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Incorporating FLT3 inhibitors in the frontline treatment of *FLT3* mutant acute myeloid leukemia

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ABSTRACT

FLT3 mutations occur in up to a third of newly diagnosed patients with acute myeloid leukemia (AML) and confer poor prognosis. Clinical development of FLT3 tyrosine kinase inhibitors for AML initially involved broad-spectrum inhibitors (midostaurin, sorafenib) targeting multiple kinases. Addition of midostaurin to upfront intensive chemotherapy for younger patients with *FLT3* mutant AML significantly improved overall survival and validated FLT3 as a therapeutic target. Other regimens such as sorafenib and hypomethylating agents (azacitidine, decitabine) have expanded the use of FLT3 inhibitors to other populations with *FLT3* mutant AML. However, emerging data on new highly potent and specific FLT3 inhibitors such as quizartinib, gilteritinib, and crenolanib suggest that these agents may soon supplant midostaurin and sorafenib in the upfront setting. Using case presentations, this review provides guidelines and practical management strategies for frontline therapy of patients with newly diagnosed *FLT3* mutant AML in the current era.

Introduction

The fms-like tyrosine kinase 3 (*FLT3*) gene encodes a transmembrane tyrosine kinase receptor normally expressed on multipotent hematopoietic progenitor cells. Interaction of the kinase with FLT3 ligand within the marrow microenvironment induces a conformational change and autophosphorylation of the ATP binding site. Activation of downstream pathways mediated by STAT, MAPK/ERK, and PI3 kinase promote proliferation, survival, and differentiation [1]. Mutations in the *FLT3* gene have been identified in 25–37% of newly diagnosed cases of acute myeloid leukemia (AML) and result in constitutively active mutant FLT3 kinases with unchecked growth [2]. The majority of mutations (25–30% of AML cases) consist of internal tandem duplications (*ITD*) in the juxtamembrane region, while a minority (7–10%) are point mutations in the tyrosine kinase domain (*TKD*), typically in the D835 aspartic acid residue.

The significance of FLT3 mutations in AML prognosis and the advent of multiple tyrosine kinase inhibitors of mutant FLT3 have transformed the therapeutic landscape for the subset of AML patients with this disease. Here we describe the incorporation of FLT3 TKIs into upfront AML therapy, with an in depth discussion of the current data obtained with midostaurin and cytarabine and anthracycline-based induction and consolidation chemotherapy and off label use with sorafenib. We will also discuss emerging data and the promise of gilteritinib, quizartinib, and crenolanib in the frontline setting in combination with intensive and non-intensive chemotherapy regimens. This review provides recommendations for the frontline management of newly diagnosed patients with *FLT3* mutant AML using two case presentations as examples for discussion of practical management strategies and future therapeutic approaches.

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Case presentation 1

Ms. B is a 55 year-old woman with a prior medical history of hypertension and high cholesterol who reports a prolonged nose bleed two weeks ago. She present to a local emergency room with multiple complaints including shortness of breath on exertion, generalized muscle weakness, a new bruise on her left thigh, and intermittent bleeding of the gums with tooth brushing. Exam demonstrates a middle-aged woman with clear lungs, rapid heart rate, and a large ecchymosis on her left thigh. Laboratory work reveals a WBC of 225,000/μL, hemoglobin 10.5 gm/dl, and platelet count of 51,000/μL with 95% peripheral blasts. The patient received oral hydroxyurea, IV fluids, and tumor lysis prophylaxis. Morphological and flow cytometric analysis of the bone marrow confirms a diagnosis of acute myeloid leukemia with 98% marrow blasts. Karyotype is female XY. Marrow samples are sent to an outside commercial laboratory for *FLT3* mutation testing. Echocardiogram demonstrates normal left ventricular ejection fraction. After discussion of the diagnosis and treatment options, the patient initiates intensive induction chemotherapy with cytarabine 100 mg/m^2 and daunorubicin 60 mg/m^2 (7 + 3). Three days later, the results of molecular testing confirms the presence of a *FLT3 ITD* mutation with an allelic ratio of 0.60. On day 8 following completion of cytotoxic chemotherapy, midostaurin 50 mg bid is started and continued for 14 days. In the interim, the patient and her five siblings are HLA-typed, and she is referred for allogeneic stem cell transplantation consideration.

