FLT3 mutations in acute myeloid leukemia: a review focusing on clinically applicable drugs

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Abstract

FMS-like tyrosine kinase 3 (FLT3) mutations, the most frequently detected genetic aberrations in patients with acute myeloid leukemia (AML), are identified in approximately 30% of patients with newly diagnosed AML and are more common in patients with normal karyotypes. Since the discovery of FLT3 mutations in AML, clinical trials have been actively conducted in patients with FLT3 mutated AML, and FLT3 inhibitors have been introduced into clinical practice. The current standard treatment for patients with newly diagnosed FLT3-mutated AML is 7+3 induction chemotherapy combined with midostaurin. Additionally, gilteritinib is more effective than salvage chemotherapy for relapsed or refractory FLT3-mutated AML. Ongoing trials are expected to provide additional treatment options depending on the disease state and patient vulnerability. This review summarizes information on clinically available FLT3 inhibitors for the management of AML with FLT3 mutations.

Key Words

Acute myeloid leukemia, FLT3-ITD, FLT3-TKD, Tyrosine kinase inhibitor, Gilteritinib, Midostaurin

INTRODUCTION

Acute myeloid leukemia (AML), the most common type of acute leukemia in adults, is characterized by poor prognosis, with a 5-year overall survival (OS) of 35% and less than 10% in patients over 65 years of age [1]. Approximately 1,300 patients are diagnosed with AML annually in South Korea [1]. In recent decades, clonal chromosomal aberrations and molecular mutations have been recognized as the most important prognostic markers in AML [2-4]. FMS-like tyrosine kinase 3 (FLT3) mutations, the most commonly observed genetic aberrations in patients with AML, are identified in approximately 30% of patients with newly diagnosed AML and are more frequently observed in patients with normal karyotypes. Since 1996, when the FLT3 internal tandem duplication (ITD) mutation was first identified in AML, numerous studies have been conducted regarding its relevance in prognosis [4-6]. Moreover, several advances have been made in targeting FLT3 mutations, and currently, FLT3 inhibitors are actively used in clinical practice [7-12]. In this review, we summarize information on clinically available FLT3 inhibitors for the management of AML with FLT3 mutations.

FLT3 MUTATIONS

FLT3 transcribes FLT3 transmembrane receptor tyrosine kinase. It is usually expressed in marrow stromal cells and hematopoietic cells and is activated by the FLT3 ligand. FLT3 plays a key role in hematopoietic cell maturation and proliferation [13, 14]. FLT3 mutations lead to the activation of tyrosine kinase by initiating FLT3 ligand-independent dimerization activation, which results in aberrant proliferation of leukemic cells.

FLT3 mutations are heterogeneous in terms of their load, size, and location [15], and are divided into two classes: ITD involving the juxtamembrane domain and that involving the tyrosine kinase domain (TKD). FLT3-ITD mutation leads to a gain-of-function by inhibiting the negative regulatory function of the juxtamembrane domain [16, 17]. FLT3-TKD mutations are point mutations in the activation loop of FLT3, mainly represented by codon D835 or deletion of codon 1836, which leads to a loss of auto-inhibition [18]. Both mutations lead to the activation of downstream proliferation
**FLT3 mutations in AML**

FLT3-ITD has a poor prognostic impact in patients with AML at diagnosis. However, FLT3-TKD mutations have not been associated with AML prognosis [4]. Owing to its prognostic significance and the choice of tyrosine kinase inhibitors, the European LeukemiaNet (ELN) recommends including molecular genetic testing for mutations of FLT3, both for ITD with allelic ratio and TKD, at diagnostic workup [4]. Testing for FLT3 mutations at relapse is also necessary because acquisition or loss of FLT3 mutations occurs due to clonal evolution in 20% of patients with relapsed AML [21, 22]. Rapid assays to identify FLT3 mutations are essential for the use of FLT3-targeting agents [4, 7, 23]. Clinically available assays include FLT3 polymerase chain reaction (PCR) and targeted DNA next-generation sequencing (NGS) [24]; however, their sensitivities and accuracies are different. Furthermore, turn-around time, an obstacle in deciding the treatment, varies according to the test. As NGS usually takes 2–4 weeks to generate mutation data, it is critical to obtain rapid results using FLT3 PCR tests when treatment decisions need to be made quickly. In addition, to determine prognosis at diagnosis, it is necessary to conduct a quantitative analysis of FLT3-ITD [4]. In the 2017 ELN risk stratification model, the presence of FLT3-ITD mutations is classified according to allelic ratio [4]. The allelic ratio is calculated as the ratio of the area under the curve of the mutant allele to the wild-type allele. However, NGS can quantify FLT3-ITD results in variant allele frequency (VAF). VAF is calculated as the fraction of mutant alleles as a percentage of all FLT3 alleles (wild-type+mutant). Therefore, there is a need for caution in the interpretation of the FLT3-ITD mutant burden based on the VAF and allelic ratio.

