International Journal of Hematology (2022) 116:351–363
https://doi.org/10.1007/s12185-022-03374-0

REVIEW ARTICLE

FLT3-targeted treatment for acute myeloid leukemia

Yasuyuki Arai[1] · SungGi Chi[2] · Yosuke Minami[2] · Masamitsu Yanada[3]

Received: 18 March 2022 / Revised: 21 April 2022 / Accepted: 21 April 2022 / Published online: 9 May 2022

Abstract

Mutations in the FMS-like tyrosine kinase 3 (*FLT3*) gene are detected in approximately 30% of acute myeloid leukemia (AML). The high frequency of *FLT3* mutations, along with their adverse effect on prognosis, makes FLT3 a promising therapeutic target, and has spurred development of FLT3 inhibitors. First-generation inhibitors, including midostaurin and sorafenib, lack specificity for FLT3 and act on multiple kinases, whereas second-generation inhibitors, including gilteritinib, and quizartinib, are highly specific to FLT3 and are more potent than first-generation inhibitors. Several FLT3 inhibitors have recently gained regulatory approval worldwide, and several others are under development. The advent of FLT3 inhibitors has changed the standard treatment for *FLT3*-mutated AML in the frontline and relapsed/refractory settings and contributed to improved outcomes for this formidable AML subtype. However, numerous unresolved issues remain owing to rapid changes in practice. These include identification of optimum FLT3 inhibitors and combination therapies, the role of maintenance therapy, and the indication for allogeneic hematopoietic cell transplantation. Furthermore, strategies to overcome resistance to FLT3 inhibitors must be pursued. Results of ongoing and future studies will improve our ability to use FLT3 inhibitors more effectively, which should provide significant benefits to a wider range of patients.

Keywords Acute myeloid leukemia · *FLT3* · Targeted therapy · Allogeneic hematopoietic cell transplantation

Introduction

The FMS-like tyrosine kinase 3 (*FLT3*) gene encodes a class III receptor tyrosine kinase that is expressed by hematopoietic stem and progenitor cells and plays a critical role in hematopoiesis [1–4]. Two distinct forms of *FLT3* mutations are as follows: internal tandem duplication (ITD) in the juxtamembrane domain [5] and a point mutation within the activation loop of the tyrosine kinase domain (TKD) [6]. Both mutations serve as a genetic driver in acute myeloid leukemia (AML) by constitutively activating FLT3 kinases, thereby leading to leukemic cell proliferation and survival [3, 4, 7, 8]. *FLT3* mutations are found in approximately 30% of patients with newly diagnosed AML [9]. The presence of *FLT3* mutations, especially *FLT3*-ITD, confers a high risk of relapse and a low probability of survival [10–15], making the treatment of *FLT3*-mutated AML a significant challenge. However, this situation has been drastically changing since the development of FLT3 inhibitors in recent years. This article reviews biological and clinical aspects of *FLT3*-mutated AML with focus on FLT3 inhibitors and discusses how the advent of FLT3 inhibitors is transforming the therapeutic landscape of *FLT3*-mutated AML.

FLT3 biology in AML

The FLT3 protein is a cell surface receptor-bound tyrosine kinase that contains extracellular immunoglobulin-like domains, a transmembrane region, a juxtamembrane region, and TKDs [4, 16]. The ligand of the extracellular receptor portion of FLT3 is produced by bone marrow stromal cells [3, 17], and binding of the FLT3 ligand to the dimerized FLT3 results in subsequent phosphorylation of tyrosine residues in the activation loop within the TKD [4]. Phosphorylated FLT3 activates multiple signaling pathways including

✉ Masamitsu Yanada
myanada@aichi-cc.jp

[1] Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[2] Department of Hematology, National Cancer Center Hospital East, Kashiwa, Japan

[3] Department of Hematology and Cell Therapy, Aichi Cancer Center, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan

RAS/MAPK, PI3K/AKT/mTOR and JAK/STAT5, and promotes cell proliferation and inhibits apoptosis [18–21].

The majority of *FLT3* mutations are in-frame insertions, that is, *FLT3*-ITD [5]. *FLT3*-ITD results in the aberrant elongation of the juxtamembrane region, which prevents its inhibitory regulation to the TKD and allows ligand-independent self-dimerization of FLT3 [22]. The conformational change in the juxtamembrane region leads to continuous TKD activation, which results in excessive proliferation and differentiation blockade of hematopoietic progenitor cells [23]. Unlike the wild-type FLT3, FLT3-ITD significantly enhances STAT5 phosphorylation [24], which leads to upregulation of BCL-XL and PIM1, both of which are involved in anti-apoptotic mechanisms on the mitochondrial outer membrane [25, 26]. Point mutations in the TKD (*FLT3*-TKD) represent the second most common *FLT3* mutations and predominantly occur within the activation loop [6]. *FLT3*-TKD causes conformational change to keep its active form even in the absence of the FLT3 ligand [27], and constitutively activates proliferative signaling cascades and are involved in leukemogenesis. Unlike *FLT3*-ITD, *FLT3*-TKD does not activate the JAK/STAT5 pathway, but enhances SHP1 and SHP2 activity that negatively regulates JAK signaling [28, 29]. This may partly explain why *FLT3*-TKD shows a less aggressive phenotype than *FLT3*-ITD [30, 31].

FLT3 mutations as a biomarker

FLT3-ITD mutations are found in up to 25% of patients with newly diagnosed AML [9]. Patients with *FLT3*-ITD are characterized by a higher white blood cell (WBC) count at diagnosis, a higher prevalence of normal karyotype, and worse outcomes than those without *FLT3*-ITD [10–15]. Although patients with *FLT3*-ITD achieve complete remission (CR) with conventional induction therapy similarly to those without *FLT3*-ITD, an increased incidence of relapse leads to inferior disease-free survival (DFS) and overall survival (OS), as demonstrated by an early meta-analysis reporting a summary hazard ratio (HR) and its 95% confidence interval (CI) of 1.86 (1.52–2.29) and 1.68 (1.29–2.03) for DFS and OS, respectively [32]. However, accumulating data suggest that not only the presence of this mutation but also allelic ratio, insertion site, ITD length, and co-mutations matter in prognostication [9]. Among them, allelic ratio and co-mutations represent the two most important factors.

The allelic ratio, which is defined as the ratio of ITD-mutated allele to wild-type allele, has been shown to differentiate the prognosis of *FLT3*-ITD AML [13, 33–37]. Thiede et al. reported that patients with a mutant/wild-type ratio above the median value of 0.78 had a significantly higher relapse incidence and shorter OS than those with a lower ratio [13]. Schlenk et al. analyzed patients enrolled in three prospective studies conducted by the German–Austrian AML Study Group (AMLSG) and found that an allelic ratio of ≥ 0.51 was associated with worse relapse-free survival (RFS) and OS [36]. Furthermore, Versluis et al. showed worse outcomes for patients with allelic ratio of > 0.50 based on the data of the Dutch–Belgian Hemato-Oncology Cooperative Group and Swiss Group for Clinical Cancer Research (HOVON/SAKK) studies [37]. Conversely, several studies argue against the prognostic significance of the allelic ratio [38, 39]. For example, Linch et al., on behalf of the United Kingdom Medical Research Council, found no significant difference in the cumulative incidence of relapse for patients with allelic ratios of $< 25\%$, 25–50%, and $> 50\%$ [38]. When interpreting these results, it is important to note that there is currently no standardized methodology for determining allelic ratios and no consensus on the optimal cutoff level. Considering the conflicting data along with the lack of assay standardization, the prognostic significance of allelic ratio remains unsettled.

The presence of certain co-mutations can influence the outcomes of patients with *FLT3*-ITD AML. *NPM1* is the most remarkable example; several studies showed that patients with *FLT3*-ITD have better outcomes in the presence of concomitant *NPM1* mutation, especially when the *FLT3*-ITD allelic ratio is low [33–35]. In contrast, investigators in the HOVON/SAKK group showed that the OS of patients with low-allelic ratio *FLT3*-ITD did not differ according to *NPM1* mutational status [37]. By conducting comprehensive genomic analysis, Papaemmanuil et al. demonstrated that the adverse influence of *FLT3*-ITD on OS was significantly greater when both *NPM1* and *DNMT3A* are concurrently mutated, which was considerably diminished in the absence of either or both mutations [40].

