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*).[3–7]

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*.[8]

Despite the fact that AML is a clinically and genetically heterogeneous disease, until recently most patients have been treated by similar chemotherapeutic regimens.[9] To date, the only approved targeted therapies for patients with AML are all-trans retinoic acid[10] and arsenic trioxide[11] for acute promyelocytic leukaemia (APL), which accounts for 10–15% of AML cases.[12]

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 aims to discuss mutations of the *FLT3* gene, as well as the therapeutic interventions targeting these mutations.

Table 1: 2017 European LeukemiaNet risk stratification by genetics[3]

Risk Category	Genetic Abnormality
Favourable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD low* Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD high* Wild type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD low* (w/o adverse risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*** Cytogenetic abnormalities not classified as favourable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype,# monosomal karyotype++ Wild type *NPM1* and *FLT3*-ITD high* Mutated *RUNX1**** Mutated *ASXL1**** Mutated *TP53*+

**Low, low allelic ratio (<0.5); high, high allelic ratio (>0.5); as determined by GeneScan analysis. **The presence of t(9;11)(p21.3;q23.3) 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(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with* BCR-ABL1. *++Defined by the presence of one single monosomy (excluding loss of X or Y) in association with at least one additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).[4] ***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.[5–7]*

Activating mutations in acute myeloid leukaemia

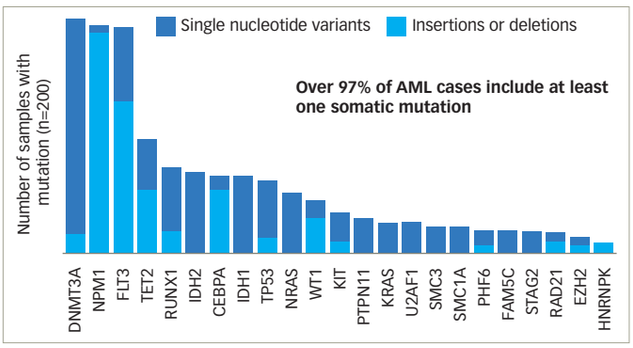
A number of cytogenetic abnormalities, mutations or epigenetic alterations, which are involved in the pathogenesis of leukaemia, have been identified (*Figure 1*).[13]

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.[14,15] *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.[16] 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) access to the FLT3 active site. This is followed by autophosphorylation and activation of numerous downstream signalling pathways.[17–19] Mutations of the *FLT3* gene lead to ligand-independent activation and dysregulation of downstream pathways such as PI3K/AKT, MAPK/ERK 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.[23]

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,[13,24] 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.[25,20]

Figure 1: Mutations in acute myeloid leukaemia[13]



AML = acute myeloid leukaemia. Reproduced with permission from The Cancer Genome Atlas Research Network.[13]

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).[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.[33] The impact of the size on outcome is still unclear with some publications stating that there is no impact on outcome,[34,35] whereas one publication found that short ITDs may impart an unfavourable outcome.[36] Nevertheless, most publications stated that longer ITDs correlate with lower complete response (CR) rates and shorter OS and event-free survival (EFS).[37–39]

Recently, the ITD insertion site within the *FLT3* gene has been shown to be an important prognostic factor.[33] 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.[33] 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.[40] 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.[33,40] 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).[33]

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.[41,42] This method is a semi-quantitative

assessment 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.[33,34,39,42,43] 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.[33,38,42,44] In addition, as AML evolves from diagnosis to relapse, the allelic ratio seems to increase.[45,46] However, in a small proportion of relapses *FLT3*-ITD was no longer detectable.[47]

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).[22] 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.[48] Other point mutations include, for instance, N676D, I836S and Y842C in the TKD1 and TKD2 domains, respectively.[49] Unlike ITDs, the incidence of point mutations is not associated with age[50] and their prognostic significance is discussed controversially.[14,44,48,51–54] Nevertheless, TKD mutations can occur after treatment with tyrosine kinase inhibitor (TKI) as a mechanism of resistance, thus implicating an adverse prognosis.[55–57]

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.[58,59] Mutations in exon 12 of the *NPM1* gene cause cytoplasmic dislocation of the NPM1 protein.[60] As a result, cytoplasmic *NPM1* is unable to undertake its normal functions as binding and transporter protein. In CN-AML, *NPM1* mutations without *FLT3*-ITD59 or a low allelic ratio are a more favourable prognostic factor.[42]

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.[61] 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* was less important.[62] 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.[63] Recent recommendations from the ELN include a revised version of the risk stratification according to genetics including the allelic ratio (*Table 1*).[3]

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,[64–66] 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.

