite of sorafenib, which was incidentally found by Pratz et al. [73] to be 15-fold more potent than the parent compound in its inhibition of FLT3 autophosphorylation in treated patients.

One phase I study of 15 patients with diagnoses of relapsed or refractory leukemia, the majority of which were AML, reported that six patients experienced transient de-

creases in bone marrow blast percentage. Preliminary data from another phase I study, from the MD Anderson Cancer Center, found that 11 of 20 evaluable patients experienced a ≥50% reduction in bone marrow blasts. Nine of the responders had FLT3-ITD disease. Those studies also revealed that sorafenib is relatively well tolerated as a single agent in AML patients [73, 74]. There has been increasing use of sorafenib on an off-label and compassionate-use basis for patients with advanced mutant *FLT3* AML, and indeed dramatic cases of CR to single-agent sorafenib have been reported [75, 76]. Sorafenib has also been studied in the setting of allogeneic stem cell transplantation of mutant *FLT3* AML. In a recent abstract presentation, six of 11 patients with refractory disease underwent allogeneic stem cell transplantation after responding to treatment with sorafenib. The same group also retrospectively reported the experience of multiple centers with daily sorafenib in the peritransplant setting. In that abstract presentation, nine of 16 patients who had relapsed after transplant experienced reductions in peripheral blood or bone marrow myeloblasts, and four experienced a complete molecular remission [77, 78]. However, in another retrospective analysis, the use of sorafenib to treat 16 patients with relapsed FLT3-ITD AML following stem cell transplantation did not appear effective, because the drug produced only two transient partial responses [79].

A phase I/II trial of 51 newly diagnosed AML patients investigated sorafenib given concurrently with cytarabine- and idarubicin-based induction. Thirty-eight patients (75%) achieved a CR following induction, but remarkably the investigators reported very high response rates in particular for patients with FLT3-ITD AML, whereby 14 of 15 patients achieved a CR. Correlative studies reported effective suppression of FLT3 phosphorylation in *FLT3* ITD mutant patients who achieved a CR, and there was a fivefold greater suppression of ITD FLT3 than WT FLT3 [80]. A European multicenter, randomized, placebo-controlled phase II trial in elderly patients is evaluating sorafenib combined with standard induction, consolidation, and maintenance chemotherapy. Data from that trial were presented at the recent ASH annual meeting. Although the combination was well tolerated in this group of 197 patients, no benefit in terms of event-free survival, OS, or the rate of CR has so far been noted, even in patients with mutant *FLT3* AML [81]. Other ongoing clinical trials are further evaluating sorafenib in combination with targeted therapies and cytotoxic regimens (ClinicalTrials.gov identifiers, NCT00516828, NCT00908167, NCT 00373373, NCT00893373, NCT00875745, NCT00943943), but results are not yet available.

Tandutinib

Tandutinib, also known as MLN518, is somewhat unique among its predecessors because of its greater selectivity against FLT3, even though it does also display some inhibitory effects on c-KIT and PDGFR, which, as mentioned above, share structural homology with FLT3 [82]. Initial preclinical assays revealed that tandutinib was preferentially cytotoxic to FLT3-ITD cells lines. However, it was not found to be a particularly potent compound, inhibiting FLT3 phosphorylation at relatively high concentrations, with an IC_{50} of ~30 nM in cell-based assays [82, 83].

A phase I trial in patients with relapsed/refractory AML and high-risk MDS revealed that a significant number of patients experienced nausea and vomiting, and that the dose-limiting toxicity was reversible muscle weakness. Correlative studies revealed that tandutinib was cleared slowly, leading to elevated plasma levels that may have exacerbated the therapy-related toxicity. Those experiencing the dose-limiting toxicity of profound muscle weakness all had persistently elevated plasma levels of tandutinib, which was felt to be responsible for the adverse therapeutic index of the agent. Nevertheless, two of eight evaluated patients with FLT3-ITD AML experienced transient decreases in blast percentage in the blood and bone marrow, both lasting <60 days. No antileukemic effects were noted in patients with WT *FLT3* [84].