Prognostic significance of *FLT3* mutations

Acute myeloid leukemia is a biologically heterogeneous disease arising from age-related clonal heterogeneity and other causes. Patients with *FLT3* mutant AML may have disease characterized by sole (*ITD* vs *TKD*) or multiple *FLT3* mutations (i.e. more than one *ITD* or *TKD* mutation or concomitant *ITD* and *TKD* mutations). Although *FLT3* mutations are among the more commonly identified molecular aberrations described at the time of AML diagnosis, the prognostic impact of *FLT3* mutations varies based on the type of mutation (*ITD* vs. *TKD*), *ITD* allelic ratio, presence of concomitant mutations, and therapeutic intervention. Multiple prior studies have demonstrated *FLT3 ITD* mutations to be most closely associated with poor outcomes, specifically higher presenting white blood cell counts, similar response rates but shorter relapse-free and overall survival. High *FLT3 ITD* allelic ratio (> 0.51) and *NPM-1* wildtype has been categorized as adverse risk prognosis by the European Leukemia Net (2017) and the National Comprehensive Cancer Network (NCCN) with the recommendation that these patients undergo allogeneic stem cell transplantation in first remission. Patients with high *FLT3* ITD allelic ratio and *NPM-1* mutations are classified as intermediate risk. Whether patients with low *FLT3 ITD* allelic ratio truly benefit from allogeneic stem cell transplant is not known. In addition, these prognostic classifications were developed in the context of chemotherapy regimens without incorporation of FLT3 inhibitors. Whether *FLT3 ITD* allelic ratio in any way predicts responses to therapy including targeted FLT3 inhibition remains controversial. The prognostic significance of *FLT3 TKD* mutations at AML diagnosis remains even less clear and under debate based on publication of many studies demonstrating no favorable or adverse risk implications; however the subsequent development of *FLT3 TKD* mutations as a mechanism of resistance to following prior FLT3 inhibition does connotate poor outcome.

FLT3 mutation testing

Incorporation of FLT3 TKIs into frontline therapy for AML necessitates that all patients suspected of new AML diagnoses undergo testing for *FLT3* mutation (both *ITD* and *TKD*) as standard of care. Turnaround time for mutation results is critical and should range from 3 to 5 business days in order to realistically allow for initiation of TKI therapy by day 8 of induction chemotherapy. Multiple commercial labs currently offer *FLT3* mutation testing meeting these criteria and should be sought out preferentially as opposed to local lab testing, which may not meet this time frame. Based on the sensitivity of the assay, peripheral blood samples containing circulating blasts may be able to be sent *in lieu* of marrow samples. Clinicians are encouraged to discuss these technical aspects with the performing laboratory. Following sample collection, it is not necessary to withhold cytarabine and daunorubicin induction chemotherapy while awaiting *FLT3* results. Cytotoxic chemotherapy may be initiated with the option to deploy TKI by day 8. Given the approval of FLT3 targeted inhibitors in this setting, *FLT3* mutation assessment is reimbursable by insurance companies. Although it may be tempting to perform broad spectrum next generation sequencing (NGS) panels for multiple gene mutations including *FLT3* on newly diagnosed patients, caution should be exercised as the results of these multi-gene panels are often not readily available in 3–5 days, thereby precluding initiation of TKI therapy on day 8. At our institute, we perform PCR for *FLT3 ITD* and *D835* mutations with a turnaround of 72 h in addition to sending samples for comprehensive NGS with a turnaround of 10–17 days.

FLT3 inhibitor and intensive chemotherapy

Since the discovery of mutant FLT3 kinases as a key driver in AML biology, significant efforts have been devoted to the development of clinically active tyrosine kinase inhibitors (TKI) of mutant FLT3. Initial studies focused on the use of known broad-spectrum multi-kinase inhibitors (i.e. sunitinib, lestaurtinib, sorafenib and midostaurin) repurposed for the treatment of *FLT3* mutant AML based on their *in vitro* inhibitory activity against FLT3 kinases. However early trials of these agents as monotherapy in patients with *FLT3* mutant relapsed and/or refractory AML were largely unsuccessful, resulting only in transient peripheral blast clearance with less than 10% of patients achieving remissions [3]. Subsequent analyses revealed that many of these multi-kinase inhibitors did not in fact exert potent or sustained *in vivo* FLT3 inhibition in most patients, potentially accounting for the lack of clinical efficacy as monotherapy. Based on these findings, clinical trials were developed combining these multi-kinase inhibitors with intensive cytotoxic

chemotherapy in the upfront and relapsed/refractory settings.

