Title page

FLT3 Inhibition in Acute Myeloid Leukaemia – Current Knowledge and Future Prospects

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Abstract

Activating mutations of FMS-like tyrosine kinase 3 (FLT3) are present in 30% of acute myeloid leukaemia (AML) patients at diagnosis and confer an adverse clinical prognosis. Mutated *FLT3* has emerged as a viable therapeutic target and a number of FLT3-directed tyrosine kinase inhibitors have progressed through clinical development over the last 10-15 years. The last two years have seen United States Food and Drug Administration (US FDA) approvals of the multi-kinase inhibitor midostaurin for newly-diagnosed *FLT3*-mutated patients when used in combination with intensive chemotherapy, and of the more FLT3-selective agent gilteritinib, used as monotherapy, for patients with relapsed or treatment-refractory *FLT3*-mutated AML. The 'second generation' agents quizartinib and crenolanib are also both at advanced stages of clinical development. Significant challenges remain, crucially in negotiating a variety of potential acquired drug resistance mechanisms and in optimising sequencing of FLT3 inhibitory drugs with existing and novel treatment approaches in different clinical settings including frontline therapy, relapsed/refractory disease and maintenance treatment. In this review, we discuss the biology of FLT3, the clinical challenge posed by *FLT3*-mutated AML, the developmental history of the key FLT3-inhibitory compounds, mechanisms of disease resistance and the future outlook for this group of agents including current and planned clinical trials.

Keywords
Acute
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Inhibitor
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Main Text

1. Introduction

After 40 years in which the '7+3' combination of cytarabine and daunorubicin chemotherapy has remained the standard treatment regimen for fitter patients the world over and survival improvements have largely been achieved through optimisation of allogeneic stem cell transplantation (SCT) and better supportive care provisions, the last two years have seen an unprecedented surge of regulatory approvals of new therapeutic agents in acute myeloid leukaemia (AML). AML is, genetically, a highly heterogeneous neoplasm; greater understanding of disease biology has led to an international drive to develop targeted therapeutic agents able to exploit molecular pathogenetic insights, potentially avoiding some of the toxicities associated with traditional therapy. Mutated in almost a third of AML patients, and associated with inferior prognosis in most of these cases, the receptor tyrosine kinase FMS-like tyrosine kinase 3 (FLT3) has formed one of the major focuses of this translational research activity. In this review, we discuss the first 10-15 years of development of FLT3 inhibitory compounds from the laboratory through to international clinical trials and the recent regulatory approvals of midostaurin and gilteritinib, reviewing the clinical data associated with each of the principal agents before going on to consider some of the biological challenges, including resistance mechanisms, that will need to be taken into account in order to optimise their future deployment amongst a widening armamentarium of new therapeutic options in AML.

2. The Biology of FLT3

2.1 FLT3 in normal haematopoiesis

FLT3 is a receptor tyrosine kinase (RTK) that plays a pivotal role in normal haematopoiesis being involved in cell survival, proliferation and differentiation. For this article a brief introduction to the biology of FLT3 is necessary, but for greater detail the reader is referred to previous excellent reviews on the subject. [1-3] In summary, the *FLT3* gene is located on chromosome 13; FLT3 is part of the class III RTK family which also includes c-KIT, PDGFR and FMS, all of which are involved in cell proliferation and survival. FLT3 is primarily expressed on normal haematopoietic precursor cells, driving the proliferation of multi-potent progenitor cells. In common with other RTKs, FLT3 is composed of an extracellular domain, a transmembrane domain, a juxtamembrane domain and split intercellular tyrosine-kinase domains. On binding its ligand, FLT3 ligand (FL), FLT3 dimerises and is activated, allowing binding of ATP and subsequent phosphorylation; this in turn results in the activation of various downstream signalling pathways including PI3K, RAS and STAT5.

2.2 FLT3 in AML

The FLT3 receptor is overexpressed on blasts in the majority of cases of acute myeloid and acute lymphoblastic leukaemia and also, to varying degrees, in other haematological cancers. [2;4] In the 1990s, activating mutations of the *FLT3* gene were described in AML, occurring in approximately 30% of newly diagnosed cases,[5;6] making them amongst the most frequently occurring molecular abnormalities in this disease.[7;8]

Of the two main *FLT3* mutations described, internal tandem duplications (*FLT3*-ITD) are much commoner, present in approximately 25% of AML patients at diagnosis.[5] ITD mutation leads to an in-frame expansion of the amino acid sequence of FLT3, resulting in a constitutively-activated kinase with consequent activation of downstream signalling pathways, dysregulation of cell survival and proliferation.[9] AML cases harbouring *FLT3*-ITDs characteristically present with a 'proliferative' clinical picture with rapid disease kinetics, high peripheral white cell counts and bone marrow blast burden; this is most commonly associated with normal karyotype disease.[8;10;11]Some studies have associated *FLT3*-ITD mutations with monocytic AML corresponding to the FAB classification M5.[12]

The presence of a *FLT3*-ITD does not appear to impact on the likelihood of remission induction, but has been repeatedly shown to be associated with an increased risk of relapse risk and poorer overall survival in comparison to *FLT3* wild type patients.[7;8;11;13] The prognostic associations of *FLT3*-ITD are considerably more nuanced than the simple presence or absence of the mutation however, with ITDs exhibiting considerable variability in terms of 'allelic burden', size, insertion site and partner mutations.

It is now widely accepted that the relative *FLT3*-ITD mutational burden, most often expressed as 'mutant to wild-type allelic ratio' substantially influences prognosis. Higher levels of mutated *FLT3* are associated with inferior disease-free and overall survival, very high mutant to wild type ratios being associated with 'loss of heterozygosity' and carrying a particularly unfavourable prognosis.[12;14;15] Although the impact of mutant allelic burden is likely to form a continuous variable, a cut-off ITD:WT ratio of 0.5 (corresponding to 33% ITD allelic burden) has been used to prognostically differentiate ITD-mutated patients, and is currently used to divide patients between risk groups in the current European LeukaemiaNet Guidelines **(Table 1)**.[16] Considerable heterogeneity is also seen in the length of the ITD insertion; the prognostic significance of this remains somewhat unclear with some groups reporting association between large ITD size and poorer prognosis, but others finding no clinical correlations.[17-20] A function of the ITD size is the 'site of the FLT3-ITD insertion'; the minority of ITDs that extend outside the juxtamembrane domain into the kinase region of the receptor appear to be associated with inferior prognosis.[21]

It is also important to consider the interaction and impact of other mutations occurring in conjunction with *FLT3*-ITD, particularly mutations in the nucleophosmin 1 gene (*NPM1*) which are seen in approximately 40% of cytogenetically-normal AML patients, associating with favourable prognosis in isolation but frequently co-occurring with *FLT3*-ITDs. Interactions between *FLT3*-ITD and *NPM1*

mutations have been widely studied and significantly impact clinical decision making around which patients should be routinely offered allogeneic stem cell transplant (SCT) in first complete remission. Again, using a cut-off *FLT3*-ITD:WT allelic ratio of >0.5 to define a 'high mutant *FLT3* allelic burden', patients with molecular genotype *NPM1*^{mut}/*FLT3*-ITD^{high} experience an increased risk of relapse, and reduced LFS and OS compared to those with either *NPM1*^{mut}/*FLT3*-ITD^{low} or *NPM1*^{mut}/*FLT3*-ITD^{WT}. [10;20;22;23]