Tandutinib in combination with the antileukemic drugs cytarabine and daunorubicin displayed synergism when incubated with FLT3-ITD leukemia samples [85]. A phase I trial of tandutinib combined with induction therapy has been completed. Although the therapeutic efficacy results are not yet available, preliminary data reported at the 2006 ASH annual meeting suggested that the combination is well tolerated [86]. In summary, the suboptimal pharmacokinetics and the relatively low potency of tandutinib have limited its clinical utility and promise as an FLT3 inhibitor.

Lestaurtinib

Lestaurtinib has perhaps been the most extensively studied FLT3 inhibitor in clinical trials. Previously known as CEP-701, it is a polyaromatic indolocarbazole compound that effectively inhibits multiple tyrosine kinases, including RET, Janus kinase 2, tropomyosin related kinase (TRK), as well as FLT3 [87–89]. Lestaurtinib was initially evaluated as therapy in solid tumor malignancies, given its activity against TRK. In this setting, although the drug was well tolerated, no objective tumor responses were noted [90].

Preclinical studies of lestaurtinib suggested that it is a potent inhibitor of FLT3, inhibiting FLT3 autophosphorylation in ITD cell lines at an IC_{50} of 2 nM and exhibiting preferential cytotoxicity against FLT3-ITD cells [38]. In

contrast to the negative results noted in clinical trials of solid tumors, a phase I/II trial of 17 patients with relapsed/refractory mutant *FLT3* AML (with all but one having ITD mutations) reported four patients with decreases in peripheral myeloblasts and one with a dramatic decrease in bone marrow blasts to <5%. Lestaurtinib was also fairly well tolerated in these patients, with common toxicities of fatigue and nausea. It was further noted that sustained suppression of FLT3 phosphorylation (over the course of 4–5 weeks of therapy) correlated strongly with the observed clinical responses [54, 91]. A subsequent phase II trial of newly diagnosed elderly patients was not restricted on the basis of *FLT3* mutational status. In that study, three of five patients with *FLT3* mutations experienced transient hematologic responses, mainly manifested as decreases in peripheral blasts. Interestingly, an additional five patients with WT *FLT3* experienced decreases in bone marrow blasts. These results were attributed to possible overexpression of FLT3 in these patients. These results may also be secondary to the multitargeted profile of lestaurtinib. In all eight patients who responded to lestaurtinib, the phosphorylation of FLT3 was continuously suppressed to <15% of baseline (as measured over time and on days 14, 28, and 56 of the study), again confirming that effective and sustained inhibition of FLT3 appears necessary for any clinical response [92].

In vitro studies demonstrated that lestaurtinib administered after cytotoxic chemotherapy led to synergistic leukemia cytotoxicity [93], which provided a rationale for this sequence. A multicenter trial of patients with relapsed AML randomized subjects to reinduction chemotherapy alone or chemotherapy followed by lestaurtinib. The results were presented at the 2009 ASH annual meeting. Unfortunately, the addition of lestaurtinib did not result in higher response rates or longer OS time in these patients with advanced disease. Correlative studies revealed that only a minority of patients achieved >85% FLT3 target inhibition by day 15 of therapy. The presenters speculated that this may, in turn, have been partly a result of elevations (as a response to cytotoxic chemotherapy) in plasma levels of FL [94]. The same investigators have indeed demonstrated a blunting of FLT3 inhibition by a variety of TKIs in the presence of increasing concentrations of FL [95].

Lestaurtinib was also incorporated into induction and consolidation chemotherapy regimens for mutant *FLT3* patients in the British Medical Research Council 15 and 17 trials. The results of those trials have not been fully reported, but unlike the phase III trial above, the British studies have not been limited to relapsed patients and include patients receiving induction and consolidation regimens. Initial reports suggest effective inhibition (>85% inhibition) of FLT3 phosphorylation in samples from a majority of evaluated patients, and, to date, 77 of 83 (93%) evaluable patients have achieved a CR. The final results of these trials are eagerly anticipated [96].