Another type of *FLT3* mutations involving the TKD accounts for 7–10% of newly diagnosed AML [9]. Consistent with *FLT3*-ITD, *FLT3*-TKD is characterized by a higher initial WBC count and normal karyotype [12, 13, 41]. However, contrasting with *FLT3*-ITD, the prognostic significance of *FLT3*-TKD is equivocal [12, 13, 41–43], and the presence or absence of this mutation does not have any influence on the current risk assessment [44]. Moreover, the prognostic impact of *FLT3*-TKD appears to depend on co-mutations. Some studies showed that prognosis was better when *NPM1* was co-mutated [41, 45], whereas worse outcomes were reported when co-mutation with partial tandem duplications of *MLL* was present [40, 41].

FLT3 inhibitors

The high frequency of *FLT3* mutations in AML along with the adverse prognostic feature makes FLT3 a promising therapeutic target. Significant research efforts have been

undertaken to develop effective FLT3 inhibitors, and several drugs, including midostaurin, gilteritinib and quizartinib, have been approved for use in one or more countries thus far, whereas several others are under development.

FLT3 inhibitors can be classified by generation and type [9]. Generation represents specificity to FLT3. First-generation inhibitors are relatively non-specific to FLT3 and act on multiple kinases. The antileukemic effects of first-generation inhibitors may well result not only from FLT3 inhibition but also from the inhibition of other kinases that are involved in AML pathogenesis. Concurrently, such off-target effects have the potential to introduce variable toxicities. The reported results of monotherapy with first-generation inhibitors were unsatisfactory, with only the modest efficacy being achieved at a tolerated dose [46–48]. Second-generation inhibitors are highly specific to FLT3 and are more potent than first-generation inhibitors. Additionally, they are characterized by less toxicity associated with off-target effects. Type represents how the drug binds to FLT3. Type I inhibitors bind to the ATP-binding site in either the active or inactive conformation, and thus have the property of inhibiting both *FLT3*-ITD and -TKD-mutated receptors. Type II inhibitors do not directly bind to the ATP-binding site, but to the hydrophobic region adjacent to the ATP-binding site that is only accessible in the inactive conformation [49]. As such, type II inhibitors are not active against TKD mutations because they favor the active conformation. Characteristics of major FLT3 inhibitors are summarized in Table 1, and selected randomized studies of FLT3 inhibitors published to date are summarized in Table 2.

Midostaurin

Midostaurin is a first-generation type I FLT3 inhibitor with activity against multiple kinases, such as FLT3, KDR, KIT, PDGFR, PKC, and VEGFR [50]. Early studies showed that midostaurin monotherapy provided moderate blast reduction in patients with relapsed/refractory AML, especially in those with mutated *FLT3*; however, complete remission (CR) was not attained in any of the patients [46, 47]. A subsequent phase IB study of midostaurin combined with standard chemotherapy showed high CR rates of 80% [51], which formed the basis of the phase III RATIFY study. This pivotal study was conducted at 215 sites in 17 countries worldwide and included 717 patients aged 18–59 years with newly diagnosed *FLT3*-mutated AML [52]. The patients were randomly assigned to either midostaurin or placebo arm both combined with induction therapy consisting of daunorubicin and cytarabine followed by four-course consolidation therapy with high-dose cytarabine. Patients remaining in CR after completion of consolidation therapy received maintenance therapy with midostaurin or placebo according to the initial randomization for up to 1 year. Maintenance therapy was not provided after allogeneic hematopoietic cell transplantation (HCT). Although CR rates were not different between the midostaurin and placebo arms (59% vs. 54%, $P=0.15$), the midostaurin arm yielded better OS (51% vs. 44% at 4 years, $P=0.009$) and event-free survival (EFS) (28% vs. 21% at 4 years, $P=0.002$) than the placebo arm. When patients were stratified into three risk groups defined by the 2017 European LeukemiaNet (ELN) guidelines, the beneficial effect of midostaurin was found across all groups [53]. Although patients in the midostaurin arm exhibited higher rates of anemia and skin rash, no significant intergroup difference was noted in the rates of severe adverse events [52]. Landmark analysis from the initiation of maintenance therapy revealed no significant difference in the cumulative incidence of relapse between arms [54]; however, interpretation of this finding requires caution considering that only 205 of the 717 patients started maintenance therapy, and approximately 40% of the patients who started the maintenance therapy did not complete the planned 12 cycles. Based on the results of the RATIFY study, midostaurin was granted approval in combination with intensive chemotherapy for newly diagnosed *FLT3*-mutated AML by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) and as maintenance treatment after conventional consolidation therapy only by the EMA. The AMLSG 16–10 study was a single-arm phase II study to evaluate midostaurin combined with standard chemotherapy for 284 patients with newly diagnosed *FLT3*-ITD AML aged ≤ 70 years [55]. CR was achieved in 76% of

Table 1 Characteristics of FLT3 inhibitors

Drug	Generation	Type	Major targets other than FLT3	Regulatory approval for AML
Midostaurin	First	I	KDR, KIT, PDGFR, PKC, VEGFR	US, EU: newly diagnosed
Sorafenib	First	II	KIT, PDGFR, RAF, RET, VEGFR	–
Lestaurtinib	First	I	JAK2, KDR, PDGFR, PKC, TRK	–
Tandutinib	First	II	KIT, PDGFR	–
Sunitinib	First	I	KIT, PDGFR, RET, VEGFR	–
Gilteritinib	Second	I	ALK, AXL, LTK	US, EU, Japan: relapsed/refractory
Quizartinib	Second	II	KIT	Japan: relapsed/refractory
Crenolanib	Second	I	PDGFR	–

Table 2 Selected randomized studies of FLT3 inhibitors

Drug	Study	*N*	Patients	Treatment	Results
Midostaurin	Stone et al. 2017 [52]	717	Untreated, *FLT3*-mutated, age 18–59	Midostaurin vs. placebo (combined with intensive chemotherapy followed by maintenance)	OS: 51% vs. 44% at 4 years ($P=0.009$); EFS: 28% vs. 21% at 4 years ($P=0.002$) for midostaurin vs. placebo
Midostaurin	Maziarz et al. 2021 [100]	60	First CR before allogeneic HCT, *FLT3*-ITD+, age 18–70	Midostaurin vs. non-maintenance	RFS: 89% vs. 76% at 18 months ($P=0.27$); OS: 85% vs. 76% at 2 years ($P=0.34$) for midostaurin vs. non-maintenance
Sorafenib	Röllig et al. 2015 [60, 61]	267	Untreated, age 18–60	Sorafenib vs. placebo (combined with intensive chemotherapy followed by maintenance)	EFS: 41% vs. 27% at 5 years ($P=0.011$); OS: 61% vs. 53% at 5 years ($P=0.282$) for sorafenib vs. placebo
Sorafenib	Serve et al. 2013 [62]	197	Untreated, age > 60	Sorafenib vs. placebo (combined with intensive chemotherapy followed by maintenance)	Median EFS: 5 months vs. 7 months ($P=0.12$); median OS: 13 months vs. 15 months ($P=0.88$) for sorafenib vs. placebo
Sorafenib	Burchert et al. 2020 [101]	83	CR after allogeneic HCT, FLT3-ITD+	Sorafenib vs. placebo	RFS: 85% vs. 53% at 2 years ($P=0.002$); OS: 91% vs. 66% at 2 years ($P=0.007$) for sorafenib vs. placebo
Sorafenib	Xuan et al. 2020 [102]	202	CR before and after allogeneic HCT, *FLT3*-ITD+, age 18–60	Sorafenib vs. non-maintenance	CIR: 7% vs. 25% at 1 year ($P=0.001$); OS: 82% vs. 68% at 2 years ($P=0.012$) for sorafenib vs. non-maintenance
Lestaurtinib	Levis et al. 2011 [66]	224	Relapsed, *FLT3*-mutated, age ≥ 18	Intensive chemotherapy w/wo lestaurtinib	CR/CRp: 26% vs. 21% ($P=0.35$) for patients w/wo lestaurtinib. No significant difference in OS between arms
Lestaurtinib	Knapper et al. 2017 [67]	500	Untreated AML or high-risk MDS, *FLT3*-mutated, age < 60	Intensive chemotherapy w/wo lestaurtinib	OS: 46% vs. 45% at 5 years ($P=0.30$); RFS: 40% vs. 36% at 5 years ($P=0.30$) for patients w/wo lestaurtinib
Gilteritinib	Perl et al. 2019 [75]	371	Relapsed/refractory, *FLT3*-mutated, age ≥ 18	Gilteritinib vs. placebo	Median OS: 9.3 months vs. 5.6 months ($P<0.001$); median EFS: 2.8 months vs. 0.7 months ($P=$ NS) for gilteritinib vs. placebo
Quizartinib	Cortes et al. 2019 [81]	367	Relapsed/refractory, *FLT3*-ITD+, age ≥ 18	Quizartinib vs. placebo	Median OS: 6.2 months vs. 4.7 months ($P=0.02$); median EFS: 1.4 months vs. 0.9 months ($P=0.11$) for quizartinib vs. placebo

OS overall survival, *EFS* event-free survival, *CR* complete remission, *HCT* hematopoietic cell transplantation, *ITD* internal tandem duplication, *RFS* relapse-free survival, *CIR* cumulative incidence of relapse, *CRp* CR with incomplete platelet recovery, *MDS* myelodysplastic syndrome, *NS* not significant

all the enrolled patients, and 47% underwent allogeneic HCT during first CR. The 2-year EFS and OS were 38% and 51%, respectively. A comparison with historical controls showed a significant improvement in EFS by adding midostaurin. Several phase I and II studies investigated midostaurin combined with azacitidine and reported the safety and efficacy of this combination [56, 57].