Current methods used to determine *FLT3*-ITD mutations have limited sensitivity and are not suitable for MRD detection. Newer techniques, such as real-time quantitative polymerase chain reaction (RT-qPCR) with patient-specific primers, aim to improve the sensitivity of *FLT3*-ITD.[67] However, this approach has limitations, since each *FLT3*-ITD mutation needs a clone-specific primer/probe set, which is time-consuming and may not be possible in every case. In addition, direct sequencing may not be possible in patients with a low allelic ratio since the wild-type sequence is competitively amplified. Recently, another PCR-based assay for *FLT3*-ITD MRD was reported.[68,69] This assay employed primers oriented in the opposite direction; hence, amplification occurred only if an *FLT3*-ITD was present. Again, this approach has limitations, since short *FLT3*-ITDs (less than 30–40 bases) are not detected due to insufficient primer annealing space, which may apply to roughly 25% of all *FLT3*-ITD cases. Both approaches are therefore not ready to be implemented in clinical routine care. Next-generation sequencing (NGS) is potentially useful[70] but generates complex data which is still expensive and requires considerable expertise to interpret.

Nevertheless, in those patients with a concurrent *NPM1* mutation, MRD can be assessed by analysis of *NPM1* mutated transcripts.[66]

In summary, *FLT3* mutational testing should be mandatory in all AML patients at diagnosis as well as at relapse for prognostic purposes and for guiding therapeutic decisions. At present, it has little utility for MRD monitoring until different methodologies can be standardised.

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.[71] However, higher allelic ratios were associated with lower CR-rates after induction therapy[42] bringing up the question of dose-intensification in these patients.[72,73] In older adults a substantial proportion of patients cannot tolerate intensive induction chemotherapy; in these cases other less intensive regimens may be used including low-dose cytarabine[74] or hypomethylating agents (e.g., azacitidine or decitabine).[75,76] 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.[77–79] 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.[42,43,80] In contrast, an ITD insertion site in the TKD1 remained an unfavourable prognostic factor regardless of the applied therapy.[42] Another important prognostic factor has been shown for the *NPM1* MRD status after the second chemotherapy[66] or before allo HSCT.[66,81]

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.[82]

Early TKIs, rather than being specifically designed to target *FLT3*, had multiple targets including KIT, PDGFR, VEGFR and JAK2.[83] Several agents have shown evidence of modest antileukemic activity as monotherapy

including midostaurin (phase IIb),[84] linifanib (phase I),[85] semaxanib (phase II)[86,87] tandutinib[88] and KW-2449 (preclinical).[89] However, responses were typically short-lived and mostly partial remissions.

Moreover, it has been suggested that the responsiveness to FLT3 TKIs seems to depend on the *FLT3* allelic ratio.[46] In an *in vitro* analysis, six different FLT3 inhibitors (lestaurtinib, midostaurin, AC220, KW-2449, sorafenib and sunitinib) were examined for potency against mutant and wild-type *FLT3*, as well as for cytotoxic effect against a series of primary blast samples obtained from *FLT3*-ITD mutated AML patients. Relapsed samples and samples with a high allelic ratio were more likely to be responsive to cytotoxicity from FLT3 inhibition as compared to the samples obtained at diagnosis or those with a low allelic ratio.[46] Therefore, it has been hypothesised that patients with newly diagnosed *FLT3*-mutant AML might be less likely to respond to highly selective FLT3 inhibition.[46] However, the results probably indicate that the presence of an *FLT3*-ITD with even a low allelic ratio cannot be excluded altogether from prognostic risk stratification.

