

Review Article

Treatment of FLT3-ITD acute myeloid leukemia

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Abstract: Acute myeloid leukemia (AML) is an aggressive hematologic malignancy which is cured in a minority of patients. A FLT3-internal tandem duplication (ITD) mutation, found in approximately a quarter of patients with de novo AML, imparts a particularly poor prognosis. Patients with FLT3-ITD AML often present with more aggressive disease and have a significantly higher propensity for relapse after remission. The therapeutic approach for these patients has traditionally included intensive induction chemotherapy, followed by consolidative chemotherapy or hematopoietic cell transplantation (HCT). In recent years, multiple small molecule inhibitors of the FLT3 tyrosine kinase have been studied preclinically and in clinical trials. The earlier generation of these agents, often non-specific and impacting a variety of tyrosine kinases, produced at best transient peripheral blood responses in early clinical trials. Additionally, the combination of FLT3 inhibitors with cytotoxic regimens has not, as of yet, demonstrated an improvement in overall survival. Nevertheless, multiple current trials, including those with sorafenib, lestaurtinib, and midostaurin, continue to study the combination of FLT3 inhibitors with standard chemotherapy. Factors such as sustained FLT3 inhibition, protein binding, pharmacokinetics, and the presence of elevated FLT3-ligand levels appear to significantly impact the potency of these agents *in vivo*. In recent years, the development of more specific and potent agents has generated hope that FLT3 inhibitors may play a more prominent role in the treatment of FLT3-ITD AML in the near future. Nevertheless, questions remain regarding the optimal timing and schedule for incorporation of FLT3 inhibitors. The suitability, type, and timing of allogeneic HCT in the therapeutic approach for these patients are also issues which require further study and definition. Recent retrospective data appears to support the efficacy of allogeneic HCT in first complete remission, possibly due to a graft versus leukemia effect. However, larger prospective studies are necessary to further elucidate the role of HCT and its potential combination with FLT3 inhibitor therapy. We are hopeful that current clinical investigation will lead to an optimization and improvement of outcomes for these patients.

Introduction

Acute myeloid leukemia (AML) is an aggressive and frequently fatal hematologic malignancy. While many patients with AML are able to achieve a complete remission (CR) with traditional induction chemotherapy, the majority of patients will relapse and eventually succumb to their disease. Rates of relapse are particularly high for patients with a FLT3 (FMS-like tyrosine kinase 3) internal tandem duplication (ITD) mutation. FLT3-ITD mutations are found in approximately one quarter of patients with AML [1-3], with a usual initial presentation of leukocytosis and normal karyotype on cytogenetic analysis. While initial remission rates are not different for patients with FLT3-ITD relative to FLT3-wildtype (WT) patients, patients with a FLT3-ITD mutation

are much more likely to relapse and do so more rapidly than those with a wildtype FLT3 gene.

Given the success of tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) and Philadelphia chromosome positive acute lymphoblastic leukemia (ALL), inhibitors of the FLT3 tyrosine kinase have been under extensive study in recent years. These include sorafenib, lestaurtinib (CEP-701), and midostaurin (PKC412), all of which were initially developed as inhibitors of other tyrosine kinases [4-9]. A more specific and potent inhibitor of FLT3, AC220, has recently entered early clinical investigation, and has been associated with dramatic responses in early-phase trials [10]. The optimal treatment for FLT3-ITD AML remains unclear with several outstanding questions: 1) Do

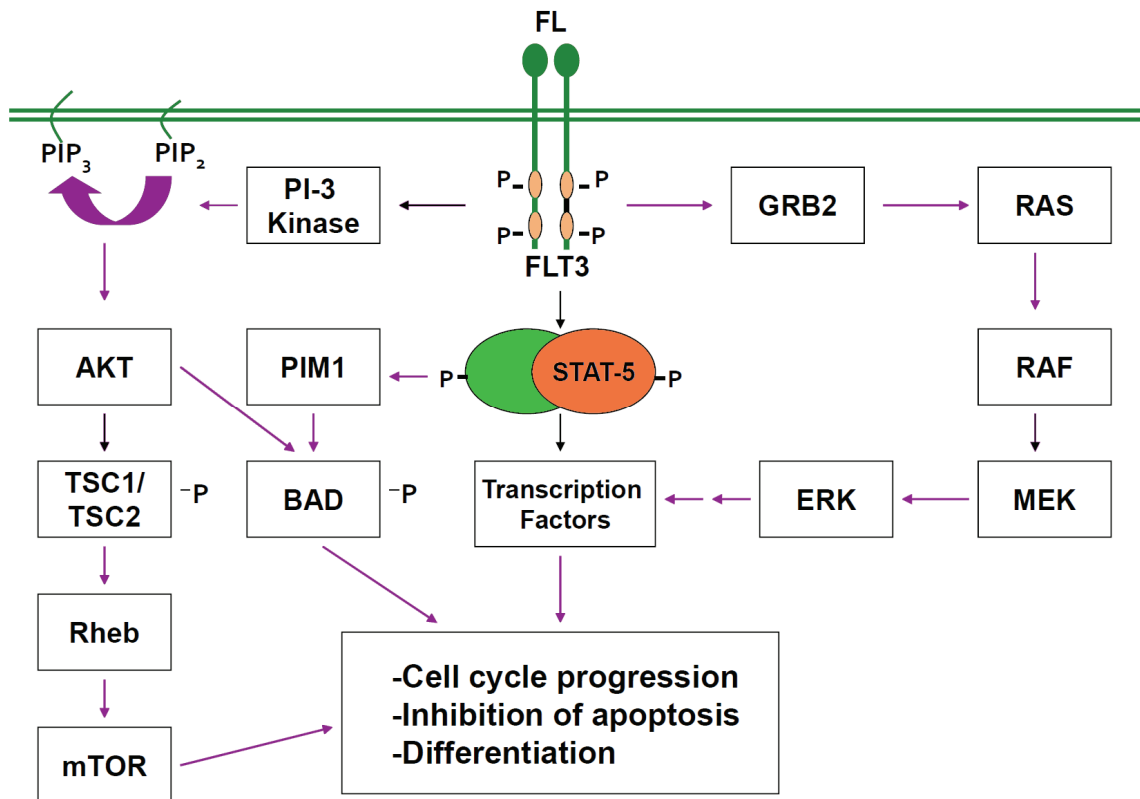


Figure 1. A simplified diagram of the signaling cascades thought to mediate the downstream effects of FLT3 activation in AML. Diagram derived and adapted from one obtained courtesy of Dr. Mark Levis, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Hospital, Baltimore, MD, USA.

FLT3 inhibitors have a role in the treatment of AML? 2) If so, what is the best agent? 3) What is the optimal way to incorporate FLT3 inhibitors into standard induction therapy? 4) What is the best consolidation therapy for FLT3-AML in first complete remission (CR1)? and 5) Is there a role for maintenance therapy with a FLT-3 inhibitor after either consolidation chemotherapy or HCT?

FLT3 as a target

The FMS-like tyrosine kinase 3 (FLT3) gene was cloned approximately 20 years ago [11, 12], and resides on chromosome 13 [13-15]. FLT3 belongs to the type III class of receptor tyrosine kinases, which also includes KIT and PDGFR [3, 16, 17]. The FLT3 receptor consists of an extracellular portion of five immunoglobulin-like domains, a trans-membrane region, a short intracellular juxtamembrane unit, and an intracellular tyrosine kinase domain. Upon binding FLT3 ligand (FL), the receptor dimerizes and the

inner leaflet of the membrane is auto-phosphorylated, which then leads to activation of the tyrosine kinase and subsequent downstream signaling, with significant mediators being PI3-kinase, AKT, MAP kinase, and STAT5 (Figure 1) [18-26]. In the normal hematopoietic environment, FLT3 expression is predominantly on CD34 expressing cells, and appears to be integrally involved in early hematopoiesis and reconstitution of multi-lineage myeloid precursors [12, 27-29]. This has been demonstrated by disruption of FLT3 signaling in murine models, which although not lethal, does bring about significant reduction of hematopoietic precursors [30].