Sorafenib

Sorafenib is a first-generation type II FLT3 inhibitor which is also active against KIT, PDGFR, RAF, RET, and VEGFR [58]. A phase I study of sorafenib monotherapy was conducted in 50 patients, 28 of whom had *FLT3*-ITD. CR or CR with incomplete platelet recovery (CRp) was achieved in five patients, and additional 17 patients showed significant blast reduction; however, the response duration was short [48]. Of note, all the responders had *FLT3*-ITD. Sorafenib was subsequently studied in combination with idarubicin and cytarabine in a phase I/II study [59]. CR rates were 75% overall, and patients with *FLT3*-mutation were more likely to achieve CR than those with wild-type *FLT3* (93% vs. 67%, $P=0.033$). The efficacy of sorafenib added to standard chemotherapy was demonstrated in the randomized phase II SORAML study conducted by the Study Alliance Leukemia (SAL) [60, 61]. This study enrolled patients aged 18–60 years with newly diagnosed AML regardless of the presence or absence of *FLT3* mutations. A total of 267 patients were randomized to receive sorafenib or placebo during induction and consolidation therapy and as maintenance for 1 year. Although CR rates were comparable for the sorafenib and placebo arms (60% vs. 59%), the sorafenib arm was superior to the placebo arm in terms of EFS (41% vs. 27% at 5 years, $P=0.011$) and RFS (53% vs. 36% at 5 years, $P=0.035$). The difference in OS did not reach statistical significance (61% vs. 53% at 5 years, $P=0.282$). Despite increased toxicities such as fever, diarrhea, bleeding, cardiac events, hand-foot-skin reaction, and rash, sorafenib proved to be useful in improving long-term outcomes. The SAL group conducted another randomized study of a similar design for 197 patients aged > 60 years with newly diagnosed AML [62]. In contrast to their study for younger patients, the addition of sorafenib did not show prolongation of EFS (5 months vs. 7 months, $P=0.12$) or OS (13 months vs. 15 months, $P=0.88$). Higher induction toxicities in the sorafenib arm resulted in higher early mortality (17% vs. 7%, $P=0.05$), lower CR rates (47% vs. 63%, $P=0.12$), and less adherence to consolidation therapy. In a smaller phase II study, investigators from the Cancer and Leukemia Group B (CALGB) also studied the combination of sorafenib with chemotherapy for patients aged ≥ 60 years [63]. The 1-year probability of OS was 62% for patients with *FLT3*-ITD, which met the primary endpoint of the study. The results appeared to be favorable compared with the results of the above-mentioned SAL study, and this difference can be at least partly explained by lower induction mortality in the CALGB study (9%). The combination of sorafenib and azacitidine is promising, with response rates reported to be 78% for untreated patients not suitable for standard chemotherapy [64] and 46% for relapsed/refractory patients [65], respectively. Further confirmatory studies are warranted for this combination. Sorafenib is currently approved for unresectable renal cell carcinoma, hepatocellular carcinoma, and thyroid carcinoma although not for AML.

Other first-generation inhibitors

Other first-generation FLT3 inhibitors, including lestaurtinib [66, 67], tandutinib [68], and sunitinib [69], were studied in clinical trials; however, their developments had been terminated owing to toxicities or lack of sufficient efficacy. Several multikinase inhibitors approved for other indications are known to possess activity against FLT3 and are currently under investigation in AML. These include ponatinib (approved for chronic myeloid leukemia and acute lymphoblastic leukemia) [70], cabozantinib (approved for medullary thyroid carcinoma and renal cell carcinoma) [71], and ibrutinib (approved for chronic lymphocytic leukemia and malignant lymphoma) (NCT03642236).

Gilteritinib

Gilteritinib is a second-generation type I FLT3 inhibitor with potent activity against both FLT3-ITD and -TKD [72]. Furthermore, this drug is active against AXL, a molecule potentially involved in a mechanism for resistance to other FLT3 inhibitors [73]. The CRYSARIS study was an open-label single-arm phase I/II study of gilteritinib monotherapy for patients with relapsed/refractory AML [74]. In this study, 252 patients received gilteritinib in dose-escalation or dose-expansion cohorts. Gilteritinib was well tolerated, and the maximum tolerated dose (MTD) was established at 300 mg/day, with doses above this level causing diarrhea and liver dysfunction. An optimal dose was decided at 120 mg/day. In total, 40% of the patients achieved response, including CR (8%), CRp (4%), CR with incomplete hematologic recovery (CRi; 18%), and partial remission (PR; 10%); the median response duration was 17 weeks. Although only 12% of the patients had received a prior FLT3 inhibitor, response was obtained regardless of previous treatment with FLT3 inhibitors. These encouraging results led to the phase III ADMIRAL study, wherein 371 patients with relapsed/refractory *FLT3*-mutated AML were randomized 2:1 to either gilteritinib at 120 mg/day or salvage chemotherapy initially chosen by the investigator [75]. The co-primary endpoints were OS and CR with full or partial hematologic

recovery. The gilteritinib arm had a longer median OS than the control arm (9.3 months vs. 5.6 months, $P < 0.001$). The gilteritinib arm had a higher percentage of patients who achieved CR with full or partial hematologic recovery (34% vs 15%) and underwent allogeneic HCT (26% vs 15%) than the control arm. In the gilteritinib arm, the most common adverse events of grade ≥ 3 were febrile neutropenia, anemia, and thrombocytopenia; however, serious adverse events occurred less frequently than the control arm. Based on these results, gilteritinib was granted regulatory approval for relapsed/refractory *FLT3*-mutated AML in the US, EU, and Japan. Currently, a phase I study is investigating gilteritinib combined with chemotherapy for newly diagnosed AML (NCT02236013). Furthermore, two phase III studies are comparing gilteritinib and midostaurin in combination with standard chemotherapy (NCT03836209 and NCT04027309). Several studies are investigating gilteritinib with doublet or triplet combination with hypomethylating agents and/or venetoclax (NCT02752035, NCT03404193, NCT03625505, NCT04140487, and NCT05010122). The phase III LACEWING study evaluated azacitidine with or without gilteritinib for unfit patients with newly diagnosed *FLT3*-mutated AML (NCT02752035); however, it was recently announced that this study failed to meet its primary endpoint of OS at interim analysis [76].

Quizartinib

Quizartinib is a second-generation type II FLT3 inhibitor with a potent inhibitory effect for FLT3-ITD but not for FLT3-TKD and is also active against KIT [77]. Quizartinib monotherapy showed an acceptable toxicity profile and encouraging efficacy. A phase I study determined an MTD at 200 mg/day, and the dose-limiting toxicity was QT prolongation [78]. Overall response and CR were achieved in 30% and 13%, respectively. Subsequent phase II studies showed that lower doses were safer and did not diminish response rates [79, 80]. When treated with 30- or 60-mg/day doses, composite CR (CR + CRp + CRi) rates were 47%, and the incidence of QT prolongation was lower than that in the earlier reports with higher doses [80]. A phase III randomized controlled study (QuANTUM-R) compared quizartinib monotherapy with salvage chemotherapy in patients with relapsed/refractory *FLT3*-ITD AML [81]. A total of 367 patients were randomized 2:1 to either quizartinib at a dose of 60 mg/day dose or salvage chemotherapy preselected by the investigator. The median OS was 6.2 and 4.7 month in the quizartinib and control arms, respectively ($P = 0.02$). The allogeneic HCT rates were higher in the quizartinib arm (32% vs. 11%). Severe adverse events were comparable between the two arms, and the frequent treatment-related serious adverse events in the quizartinib group were febrile neutropenia, sepsis, QT prolongation, and nausea. The rate of grade 3 QT prolongation was 2%. The study results demonstrated a survival advantage with quizartinib, which led to the regulatory approval of this drug for relapsed/refractory *FLT3*-ITD AML in Japan. However, approval was not granted by FDA or EMA on the argument that the benefits of this drug do not outweigh its risks. Several studies reported the efficacy of quizartinib in combination with azacitidine [82], low-dose cytarabine [82, 83], and standard chemotherapy [84]. The QuANTUM-First study is a phase III study that compares quizartinib and placebo in combination with standard chemotherapy for patients with newly diagnosed *FLT3*-ITD AML (NCT02668653). According to a recent press release, this study has met the primary endpoint of OS [85], and the publication of the study results is eagerly awaited.