The second principal category of *FLT3* mutations are *FLT3*-TKDs, single base substitutions, small deletions or insertions within the activation loop of the second tyrosine kinase domain detectable in approximately 7-9% of AMLs at diagnosis, most frequent being the D835Y mutation.[6] Although *FLT3*-TKDs also lead to constitutive FLT3 kinase activation and are associated with a high presenting WBC, TKDs have much less well-defined prognostic associations, with conflicting studies reporting association with adverse, intermediate or even improved clinical outcomes.[12;24-26]

Current European LeukaemiaNet Guidelines (2017) recommend that all new cases of AML should be tested for presence of *FLT3*-ITD and TKD mutations and *NPM*1 mutations (including reporting of *FLT3*-ITD mutant to wild-type allelic ratio), results being made available within 48-72 hours in order to improve choice of induction therapy including access to FLT3 inhibitors.[16] **Table 1** summarises the ELN risk classification according to *FLT3* and *NPM1* molecular genotypes, with stated recommendation that patients in the favourable risk genetic group, which includes patients with mutated *NPM1* lacking *FLT3*-ITD or with mutated *NPM1* accompanied by *FLT3*-ITD with allelic ratio of <0.5 should not be routinely assigned to allogeneic SCT in first remission.[16]

The most commonly used methods for detecting *FLT3* mutations are based on PCR technology; the most widely employed technique is based on multiplex PCR assays that can detect both *FLT3* ITD and TKD mutations, utilising capillary electrophoresis to analyse the labelled PCR products compared to the product of a wild type control sample. This technique allows measurement of the ITD:WT ratio.[27] More recently, next generation sequencing (NGS) techniques have been employed, particularly multiplex-targeted NGS panels where large numbers of genes can be sequenced simultaneously, offering highly sensitive, relatively low cost diagnosis with turnaround times that are becoming sufficiently rapid to make this technique relevant to daily clinical practice in light of the AML guidelines outlined above. Disadvantages of the NGS approach include greater difficulty in detecting structural variants; additionally any mutations not included in the specified panel will not be detected.[28]

2.3 FLT3 in relapsed AML

It is well-established that *FLT3*-ITD-mutated AML is associated with a particularly high risk of early relapse. Studies using paired samples obtained at first presentation and relapse demonstrate relative instability of *FLT3* mutations as the clonal structure evolves. The majority of patients with *FLT3*-ITD

at diagnosis will retain the mutation at the time of relapse at which point increased mutant to wild-type ratio is frequently observed with expansion of the *FLT3*-ITD clone, higher levels of biallelic loss and tumours exhibiting 'oncogenic addiction' to FLT3 signalling; as will be discussed later in this review, this phenomenon may have a bearing on the preferential activity of highly-selective FLT3 inhibitors over less-specific multi-kinase inhibitors in the relapse setting.[29] New *FLT3* mutations may, however, sometimes be acquired at relapse, or previously-detected mutations sometimes lost; the latter phenomenon applying particularly to *FLT3*-TKDs.[30-32]

3. FLT3 inhibitors - Introduction

Unsurprisingly, with expanding awareness both of the role that FLT3 plays in AML oncogenesis, and of the frequency and negative prognostic conations of *FLT3* mutations, FLT3 has, for well over a decade, been a heavily-investigated target in AML treatment. So-called 'first generation FLT3 inhibitors', primarily developed to target non-FLT3 kinases in the treatment of solid tumours, were initially repurposed to target FLT3. First generation compounds thus tended to be relatively non-specific with considerable off-target activity.[33] In an attempt to create more selective agents, bespoke 'second generation' inhibitors were subsequently designed with FLT3-targeting in mind. FLT3-inhibitory compounds may also be classified according to their ability to bind to, and inhibit, the active or inactive FLT3 receptor. 'Type I' inhibitors such as midostaurin, gilteritinib and crenolanib are able, through their three-dimensional configuration, to bind to the gatekeeper domain of FLT3 near the activation loop or ATP-binding pocket, irrespective of whether the receptor is in active or inactive conformation, whilst 'type 2' inhibitors such as sorafenib and quizartinib bind to the hydrophobic region directly adjacent to the ATP-binding domain, but only when the protein is in its inactive form.[34] **Table 2** summarises the main properties of the principal FLT3 inhibitors development.

We will now review the published data for each these agents including their clinical investigation in the settings of relapsed/refractory disease, front-line treatment of newly-diagnosed patients and as maintenance therapy, including, where applicable, a summary of current and pending regulatory approvals and ongoing/planned studies. **Table 3** gives an overview of published clinical studies.

4. First generation FLT3 inhibitors

4.1 Midostaurin

Midostaurin (previously PKC412) is an indolocarbazole kinase inhibitor originally developed to treat solid tumours through its inhibition of protein kinase C (PKC) but subsequently shown to have more potent activity against a number of other tyrosine kinases including PDGFR, c-KIT, VEGFR and FLT3. Midostaurin's metabolite CGP52541 also has significant kinase inhibitory activity with longer

half-life and lower protein binding than its parent compound. Midostaurin was shown to cause G1-arrest and subsequent apoptosis of cells expressing mutated FLT3 *in vitro* [35] this discovery leading to extensive clinical investigation, primarily in patients with *FLT3*-mutated AML.

The first trial to demonstrate biological activity of midostaurin was a phase II study in patients with advanced MDS or relapsed/refractory AML and *FLT3*-ITD or TKD mutations, one of the first AML studies to restrict entry to this tumour-specific genotype.[36] 20 patients received oral midostaurin 75 mg orally three times daily until toxicity or disease progression. Transient, greater than 50% reductions in peripheral blood blasts were observed in 14 of the 20 patients; 6 also had >50% reduction in bone marrow blast counts with correlative pharmacodynamic studies demonstrating inhibition of FLT3 phosphorylation in most responding patients. A second, larger phase II trial examined midostaurin monotherapy in relapsed/refractory AML patients including both FLT3-mutated and wild-type cases, blast reductions being observed in 71% of FLT3-mutated and 42% of wild type patients.[37] In both of these monotherapy studies, midostaurin was well-tolerated but responses were shallow (no complete remissions [CRs] observed) and transient.

Subsequent trials have focussed on the use of midostaurin in combination with chemotherapy. In an initial phase Ib trial examining midostaurin in combination with cytarabine/daunorubicin induction and high-dose cytarabine consolidation in newly-diagnosed AML, significant gastrointestinal toxicity emerged at a dose of 100mg bd before the dose of 50mg twice-daily for 14 days starting on day eight of treatment was established as a tolerable schedule, associated with high CR and overall survival (OS) rates, to take forward into phase III investigation.[38]

The Cancer and Leukemia Group B (CALGB) 10603 (RATIFY) trial was a multinational, randomised, double-blind, placebo-controlled trial that enrolled 717 patients, aged 18-59, with newly-diagnosed, previously-untreated FLT3-mutated AML.[39] Patients received daunorubicin/cytarabine '7+3' induction and, following confirmation of *FLT3* status, were randomised to receive either midostaurin 50mg twice a day or placebo on days 8 to 21, randomisation being stratified according to the subtype of *FLT3* mutation: high (>0.7) or low (0.05-0.7) ITD:WT allelic ratio or TKD. A second identical induction cycle was administered if residual leukaemia remained at the time of a day 21 repeat marrow examination. Induction therapy was then followed by four cycles of consolidation therapy with high-dose cytarabine, again with midostaurin or placebo. Non-transplanted patients who remained in remission after completing consolidation therapy were eligible to receive twelve 28-day cycles of midostaurin/placebo maintenance.

