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***FLT3* tyrosine kinase inhibitors in acute myeloid leukemia: clinical implications and limitations**

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Abstract

Internal tandem duplications of the FMS-like tyrosine kinase 3 (*FLT3*) gene are one of the most frequent gene mutations in acute myeloid leukemia (AML) and are associated with poor clinical outcome. The remission rate is high with intensive chemotherapy, but most patients eventually relapse. During the last decade, *FLT3* mutations have emerged as an attractive target for a molecularly specific treatment strategy. Targeting *FLT3* receptor tyrosine kinases in AML has shown encouraging results in the treatment of *FLT3* mutated AML, but in most patients responses are incomplete and not sustained. Newer, more specific compounds seem to have a higher potency and selectivity against *FLT3*. During therapy with *FLT3* tyrosine kinase inhibitors (TKIs) the induction of acquired resistance has emerged as a clinical problem. Therefore, optimization of the targeted therapy and potential treatment options to overcome resistance is currently the focus of clinical research. In this review we discuss the use and limitations of TKIs as a therapeutic strategy for the treatment of *FLT3* mutated AML, including mechanisms of resistance to TKIs as well as possible novel strategies to improve *FLT3* inhibitor therapy.

Keywords

Acute myeloid leukemia; *FLT3* mutations; molecular tailored therapy; tyrosine kinase inhibitors; mechanism of resistance

Introduction

Acute myeloid leukemia (AML) is a malignant clonal disorder of the hematopoietic system characterized by an enormous heterogeneity of acquired genetic and epigenetic changes in hematopoietic precursor cells and by impaired mechanisms of self-renewal, proliferation and differentiation. To date, more than 200 structural and numerical cytogenetic abnormalities have been described in this disease, with incidences ranging from <0.1% to 10% [1]. More recently, a variety of gene mutations, deregulated expression of genes, and non-coding

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RNAs (microRNAs) and epigenetic changes have also been identified in AML over the past few years [2,3].

In a model of the pathogenesis of AML proposed a decade ago, it was postulated that there are at least two classes of mutations that are necessary for the development of AML, with one class affecting the growth factor pathways and the other class resulting in a block in differentiation [4]. More recently, with the advent of whole genome sequencing of AML specimens, a variation on this two-step model has been suggested, in which an initiating driver mutation, such as *NPM1*, *DNMT3A*, *IDH2* or *TET2*, confers a survival advantage to a hematopoietic stem/progenitor cell. This is followed by a cooperating driver mutation, which results in full-blown transformation to AML [5]. This model will undoubtedly evolve, in light of the evidence that AML is polyclonal at presentation, but changes its clonality and mutational profile over time in the setting of chemotherapy and eventual relapse [6]. The most common cooperating mutation in both models is an internal tandem duplication mutation of the FMS-like tyrosine kinase 3 gene (*FLT3*-ITD mutation). *FLT3*-ITD mutations constitutively activate the receptor tyrosine kinase (RTK) activity and its downstream signaling pathways, leading to dysregulation of cellular proliferation [7–9].

Since RTK mutations play a crucial role in the pathogenesis of AML and are relatively common, they seem to be an ideal target for a molecularly tailored treatment approach. Targeting *FLT3* RTK signaling in particular is very attractive given that *FLT3* mutations are found in approximately one-third of patients with AML [10].

In this article, we discuss the use and limitations of tyrosine kinase inhibitors (TKIs) as a therapeutic strategy for the treatment of *FLT3* mutated AML. Mechanisms of resistance to TKIs are highlighted as well as possible novel strategies to improve *FLT3* inhibitor therapy.

***FLT3* mutated acute myeloid leukemia**

FLT3, located on chromosome 13q12, is grouped into the class III RTK family, and was first described by Nakao *et al.* in 1996 [11]. The *FLT3* gene plays an important role in growth and differentiation of hematopoietic stem cells [10]. *FLT3* mutations are found in about one-third of all patients with AML, and are one of the most frequent genetic abnormalities found in AML [2]. At present, three different activating *FLT3* gene mutations are known: *FLT3*-ITD mutations, which can be detected in approximately 20% [11–13], point mutations in the activation loop of the second tyrosine kinase domain (*FLT3*-TKD), detectable in about 6–8% [14,15], and point mutations in the juxtamembrane (JM) as well as extracellular domain of the receptor, which are very rare (approximately 2%) [16]. The most common mutation, *FLT3*-ITD, leads to a ligand-independent dimerization and constitutive activation of the tyrosine kinase domain, thus prompting factor-independent cell growth [7]. These mutations are associated with constitutive activation of multiple downstream pathways, such as phosphatidylinositol 3-kinase/v-akt murine thymoma viral oncogene homolog 1 (PIK3/AKT), mitogen-activated protein kinase/extracellular signal regulated kinase (MAPK/ERK) and signal transducer and activator of transcription 5 (STAT5) [8,9]. Previously, it was thought that *FLT3*-ITD mutations insert exclusively within the JM domain. Recently, however, we and others have shown that about one-third of *FLT3*-ITD

mutations insert outside the JM domain, in particular in the β 1-sheet of the tyrosine kinase domain 1 (TKD1; Figure 1) [17– 19]. In cell culture analyses, a prototypic *FLT3*-ITD with insertion site in the β 2-sheet of the TKD1 (*FLT3*-ITD627E) mediated phosphorylation of FLT3 and STAT5, suggesting that non-JM domain *FLT3*-ITD mutations confer constitutive activation of the receptor [18]. Additionally, *FLT3*-ITD627E induced transformation of hematopoietic 32D cells and led to a lethal myeloproliferative disease in a syngeneic mouse model [18]. A dramatic up-regulation of the anti-apoptotic myeloid cell leukemia 1 (MCL-1) protein expression could be shown after expression of the FLT3-ITD627E receptor in a cell culture model [20]. Increased binding capacity of the adaptor protein Grb2 to the FLT3-receptor was shown to be involved in MCL-1 up-regulation and was independent from TKI (midostaurin)-induced inhibition of the receptor kinase [20].

Besides the insertion site, *FLT3*-ITD mutations show a broad variability in the mutant to wild-type (WT) allelic ratio, in the size of the insertion (ranging from three to over 100 base pairs), and the number of affected clones per patient [12, 17, 19, 21–28].

