Abstract: Quizartinib is an effective therapy for patients with FLT3-ITD acute myeloid leukemia (AML) by continuing to inhibit the activity of FLT3 gene, leading to apoptosis of tumor cells. Multiple clinical trials have proved that it is effective in relapsed or refractory AML with an FLT3-ITD mutation. In this review, we focus on the characteristics of FLT3-ITD mutations, the mechanism and pharmacokinetics of quizartinib, and the mechanisms of resistance to quizartinib. We also summarize clinical experiences and adverse effects with quizartinib and recommend crucial approaches of quizartinib in the therapy of patients with newly diagnosed AML and patients with relapsed/refractory AML, particularly those with FLT3-ITD mutation. Quizartinib presents its advantages as a very promising agent in the treatment of AML, especially in patients with FLT3-ITD mutations. FLT3/ITD mutation can lead to constitutive autophosphorylation of FLT3 and activation of its downstream effectors including RAS/RAF/MEK, MAPK/ERK, PI3K/AKT/mTOR and JAK/STAT5 signal pathways, while Quizartinib can inhibit these downstream pathways through specific FLT3 inhibition. Quizartinib has received US Food and Drug Administration breakthrough therapy designation in patients with relapsed/refractory FLT3-ITD AML based on clinical trials. A larger sample of clinical trials are needed to verify its safety and efficacy, and the efficacy of quizartinib combined with chemotherapy or allogeneic hematopoietic cell transplantation should also be estimated in clinical trials. Meanwhile, for the side effects of quizartinib, further studies are needed to find a way to reduce its toxicity.

Keywords: quizartinib, FLT3 inhibitor, FLT3-ITD mutation, AML, clinical trials, targeted therapy

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by multiple genetic aberrations.1 Some progress has been made in the pathogenesis of AML in recent years, there is a deeper understanding in molecular biology, immunology, clinical features and prognosis. Some breakthroughs have been made in the treatment of AML compared with the past. Traditional treatment regimens include the so-called “7+3” regimen, specifically 7 days of cytarabine +3 days of daunorubicin, and the 5-year survival rate of AML patients is still not optimistic. Besides, due to age and health conditions, many elderly patients find it difficult to adhere to the “7+3” treatment.2–4 Studies have shown that venetoclax in combination with the hypomethylating regimen can be used for the treatment of AML patients who ineligible for standard induction chemotherapy, with high response rate and long duration. Venetoclax in combination with the hypomethylating agent was approved by the US Food and Drug Administration (FDA) as a new treatment for the elderly.5,6 Moreover, approximately 20–30% of AML patients carry an internal tandem duplication (ITD)
mutation in the \textit{FLT3} gene, carrying a dismal prognosis,\textsuperscript{7} and is considered as one of the adverse risk groups in the 2017 European LeukemiaNet risk stratification.\textsuperscript{8} All of these bring great challenges to the treatment of AML.

Quizartinib, a specific oral \textit{FLT3} inhibitor which can continue inhibiting the activity of \textit{FLT3} gene, leading to apoptosis of tumor cells.\textsuperscript{9–12} Currently, it has been granted as fast-track status to treat recurrent/refractory AML, as well as orphan status to treat AML by the US FDA and the European Drug Administration. In multiple clinical trials, quizartinib has shown its efficiency and security in patients with relapsed/refractory \textit{FLT3-ITD} mutant AML or patients who have undergone transplantation or a second-line treatment.\textsuperscript{13,14} In this review, we address the characteristics of \textit{FLT3/ITD} mutations, the mechanisms and pharmacokinetics of quizartinib and the mechanisms of resistance to quizartinib. We also review clinical studies and adverse effects with quizartinib and suggest critical approaches of quizartinib in the treatment of relapsed/refractory AML.

**Characteristics of \textit{FLT3} mutations**

The human \textit{FLT3} gene (Fms Related Tyrosine Kinase 3) is a Protein-Coding gene which is located on band 13q12 and organized in 24 exons, and is ordinarily only expressed in primitive hematopoietic precursors, plays an important role in normal growth and differentiation of hematopoietic antecedent cells.\textsuperscript{15–18} It encodes a protein of 993 amino acids with four domains\textsuperscript{19,20} (Figure 1). Several different mutations can occur in the \textit{FLT3} gene and the \textit{ITD} mutation on exon 14 is the most common mutation, which occurs in about 23% of de novo AML patients.\textsuperscript{21,22} \textit{ITD} mutations occur in the juxtamembrane region of the receptor, which can damage its negative regulatory function, resulting in constitutive autophosphorylation of \textit{FLT3} and activation of its downstream effectors including \textit{RAS/RAF/MEK}, \textit{MAPK/ERK}, \textit{PI3K/AKT/mTOR} and \textit{JAK/STAT5} signal pathways, all of which plays a significant role in the development of cell cycle progression, cell proliferation, survival, and differentiation, and cooperates with other recurrent molecular abnormalities to induce acute leukemia.\textsuperscript{21,23–28} \textit{ITD} mutations range in length from 3 to 400 base pairs. It has been reported that the length of the \textit{ITD} mutation has prognostic significance, and there is a correlation between the increase in \textit{ITD} length and the decrease in overall survival.\textsuperscript{18,29,30}