Crenolanib

Crenolanib is a second-generation type I FLT3 inhibitor and has an inhibitory activity against PDGFR in addition to FLT3-ITD and FLT3-TKD [86]. Following promising results of phase II studies in the frontline setting [87], three phase III studies have been conducted, including two studies comparing crenolanib with placebo for relapsed/refractory AML (NCT02298166 and NCT03250338) and one study comparing crenolanib with midostaurin both in combination with standard chemotherapy for untreated AML (NCT03258931).

FLT3 inhibitors before or after allogeneic HCT

Allogeneic HCT is a therapy with maximal antileukemic effect and is generally recommended for young and fit patients with *FLT3*-ITD AML in first CR [88]. Multiple studies revealed the beneficial effect of allogeneic HCT for patients with *FLT3*-ITD AML in first CR [35, 37, 89–93]. Some studies reported that patients with favorable risk profiles, i.e., a low allelic ratio of *FLT3*-ITD and concomitant *NPM1* mutation, did not benefit from allogeneic HCT [36, 94], whereas others showed that allogeneic HCT improved outcomes regardless of the allelic ratio or concomitant *NPM1* mutation [92, 93, 95]. By analyzing data of a Japanese patient cohort, Sakaguchi et al. showed that allogeneic HCT in first CR provided a significant survival advantage even for patients with low-allelic ratio *FLT3*-ITD and concomitant *NPM1* mutations [93]. Although these patients were classified as having favorable risk following the updated ELN risk stratification [44], their outcomes were poor without allogeneic HCT (the 4-year RFS and OS rates of 15% and 16%, respectively) [93], which possibly constitutes a major factor contributing to the superiority of allogeneic HCT shown in this study. When discussing the role of

allogeneic HCT in *FLT3*-ITD AML, it is important to note that most of the available evidence are based on data before the widespread use of FLT3 inhibitors and may not apply to the current clinical practice. Presently, there is very limited information to ascertain the role of allogeneic HCT during first CR in the era of FLT3 inhibitors. In the RATIFY study, allogeneic HCT was performed at the discretion of the investigator, and 28% and 23% of the patients in the midostaurin and placebo arms, respectively, underwent allogeneic HCT during first CR [52]. When the analysis was confined to this patient population, a trend for better OS in the midostaurin arm remained ($P=0.07$). In a post-hoc analysis of the study, the prognostic impact of allogeneic HCT was evaluated by considering allogeneic HCT conducted during first CR as a time-dependent covariate [53]. Multivariate analysis revealed that the beneficial effect of allogeneic HCT on OS was significant overall (HR, 0.57; 95% CI, 0.42–0.94; $P=0.021$). However, after patients were stratified by the ELN risk, allogeneic HCT was associated with a significant survival advantage for patients in the adverse-risk group (HR, 0.39; 95% CI, 0.21–0.73; $P=0.003$) although not for those in the favorable- (HR, 0.78; 95% CI, 0.28–2.13; $P=0.621$) or intermediate-risk groups (HR, 0.81; 95% CI, 0.41–1.58; $P=0.535$). These results suggest that patients in the adverse-risk group may still benefit from allogeneic HCT during first CR; however, no firm conclusion can be drawn especially for those in the favorable- and intermediate-risk groups because the study was insufficiently powered for this kind of analysis.

The prognosis of AML is extremely poor once patients have developed a post-transplant relapse [96], and this occurs at > 30% even after allogeneic HCT during first CR in patients with *FLT3*-ITD AML [97–99]. The development of effective post-transplant maintenance therapy is an unmet medical need and FLT3 inhibitors have been investigated for this purpose. In the phase II AMLSG 16–10 study, maintenance with midostaurin was initiated in 75 of 134 patients after allogeneic HCT [55]. The landmark analysis showed that the patients who started maintenance therapy within 100 days post-transplant had significantly better EFS ($P=0.004$) and OS ($P=0.01$) than those who did not. In this study, maintenance therapy was planned to be implemented for 1 year; however, the therapy was terminated early owing to toxicity in 24 patients. The most common adverse events of grade ≥ 3 were gastrointestinal toxicity, infections, and blood count changes. In the phase II RADIUS study, 60 patients with *FLT3*-ITD AML in first CR were randomly assigned to a 12-month therapy of midostaurin maintenance or no maintenance [100]. Although statistical significance was not reached due to the small sample size, RFS as the primary endpoint was higher in the maintenance arm than that in the non-maintenance arm (89% vs. 76% at 18 months, $P=0.27$). The frequently reported adverse events in the midostaurin arm included diarrhea, nausea, and vomiting; dose adjustment and discontinuation were required in 63% and 27%, respectively. The efficacy of post-transplant maintenance with sorafenib was demonstrated in two randomized studies. The SORMAIN study was a randomized phase II study wherein patients with *FLT3*-ITD in CR after allogeneic HCT were randomly assigned to receive sorafenib or placebo for up to 2 years [101]. Although the study was prematurely terminated due to slow accrual when 83 of the planned 200 patients were enrolled, an analysis of the 83 patients showed the superiority of sorafenib maintenance in terms of RFS (85% vs. 53% at 2 years, $P=0.002$) and OS (91% vs. 66% at 2 years, $P=0.007$). Of note, sorafenib maintenance was beneficial particularly for patients who were negative for measurable residual disease (MRD) pre-transplant and those with positive MRD post-transplant. Sorafenib was not associated with higher toxicity than placebo, and graft-versus-host disease, infections, gastrointestinal toxicity, electrolyte alterations, and skin toxicity were the most common adverse events. An open-label randomized phase III study conducted at seven hospitals in China allocated 202 patients with *FLT3*-ITD AML who underwent allogeneic HCT during CR to either sorafenib maintenance from day 30 to day 180 post-transplant or no maintenance [102]. The sorafenib arm had a lower cumulative incidence of relapse than the non-maintenance arm (7% vs. 35% at 1 year, $P=0.001$), which translated into better OS (82% vs. 68% at 2 years, $P=0.012$). Sorafenib was well tolerated, and the frequencies of grade ≥ 3 adverse events were similar between treatment groups. In addition to FLT3 inhibition, preclinical studies suggested that sorafenib enhances the activity of cytotoxic T cells and graft-versus-leukemia effects through IL-15 activation [103]. Second-generation FLT3 inhibitors for post-transplant maintenance therapy are currently investigated in prospective studies; some are focusing on post-transplant maintenance, such as a phase III study comparing gilteritinib and placebo (MORPHO, NCT02997202) and a phase II study of crenolanib (NCT02400255), and others are evaluating a sequence of treatment including post-transplant maintenance such as a phase III study comparing midostaurin and gilteritinib (NCT04027309), a phase III study comparing midostaurin and crenolanib (NCT03258931), and a phase III study comparing quizartinib and placebo (QuANTUM-First, NCT02668653). These ongoing studies are expected to provide insights into the current clinical questions and refine the standard of care for *FLT3*-ITD AML.

Mechanism of resistance to FLT3 inhibitors

Primary resistance

Several mechanisms of primary resistance to FLT3 inhibitors have been suggested, such as FLT3 ligand bypassing, FLT3-independent MAPK activation, cell adhesion in the

microenvironment, and degradation of FLT3 inhibitors [16]. Since FLT3 inhibitors barely act on wild-type FLT3, FLT3 ligand can bind with wild-type FLT3 to initiate FLT3-mediated activation of the MAPK signaling pathway, which militates leukemic cell survival [104]. Moreover, the MAPK signaling pathway is activated by signals from FGFR1 by binding with its ligand FGF2. Traer et al. demonstrated that FGF2 promotes resistance to quizartinib through the activation of MAPK effectors in leukemic cell lines and enhances the FGF2 expression in bone marrow stromal cells of patients with *FLT3*-ITD AML who had been treated with quizartinib [105]. The hepatic CYP3A4 enzyme inactivates most tyrosine kinase inhibitors. Chang et al. showed that the CYP3A4 expression in bone marrow stromal cells attenuates the activity of three different FLT3 inhibitors in *FLT3*-ITD AML [106].