Midostaurin is a broad-spectrum multi-kinase inhibitor with activity against *FLT3 ITD* and *TKD* mutations as well as multiple other kinases including c-KIT, VEGF, and PDGF receptors. Although not specifically developed as a FLT3 inhibitor, this drug constitutes the first agent approved for the treatment of *FLT3* mutant AML in combination regimens. Multiple phase 1 trials of midostaurin in relapsed/refractory *FLT3* mutant AML led to transient peripheral blast reductions but < 10% partial or complete remissions. However, the addition of midostaurin to induction chemotherapy with infusional cytarabine and daunorubicin followed by high dose cytarabine consolidation significantly enhanced clinical outcomes of patients with *de novo FLT3 ITD* and *TKD* mutant AML [4]. In the international multi-center RATIFY trial, over 2000 patients were screened to identify 717 adult patients aged 18–60 years old with newly diagnosed *FLT3* mutant AML. These individuals were randomized to either placebo or midostaurin 50 mg orally twice daily given on days 8–21 of induction (daunorubicin 60 mg/m^2 D1-3 and cytarabine 200 mg/m^2 D1-7) and multiple cycles of consolidation (cytarabine 3 g/m^2 q 12 h on days 1, 3, and 5) chemotherapy. Patients not proceeding to allogeneic stem cell transplant (alloSCT) received up to 12 months of additional maintenance therapy with midostaurin 50 mg bid or placebo. Midostaurin and chemotherapy did not significantly improve complete remission (CR) rates over placebo plus chemotherapy with a protocol-defined complete remission (CR) rate of 58.9% (95% CI, 53.6 to 64.0) in the midostaurin group and 53.5% (95% CI, 48.2 to 58.8) in the placebo group (P = 0.15 by Fisher's exact test). However, using an expanded CR definition (CRs during protocol treatment and those in the 30 days following treatment discontinuation), a significantly higher CR rate was noted in midostaurin-treated patients compared to placebo (68% vs 61%, two-sided Fisher's exact p = 0.04) Moreover, both overall (hazard ratio for death, 0.78; one-sided P = 0.009) and event-free survival (EFS) (hazard ratio for event or death, 0.78; one-sided P = 0.002) was significantly prolonged in the midostaurin arm vs. the placebo arm. Benefit was seen in both *FLT3 ITD* mutant patients (regardless of variant allelic ratio, believed to reflect *FLT3* mutant clonal burden) and *FLT3 TKD* mutant patients with and without censoring for transplant. These results translated into a 7.2% absolute improvement in 4-year OS with 51.4% of midostaurin treated patients vs. 44.2% of placebo surviving to this time point [4].

Treatment with midostaurin and chemotherapy for *FLT3* mutant AML is not ideal. Notably, the risk of relapse from CR remained high despite FLT3 TKI therapy. Only 46.4% of patients achieving CR after midostaurin remained disease free after 4 years with an ongoing risk of relapse, especially during the first year after treatment end. Also, in the original trial design, midostaurin was planned to be administered at twice the dose (100 mg BID) continuously following chemotherapy; both the dose and duration of TKI therapy had to be reduced to 50 mg BID for 14 days due to adverse events, largely gastrointestinal [4]. Midostaurin also Interacted with other concomitant medications, resulting in alterations in levels and QTc prolongation, particularly in combination with azole drugs. A higher proportion of patients in the midostaurin arm were noted to have QTc prolongation as compared to placebo (QTcF > 480 ms: 10.1% vs 5.7%; QTcF > 500 ms: 6.2% vs 2.6%; QTcF > 60 ms: 18.4% vs 10.7%) [5]. Moreover, issues with nausea, vomiting and diarrhea have limited the ability of patients to remain on long-term midostaurin maintenance without dose reductions or interruptions. Although midostaurin was not approved for maintenance therapy of *FLT3* mutant AML, recent post-hoc analysis supported the importance of continued midostaurin dosing as well as alloSCT in reducing cumulative risk of relapse [6].

Other first generation multi-kinase inhibitors have been investigated in combination with cytarabine and anthracycline based induction and consolidation chemotherapy for newly diagnosed FLT3 mutant AML patients. Two randomized phase 3 trials of lestaurtinib added to induction and salvage chemotherapy, respectively, for newly diagnosed and relapsed/refractory *FLT3* mutant AML patients resulted in no significant difference in OS between the two arms [7,8]. However, in both trials, those lestaurtinib treated patients shown to achieve potent (> 85%) *in vivo* FLT3 inhibition via correlative assays demonstrated significantly improved remission rates [8] and OS [7]. These data suggested that the development of newer generation FLT3 TKIs exhibiting more potent inhibition of FLT3 kinases could further improve outcomes.