*Figure 1* The protein structure encoded by the \textit{FLT3} gene and the relevant pathways initiated by the activation of the \textit{FLT3} receptor. \textit{FLT3} encodes for a protein with 993 amino acids, which is a member of class III receptor tyrosine kinase family, containing an extracellular ligand binding domain, a transmembrane domain, and, intracellularly, a tyrosine kinase domain and juxtamembrane domain. \textit{ITD} mutations occur in the juxtamembrane region of the receptor, which can damage its negative regulatory function, resulting in constitutive autophosphorylation of \textit{FLT3} and activation of its downstream effectors including \textit{RAS/RAF/MEK}, \textit{MAPK/ERK}, \textit{PI3K/AKT/mTOR} and \textit{JAK/STAT5} pathways, all of which plays a significant role in the promotion of cell cycle progression, cell proliferation, survival and differentiation.
Mechanism and pharmacokinetics of quizartinib

FLT3 is an essential therapeutic target for the treatment of AML. It is a member of class III receptor tyrosine kinase family and is generally only expressed in primordial hematopoietic ancestors within the bone marrow. Tyrosine kinase inhibitors were incipiently developed for the therapy of solid neoplasms. The original intention of the researchers was to inhibit other kinases but then stumbled upon that they could also inhibit the activity of FLT3 gene. Therefore, in addition to inhibiting FLT3, the original tyrosine kinase inhibitors also inhibit other various kinases, resulting in poor specificity, high toxic side effects and low efficacy when used in the treatment of FLT3-ITD AML. And previous experiments have also shown that the pharmacokinetics of the first-generation tyrosine kinase inhibitors are not ideal enough to continue to exert inhibition. In order to solve the problem of tyrosine kinase production, the second generation of tyrosine kinase came into being. Quizartinib is the first drug designed as a selective FLT3 inhibitor with better pharmacokinetic characteristics, high specificity, more effective, better tolerance and other characteristics. The mechanism of quizartinib is that binding to FLT3 tyrosine kinase receptor isoform, thus leading to the inhibition of cancer cell proliferation and causes cancer cell death.

Quizartinib is a selective and efficient FLT3 inhibitor with IC50 ≤1 nmol/L. Its half-life is relatively long, approximately more than 1.5 days. AC886 is a pharmacologically effective metabolite of quizartinib, which was recognized in early experiments. Like quizartinib, their systemic exposure was dose-dependent. Existing clinical trials have shown that for R/R AML patients, the maximum tolerated dose (MTD) was 60 mg/day when combined with induction and strengthening chemotherapy. Besides, another Phase I study administered by Altman et al demonstrated that the MTD was 60 mg/day when combined with induction and strengthening chemotherapy. In another Phase I clinical trial of quizartinib monotherapy, quizartinib was used for supporting therapy in patients with AML after allogeneic hematopoietic stem cell transplantation. In this clinical trial, quizartinib was given orally in a 28-day cycle up to 24 cycles and the maximum dose of quizartinib for constant daily treatment is 60 mg. All of these clinical studies have shown that quizartinib has good tolerance and controllable safety. Whether as a single drug or in combination with chemotherapy or as supporting therapy after allogeneic hematopoietic stem cell transplantation, quizartinib showed a promising antileukemia activity in newly diagnosed AML patients and R/R AML patients, especially those with FLT3-ITD mutations.

Preclinical studies

In the leukemia cell lines with a homozygous FLT3-ITD mutation and cell lines which express wild-type FLT3, AC220 has been proved to restrain FLT3 autophosphorylation and cell proliferation. In vivo, AC220 can significantly prolong survival at a dose of 1 mg/kg orally once a day and eradicate tumors at 10 mg/kg. It has also been demonstrated to inhibit FLT3 activity in primary leukemia cells. In a preclinical model using immortalized MV4-11 cells, quizartinib-treated mice showed rapid and complete tumor regression. In another leukemia model transplanted into the bone marrow, quizartinib achieved an increase in overall survival at 10 mg/kg, which was 25% longer than the control group.