Secondary resistance

Considering the lack of activity of type II FLT3 inhibitors against FLT3-TKD, the emergence of additional mutations in the TKD confers on-target resistance in patients treated with type II FLT3 inhibitors [49, 107, 108]. The F691L gatekeeper mutation in the TKD is exclusively found as a secondary mutation upon pre-existing *FLT3* mutations [109, 110]. The F691 residue is not involved in the activation loop; however, it is located just adjacent to the ATP-binding site. Altered F691 residue prevents FLT3 inhibitors from binding to their target regions, which renders AML with this mutation highly resistant to most FLT3 inhibitors. As mentioned earlier, signals from mutant *FLT3* mainly rely on the RAS/MAPK, PI3K/AKT/mTOR, and JAK/STAT5 pathways [7, 18–21]. Thus, additional mutations leading to alternative activation of these pathways are theoretically responsible for off-target resistance to FLT3 inhibitors. In a comparative genetic analysis before and after relapse in patients who had been treated with gilteritinib, mutations were frequently found in the RAS/MAPK pathway-related genes, such as *NRAS, KRAS, PTPN11, CBL,* and *BRAF* [111]. Additionally, upregulation of effector proteins involved in the PI3K/AKT/mTOR pathway was observed in sorafenib-resistant cell lines [112]. Similarly, JAK/STAT5 signaling is bypassed by the overexpression of the downstream effector PIM1 in resistant leukemic cell lines [26, 113]. Other mutations that are not associated with the FLT3-related pathways, including *TET2*, *IDH1*, and *TP53*, may also be involved in the mechanism of resistance to FLT3 inhibitors [114]. The status of *FLT3* mutations occasionally changes during relapse because of the clonal evolution. An analysis of paired samples collected at diagnosis and relapse showed that 11% and 9% of the patients with AML gained and lost the *FLT3* mutation during relapse, respectively [115]. In patients with *FLT3*-ITD AML, who were refractory to or relapsed after chemotherapy plus midostaurin, 46% became negative for *FLT3*-ITD under the selection pressure exercised by the FLT3 inhibitor [116]. These findings highlight the importance of reassessing mutational profiles, including those of *FLT3*, whenever a decision regarding a change in treatment is required.

Future perspectives

FLT3 inhibitors have now become an essential component of the treatment for *FLT3*-mutated AML. However, owing to rapid changes in practice, many unresolved issues are present. First, insufficient data to determine which one is preferable exists among several approved or unapproved FLT3 inhibitors. For example, midostaurin is used in combination with intensive chemotherapy for newly diagnosed patients as a de facto standard; however, second-generation FLT3 inhibitors may be more useful considering their property of more potent and selective FLT3 inhibition. Second, optimum combination therapies need to be pursued. AML is a disease that predominantly affects older adults, and intensive chemotherapy is highly toxic for a significant proportion of patients [117]. Azacitidine plus venetoclax has recently become the treatment of choice for patients with newly diagnosed AML who are ineligible for intensive chemotherapy [118]. In this context, azacitidine, venetoclax, or both is a promising candidate to be combined with FLT3 inhibitors and other molecularly targeted drugs with favorable toxicity profiles may be a good partner. Such low-intensity therapies will expand the applicability of the use of FLT3 inhibitors. Third, limited data are available regarding the usefulness of maintenance therapy with FLT3 inhibitors. For patients undergoing allogeneic HCT, sorafenib maintenance reduces post-transplant relapse and improves OS [101, 102]. However, whether patients who have received FLT3 inhibitors before transplantation still benefit from post-transplant FLT3 inhibitors is unknown, as is which FLT3 inhibitor is optimal for this indication. The role of maintenance therapy is much less clear in the non-transplant setting. It is hoped that these uncertainties will be addressed by ongoing and future studies. Furthermore, the role of allogeneic HCT must be reappraised following the significant change in practice. Historically, young patients with *FLT3*-mutated AML are encouraged to proceed to allogeneic HCT during first CR based on the concept that it is the only established treatment with curative potential [88]. It is reasonable to consider that this principle remains valid at present, because, to date, there has been no clear evidence so far to show that a non-transplant treatment is better than or at least comparable with allogeneic HCT. When comparing non-transplant treatment with allogeneic HCT, it should be considered that outcomes of allogeneic HCT may also be improved by the

introduction of FLT3 inhibitors. Therefore, this issue needs to be re-evaluated in contemporary patient populations in the form of a prospective randomized study wherever feasible or a retrospective study adopting the appropriate statistical methodology.

Despite encouraging response rates achieved with FLT3 inhibitors, the emergence of acquired resistance represents a significant challenge, and novel FLT3 inhibitors designed to overcome common resistance mechanisms are anticipated. FF-10101 is the first FLT3 inhibitor that covalently binds to the C695 residues of FLT3 [119]. FF-10101 is unaffected by the F691L gatekeeper mutations and has demonstrated potent activity in quizartinib-resistant AML cells with F691 mutations. A phase I/II study of this drug for relapsed/refractory AML is ongoing (NCT03194685). Furthermore, several other highly selective FLT3 inhibitors with the potential to overcome resistance are in development [16]. Finally, some comments were made regarding the special situation in Japan. Unlike Western countries, midostaurin is not approved for use at the time of writing, and two FLT3 inhibitors, namely, gilteritinib and quizartinib, gained regulatory approval for relapsed/refractory patients. Recently, an analysis of patients consecutively treated at an academic center in the United States reported that the presence of *FLT3* mutations no longer has an adverse prognostic impact on OS [120]. However, this finding cannot be generalized to Japanese patients because of the above-mentioned differences in practice. To clarify the clinical picture of *FLT3*-mutated AML in Japan, including how the advent of FLT3 inhibitors has altered the outcomes, it is imperative to aggregate the clinical data of many Japanese patients within the framework of national collaboration.

Conclusions

The advent of FLT3 inhibitors has changed the standard treatment for *FLT3*-mutated AML in the frontline and relapsed/refractory settings and contributed to better outcomes of this formidable AML subtype. Results of ongoing and future studies will improve our ability to use FLT3 inhibitors more effectively, which is expected to provide significant benefits to a wider range of patients.

Acknowledgements This work was supported in part by the National Cancer Research and Development expenses grant, grant number: 2021-A-11 (YM), and the Aichi Cancer Research Foundation, grant number: 2021-1-19 (MY).

Declarations

Conflict of interest YM received honoraria from Bristol-Myers Squibb, Novartis, and Pfizer. MY received research funding from AbbVie and Novartis; and honoraria from AbbVie, Daiichi Sankyo, and Kyowa Kirin.

References

1. Matthews W, Jordan CT, Wiegand GW, Pardoll D, Lemischka IR. A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations. Cell. 1991;65:1143–52.
2. Rosnet O, Mattei MG, Marchetto S, Birnbaum D. Isolation and chromosomal localization of a novel FMS-like tyrosine kinase gene. Genomics. 1991;9:380–5.
3. Small D, Levenstein M, Kim E, Carow C, Amin S, Rockwell P, et al. STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. Proc Natl Acad Sci U S A. 1994;91:459–63.
4. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood. 2002;100:1532–42.
5. Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia. 1996;10:1911–8.
6. Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood. 2001;97:2434–9.
7. Lyman SD, James L, Vanden Bos T, de Vries P, Brasel K, Gliniak B, et al. Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell. 1993;75:1157–67.
8. Drexler HG, Meyer C, Quentmeier H. Effects of FLT3 ligand on proliferation and survival of myeloid leukemia cells. Leuk Lymphoma. 1999;33:83–91.
9. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. Leukemia. 2019;33:299–312.
10. Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood. 1999;93:3074–80.
11. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001;98:1752–9.
12. Frohling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood. 2002;100:4372–80.
13. Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002;99:4326–35.
14. Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood. 2002;100:59–66.
15. Moreno I, Martin G, Bolufer P, Barragan E, Rueda E, Roman J, et al. Incidence and prognostic value of FLT3 internal tandem