RATIFY met its primary endpoint with median overall survival (OS) being significantly longer (74.7 months) in the midostaurin-treated group compared to placebo (25.6 months), the corresponding hazard ratio for death being 0.78 (p=0.009). Median event-free survival (EFS) was also improved with midostaurin (8.2 vs 3.0 months, p=0.002), as was the median disease-free survival (DFS) (26.7 months vs 15.5 months, p=0.01). RATIFY was not appropriately powered for subgroup analysis, but results

suggested benefit with midostaurin irrespective of *FLT3* mutation-type or allelic burden. 57% of RATIFY patients underwent an allogeneic stem cell transplant, including, in first CR, a greater number of patients in the midostaurin arm than the placebo group. Although patients stopped the trial drug at transplant which may have reduced the time of exposure to midostaurin, a sensitivity analysis of the primary end point (OS), censored at transplant, showed a 24.3% lower risk of death in the midostaurin group compared with the placebo. Both groups in the trial achieved a similar CR rate (59% midostaurin vs 54% placebo, p=0.15), but there is speculation, based on the significantly better outcomes seen in midostaurin-treated patients transplanted in first CR, that depths of remission going into transplant were likely to have been deeper in the midostaurin group; unfortunately no MRD or pharmacodynamic data were collected to back up this thesis.

The RATIFY results led to the April 2017 US Food and Drug Administration (FDA) approval of midostaurin (in combination with induction and consolidation chemotherapy) for patients with newly-diagnosed *FLT3*-mutated AML.[40] In the same year midostaurin was similarly approved by the European Medicines Agency (EMA) and, in 2018, by the National Institute of Clinical Excellence (NICE) for reimbursed use in the UK.[41;42]

Evidence to support the routine use of midostaurin in maintenance therapy remains limited. In RATIFY, maintenance therapy was only administered to 19.2% of the midostaurin group and 14.3% of the placebo group.[39] A post-hoc analysis of RATIFY data presented at the 2017 ASH meeting showed no difference in DFS, or OS from the time of starting maintenance, between the midostaurin and placebo groups.[43] Midostaurin as 12 months post-chemotherapy (but not post-allogeneic SCT) maintenance therapy was, however, approved by both EMA and NICE but not by the FDA.[41;42]

There is, to date, minimal published data to support use of midostaurin in post-transplant maintenance. RADIUS is a small, as-yet-unpublished randomised phase 2 trial in which FLT3-mutated patients were randomised to midostaurin maintenance versus 'standard of care' following allogeneic SCT in the absence of active grade 2-4 graft versus host disease. Preliminary results presented in abstract form reported relapse rates of 24% in the standard of care arm in comparison to 11% in the midostaurin arm, a 46% relative reduction in risk of relapse, although the trial is unlikely to have been sufficiently powered to detect significant differences.[44]

The largest published experience of midostaurin maintenance therapy post-transplant, and also the only published outcomes for patients aged 60+ treated with intensive chemotherapy plus midostaurin was reported by Schlenk and colleagues in the AMLSG 16-10 phase 2 'hypothesis generating' trial.[45] 284 newly-diagnosed FLT3-ITD-mutated patients, including 86 aged >60yrs received an intensive chemotherapy-midostaurin combination, with 72% of patients going on to allogeneic-SCT. Patients aged >60yrs achieved similar rates of CR/CRi to younger patients (78% vs 76%) with significant improvement in EFS in comparison to historical controls from the AMLSG. Within the 16-10 study, 97 patients received midostaurin maintenance therapy, including 75 post-allograft, although the non-

randomised nature of the 16-10 study limits the drawing of any formal conclusions relating to the use of midostaurin maintenance post-SCT, which is still to be addressed in a prospective randomised fashion.

Further trials have assessed the addition of midostaurin to the hypomethylating agent azacitidine, primarily in patients unfit for intensive treatment. An initial phase I trial, unrestricted by *FLT3* mutation status, showed the combination to be well-tolerated, with similar response rates to azacitidine monotherapy.[46] A second phase I/II trial recruited either newly-diagnosed high-risk MDS / AML patients who were unsuitable for intensive therapy or those with relapsed/refractory disease, both irrespective of *FLT3* status.[47] >50% reduction in bone marrow blasts was seen in 53% with best responses seen in FLT3-mutated patients with no history of prior FLT3 inhibitor exposure or previous allogeneic stem cell transplant. The overall response rate (ORR) for the combination was only 26% which was felt by the authors to be higher than the response rates seen in a similar historical population treated with azacitidine monotherapy.

4.2 Lestaurtinib

Lestaurtinib (formerly CEP701) is another first generation indolocarbazole drug that was repurposed as a FLT3 (and JAK2) inhibitor after initial development as a TrkA inhibitor.[33] It was initially investigated as monotherapy in patients with FLT3-mutated relapsed/refractory AML; a phase I/II trial saw significant but short-lived reductions in blood and bone marrow blast counts in 36% of patients,[48] and also in a phase II study in newly-diagnosed older patients unsuitable for intensive therapy in which transient reductions in blood/bone marrow blasts were seen in 60% of patients with mutated FLT3 and 22% of FLT3-WT cases.[49] Importantly, in both of these studies, clinical responses were shown to be associated with laboratory evidence of sustained in vivo FLT3 inhibition.[50]

Lestaurtinib was subsequently investigated in two phase 3 randomised studies in combination with chemotherapy, in the settings of relapsed/refractory and newly-diagnosed *FLT3*-mutated AML. In the Cephalon-204 study, 224 patients in first relapse were randomised to receive chemotherapy alone or in combination with 80mg lestaurtinib twice daily; no difference in 2nd CR rate or OS was shown between the two groups, suggested in part to be due to only small numbers of patients achieving sustained FLT3 inhibitory levels.[51] The effects of the randomised addition of lestaurtinib to intensive chemotherapy for previously-untreated patients *FLT3*-mutated AML was assessed in 500 patients treated in the UK NCRI AML15&17 trials; in a pre-planned meta-analysis no significant differences in either 5-year OS or 5-year relapse-free survival (RFS) were seen across the studies, although significantly improved OS and RFS *were* seen in patients in whom sustained FLT3-inhibitory drug levels were achieved, providing further proof of principle of the potential clinical benefits of FLT3 inhibition in front-line management of *FLT3*-mutated AML.[52] There are currently no plans for the further clinical development of lestaurtinib.

4.3 Sorafenib

Sorafenib has FDA approval for the treatment of a number of solid tumours including advanced hepatocellular and renal cell carcinomas. It was initially designed as a c-Raf kinase inhibitor but has also been shown to inhibit VEGFR, PDGFR, c-Kit and FLT3. An early *in vitro* study demonstrated that sorafenib reduced FLT3 phosphorylation and induced apoptosis more effectively in cells with *FLT3*-ITD or D835G mutations than in those *FLT3*-D835Y-mutated or wild-type *FLT3*. An accompanying phase I trial showed decreases in circulating and bone marrow blasts in patients with *FLT3*-ITD mutations that were not seen in wild type *FLT3* cases.[53]

Initial clinical trials investigated sorafenib monotherapy. 15 patients with relapsed/refractory AML or ALL received sorafenib in a phase I dose escalation trial; in contrast to midostaurin, sorafenib was observed to have a slow terminal elimination phase with plasma inhibitory assays confirming excellent inhibition of FLT3 and downstream pathways; despite which clinical responses were limited.[54] In another phase I sorafenib trial, CR or CRi was achieved in 5 *FLT3*-mutated patients (10% of the total trial population);[55] responses were again transient; subsequent studies moved to assessing the combination of sorafenib with chemotherapy.

