Targeting the FLT3 Mutation in Acute Myeloid Leukaemia

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Acute myeloid leukaemia (AML) exhibiting an internal tandem duplication of the FLT3 gene (FLT3-ITD) is an aggressive haematologic malignancy with a poor prognosis due to a high relapse rate and very limited options after relapse with conventional salvage regimens, whereas the prognostic impact of point mutations in the tyrosine kinase domain of the FLT3 gene (FLT3-TKD) are less clear. A number of tyrosine kinase inhibitors (TKIs) have been developed that inhibit the constitutively activated kinase activity caused by the FLT3 mutation, thus interrupting signalling pathways. Early clinical trials of these agents as monotherapy failed to elicit enduring complete responses, leading to clinical testing of FLT3 TKI in combination with conventional chemotherapy. Midostaurin has demonstrated improved survival in combination with standard intensive chemotherapy as compared to standard chemotherapy alone in younger adult patients with newly diagnosed FLT3-mutated AML and is the first and currently the only approved FLT3 TKI. Newer, more selective compounds, such as gilteritinib and crenolanib, have also demonstrated significant potency and specificity. Several combination trials are ongoing or planned in both relapsed and newly diagnosed AML patients with activating FLT3 mutations.

Keywords

Acute myeloid leukaemia, FLT3 mutations, tyrosine kinase inhibitors, intensive chemotherapy, allogeneic stem cell transplantation

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Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder of haematopoietic progenitor cells resulting in uncontrolled growth and accumulation of malignant white blood cells. It is the most common myeloid leukaemia in adults, with a prevalence of 3–8 cases per 100,000 adults rising to 9–17 cases per 100,000 adults aged 65 years and older. The median age at presentation is about 70 years.¹ AML affects both male as well as female patients with a slight predominance of the male gender (m/f: 3:2). According to the American Cancer Society, AML accounted for approximately 33% of all new leukaemia cases in the United States in 2016. Almost 20,000 patients had been newly diagnosed with AML in 2016 in the United States and over 10,000 died of the disease (www.cancer.org). The median overall survival (OS) after 5 years in younger (18–60 years) adult AML patients is roughly 40% with the disease being even more detrimental in older individuals with only around 10% surviving patients above the age of 60 years.² Hence, there is a high medical need to improve the outcome of AML patients.

The prognosis for patients with AML is determined to a large degree by the biology of the disease. In recent years, the identification and characterisation of genetic aberrations has vastly improved our understanding of the pathogenesis of AML. These genetic alterations allow for the stratification of patient populations into different risk groups, thus guiding treatment. Based on the currently updated version of the European LeukemiaNet (ELN) risk stratification by genetics, the risk groups consist of the favourable, intermediate and adverse risk categories (Table 1).³,⁴ AML with normal karyotype (accounting for roughly 50% of the patients) can be categorised according to molecular abnormalities. Of these, the most frequently affected gene mutations are NPM1 and FLT3.⁵

Despite the fact that AML is a clinically and genetically heterogeneous disease, until recently most patients have been treated by similar chemotherapeutic regimens.⁶ To date, the only approved targeted therapies for patients with AML are all-trans retinoic acid (ATRA) and arsenic trioxide⁷ for acute promyelocytic leukaemia (APL), which accounts for 10–15% of AML cases.⁸ There is a clear need for more targeted therapies and a more individualised approach in the treatment of AML. However, in the last decade the treatment options for AML have expanded as a result of the discovery of cytogenetic abnormalities as well as molecular mutations, but only two new nontargeted drugs have been approved in the EU in this period. This article discusses differences in the FLT3 gene, as well as the therapeutic interventions targeting these mutations.
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Table 1: 2017 European LeukemiaNet risk stratification by genetics

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>Genetic Abnormality</th>
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<tbody>
<tr>
<td>Favourable</td>
<td>t(8;21)(q22;q22.1); RUNX1-RUNX1T1</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13.1;q22) or t(16;1p13.1;q22); CBFβ-MYH11</td>
</tr>
<tr>
<td></td>
<td>Mutated NPM1 without FLT3-ITD or with FLT3-ITD low*</td>
</tr>
<tr>
<td></td>
<td>Bi-allelic mutated CEBPA</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Mutated NPM1 and FLT3-ITD high*</td>
</tr>
<tr>
<td></td>
<td>Wild type NPM1 without FLT3-ITD or with FLT3-ITD low* (w/o adverse risk genetic lesions)</td>
</tr>
<tr>
<td></td>
<td>t(9;11)(p21.3;q23.3); MLL3-KMT2A**</td>
</tr>
<tr>
<td></td>
<td>Cyto genetic abnormalities not classified as favourable or adverse</td>
</tr>
<tr>
<td>Adverse</td>
<td>t(6;9)(p23;q34.1); DDX5-WUP124</td>
</tr>
<tr>
<td></td>
<td>t(11q23.3); KMT2A rearranged</td>
</tr>
<tr>
<td></td>
<td>t(2;21)(p11.2;q22); BCR-ABL1</td>
</tr>
<tr>
<td></td>
<td>inv(3)(q26.2) or t(3;3)(q26.2); GATA2, MECOM, ETV6</td>
</tr>
<tr>
<td></td>
<td>-5 or del(5q); -7; -17/18(17p)</td>
</tr>
<tr>
<td></td>
<td>Complex karyotype;# monosomal karyotype++</td>
</tr>
<tr>
<td></td>
<td>Wild type NPM1 and FLT3-ITD high*</td>
</tr>
<tr>
<td></td>
<td>Mutated RUNX1***</td>
</tr>
<tr>
<td></td>
<td>Mutated ASXL1****</td>
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<tr>
<td></td>
<td>Mutated TP53***</td>
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</table>