Patients with AML harboring *FLT3*-ITD mutations have characteristic pretreatment features including increased white blood cell count, a higher percentage of blood and bone marrow blasts, and a more frequent diagnosis of *de novo* rather than secondary AML [13, 21]. *FLT3*-ITD mutations are most frequently associated with a normal karyotype as well as with the translocations t(6;9)(p23;q34) [29] and t(15;17)(q22;q12) [30]. In cytogenetically normal AML, *FLT3*-ITD mutations confer a worse prognosis due to a high relapse rate and adverse overall survival (OS) [13, 21, 22, 27]. Furthermore, previous studies suggested a prognostic role for the mutant to WT allelic ratio, as quantified by Gene-Scan analysis, and the size of the ITD [21–27]. So far, data on the allelic ratio consistently show an association of high allelic burden with worse prognosis, whereas the prognosis of patients with a low *FLT3*-ITD allelic ratio (allelic ratio either < 0.5 or < 0.78) is discussed controversially [21, 22, 27]. The data published by Gale *et al.* are in contrast to another study, where no significant difference in outcome was found between lower level mutants and WT *FLT3*, although the exact cut-offs for the allelic ratio varied [21,27]. One possible explanation for this finding could be that in these patients the *FLT3*-ITD mutation occurred in a late stage of leukemogenesis, including a lower addiction to *FLT3*-ITD signaling. And even the responsiveness to FLT3 TKIs seems to depend on the *FLT3* allelic ratio [31]. Patients at diagnosis seem to present more often with lower allelic ratios, which are relatively less addicted to *FLT3*-ITD signaling for survival as compared to those at the time point of relapse [31]. In an *in vitro* analysis, relapsed samples and samples with a high mutant allelic ratio were more likely to be responsive to cytotoxicity from FLT3 TKIs as compared to the samples obtained at diagnosis or those with a low mutant allelic ratio [31]. However, the results probably indicate that the presence of a *FLT3*-ITD with even a low allelic ratio cannot be excluded altogether from prognostic risk stratification. For instance, if the presence of a *FLT3*-ITD provides resistance to chemotherapy by enhancing DNA repair and salvage of damaged cells [32], then mutant-carrying cells will have a survival advantage on treatment with conventional chemotherapy, irrespective of their relative proportion in the total population.

Besides the allelic ratio, the prognostic impact of the *FLT3*-ITD size is also a matter of debate. Both longer and shorter *FLT3*-ITD mutations have been associated with an inferior outcome, or have been shown to have no prognostic impact [23–27]. Moreover, we were able to show that a *FLT3*-ITD insertion site in the β 1-sheet of the TKD1 was associated with an inferior outcome as compared to all other insertion sites for younger adult patients with AML who had been treated with intensive chemotherapy, even after allogeneic hematopoietic stem cell transplant [17, 33]. Recently, these results have been confirmed by Schnittger and colleagues, who could show that *FLT3*-ITD insertion sites localized more C-terminally within the *FLT3* gene were associated with an adverse outcome [19].

Furthermore, the molecular background of cooperating mutations, such as *NPM1* and *DNMT3A*, may influence the prognostic impact of *FLT3*-ITD positive AML. Yet again, the prognostic impact of a concurrent *NPM1* mutation in *FLT3*-ITD positive AML is discussed controversially. In a publication by Gale and co-workers, a better outcome in *FLT3*-ITD positive patients harboring a concurrent *NPM1* mutation was stated [27], whereas according to other authors the “protective effect” of *NPM1* in AML with a higher *FLT3*-ITD allelic ratio (> 0.5) seemed at least to be diminished or to get lost [34]. Recently published data suggest that *FLT3*-ITD retains its negative prognostic impact in intermediate-risk AML, even in the context of other genetic abnormalities, such as *DNMT3A* and *TET2* [35].

For *FLT3*-TKD mutations, point mutations, small insertions or deletions can be found in exon 20 of the *FLT3* gene, most commonly a substitution of aspartic acid by tyrosine at codon 835, which affect the activation loop of the carboxy terminal part of the TKD [2]. *FLT3*-TKD mutations stabilize the activation loop of the open adenosine-5-triphosphate (ATP)-binding configuration, thus leading to constitutive activation of the *FLT3* gene. When transduced into murine hematopoietic stem cells, *FLT3*-TKD mutations induce an oligoclonal lymphoid disorder, suggesting differences in cell signaling between *FLT3*-TKD mutants and *FLT3*-ITDs [36]. Indeed, strong STAT5 activation could only be demonstrated for *FLT3*-ITD mutations [36,37]. Currently, the prognostic value of a *FLT3*-TKD mutation is still unclear [2,38,39].

Treatment with *FLT3* tyrosine kinase inhibitors

Activation of signaling pathways via RTKs plays a central role in the pathogenesis of AML, and inhibition of these tyrosine kinases using small molecules represents an attractive therapeutic concept. One option to interfere with *FLT3* activity is to inhibit its kinase activity. TKIs compete with ATP for binding to the active pocket of the kinases, resulting in the inability to autophosphorylate or phosphorylate substrate proteins by transferring the terminal phosphate of ATP. Thus, signal transduction initiated by the mutated RTK is interrupted [40]. Several small molecule kinase inhibitors with activity against *FLT3* have been evaluated in patients with AML as single agents and in combination with intensive chemotherapy, e.g. midostaurin (PKC412), lestaurtinib (CEP-701), tandutinib (MLN-518), sunitinib (SU11248) and sorafenib (BAY 43-9006) [41]. These compounds were not specifically developed as *FLT3* inhibitors, but exhibit activity against other RTKs, as summarized in Table I. In single-agent phase I and II trials in relapsed or refractory AML,

responses were generally limited and not durable. Clinical experience using FLT3 TKIs is outlined below and summarized in Table II.

Midostaurin

In a phase II trial, midostaurin has been evaluated as single-agent therapy in 20 patients with relapsed or refractory *FLT3*-mutated AML, including one patient with advanced myelodysplastic syndrome (MDS), at a dose of 75 mg orally three times daily [42]. Fourteen of the 20 patients (70%) showed a decrease of peripheral blast counts by 50%, and seven of the 20 patients (35%) experienced a greater than 2-log reduction in peripheral blast counts for at least 4 weeks (median response duration, 13 weeks; range, 9 – 47 weeks). In six of the 20 patients (30%) bone marrow blast counts were reduced by 50%, and two of these achieved a complete clearance of bone marrow blasts [42]. In combination with chemotherapy, midostaurin induced high complete remission (CR) rates in *FLT3* mutated AML [43], and based on these favorable results the impact of midostaurin in AML with *FLT3*-ITD was evaluated in a large international randomized phase III study (Table III). This trial reached its accrual goal of over 700 patients with *FLT3* mutated AML in October 2011, and first results are expected in 2014. An important issue relates to the scheduling and duration of treatment with the TKI. Administration of midostaurin simultaneously with chemotherapy appeared to be more toxic than a sequential approach, where midostaurin was given after chemotherapy [43]. Therefore, in the completed randomized phase III trial, midostaurin was administered sequentially following chemotherapy. Currently, the German - Austrian AML Study Group (AMLSG) is evaluating the impact of midostaurin given in combination with intensive induction, consolidation including allogeneic hematopoietic stem cell transplant (HSCT) and single-agent maintenance therapy on event-free survival in a single-arm, multicenter phase II study in adult patients with AML with *FLT3*-ITD (Table III).