Second generation FLT3 TKIs, consisting of gilteritinib, quizartinib, and crenolanib (Table 1) have been specifically developed for clinical use on AML patients based on their ability to selectively and potently inhibit mutant FLT3 kinases. In addition to their kinase profiles, these agents can also be distinguished by their pharmacokinetics, ability to inhibit FLT3 ITD vs TKD mutations, and toxicity profile. Two agents (gilteritinib and crenolanib) exhibit activity against both *FLT3 ITD* and *TKD* mutant AML cells. In contrast, quizartinib and sorafenib solely bind the active formulation of FLT3 kinase and therefore only inhibitor *FLT3 ITD*, not *TKD*, mutant AML cells. All three newer generation TKIs have demonstrated significantly more clinical activity as monotherapy in the relapsed/refractory AML setting with overall response rates of 40–50% in small studies [9–11]. For example, two randomized phase 3 trials of gilteritinib or quizartinib compared with salvage chemotherapy in patients with first relapsed *FLT3* mutant AML have both convincingly demonstrated significant improvement in response rates as well as OS following FLT3 inhibitor monotherapy. Salvage chemotherapy regimens consisted of cytarabine and mitoxantrone based chemotherapy, azacitidine, or low dose cytarabine [12,13]. Based on these data, gilteritinib was recently approved by in the United States for relapsed/refractory *FLT3* mutant AML, with quizartinib expected to undergo regulatory review later in 2019.

New generation FLT3 inhibitors have actively been explored in combination with intensive chemotherapy to potentially supplant midostaurin in the upfront setting. Early results in small phase 1 and 2 trials confirm that all three TKIs (quizartinib, crenolanib, and gilteritinib) can safely be administered in these settings [14–16]. More importantly, all three TKIs combined with 7 + 3 resulted in encouragingly high CR rates of 80–90% which compare favorably with the 55–59% CR rate reported in the RATIFY trial [4]. However randomized phase 3 trials comparing these agents directly with midostaurin are needed to determine the “best” TKI to be used in the upfront setting (Table 2).

Quizartinib is a highly potent, type 2 inhibitor of FLT3 ITD without activity against FLT3 TKD. Altman and colleagues reported an 84% overall response rate in 16 evaluable AML patients treated with quizartinib plus intensive chemotherapy in a phase 1 trial. The maximum tolerated dose of quizartinib was 40 mg for two weeks with no unexpected toxicities [15]. Based on these results, the

Table 1
Summary of FLT3 inhibitors used in upfront therapy of FLT3 mutant AML.

Agent	Kinase inhibitory profile	Dose	Half-life in humans	Activity against FLT3 TKD mutation	Adverse side effects of interest
Midostaurin	VEGFR2, cKIT, PDGFR, FLT3	50 mg BID	21 h	Yes	Nausea, vomiting, diarrhea
Sorafenib	RAF, MEK, ERK, BRAF, KIT, VEGFR, PDGFR, FLT3	400 mg BID	24–38 h	No	Oral ulcers, rash, liver abnormalities
Quizartinib	CKIT, PDGFR, CSF1R, FLT3	30 mg QD	1.5–3.5 days	No	QTC prolongation, myelosuppression
Gilteritinib	AXL, ALK, FLT3	120 mg QD	45–159 h	Yes	Myelosuppression,
Crenolanib	PDGFR, FLT3	100 mg TID	6–8 h	Yes	Liver abnormalities, nausea

Table 2
Clinical trials of FLT3 inhibitors combined with intensive chemotherapy for frontline AML therapy.

FLT3 inhibitor + chemotherapy	Trial design	Enrolled/goal	Primary results to date
Midostaurin vs Placebo plus 7 + 3 (cytarabine + daunorubicin) [15]	Phase 3	717 patients	CR/CRi: Midostaurin 54% vs placebo 59% (p = NS) 4 yr OS: Mido 51.4% vs 44.3% (7.1% difference)
Midostaurin plus 7 + 3 (cytarabine + daunorubicin or idarubicin) and maintenance	Phase 3b	300 patients	Currently accruing
Quizartinib plus 7 + 3 [18]	Phase 1	19 patients	CR/CRi = 84%; well tolerated
Quizartinib vs. Placebo plus 7 + 3 (cytarabine + daunorubicin)	Phase 3	536 patients	Completed accrual; Results pending
Crenolanib plus 7 + 3 (cytarabine + daunorubicin/idarubicin) [17]	Phase 2	38 patients	CR/CRi = 88%; 2 year OS = 79%
Crenolanib vs. Midostaurin plus 7 + 3 (cytarabine + daunorubicin)	Phase 3	510 patients	Currently accruing
Gilteritinib plus 7 + 3 (cytarabine + idarubicin or daunorubicin) [19]	Phase 1	70 patients	CR/CRi = 93% (n = 30)