*Low, low allelic ratio (<0.5); high, high allelic ratio (>0.5); as determined by GeneScan analysis. **The presence of t(11q23.3) takes precedence over rare, concurrent adverse-risk gene mutations. ***Three or more unrelated chromosome abnormalities in the absence of one of the World Health Organization-designated recurring translocations or inversions, i.e., t(8;21), inv(16) or t(15;17), t(9;11), t(4;11), t(12;21), t(8;14), t(8;16), t(9;19), t(9;22), t(1;19), t(9;22), t(11;19) or t(16;16). Neural tube defects are not included for these purposes. ****These markers should not be used as an adverse prognostic marker if they co-occur with favourable-risk AML subtypes. #TP53 mutations are significantly associated with AML with complex and monosomal karyotype.##

Activating mutations in acute myeloid leukaemia

A number of cytogenetic abnormalities, mutations or epigenetic alterations, which are involved in the pathogenesis of leukaemia, have been identified (Figure 1).13

Activating mutations of FLT3, resulting in the constitutive activation of this receptor tyrosine kinase, are among the most frequent molecular abnormalities in AML and are present in about 30% of newly diagnosed patients.11,12 FLT3 is a member of the class III receptor tyrosine kinase family and has an established role in normal growth and differentiation of haematopoietic precursor cells.13 Following ligand binding, the FLT3 receptor dimerizes at the plasma membrane, leading to a conformational change in its activation loop that allows adenosine triphosphate (ATP) receptor dimerization at the plasma membrane, leading to a conformational change in its activation loop that allows adenosine triphosphate (ATP) access to the FLT3 active site. This is followed by autophosphorylation of haematopoietic precursor cells.16 Following ligand binding, the FLT3 gene lead to ligand-independent activation and STAT5.20–22 These pathways inhibit apoptosis and differentiation, and promote proliferation. The frequency of mutated FLT3 in AML, its location on the cell surface and its association with an adverse prognosis make it an attractive target.22

Internal tandem duplications

The most common FLT3 mutations are internal tandem duplications (ITDs), which occur in roughly 20–30% of all AML patients, particularly, not only in cytogenetically normal AML,10,11 but also in APL with t(15;17) (q22;q12) and AML with t(6;9)(p23;q34). Its incidence is associated with age: it can only be rarely found in children, whereas its incidence is highest in young adults up to the age of 60 years and declines in the elderly.24 Clinically, FLT3-ITD mutations are associated with a high white blood cell count, a high percentage of myeloid blast cells in the peripheral blood and bone marrow, and a more frequent diagnosis of de novo rather than secondary AML.25,26

In cytogenetically normal AML the presence of an ITD is associated with an increased relapse rate and reduced OS as compared to wild type FLT even after allogeneic haematopoietic stem cell transplantation (allo HSCT).27,28 FLT3-ITD is also a negative prognostic factor for survival in patients with either refractory or relapsed AML as they have a poor response to salvage therapy.29–32

Regarding specific ITD characteristics, the size of these duplications varies widely, typically ranging from 3 to over 100 base pairs (bps) with a median of 48 bps.33 In addition, size and ITD insertion site in the FLT3 gene seem to be correlated in that the more 3s the insertion site, the longer the ITD is.34 The impact of the size on outcome is still unclear with some publications stating that there is no impact on outcome,35,36 whereas one publication found that short ITDs may impart an unfavourable outcome.37 Nevertheless, most publications stated that longer ITDs correlate with lower complete response (CR) rates and shorter OS and event-free survival (EFS).38–40