Lestaurtinib

The TKI lestaurtinib has been evaluated as a single-agent therapy in a phase I – II trial in patients with relapsed or refractory *FLT3* mutated AML [44]. Fourteen patients with AML were treated with lestaurtinib at an initial dose of 60 mg orally twice daily. Of those, five patients (36%) had clinical evidence of biologic activity and measurable clinical response, including significant reductions of myeloid blast cells in peripheral blood and bone marrow. However, responses were generally short, lasting from 2 weeks to 3 months. In a follow-up phase II study, lestaurtinib was administered as first-line single-agent treatment for older patients with AML not considered fit for intensive chemotherapy, irrespective of *FLT3* mutation status. Lestaurtinib was given orally for 8 weeks, initially at a dose of 60 mg twice daily, escalating to 80 mg twice daily, and was generally well tolerated. Clinical activity, manifest as transient reductions of myeloid blast cells in peripheral blood and bone marrow or longer periods of transfusion independence, was seen in three of five *FLT3* mutated patients (60%) and five of 22 evaluable *FLT3* WT patients (23%) [45]. In a randomized trial of *FLT3* mutated AML in first relapse, 224 patients received chemotherapy alone or followed by 80 mg of lestaurtinib twice daily. Twenty-nine patients in the lestaurtinib arm achieved less than 5% myeloid blast cells in bone marrow as compared to 23 patients in the control arm (26% vs. 21%; $p = 0.35$). In this study, lestaurtinib treatment after chemotherapy

did not increase response rates or prolong survival. Correlative studies from this trial, however, indicated that only a limited number of patients achieved FLT3 target inhibition with lestaurtinib [46]. Similar to midostaurin, the scheduling of lestaurtinib administration seems to be important, since lestaurtinib induced cytotoxicity only if it was used simultaneously or immediately following exposure to chemotherapy. This effect appeared to be attributable to the cell cycle arrest induced by lestaurtinib [47].

Sunitinib

Pharmacodynamic and pharmacokinetic effects of the multi-targeted oral TKI sunitinib have been evaluated in a single-dose phase I study in 29 patients with AML. The dose of sunitinib was escalated from 50 to 350 mg, in increments of 50 mg and cohorts of three to six patients. In this clinical trial, only five of the 29 (17%) patients harbored a *FLT3* mutation: three patients had a *FLT3*-ITD and two patients a *FLT3* -TKD. Inhibition of FLT3 phosphorylation was dependent on dose and plasma drug levels. The primary end point, strong inhibition of FLT3 phosphorylation in > 50% patients, was reached in 200 mg and higher dose cohorts [48]. Furthermore, 15 patients with refractory AML were treated in a phase I study with sunitinib. Contrary to the encouraging results of midostaurin or sorafenib, single-agent therapy with sunitinib induced only partial responses (PRs) of short duration (4 – 16 weeks) in this cohort of patients with AML [49]. However, much better results have been reported recently on a phase I/II clinical study, which evaluated the feasibility of standard induction and consolidation therapy in combination with orally administered sunitinib in elderly patients with AML with activating FLT3 mutations. Median age in this trial was 70 years (range, 60 – 78 years). Interestingly, the CR rate in AML with *FLT3*-ITD was 53% (8/15) and was 71% (5/7) in those with *FLT3* -TKD. All 13 patients who achieved a CR received repetitive cycles of high-dose cytarabine consolidation therapy, and seven proceeded to single-agent sunitinib maintenance therapy (median duration, 11 months; range, 1 – 24 months). Within this study, median survival was 18.8 months, median relapse-free survival 11 months, and two patients are in sustained CR [50].

Sorafenib

The anti-leukemic activity of sorafenib was investigated in a phase I study in 16 patients with refractory or relapsed AML. Sorafenib was administered orally with dose levels from 200 to 400 mg twice daily. In this study, no dose limiting toxicity was observed. Sorafenib significantly reduced the percentage of myeloid blast cells in peripheral blood and bone marrow in patients with *FLT3*-ITD AML ($n = 6/6$), but showed only modest activity in *FLT3* WT patients ($n = 3/7$) and no clinical response in patients with a *FLT3* -TKD mutation ($n = 0/3$) [51]. A recently published study on 65 patients with *FLT3*-ITD AML treated with sorafenib monotherapy either after chemotherapy or allogeneic HSCT further highlights these encouraging results [52]. In a pharmacodynamic phase I dose escalation study, evaluating sorafenib in 15 patients with relapsed or refractory leukemia, of whom only two harbored a *FLT3*-ITD mutation, no significant clinical responses were noted [53]. Sorafenib was further evaluated in combination with intensive chemotherapy in a combined phase I/II study [54]. In this study, sorafenib induced high CR rates in younger *FLT3* mutated as well as *FLT3* WT patients with AML. The survival rate at 1 year was 74% (median follow-up of 54 weeks; range, 8 – 87 weeks). Accompanying plasma inhibitory assays in 10 patients

demonstrated an on-target effect on FLT3 kinase activity due to complete inhibition of phosphorylated FLT3 [54]. So far, there are only limited results for sorafenib treatment in older patients with AML (> 60 years). A completed randomized double-blinded study of 197 older patients with AML could not show a beneficial effect of sorafenib in combination with standard chemotherapy on clinical outcome [55]. In this study, however, there might have been biases, since the study was not selected for the target population and the proportion of *FLT3*-ITD was very low in the study cohort (28 of 197 patients; 14%). In the context of allogeneic HSCT, sorafenib has shown clinical activity in some cases of *FLT3*-ITD positive AML [52,56,57]. A case report of a patient with *FLT3* -ITD positive AML after allogeneic HSCT illustrates that sorafenib in conjunction with graft-versus-leukemia effects could induce molecular negativity of *FLT3*-ITD after a second relapse [56]. Encouraging results had also been reported on the monotherapy with sorafenib in 65 patients with *FLT3*-ITD AML, who had been relapsed or were refractory after chemotherapy or allogeneic HSCT [52]. Within this study, a response could be achieved in 83% of the patients ($n = 54/65$), including the achievement of a CR (with or without normalization of peripheral blood counts; $n = 15$). Furthermore, patients after prior allogeneic HSCT had a significantly lower rate of resistance toward sorafenib treatment as compared to those patients after chemotherapy (38% vs. 47%), as well as a longer remission duration (median, 197 days vs. 136 days; $p = 0.03$), suggesting that sorafenib therapy in the context of allogeneic HSCT may synergize with allogeneic immune effects [52]. However, these encouraging results could not be obtained in 16 patients with *FLT3*-ITD AML treated at the M. D. Anderson Cancer Center, who were relapsed after allogeneic HSCT [58]. The patients received either sorafenib alone ($n = 8$) or in combination with chemotherapy ($n = 8$). Although the number of circulating blasts decreased in 80% of cases, only three patients achieved a partial remission. Two patients were bridged to a second allogeneic HSCT, but both relapsed within 3 months and the median OS was only 83 days [58]. Currently, several studies evaluating the feasibility as well as efficacy of sorafenib maintenance after allogeneic HSCT are under way (Table III).