Sorafenib was safely added to cytarabine/idarubicin induction chemotherapy in a phase II study in 51 previously-untreated AML patients; higher CR rates were observed in *FLT3*-mutated patients (93% vs 66% in *FLT3* wild type) with any formal conclusions being limited by the small sample size. [56] More extended follow-up data from this study showed no difference in OS or DFS between *FLT3*-ITD-mutated and *FLT3*-WT patients, leading to the postulation that sorafenib might be offsetting the poorer prognosis normally associated with *FLT3*-ITD and allowing more patients to proceed to allogeneic SCT.[57]

SORAML was a much larger, randomised, double-blind, placebo-controlled phase II trial in which 267 newly-diagnosed AML patients, aged 18-60 years were randomised between the addition of sorafenib (400mg twice daily) or placebo following cycles of '7+3' induction and high dose cytarabine consolidation chemotherapy, followed by 12 months maintenance therapy; eligible patients proceeded to allogeneic SCT.[58] Notably, SORAML study eligibility was *not FLT3*-restricted with only 17% of patients harbouring a *FLT3*-ITD mutation. The 3-year event-free survival (EFS) was statistically higher in the sorafenib group (40% versus 22%, p=0.013) but this did not translate into improved OS. Subgroup analysis showed only trends towards better RFS and OS in the *FLT3*-mutated group. Greater toxicity was seen in the sorafenib-treated group including rash, hand-foot syndrome, diarrhoea, bleeding and cardiac events. A second randomised phase 2 trial examined sorafenib (vs placebo) in combination with intensive chemotherapy in 201 newly-diagnosed patients, this time aged >60 years; again the study was unrestricted by *FLT3* mutation status.[59] Disappointingly, no improvements in clinical outcome were demonstrated, either across the whole study population or in the *FLT3*-mutated

subgroup. Again, significantly greater toxicity, including higher treatment-related mortality was observed in sorafenib-treated patients.

Sorafenib has also been fairly extensively investigated in combination with azacitidine. In the largest published experience to date, 37 evaluable patients (93% with *FLT3*-ITD) either with relapsed/refractory AML or aged >60 years and deemed unsuitable for intensive treatment were included.[60] The overall response rate was 46%, including 16% CR and 27% CRi, although response durations were short (median 2.3 months). Satisfactory FLT3 inhibition was only seen in 64% of assayed patients, this being attributed in part to the frequent need for sorafenib dose reductions due to toxicity.

More encouraging findings have emerged from studies of sorafenib in the peri-transplant and maintenance therapy settings. A retrospective study evaluated sorafenib monotherapy in 65 *FLT3*-ITD-mutated patients with relapsed/refractory AML, 45% of whom had undergone prior allogeneic SCT.[61] CR/CRi was seen in 23% of patients, including 15% in whom *FLT3*-ITD mRNA became undetectable; interestingly sustained remissions were seen primarily in the post-allograft cohort leading to the hypothesis that sorafenib may synergize with alloimmune effects to induce durable remissions. This investigation has been continued in the SORMAIN Trial, a German/Austrian group phase 2b study, in which 83 patients with *FLT3*-ITD-mutated AML in first remission were randomised at day 60-100 post allogeneic SCT, in the absence of grade 2-4 graft versus host disease, to either 2 years of sorafenib maintenance therapy or placebo.[62] Data presented at the 2018 ASH meeting showed significantly improved 2-year RFS (85% vs 53%, p=0.013) and overall survival with sorafenib maintenance which appears to be extend beyond the period of maintenance therapy. Notably, the vast majority of patients in SORMAIN had not been exposed to other FLT3 inhibitory agents at earlier stages of their treatment.

Despite relatively mixed study results, sorafenib continues to be utilised fairly extensively by AML-treating clinicians accessed off-label through its license as a RAF-inhibitor, used especially in the relapse/refractory setting either as monotherapy, or in combination with azacitidine as a potential 'bridge to transplant'. Pending regulatory approvals of second generation inhibitors are likely to impact on this practice.

5. Second generation FLT3 inhibitors

Although first generation FLT3 inhibitors have demonstrated biological efficacy that has been proven to correlate with inhibition of FLT3 they lack specificity, show limited potency and, when used as monotherapy, have been generally been associated with transient clinical responses. Additionally, the development of resistance due to acquisition of tyrosine kinase domain mutations is well documented.[63] Second generation drugs have been developed based around more selective activity

against FLT3 and carry the potential for deeper and more sustained clinical outcomes. They have, to date, primarily been studied in the setting of relapsed/refractory *FLT3*-mutated disease. The development and prospects of the three leading second generation compounds, quizartinib, gilteritinib and crenolanib, will now be summarised.

5.1 Quizartinib

Quizartinib (formerly AC220) was first investigated as a FLT3 inhibitor after it was found to have a high binding affinity for FLT3 during high throughput molecular screening; it is a highly potent inhibitor with favourable pharmacokinetics, and relatively little off-target activity, excepting notable inhibition of c-KIT.[64] *In vitro* and *in vivo* studies showed prolonged inhibition of FLT3 phosphorylation irrespective of *FLT3* genotype, but with apoptosis only being observed in *FLT3*-ITD-mutated cells;[65] quizartinib is well-recognised to have minimal activity against *FLT3*-TKD-mutated AML. It is a type II FLT3 inhibitor, only binding to the inactive configuration of the FLT3 receptor.

Initial clinical investigation of quizartinib was as monotherapy, primarily in relapsed/refractory AML. A large phase II trial enrolled 333 patients in two main cohorts; the first group included patients aged >60 years with relapsed/refractory AML within one year of first-line treatment; the second group consisted of patients aged >18 years with relapsed/refractory AML following second line chemotherapy or allogeneic stem cell transplant; both groups included both *FLT3*-mutated and wild type cases.[66] In the first cohort, 'composite complete responses' were seen in 56% of *FLT3*-ITD patients and 36% of *FLT3*-wild-type patients; notably the vast majority of responses were 'CRis' with only 3% of the *FLT3*-ITD-mutated and 5% wild type patients achieving traditional CR with full count recovery. Similarly, in the second cohort, composite complete responses were observed in 46% *FLT3*-ITD-mutated and 30% wild type patients with only 4% and 3%, respectively, achieving CR. Although this phase II study was not formally designed to assess survival, across the trial median OS was 25.4 weeks in cohort 1 and 24.0 weeks in cohort 2; this is significantly longer than previously observed with relapsed/refractory *FLT3*-mutated patients receiving salvage chemotherapy alone.[51] Quizartinib treatment enabled 35% of patients to proceed to allogeneic SCT. Although clinical responses were generally transient, they were deeper than those seen in 'first generation FLT3 inhibitor' monotherapy trials, and were supported by laboratory evidence of sustained, complete FLT3 PIA. At higher doses, quizartinib was found to be associated with troublesome QTc prolongation, leading investigators to take forward the lower dose of 60mg into subsequent phase III studies.