Midostaurin

Midostaurin, also known as PKC412, is a staurosporine derivative, described initially as an inhibitor of protein kinase C. However, midostaurin was subsequently found to suppress the tyrosine kinases VEGFR, PDGFR, c-KIT, as well as FLT3. This multitargeted potential suggested promise in a variety of malignancies as an antiangiogenic and antiproliferative agent [97]. A phase I trial of midostaurin in solid tumors revealed minimal responses, that the primary toxicities were gastrointestinal, and that the drug was generally well tolerated [98].

Midostaurin was subsequently confirmed to be a potent inhibitor of FLT3 autophosphorylation, with an IC_{50} of 10 nM in FLT3-ITD cell lines [99]. A phase I trial of midostaurin was performed in patients with relapsed/refractory AML. Seven of 20 patients experienced transient decreases in peripheral blasts and five experienced decreases in bone marrow blasts, similar to results seen with other inhibitors of FLT3 [100].

Data from a phase Ib trial of midostaurin combined with induction chemotherapy in newly diagnosed patients was presented at the 2009 ASH annual meeting, revealing that mutant *FLT3* patients had a rate of OS at 2 years similar to that of WT *FLT3* AML patients [101]. Earlier this year, results of a phase IIb trial of midostaurin were published comparing two different dosages (50 mg and 100 mg daily) of the agent in patients with AML and MDS. Sixty-five of 92 patients (71%) with an *FLT3* mutation experienced a significant decrease in marrow or peripheral blasts (≥50%) on therapy, as did 39 patients (42%) with WT *FLT3* disease. Response rates did not differ according to dose. These results suggest that some patients with WT *FLT3* disease may derive clinical benefit from this agent, perhaps explained by the multitargeted profile of midostaurin [102]. A randomized, multicenter, phase III study of midostaurin with induction and consolidation chemotherapy followed by midostaurin maintenance in newly diagnosed patients, is currently ongoing (ClinicalTrials.gov identifier, NCT00651261).

Inhibitors of FLT3: Newer Agents

Agents in the earlier generation of FLT3 inhibitors were initially developed against other tyrosine kinase targets for use in a variety of nonhematologic malignancies. Indeed, their relative nonselectivity could explain their efficacy in newly diagnosed mutant *FLT3* AML patients, especially because multiple upregulated pathways, in addition to FLT3, may

drive the proliferation of myeloblasts. This is especially true in the case of the broad TKIs sorafenib, lestaurtinib, and midostaurin. However, nonselectivity may also be associated with a broader range of toxicity, and in some cases a lesser degree of potency.

The newer generation of FLT3 inhibitors exhibit, in part, greater relative specificity for FLT3. This specificity may hold greater promise, especially in the setting of relapsed disease, wherein leukemic cells have been characterized as having a greater mutant *FLT3* allele burden. These blasts appear addicted to and driven primarily by constitutively active FLT3 [58]. In such a setting, specific and potent FLT3 inhibitors, such as AC220 (currently in clinical trials), may hold greater promise.

KW-2449

KW-2449, a TKI from Kyowa Hakko Kirin Pharma Inc. (Princeton, NJ), effectively suppresses FLT3 phosphorylation, but also has activity against the Abl and aurora kinases. It has been studied in mutant *FLT3* cell lines, where it was found to effectively suppress the phosphorylation of FLT3 and its downstream target STAT-5 at an IC_{50} of approximately 15 nM [103]. Given these promising preclinical findings, a clinical trial followed soon thereafter.

A phase I trial of KW-2449 reported that eight of 31 enrolled patients achieved a 50% reduction in peripheral or bone marrow blasts, with the majority (five) of responders harboring FLT3-activating mutations [104]. The responses consisted of transient decreases in blast percentage, and as with other FLT3 inhibitors, correlated with in vivo FLT3 inhibition. However, the plasma half-life of this agent is quite short (2.5–3.5 hours), leading to difficulty in maintaining plasma levels of the drug, and thus requiring frequent dosing for sustained FLT3 inhibition. This may be a limiting factor in the clinical use of this drug [56].