Quizartinib

While the first-generation TKIs showed only limited activity as a single agent, the second-generation TKI quizartinib (AC220) was effective as monotherapy in early phase I and II studies of patients with relapsed or refractory AML. Initially, quizartinib was identified due to screening of a scaffold-based library of compounds against several kinases [59]. Based on binding affinity, quizartinib was identified as a compound with high potency and selectivity for FLT3 [60]. In a phase I study, quizartinib was administered once daily as an oral solution in an outpatient setting for 14 days every 28 days at a starting dose of 12 mg, with dose escalations of 50% in subsequent cohorts. A later amendment included the ability to administer quizartinib continuously for 28 days without interruption. Observed adverse side effects were mainly gastrointestinal symptoms as well as prolongation of the QTc interval. The phase I study included 76 patients, of whom 18 (24%) were *FLT3*-ITD positive, 45 (59%) were *FLT3* WT and 13 (17%) were indeterminate. Ten of 18 (56%) *FLT3*-ITD patients as compared to nine of 45 (20%) *FLT3* WT patients responded. The median duration of response was 14 weeks. Of note, responses were observed in cohorts treated with doses as low as 18 and 40 mg/day. The *FLT3* WT group comprised three patients with a

FLT3 -TKD mutation, none of whom responded [60]. Very recently, the phase II study of quizartinib has reached its accrual goal of approximately 300 patients with AML. The trial consisted of two treatment cohorts: the first one comprised older patients (> 60 years) who had relapsed after one first-line chemotherapy regimen within 1 year or were primary refractory to first-line chemotherapy. The second cohort consisted of patients who were < 18 years of age and had relapsed or were refractory after one second-line (salvage) regimen or HSCT. Most patients in this study were *FLT3*-ITD positive, but a small number in each cohort lacked the mutation. The primary objective of the trial was to evaluate the composite CR rate, defined as the combination of CR, CR with incomplete platelet recovery and CR with incomplete hematologic recovery. Within both treatment cohorts, quizartinib as a single agent has shown promising efficacy, leading to a composite CR rate (CRc) of 46 – 54% in relapsed/refractory *FLT3*-ITD positive AML [61,62]. Notably, remission rates were highest in patients who had relapsed after allogeneic HSCT, and a high percentage (37%) of cohort II patients could be successfully bridged to allogeneic HSCT [62]. Patients with *FLT3* wild-type also responded to quizartinib, but to a lower extent (CRc rates of 31% and 32% for cohort I and cohort II, respectively). Overall, quizartinib was well tolerated, with manageable toxicities; adverse events included mainly gastrointestinal symptoms, reversible QT prolongation and myelosuppression, possibly related to KIT inhibition [61,62]. These data represent the highest level of single-agent activity observed to date for *FLT3*-targeted therapy in patients with relapsed/ refractory *FLT3*-ITD positive AML. The encouraging efficacy results as well as an acceptable safety profile in this population with high-risk AML support continued clinical evaluation in mono- and combination therapy as well as for maintenance after allogeneic HSCT (currently ongoing clinical trials are summarized in Table III).

Allogeneic hematopoietic stem cell transplant

In addition to TKIs, allogeneic HSCT seems to be clinically effective in patients with AML with *FLT3* -ITD: both relapse-free and OS rates are significantly improved by allogeneic HSCT [33,63– 68]. Notably, the relapse risk after allogeneic HSCT could be reduced to roughly 30% [64,66,67,69]. The reported data suggest the existence of a strong anti-leukemic effect after myeloablative, but also after reduced-intensity conditioning in combination with a graft-versus-leukemia reaction. In contrast, in a single-center experience, a lower disease-free survival and increased risk of relapse after allogeneic HSCT in *FLT3*-ITD patients was reported as compared to patients with wild-type *FLT3* [70]. However, the data might have been biased due to the low patient number and long median time to transplant, requiring several courses of chemotherapy before allogeneic HSCT. Within this survey of patients after allogeneic HSCT, any source of donor (including umbilical cord blood and haploidentical) had been used [70], which contrasts with other publications where the source of donor was restricted to matched-related or matched-unrelated donors [63,64,66,68,69]. Furthermore, almost half of the patients received reduced-intensity conditioning [70]. Therefore, these data should be interpreted cautiously. An overview of the outcome results for *FLT3*-ITD patients after allogeneic HSCT is given in Table IV. Yet again, the molecular characteristics of *FLT3*-ITD may further influence its prognostic impact even in the setting of allogeneic HSCT [71,72]. Recently presented data in *FLT3*-

ITD positive AML suggest that a high allelic ratio and/or a *FLT3*-ITD insertion site in the β 1-sheet of the *FLT3* gene and, in particular, the presence of both seem to be associated with a very unfavorable prognosis, which could not be overcome by an allogeneic HSCT performed in first CR [72]. In contrast, allogeneic HSCT performed in first CR had a beneficial effect on relapse-free and OS in all other patients with *FLT3*-ITD positive AML [72]. Whether the recent introduction of FLT3 kinase inhibitors will significantly improve outcome after chemotherapy remains to be answered by ongoing clinical trials. TKIs may increase the proportion of patients with *FLT3*-ITD who achieve CR and reach allografting in the best condition. Therefore, FLT3 inhibitors may be a strategy early on in therapy to improve remission rate and to maintain patients in remission after allogeneic HSCT. Several ongoing clinical trials are now investigating this strategy (Table III).

Mechanisms of resistance during treatment with tyrosine kinase inhibitors

During therapy with FLT3 TKIs the induction of secondary *FLT3* -TKD mutations seems to emerge in a substantial proportion of patients with *FLT3*-ITD AML, which is associated with loss of response [73,74]. The emergence of a *FLT3*-TKD during treatment with midostaurin had first been described in an index patient with AML by Heidel and colleagues [75] and has now been shown in several patients with AML who had been treated within the AC220-002 trial [73]. The *FLT3* -TKD mutation might possibly lead to a change in the RTK structure, thus hampering the binding of the TKI and therefore leading to resistance similar to that described for resistance to imatinib in chronic myeloid leukemia (CML) [76]. Recently, it has been shown that different FLT3 TKIs, e.g. midostaurin, sorafenib and SU5614, displayed distinct and non-overlapping resistance profiles in *FLT3*-ITD expressing cell lines [77]. Interestingly, the profile of resistance mutations emerging with SU5614 was limited to exchanges in the second part of the kinase domain, predominantly exchanges of D835. In contrast, midostaurin exclusively produced mutations within the tyrosine kinase domain 1 at codon N676 [77], which is in line with the findings reported by Heidel and colleagues [75]. Similarly, in a cell based model, saturation mutagenesis of *FLT3*-ITD followed by selection of transfected cells in FLT3 TKIs revealed a growing number of *FLT3* -TKD point mutations that are likely to contribute to TKI resistance [78]. Therefore, combinations of FLT3 inhibitors may be useful to prevent the induction of resistance mutations. Very recently, preliminary encouraging results were presented for the TKI crenolanib [79], which showed activity against platelet-derived growth factor receptor (PDGFR) and FLT3 and is currently being tested in clinical trials on solid tumors. Strikingly, crenolanib displayed cytotoxic activity against primary AML cells from patients who had developed D835 resistance mutations during FLT3 TKI treatment. *In vitro*, the 50% inhibitory concentration (IC₅₀) of crenolanib for inhibition of *FLT3*-ITD in plasma was 34 nM, and from pharmacokinetic studies of crenolanib in patients with solid tumors, steady state trough plasma levels of roughly 500 nM were found to be safe and tolerable [79]. A phase II study of crenolanib in relapsed or refractory patients with AML with *FLT3* -TKD mutations at codon D835 started recruitment quite recently (ClinicalTrials.gov Identifier: NCT01522469). Similarly, the multi-targeted kinase inhibitor ponatinib (AP24534), primarily created to inhibit BCR – ABL including the gatekeeper resistance mutation T315I [80], but also certain other tyrosine kinases including FLT3, showed

promising activity not only in *FLT3*-ITD cell lines but also in transfected subclones harboring additional *FLT3* -TKD point mutations (N676D, F691I or G697R) [81,82]. Additionally, there seems to be evidence of clinical activity in patients with *FLT3*-ITD positive AML: in a first phase I study on patients with relapsed or refractory AML the response rate was 43% ($n = 3/7$). All responses observed were in *FLT3*-ITD positive patients who were naive to FLT3 inhibitors [83]. Further evaluation of the activity of ponatinib in patients with AML with *FLT3*-ITD and/or acquired TKD resistance mutations may be warranted.