**PROGNOSTIC SIGNIFICANCE OF FLT3-ITD IN AML**

The prognostic impact of FLT3-ITD in AML is affected by the mutant allelic ratio and co-mutation status of nucleophosmin 1 (NPM1) [4]. The 2017 ELN guidelines stratify FLT3-ITD AML into three risk groups: 1) AML with an FLT3-ITD high allelic ratio (≥0.5) in the absence of NPM1 mutations is stratified as an adverse risk category; 2) FLT3-ITD low allelic ratio (≤0.5) is associated with favorable risk in patients with NPM1 co-mutation; and 3) intermediate risk is observed in patients with NPM1 wild-type and FLT3-ITD low allelic ratio or NPM1 mutated and FLT3-ITD high allelic ratio [4, 25]. However, a study on patients with intermediate cytogenetic risk showed a high relapse rate (68–79%) regardless of the allelic burden of FLT3-ITD in patients with NPM1 and FLT3-ITD mutated AML [26]. Oran et al. [27] also reported that allogeneic hematopoietic cell transplantation (HCT) improves relapse-free survival (RFS) and OS compared with those with consolidation chemotherapy, regardless of the allelic ratio in FLT3-ITD mutated AML. An FLT3-ITD low allelic ratio is not as favorable as the FLT3-ITD wild-type in patients with AML and NPM1 mutations, as seen previously. Allogeneic HCT for post-remission therapy may be considered to reduce the relapse risk even in patients with FLT3-ITD low allelic ratio, regardless of NPM1 mutation status [12, 26-28].

The prognostic relevance of FLT3-TKD mutations is conflicting [29]. However, the importance of FLT3-TKD mutations is emerging as targetable FLT3 inhibitors have been introduced [7, 23, 30].

**CLINICALLY APPLICABLE FLT3 INHIBITORS**

FLT3 tyrosine kinase inhibitors differ in potency, selectivity, mode of binding, and protein binding [31]. Type I FLT3 inhibitors bind in the kinase-active conformation, whereas type II inhibitors bind in the inactive conformation [24]. Representative type I inhibitors include midostaurin, gilteritinib, and crenolanib, whereas type II inhibitors include quizartinib and sorafenib. In general, type II FLT3 inhibitors have increased selectivity compared with that for type I FLT3 inhibitors (Table 1) [32].

**MIDOSTAURIN**

Midostaurin was one of the first FLT3 inhibitors to be studied in patients with AML. In the phase 3 RATIFY trial, midostaurin was evaluated in combination with standard induction and consolidation therapy and maintenance in young adults (<60 yr) with newly diagnosed FLT3-mutated AML [7]. This regimen was used for FLT3-ITD- and TKD-mutated AML. The combination of midostaurin with standard 7+3 induction chemotherapy has been shown to improve OS significantly, with a median OS of 74.7 months in patients receiving midostaurin plus chemotherapy vs. 25.6 months in patients receiving 7+3 chemotherapy alone (hazard ratio=0.78, 95% CI, 0.61). However, the FDA has not approved midostaurin for maintenance therapy after consolidation or allogeneic transplantation. Based on the results of this study, midostaurin was approved for clinical use by the U.S. Food and Drug Administration (FDA) in April 2017. The RATIFY trial was designed for maintenance with midostaurin for 12 months in young patients (18–60 yr). Older patients (≥60 yr) were not enrolled in the RATIFY trial; however, no age restrictions were imposed for midostaurin combination therapy in the FDA approval. A phase 2 trial was extended to patients up to 70 years of age for midostaurin plus intensive chemotherapy [33]. Compared with that for historical controls, midostaurin significantly improved event-free survival in overall age (hazard ratio=0.58, 95% CI, 0.48-0.70) and in older patients (hazard ratio=0.42, 95% CI, 0.29-0.61). However, the FDA has not approved midostaurin for maintenance therapy after consolidation or allogeneic transplantation. The European Medicines Agency (EMA) granted marketing authorization for midostaurin in 2017. The EMA included an indication for midostaurin maintenance therapy until relapse for up to 12 months in adult patients in complete remission following induction and consolidation.
### Table 1. Summary of clinically applicable FLT3 inhibitors for FLT3-mutated AML.