QUANTUM-FIRST trial was initiated randomizing newly diagnosed *FLT3* ITD mutant patients to quizartinib or placebo plus intensive chemotherapy. Results of this study which has completed accrual are pending [15].

Gilteritinib and crenolanib represent type 1 inhibitors of mutant FLT3 with activity against the mutant *FLT3 ITD* and *TKD*. Pratz and colleagues recently reported on the results of 50 *FLT3* wildtype and mutant AML patients treated with gilteritinib plus intensive chemotherapy in the upfront setting. Two patients developed dose-limiting toxicities of neutropenia, thrombocytopenia, and decreased ejection fraction, requiring schedule modification. In the dose expansion cohort, gilteritinib (120 mg daily) and chemotherapy resulted in a CR rate of 91.3% in *FLT3* mutant patients [16]. Plans for a randomized phase 3 study comparing gilteritnib vs. midostaurin added to intensive chemotherapy are underway [4]. Crenolanib is a novel highly potent inhibitor of mutant *FLT3* with limited activity against cKIT and other kinases. A phase 2 study of crenolanib (100 mg TID) added to induction therapy in 38 adult patients with newly diagnosed *FLT3* mutant AML demonstrated promising results. In this study, patients received 7 + 3 with crenolanib starting on day 9 of induction continuously until 24 h prior to consolidation chemotherapy. Notably, 84% of patients were able to continue on full dose crenolanib 100 mg TID during induction, and crenolanib was not held or delayed due to low counts during therapy. In the 32 evaluable patients aged 18–60 years old, Wang et al. reported an overall CR rate of 88% with 42% bridged to alloSCT. After a median follow-up of 17.6 months, 79% of patients will still alive [14].

Of note, the dose and choice of anthracycline drug to be used in upfront 7 + 3 intensive induction chemotherapy regimens for *de novo FLT3* mutant AML patients is still under debate. The phase 3 ECOG-ACRIN E1900 trial demonstrated significantly improved outcome for FLT3 mutant AML patients receiving higher dose (90 mg/m^2) of daunorubicin as compared with 45 mg/m^2 (hazard ratio 0.61) with a 10% improvement in 4-year overall survival [17]. A United Kingdom national AML study determined that *FLT3* mutant AML patients receiving 90 mg/m^2 as opposed to 45 mg/m^2 experienced fewer risk of relapse and higher overall survival; however all patients received two induction cycles [18]. A recent Korean cooperative group study recently demonstrated that high dose daunorubicin (90 mg/m^2) resulted in better outcomes that idarubicin (12 mg/m^2) in FLT3 mutant disease [19]. To add to this conflicting data, different anthracycline-based regimens have been employed in combination with FLT3 kinase inhibitors. The RATIFY trial utilized daunorubicin 60 mg/m^2 [4], while a phase 2 trial of crenolanib plus 7 + 3 allowed varying doses of daunorubicin 60 or 90 mg/m^2 or idarubicin based on institutional standard at the various accruing sites [14]. A phase 1 trial of gilteritinib plus 7 + 3 originally utilized idarubicin 12 mg/m^2 in the dose escalation phase but has recently opened cohorts substituting daunorubicin 90 mg/m^2 in induction [16]. So far, the use of higher dose daunorubicin together with new generation FLT3 inhibitors in younger patients appears safe without increased cardiotoxicity. Whether the improved response rates seen following these regimens result from the new generation FLT3 inhibitor and/or the high dose daunorubicin is not known. A current clinical trial is evaluating the safety and efficacy of midostaurin added to cytarabine and either high dose (90 mg/m^2) vs. standard dose (60 mg/m^2) daunorubicin or idarubicin (12 mg/m^2) in younger patients with *de novo* FLT3 mutant disease (ClinicalTrials.gov: NCT03379727).