Besides ponatinib, the oral multi-targeted TKI PLX3397 showed potential activity against *FLT3*, including the *FLT3*-TKD point mutation F691; however, it conferred cross-resistance to other quizartinib-resistant *FLT3* -TKD point mutations, such as D835V/Y as well as Y842C/H [84]. Evaluation of the safety and efficacy of PLX3397 in a phase I/II study in adult patients with relapsed or refractory AML is currently ongoing (ClinicalTrials.gov Identifier: NCT01349049).

Another mechanism of resistance might be related to the *FLT3*-ITD insertion site, since an insertion site outside the JM domain of the FLT3 receptor was shown to be associated with rewired signaling, resulting in an up-regulation of the anti-apoptotic protein MCL-1 [20]. This resulted in a significant reduction of apoptosis as well as resistance toward treatment with midostaurin. Decreasing MCL-1 expression levels by small interfering RNA knockdown resulted in rescue of TKI-sensitivity, indicating the pivotal role of MCL-1 in mediating resistance [20]. Clinically, a *FLT3*-ITD insertion site within the β 1-sheet of the TKD1 was associated with an inferior outcome as compared to all other insertion sites, even after allogeneic HSCT [17,33]. Moreover, overexpression of survivin with concomitant activation of enhanced signaling pathways has been found to mediate resistance to FLT3 inhibition as well [85]. Enhanced signaling seems to be one mechanism to escape from inhibition of TKI treatment, since FLT3 TKI-resistant cell lines and primary samples showed continued activation of downstream PI3K/AKT and/or RAS/MEK/ MAPK signaling pathways as well as continued expression of genes involved in FLT3-mediated cellular transformation [86]. Inhibition of these downstream signaling pathways by PI3K or MEK inhibitors restored partial sensitivity to FLT3 TKIs [86]. Therefore, an approach combining FLT3 TKIs with anti-FLT3 antibodies and/or inhibitors of important pathways downstream of FLT3 may reduce the chances of developing resistance.

Stromal cells of the bone marrow environment may also contribute to resistance against TKIs due to a possible cyto-protective effect [87,88]. In a cell culture experiment, FLT3 inhibition did not induce apoptosis in *FLT3*-ITD AML blast cells after being cultured for several days on stroma [88]. Therefore, the bone marrow stroma may provide survival signals to *FLT3*-ITD AML blast cells, thus enabling survival despite the presence of potent FLT3 inhibition. ERK signaling was strongly activated in primary *FLT3*-ITD AML blast cells when the cells were co-cultured with stroma, and this activation was unaffected by FLT3 ligand or by FLT3 inhibition [88]. These observations may account for the difference in response to FLT3 inhibition observed between peripheral blood and bone marrow blast cells. Recent data suggest that a combination of FLT3 inhibitors and Janus kinase (JAK)

inhibitors might be effective in overriding stromal protection and potentiating FLT3 inhibition [89].

One further clinically relevant aspect may simply be related to the timing of TKI therapy in relation to conventional chemotherapy regimens. The sequence of combination of TKI with chemotherapy may be important, since *in vitro* studies indicate that the use of TKI prior to chemotherapy results in antagonism, whereas the use of TKI adjacent to chemotherapy results in synergistic effects [47]. Furthermore, it has been shown that FLT3 ligand (FL) levels increase dramatically following intensive chemotherapy [90]. On the one hand, such increased FL levels appear to shift the cytotoxicity IC₅₀ of FLT3 inhibitors upward by 2 – 4-fold; on the other hand, FL up-regulation immediately after chemotherapy may be an important driver of residual *FLT3*-mutant cells, particularly if the inhibitor is only given intermittently. Clinical responses to FLT3 TKIs appear to be dependent upon sustained inhibition, as determined by the plasma inhibitory activity for FLT3, but this is a difficult obstacle to overcome when the agents are administered immediately following chemotherapy, when FL levels are highest – and yet it may be the most important time period to try [91].

Conclusions

Our progress in unraveling the heterogeneity of AML has allowed us to identify a number of potential molecular targets such as *FLT3*, and has provided insight into the basis for the enormous diversity in the response to treatment. Complete and sustained inhibition of *FLT3*-mutated AML will probably require a combination of agents, both targeted and conventional chemotherapy. The ongoing investigations into the mechanisms of resistance to FLT3 inhibition will enable us to gain a deeper insight into the pathobiology of *FLT3*-ITD mutated AML and will probably continue to lead to the development of even more novel target-specific agents.

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Figure 1. Schematic structure of the *FLT3* receptor displaying the frequency of *FLT3*-ITD insertion sites in the juxtamembrane (JM) domain as well as in the tyrosine kinase (TK) domain 1 (according to [17]).

Table 1
Overview of preclinical characteristics of FLT3-tyrosine kinase inhibitors used in clinical trials.

TKI	Structural class	IC ₅₀ [*] (nM) (medium)	IC ₅₀ [*] (nM) (plasma)	T _{1/2} (plasma)	Additional targets
Midostaurin (PKC412)	Indolocarbazole alkaloid	6	1800	~24 h	cKIT, FMS, PDGFR
Lestauritinib (CEP-701)	Indolocarbazole alkaloid	2	700	8 – 12 h	JAK2, VEGFR, TrkA
Tandutinib (MLN-518)	Piperazinyl quinazoline	30	NA	25 – 29 h	cKIT, PDGFR
Sunitinib (SU11248)	Indolinone derivative	50	NA	40 – 86 h	cKIT, CSF, PDGFR, RET, VEGFR
Sorafenib (BAY 43-9006)	Bis-aryl urea derivative	3	265	25 – 48 h	cKIT, cRAF, FLT3 wt, PDGFR, VEGFR
Quizartinib (AC220)	Bis-aryl urea derivative	1	18	36 – 48 h	cKIT, FLT3 wt

AML, acute myeloid leukemia; cKIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; cRAF, cellular rapidly accelerated fibrosarcoma; CSF, colony stimulating factor; FLT3, FMS-like tyrosine kinase 3; IC₅₀, 50% inhibitory concentration; JAK2, janus kinase 2; NA, not available; PDGFR, platelet derived growth factor receptor; RET, rearranged during transfection; T_{1/2}, half-life; TKI, tyrosine kinase inhibitor; TrkA, tropomyosin-related kinase A; VEGFR, vascular endothelial growth factor receptor; wt, wild type.

* IC₅₀ indicates the concentration of drug that inhibits the autophosphorylation of FLT3 *in vitro* to 50% of its baseline value (according to [31,41,91,97]).