| Patient eligibility | Disease status | Drug | Target mutated lesion | Representative trial | Usage | Benefit | Approval | In Korea |
|---------------------|---------------|------|-----------------------|-----------------------|-------|---------|----------|---------|
| Intensive induction eligible | Newly diagnosed | Midostaurin | FLT3-ITD/TKD | RATIFY (Phase3) [7] | Combination with 7+3 induction and consolidation chemotherapy Maintain until relapse for up to 12 months | Median OS (74.7 vs. 25.6 mo), P=0.009 | FDA, EMA Available |
| Maintenance | Midostaurin | FLT3-ITD/TKD | RATIFY (Phase3) [7] | Maintain until relapse for up to 24 months | Monotherapy | 2-year RFS (85 vs. 53%), P=0.002 | Off-label Not available |
| Post-HCT maintenance | Sorafenib | FLT3-ITD | SORMAIN (Phase 2) [9] | Maintain until relapse for up to 24 months | Monotherapy | Median OS (9.3 vs. 5.6 mo), P<0.001 | FDA, EMA Available |
| Relapsed or refractory | Gililtersinib | FLT3-ITD/TKD | ADMIRAL (Phase 3) | Monotherapy | Median OS (8.3 mo) | Off-label Not available |
| Intensive induction ineligible | Newly diagnosed | Sorafenib | FLT3-ITD | NCT02196857 (Phase 2) and NCT01254890 (Phase 1/2) [35] | Combination with azacitidine | Response rate: 46% | Off-label Not available |
| Relapsed or refractory | Sorafenib | FLT3-ITD | NCT01254890 [36] | Combination with azacitidine | Off-label Not available |

Abbreviations: AML, acute myeloid leukemia; EMA, European Medicines Agency; FDA, Food and Drug Administration; FLT3, FMS-like tyrosine kinase 3; HCT , hematopoietic cell transplantation; ITD, internal tandem duplication; mo, months; OS, overall survival; RFS, relapse-free survival; TKD, tyrosine kinase domain.

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**Gilteritinib**

Gilteritinib is a potent type I \textit{FLT3} inhibitor that targets \textit{FLT3}-ITD and \textit{FLT3}-TKD mutations. The FDA and EMA approved gilteritinib monotherapy for relapsed or refractory (R/R) \textit{FLT3}-mutated AML based on the interim data of the Phase 3 ADMIRAL trial [23]. The ADMIRAL trial evaluated gilteritinib monotherapy vs. investigator-choice salvage chemotherapy in patients with R/R \textit{FLT3}-ITD-mutated AML. Compared with that for salvage chemotherapy, gilteritinib showed significantly superior complete remission (CR) and CR with hematologic improvement (CRh) rate (34% vs. 15%, \(P=0.0001\)) and decreased the death rate by 36%, with a median OS of 9.3 months vs. 5.6 months (\(P<0.001\)).

A phase 3 trial is ongoing to determine whether gilteritinib has therapeutic benefits similar to those of midostaurin in newly diagnosed AML with \textit{FLT3} mutations (NCT04027309, HOVON 156 AML trial). The HOVON 156 AML trial compared gilteritinib with midostaurin combined with intensive chemotherapy, followed by maintenance therapy. Additionally, an ongoing phase 3 trial is being conducted to elucidate the role of gilteritinib in post-transplant maintenance (NCT02997202, MORPHO trial) and following induction/consolidation therapy (NCT02927262) in patients with \textit{FLT3}-ITD-mutated AML.