duplication and D835 mutations in acute myeloid leukemia. Haematologica. 2003;88:19–24.
16. Eguchi M, Minami Y, Kuzume A, Chi S. Mechanisms Underlying Resistance to FLT3 Inhibitors in Acute Myeloid Leukemia. Biomedicines. 2020;8:245.
17. Hannum C, Culpepper J, Campbell D, McClanahan T, Zurawski S, Bazan JF, et al. Ligand for FLT3/FLK2 receptor tyrosine kinase regulates growth of haematopoietic stem cells and is encoded by variant RNAs. Nature. 1994;368:643–8.
18. Mizuki M, Fenski R, Halfter H, Matsumura I, Schmidt R, Muller C, et al. Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. Blood. 2000;96:3907–14.
19. Hayakawa F, Towatari M, Kiyoi H, Tanimoto M, Kitamura T, Saito H, et al. Tandem-duplicated Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. Oncogene. 2000;19:624–31.
20. Brandts CH, Sargin B, Rode M, Biermann C, Lindtner B, Schwable J, et al. Constitutive activation of Akt by Flt3 internal tandem duplications is necessary for increased survival, proliferation, and myeloid transformation. Cancer Res. 2005;65:9643–50.
21. Ekim B, Magnuson B, Acosta-Jaquez HA, Keller JA, Feener EP, Fingar DC. mTOR kinase domain phosphorylation promotes mTORC1 signaling, cell growth, and cell cycle progression. Mol Cell Biol. 2011;31:2787–801.
22. Kiyoi H, Ohno R, Ueda R, Saito H, Naoe T. Mechanism of constitutive activation of FLT3 with internal tandem duplication in the juxtamembrane domain. Oncogene. 2002;21:2555–63.
23. Chung KY, Morrone G, Schuringa JJ, Wong B, Dorn DC, Moore MA. Enforced expression of an Flt3 internal tandem duplication in human CD34+ cells confers properties of self-renewal and enhanced erythropoiesis. Blood. 2005;105:77–84.
24. Spiekermann K, Bagrintseva K, Schwab R, Schmieja K, Hiddemann W. Overexpression and constitutive activation of FLT3 induces STAT5 activation in primary acute myeloid leukemia blast cells. Clin Cancer Res. 2003;9:2140–50.
25. Nosaka T, Kawashima T, Misawa K, Ikuta K, Mui AL, Kitamura T. STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. EMBO J. 1999;18:4754–65.
26. Kim KT, Baird K, Ahn JY, Meltzer P, Lilly M, Levis M, et al. Pim-1 is up-regulated by constitutively activated FLT3 and plays a role in FLT3-mediated cell survival. Blood. 2005;105:1759–67.
27. Al-Subaie AM, Kamaraj B. The structural effect of FLT3 mutations at 835th position and their interaction with acute myeloid leukemia inhibitors: in silico approach. Int J Mol Sci. 2021;22:7602.
28. Klingmuller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. Cell. 1995;80:729–38.
29. Zhang Y, Askenazi M, Jiang J, Luckey CJ, Griffin JD, Marto JA. A robust error model for iTRAQ quantification reveals divergent signaling between oncogenic FLT3 mutants in acute myeloid leukemia. Mol Cell Proteom. 2010;9:780–90.
30. Grundler R, Miething C, Thiede C, Peschel C, Duyster J. FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. Blood. 2005;105:4792–9.
31. Bailey E, Li L, Duffield AS, Ma HS, Huso DL, Small D. FLT3/D835Y mutation knock-in mice display less aggressive disease compared with FLT3/internal tandem duplication (ITD) mice. Proc Natl Acad Sci U S A. 2013;110:21113–8.
32. Yanada M, Matsuo K, Suzuki T, Kiyoi H, Naoe T. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. Leukemia. 2005;19:1345–9.
33. Gale RE, Green C, Allen C, Mead AJ, Burnett AK, Hills RK, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008;111:2776–84.
34. Schnittger S, Bacher U, Kern W, Alpermann T, Haferlach C, Haferlach T. Prognostic impact of FLT3-ITD load in NPM1 mutated acute myeloid leukemia. Leukemia. 2011;25:1297–304.
35. Pratcorona M, Brunet S, Nomdedeu J, Ribera JM, Tormo M, Duarte R, et al. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. Blood. 2013;121:2734–8.
36. Schlenk RF, Kayser S, Bullinger L, Kobbe G, Casper J, Ringhoffer M, et al. Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. Blood. 2014;124:3441–9.
37. Versluis J, In't Hout FE, Devillier R, van Putten WL, Manz MG, Vekemans MC, et al. Comparative value of post-remission treatment in cytogenetically normal AML subclassified by NPM1 and FLT3-ITD allelic ratio. Leukemia. 2017;31:26–33.
38. Linch DC, Hills RK, Burnett AK, Khwaja A, Gale RE. Impact of FLT3(ITD) mutant allele level on relapse risk in intermediate-risk acute myeloid leukemia. Blood. 2014;124:273–6.
39. Boddu PC, Kadia TM, Garcia-Manero G, Cortes J, Alfayez M, Borthakur G, et al. Validation of the 2017 European LeukemiaNet classification for acute myeloid leukemia with NPM1 and FLT3-internal tandem duplication genotypes. Cancer. 2019;125:1091–100.
40. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–21.
41. Bacher U, Haferlach C, Kern W, Haferlach T, Schnittger S. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters–an analysis of 3082 patients. Blood. 2008;111:2527–37.
42. Mead AJ, Linch DC, Hills RK, Wheatley K, Burnett AK, Gale RE. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. Blood. 2007;110:1262–70.
43. Whitman SP, Ruppert AS, Radmacher MD, Mrozek K, Paschka P, Langer C, et al. FLT3 D835/I836 mutations are associated with poor disease-free survival and a distinct gene-expression signature among younger adults with de novo cytogenetically normal acute myeloid leukemia lacking FLT3 internal tandem duplications. Blood. 2008;111:1552–9.
44. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129:424–47.
45. Boddu P, Kantarjian H, Borthakur G, Kadia T, Daver N, Pierce S, et al. Co-occurrence of FLT3-TKD and NPM1 mutations defines a highly favorable prognostic AML group. Blood Adv. 2017;1:1546–50.
46. Stone RM, DeAngelo DJ, Klimek V, Galinsky I, Estey E, Nimer SD, et al. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. Blood. 2005;105:54–60.
47. Fischer T, Stone RM, Deangelo DJ, Galinsky I, Estey E, Lanza C, et al. Phase IIB trial of oral midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and