Recently, the ITD insertion site within the FLT3 gene has been shown to be an important prognostic factor.41 About three-quarters of FLT3-ITDs occur in the juxtamembrane domain (JMD) whereas one-quarter in the tyrosine kinase domain 1 (TKD1) of the FLT3 gene, particularly in the β1-sheet.42 In cell culture analyses, a prototypic FLT3-ITD with insertion site in the β2-sheet of the TKD1 (FLT3-ITD627E) mediated phosphorylation of FLT3 and STAT5, suggesting that non-JMD FLT3-ITD mutations confer constitutive activation of the receptor.43 In addition, FLT3-ITD627E induced transformation of haematopoietic 32D cells and led to a lethal myeloproliferative disease in a syngeneic mouse model. Insertions in the β1-sheet of TKD1 may introduce a greater instability into the protein structure and may therefore be associated with a pronounced adverse prognosis.44 In DNA-based Sanger sequencing analysis of diagnostic samples from 241 FLT3-ITD mutated patients, an ITD insertion site in the β1-sheet of the TKD1 was associated with an inferior prognosis as compared to other insertions sites in terms of achievement of complete remission (CR; odds ratio [OR], 0.22; p=0.01), relapse-free survival (RFS; hazard ratio [HR], 1.86; p=0.001) and OS (HR, 1.59; p=0.008).31

Besides the insertion site, further prognostic and predictive impact has been shown for the allelic ratio, which is quantified by GeneScan analysis using DNA fragment analysis.45,46 This method is a semi-quantitative
assessments of the FLT3-ITD allelic ratio, expressing the allelic ratio as a percentage of the area under the curve for FLT3-ITD divided by the area under the curve for wild-type FLT3. According to different publications the distribution of the allelic ratio varies widely. Therefore, the question arises where the optimal cut-off value should be to distinguish patients with high versus low allelic ratio. Currently, there is still a lack of consensus on clinically relevant cut-offs between high/low allelic ratios, which might be due to different methodologies of testing that had been applied. Nevertheless, there is a clear association of an inferior OS and EFS in patients with higher allelic ratios. In addition, as AML evolves from diagnosis to relapse, the allelic ratio seems to increase. However, in a small proportion of relapses FLT3-ITD was no longer detectable.

### Point mutations of the tyrosine kinase domain

In addition, approximately 5–10% of AML patients harbour point mutations within exon 20 of the FLT3 gene (FLT3-TKD). TKD mutations most frequently occur at codon 835 where a tyrosine residue replaces aspartic acid, stabilising the activation loop in the ATP-bound configuration and promoting activation. Other point mutations include, for instance, N676D, I836S and Y842C in the TKD1 and TKD2 domains, respectively. Unlike ITDs, the incidence of point mutations is not associated with age and their prognostic significance is discussed controversially. Nevertheless, TKD mutations can occur after treatment with tyrosine kinase inhibitor (TKI) as a mechanism of resistance, thus implicating an adverse prognosis.

### Concurrent mutations

The prognostic impact of FLT3-ITD is also affected by concurrent mutations, such as nucleophosmin 1 (NPM1) and DNMT3A. In normal karyotype AML (CN-AML) with NPM1 mutation, FLT3-ITDs are present in about 45% of patients. Mutations in exon 12 of the NPM1 gene cause cytoplasmic dislocation of the NPM1 protein. As a result, cytoplasmic NPM1 is unable to undertake its normal functions as binding and transporter protein. In CN-AML, NPM1 mutations without FLT3-ITDS9 or a low allelic ratio are a more favourable prognostic factor.

The prognostic effect of concurrent FLT3 and NPM1 mutations is a matter of controversy. A cohort study of young adult AML patients identified three prognostic groups: good (FLT3-ITD−[NPM1+]), intermediate (FLT3-ITD+[NPM1−]) or FLT3-ITD+[NPM1−] and poor (FLT3-ITD+[NPM1−]). The authors concluded that patients with an FLT3-ITD mutation burden greater than 50% or (FLT3-ITD+[NPM1−]) have a poor prognosis and may be good candidates for experimental therapeutic approaches. However, another study found that the FLT3-ITD load also has to be taken into account: in patients with a high FLT3-ITD allelic burden, the effect of an NPM1 mutation was less important. A study of older AML patients suggested that NPM1(+)/FLT3-ITD(−) confers a favourable prognosis for patients with AML of ages 55–65 years but not in those of age >65 years. Recent recommendations from the ELN include a revised version of the risk stratification according to genetics including the allelic ratio (Table 1).

### Detection of minimal residual disease

Pretherapeutic molecular testing for NPM1 and FLT3 is considered a standard of care to determine the best treatment option. Whereas NPM1 has been shown to be a reliable marker for minimal residual disease (MRD) detection with high sensitivity, the suitability of FLT3-ITD for MRD detection has been questioned. First, FLT3-ITD mutations display substantial heterogeneity in terms of size, number of clones per patient, allelic ratio and insertion site within the FLT3 gene and second, its proposed instability (reported on about 25% of paired diagnosis-relapse samples) during the course of treatment.