Following the promising monotherapy results, the phase III QuANTUM-R study followed the, at the time, relatively bold design of randomising patients between quizartinib monotherapy and 'standard of care' salvage chemotherapy.[67] 367 patients aged ≥18 years with *FLT3*-ITD-mutated disease that was either treatment-refractory or relapsed within 6 months of achieving first complete remission, were randomly assigned (on a 2:1 basis) between quizartinib or an investigator's choice of three chemotherapy regimens (subcutaneous low-dose cytarabine, MEC [mitoxantrone, etoposide and

cytarabine] or FLAG-Ida [fludarabine, cytarabine, idarubicin and granulocyte colony-stimulating factor]). Patients with prior exposure to midostaurin during front-line therapy were eligible. Although clinical responses to quizartinib were again largely CRis, QUANTUM-R met its primary endpoint with median overall survival of 6.2 months in the quizartinib group compared with 4.7 months in the chemotherapy group (HR 0·76, one-sided p=0·02). In this high-risk disease setting where a key aim is to consolidate any remission achieved with allogeneic SCT, 32% of the quizartinib group were able to proceed to transplant in comparison with 12% of the chemotherapy group. Treatment-emergent adverse events were less frequent in the quizartinib arm than with chemotherapy. A criticism of the QUANTUM-R study has been that a number of patients withdrew from the trial before receiving chemotherapy in the standard of care group. At the 2018 ASH meeting the NCRI group presented data from 264 patients from the UK AML15, 16 and 17 trials who had developed relapsed/refractory *FLT3*-ITD-mutated AML and had gone on to be treated with salvage chemotherapy and described similarly-poor clinical outcomes to those in the QuANTUM-R control group.[68]

Although QuANTUM-R was the first trial to show a survival benefit with FLT3-inhibitor monotherapy in relapsed/refractory FLT3-mutated disease, the FDA have recently recommended against approval of quizartinib in this disease setting, primarily based on relatively modest survival benefits, the perceived high proportion of randomly assigned but untreated patients in the study control arm and continuing concerns over risk of QT prolongation. Quizartinib has, however, recently been approved for this indication in Japan, and is currently undergoing accelerated EMA assessment in Europe.

There are, so far, relatively few published data on the combination of quizartinib with chemotherapy. A phase I trial showed it to be safe and effective in combination with intensive chemotherapy as first-line treatment [69] and quizartinib has also been safely combined with intensive chemotherapy in adults aged >60 years (irrespective of *FLT3* status) by the NCRI group who continue to perform a randomised investigation of its use in this setting in the AML18 study.[70] QuANTUM-First (NCT02668653) is an important phase III randomised study, designed along broadly similar lines to the RATIFY trial of midostaurin, in which 536 newly-diagnosed *FLT3*-ITD-mutated patients are being treated with intensive induction and consolidation chemotherapy accompanied by either quizartinib or placebo; the study has recently completed recruitment and the eagerly-anticipated results will provide the first randomised data on the addition of a highly-targeted second generation FLT3 inhibition to chemotherapy in the frontline disease setting, outcomes inevitably being compared with the 'multi-kinase' inhibitory approach used with midostaurin in RATIFY.

Quizartinib also remains under investigation both in combination with azacitidine and low dose cytarabine [71] as well as in the maintenance setting following allogeneic stem cell transplant.[72]

5.2 Gilteritinib

Gilteritinib (formerly ASP2215) is a pyrazinecarboxamide derivative that has shown activity against all types of *FLT3* positive AML including *FLT3*-D835 which is both the most prevalent *FLT3*-TKD mutation seen at diagnosis and the most commonly-acquired mutation associated with the development of resistance to other FLT3 inhibitors. Gilteritinib is a type I FLT3 inhibitor that also has activity against the RTK Axl.[73] Axl appears to play an important role in the pathogenesis of *FLT3*-ITD positive AML; pre-clinical studies have demonstrated removal of myeloid differentiation block and triggering of apoptosis through Axl blockade in FLT3-ITD-mutated AML, with decreased growth in an in vivo *FLT3*-ITD model.[74]

In recent years gilteritinib has progressed rapidly through an extensive clinical development programme in AML. 265 patients were treated in CHRYSALIS, a large phase I/II dose-escalation trial of gilteritinib monotherapy in which robust FLT3 inhibition and anti-leukaemic effects were shown at all dose levels.[75] Overall response rates (ORR) across the trial were 40% (49% in *FLT3*-mutated patients compared to only 12% in *FLT3* wild type cases) with median response duration of 17 weeks and median OS of 25 weeks. *FLT3*-mutated patients who received gilteritinib doses of ≥80mg/day achieved better responses with ORR 52%, median response duration of 20 weeks and median OS of 31 weeks. Encouragingly, patients who had previously been treated with FLT3 inhibitors and relapsed still achieved an ORR of 37%.

These promising monotherapy results led to ADMIRAL, a phase III trial broadly similar in design to QuANTUM-R. 371 patients with relapsed or refractory AML, this time including both *FLT3*-ITD and TKD-mutated cases, were randomised (in 2:1 ratio) between gilteritinib monotherapy at a daily dose of 120mg and 'standard of care' salvage chemotherapy which could either be intensive (FLAG-Ida, MEC) or non-intensive (LDAC, azacitidine). Prior exposure to midostaurin or sorafenib was permitted. Outcome data, presented in abstract form at the American Association of Cancer Research (AACR) 2019 Annual Meeting showed that the study met its primary endpoint with respective median OS and one-year survival rate of 9.3 months and 37.1% with gilteritinib arm in comparison to 5.6 months and 16.7% with salvage chemotherapy (p=0.0007).[76] 34% of patients in the gilteritinib arm achieved CR/CRi, compared to 15% in the control arm, notably a higher proportion of patients achieving 'full CR' with gilteritinib in ADMIRAL compared quizartinib in QuANTUM-R (21% vs 4%). The greatest survival benefit appears to have been in gilteritinib-treated patients who proceeded to allogeneic SCT and were then able to resume therapy as post-SCT maintenance. Overall, gilteritinib was well-tolerated, most commonly-reported non-haematological adverse events were minor elevations in aspartate aminotransferase (AST) or alanine aminotransferase (ALT), diarrhoea and fatigue.

Analysis of interim ADMIRAL study data led to the FDA's November 2018 decision to approve gilteritinib as monotherapy for adult patients with *FLT3*-mutated relapsed or refractory AML.[77] In Europe, gilteritinib is currently undergoing both accelerated EMA assessment (decision pending) and NICE appraisal in the UK. Outside the relapsed/refractory disease setting, gilteritinib is being assessed

in a number of current and planned trials, both in combination with chemotherapy and in different maintenance settings.

Gilteritinib has been shown to be well-tolerated in combination with intensive chemotherapy in a phase I study in which patients with *FLT3* mutations were observed to achieve non-significantly higher CRc rates and longer EFS and DFS than their *FLT3*-wild type counterparts.[78] The shortly-to-open HOVON156 phase III European intergroup trial is an highly-anticipated study that will compare the addition of either midostaurin or gilteritinib to standard induction and consolidation chemotherapy, then continuing for 12 months as maintenance in 768 patients aged ≥18 years, with newly-diagnosed FLT3-mutated AML; this trial should shed light on the crucial question of whether a relatively non-targeted multi-kinase approach or more highly FLT3-selective approach is superior in the newly-diagnosed disease setting.