FLT3 ligand (FL) and the FLT3 receptor appear to be upregulated in the majority of human leukemia cell lines [31, 32]. In myeloid blasts, FLT3 expression is no longer tightly associated with CD34 expression, as it is in normal precursors. Some AML cell lines exhibit overexpression of wild-type FLT3, but others have activat-

ing mutations which render the FLT3 tyrosine kinase hyperactive such as point mutations or the ITD alteration [5, 13, 33-35]. As has been shown in several large series of AML patients, internal tandem duplications are found in approximately 23% of patients with de novo AML [1, 2, 36, 37]. Point mutations within the activation loop of the kinase domain are found in an additional 7% of patients [13, 38]. These alterations result in increased and constitutive FLT3 activation. This then leads to triggering of STAT5 and downstream MAP kinase and AKT signaling cascades, causing suppression of apoptosis and dysregulated cell proliferation [5, 39, 40]. The ITD mutations have been uniformly associated with an adverse prognosis, as demonstrated in multiple clinical studies, but, interestingly, the prognostic impact of the tyrosine kinase point mutations remains controversial [41-45].

The adverse clinical impact of FLT3-ITD alterations in AML has led to efforts to develop effective FLT3 inhibitors as targeted therapy for these patients. Multiple candidate compounds have been investigated and reported as effective FLT3 kinase inhibitors *in vitro*. Most of these compounds are structural mimics of the purine component of ATP, and occupy the ATP-binding pocket of the tyrosine kinase [46, 47]. Studies have suggested that specific FLT3 inhibitors induce preferential cytotoxicity in FLT3-mutant AML cells, and that sustained and potent FLT3 inhibition appear essential in bringing about cytotoxicity against myeloblasts [4, 48]. In recent years, multiple inhibitors of FLT3, some more potent and specific than others, have been transitioned from the laboratory and studied in clinical trials. Those which are most advanced in clinical trials are summarized in **Table 1**, and outlined in detail below.

Inhibitors of FLT3 currently under clinical investigation

Sorafenib

Sorafenib is approved by the FDA and widely used in advanced renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC) [49, 50]. It is a potent inhibitor of many receptor tyrosine kinases, including c-KIT, NRAS, RAF kinase, and FLT3 [51, 52]. Sorafenib effectively suppresses FLT3 auto-phosphorylation and downstream signaling, leading to leukemic cell death [53, 54]. It is relatively well tolerated as a single

agent in AML, and can effect transient decreases in bone marrow blasts, particularly in those patients with FLT3-ITD mutations [55, 56]. Given its commercial availability, there has been increasing use of sorafenib on an off-label basis for patients with advanced FLT3-mutant AML. Case reports of dramatic responses to single agent sorafenib have been published, including reports of complete remission [57, 58]. In a recent abstract presentation, six of 11 patients with refractory AML were able to proceed to HSCT after responding to treatment with sorafenib. The same group also described prolonged complete remissions when sorafenib was delivered in the relapsed post-transplant setting [59, 60].

A phase I/II trial of 61 newly-diagnosed cases of AML investigated sorafenib combined with cytarabine and idarubicin based induction therapy. The phase I portion of this study evaluated the safety of sorafenib in cohorts of escalating dose, including an initial dose of 400 mg by mouth every other day, then at 400 mg daily, and finally at 400 mg twice daily. As the 400 mg twice daily regimen was well-tolerated, this dose was administered during the phase II portion of the trial, and given concurrently during the first seven days of induction therapy, throughout each cycle of consolidation, and continued as maintenance for a total of one year. High rates of complete remission (CR) were reported, with 38 patients (75%) in total, and 14 of 15 FLT3-ITD patients (93%), achieving a CR following induction. Among the FLT3-mutated patients, 10 patients relapsed and five remained in CR with a median follow-up of 62 weeks. Correlative studies from the study reported effective suppression of FLT3-phosphorylation in the FLT3-ITD patients [7].

Results from a recent European randomized, placebo-controlled phase II trial in elderly patients, receiving sorafenib or placebo with standard induction, consolidation, and maintenance chemotherapy, were also recently presented. These investigators also employed the 400 mg twice daily dosing of sorafenib, administered after chemotherapy and in between cycles of consolidation, and continued for a period of one year. The combination was well-tolerated, but no benefit in survival parameters or rate of CR were found, including the subset of patients with FLT3-ITD AML [61]. There are other trials currently evaluating sorafenib combined with cytotoxic therapies. A CALGB-led phase II clini-

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Table 1. A summary of the advanced phase trials of FLT3 inhibitors in AML

Compound	Clinical Trial Stage	Significant Findings
Sorafenib (BAY 43-9006)	Phase II [62]	European, randomized, placebo-controlled, double-blind trial of induction/consolidation chemotherapy with or without sorafenib in older patients, regardless of FLT3-status. Event free survival (EFS) and overall survival (OS) not significantly different between the two groups. No differences in CR, EFS or OS were noted in 14% of patients with FLT3-ITD mutations.
Lestaurtinib (CEP-701)	Phase III [70]	Randomized trial of 224 FLT3-mutant patients in first relapse. No difference in CR rate or overall survival between cohorts which receiving chemotherapy alone or followed by lestaurtinib. FLT3 inhibition correlated with RR, but target inhibition achieved in only 58% of patients.
	Phase III [71]	British MRC trials are investigating the combination of lestaurtinib with induction and consolidation chemotherapy. Interim results suggest FLT3 target inhibition of greater than 85% in 82% of measured time-points for evaluable patients. Additionally, 77 of 83 evaluable patients (93%) achieved a complete remission.
Midostaurin (PKC412)	Phase IIB [75]	Ninety five patients with AML or MDS, regardless of FLT3 status, randomly assigned to receive oral midostaurin at 50 or 100 mg twice daily. The bone marrow response rate was 71% in FLT3-mutant patients and 42% in FLT3-wt patients.
	Phase III [76]	RATIFY is a CALGB-led multi-national, placebo controlled, phase III trial, which randomizes patients with newly diagnosed FLT3-mutant AML, to induction and consolidation therapy with or without midostaurin. This CALGB-led trial is currently accruing patients.
AC220	Phase II [80]	An interim analysis of this open label, mono-therapy study was presented at the 2011 Congress of the EHA. Based on response data from 62 relapsed/refractory FLT3-mutant patients, the investigators reported a CR rate of 45%, with the majority being CRi. In addition, an additional 25% achieved a partial response (PR). The median survival was 24.7 weeks. A significant number of patients who had previously failed chemotherapy successfully transitioned to HSCT.

cal trial will assess the efficacy of sorafenib combined with 7+3 induction therapy in older FLT3-ITD patients, and administer the drug on days 1-7 of induction, throughout consolidation, and as maintenance therapy (clinicaltrials.gov, NCT01253070). Other ongoing combination trials include that of sorafenib with low-dose cytarabine in older patients (NCT00516828), and with clofarabine and cytarabine in the relapsed/refractory setting (NCT00893373).

Lestaurtinib

Lestaurtinib is a polyaromatic indolocarbazole compound which was initially found to effectively inhibit a variety of tyrosine kinases, including RET, JAK2, and TRK, as well as FLT3 [62-64]. Given this activity, lestaurtinib was first clinically evaluated as therapy for solid tumors. Although well-tolerated, the drug was not effective

in achieving objective responses [65]. Pre-clinical studies of lestaurtinib, however, found it to be a potent inhibitor of FLT3, and preferentially cytotoxic to FLT3-ITD cell lines and primary samples [5]. Interestingly, early *in vitro* studies of lestaurtinib combined with traditional cytotoxic chemotherapy found synergistic cytotoxicity when it was used concurrently or subsequent to chemotherapy. In contrast, when leukemia cells were exposed to lestaurtinib followed by exposure to chemotherapy, antagonism was noted. The biological basis for this observation was postulated to be G1 cell cycle arrest in leukemic cells exposed to lestaurtinib, leading to a decreased efficacy of chemotherapeutic agents [66].