Selection of FLT3 inhibitor based on mutation type

Currently available FLT3 inhibitors may be divided into two types based on their ability to inhibit different formulations of FLT3 kinase. Type 1 inhibitors such as midostaurin, crenolanib, and gilteritinib bind active and inactive receptors near the activation loop and/or the ATP binding pocket and therefore exert activity against both *FLT3 ITD* and *TKD* mutations. In contrast, type 2 inhibitors such as sorafenib and quizartinib bind inactive FLT3 receptors near the ATP-binding domain and only inhibit *FLT3 ITD* mutations, not *TKD*. In the RATIFY trial, patients with *FLT3 ITD* mutant disease were divided into two groups based on the *ITD* allelic ratio reflecting mutant over wildtype FLT3 expression levels. Although patients with AML characterized by higher *FLT3 ITD* allelic ratio were hypothesized to have improved response rates to FLT3 inhibitors, midostaurin plus intensive chemotherapy was equally effective in *FLT3 ITD* mutant patients with high and low allelic ratios as well as in *TKD* mutant disease [4]. In contrast, sorafenib and quizartinib are ineffective against *FLT3 TKD* mutant disease present at AML diagnosis or developing over time following exposure to these TKIs. Identification of the type of *FLT3* mutation therefore is important in selection of the appropriate TKI for upfront therapy.

Discussion of case 1

In this case, the patient was an appropriate candidate for upfront therapy of newly diagnosed AML. Prompt testing for FLT3 mutation testing at diagnosis allowed identification of a FLT3 ITD mutation associated with poor prognosis, particularly given the high allelic ratio. Rapid turnaround of FLT3 test results allows for the addition of midostaurin, the only approved FLT3 inhibitor in the frontline setting, to be added to her treatment regimen on day 8 of induction chemotherapy. Given the results of the RATIFY trial, strong consideration should be given to allogeneic stem cell transplantation in first complete remission following midostaurin therapy for optimal long-term survival. If available, the patient would also be a candidate for clinical trials of second generation FLT3 inhibitors vs. midostaurin in addition to intensive chemotherapy in the upfront setting.

Case presentation 2

Mr H is a 70 year-old man with a prior medical history of coronary artery disease and previous coronary bypass surgery who presented to his outside physician with complaints of dyspnea on exertion and intermittent chest pain. Exam was unremarkable. EKG and chest X-ray as well as chest CT revealed no new cardiopulmonary issues. However laboratory work demonstrates an elevated white blood cell count of 58,000/μL, hemoglobin 9.4 gm/dl and platelet count 121,000/μL with 11% circulating blast cells. Bone marrow biopsy reveals an acute monoblastic leukemia with 88% myeloid blasts. Cytogenetics are normal male karyotype. However molecular profiling reveals a FLT3 ITD mutation. The patient is not interested in allogeneic stem cell transplantation and is not considered a candidate for intensive chemotherapy given his prior coronary article disease. Therefore, after receiving hydroxyurea for several days, he begins therapy with off label sorafenib and azacitidine chemotherapy.

FLT3 inhibitor and low intensity chemotherapy

A proportion of patients with newly diagnosed *FLT3* mutant AML are not candidates for intensive chemotherapeutic approaches due to advanced age, medical comorbidities, organ dysfunction, poor performance status, or personal preference. For these individuals, regimens combining FLT3 inhibitors with lower dose chemotherapy approaches have been evaluated as upfront therapy (Table 3).

The combination of FLT3 TKI with hypomethylating agents (HMA), either azacitidine or decitabine, is appealing for many reasons. Both drugs may be administered in the ambulatory setting, and the efficacy and tolerability of HMA in older unfit individuals with AML is well established. Moreover, HMA therapy does not appear to result in upregulation of FLT3 ligand in patients with *FLT3* mutant AML, a phenomenon reported in patients receiving intensive chemotherapy, which theoretically could negate the ability of FLT3 TKIs to suppress growth of *FLT3* mutant AML cells in patients [20]. Midostaurin added following azacitidine therapy in a small number (n = 17) of AML patients with *de novo* FLT3 wildtype disease resulted in no reported dose limiting toxicities but was associated with only a 21% complete remission rate (3 out of 14 patients) and a median overall survival of only 6 months similar to

Table 3
Clinical studies of FLT3 inhibitors combined with low intensity chemotherapy.