Gilteritinib-based maintenance therapy approaches are also being assessed in two further international studies. GOSSAMER (NCT02927262), a randomised phase 2b study of gilteritinib (versus placebo) given for 2 years following completion of intensive chemotherapy for *FLT3*-mutated patients not intended for allogeneic SCT has recently closed to recruitment, patient enrolment becoming challenging following the generalised regulatory approval of midostaurin alongside front-line therapy. Meanwhile, the MORPHO study (NCT02997202) continues to assess the important question of whether long term outcomes will be improved by the addition of gilteritinib (versus placebo) as maintenance therapy for 2 years following allogeneic SCT in 346 *FLT3*-ITD-mutated patients (in the absence of grade 2-4 graft versus host disease at 30-90 days post-transplant). Gilteritinib plus hypomethylating agent approaches are also the subject of an ongoing phase II study (NCT02752035)

5.3 Crenolanib

Crenolanib, the third 'second generation' FLT3 inhibitor currently in advanced clinical development is, like gilteritinib, a 'type I' kinase inhibitor. Although initially developed primarily for its inhibition of PDGFR, crenolanib has been shown to be a potent and selective inhibitor of *FLT3*-ITD as well as several *FLT3*-TKD mutations that have previously conferred resistance to other FLT3 inhibitors, including mutations at D835.[63;79] Inhibition of c-KIT appears minimal with crenolanib, suggesting less potential for causing the myelosuppression that is associated with quizartinib; less favourable pharmacokinetics of crenolanib, however, necessitate thrice-daily dosing.

Although several clinical trials of crenolanib are underway, including phase III studies either recruiting or soon to open, relatively little clinical data have been published to date, with the majority of findings being presented as conference abstracts.

In a phase II study, 69 patients with *FLT3*-mutated, relapsed/refractory AML received thrice-daily crenolanib monotherapy, equating to 200mg/m^{2}/24hrs.[80] Many of the patients had received two or

more prior FLT3 inhibitors. Amongst 18 FLT3 inhibitor naïve patients, 39% achieved CRi, with an additional 11% achieving PR with a median overall survival of 234 days. An encouraging overall response rate of 31% (17% CRi, 14% PR) was seen in 36 relapsed/refractory patients previously exposed to FLT3 inhibitors, including 19 patients with dual ITD and D835 mutations, supporting the potential activity of crenolanib in patients with resistance mutations.

Conference abstracts have also reported on the safe combination of crenolanib with salvage chemotherapy in the setting of relapsed/refractory *FLT3*-mutated AML. In a phase Ib study, 13 patients received idarubicin and high dose cytarabine followed by crenolanib; an ORR of 36% was achieved, with clinical activity demonstrated in patients with combined *FLT3*-ITD and D835 mutations, including those who had received multiple lines of prior therapy including FLT3 inhibitors. In a separate study, efficacy and safety was also demonstrated for the combination of crenolanib with HAM (cytarabine and mitoxantrone) in a group of older patients with primary refractory or relapsed AML.[81] These studies have led to the randomised phase III investigation of crenolanib (versus placebo) in combination with an investigator's choice of either HAM or FLAG-Ida salvage chemotherapy in patients aged 18-75 with relapsed / refractory AML with *FLT3*-activating mutations in the recently-opened international ARO-013 study (NCT02298166).

Crenolanib has also been safely combined with intensive induction and consolidation chemotherapy in newly-diagnosed *FLT3*-mutated AML; in a phase Ib/II trial the combination achieved a CR/CRi rate of 96% and there were noted to be no delays in administering consolidation therapy.[82] ARO-021 (NCT03258931), a phase III study in which 510 newly-diagnosed patients with *FLT3*-mutated AML are to be randomised between crenolanib and midostaurin in combination with standard front-line chemotherapy has recently opened at centres in the US.

Finally, recent 'proof of concept' data have suggested a role for crenolanib (and, by extension, FLT3-inhibitory agents in general) used as an adjunct to FLT3-specific chimeric antigen receptor (CAR) T-cells; co-treatment with crenolanib led to increased surface expression of FLT3 on *FLT3*-ITD-mutated AML cells, enhancing target recognition by engineered FLT3-CAR T-cells both *in vitro* and in vivo.[83]

6. Understanding responses to FLT3 inhibitors – mechanisms of resistance

The clinical trials described in preceding sections of this review are now starting to provide a clearer vision of the clinical benefits that may be achievable when FLT3 inhibitors are employed optimally, either with chemotherapy in newly-diagnosed *FLT3*-mutated AML, as monotherapy in relapsed disease, as maintenance agents following chemotherapy / allogeneic SCT or (most probably) through future strategies that combine these approaches. As we have seen, typical biological responses to FLT3-inhibitors are rapid but transient; in order to make further progress it is important that we understand the barriers that prevent achievement of optimal FLT3 inhibition and the diverse resistance

mechanisms that may lessen drug efficacy. Factors that need to be taken into account here include the pharmacokinetic properties of individual inhibitors (such as plasma protein binding, drug metabolism, CYP3A4 and drug interactions), acquired resistance due to up-regulation of FLT3 ligand (FL) or via increased activity of parallel pro-survival pathways, and through the impact of *FLT3*-TKD mutations that are present at the point of diagnosis or emerge as a consequence of TKI therapy.

In order to achieve meaningful FLT3 inhibition and induce cell death, sufficient active drug levels must be maintained in the plasma; the 'plasma inhibitory activity' assay technique pioneered by Mark Levis at Johns Hopkins, has enabled us to measure drug activity and correlate this with clinical responses alongside a number of the reported trials.[50] Many of the FLT3 inhibitors studied to date are highly protein bound; in some cases <1% of drug is free (and thereby biologically active) in the plasma, a particular problem in trials of the first generation of FLT3 inhibitors where it was frequently difficult to maintain FLT3-inhibitory concentrations at drug doses that were tolerable to the patient.[51;52] Both midostaurin and lestaurtinib are primarily metabolised hepatically via CYP3A4; co-administration with CYP3A4 inhibitors (in the context of AML therapy this is most prominently azole anti-fungal agents) can cause several-fold increases in drug concentration, impacting significantly on both tolerability and the FLT3-inhibitory activity achieved.[52;84] These issues have proved less problematic with the more potent second generation of drugs which have consistently demonstrated sustained abrogation of FLT3 kinase activity by PIA at readily pharmacologically-achievable doses.