high-risk myelodysplastic syndrome with either wild-type or mutated FLT3. J Clin Oncol. 2010;28:4339–45.
48. Borthakur G, Kantarjian H, Ravandi F, Zhang W, Konopleva M, Wright JJ, et al. Phase I study of sorafenib in patients with refractory or relapsed acute leukemias. Haematologica. 2011;96:62–8.
49. Smith CC, Lin K, Stecula A, Sali A, Shah NP. FLT3 D835 mutations confer differential resistance to type II FLT3 inhibitors. Leukemia. 2015;29:2390–2.
50. Weisberg E, Boulton C, Kelly LM, Manley P, Fabbro D, Meyer T, et al. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. Cancer Cell. 2002;1:433–43.
51. Stone RM, Fischer T, Paquette R, Schiller G, Schiffer CA, Ehninger G, et al. Phase IB study of the FLT3 kinase inhibitor midostaurin with chemotherapy in younger newly diagnosed adult patients with acute myeloid leukemia. Leukemia. 2012;26:2061–8.
52. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. N Engl J Med. 2017;377:454–64.
53. Dohner K, Thiede C, Jahn N, Panina E, Gambietz A, Larson RA, et al. Impact of NPM1/FLT3-ITD genotypes defined by the 2017 European leukemianet in patients with acute myeloid leukemia. Blood. 2020;135:371–80.
54. Larson RA, Mandrekar SJ, Huebner LJ, Sanford BL, Laumann K, Geyer S, et al. Midostaurin reduces relapse in FLT3-mutant acute myeloid leukemia: the alliance CALGB 10603/RATIFY trial. Leukemia. 2021;35:2539–51.
55. Schlenk RF, Weber D, Fiedler W, Salih HR, Wulf G, Salwender H, et al. Midostaurin added to chemotherapy and continued single-agent maintenance therapy in acute myeloid leukemia with FLT3-ITD. Blood. 2019;133:840–51.
56. Strati P, Kantarjian H, Ravandi F, Nazha A, Borthakur G, Daver N, et al. Phase I/II trial of the combination of midostaurin (PKC412) and 5-azacytidine for patients with acute myeloid leukemia and myelodysplastic syndrome. Am J Hematol. 2015;90:276–81.
57. Tomlinson BK, Gallogly MM, Kane DM, Metheny L, Lazarus HM, William BM, et al. A Phase II Study of midostaurin and 5-azacitidine for untreated elderly and unfit patients with FLT3 wild-type acute myelogenous leukemia. Clin Lymphoma Myeloma Leuk. 2020;20(226–33): e1.
58. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov. 2006;5:835–44.
59. Ravandi F, Cortes JE, Jones D, Faderl S, Garcia-Manero G, Konopleva MY, et al. Phase I/II study of combination therapy with sorafenib, idarubicin, and cytarabine in younger patients with acute myeloid leukemia. J Clin Oncol. 2010;28:1856–62.
60. Rollig C, Serve H, Huttmann A, Noppeney R, Muller-Tidow C, Krug U, et al. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukaemia (SORAML): a multicentre, phase 2, randomised controlled trial. Lancet Oncol. 2015;16:1691–9.
61. Rollig C, Serve H, Noppeney R, Hanoun M, Krug U, Baldus CD, et al. Sorafenib or placebo in patients with newly diagnosed acute myeloid leukaemia: long-term follow-up of the randomized controlled SORAML trial. Leukemia. 2021;35:2517–25.
62. Serve H, Krug U, Wagner R, Sauerland MC, Heinecke A, Brunnberg U, et al. Sorafenib in combination with intensive chemotherapy in elderly patients with acute myeloid leukemia: results from a randomized, placebo-controlled trial. J Clin Oncol. 2013;31:3110–8.
63. Uy GL, Mandrekar SJ, Laumann K, Marcucci G, Zhao W, Levis MJ, et al. A phase 2 study incorporating sorafenib into the chemotherapy for older adults with FLT3-mutated acute myeloid leukemia: CALGB 11001. Blood Adv. 2017;1:331–40.
64. Ohanian M, Garcia-Manero G, Levis M, Jabbour E, Daver N, Borthakur G, et al. Sorafenib combined with 5-azacytidine in older patients with untreated FLT3-ITD mutated acute myeloid leukemia. Am J Hematol. 2018;93:1136–41.
65. Ravandi F, Alattar ML, Grunwald MR, Rudek MA, Rajkhowa T, Richie MA, et al. Phase 2 study of azacytidine plus sorafenib in patients with acute myeloid leukemia and FLT-3 internal tandem duplication mutation. Blood. 2013;121:4655–62.
66. Levis M, Ravandi F, Wang ES, Baer MR, Perl A, Coutre S, et al. Results from a randomized trial of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. Blood. 2011;117:3294–301.
67. Knapper S, Russell N, Gilkes A, Hills RK, Gale RE, Cavenagh JD, et al. A randomized assessment of adding the kinase inhibitor lestaurtinib to first-line chemotherapy for FLT3-mutated AML. Blood. 2017;129:1143–54.
68. DeAngelo DJ, Stone RM, Heaney ML, Nimer SD, Paquette RL, Klisovic RB, et al. Phase 1 clinical results with tandutinib (MLN518), a novel FLT3 antagonist, in patients with acute myelogenous leukemia or high-risk myelodysplastic syndrome: safety, pharmacokinetics, and pharmacodynamics. Blood. 2006;108:3674–81.
69. Fiedler W, Kayser S, Kebenko M, Janning M, Krauter J, Schittenhelm M, et al. A phase I/II study of sunitinib and intensive chemotherapy in patients over 60 years of age with acute myeloid leukaemia and activating FLT3 mutations. Br J Haematol. 2015;169:694–700.
70. Shah NP, Talpaz M, Deininger MW, Mauro MJ, Flinn IW, Bixby D, et al. Ponatinib in patients with refractory acute myeloid leukaemia: findings from a phase 1 study. Br J Haematol. 2013;162:548–52.
71. Fathi AT, Blonquist TM, Hernandez D, Amrein PC, Ballen KK, McMasters M, et al. Cabozantinib is well tolerated in acute myeloid leukemia and effectively inhibits the resistance-conferring FLT3/tyrosine kinase domain/F691 mutation. Cancer. 2018;124:306–14.
72. Mori M, Kaneko N, Ueno Y, Yamada M, Tanaka R, Saito R, et al. Gilteritinib, a FLT3/AXL inhibitor, shows antileukemic activity in mouse models of FLT3 mutated acute myeloid leukemia. Invest New Drugs. 2017;35:556–65.
73. Park IK, Mundy-Bosse B, Whitman SP, Zhang X, Warner SL, Bearss DJ, et al. Receptor tyrosine kinase Axl is required for resistance of leukemic cells to FLT3-targeted therapy in acute myeloid leukemia. Leukemia. 2015;29:2382–9.
74. Perl AE, Altman JK, Cortes J, Smith C, Litzow M, Baer MR, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study. Lancet Oncol. 2017;18:1061–75.
75. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. N Engl J Med. 2019;381:1728–40.
76. Astellas Pharma, Inc. Press release (21 Dec 2020): Astellas Reports XOSPATA® (gilteritinib) in combination with azacitidine did not meet endpoint of overall survival in newly diagnosed FLT3 mutation-positive acute myeloid leukemia patients ineligible for intensive induction chemotherapy. Available at: https://www.astellas.com/us/news/5306 (Accessed 7 Mar 2022).
77. Zarrinkar PP, Gunawardane RN, Cramer MD, Gardner MF, Brigham D, Belli B, et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). Blood. 2009;114:2984–92.