A phase I/II trial of lestaurtinib in FLT3-mutant AML patients demonstrated that lestaurtinib was well-tolerated and that it produced clinical

responses, although mostly just reductions in the peripheral blast count. Additionally, a sustained and effective suppression of FLT3 phosphorylation, as measured with an *ex vivo* assay, correlated strongly with these clinical responses [48, 67]. In a phase II trial of newly diagnosed elderly patients, three of five patients with FLT3 mutations experienced transient hematologic responses. Interestingly, a number of patients with wildtype FLT3 experienced decreases in bone marrow blasts as well, which was attributed to possible over-expression of FLT3 in these patients [68].

A phase II trial of relapsed FLT3-mutant AML randomized patients to re-induction chemotherapy alone or re-induction followed by lestaurtinib. The study was subsequently expanded to a phase III trial, the results of which were recently reported by Levis et al. In contrast to the sequence used in the combination sorafenib studies, lestaurtinib, at a dose of 80mg twice daily, was initiated two days after conclusion of induction chemotherapy and continued until day 112. Unfortunately, the investigators reported no benefit in any survival parameters or response rate with the addition of lestaurtinib to induction chemotherapy. However, effective and sustained inhibition of FLT3 was achieved in only 58% of patients by day 15 of treatment, and therefore definitive conclusions regarding the efficacy of FLT3 inhibition in combination with chemotherapy could not be made and argued for a different dosing schedule of lestaurtinib [69].

Lestaurtinib has also been incorporated into induction and consolidation chemotherapy regimens for FLT3-mutated patients in the British MRC AML17 trial. Similar to the above study, lestaurtinib in this trial was not administered concurrently with chemotherapy, but rather initiated two days after conclusion of and discontinued two days prior to initiation of consecutive cycles of cytotoxic chemotherapy. Preliminary reports have suggested effective inhibition of FLT3 activity in the large majority of evaluated patients. Additionally, to date, more than 90% of the evaluated patients have achieved a CR, which is higher than historical response rates and final results are eagerly anticipated [70].

Midostaurin

Midostaurin, a staurosporine derivative, was initially described as an inhibitor of protein

kinase C. However, like other similar agents, it was subsequently found to suppress the tyrosine kinases VEGFR, PDGFR, c-KIT, as well as FLT3 with significant cytotoxicity in FLT3-ITD cell lines [71, 72]. A phase I trial of midostaurin in patients with relapsed/refractory AML showed that seven of twenty patients experienced transient decreases in peripheral blasts and five showed reductions in bone marrow blasts as well [8]. A phase I trial of midostaurin with induction chemotherapy was also conducted, with preliminary data revealing that FLT3-mutant patients had similar rates of overall survival at 2 years when compared to those with FLT3-wildtype AML. In this study, midostaurin was administered both concomitantly (days 1-7) and sequentially (days 8-21) with chemotherapy, and both regimens were shown to be well-tolerated [73]. A phase IIb trial of single-agent midostaurin, at two different dosages (50mg or 100mg twice daily), in patients with AML and myelodysplastic syndrome (MDS) was also recently reported. In this study, 71% of patients with FLT3-mutant AML experienced a $\geq 50\%$ decrease in marrow or peripheral blasts, as did 42% of patients with FLT3-wildtype disease. The results suggested that MDS/AML patients, regardless of FLT3 status, can potentially benefit from the multi-targeted profile of midostaurin [74]. A multi-center, phase III study of midostaurin with induction and consolidation chemotherapy, followed by midostaurin maintenance in newly diagnosed patients, is currently ongoing (clinicaltrials.gov #NCT00651261). In this trial, midostaurin, at a dose of 50 mg twice daily, has been administered sequentially following conclusion of induction therapy, on days 8-21 of each cycle, followed by one year of midostaurin maintenance [75].

AC220

AC220 (Ambit Biosciences, San Diego, CA) is a potent and specific inhibitor of FLT3, and has only recently been under clinical investigation. The selective profile of AC220 was demonstrated in preclinical studies. The agent also displays higher potency, by 1-2 orders of magnitude, over other FLT3 inhibitors [76, 77]. In addition, AC220 has a long plasma half-life with sustained FLT3 inhibition. Another remarkable feature of AC220 is its retained potency in plasma, where protein binding and metabolism are often limiting factors. Pratz et al. recently surveyed a series of FLT3 inhibitors, including lestaurtinib, midostaurin, sorafenib, and AC220,

and found that all agents inhibited FLT3-ITD phosphorylation effectively in culture medium, with an IC50 ranging from 1-10nM. However, potency in plasma varied across orders of magnitude, from 18 to 1700 nM, with AC220 being the most potent [78]. A phase I study of single-agent AC220 in relapsed/refractory AML confirmed the potency of AC220, with 11 of 45 evaluated patients experiencing transient clinical responses. Intriguingly, 4 patients experienced a CR, three of whom were FLT3-mutated [10].

An open-label phase II monotherapy trial of AC220 in relapsed/refractory patients with FLT3-mutant AML is currently enrolling. Promising interim results were recently reported at the 2011 Congress of the European Hematology Association (EHA). In 53 relapsed/refractory FLT3-mutant patients, a CR rate of 45% was reported, with the majority of these being complete remission with incomplete hematologic recovery (CRi). An additional 25% of patients achieved partial responses (PR) on monotherapy with AC220. The median duration of responses was 12.1 weeks. A significant number of patients who had failed previous therapies went on to HCT after receiving AC220 [79]. Additionally, British investigators plan to conduct a pilot trial in older patients with AML, combining AC220 with cytotoxic chemotherapy. In this regimen, AC220 will be administered daily starting two days after the conclusion of each course of chemotherapy during induction (clinicaltrials.gov #NCT01236144).

Other FLT3 inhibitors

Several other FLT3 inhibitors have been studied in clinical trials over the last decade and merit mention. These include the agents semaxinib [80], sunitinib [81, 82], tandutinib [83] and KW-2449 [84]. Some of these compounds have produced transient hematologic responses in a fraction of studied patients, but have largely not progressed beyond early-phase clinical trials for a variety of reasons. These have included inadequate activity [80], significant non-hematologic toxicities, [81, 85] or suboptimal pharmacokinetic parameters [83, 86].

The Evolution of FLT3 inhibitors

The majority of FLT3 inhibitors were developed against tyrosine kinases other than FLT3, and

were initially studied in solid tumors. This non-selectivity could explain some of the observed efficacy in all patients with AML, regardless of FLT3 mutational status as multiple up-regulated pathways, in addition to FLT3, undoubtedly drive the proliferation of myeloblasts [68, 74]. However, it is important to note that this non-selectivity may also be associated with a broader range of toxicity. Recently, newer, more effective FLT3 inhibitors have exhibited greater relative specificity and potency against the FLT3 target. This greater specificity may hold promise particularly in the setting of relapsed disease, where leukemic cells have been characterized as having a greater FLT3-mutant allele burden, and thus are more "addicted" to a constitutively active FLT3 kinase rather than alternative pathways [78].

Combining FLT3 inhibition with cytotoxic chemotherapy

As detailed above, multiple attempts have been made to combine FLT3 inhibitors with traditional cytotoxic induction and consolidation chemotherapy [7, 61, 70, 73, 87]. There are ongoing randomized studies of FLT3 inhibitors combined with chemotherapy and these include the British MRC trials, which have incorporated lestaurtinib, and the CALGB-led RATIFY trial, which is studying midostaurin. Thus far, however, randomized trials of FLT3 inhibitors in combination with chemotherapy have not demonstrated any improvement in disease-free or overall survival outcomes for patients with FLT3-mutant AML [61, 87]. Recently, it has been suggested that FLT3 ligand (FL) levels rise significantly after each successive administration of intensive chemotherapy [88]. The main source of FL may be bone marrow stromal cells with FL production induced by marrow aplasia [89]. These investigators further demonstrated that the presence of FLT3-ligand (FL) *in vitro* blunts the inhibition of FLT3 phosphorylation by a variety of tyrosine kinase inhibitors, including lestaurtinib, midostaurin, sorafenib, and AC220. They thus hypothesized that a dramatic rise in FL following chemotherapy may be responsible for suppressing sustained FLT3 inhibition, possibly explaining the unimpressive outcomes, to date, in clinical trials of FLT3 inhibitors combined with chemotherapy [88]. Potentially, the traditional schedule of FLT3-inhibitors given concurrently with chemotherapy may be adding toxicity without any benefit of inhibiting FLT3 due to over-

whelming levels of FL. A more effective approach in future trials may be the use of FLT3 inhibitors at times when the FL levels are not so high – i.e. during the first week of induction therapy and/or as maintenance following consolidative chemotherapy or HCT once cytopenias have resolved and FL levels are lower.