Table II

Overview on reported data from clinical trials investigating the efficacy of FLT3 tyrosine kinase inhibitor treatment.

TKI	Trial phase [reference]	Patient population	FLT3 status	Schedule	Clinical response
Midostaurin (PKC412)	Phase II [42]	<i>n</i> = 20, relapsed/refractory AML (<i>n</i> = 19/20) or advanced MDS (<i>n</i> = 1/20)	ITD <i>n</i> = 18; TKD <i>n</i> = 2	Oral, single agent, 75 mg, 3X/day	PB blasts < 50%; in 70% of patients; BM blasts < 50%; in 30% of patients
Midostaurin (PKC412)	Phase IIb [92]	<i>n</i> = 95, relapsed/refractory AML or newly diagnosed unfit for intensive CTX (<i>n</i> = 85/95); high-risk MDS (<i>n</i> = 10/95)	ITD <i>n</i> = 26; TKD <i>n</i> = 9; wt <i>n</i> = 60	Oral, single agent, randomized, 50 or 100 mg, 2X/day	PB or BM blasts < 50%; in 71% of FLT3 mutated patients and in 42% of wt patients
Midostaurin (PKC412)	Phase Ib [43]	<i>n</i> = 69, newly diagnosed AML, age 18–60 years (<i>n</i> = 40/69 patients: 50 mg dose schedule; <i>n</i> = 29 patients: 100 mg dose schedule)	FLT3 mutated, <i>n</i> = 19; wt, <i>n</i> = 50; evaluable patients: FLT3 mutated, <i>n</i> = 13; wt, <i>n</i> = 27	Oral, 50 or 100 mg, 2X/day, either concomitantly (days 1–7 and 15–21) or sequentially (days 8–21) with CTX: induction: daunorubicin 60 mg/m ² i.v. on days 1–3 and cytarabine 200 mg/m ² continuously i.v. on days 1–7; consolidation: 3 cycles AraC 3 g/m ² i.v. every 12 h (days 1, 3, 5)	FLT3 wt: 74% CR, 78% 1-year OS, 52% 2-year OS; FLT3 mutated: 92% CR, 85% 1-year OS, 62% 2-year OS
Lestauritinib (CEP-701)	Phase I/II [44]	<i>n</i> = 17, relapsed/refractory AML, age 18–74 years (median: 61 years)	ITD, <i>n</i> = 16; TKD, <i>n</i> = 1	Oral monotherapy 40 mg 2X/day in a 28-day cycle, dose escalation to 80 mg 2X/day	6% CR; 24% PB blast clearance; short response duration (2 weeks to 3 months)
Lestauritinib (CEP-701)	Phase II [45]	<i>n</i> = 29, newly diagnosed AML, age > 70 years (or 60–70 years with comorbidity)	ITD, <i>n</i> = 2; TKD, <i>n</i> = 3; wt, <i>n</i> = 24	Oral monotherapy, 60 mg 2X/day, escalating to 80 mg 2X/day	PB blast clearance or at least PR: in 60% of FLT3 mutant and in 23% of wt patients
Lestauritinib (CEP-701)	Phase III [46]	<i>n</i> = 224, patients with AML in first relapse, age 20–81 years	ITD, <i>n</i> = 198; TKD, <i>n</i> = 17; both, <i>n</i> = 8; not confirmed <i>n</i> = 1	Randomized with (<i>n</i> = 112) or without (<i>n</i> = 112) lestauritinib; oral 80 mg 2X/day in combination with CTX: either mitoxantrone 8 mg/m ² , etoposide 100 mg/m ² , AraC 1 g/m ² days 1–5, if 1st CR < 6 months or AraC 1.5 g/m ² days 1–5, if 1st CR lasted from 6 to 24 months	CTX + lestauritinib: 26% CR; CTX only: 21% CR (no difference in OS between 2 treatment arms)
Tandutinib (MLN-518)	Phase I [93]	<i>n</i> = 40, relapsed/refractory AML or AML unfit for intensive CTX (<i>n</i> = 39) as well as high-risk MDS (<i>n</i> = 1), age > 18 years	ITD, <i>n</i> = 8; wt, <i>n</i> = 32	Oral monotherapy, dose escalation from 50 mg to 700 mg 2X/day	No CR or PR; 25% antileukemic effect (at 525 mg and 700 mg 2X/day, respectively)
Tandutinib (MLN-518)	Phase II [94]	<i>n</i> = 20, relapsed/refractory AML or AML unfit for intensive CTX	ITD, <i>n</i> = 20	Oral monotherapy, 525 mg 2X/day	No CR or PR; 30% antileukemic effect

TKI	Trial phase [reference]	Patient population	FLT3 status	Schedule	Clinical response
Tandutinib (MLN-518)	Phase I/II [95]	<i>n</i> = 29, newly diagnosed AML, age 26–83 years	ITD, <i>n</i> = 5; wt, <i>n</i> = 24	Oral 200 mg 2×/day (continuously or days 1–14) or 500 mg 2×/day in combination with CTX: induction: AraC 200 mg/m ² , days 1–7, + daunorubicin 60 mg/m ² , days 1–3; consolidation: 2–4 cycles AraC 3 g/m ² every 12 h, days 1, 3, 5	73% CR; not subsequently reported
Sunitinib(SU11248)	Phase I [48]	<i>n</i> = 29, AML (unrestricted)	ITD, <i>n</i> = 3; TKD, <i>n</i> = 2; wt, <i>n</i> = 24	Oral, 50–350 mg (dose escalation, single dose)	Translational study; strong inhibition of FLT3 phosphorylation in > 50% of patients (with 200 mg)
Sunitinib(SU11248)	Phase I [49]	<i>n</i> = 15, relapsed/refractory AML, unfit for intensive CTX	ITD, <i>n</i> = 2; TKD, <i>n</i> = 2; wt, <i>n</i> = 10; undetermined, <i>n</i> = 1	Oral monotherapy, 50–75 mg	PR or antileukemic effect: in 100% of FLT3 patients, in 20% of wt patients; short response duration (4–16 weeks)
Sunitinib(SU11248)	Phase I/II [50]	<i>n</i> = 22, newly diagnosed AML, fit for intensive therapy, age 60–78 years	ITD, <i>n</i> = 15; TKD, <i>n</i> = 7	Sunitinib 25 mg, oral in combination with: induction: cytarabine 100 mg/m ² days 1–7, daunorubicin 60 mg/m ² days 1–3; consolidation: 3 cycles cytarabine 1 g/m ² i.v. bid, days 1, 3, 5	59% CR, 4.5% PR (CR in ITD: 53%, CR in TKD: 71%); median OS: 18.8 months, median RFS: 11 months; 2-year OS: 36%
Sorafenib (BAY 43–9006)	Phase I [51]	<i>n</i> = 16, relapsed/refractory AML, age 48–81 years	ITD, <i>n</i> = 6; TKD, <i>n</i> = 3; wt, <i>n</i> = 7	Oral monotherapy, dose escalation, 20–60 mg 2×/day	PB and BM blast cell decrease: in 100% of ITD patients, in 43% of wt patients
Sorafenib (BAY 4–006)	Phase I [52]	<i>n</i> = 65, relapsed/refractory AML; two cohorts: (a) <i>n</i> = 29 patients after allogeneic HSCT, (b) <i>n</i> = 36 patients after intensive CTX	ITD, <i>n</i> = 65	Oral monotherapy, 400 mg 2×/day	37% PB remission, 8% CRi, 23% CR, 15% molecular remission
Sorafenib (BAY 4–006)	Phase I [53]	<i>n</i> = 15, relapsed/refractory acute leukemia (AML, <i>n</i> = 12; ALL, <i>n</i> = 2, biphonotypic, <i>n</i> = 1), age 3–5 years	ITD, <i>n</i> = 2	Oral monotherapy, dose escalation, MTD 400 mg 2×/day	73% SD
Sorafenib (BAY 4–006)	Phase I/II [54]	<i>n</i> = 61, phase I: relapsed/refractory (<i>n</i> = 10); phase II: newly diagnosed (<i>n</i> = 51); age 1–5 years	ITD, <i>n</i> = 20; TKD, <i>n</i> = 2; wt, <i>n</i> = 39	Oral, 400 mg 2×/day, days – in combination with CTX: AraC 1.5 g/m ² days –, idarubicin 12 mg/m ² days –	CR/CRp: in 93% of ITD, in 75% of wt patients; 1-year OS: 74%
Sorafenib (BAY 4–006)	Phase II [55]	<i>n</i> = 197, newly diagnosed AML, age > 60 years	ITD, <i>n</i> = 28	Randomized, placebo-controlled, 400 mg 2×/day in combination with CTX: induction: AraC/daunorubicin (“7 + 3”), consolidation: 2 cycles AraC 1 g/m ² ; 1 year sorafenib maintenance	CR/CRi: in 48%/9% with sorafenib, in 60%/4% with placebo, respectively; no difference in EFS and OS between placebo and sorafenib treatment cohort
Sorafenib (BAY 4–006)	[58]	<i>n</i> = 16, relapsed after HSCT, age 2–3 years (median 34 years); retrospective chart review, two treatment cohorts	ITD, <i>n</i> = 16	Cohort I: oral sorafenib as single agent 2×/day 400 mg (<i>n</i> = 6), or 600 mg (<i>n</i> = 2) on a 3-week cycle, either 5 days on therapy and 2 days off	81% PB blast cell decrease; 19% PR; 13% bridge to 2nd HSCT; median OS: 83 days