FLT3 inhibitor + other agents	Trial Design	Patient Numbers	Summary of results/status
Midostaurin + azacitidine(Cooper et al.)	Phase 1	17 patients (all *FLT3* wildtype)	Midostaurin dosed 25, 50, and 75 mg bid on days 8–21 Median overall survival = 6 months 3/14 complete remissions
Sorafenib + azacitidine(Ravandi et al.)	Phase 2	43 patients *FLT3 ITD* RR-AML	*FLT3 ITD* mutant patients > 60 yo Overall response rate = 46% Median overall survival = 6.2 months
Sorafenib + decitabine (Muppidi et al.)	Case series	6 patients *FLT3 ITD* RR-AML	Overall response rate = 83% CR = 16%, CRi = 66% Median overall survival = 155 days
Gilteritinib ± Azaciitidine vs Azacitidine alone (Esteve et al.)	Phase 3	540 patients *FLT3* mut DN-AML	10 of 15 patients responded Overall response rate = 67%
Quizartinib + azacitidine or low dose cytarabine (Swaminathan et al.)	Phase 1/2	61 patients with *FLT3 ITD* mutant DN-AML and RR-AML	Overall response rate = 70% DN-AML: ORR 92%, median overall survival = 18.6 mos RR-AML: ORR 68%, median overall survival 11.3 mos
Quizartinib + decitabine	Phase 1/2	52 patients *FLT3 ITD* mut RR-AML	Currently accruing
Quizartinib + Omacetaxine	Phase 1	40 patients *FLT3 ITD* mut RR-AML	Currently accruing
Quizartinib + DS-3032 (mdm2 inhibitor)	Phase 1	156 patients *FLT3 ITD* mut RR-AML	Currently accruing

the results of HMA therapy alone [21].

The multi-kinase inhibitor, sorafenib, has been evaluated in combination with HMA therapy with variable results. Ravandi and colleagues investigated the efficacy of sorafenib (400 mg bid) combined with azacitidine (Aza) In a phase 2 study of 43 older patients (> 60 years) with relapsed and refractory AML failing a median of two prior therapies including prior TKI therapy. Over 90% of patients had *FLT3 ITD* mutant AML. The overall response rate for *FLT3* mutant patients was 46%, with 16% of patients proceeding to alloSCT. While encouraging, the median overall survival of these patients was only 6.2 months; however all of these patients had relapsed disease, suggesting that results in the upfront setting may be much improved [22]. In another series, 6 patients with *FLT3 ITD* mutant relapsed/refractory AML treated with sorafenib and decitabine had an overall response rate of 83%, including 16% CR and 66% CRi [23]. Based on these results and the lack of other approved regimens for such patients, the NCCN AML therapy panel has listed sorafenib and azacitidine as a potential treatment option for older individuals with *FLT3 ITD* mutant AML who are not considered candidates for intensive therapy.

The newer FLT3 inhibitors, gilteritinib and quizartinib, are currently being investigated in combination with HMA therapy as well. Based on promising preclinical studies of gilteritinib combined Aza in *FLT3 ITD* mutant AML mouse models [24], a phase 2/3 study of gilteritinib and Aza in newly diagnosed *FLT3* mutant AML patients is accruing (NCT02752035). Early results from the first 15 patients enrolled on trial established a gilteritinib dose of 120 mg for further study with tolerable adverse events of anemia, febrile neutropenia, cytopenias, and nausea. Overall response rate was an impressive 67% (10 of 15 patients) with 4 CR and 6 CRi. Encouragingly, 8 (56%) patients have remained on therapy for > 6 months with three patients on therapy for 317–377 days and counting with clinical responses. Patients are now being accrued onto the randomization arm of gilteritinib plus azacitidine versus azacitidine alone [25]. Similiarly high response rates were reported in a phase 1/2 trial of quizartinib (60 or 80 mg) combined with azacitidine (n = 38) or low dose cytarabine (LDAC)(n = 23) in *FLT3 ITD* mutant AML patients. Overall response rate in all 61 patients was 73%. Of note, outcomes in previously untreated patients were particularly notable with an overall response rate of 92% (11 of 12 patients) and a median OS of 18.6 months. Treatment of relapsed patients resulted in a lower but still excellent response rate of 68% with median OS of 11.3 months [26]. A phase 1/2 trial of quizartinib and decitabine has also recently opened (NCT03661307).

Novel combinations of FLT3 inhibitors for upfront therapy

Given the rapidly evolving therapeutic landscape of AML and the tolerability of these oral agents, FLT3 TKIs are increasingly is being explored in combination with other agents. Ongoing clinical trials are assessing the efficacy of crenolanib combined with intensive salvage chemotherapy for fit patients with relapsed/refractory *FLT3* mutant AML (NCT2626338).