### Treatment options for FLT3-ITD acute myeloid leukaemia

In younger patients with newly diagnosed AML considered suitable for intensive induction therapy, the combination of an anthracycline and cytarabine (“7+3” regimen) remains the standard of care also for patients with activating FLT3 mutations. However, higher allelic ratios were associated with lower CR-rates after induction therapy bringing up the question of dose-intensification in these patients. In older patients a substantial proportion of patients cannot tolerate intensive induction chemotherapy, in these cases other less intensive regimens may be used including low-dose cytarabine or hypomethylating agents (e.g., azacitidine or decitabine). Based on the assessment of the risk-benefit ratio (i.e., non-relapse mortality/morbidity versus reduction of relapse risk) allo HSCT from matched-related or unrelated donor in early first CR is the treatment option for patients with intermediate and adverse risk genetics. In addition, allo HSCT has been shown to improve outcomes in FLT3-ITD AML, particularly in patients with a high allelic ratio. Nevertheless, recent studies indicate that AML patients with NPM1 mutation and low FLT3-ITD allelic ratio may have a more favourable prognosis and should therefore not routinely be assigned to allo HSCT. In contrast, an ITD insertion site in the TKD1 remained an unfavourable prognostic factor regardless of the applied therapy. Another important prognostic factor has been shown for the NPM1 MRD status after the second chemotherapy or before allo HSCT.

### New therapies targeting FLT3

In the last decade, numerous small molecule FLT3-TKIs have been developed to disrupt oncogenic signalling. Most compete for the ATP binding site in the active domain of the kinase, inhibiting protein phosphorylation. Early TKIs, rather than being specifically designed to target FLT3, had multiple targets including KIT, PDGFR, VEGFR and Jak2. Several agents have shown evidence of modest antileukemic activity as monotherapy...
Results of clinical trials with tyrosine kinase inhibitor treatment

The major clinical studies investigating TKI treatment in FLT3-mutated AML are summarised in Table 2. In a phase I/II study, monotherapy with lestaurtinib demonstrated biologic and clinical activity in five out of 14 heavily pretreated patients with relapsed or refractory FLT3-mutated AML, including reductions of blast cells from bone marrow and peripheral blood. In addition, in a phase II trial, single agent lestaurtinib has shown modest activity as first-line treatment for older AML patients who were unfit for intensive chemotherapy. Within this trial, lestaurtinib was given orally for 8 weeks, starting with 60 mg twice daily (bid), escalating to 80 mg bid, and was generally well tolerated. Clinical activity included transient reductions in bone marrow and peripheral blast cells in three of five patients with mutated FLT3 and five of 22 evaluable wild-type FLT3 patients. In both studies, FLT3 inhibition correlated with clinical response. These findings prompted a large, multicentre phase III clinical trial evaluating lestaurtinib in combination with chemotherapy in relapsed/refractory patients. However, no increase in response rates or prolongation of survival of AML patients with activating FLT3 mutations was found. In addition, it has been shown that plasma FLT3 ligand levels rise dramatically after chemotherapy and this has been suggested to interfere with the bioavailability of FLT3 TKIs. This issue has been evaluated in a meta-analysis of two consecutive phase III trials of the Medical Research Council (AML15 and AML17 trials), including n=500 FLT3-mutated AML patients. Within this trial, newly diagnosed AML patients with activating FLT3 mutations (median age, 49 years; range, 5–68 years) were randomised to receive either oral lestaurtinib or placebo, for up to 28 days after each of the four courses of chemotherapy. Recently published data showed that lestaurtinib yielded no improvements in 5-year RFS and OS when added to first-line chemotherapy. Nevertheless, subgroup analysis indicated improved OS and significantly reduced rates of relapse in lestaurtinib-treated patients who sustained >85% FLT3 inhibition as assessed by the plasma inhibitory activity assay. In addition, elevated FLT3 ligand had no impact on lestaurtinib plus chemotherapy treatment.

Three multtargeted TKIs currently approved for other malignancies have demonstrated activity against FLT3: ponatinib, sunitinib and sorafenib. In a phase I study of 12 previously treated patients with AML (58% had FLT3-ITD), ponatinib gave an overall response rate (ORR) of 25%. Following safety concerns, ponatinib was temporarily removed from the market in 2013. Since December 2013 the FDA has granted ponatinib full approval for the treatment of adult patients with chronic phase, accelerated phase or blast phase chronic myeloid leukaemia (CML) or (p,22)-positive acute lymphoblastic leukaemia (ALL) for whom no other TKI therapy is indicated; and for the treatment of adult patients with T315I-positive CML or T315I-positive and (p,22)-positive ALL. Currently, a dose escalation study of ponatinib, alone and in combination with 5-azacytidine, in patients with FLT3-mutated AML is planned at the MD Anderson Center but not yet recruiting. In addition, sunitinib in combination with intensive chemotherapy (cytotoxic arabinoside/daunorubicin induction followed by three cycles of intermediate-dose cytotoxic arabinoside) as maintenance therapy for 2 years showed promising findings in a phase II trial of 22 AML patients with activating FLT3 mutations.