The role of stem cell transplantation

Standard approaches to treatment of FLT3-ITD AML are poor with long-term disease-free survival probabilities of 20-30%. While this prognosis is comparable to patients with poor risk cytogenetics, it is unclear if consolidation in first remission with HCT should be the standard of care as there has been no prospective clinical trial which shows that HCT improves overall outcomes patients with FLT3-ITD AML. Ideally, a multi-center prospective clinical trial randomizing patients between standard consolidation chemotherapy and HCT would be able to provide answers to this question, however, logistics with expense, donor availability, physician preference, and competing research avenues make such a trial unlikely. Indeed, many patients enrolled on up-front studies of chemotherapy in combination with FLT3 inhibitors come off protocol to undergo HCT in 1st remission at the direction of their treating physicians.

Autologous transplantation

High-dose chemotherapy with autologous stem cell transplantation (ASCT) has never shown an overall survival benefit when compared to standard high-dose cytarabine based consolidation for patients with AML and has not been favored by many practitioners due to the lack of the graft-vs-leukemia effect and fears over malignant cell contamination in the autologous stem cell graft. Nevertheless, ASCT offers the delivery of a more intense dose of chemotherapy compared with standard consolidation, and thus, some investigators have retrospectively studied if ASCT has a possible benefit in FLT3-ITD AML. Investigators from Harvard retrospectively analyzed 56 younger patients with either a FLT3-ITD or TKD mutation and compared them based on choice of consolidation therapy. Analysis showed that ASCT appeared to have a benefit in both DFS and OS when compared to high-dose cytarabine based consolidation for patients with the FLT3-ITD mutation [90]. A larger German analysis was performed on 175

FLT3-ITD AML patients who were treated on a protocol where patients with a matched sibling donor underwent allogeneic HCT for consolidation and those who did not have a sibling donor were treated with ASCT if autologous stem cells could be mobilized, and standard cytarabine consolidation was given to patients if stem cells could not be successfully mobilized. Results suggested a similar prognosis between those who underwent allogeneic HSCT and ASCT, both being superior to cytarabine-based consolidation [91]. However, ASCT has gradually fallen out of favor as the consolidation therapy of choice for FLT3-AML as allogeneic HCT has increasingly gained support.

Allogeneic transplantation

Many centers currently support the use of allogeneic stem cell transplantation as the most effective consolidation therapy for patients with FLT3-ITD AML in CR1, although this remains controversial [92-94]. If elevated levels of FL do lead to disease relapse and graft-vs-leukemia (GVL) is effective for FLT3-ITD AML, then logic dictates allogeneic HCT should be pursued expeditiously as soon as CR1 is achieved [93]. Nevertheless, there are no prospective clinical trials for the FLT3-ITD population exclusively, either randomized or genetically randomized by donor availability, to guide treatment recommendations. However, several retrospective analyses have been performed to attempt to answer this question.

Gale et al. first analyzed patients treated on the UK MRC AML 10 and 12 trials where a FLT3-ITD mutation was found in 283 of 1135 patients and choice of consolidation therapy had not been prospectively guided by FLT3 mutational status as it was unknown at the time of the trial. Of the 1135 patients in this cohort, 186 were prospectively randomized to ASCT vs chemotherapy consolidation. Of the patients randomized to ASCT, 35 were FLT3-ITD+ and 26 patients in the group undergoing chemotherapy consolidation were FLT3-ITD+. Analysis showed a benefit for decreased relapse for those undergoing ASCT, but this did not translate into a benefit for overall survival. 683 patients in this cohort were treated on a donor vs. no donor basis, where patients with an available matched sibling donor underwent allogeneic HCT, while those without a donor underwent chemotherapy consolidation. 68 of the 273 patients who had

a sibling donor had the FLT3-ITD while 114 of the 410 patients without a donor had the FLT3-ITD. A benefit in the risk of relapse for HCT was demonstrated, but this again did not translate into a significant difference in overall survival. Based on these data, the authors concluded that the presence of the FLT3-ITD should not factor into the decision to offer a patient allogeneic HCT in CR1 [95].

Schlenk et al. reported an analysis on 872 adult patients with normal karyotype AML treated consecutively on 4 clinical trials as part of the German-Austrian AML Study Group. In each study, patients who had a matched related stem cell donor were assigned to undergo allogeneic HCT as consolidation therapy. 31% of the 872 patients had a FLT3-ITD mutation and a total of 150 of the 663 patients eligible for post-remission therapy proceeded to HCT. Patients who underwent ASCT appeared to have a similar prognosis to those who were treated with chemotherapy alone and were combined into the no-donor group. Analysis by molecular mutational status showed that patients with the FLT3-ITD mutation and those without NPM1 or CEBP α mutation had a benefit in terms of relapse-free survival (RFS) from allogeneic HCT in CR1, although comparisons of overall survival were not presented. When compared to the analysis by Gale et al., there was more compliance with HCT in the donor group (82% vs 63%) and less transplant-related mortality (21% vs 30%) [44].

More recently, two studies have been presented which have illustrated the outcomes of patients with FLT3-ITD AML who were treated with an aggressive strategy involving early allogeneic HCT after achieving CR1. The German-Austrian group described 437 adult patients with FLT3-ITD, including some who were included in the above study. From 1993-2006, patients underwent allogeneic HCT only if a matched sibling was available, but in 2006, patients with a matched unrelated donor were treated with allogeneic HCT as well. No significant differences between the two transplant cohorts were observed. Landmark analyses for relapse-free survival at 5 months revealed a beneficial impact of allogeneic HSCT from both MRD and MUD, with longer follow-up and complete results eagerly awaited. Interestingly, it was noted that patients who received a transplant sooner rather than later had better outcomes, which is

the opposite of what is usually observed in AML HCT studies, and suggests, that repeated courses of consolidation could potentially be harmful, which would be consistent with the recently described FLT ligand data [96].

Investigators at Johns Hopkins University recently described the outcomes of 133 consecutive newly diagnosed patients with AML under the age of 60, of whom 31 (23%) had a FLT3-ITD. Patients with FLT3-ITD were given standard induction chemotherapy and then taken to allogeneic HCT upon remission with any available donor. In comparing the FLT3-ITD with FLT3-WT patients in this single institution cohort, median OS was similar (19.3 months vs. 15.5 months, respectively, $p=0.56$). Of the 20 FLT3-ITD patients achieving CR1, 11 underwent allogeneic HCT in CR1 (4 myeloablative MRD, 5 myeloablative MUD, 2 RIC haploidentical) and 9 did not undergo HCT due to comorbidities or lack of a suitable donor. The median RFS of the FLT3-ITD patients who did not receive HCT was 8.6 months, which was significantly shorter than the median of 54.1 months ($p=.03$) for those that were able to undergo allogeneic HCT [97].

Conclusion

It is clear that FLT3-ITD AML represents a subset of patients with a particularly poor prognosis, marked by an aggressive presentation, and significantly higher rates of relapse. Defining the optimal therapy is not easy, although there is clearly room for improvement. FLT3 remains an attractive target, at initial presentation, in remission, and especially at relapse, when the disease appears to be more dependent upon the FLT3 pathway.

In the last decade, multiple inhibitors of FLT3 have been investigated in clinical trials though none has yet been approved for routine clinical use. Many of these agents were potent inhibitors of a variety of tyrosine kinases, in addition to FLT3, and this could have been responsible for the observed toxicities. Unfortunately, no agent has yet demonstrated a significant clinical benefit in advanced clinical trials. These results may be related to pharmacokinetic parameters, protein binding, metabolism and fluctuating drug levels, or the co-presence of high levels of FLT3 ligand induced by marrow aplasia. Currently, the agents midostaurin, lestauritinib, and sorafenib are in advanced phases of

clinical investigation and may play a role as adjunctive treatment in FLT3-ITD AML in the future. Recently, a more potent and selective agent, AC220, has entered into early clinical trials, and may hold greater promise.