TKI	Trial phase [reference]	Patient population	FLT3 status	Schedule	Clinical response
Quizartinib (AC220)	Phase I [60]	<i>n</i> = 76, relapsed/refractory AML, unselected for FLT3 mutations, age 2–6 years	ITD, <i>n</i> = 18; wt, <i>n</i> = 45; undetermined, <i>n</i> = 13	weekly, or 14 days on and 7 days off; cohort II: combined with CTX: sorafenib 400 mg/day (<i>n</i> = 4) or 800 mg 2x/day (<i>n</i> = 4) with AraC and idarubicin (<i>n</i> = 7), or azacitidine	PR + CR: ITD: 56%, wt: 20%; (cCR: ITD: 28%; wt: 7%); median response duration: 14 weeks
Quizartinib (AC220)	Phase II [61]	Cohort I, <i>n</i> = 133, 60 years, 1st relapse within 1 year, or refractory to 1st line treatment	ITD, <i>n</i> = 90; wt, <i>n</i> = 42	Oral solution, monotherapy, 135 mg/day for men, 90 mg/day for women	cCR: ITD: 54%, wt: 31%; median response duration: 12.1 weeks for ITD and 22.1 weeks for wt patients
Quizartinib (AC220)	Phase II [62]	Cohort II, <i>n</i> = 138, 18 years, relapsed after, or refractory to 2nd line treatment or HSCT	ITD, <i>n</i> = 100; wt, <i>n</i> = 38	Oral solution, monotherapy, 135 mg/day for men, 90 mg/day for women	cCR: ITD: 46%, wt: 32%, median response duration: 12.1 weeks for ITD and 7.0 weeks for wt patients; 37% of patients bridged to HSCT

ALL, acute lymphatic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CR, complete remission; cCR, composite complete remission, defined as the combination of CR, CR with incomplete platelet recovery, and CR with incomplete hematologic recovery; CTX, chemotherapy; FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; MDS, myelodysplastic syndrome; MRC, Medical Research Council; MTD, maximum tolerated dose; OS, overall survival; PB, peripheral blood; RFS, relapse-free survival; SD, stable disease; TKD, tyrosine kinase domain; TKI, tyrosine kinase inhibitor; wt, wild type.

Table III

Overview of currently ongoing clinical trials evaluating FLT3-tyrosine kinase inhibitor treatment in acute myeloid leukemia.

TKI	Title	Trial phase	Patient population	Schedule	Trial reference
Midostaurin (PKC412)	Phase II study evaluating midostaurin in induction, consolidation and maintenance therapy also after allogeneic blood stem cell transplant in patients with newly diagnosed acute myeloid leukemia exhibiting <i>FLT3</i> internal tandem duplication	Phase II	<i>n</i> = 142, newly diagnosed patients with AML, <i>FLT3</i> -ITD positive, multicenter, age 1–0 years	Induction: AraC 200 mg/m ² /day, days 1–7; daunorubicin 60 mg/m ² /day, days 1–3; midostaurin: 50 mg, oral, twice daily; consolidation: 1st priority: allogeneic HSCT, 2nd priority: aged-adapted AraC 3 g/m ² /day, days 1, 3, 5 with midostaurin 50 mg, oral twice daily, followed by 1 year maintenance with midostaurin 50 mg twice daily	NCT01477606; recruiting
Midostaurin (PKC412)	Phase III randomized, double-blind study of induction (daunorubicin/cytarabine) and consolidation (high-dose cytarabine) chemotherapy + midostaurin or placebo in newly diagnosed patients < 60 years of age with <i>FLT3</i> mutated AML	Phase III	<i>n</i> = 714, newly diagnosed patients with AML, <i>FLT3</i> mutated, international, age 1–0 years	Induction: AraC 200 mg/m ² /day, days –; daunorubicin 60 mg/m ² /day, days –; consolidation: AraC 3 g/m ² /day, days 1, 3, 5 either with midostaurin or placebo, followed by 12 cycles of maintenance with midostaurin/placebo	NCT00651261; ongoing, not recruiting
Midostaurin (PKC412)	Combination of PKC412 and 5-azacytidine for the treatment of patients with refractory or relapsed acute leukemia and MDS	Phase I/II	<i>n</i> = 54, refractory/relapsed MDS, CMML or AML, age 18 years	5-Azacytidine 75 mg/m ² /day subcutaneously or by vein on days – of a 28-day cycle; midostaurin 25 mg, oral, twice daily for 14 days (days –1), of every 28-day cycle	NCT01202877; recruiting
Lestaurtinib (CEP-701)	MRC AML 15: a trial of directed therapy in younger patients with AML	Phase III	Newly diagnosed patients with AML, <i>FLT3</i> mutated, age 1–0 years	Induction: AraC plus daunorubicin, etoposide; consolidation: 2 cycles HD-AraC or MACE plus MidAC; lestaurtinib for <i>FLT3</i> -mutated patients	ISRCTN 17161961; recruitment completed (12/200–1/2009) (first results according to [90])
Lestaurtinib (CEP-701)	MRC AML 17: randomized, controlled, open label phase III trial for patients with AML and high-risk MDS	Phase III	Newly diagnosed patients with AML, <i>FLT3</i> mutated, age 1–0 years	Induction: 2 cycles AraC, daunorubicin and etoposide ± mylotarg; consolidation: 1 cycle MACE ± MidAC; lestaurtinib for <i>FLT3</i> -mutated patients	ISRCTN 55675535; recruitment from 04/2009 onward (first results according to [96])
Sorafenib (BAY 4–006)	Phase I trial of sorafenib maintenance therapy for patients with <i>FLT3</i> -ITD AML after allogeneic HSCT	Phase I	<i>n</i> = 28, <i>FLT3</i> -ITD mutated patients with AML after allogeneic HSCT, age 1–5 years	Oral, monotherapy, 20–00 mg QD or BID, duration of maintenance after allogeneic HSCT max. 3 years	NCT01398501; recruiting
Sorafenib (BAY 4–006)	G-CSF and plerixafor with sorafenib for AML with <i>FLT3</i> mutations	Phase I	<i>n</i> = 28, patients with relapsed/refractory AML with <i>FLT3</i> -ITD mutations, age 18 years	Oral, 400 mg twice daily, dose-escalation, in combination with G-CSF and plerixafor 240 µg/kg subcutaneous injection, days –, repeated every 28 days	NCT00943943; recruiting