AC220

AC220, developed by Ambit Biosciences (San Diego, CA), is the most recent addition to the group of FLT3 inhibitors currently under clinical investigation. Preclinical studies of AC220 demonstrated significant selectivity in the inhibition of FLT3, but also higher potency, by one to two orders of magnitude, than other FLT3 inhibitors [105, 106]. AC220 also has a long plasma half-life of approximately 1.5 days, allowing sustained FLT3 inhibition and more practical dosing for patients. A recent survey of FLT3 inhibitors currently in clinical investigation (lestaurtinib, midostaurin, sorafenib, sunitinib, KW-2449, and AC220) found that all agents inhibited FLT3-ITD phosphorylation effectively in media-based cell lines, with an IC_{50} in the range of 1–10 nM, and with AC220 exhibiting the greatest potency (1 nM). However, the potency of these agents in plasma varied across two orders of magnitude (IC_{50} values in the range of 18–1,700 nM). AC220 again was the most potent, by a significant margin (Table 1). These results accentuate the importance of plasma protein binding and other pharmacokinetic factors affecting target inhibition in vivo, and suggest that AC220 most effectively maintains its potency in vivo, in comparison with other agents [58].

A phase I study of AC220 in patients with relapsed/refractory AML reported very promising preliminary results. Eleven of 45 evaluated patients (24%) experienced transient clinical responses, and four patients achieved a CR with single-agent AC220. Three of the responders harbored FLT3 mutations, but the other responders had WT *FLT3* [9]. A phase II trial of AC220 in relapsed/refractory patients with mutant *FLT3* AML is currently enrolling patients at multiple institutions (ClinicalTrials.gov identified, NCT00651261). The high specificity and potency against FLT3, along with a favorable pharmacokinetic profile, indicate significant promise for this new agent as effective therapy for patients with mutant *FLT3* AML.

Conclusion

Patients with AML and an *FLT3* ITD mutation have a particularly poor prognosis. Their disease is marked by an aggressive presentation and a high propensity for relapse. In fact, the large majority of patients relapse after induction therapy and most ultimately succumb to their disease. Since the cloning of FLT3 more than 15 years ago, we have learned much regarding the structure and function of this receptor tyrosine kinase and the mutations that affect its function. ITD alterations, the most common *FLT3* mutations in AML, render the receptor constitutively active and lead to uncontrolled proliferation of blasts. Inhibition of FLT3 in model systems suppresses the autophosphorylation of FLT3, inhibits downstream signaling, and leads to apoptosis.

In the last decade, multiple inhibitors of FLT3 have been investigated in clinical trials. Some of the earlier agents were initially developed as inhibitors of other tyrosine kinases, and some, such as sorafenib and sunitinib, are effectively used today as therapy in solid tumor malignancies. These compounds were found to also be potent inhibitors of FLT3 and displayed clinical promise in mutant *FLT3* AML, with a proportion of treated patients experiencing transient clinical responses in the peripheral blood and bone marrow. However, the relative nonselectivity of some FLT3 inhibitors and the suboptimal pharmacokinetics of others may have been responsible for the disappointing results in clinical trials. Despite the lower selectivity and modest potency of most available FLT3 inhibitors, compounds such as lestaurtinib and midostaurin are currently

being studied in advanced phases of clinical investigation and may still hold promise as future adjuncts to the treatment of AML. AC220, a more selective and potent FLT3 inhibitor, has been demonstrated to have promising activity against AML in phase I studies. However, it is still in early phases of clinical investigation as single-agent therapy and in combination with traditional cytotoxic regimens. Therefore, despite encouraging preclinical results that appear to validate FLT3 as a target and the emergence of newer compounds with more therapeutic promise, it has yet to be established that FLT3 inhibitors will add clinical benefit to our current management of AML.

Author Contributions

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