Given the historically poor prognosis with approaches using cytotoxic chemotherapy in FLT3-ITD AML, and the lack of proven benefit with an additional effective targeted agent, our current approach to the management of FLT3-ITD AML is induction chemotherapy, followed, in appropriate patients, by allogeneic HCT in first remission with related, unrelated, or alternative donors. It is still unproven if courses of consolidation chemotherapy should be given if there are delays in donor availability, especially when choosing between a matched unrelated donor (where we would favor a myeloablative approach) vs. an alternative donor such as umbilical cord blood or haploidentical source (where we would favor a reduced intensity conditioning approach).

Additional data regarding FLT3 ligand are required to optimize treatment, particularly regarding when to incorporate FLT3 inhibitors during induction and consolidation. The post-HCT setting would seem to be ideal to deliver specifically targeted therapy against FLT3, once cytopenias have resolved and FLT3 ligand levels are presumably low. This would be similar to the current widespread use of tyrosine kinase inhibitors such as imatinib, nilotinib, and dasatinib to patients with CML or Ph+ ALL who have undergone HSCT. Along these lines, there is currently a phase I clinical trial at our institution administering maintenance sorafenib in the post-HCT setting for patients with FLT3-ITD AML in CR.

In the setting of relapsed or refractory disease, FLT3-ITD AML should be managed similar to other patients with AML and the patient should be expeditiously treated with allogeneic HCT. It is likely that some re-induction therapy will be required given the proliferative nature of FLT3-ITD AML, and all efforts should be made to enroll such patients on clinical trials which include FLT3 targeted agents. It is notable that there are increasing anecdotes of patients being receiving allogeneic HCT, after having their relapsed / refractory disease treated with single-agent sorafenib or AC220.

In summary, the appropriate approach to the treatment of FLT3-ITD AML remains undefined. Multiple clinical trials are currently investigating the incorporation of FLT3 inhibitors into traditional cytotoxic regimens and transplant approaches, and these may very well become useful and effective adjuncts in the near future. Nevertheless, given the consistently poor prognosis of these patients, we believe that appropriate approaches include either aggressive multi-agent induction therapy followed by consolidative allogeneic HCT, or enrollment in appropriate clinical trials which expand our experience with FLT3 inhibitor therapy.

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References

- [1] Gilliland DG and Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood* 2002; 100: 1532-1542.
- [2] Kiyoi H, Towatari M, Yokota S, Hamaguchi M, Ohno R, Saito H and Naoe T. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. *Leukemia* 1998; 12: 1333-1337.
- [3] Levis M and Small D. FLT3: ITDoes matter in leukemia. *Leukemia* 2003; 17: 1738-1752.
- [4] Knapper S, Mills KI, Gilkes AF, Austin SJ, Walsh V and Burnett AK. The effects of lestaurtinib (CEP701) and PKC412 on primary AML blasts: the induction of cytotoxicity varies with dependence on FLT3 signaling in both FLT3-mutated and wild-type cases. *Blood* 2006; 108: 3494-3503.
- [5] Levis M, Allebach J, Tse KF, Zheng R, Baldwin BR, Smith BD, Jones-Bolin S, Ruggeri B, Dionne C and Small D. A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. *Blood* 2002; 99: 3885-3891.

Treatment of FLT3-ITD acute myeloid leukemia

- [6] Levis M, Smith BD, Beran M, Baer MR, Erba HP, Cripe L, Coutre S, Advani A, Perl A, Devetten M, Stuart R, Tallman MS, Brown P, Tremmel L and Small D. A randomized, open-label study of lestaurtinib (CEP-701), an oral FLT3 inhibitor, administered in sequence with chemotherapy in patients with relapsed AML harboring FLT3 activating mutations: Clinical response correlates with successful FLT3 inhibition. *Blood* 2005; 106: 121a.
- [7] Ravandi F, Cortes JE, Jones D, Faderl S, Garcia-Manero G, Konopleva MY, O'Brien S, Estrov Z, Borthakur G, Thomas D, Pierce SR, Brandt M, Byrd A, Bekele BN, Pratz K, Luthra R, Levis M, Andreeff M and Kantarjian HM. Phase I/II study of combination therapy with sorafenib, idarubicin, and cytarabine in younger patients with acute myeloid leukemia. *Journal of Clinical Oncology* 2010; 28: 1856-1862.
- [8] Stone RM, DeAngelo DJ, Klimek V, Galinsky I, Estey E, Nimer SD, Grandin W, Lebwohl D, Wang Y, Cohen P, Fox EA, Neuberg D, Clark J, Gilliland DG and Griffin JD. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood* 2005; 105: 54-60.
- [9] Yee KW, O'Farrell AM, Smolich BD, Cherrington JM, McMahon G, Wait CL, McGreevey LS, Griffith DJ and Heinrich MC. SU5416 and SU5614 inhibit kinase activity of wild-type and mutant FLT3 receptor tyrosine kinase. *Blood* 2002; 100: 2941-2949.
- [10] Cortes J, Foran J, Ghirdaladze D, DeVetten MP, Zodelava M, Holman P, Levis MJ, Kantarjian HM, Borthakur G, James J, Zarrinkar PP, Gunawardane RN, Armstrong RC, Padre NM, Wierenga W, Corringham R and Trikha M. AC220, a potent, selective, second generation FLT3 receptor tyrosine kinase (RTK) inhibitor, in a first-in-human (FIH) phase 1 AML study. *Blood (ASH Annual Meeting Abstracts)* 2009; 114: Abstract 636.
- [11] Rosnet O, Schiff C, Pebusque MJ, Marchetto S, Tonnelle C, Toiron Y, Birg F and Birnbaum D. Human FLT3/FLK2 gene: cDNA cloning and expression in hematopoietic cells. *Blood* 1993; 82: 1110-1119.
- [12] Small D, Levenstein M, Kim E, Carow C, Amin S, Rockwell P, Witte L, Burrow C, Ratajczak MZ and Gewirtz AM. STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 1994; 91: 459-463.
- [13] Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR and Reilly JT. Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. *British Journal of Haematology* 2001; 113: 983-988.
- [14] Agnes F, Shamoan B, Dina C, Rosnet O, Birnbaum D and Galibert F. Genomic structure of the downstream part of the human FLT3 gene: exon/intron structure conservation among genes encoding receptor tyrosine kinases (RTK) of subclass III. *Gene* 1994; 145: 283-288.
- [15] Carow CE, Kim E, Hawkins AL, Webb HD, Griffin CA, Jabs EW, Civin CI and Small D. Localization of the human stem cell tyrosine kinase-1 gene (FLT3) to 13q12->q13. *Cytogenetics & Cell Genetics* 1995; 70: 255-257.
- [16] Blume-Jensen P and Hunter T. Oncogenic kinase signalling. *Nature* 2001; 411: 355-365.
- [17] van der Geer P, Hunter T and Lindberg RA. Receptor protein-tyrosine kinases and their signal transduction pathways. *Annual Review of Cell Biology* 1994; 10: 251-337.
- [18] Dosil M, Wang S and Lemischka IR. Mitogenic signalling and substrate specificity of the Flk2/Flt3 receptor tyrosine kinase in fibroblasts and interleukin 3-dependent hematopoietic cells. *Molecular & Cellular Biology* 1993; 13: 6572-6585.
- [19] Lavagna-Sevenier C, Marchetto S, Birnbaum D and Rosnet O. FLT3 signaling in hematopoietic cells involves CBL, SHC and an unknown P115 as prominent tyrosine-phosphorylated substrates. *Leukemia* 1998; 12: 301-310.
- [20] Lavagna-Sevenier C, Marchetto S, Birnbaum D and Rosnet O. The CBL-related protein CBLB participates in FLT3 and interleukin-7 receptor signal transduction in pro-B cells. *Journal of Biological Chemistry* 1998; 273: 14962-14967.
- [21] Marchetto S, Fournier E, Beslu N, Aurrans-Schleinitz T, Dubreuil P, Borg JP, Birnbaum D and Rosnet O. SHC and SHIP phosphorylation and interaction in response to activation of the FLT3 receptor. *Leukemia* 1999; 13: 1374-1382.
- [22] Mizuki M, Schwable J, Steur C, Choudhary C, Agrawal S, Sargin B, Steffen B, Matsumura I, Kanakura Y, Bohmer FD, Muller-Tidow C, Berdel WE and Serve H. Suppression of myeloid transcription factors and induction of STAT response genes by AML-specific Flt3 mutations. *Blood* 2003; 101: 3164-3173.
- [23] Scheijen B, Ngo HT, Kang H and Griffin JD. FLT3 receptors with internal tandem duplications promote cell viability and proliferation by signaling through Foxo proteins. *Oncogene* 2004; 23: 3338-3349.
- [24] Levis M. Recent advances in the development of small-molecule inhibitors for the treatment of acute myeloid leukemia. *Current Opinion in Hematology* 2005; 12: 55-61.
- [25] Fathi AT, Grant S and Karp JE. Exploiting cellular pathways to develop new treatment strategies for AML. *Cancer Treatment Reviews* 2010; 36: 142-150.
- [26] Fathi AT, Swinnen I, Rajkhowa T, Small D, Marmsater F, Robinson JE, Gross SD,