Sorafenib demonstrated efficacy in phase I studies, and no dose-limiting toxicity was observed. The addition of sorafenib to chemotherapy has also yielded positive data in a phase I and II study. In the phase II study in younger adult (age range, 18–60 years) AML patients (n=267, of whom n=46 were positive for FLT3-ITD), median EFS was 9 months in the placebo group as compared to 21 months in the sorafenib group, corresponding to a 3-year EFS of 22% in the placebo group as compared to 40% in the sorafenib group (HR 0.64, 95% confidence interval [CI]: 0.45–0.91; p=0.013). In the subgroup analysis of FLT3-ITD positive AML, EFS (18 versus 6 months) and OS (not reached versus 19 months) were higher in the sorafenib group as compared to the placebo group.

The results in elderly AML patients with sorafenib in combination with standard intensive chemotherapy are controversial. Whereas one randomised double-blinded study in 197 elderly AML patients found no beneficial effect of the addition of sorafenib as compared to placebo, the opposite was the case in a recently published trial. Within this study, sorafenib was added to daunorubicin and cytarabine-based induction and consolidation chemotherapy and was also continued for 12 months of maintenance therapy. Fifty-four patients with a median age of 67 years (range, 60–83 years) were enrolled (n=99 were FLT3-ITD mutated (71%) and n=15 were FLT3-TKD (29%) mutated). The observed 1-year OS was 62% (95% CI, 45–78%) for the FLT3-ITD patients (meeting the primary end point 62% versus 30% for a historical control group, p<0.0001) and 71% (95% CI, 42–92%) for the FLT3-TKD patients. Nevertheless, the study by Serve et al. might have been biased, since the trial 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 a phase II study of previously treated patients with AML (n=37, FLT3-ITD in 93%), a lower intensity regime with azacytidine yielded promising results: an ORR for response of 46% including incomplete count recovery (CRi) in 27%, CR in 16% and partial response (PR) in 3%. The median time to achieve CR/CRi was two cycles and the median duration of CR/CRi was 2.3 months. These findings suggest that further investigation of sunitinib and sorafenib in this treatment setting is warranted.