TKI	Title	Trial phase	Patient population	Schedule	Trial reference
Sorafenib (BAY 4-006)	Phase II study incorporating sorafenib into therapy of patients a 60 years of age with <i>FLT3</i> mutated AML	Phase II	<i>n</i> = 49, newly diagnosed patients with <i>FLT3</i> mutated AML, age a 60 years	Oral sorafenib in combination with CTX: induction: daunorubicin i.v. days –, AraC i.v. continuously days – and sorafenib twice daily on days –; consolidation: 2 cycles AraC i.v. days – and sorafenib twice daily on days –8, repeated every 28 days; maintenance: sorafenib twice daily for 1 year	NCT01253070; recruiting
Sorafenib (BAY 4-006)	Pilot study of sorafenib in patients with AML as peri-transplant remission maintenance	Phase I	<i>n</i> = 36, patients with <i>FLT3</i> -ITD mutated AML, eligible to undergo a bone marrow transplant, age 18 years	Oral sorafenib twice daily, 200 mg (dose adoption possible) pre-transplant; maintenance: sorafenib 20–00 mg twice daily continuously after BM transplant for up to 2 years	NCT01578109; recruiting
Quizartinib (AC220)	Phase II, randomized, open-label study of safety and efficacy of two doses of quizartinib in subjects with <i>FLT3</i> -ITD positive relapsed or refractory AML	Phase II	<i>n</i> = 64, patients with relapsed or refractory AML, <i>FLT3</i> -ITD mutated, age 18 years	Oral, monotherapy, equally randomized between 30 or 60 mg per day; dose adoption according to toxicity and response	NCT01565668; recruiting
Quizartinib (AC220)	Phase I study of AC220 in combination with induction and consolidation chemotherapy in patients with newly diagnosed AML	Phase I	<i>n</i> = 58, newly diagnosed patients with AML, <i>FLT3</i> -ITD and <i>FLT3</i> wt are eligible, age 1–0 years	Induction: escalating doses of quizartinib + standard 7 + 3 cytarabine and daunorubicin; consolidation: quizartinib + 3 cycles high dose cytarabine; maintenance: quizartinib alone for up to 1 year	NCT01390337; recruiting
Quizartinib (AC220)	Phase I study of quizartinib as maintenance therapy in subjects with AML who have been treated with allogeneic hematopoietic stem cell transplant	Phase I	<i>n</i> = 30, patients with AML after allogeneic HSCT, performed in first or second remission	2 Years maintenance with quizartinib in patients after allogeneic HSCT	NCT01468467; recruiting

AML, acute myeloid leukemia; BM, bone marrow; CR, complete remission; CTX, chemotherapy; *FLT3*, FMS-like tyrosine kinase 3; G-CSF, granulocyte colony stimulating factor; HSCT, hematopoietic stem cell transplant; ITD, internal tandem duplication; i.v., intravenously; MDS, myelodysplastic syndrome; MRC, Medical Research Council; OS, overall survival; PB, peripheral blood; TKD, tyrosine kinase domain; TKI, tyrosine kinase inhibitor; wt, wild type.

Table IV

Outcome after hematopoietic stem cell transplant in *FLT3-YID* AML.

Reference	Treatment	No. of <i>FLT3</i> ITD patients	RR(%)	RFS (%)	OS (%)	Comments
63	Allo-HSCT (MRD) [†] ; CTX	60 [‡] 148 [‡]	–	(5 years) 34 [‡] ; 18 [‡]	No statistically significant differences	Intention to treat analysis; [‡] combined analyses of patients with <i>FLT3</i> -ITD and genotype “ <i>NPM1</i> wt, <i>CEBPA</i> wt, <i>FLT3</i> -ITD negative”
64	Allo-HSCT (MRD and MUD)	120	30 (2 years)	–	–	As-treated analysis, survey of patients after allo-HSCT
65	Allo-HSCT (all donor types); CTX	11; 9		Median: 54.1 months; 8.6 months	35 [‡] (5 years); 23 [‡] (3 years)	As-treated analysis, single-center experience; patients after allo-HSCT as compared to historical controls
66	Allo-HSCT (MRD) [†] ; auto-HSCT; CTX	40; 46; 38	(5 years) 35 [‡] ; 53 [‡] ; 95 [‡]	–	(5 years) 58 [‡] ; 48 [‡] ; 20 [‡]	As-treated analysis, therapy allocated by prioritization, not randomized
69	Allo-HSCT (MRD) [†] ; auto-HSCT	35; 37	(5 years) 31; 56	–	(5 years) 44; 43	Intention to treat analysis
67	RIC allo-HSCT (all donor types); CTX	37; 29	(3 years) 25; 61	–	(3 years) 52; 44	As-treated analysis, patients after allo-HSCT as compared to historical controls
68	Allo-HSCT (MRD); CTX	13; 20	–	–	Median OS: 42.5 weeks; 29.6 weeks	As-treated analysis, patients after allo-HSCT as compared to historical controls
70	Allo-HSCT (all donor types)	16	59 (1 year)	–	–	As-treated analysis, single-center experience; survey of patients after allo-HSCT

allo, allogeneic; AML, acute myeloid leukemia; auto, autologous; CTX, chemotherapy; HSCT, hematopoietic stem cell transplant; ITD, internal tandem duplication; MRD, matched related donor; MUD, matched unrelated donor; OS, overall survival; RFS, relapse-free survival; RIC, reduced-intensity conditioning; RR, relapse risk.

[‡] With respect to allo-HSCT genetic randomization by donor availability.

[†] Estimated from Kaplan-Meier curves.