- Martinson M, Allen S, Kallan N and Levis M. PIM: An integral component of FLT3 signaling and a potential therapeutic target in acute myeloid leukemia. *Blood (ASH Annual Meeting Abstracts)* 2010; 114: Abstract 1735.
- [27] Broxmeyer HE, Lu L, Cooper S, Ruggieri L, Li ZH and Lyman SD. Flt3 ligand stimulates/costimulates the growth of myeloid stem/progenitor cells. *Experimental Hematology* 1995; 23: 1121-1129.
- [28] Gotze KS, Ramirez M, Tabor K, Small D, Matthews W and Civin CI. Flt3high and Flt3low CD34+ progenitor cells isolated from human bone marrow are functionally distinct. *Blood* 1998; 91: 1947-1958.
- [29] Veiby OP, Jacobsen FW, Cui L, Lyman SD and Jacobsen SE. The flt3 ligand promotes the survival of primitive hemopoietic progenitor cells with myeloid as well as B lymphoid potential. Suppression of apoptosis and counteraction by TNF-alpha and TGF-beta. *Journal of Immunology* 1996; 157: 2953-2960.
- [30] McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, Maliszewski CR, Lynch DH, Smith J, Pulendran B, Roux ER, Teepe M, Lyman SD and Peschon JJ. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* 2000; 95: 3489-3497.
- [31] Drexler HG. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia* 1996; 10: 588-599.
- [32] Meierhoff G, Dehmel U, Gruss HJ, Rosnet O, Birnbaum D, Quentmeier H, Dirks W and Drexler HG. Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. *Leukemia* 1995; 9: 1368-1372.
- [33] Armstrong SA, Kung AL, Mabon ME, Silverman LB, Stam RW, Den Boer ML, Pieters R, Kersey JH, Sallan SE, Fletcher JA, Golub TR, Griffin JD and Korsmeyer SJ. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell* 2003; 3: 173-183.
- [34] Quentmeier H, Reinhardt J, Zaborski M and Drexler HG. FLT3 mutations in acute myeloid leukemia cell lines. *Leukemia* 2003; 17: 120-124.
- [35] Yokota S, Kiyoi H, Nakao M, Iwai T, Misawa S, Okuda T, Sonoda Y, Abe T, Kahsima K, Matsuo Y and Naoe T. Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines. *Leukemia* 1997; 11: 1605-1609.
- [36] American Cancer Society *Cancer Facts & Figures* 2010.
- [37] Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, Sonoda Y, Fujimoto T and Misawa S. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. *Leukemia* 1996; 10: 1911-1918.
- [38] Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Koda Y, Miyawaki S, Asou N, Kuriyama K, Yagasaki F, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Takeshita A, Saito H, Ueda R, Ohno R and Naoe T. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* 2001; 97: 2434-2439.
- [39] Hayakawa F, Towatari M, Kiyoi H, Tanimoto M, Kitamura T, Saito H and Naoe T. Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. *Oncogene* 2000; 19: 624-631.
- [40] Mizuki M, Fenski R, Halfter H, Matsumura I, Schmidt R, Muller C, Gruning W, Kratz-Albers K, Serve S, Steur C, Buchner T, Kienast J, Kanakura Y, Berdel WE and Serve H. Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. *Blood* 2000; 96: 3907-3914.
- [41] Frohling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, Dohner H and Dohner K. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood* 2002; 100: 4372-4380.
- [42] Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, Walker H, Wheatley K, Bowen DT, Burnett AK, Goldstone AH and Linch DC. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood* 2001; 98: 1752-1759.
- [43] Rombouts WJ, Blokland I, Lowenberg B and Ploemacher RE. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the Flt3 gene. *Leukemia* 2000; 14: 675-683.
- [44] Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, Habdank M, Spath D, Morgan M, Benner A, Schlegelberger B, Heil G, Ganser A, Dohner H and German-Austrian Acute Myeloid Leukemia Study G. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *New England Journal of Medicine* 2008; 358: 1909-1918.
- [45] Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, Loffler H, Sauerland CM, Serve H, Buchner T, Haferlach T and Hiddemann W. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker

Treatment of FLT3-ITD acute myeloid leukemia

- for the detection of minimal residual disease. *Blood* 2002; 100: 59-66.
- [46] Bohmer FD, Karagoyozov L, Uecker A, Serve H, Botzki A, Mahboobi S and Dove S. A single amino acid exchange inverts susceptibility of related receptor tyrosine kinases for the ATP site inhibitor STI-571. *Journal of Biological Chemistry* 2003; 278: 5148-5155.
- [47] Lamers MB, Antson AA, Hubbard RE, Scott RK and Williams DH. Structure of the protein tyrosine kinase domain of C-terminal Src kinase (CSK) in complex with staurosporine. *Journal of Molecular Biology* 1999; 285: 713-725.
- [48] Smith BD, Levis M, Beran M, Giles F, Kantarjian H, Berg K, Murphy KM, Dausers T, Allebach J and Small D. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. *Blood* 2004; 103: 3669-3676.
- [49] Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R and Bukowski RM. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; 356: 125-134.
- [50] Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D and Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; 359: 378-390.
- [51] Christiansen DH, Andersen MK, Desta F and Pedersen-Bjergaard J. Mutations of genes in the receptor tyrosine kinase (RTK)/RAS-BRAF signal transduction pathway in therapy-related myelodysplasia and acute myeloid leukemia. *Leukemia* 2005; 19: 2232-2240.
- [52] Clark JW, Eder JP, Ryan D, Lathia C and Lenz HJ. Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43-9006, in patients with advanced, refractory solid tumors. *Clinical Cancer Research* 2005; 11: 5472-5480.
- [53] Auclair D, Miller D, Yatsula V, Pickett W, Carter C, Chang Y, Zhang X, Wilkie D, Burd A, Shi H, Rocks S, Gedrich R, Abriola L, Vasavada H, Lynch M, Dumas J, Trail PA and Wilhelm SM. Antitumor activity of sorafenib in FLT3-driven leukemic cells. *Leukemia* 2007; 21: 439-445.
- [54] Zhang W, Konopleva M, Shi YX, McQueen T, Harris D, Ling X, Estrov Z, Quintas-Cardama A, Small D, Cortes J and Andreeff M. Mutant FLT3: a direct target of sorafenib in acute myelogenous leukemia. *Journal of the National Cancer Institute* 2008; 100: 184-198.
- [55] Delmonte J, Kantarjian HM, Andreeff M, Faderl S, Wright JJ, Zhang W, Konopleva M, Verstovsek S, Borthakur G, Ravandi F and Cortes J. Update of a Phase I Study of Sorafenib in Patients with Refractory/Relapsed Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome. *Blood (ASH Annual Meeting Abstracts)* 2007; 110: Abstract 893.
- [56] Pratz KW, Cho E, Levis MJ, Karp JE, Gore SD, McDevitt M, Stine A, Zhao M, Baker SD, Carducci MA, Wright JJ, Rudek MA and Smith BD. A pharmacodynamic study of sorafenib in patients with relapsed and refractory acute leukemias. *Leukemia* 2010; 24: 1437-1444.
- [57] Lee SH, Paietta E, Racevskis J and Wiernik PH. Complete resolution of leukemia cutis with sorafenib in an acute myeloid leukemia patient with FLT3-ITD mutation. *American Journal of Hematology* 2009; 84: 701-702.
- [58] Safaian NN, Czibere A, Bruns I, Fenk R, Reinecke P, Dienst A, Haas R and Kobbe G. Sorafenib (Nexavar) induces molecular remission and regression of extramedullary disease in a patient with FLT3-ITD+ acute myeloid leukemia. *Leukemia Research* 2009; 33: 348-350.
- [59] Metzelder S, Wang Y, Wollmer E, Wanzel M, Teichler S, Chaturvedi A, Eilers M, Enghofer E, Neubauer A and Burchert A. Compassionate use of sorafenib in FLT3-ITD-positive acute myeloid leukemia: sustained regression before and after allogeneic stem cell transplantation. *Blood* 2009; 113: 6567-6571.
- [60] Metzelder S, Finck A, Fey M, Scholl S, Kroger M, Reiter A, Salih HR, Gotze K, Meyer RG, Giagounidis A, Brugger W, Vohringer M, Muller L, Ching LY, Dreger P, Masanori M, Basara N, Schaefer-Eckart K, Schultheis B, Baldus C, Neubauer A and Burchert A. Sorafenib Monotherapy Is Effective In Relapsed and Refractory FLT3-ITD Positive Acute Myeloid Leukemia, Particularly After Allogeneic Stem Cell Transplantation. *Blood (ASH Annual Meeting Abstracts)* 2010; 116: Abstract 3314.
- [61] Serve H, Wagner R, Sauerland C, Brunnberg U, Krug U, Schaich M, Ottmann OG, Duyster J, Wandt H, Herr W, Giagounidis A, Neubauer A, Reichle A, Aulitzky WE, Noppeney R, Blau IW, Kunzmann V, Schmitz N, Kreuzer KA, Krämer A, Brandts C, Steffen B, Heinecke A, Thiede C, Müller-Tidow C, Ehninger G and Berdel WE. Sorafenib in combination with standard induction and consolidation therapy In elderly AML patients: Results from a randomized, placebo-controlled phase II trial. *Blood (ASH Annual Meeting Abstracts)* 2010; 116: Abstract 333.
- [62] Camoratto AM, Jani JP, Angeles TS, Maroney AC, Sanders CY, Murakata C, Neff NT, Vaught JL, Isaacs JT and Dionne CA. CEP-751 inhibits TRK receptor tyrosine kinase activity in vitro exhibits anti-tumor activity. *International Journal of Cancer* 1997; 72: 673-679.
- [63] Hexner EO, Serdikoff C, Jan M, Swider CR, Robinson C, Yang S, Angeles T, Emerson SG, Carroll

Treatment of FLT3-ITD acute myeloid leukemia

- M, Ruggeri B and Dobrzanski P. Lestaurtinib (CEP701) is a JAK2 inhibitor that suppresses JAK2/STAT5 signaling and the proliferation of primary erythroid cells from patients with myeloproliferative disorders. *Blood* 2008; 111: 5663-5671.
- [64] Strock CJ, Park JI, Rosen M, Dionne C, Ruggeri B, Jones-Bolin S, Denmeade SR, Ball DW and Nelkin BD. CEP-701 and CEP-751 inhibit constitutively activated RET tyrosine kinase activity and block medullary thyroid carcinoma cell growth. *Cancer Research* 2003; 63: 5559-5563.
- [65] Marshall JL, Kindler H, Deeken J, Bhargava P, Vogelzang NJ, Rizvi N, Luhtala T, Boylan S, Dordal M, Robertson P, Hawkins MJ and Ratain MJ. Phase I trial of orally administered CEP-701, a novel neurotrophin receptor-linked tyrosine kinase inhibitor. *Investigational New Drugs* 2005; 23: 31-37.
- [66] Levis M, Pham R, Smith BD and Small D. In vitro studies of a FLT3 inhibitor combined with chemotherapy: sequence of administration is important to achieve synergistic cytotoxic effects. *Blood* 2004; 104: 1145-1150.
- [67] Levis M, Brown P, Smith BD, Stine A, Pham R, Stone R, Deangelo D, Galinsky I, Giles F, Estey E, Kantarjian H, Cohen P, Wang Y, Roesel J, Karp JE and Small D. Plasma inhibitory activity (PIA): a pharmacodynamic assay reveals insights into the basis for cytotoxic response to FLT3 inhibitors. *Blood* 2006; 108: 3477-3483.
- [68] Knapper S, Burnett AK, Littlewood T, Kell WJ, Agrawal S, Chopra R, Clark R, Levis MJ and Small D. A phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. *Blood* 2006; 108: 3262-3270.
- [69] Levis M, Ravandi F, Wang ES, Baer MR, Perl A, Coutre S, Erba H, Stuart RK, Baccharani M, Cripe LD, Tallman MS, Meloni G, Godley LA, Langston AA, Amadori S, Lewis ID, Nagler A, Stone R, Yee K, Advani A, Douer D, Wiktor-Jedrzejczak W, Juliusson G, Litzow MR, Petersdorf S, Sanz M, Kantarjian HM, Sato T, Tremmel L, Bensen-Kennedy DM, Small D and Smith BD. Results from a randomized trial of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. *Blood* 2011; 117: 3294-3301.
- [70] Knapper S, Burnett AK, Hills RK, Small D and Levis M. Lestaurtinib FLT3 Inhibitory Activity Is Modulated by Concomitant Azole Therapy and May Influence Relapse Risk. *Blood* (ASH Annual Meeting Abstracts) 2009; 114: Abstract 789
- [71] Fabbro D, Ruetz S, Bodis S, Pruschy M, Csermak K, Man A, Campochiaro P, Wood J, O'Reilly T and Meyer T. PKC412—a protein kinase inhibitor with a broad therapeutic potential. *Anti-Cancer Drug Design* 2000; 15: 17-28.
- [72] Weisberg E, Boulton C, Kelly LM, Manley P, Fabbro D, Meyer T, Gilliland DG and Griffin JD. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. *Cancer Cell* 2002; 1: 433-443.
- [73] Stone RM, Fischer T, Paquette R, Schiller G, Schiffer CA, Ehninger G, Cortes J, Kantarjian HM, DeAngelo DJ, Huntsman-Labed A, Dutreix C, Rai S and Giles F. A phase 1b study of midostaurin (PKC412) in combination with daunorubicin and cytarabine induction and high-dose cytarabine consolidation in patients under age 61 with newly diagnosed de novo acute myeloid leukemia: Overall survival of patients whose blasts have FLT3 mutations is similar to those with wild-type FLT3. *ASH Annual Meeting Abstracts* 2009; 114: Abstract 634.
- [74] Fischer T, Stone RM, Deangelo DJ, Galinsky I, Estey E, Lanza C, Fox E, Ehninger G, Feldman EJ, Schiller GJ, Klimek VM, Nimer SD, Gilliland DG, Dutreix C, Huntsman-Labed A, Virkus J and Giles FJ. Phase IIB trial of oral Midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated FLT3. *J Clin Oncol* 2010; 28: 4339-4345.
- [75] Stone RM, Dohner H, Ehninger G, Villeneuve M, Teasdale T, Virkus JD, Bressler LR, Seiler MM, Marcucci G and Larson RA. CALGB 10603 (RATIFY): A randomized phase III study of induction (daunorubicin/cytarabine) and consolidation (high-dose cytarabine) chemotherapy combined with midostaurin or placebo in treatment-naive patients with FLT3 mutated AML. *J Clin Oncol* 2011; 29: Abstract TPS199.
- [76] Chao Q, Sprankle KG, Grotzfeld RM, Lai AG, Carter TA, Velasco AM, Gunawardane RN, Cramer MD, Gardner MF, James J, Zarrinkar PP, Patel HK and Bhagwat SS. Identification of N-(5-tert-butyl-isoxazol-3-yl)-N'-[4-[7-(2-morpholin-4-yl-ethoxy)imidazo[2,1-b][1,3]benzothiazol-2-yl]phenyl]urea dihydrochloride (AC220), a uniquely potent, selective, and efficacious FMS-like tyrosine kinase-3 (FLT3) inhibitor. *Journal of Medicinal Chemistry* 2009; 52: 7808-7816.
- [77] Zarrinkar PP, Gunawardane RN, Cramer MD, Gardner MF, Brigham D, Belli B, Karaman MW, Pratz KW, Pallares G, Chao Q, Sprankle KG, Patel HK, Levis M, Armstrong RC, James J and Bhagwat SS. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). *Blood* 2009; 114: 2984-2992.
- [78] Pratz KW, Sato T, Murphy KM, Stine A, Rajkhowa T and Levis M. FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. *Blood* 2010; 115: 1425-1432.
- [79] Cortes J, Perl A, Smith C, Kovacovics T, Dombret H, Döhner H, Steffen B, Pigneux A, Rous-