In other studies, gilteritinib is being evaluated in combination with immune checkpoint antibody therapy (NCT03730012) or the bcl-2 inhibitor, venetoclax (NCT03625505). Last but not lease, quizartinib is also being explored with mdm2 inhibition (NCT03552029) as well as in combination with venetoclax (NCT03735875.

Post transplant maintenance with FLT3 inhibitors

FLT3 inhibitors have also been explored as maintenance therapy following allogeneic stem cell transplant to prevent subsequent relapse in *FLT3* mutant AML patients. The RADIUS trial was a randomized phase 2 trial of standard of care (SOC) therapy vs. midostaurin plus SOC therapy starting between 28 and 60 days after transplant. In this study, there was no statistically significant benefit to receiving midostaurin based on 2-year relapse-free survival (HR 0.60, CI 0.17–2.14, p = 0.43) or overall survival (HR 0.58, CI 0.19–1.79, p = 0.34) [27]. The German Sormain trial employed a similar schema in randomizing *FLT3 ITD* mutant patients to receive either sorafenib or SOC following allogeneic transplant. In this study of 83 patients, both relapse-free survival (HR 0.39, CI 0.18–0.85, p = 0.013) and overall survival (HR 0.447, CI 0.20–0.97, p = 0.03) were significantly enhanced by sorafenib therapy in association with lower non-relapse mortality [28]. Both the RADIUS and the SORMAIN trial noted no significant exacerbation or worsening of acute graft vs. host disease with FLT3 TKI therapy. Of note, patients in both trials did not receive FLT3 TKI therapy as part of upfront induction and consolidation unlike current practice. At present, the ongoing MORPHO clinical trial is a randomized phase 3 study comparing the outcomes and toxicities of gilteritinib versus placebo therapy following allogeneic stem cell transplant for *FLT3* mutant AML.

Summary

FLT3 mutations are among the most frequently identified mutations in newly diagnosed AML patients. Repurposing broad-spectrum multi-kinase inhibitors as FLT3 TKIs led to the approval of midostaurin for younger patients with *FLT3* mutant AML in combination with intensive chemotherapy. New generation FLT3 inhibitors such as quizartinib, gilteritinib and crenolanib demonstrate improved potency and specificity for mutant FLT3. Incorporation of these agents into frontline intensive chemotherapy regimens have resulted in high response rates and may eventually replace midostaurin in the upfront setting pending the results of ongoing phase 3 trials. The combination of sorafenib with hypomethylating agents (azacitidine or decitabine) has resulted in high response rates in patients with *FLT3* mutant AML inappropriate for intensive chemotherapy. FLT3 inhibitors are actively being explored in combination with other targeted agents.

Practice points

- *FLT3* mutation testing for both ITD and TKD mutations should be performed with rapid turn-around time (< 3–5 days) should be performed as standard of care in all patients with suspected acute myeloid leukemia.
- All younger fit patients with *FLT3* mutant AML should receive midostaurin and intensive induction and consolidation chemotherapy.
- *FLT3* mutant AML patients receiving FLT3 TKI therapy and cytotoxic chemotherapy should receive allogeneic stem cell transplantation in first complete remission if at all possible to achieve best long-term outcome. Patients ineligible for transplant should consider maintenance TKI monotherapy for 12 months following consolidation chemotherapy.

Research agenda

- Younger fit patients should be encouraged to participate in ongoing clinical trials of newer generation FLT3 TKI plus intensive chemotherapy.
- Older and/or unfit patients with newly diagnosed *FLT3* mutant AML should be encouraged to partake in clinical trials of FLT3 TKI therapy plus low dose chemotherapy or other experimental agents.

Conflicts of interest

Eunice Wang has reported participation in speaker bureaus for Novartis, Jazz, and Astellas. She has also participated in an advisory role/consultant for Pfizer, Amgen, Agios, Celyad, and Abbvie.

Dr. Wang is also leading clinical trials at her institute funded by the following pharmaceutical sponsors: Stemline Therapeutics, Eisai/H3B Biosciences, Astellas, Amgen, Agios, Incyte, Forma Therapeutics, Tolero, Arog Pharmaceuticals, Pfizer, Immunogen, Trovagene, Daiichi, Oscotec, Ono Pharmaceuticals.

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