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**Quizartinib**

Quizartinib is a second-generation potent type II \textit{FLT3} inhibitor. A phase 3 randomized controlled trial (QuANTUM-R) evaluated quizartinib monotherapy vs. investigator choice salvage chemotherapy in patients with R/R \textit{FLT3}-ITD-mutated AML [8]. OS was longer in the quizartinib group than that in the chemotherapy group [hazard ratio 0.76 (95% CI, 0.58-0.98; \(P=0.02\)]. The median OS was 6.2 months (5.3-7.2) in the quizartinib group and 4.7 months (4.0-5.5) in the chemotherapy group, and 32% of the patients in the quizartinib group underwent allogeneic transplantation compared with 11% of the patients in the salvage chemotherapy group. Despite these positive results, both the FDA and EMA have rejected the marketing authorization for quizartinib because of various reasons, such as dropouts (23% of the control group did not receive chemotherapy) and concerns about cardiac and infection adverse events. However, Japan has approved quizartinib as a monotherapy for R/R \textit{FLT3}-ITD-mutated AML. In patients with newly diagnosed AML, quizartinib is currently being evaluated in the Phase 3 QuANTUM-First trial, which compares quizartinib vs. placebo with 7+3 induction, consolidation, and maintenance (NCT02668653). Owing to improved efficacy and selectivity, the second-generation type II \textit{FLT3} inhibitor quizartinib achieved much higher single-agent clinical response rates than those with the first-generation type I \textit{FLT3} inhibitor midostaurin. Despite the high response rates achieved
with quizartinib in patients with R/R FLT3-ITD-mutated AML, the responses were not strong. Most patients relapsed because of secondary FLT3-TK mutations, impairing quizartinib binding [34].

SORAFENIB

Sorafenib is a first-generation, type II FLT3 inhibitor. It remains unapproved for use in patients with AML; however, several studies have shown its potential in FLT3-ITD-mutated AML. A phase 2, randomized, placebo-controlled trial (SORAML) evaluated 7+3 induction and consolidation with or without sorafenib in young individuals (≤60 yr) with newly diagnosed AML, regardless of FLT3-ITD mutation status. Sorafenib improved event-free survival (21 mo vs. 9 mo, P=0.013) but not OS. Sorafenib with azacitidine, as a front-line strategy in older patients (>60 yr) with FLT3-ITD-mutated AML who could not tolerate intensive induction, reported an overall response rate of 78% (CR: 26%, CR with incomplete count recovery (CRI)/CR with incomplete platelet recovery (CRp): 44%, and partial response (PR): 7%) [35]. Sorafenib combined with azacitidine demonstrated an overall response rate of 46% (CR: 16%, CRI: 27%, PR: 3%) for FLT3-ITD-mutated R/R AML [36].

The SORMAIN trial (a placebo-controlled, randomized, phase 2 trial) evaluated sorafenib maintenance therapy in patients with FLT3-ITD-mutated AML undergoing allogeneic HCT. The hazard ratio (HR) for relapse or death for sorafenib vs. placebo was 0.39 (95% CI, 0.18-0.85; log-rank P=0.013). The probability of 24-month RFS was 85.0% (95% CI, 0.70-0.93) with sorafenib (HR, 0.256; 95% CI, 0.10-0.65) and 53.3% (95% CI, 0.36-0.68) with placebo (log-rank P=0.002) [9]. In another phase 3 trial, sorafenib also demonstrated a decreased 1-year cumulative incidence of relapse (7.0% vs. 24.5%, P=0.001) and improved OS (82.1% vs. 68%, P=0.012) without treatment-related deaths [11]. The post-transplant maintenance results for sorafenib suggest potential synergy with post-transplant alloimmune effects [12, 37].

CONCLUSION

Rapid determination of FLT3 mutations during diagnosis or relapse is essential for making treatment decisions to manage AML and for the early selection of FLT3-targeting agents. The current standard treatment for a patient with a newly diagnosed FLT3-mutated AML is 7+3 induction chemotherapy combined with midostaurin [10]. In FLT3-mutated AML, allogeneic HCT as a post-remission therapy is considered to lower the risk of relapse. Although the role of post-transplant maintenance with FLT3 inhibitors has not been established, experts recommend maintenance therapy to reduce relapse risk [10, 12]. Gilteritinib is more effective than salvage chemotherapy for R/R FLT3-mutated AML. Ongoing trials are expected to provide additional treatment options depending on the disease state and patient vulnerability to FLT3-mutated AML.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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