78. Cortes JE, Kantarjian H, Foran JM, Ghirdaladze D, Zodelava M, Borthakur G, et al. Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukemia irrespective of FMS-like tyrosine kinase 3-internal tandem duplication status. J Clin Oncol. 2013;31:3681–7.
79. Cortes J, Perl AE, Dohner H, Kantarjian H, Martinelli G, Kovacsovics T, et al. Quizartinib, an FLT3 inhibitor, as monotherapy in patients with relapsed or refractory acute myeloid leukaemia: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol. 2018;19:889–903.
80. Cortes JE, Tallman MS, Schiller GJ, Trone D, Gammon G, Goldberg SL, et al. Phase 2b study of 2 dosing regimens of quizartinib monotherapy in FLT3-ITD-mutated, relapsed or refractory AML. Blood. 2018;132:598–607.
81. Cortes JE, Khaled S, Martinelli G, Perl AE, Ganguly S, Russell N, et al. Quizartinib versus salvage chemotherapy in relapsed or refractory FLT3-ITD acute myeloid leukaemia (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2019;20:984–97.
82. Swaminathan M, Kantarjian HM, Levis M, Guerra V, Borthakur G, Alvarado Y, et al. A phase I/II study of the combination of quizartinib with azacitidine or low-dose cytarabine for the treatment of patients with acute myeloid leukemia and myelodysplastic syndrome. Haematologica. 2021;106:2121–30.
83. Dennis M, Thomas IF, Ariti C, Upton L, Burnett AK, Gilkes A, et al. Randomized evaluation of quizartinib and low-dose ara-C vs low-dose ara-C in older acute myeloid leukemia patients. Blood Adv. 2021;5:5621–5.
84. Altman JK, Foran JM, Pratz KW, Trone D, Cortes JE, Tallman MS. Phase 1 study of quizartinib in combination with induction and consolidation chemotherapy in patients with newly diagnosed acute myeloid leukemia. Am J Hematol. 2018;93:213–21.
85. Daiichi Sankyo, Inc. Press release (18 Nov 2021): Quizartinib added to chemotherapy demonstrates superior overall survival compared to chemotherapy alone in adult patients with newly diagnosed FLT3-ITD positive AML. https://daiichisankyo.us/press-releases/-/article/364091/11880925. Accessed 7 Mar 2022.
86. Lewis NL, Lewis LD, Eder JP, Reddy NJ, Guo F, Pierce KJ, et al. Phase I study of the safety, tolerability, and pharmacokinetics of oral CP-868,596, a highly specific platelet-derived growth factor receptor tyrosine kinase inhibitor in patients with advanced cancers. J Clin Oncol. 2009;27:5262–9.
87. Galanis A, Ma H, Rajkhowa T, Ramachandran A, Small D, Cortes J, et al. Crenolanib is a potent inhibitor of FLT3 with activity against resistance-conferring point mutants. Blood. 2014;123:94–100.
88. Yanada M. The evolving concept of indications for allogeneic hematopoietic cell transplantation during first complete remission of acute myeloid leukemia. Bone Marrow Transplant. 2021;56:1257–65.
89. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358:1909–18.
90. DeZern AE, Sung A, Kim S, Smith BD, Karp JE, Gore SD, et al. Role of allogeneic transplantation for FLT3/ITD acute myeloid leukemia: outcomes from 133 consecutive newly diagnosed patients from a single institution. Biol Blood Marrow Transplant. 2011;17:1404–9.
91. Kurosawa S, Yamaguchi H, Yamaguchi T, Fukunaga K, Yui S, Wakita S, et al. Decision analysis of postremission therapy in cytogenetically intermediate-risk acute myeloid leukemia: the impact of FLT3 internal tandem duplication, nucleophosmin, and CCAAT/enhancer binding protein alpha. Biol Blood Marrow Transplant. 2016;22:1125–32.
92. Oran B, Cortes J, Beitinjaneh A, Chen HC, de Lima M, Patel K, et al. Allogeneic transplantation in first remission improves outcomes irrespective of FLT3-ITD allelic ratio in FLT3-ITD-positive acute myelogenous leukemia. Biol Blood Marrow Transplant. 2016;22:1218–26.
93. Sakaguchi M, Yamaguchi H, Najima Y, Usuki K, Ueki T, Oh I, et al. Prognostic impact of low allelic ratio FLT3-ITD and NPM1 mutation in acute myeloid leukemia. Blood Adv. 2018;2:2744–54.
94. Ho AD, Schetelig J, Bochtler T, Schaich M, Schafer-Eckart K, Hanel M, et al. Allogeneic stem cell transplantation improves survival in patients with acute myeloid leukemia characterized by a high allelic ratio of mutant FLT3-ITD. Biol Blood Marrow Transplant. 2016;22:462–9.
95. Kawashima N, Ishikawa Y, Atsuta Y, Sawa M, Ozawa Y, Hayashi M, et al. Allogeneic hematopoietic stem cell transplantation at the first remission for younger adults with FLT3-internal tandem duplication AML: the JALSG AML209-FLT3-SCT study. Cancer Sci. 2020;111:2472–81.
96. Yanada M, Konuma T, Yamasaki S, Kondo T, Fukuda T, Shingai N, et al. Relapse of acute myeloid leukemia after allogeneic hematopoietic cell transplantation: clinical features and outcomes. Bone Marrow Transplant. 2021;56:1126–33.
97. Brunet S, Labopin M, Esteve J, Cornelissen J, Socie G, Iori AP, et al. Impact of FLT3 internal tandem duplication on the outcome of related and unrelated hematopoietic transplantation for adult acute myeloid leukemia in first remission: a retrospective analysis. J Clin Oncol. 2012;30:735–41.
98. Deol A, Sengsayadeth S, Ahn KW, Wang HL, Aljurf M, Antin JH, et al. Does FLT3 mutation impact survival after hematopoietic stem cell transplantation for acute myeloid leukemia? A Center for International Blood and Marrow Transplant Research (CIBMTR) analysis. Cancer. 2016;122:3005–14.
99. Bazarbachi A, Labopin M, Battipaglia G, Djabali A, Forcade E, Arcese W, et al. Allogeneic stem cell transplantation for FLT3-mutated acute myeloid leukemia: in vivo T-Cell depletion and posttransplant sorafenib maintenance improve survival. A retrospective acute leukemia working party-European Society for Blood and Marrow Transplant Study. Clin Hematol Int. 2019;1:58–74.
100. Maziarz RT, Levis M, Patnaik MM, Scott BL, Mohan SR, Deol A, et al. Midostaurin after allogeneic stem cell transplant in patients with FLT3-internal tandem duplication-positive acute myeloid leukemia. Bone Marrow Transplant. 2021;56:1180–9.
101. Burchert A, Bug G, Fritz LV, Finke J, Stelljes M, Rollig C, et al. Sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with FLT3-internal tandem duplication mutation (SORMAIN). J Clin Oncol. 2020;38:2993–3002.
102. Xuan L, Wang Y, Huang F, Fan Z, Xu Y, Sun J, et al. Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. Lancet Oncol. 2020;21:1201–12.
103. Mathew NR, Baumgartner F, Braun L, O'Sullivan D, Thomas S, Waterhouse M, et al. Sorafenib promotes graft-versus-leukemia activity in mice and humans through IL-15 production in FLT3-ITD-mutant leukemia cells. Nat Med. 2018;24:282–91.
104. Sato T, Yang X, Knapper S, White P, Smith BD, Galkin S, et al. FLT3 ligand impedes the efficacy of FLT3 inhibitors in vitro and in vivo. Blood. 2011;117:3286–93.
105. Traer E, Martinez J, Javidi-Sharifi N, Agarwal A, Dunlap J, English I, et al. FGF2 from marrow microenvironment promotes resistance to FLT3 inhibitors in acute myeloid leukemia. Cancer Res. 2016;76:6471–82.

106. Chang YT, Hernandez D, Alonso S, Gao M, Su M, Ghiaur G, et al. Role of CYP3A4 in bone marrow microenvironment-mediated protection of FLT3/ITD AML from tyrosine kinase inhibitors. Blood Adv. 2019;3:908–16.
107. Heidel F, Solem FK, Breitenbuecher F, Lipka DB, Kasper S, Thiede MH, et al. Clinical resistance to the kinase inhibitor PKC412 in acute myeloid leukemia by mutation of Asn-676 in the FLT3 tyrosine kinase domain. Blood. 2006;107:293–300.
108. Alvarado Y, Kantarjian HM, Luthra R, Ravandi F, Borthakur G, Garcia-Manero G, et al. Treatment with FLT3 inhibitor in patients with FLT3-mutated acute myeloid leukemia is associated with development of secondary FLT3-tyrosine kinase domain mutations. Cancer. 2014;120:2142–9.
109. Baker SD, Zimmerman EI, Wang YD, Orwick S, Zatechka DS, Buaboonnam J, et al. Emergence of polyclonal FLT3 tyrosine kinase domain mutations during sequential therapy with sorafenib and sunitinib in FLT3-ITD-positive acute myeloid leukemia. Clin Cancer Res. 2013;19:5758–68.
110. Smith CC, Zhang C, Lin KC, Lasater EA, Zhang Y, Massi E, et al. Characterizing and overriding the structural mechanism of the quizartinib-resistant FLT3 "Gatekeeper" F691L Mutation with PLX3397. Cancer Discov. 2015;5:668–79.
111. McMahon CM, Ferng T, Canaani J, Wang ES, Morrissette JJD, Eastburn DJ, et al. Clonal selection with RAS pathway activation mediates secondary clinical resistance to selective FLT3 inhibition in acute myeloid leukemia. Cancer Discov. 2019;9:1050–63.
112. Lindblad O, Cordero E, Puissant A, Macaulay L, Ramos A, Kabir NN, et al. Aberrant activation of the PI3K/mTOR pathway promotes resistance to sorafenib in AML. Oncogene. 2016;35:5119–31.
113. Green AS, Maciel TT, Hospital MA, Yin C, Mazed F, Townsend EC, et al. Pim kinases modulate resistance to FLT3 tyrosine kinase inhibitors in FLT3-ITD acute myeloid leukemia. Sci Adv. 2015;1: e1500221.
114. Zhang H, Savage S, Schultz AR, Bottomly D, White L, Segerdell E, et al. Clinical resistance to crenolanib in acute myeloid leukemia due to diverse molecular mechanisms. Nat Commun. 2019;10:244.
115. McCormick SR, McCormick MJ, Grutkoski PS, Ducker GS, Banerji N, Higgins RR, et al. FLT3 mutations at diagnosis and relapse in acute myeloid leukemia: cytogenetic and pathologic correlations, including cuplike blast morphology. Arch Pathol Lab Med. 2010;134:1143–51.
116. Schmalbrock LK, Dolnik A, Cocciardi S, Strang E, Theis F, Jahn N, et al. Clonal evolution of acute myeloid leukemia with FLT3-ITD mutation under treatment with midostaurin. Blood. 2021;137:3093–104.
117. Yanada M, Naoe T. Acute myeloid leukemia in older adults. Int J Hematol. 2012;96:186–93.
118. DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med. 2020;383:617–29.
119. Yamaura T, Nakatani T, Uda K, Ogura H, Shin W, Kurokawa N, et al. A novel irreversible FLT3 inhibitor, FF-10101, shows excellent efficacy against AML cells with FLT3 mutations. Blood. 2018;131:426–38.
120. Reville PK, Sasaki K, Kantarjian HM, Daver NG, Yilmaz M, Dinardo CD, et al. Improved outcomes among newly diagnosed patients with FMS-like tyrosine kinase 3 internal tandem duplication mutated acute myeloid leukemia treated with contemporary therapy: revisiting the European leukemianet adverse risk classification. Am J Hematol. 2022;97:329–37.