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.[90] 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.[91] 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.[92,93] 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.[94] 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.[95] Recently published data showed that lestaurtinib yielded no improvements in 5-year RFS and OS when added to first-line chemotherapy.[95] 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.[95] In addition, elevated FLT3 ligand had no impact on lestaurtinib plus chemotherapy treatment.[95]

Three multitargeted 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%.[96] Following safety concerns, ponatinib was temporarily removed from the market in 2013.[97] 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 t(9;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 t(9;22)-positive ALL. The full approval and label update was based on a 48-month follow-up data from the pivotal phase II PACE clinical trial of ponatinib in heavily pretreated patients with resistant or intolerant CML or t(9;22)-positive ALL.[98,99] 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.[100] In addition, sunitinib in combination with intensive chemotherapy (cytosine arabinoside/daunorubicin induction followed by three cycles of intermediate-dose cytosine arabinoside) as maintenance therapy for 2 years showed promising findings in a phase I/II trial of 22 AML patients with activating *FLT3* mutations.[101]

Sorafenib demonstrated efficacy in phase I studies, and no dose-limiting toxicity was observed.[102,103] The addition of sorafenib to chemotherapy has also yielded positive data in a phase I and II study.[104,105] 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).[104] In the subgroup analysis of *FLT3*-ITD positive AML, RFS (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,[106] the opposite was the case in a recently published trial.[107] 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=39 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.[106] 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.[108] 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.[109] Midostaurin affects multiple targets including c-Kit, platelet-derived growth factor receptors (PDGFR), as well as *FLT3*.[110] 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.[111] These encouraging results provided rationale to move on to a randomised

Table 2: Clinical studies of tyrosine kinase inhibitors (phase I-III)

Agent	Study type	Outcomes	Reference
Crenolanib	Phase II, n=34, heavily pretreated relapsed/refractory patients, median duration 9 weeks.	ORR=47%. Median EFS was 8 weeks and OS was 19 weeks for the whole cohort. Well-tolerated.	Randhawa et al., 2014[122]
Gilteritinib (ASP2215)	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.	ORR=52% with CR in 11%. Treatment-related AEs of all grades, reported in ≥10% of the safety population were diarrhoea (16%), fatigue (13%) and increased AST (11%).	Perl et al., 2016[125]
Lestaurtinib	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.	Knapper et al., 2006[91]
Lestaurtinib	Phase III, n=500, newly diagnosed AML, duration 5 years.	No difference in CR, RFS or OS between the arms.	Knapper et al., 2014[92]
Midostaurin	Phase Ib, n=20, relapsed/refractory AML.	PB blasts <50%: in 70% of patients; BM blasts <50%: in 30% of patients.	Stone et al.,2012[111]
Midostaurin + chemotherapy	Phase III, n=717, previously untreated AML, median follow-up 57 months.	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.	Stone et al., 2015[112]
Sorafenib + chemotherapy	Phase II, n=276, previously untreated AML, median follow-up 36 months.	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), hand-foot-skin reaction (only in sorafenib group), and rash (RR 4.06).	Röllig et al., 2015[104]
Sorafenib	Phase II, n=197, newly diagnosed AML, age >60 years.	CR in 48% with sorafenib, 60% with placebo, no difference in EFS or OS between placebo and sorafenib treatment cohort.	Serve et al., 2013[106]
Sunitinib + chemotherapy	Phase I/II, age >60 years, n=22, duration 2 years.	CR in 59%. At lower dose, median OS and EFS were 1.6, and 0.4 years, respectively. Dose-limiting toxicities at higher dose.	Fiedler, 2015[101]
Tandutinib (MLN-518)	Phase II, n=20, relapsed/refractory AML.	No CR or PR, antileukemic effect in 30%.	DeAngelo et al., 2006[88]
Quizartinib (AC220)	Phase II, n=137, relapsed or refractory to second-line, salvage chemotherapy or relapsed after HSCT.	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%).	Levis et al., 2012[119]
Quizartinib (AC220)	Phase II, n=76, relapsed or refractory AML after either one second-line therapy or HSCT, median treatment duration 10.9 weeks Group A (30 mg/day) and 11.0 weeks Group B (60 mg/day).	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: diarrhoea (18%), febrile neutropenia (16%) and QT prolongation (15%).	Schiller et al., 2014[120]