Midostaurin is currently the only TKI that has demonstrated convincingly superior results as compared to standard intensive therapy in younger FLT3-mutated AML patients for all survival end points including OS. Midostaurin affects multiple targets including c-KIT, platelet-derived growth factor receptors (PDGFR), as well as FLT3. In a phase I-II trial midostaurin 50 mg orally 2×/day given for 14 days was safely combined with standard induction therapy of daunorubicin and cytarabine in patients with newly diagnosed AML and a CR-rate of 80% could be achieved. These encouraging results provided rationale to move on to a randomised...
Table 2: Clinical studies of tyrosine kinase inhibitors (phase I-III)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Study type</th>
<th>Outcomes</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Crenolanib</td>
<td>Phase II, n=34</td>
<td>Heavily pretreated relapsed/refractory patients, median duration 9 weeks.</td>
<td>Randhawa et al., 201412</td>
</tr>
<tr>
<td>Gilteritinib (ASP2211)</td>
<td>Phase I/II, n=215, relapsed or refractory AML, 65% received ≥2 prior lines of AML therapy, 29% had prior HSCT and 23% had prior TKI.</td>
<td>Perel et al., 201615</td>
<td></td>
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<tr>
<td>Lestaurtinib</td>
<td>Phase II, n=29, newly diagnosed AML, age ≥70 years (or 60–70 years with comorbidity). Clinical activity transient reductions in bone marrow and peripheral-blood blasts or longer periods of transfusion independence in 60% of patients with mutated FLT3 and 23% of wild-type FLT3 patients.</td>
<td>Knapper et al., 200614</td>
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</tr>
<tr>
<td>Lestaurtinib</td>
<td>Phase III, n=500, newly diagnosed AML, duration 5 years. No difference in CR, RFS or OS between the arms.</td>
<td>Knapper et al., 201414</td>
<td></td>
</tr>
<tr>
<td>Midostaurin</td>
<td>Phase Ib, n=20, relapsed/refractory AML.</td>
<td>PB blasts &lt;50%: in 70% of patients; BM blasts &lt;50%: in 30% of patients.</td>
<td>Stone et al., 201214</td>
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<tr>
<td>Midostaurin + chemotherapy</td>
<td>Phase III, n=717, previously untreated AML, median follow-up 57 months.</td>
<td>CR in 59% versus 54% placebo, p=0.18; median OS=74.7 months versus 26.0%. EFS 8 months versus 3 months. No difference in AEs between two groups.</td>
<td>Stone et al., 201512</td>
</tr>
<tr>
<td>Sorafenib + chemotherapy</td>
<td>Phase II, n=276, previously untreated AML, median follow-up 36 months.</td>
<td>Median EFS=21 months versus 9 months in placebo group. Grade ≥3 AEs that were significantly more common in the sorafenib group than the placebo group were fever (RR 1.54), diarrhoea (RR 7.89), bleeding (RR 3.75), 1.5–10.0, cardiac events (RR 3.46) and rash (RR 4.06).</td>
<td>Röllig et al., 201514</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Phase II, n=197, newly diagnosed AML, age ≥60 years.</td>
<td>CR in 48% with sorafenib, 60% with placebo, no difference in EFS or OS between placebo and sorafenib treatment cohort.</td>
<td>Serve et al., 201314</td>
</tr>
<tr>
<td>Sunifibin + chemotherapy</td>
<td>Phase III, age ≥60 years, n=22, duration 2 years.</td>
<td>CR in 59%. At lower dose, median OS and EFS were 1.6, and 0.4 years, respectively. Dose-limiting toxicities at higher dose.</td>
<td>Fiedler, 201513</td>
</tr>
<tr>
<td>Tandutinib (MLN-518)</td>
<td>Phase II, n=20, relapsed/refractory AML.</td>
<td>No CR or PR, antileukemic effect in 30%.</td>
<td>DeAngelis et al., 201014</td>
</tr>
<tr>
<td>Quizartinib (AC220)</td>
<td>Phase II, n=137, relapsed or refractory to second-line, salvage chemotherapy or relapsed after HSCT.</td>
<td>CT rate 44% with median duration of response of 11.3 weeks and median OS of 23.1 weeks. Most common AEs were nausea (38%), anaemia (29%), QT interval prolongation (26%), vomiting (26%), febrile neutropenia (25%), diarrhoea (20%) and fatigue (20%). Most common Grade 3 or 4 AEs were anaemia (26%), febrile neutropenia (25%), thrombocytopenia diarrhoea, neutropenia (12%) and QT interval prolongation (10%).</td>
<td>Levis et al., 201214</td>
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<tr>
<td>Quizartinib (AC220)</td>
<td>Phase II, n=76, relapsed or refractory AML after either one second-line therapy or HSCT; median treatment duration 10.9 weeks Group A (60 mg/day) and 11.0 weeks Group B (60 mg/day).</td>
<td>CR rate in both groups=47%; ORR=61% in Group A and 71% in Group B. Median OS was 20.7 weeks in Group A and 25.4 weeks in Group B: AEs of diarrhoea (18%), febrile neutropenia (16%) and QT prolongation (15%).</td>
<td>Schiller et al., 201413</td>
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</table>

AEs = adverse events; AML = acute myeloid leukaemia; AST = aspartate aminotransferase; CR = complete response; EFS = event-free survival; HSCT = haematopoietic stem cell transplantation; ORR = overall response rate; OS = overall survival; PR = partial response; RFS = relapse-free survival; RR = relative risk; TKI = tyrosine kinase inhibitor.

phase III trial CALGB 10603 (RATIFY; NCT00651261). The trial was activated in May 2008 and recruitment of over 700 younger adult FLT3-mutated, including FLT3-ITD and FLT3-TKD, AML patients (18–59 years) was finally achieved in October 2011. The study scheme consisted of the addition of midostaurin or placebo to standard intensive “7+3” induction chemotherapy as well as four cycles of high-dose cytarabine (HiDAC) as consolidation therapy. In all patients, maintenance therapy of 1 year with midostaurin or placebo according to initial randomisation was intended. Although not specifically mandated, allo HSCT was performed in 57% of the overall study cohort including transplants in refractory and relapsed patients. The combination of midostaurin to intensive chemotherapy significantly improved OS in younger adults with FLT3-mutated AML with a HR of 0.77 (95%-CI: 0.63–0.95, p=0.008), translating into a median OS of 74.7 months for the midostaurin arm (range, 31.7 months – not reached) as compared to 25.6 months for the placebo-arm (range, 18.6–42.9 months), respectively. Interestingly, this improvement was regardless of the FLT3 mutational status (either ITD or TKD) or the FLT3-ITD allelic ratio.143 Based on these results, on April 28, 2017, the US Food and Drug Administration (FDA) approved midostaurin (Rydapt®, Novartis, Basel, Switzerland) for the treatment of AML in newly diagnosed patients who are FLT3-mutation-positive as detected by an FDA-approved test, in combination with chemotherapy.115 In Europe, the marketing authorisation application for midostaurin is still under review by the European Medicines Agency (EMA). Based on a phase II follow-up study of the RATIFY trial in AML patients (age 18–70 years) with FLT3-ITD evaluating midostaurin in combination with intensive induction, consolidation- including allo HSCT and maintenance therapy in all patients, the approval may be extended to older patients aged between 60 and 70 years.116