Importantly, high levels of FL also reduce the clinical efficacy of FLT3 inhibitors; it is now well-established that treatment of *FLT3*-mutated patients with chemotherapy leads to sustained rises in FL levels through induction and consolidation, increasing FLT3 receptor activation and thereby promoting blast survival.[85] *In vitro* studies have demonstrated that addition of FL at the concentrations encountered in patients significantly shifts the IC_{50} (50% inhibitory concentration) of FLT3 inhibitors upwards, blunting their efficacy against cell lines and primary AML blasts.[86] These observations should be borne in mind when sequencing FLT3 inhibitors with chemotherapy; it may be prudent to commence FLT3 inhibition early after induction chemotherapy prior to the anticipated surge in FL levels whilst, in the FLT3-driven disease relapse setting which is associated with even higher FL levels, it may be preferable to use FLT3 inhibitors as monotherapy, or to consider combination with therapies that appear less likely to increase FL, such as hypomethylating agents or other targeted agents.[60]

FLT3-dependent blasts may develop secondary resistance through up-regulation of compensatory signalling pathways following prolonged exposure to FLT3 inhibitors. Resistant cell lines and primary AML samples have shown up-regulation of PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways in TKI-resistant cases, suggesting potential efficacy of PI3K or MEK inhibitors in this setting.[87;88] A recently-published study in which targeted next generation sequencing was performed in 41 patients developing secondary resistance to gilteritinib monotherapy highlighted the emergence of mutations that activate RAS/MAPK signalling (most commonly in NRAS or KRAS) in 37% of cases; single-cell

targeted DNA sequencing illustrated diverse patterns of clonal selection and evolution in response to FLT3 inhibition.[89] The bone marrow microenvironment also appears to play an important role by rescuing *FLT3*-ITD positive blasts from FLT3 inhibition through mesenchymal stromal interactions or through secretion of fibroblast growth factor 2 (FGF2) and CXCL12-CXCR4; the overexpression of the oncogenic serine/threonine kinase Pim1 appears to be a key regulator of this process. Approaches of combining FLT3 inhibition with either direct inhibition of Pim-1 or inhibition of CDKs 4 and 6 (kinases that regulate transcription of both FLT3 and Pim-1) are currently the subject of early phase studies (NCT02078609, NCT03132454). Anti-apototic proteins including Bcl-2, Bcl-xL and MCL-1 have also been observed to be up-regulated in TKI-resistant *FLT3*-ITD mutated AML;[90;91] there is now considerable interest in incorporation of Bcl-2 inhibition into AML treatment schedules and a phase 1b study combining gilteritinib with venetoclax is currently in progress (NCT03625505).

FLT3-TKD mutations are generally associated with single amino acid changes in FLT3 activating loop (eg D835, I836, D839, y842) or gatekeeper residues (eg. F691) that impact on the binding affinity of FLT3 inhibitors and tend to confer cross-resistance within the same class of TKI; while all FLT3 inhibitors have activity against ITD-mutated FLT3, type II inhibitors such as sorafenib and quizartinib do not have significant activity in patients with TKD point mutations. Treatment-emergent TKD mutations are an important mechanism of FLT3 inhibitor resistance; their emergence may be related to the specific properties of a particular TKI that selects pre-existent resistant cellular sub-clones that are able to evade the inhibitor. As far back as 2004, results of a laboratory drug resistance screen identified point mutations at 4 positions within the ATP-binding pocket of FLT3 that conferred *in vitro* resistance to midostaurin;[92] one of these (N676K) was subsequently described in a clinically-responsive *FLT3*-ITD AML patient who became resistant to the drug after 280 days of treatment.[93] Different FLT3 inhibitors generate clearly distinct patterns of secondary resistance mutations [94;95]; combination therapy may be a way of overcoming this. Reassuringly, the newer TKIs have demonstrated clinical activity in the setting of prior FLT3 inhibitor exposure and resistance [76;96]. Crenolanib, for example, appears to cover both activating loop and gatekeeper (D691) mutations, showing *in vitro* efficacy against quizartinib-resistance mutations and clinical activity in D835-mutated relapsed patients. [79;80] It should be emphasised, however, that, while important to factor into our thinking about best application of FLT3 inhibitor therapy, treatment-emergent TKD mutations are by no means the exclusive mechanism through which patients become resistant to FLT3 inhibitors; in one study only 22% of patients receiving FLT3 inhibitor treatment had a newly-detectable TKD mutation at the point of disease progression.[97]

7. Current Position, Prospects and Future Directions

After well over a decade of pre-clinical and clinical development, the use of FLT3-directed therapy in AML can finally begin to be considered to form part of 'standard of care' following recent approvals by the FDA, firstly of midostaurin for the treatment of *FLT3*-mutated AML in frontline combination with intensive daunorubicin and cytarabine-containing induction and consolidation regimens,[40] and

more recently of gilteritinib, used as monotherapy for the treatment of relapsed/refractory *FLT3*-mutated AML[77]. Additionally, sorafenib continues to be accessed 'off-label' by many AML-treating physicians and quizartinib remains under accelerated regulatory assessment. Despite these significant developments, many fundamental questions remain over how we should best apply these drugs in the real world clinical setting. How should FLT3 inhibitors be timed and sequenced with chemotherapy and when are they better used alone? When might a multi-targeted first generation inhibitor conceivably hold advantages over a more FLT3-specific second generation agent? Should these agents impact upon the selection of patients for allogeneic SCT in first remission and how might maintenance therapy be best incorporated around this: with which drug and for how long? How should we seek to incorporate FLT3 inhibitors alongside a range of other recently-approved AML therapies when combination safety and efficacy data are currently limited or non-existent?

AML is a complex, highly heterogeneous and usually polyclonal disease; *FLT3* mutations tend to be relatively late events in leukaemogenesis meaning that, at the time of initial diagnosis only a relatively small subset of leukaemic blasts may be dependent on FLT3 signalling. In this setting, a relatively 'broad spectrum' TKI which has additional anti-leukaemic effects against c-KIT, PKC, VEGF and PDGFR may actually be more advantageous than a more highly FLT3-specific agent. Midostaurin is one such agent and, to date, the only TKI to have demonstrated survival benefit over placebo in frontline treatment of *FLT3*-mutated AML.[39]. Given the breadth of kinases inhibited by midostaurin and the relative lack of correlative pharmacodynamic data obtained within RATIFY, the degree of survival benefit actually attributable to FLT3 inhibition within that study remains debatable, indeed an international phase III study is currently assessing the benefits of adding midostaurin (versus placebo) to intensive chemotherapy in newly-diagnosed patients with *wild type* FLT3 (NCT03512197). At the time of writing, midostaurin represents 'standard of care' and it remains far from clear whether second generation drugs will prove superior in the context of newly diagnosed *FLT3*-mutated disease. This question will be at least partly addressed by the soon-to-complete phase III QuANTUM-First study (quizartinib vs placebo with chemotherapy) and should be comprehensively answered by the upcoming phase III HOVON156 (gilteritinib vs midostaurin) and ARO-021 (crenolanib vs midostaurin) studies which will recruit over the next 4-5 years.

Following achievement of first complete remission, allogeneic SCT remains a key part of therapy for many *FLT3*-ITD-mutated patients, although its role remains controversial and practice is by no means uniform in this area. Given the repeated observation that FL levels rise through induction and consolidation chemotherapy, potentially protecting cells that are 'FLT3 addicted' and chemotherapy resistant which are then able to re-emerge at relapse, there is certainly a strong argument to start planning towards allogeneic SCT right from the point at which *FLT3*-ITD-mutated AML is diagnosed and to then move rapidly towards transplant upon achievement of remission.[85] Benefits from transplant may not be universal, however, the AML-SG reported that in 323 consecutively presenting patients with *FLT3*-ITD-mutated AML only those with presenting *FLT3* ITD to WT allelic ratios >0.5 derived an overall survival benefit from allogeneic SCT [10]; this is reflected in the European

LeukaemiaNet Guidelines which currently recommend that allogeneic SCT is *not* routinely performed in first CR for those patients with low *FLT3*-ITD allelic burden (see Table 1).[16] Additional information provided by minimal residual disease (MRD) assays can further inform transplant decision making in this context; in the NCRI AML17 study relapse rates in *FLT3*-ITD patients with co-mutation of *NPM1* who tested *NPM1* qPCR negative in the blood upon recovery from a second cycle of intensive chemotherapy fell to the same level as that seen in favourable risk *FLT3* wild type, *NPM1*-mutated patients. [98]