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.[112] 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.[113] 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.[114]

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

was 6 months (range, 1 to ≥19 months). Interestingly, none of the patients harboured an *FLT3* mutation. The authors concluded that the combination of sequential azacitidine and midostaurin was safe and tolerable with response rates comparable with azacitidine alone.[115]

The combination therapy of midostaurin and azacitidine was also evaluated in a phase I/II study (n=54; 74% had a *FLT3* 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, after 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,[112] raising the question as to whether or not more selective TKIs would be more beneficial.

Second-generation FLT3 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 *FLT3*, has a high capacity for sustained FLT3 inhibition and an acceptable toxicity profile.[118] In a phase II study quizartinib demonstrated particular efficacy in patients with *FLT3*-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 *FLT3*-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 *FLT3*-ITD AML, is ongoing (age range: 18–75 years, planned inclusion number: n=536; ClincalTrials.gov identifier: NCT02668653).[121] However, resistance to quizartinib in *FLT3*-TD has been reported; this has been attributed to acquired D835Y TKD mutation on the *FLT3*-ITD allele.[57]

Other second-generation FLT3 inhibitors have also yielded positive findings. Gilteritinib and crenolanib are able to inhibit both *FLT3*-ITD and *FLT3*-TKD mutations. High response rates have been reported in two clinical studies of crenolanib, particularly in FLT3 inhibitor-naive 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 FLT3 TKIs, respectively). At a median follow-up of 14 weeks, the ORR was 47%: 12% achieved CRi, 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 I/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, *FLT3*-ITD patients showed an ORR of 52%, with CR in 11%. Clinical responses occurred in *FLT3*-mutated patients with ITD, D835 and both mutations (ORR: 55%, 17% and 62%, respectively). The median OS for *FLT3*-mutated patients receiving gilteritinib ≥80 mg was around 31 weeks.[124] A phase III trial of gilteritinib is currently ongoing (NCT02421939, estimated enrolment 369). Patients with *FLT3*-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.[125]

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 37%, bone marrow remission in 8%, CR (with and without normalisation of peripheral blood counts) in 23% and molecular remission with undetectable *FLT3*-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=0.03) than those without prior HSCT, and sustained remissions were seen only in the allo HSCT cohort.[102] 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 *FLT3*-ITD mutant to wild type ratio.[114] 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.[57,126] 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 *FLT3*-ITD mutations and niche cells has been reported in another publication.[128] This interaction enables the maintenance of leukemic progenitors in the presence of a TKI.[128]

Different FLT3 TKIs display distinct and nonoverlapping resistance profiles *in vitro*; TKD1 mutations showed a response to SU5614, sorafenib and sunitinib but diminished response to PKC412, whereas TKD2 mutations were sensitive to PKC412, sunitinib or sorafenib.[56]

Another mechanism of resistance might be related to the *FLT3*-ITD insertion site.[129] 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.[49]

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.[130] Homoharringtonine has been shown to act synergistically with FLT3 inhibitors.[131] In addition, preclinical data suggest that a number of PI3K, AKT, mTOR and MEK inhibitors may act synergistically with FLT3 inhibition.[132–134] Recently, dual inhibition of FLT3 and Pim kinases has been found to

eradicate *FLT3*-ITD mutated AML cells *in vitro*.[135] However, concern has been expressed that targeting multiple pathways may result in increased toxicity; therefore, more clinical data are needed on these combinations.

Other multitargeted TKIs are also in early stage clinical development. Pacritinib (formerly SB1518) is a TKI with activity against *FLT3* and Janus kinase 2.[136] The first clinical study of pacritinib showed promising results.[137]

Summary and concluding remarks

Increased understanding of *FLT3* mutations in AML has presented an opportunity for the use 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 a significant improvement 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 allo HSCT including maintenance therapy in younger, adult AML with *FLT3*-mutations. Since midostaurin 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 allo HSCT 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 expand our understanding of *FLT3* mutations and the mechanisms of resistance to FLT3 TKIs. ❑

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