In addition, the combination of sequential azacitidine (intravenous 75 mg/m² daily for 7 days) and escalating doses of oral midostaurin (25, 50 and 75 mg bid) on days 8–21 of a 28-day cycle has been investigated in a phase I study in untreated, elderly (median age: 73, range 57–83 years) and/or relapsed AML patients. No dose-limiting toxicities occurred. Seventeen patients were enrolled and 14 patients were evaluable for response: three attained a CR and two had haematologic improvement. Median survival from enrolment
The combination therapy of midostaurin and azacitidine was also evaluated in a phase II study (n=54; 74% had a FL3T mutation; 76% had been previously treated). During the dose-finding part of the study, six patients received midostaurin at a dose of 25 mg bid and eight at a dose of 50 mg bid. No dose-limiting toxicities occurred. Among the 54 patients in the phase II study, a median of 12 weeks (range, 1–31), the ORR was 26%. One patient (2%) achieved a CR, six (11%) achieved a CR with CRi, six (11%) a morphologic leukaemia-free status (defined as <5% blasts in the bone marrow regardless of neutrophil and platelet count in the peripheral blood) and one patient (2%) a PR.115,116 Nevertheless, even with the addition of midostaurin to intensive therapy including allo HSCT and maintenance therapy within the RATIFY trial, a significant proportion of the patients still relapsed within the first 2 years,117 raising the question as to whether or not more selective TKIs would be more beneficial.

Second-generation FL3T TKIs including quizartinib, crenolanib, PLX3397 and gilteritinib (formerly ASP2215), are more potent and selective based on cell cultures and animal models than the first-generation inhibitors.117 Quizartinib, a novel bis-aryl urea, is very specific for FL3T, has a high capacity for sustained FL3T inhibition and an acceptable toxicity profile.118 In a phase II study quizartinib demonstrated particular efficacy in patients with FL3T-ITD mutations (n=137) who were relapsed or refractory to second-line, salvage chemotherapy or relapsed after allo HSCT.119 The CR rate was 44% with a median duration of response of 11.3 weeks and median OS of 23.1 weeks. Of note, one-third of patients were successfully bridged to allo HSCT. A subsequent phase II study recruited 76 patients with FL3T-ITD mutations, with relapsed or refractory AML after either one second-line therapy or allo HSCT. Patients were randomised to quizartinib 30 mg/day (Group A) or 60 mg/day (Group B) given orally during 28-day continuous treatment cycles, until relapse, intolerance or proceeding to allo HSCT. The ORR was 61% in Group A and 71% in Group B. In addition, 32% of patients in Group A and 42% in Group B could be successfully bridged to allo HSCT.120 A phase III study of quizartinib or placebo with induction and consolidation chemotherapy, and as maintenance in patients with newly diagnosed FL3T-ITD AML, is ongoing (age range: 18–75 years, planned inclusion number: n=536; ClinicalTrials.gov identifier: NCT02668653).121 However, resistance to quizartinib in FL3T-ITD has been reported; this has been attributed to acquired DB3SY TKD mutation on the FL3T-ITD allele.22

Other second-generation FL3T inhibitors have also yielded positive findings. Gilteritinib and crenolanib are able to inhibit both FL3T-ITD and FL3T-TKD mutations. High response rates have been reported in two clinical studies of crenolanib, particularly in FL3T inhibitor-naïve patients (phase II).122,123 In a single centre phase II study (n=34), patients had received a median of 3.5 prior therapies (sorafenib in 57%, quizartinib in 14%, PLX3397 in 5% and midostaurin in 10%; 9% and 5% had received two and three FL3T TKIs, respectively). At a median follow-up of 14 weeks, the ORR was 47%; 12% achieved CR, and 3% morphologic leukaemia-free state. The median EFS was 8 weeks and OS was 19 weeks for the whole cohort.122 In another phase II study of relapsed/refractory patients (n=19, median age 47 years), one patient had a CR while two had a CRi and four patients were bridged to transplant.123 These preliminary data suggest that crenolanib is very promising in relapsed and refractory AML patients, and further trials are being initiated (e.g., NCT02298166, NCT02400281, NCT02270788, NCT02283177).