Relapses of *FLT3*-ITD-mutated AML frequently occur following first remission durations of under 6 months and are generally associated with dismal clinical outcomes; rates of CR in response to salvage chemotherapy regimens are only 11-13% with poor associated long term survival.[51;99] At relapse, disease tends to be 'less polyclonal' with higher *FLT3* mutant allelic burden and greater 'addiction' to FLT3 signalling; this relative chemotherapy-resistance may be related to upregulation of anti-apoptotic proteins such as MCL-1.[29;91] In this setting, the recent results of QuANTUM-R and ADMIRAL have now demonstrated the relative merits of highly FLT3-targeted quizartinib or gilteritinib monotherapy over salvage chemotherapy, both drugs being generally well-tolerated and extending survival through bridging to transplant although long-term responses remain relatively elusive.[67;76;96] It seems relatively unlikely that combining a second generation FLT3 inhibitor with traditional salvage chemotherapy will bring additional benefit; chemotherapy-induced surges in FL level could blunt the efficacy of the FLT3 inhibitor in these circumstances, along with an expected cumulative increase in toxicity; the merits of such an approach will be clarified by the currently-open ARO-021 study of salvage chemotherapy with either crenolanib or placebo (NCT03258931). Outside ongoing trials, current clinical data appear to favour treating relapsed/refractory *FLT3*-ITD mutated patients with an approved, highly FLT3-selective inhibitor (selected according to local regulatory approvals [77] or available 'compassionate access' programmes, with sorafenib remaining as an off-label alternative) and then moving promptly to allogeneic SCT once a marked reduction in bone marrow blasts has been achieved. It remains to be seen whether combination of FLT3 inhibitors with hypomethylating agents or other targeted therapeutic agents will bring additional benefit in this setting.

Although there is clearly a strong biological rationale to use FLT3 inhibitors as maintenance therapy following achievement of remission to prevent re-emergence of residual *FLT3*-mutated clones and reduce the incidence of relapse there are, as yet, very limited clinical data to support this. Midostaurin has received regulatory approval in some jurisdictions for maintenance use for 12 months following chemotherapy (but not post-SCT). Although the results of the SORMAIN study, presented to date only in abstract form, have provided encouragement that sorafenib (and by extension, other FLT3 inhibitors) will provide significant benefit in reducing rate of relapse and extending overall survival post-SCT, the absence of frontline midostaurin therapy for patients treated in that trial may limit full extrapolation of its results to patient management.[62] It is unclear whether second generation drugs will hold advantages in the maintenance setting; again there is a biological argument that more FLT3-selective agents may be more effective in eliminating low level 'FLT3-addicted' sub-clones that are attempting

to cause relapse. Two important placebo-controlled trials of gilteritinib, following completion of chemotherapy (GOSSAMER) and after allogeneic SCT in first CR (MORPHO) are ongoing and will hopefully provide clarification on the relative contribution of maintenance FLT3 inhibition in either setting. Maintenance therapy may ultimately need to be applied more strategically; the presence/absence of MRD following completion of 'standard therapy' may be important in delineating which sub-groups of patients stand to benefit most (and in which groups it would be better to avoid the potential additional toxicities of extended TKI treatment); it is also important that clinical trials pay heed of the effects of maintenance therapy on evolving clonal architecture and the acquisition of *FLT3*-resistance mutations.

The recent regulatory approvals of midostaurin and gilteritinib have happened during a time of unprecedented wider advancement in AML therapeutics, occurring within 12-18 months of FDA approvals of the anti-CD33-chemotherapy conjugate gemtuzumab ozogamicin (GO, Mylotarg) and the liposomally-delivered combination cytotoxic CPX-351 (Vyxeos) for use in front-line intensive therapy[100;101]; the IDH1 and IDH2 inhibitors ivosidenib and enasidenib, BCL-2 inhibitor venetoclax and smoothened inhibitor glasdegib have also been approved for non-intensive treatment indications. In some cases, these approvals create clinical conflicts where more than one novel agent may be indicated and approved for a particular clinical situation (for example GO and midostaurin in a newly-diagnosed AML patient expressing CD33 with non-adverse cytogenetics and *FLT3* mutation) but with an absence of safety data to support combining the treatments. The observations that *FLT3*-ITD mutated patients potentially benefit from intensification of induction chemotherapy [102] and that *FLT3*-mutated AML blasts express high levels of CD33[103] has stimulated interest in combining FLT3-directed TKI therapy with GO; the UK NCRI AML19 study is seeking to collect safety and efficacy data on the joint combination of GO and midostaurin with daunorubicin/cyatarbine induction chemotherapy in the setting of newly-diagnosed *FLT3*-mutated AML and this may inform subsequent randomised assessment.

Recently-published phase 2 studies have demonstrated highly promising activity of the Bcl-2 inhibitor venetoclax when combined with either low dose cytarabine or hypomethylating agents in the treatment of newly-diagnosed AML in older adults who are not candidates for intensive chemotherapy and, following approval by the FDA, venetoclax-containing combinations are rapidly emerging as a new standard of care in this setting.[104;105] As discussed above, there is considerable biological rationale for assessing Bcl-2 inhibition in *FLT3*-mutated AML; a phase 1b study combining gilteritinib with venetoclax is already in progress (NCT03625505) and it seems probable that trials of 'triplet combinations' including hypomethylating agents (or low dose cytarabine), venetoclax and FLT3 inhibitors will follow, moving towards the frontline treatment setting for less fit, older *FLT3*-mutated patients (and potentially, with time, potentially impacting on treatment of younger, fitter patients). There is now also a strong biological rationale to support clinical combination of FLT3 inhibitors and IDH-directed therapies in *FLT3*, *IDH1/2* co-mutated patients.

8. Conclusion

Writing in 2019, it is now more than 20 years since the first description of *FLT3*-ITD mutations in AML [5] and 15 years since publication of the first early phase studies of FLT3-directed TKI therapies. [36;48] While there has been no shortage of bumps along the road to their subsequent clinical development, FLT3 inhibitors are now approved therapeutic options, for both newly-diagnosed and relapsed/refractory *FLT3*-mutated AML patients. The field continues to evolve rapidly and our research priorities now lie in trying to better-define the optimal deployment of the range of currently-available inhibitors, and particularly to learn how to best strategically combine and sequence them with established and recently-approved therapies and more newly-emerging therapeutic approaches.

Abbreviations

Have been defined within the text where first used.

Conflicts of Interest

SK: Astellas: advisory board membership. Daiichi Sankyo: advisory board membership, support for conference attendance. Novartis: advisory board membership, research funding, speaker bureau, support for conference attendance.

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The manuscript is well written to review FLT3 inhibitors including the latest data. However, several reviews have already been made and it was difficult to find authors' original viewpoint. In the chapter on resistance mechanisms, the paper by McMahon et al. (Cancer Discov. 2019) should be cited and discussed. For readers, it is better to describe the detection method of FLT3 mutations.

Minor:
1. P.3, line 3, p. 7, line 11. "3+7" and "7+3" are confused. "7+3"is more common.
2. P.10, line 1. "PIAs" here should be written in full spell.
3. P10. line 14. "addition or" should be "addition of".
4. English words had better to be considered for readers whose native language is not English. For example: p.18, "moot": p. 20, "nuanced"; p.21, "armamentarium".

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