Treatment of FLT3-ITD acute myeloid leukemia

- selot P, Krauter J, Martineli G, Estey E, Burnett A, Ho A, Ifrah I, de Witte T, Corringham R, James J, Lillienfeld D, Leo E, Trikha M and Levis M. A Phase II open-label, AC220 monotherapy efficacy (ACE) study in patients with acute myeloid leukemia (AML) with FLT3-ITD activating mutations: Interim results. 16th Congress of the European Hematology Association 2011; Abstract 1019.
- [80] Giles FJ, Stopeck AT, Silverman LR, Lancet JE, Cooper MA, Hannah AL, Cherrington JM, O'Farrell AM, Yuen HA, Louie SG, Hong W, Cortes JE, Verstovsek S, Albitar M, O'Brien SM, Kantarjian HM and Karp JE. SU5416, a small molecule tyrosine kinase receptor inhibitor, has biologic activity in patients with refractory acute myeloid leukemia or myelodysplastic syndromes. *Blood* 2003; 102: 795-801.
- [81] Fiedler W, Krauter J, Götze K, Salih HR, Bokemeyer C, Spaeth D, Döhner K, Döhner H and Schlenk RF. A phase I/II study combining sunitinib with standard ara-C/daunorubicin chemotherapy in patients 60 years or older with FLT3 mutated AML. *Blood (ASH Annual Meeting Abstracts)* 2010; 116: Abstract 3285.
- [82] O'Farrell AM, Foran JM, Fiedler W, Serve H, Paquette RL, Cooper MA, Yuen HA, Louie SG, Kim H, Nicholas S, Heinrich MC, Berdel WE, Bello C, Jacobs M, Scigalla P, Manning WC, Kelsey S and Cherrington JM. An innovative phase I clinical study demonstrates inhibition of FLT3 phosphorylation by SU11248 in acute myeloid leukemia patients. *Clin Cancer Res* 2003; 9: 5465-5476.
- [83] DeAngelo DJ, Stone RM, Heaney ML, Nimer SD, Paquette RL, Klisovic RB, Caligiuri MA, Cooper MR, Lecerf JM, Karol MD, Sheng S, Holford N, Curtin PT, Druker BJ and Heinrich MC. Phase 1 clinical results with tandutinib (MLN518), a novel FLT3 antagonist, in patients with acute myelogenous leukemia or high-risk myelodysplastic syndrome: safety, pharmacokinetics, and pharmacodynamics. *Blood* 2006; 108: 3674-3681.
- [84] Cortes J, Roboz GJ, Kantarjian H, Feldman E, Karp JE, Pratz KW, Rao N, Akinaga S and Levis M. A phase I dose escalation study of KW-2449, an oral multi-kinase inhibitor against FLT3, Abl, FGFR1 and Aurora in patients with relapsed/refractory AML, ALL and MDS or resistant/intolerant CML. *Blood* 2008; 112 Abstract 2967.
- [85] O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW, Wong LM, Hong W, Lee LB, Town A, Smolich BD, Manning WC, Murray LJ, Heinrich MC and Cherrington JM. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood* 2003; 101: 3597-3605.
- [86] Pratz KW, Cortes J, Roboz GJ, Rao N, Arowojolu O, Stine A, Shiotsu Y, Shudo A, Akinaga S, Small D, Karp JE and Levis M. A pharmacodynamic study of the FLT3 inhibitor KW-2449 yields insight into the basis for clinical response. *Blood* 2009; 113: 3938-3946.
- [87] Levis M, Ravandi F, Wang ES, Baer MR, Perl A, Coutre S, Erba H, Stuart RK, Baccharani M, Cripe LD, Tallman MS, Meloni G, Godley LA, Langston AA, Amadori S, Lewis ID, Nagler A, Stone R, Yee K, Advani A, Douer D, Wiktor-Jedrzejczak W, Juliusson G, Litzow MR, Petersdorf S, Sanz M, Kantarjian HM, Sato T, Tremmel L, Bensen-Kennedy DM, Small D and Smith BD. Results from a randomized trial of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. *Blood* 2010; 117: 3294-3301.
- [88] Sato T, Yang X, Knapper S, White P, Smith BD, Galkin S, Small D, Burnett A and Levis M. FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. *Blood* 2010; 117: 3286-3293.
- [89] Haidar JH, Bazarbachi A, Mahfouz R, Haidar HA, Jaafar H and Daher R. Serum Flt3 ligand variation as a predictive indicator of hematopoietic stem cell mobilization. *Journal of Hematology & Stem Cell Research* 2002; 11: 533-538.
- [90] Singh H, Werner LL, Asali S, Deangelo DJ, Ballen KK, Amrein PC, Wadleigh M, Galinsky I, Wolpin B, Pidala J, Neuberg DS, Fox EA, Stone RM and Attar EC. Comparison of autologous stem cell transplantation versus consolidation chemotherapy for patients with cytogenetically normal acute myeloid leukemia (CN-AML) and FLT3ITD. *Am J Hematol* 2010; 86: 625-627.
- [91] Bornhauser M, Illmer T, Schaich M, Soucek S, Ehninger G and Thiede C. Improved outcome after stem-cell transplantation in FLT3/ITD-positive AML. *Blood* 2007; 109: 2264-2265; author reply 2265.
- [92] Gupta V, Tallman MS and Weisdorf DJ. Allogeneic hematopoietic cell transplantation for adults with acute myeloid leukemia: myths, controversies, and unknowns. *Blood* 117: 2307-2318.
- [93] Levis M. FLT3/ITD AML and the law of unintended consequences. *Blood* 2011; 117: 6987-6990.
- [94] Rowe JM and Tallman MS. How I treat acute myeloid leukemia. *Blood* 2011; 116: 3147-3156.
- [95] Gale RE, Hills R, Kottaridis PD, Srirangan S, Wheatley K, Burnett AK and Linch DC. No evidence that FLT3 status should be considered as an indicator for transplantation in acute myeloid leukemia (AML): an analysis of 1135 patients, excluding acute promyelocytic leukemia, from the UK MRC AML10 and 12 trials. *Blood* 2005; 106: 3658-3665.
- [96] Kayser S, Dohner K, Krauter J, Casper J, Horst H, Held G, Wilhelm S, Kobbe G, Lubbert M, Salih H, Gotze K, Rummel M, Fiedler W, von Lilienfeld-Toal M, Nachbaur D, Wattad M, Spath D, Erdmann P, Ganser A, Dohner H and Schlenk R. Impact of Allogeneic Transplantation From

Treatment of FLT3-ITD acute myeloid leukemia

Matched Related and Unrelated Donors on Clinical Outcome in Younger Adult AML Patients with FLT3 Internal Tandem Duplications. *Blood* 2010; 116: Abstract 909.

[97] Dezern AE, Sung A, Kim S, Smith BD, Karp JE, Gore SD, Jones RJ, Fuchs E, Luznik L, McDevitt

M, Levis M. Role of allogeneic transplantation for FLT3/ITD acute myeloid leukemia: Outcomes from 133 consecutive newly diagnosed patients from a single institution.