In a phase II study (n=252) of heavily pretreated patients (70% had ≥2 prior AML therapies, 29% had prior HSCT, and 25% had prior TKI treatment, most commonly sorafenib) receiving gilteritinib, FL3T-ITD patients showed an ORR of 52%, with CR in 11%. Clinical responses occurred in FL3T-mutated patients with ITD, D835 and both mutations (ORR: 55%, 17% and 62%, respectively). The median OS for FL3T-mutated patients receiving gilteritinib >80 mg was around 31 weeks.124 A phase III trial of gilteritinib is currently ongoing (NCT02421999, estimated enrolment 369). Patients with FL3T-mutated AML in first relapse or refractory to frontline therapy are being recruited and randomised to treatment with either gilteritinib or to investigator’s prerandomisation choice of specified salvage chemotherapy. The primary objective is OS; key secondary objectives are EFS and CR rate.121

TKI treatment postallogeneic HSCT

In addition, the efficacy of TKIs following allo HSCT is being investigated. A retrospective multicentre study of 29 patients who had undergone allo HSCT, treatment with sorafenib led to haematological remission in 79%, bone marrow remission in 8%, CR (with and without normalisation of peripheral blood counts) in 23% and molecular remission with undetectable FL3T-ITD mRNA in 15%, respectively. Allo HSCT patients developed sorafenib resistance less frequently (38% versus 47%) and significantly later (197 days versus 136 days, p=0.08) than those without prior HSCT, and sustained remissions were seen only in the allo HSCT cohort.125 The addition of midostaurin to intensive induction therapy and as maintenance after allo HSCT or HiDAC is currently being investigated. Preliminary data indicate that this approach was feasible and outcomes were favourable compared with historical data, particularly in patients with a high FL3T-ITD mutant to wild type ratio.126 An ongoing trial is also investigating the efficacy and safety of quizartinib, postallogeneic transplant (NCT02668653).121

Mechanisms of resistance

Patients who relapse after treatment with a TKI can develop point mutations in the target kinase domain as a mechanism of resistance.127,128 Resistance has also been associated with upregulation of parallel and downstream signal transduction pathways, and may also involve stromal cells of the bone marrow.127 In addition, an interaction between CD34+ progenitor cells from patients with FL3T-ITD mutations and niche cells has been reported in another publication.129 This interaction enables the maintenance of leukemic progenitors in the presence of a TKI.128 Different FL3T TKIs display distinct and nonoverlapping resistance profiles in vitro; TKD1 mutations showed a response to SU664, sorafenib and sunitinib but diminished response to PKC412, whereas TKD2 mutations were sensitive to PKC412, sunitinib or sorafenib.130

Another mechanism of resistance might be related to the FLT3 insertion site. These data suggest that combinations of FLT3 inhibitors may be required to prevent FLT3 resistance mutations in FLT3-ITD-positive AML. Some research has suggested that FLT3 inhibitor therapy combined with crenolanib may prevent the emergence of resistance.131

Future developments

Targeting multiple pathways may be necessary to ensure enduring responses, leading to a focus on combined treatment regimens. A phase I clinical trial evaluating the combination of the mammalian target of rapamycin (mTOR)-inhibitor RAD001 with midostaurin is ongoing.132 Homoharringtonine has been shown to act synergistically with FLT3 inhibitors.133,134 In addition, preclinical data suggest that a number of PI3K, AKT, mTOR and MEK inhibitors may act synergistically with FLT3 inhibition.135,136 Recently, dual inhibition of FL3T and Pim kinases has been found to...
eradicate FLT3-ITD mutated AML cells in vitro. However, concern has been expressed that targeting multiple pathways may result in increased toxicity; therefore, more clinical data are needed on these combinations.

Other multtargeted TKIs are also in early stage clinical development. Pacritinib (formerly SB1518) is a TKI with activity against FLT3 and Janus kinase 2. The first clinical study of pacritinib showed promising results.

**Summary and concluding remarks**

Increased understanding of FLT3 mutations in AML has presented an opportunity for the identification of targeted therapies, and thus broadened a treatment landscape that has remained unchanged for decades. The incorporation of FLT3 TKIs into current treatment paradigms should lead to significant improvements in the prognosis for AML patients with activating FLT3 mutations. Of the several promising therapeutic agents that are in clinical development, midostaurin is at the most advanced stage and is the first targeted agent to improve survival in AML with FLT3 mutations in combination with intensive chemotherapy and/or alloHCT including maintenance therapy in younger, adult AML with FLT3-mutations. Since midosaurin was FDA approved on April 28, 2017, its use according to the CALGB 10603/RATIFY trial to treat younger adult AML patients with FLT3 mutations seems currently to be the best approach for this patient group. Whether newer, more selective TKIs might be clinically more beneficial is currently being tested in clinical trials.

The optimum use of FLT3 TKIs remains an active area of research. Numerous questions remain unanswered, including the optimal sequencing of second-generation FLT3-specific agents and multikinase TKIs. Complete and sustained inhibition of FLT3-mutated AML may require a combination of agents, both targeted and conventional chemotherapy, but at present the optimal schedule is not known. The optimum role of FLT3 TKIs in relapsed/refractory patients as a bridge to alloHSCT or as post-HSCT maintenance remains to be established. There is a need for further randomised clinical trials to investigate these questions. Further research will examine our understanding of FLT3 mutations and the mechanisms of resistance to FLT3 TKIs.

**Targeting the FLT3 Mutation in Acute Myeloid Leukaemia**


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