Clinical studies

AC220 has been studied in a number of clinical trials as a single drug, as well as in combination with chemotherapy drugs for the treatment of AML and satisfactory results have been reported in multiple clinical trial centers. (Tables 1 and 2)

Phase I

In a Phase I study, quizartinib was given as a single agent, 6 healthy male volunteers were treated at a dose of 60 mg oral solution, and the study confirmed that quizartinib has excellent safety and tolerance in healthy men. In another Phase I, multicenter dose-escalation research, Jorge et al conducted quizartinib at an escalating dose of 12–450 mg/day in 28-day cycles to 76 patients with R/R AML. Concomitant use of hydroxyurea was allowed for up to 5 days during the first 28 days of this study, up to a maximum dose of 5 g/day. This study revealed that the maximum-tolerated dose (MTD) was 200 mg/day. Besides, another Phase I study administered by Altman et al demonstrated that the MTD was 60 mg/day when combined with induction and strengthening chemotherapy. In another Phase I clinical trial of quizartinib monotherapy, quizartinib was used for supporting therapy in patients with AML after allogeneic hematopoietic stem cell transplantation. In this clinical trial, quizartinib was given orally in a 28-day cycle up to 24 cycles and the maximum dose of quizartinib for constant daily treatment is 60 mg. All of these clinical studies have shown that quizartinib has good tolerance and controllable safety. Whether as a single drug or in combination with chemotherapy or as supporting therapy after allogeneic hematopoietic stem cell transplantation, quizartinib showed a promising antileukemia activity in newly diagnosed AML patients and R/R AML patients, especially those with FLT3-ITD mutations.
mutation and two were for relapsed/refractory AML. Furthermore, one of the six clinical trials have been completed and the other five are underway. Jorge et al divided 333 patients with relapsed/refractory AML into two groups to evaluate the effect of quizartinib. Patients aged 60 or older who received first-line treatment within 1 year were listed in group 1, and those aged 18 or older who received salvage chemotherapy or hematopoietic stem cell transplantation were listed in group 2. *FIT3-ITD* mutation was detected among all patients; the doses of quizartinib were 135 mg/d for male and 90 mg/d for female in 28-day treatment cycles continued until relapse. Among 332 evaluable patients, the composite complete remission rates were 47% in both groups. The bridge to graft rate for the two group patients was 32% and 42%, the continuation of composite complete remission was 4.2 and 9.1 weeks and the median overall survival time was 20.9 and 27.3 weeks, respectively. It showed that a daily dose of 60 mg is more effective than a daily dose of 30 mg.51

**Table 1** Summary of clinical trials using quizartinib as single agent

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier</th>
<th>Trial</th>
<th>Number (ages eligible for study)</th>
<th>Disease characteristics</th>
<th>Dosage</th>
<th>Clinical response rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02675478</td>
<td>Phase I</td>
<td>17 (20 years and older)</td>
<td>Relapsed or refractory AML</td>
<td>Escalating doses of AC220</td>
<td>Without results</td>
</tr>
<tr>
<td>NCT00462761&lt;sup&gt;1,3&lt;/sup&gt;</td>
<td>Phase I</td>
<td>76 (18 years and older)</td>
<td>Relapsed or refractory AML regardless of FLT3 status</td>
<td>14 days</td>
<td>OR: 30% (CR: 13%, PRs: 17%)</td>
</tr>
<tr>
<td>NCT01468467&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>Phase I</td>
<td>13 (18 years and older)</td>
<td>As maintenance therapy after treatment with an allogeneic stem cell transplant</td>
<td>28 consecutive days a cycle with 24 continuous treatment cycles</td>
<td>MS: 14 weeks</td>
</tr>
<tr>
<td>NCT02984995</td>
<td>Phase II</td>
<td>38 (20 years and older)</td>
<td>Relapsed or refractory AML with FLT3-ITD(+)</td>
<td>Once-daily</td>
<td>OS: 13–142 weeks 69% patients ≥50 weeks 31% patients ≥2 year</td>
</tr>
<tr>
<td>NCT01565668&lt;sup&gt;51&lt;/sup&gt;</td>
<td>Phase IIb</td>
<td>76 (18 years and older)</td>
<td>Relapsed or refractory AML with FLT3-ITD(+)</td>
<td>28-day cycles</td>
<td>OR: 30 mg group: 61% 60 mg group: 71%</td>
</tr>
<tr>
<td>NCT02039726&lt;sup&gt;57&lt;/sup&gt;</td>
<td>Phase III</td>
<td>367 (18 years and older)</td>
<td>Relapsed or refractory AML with FLT3-ITD(+)</td>
<td>20 or 30 mg quizartinib</td>
<td>Median OS: 27 weeks</td>
</tr>
<tr>
<td>NCT03746912</td>
<td>Expanded treatment</td>
<td>18 years and older (adult, older adult)</td>
<td>Relapsed or refractory AML with FLT3-ITD mutations</td>
<td>Once-daily oral administration at doctor-recommended dose in continuous 28-day cycles</td>
<td>Recruiting without results</td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; CRs, complete remissions; OR, overall response; MS, median survival; OS, overall survival; PRs, partial remissions.

**Phase III**

The current Phase III clinical trials of quizartinib as a promising drug for AML, compared the efficacy of quizartinib with induction/consolidation chemotherapy and salvage chemotherapy and further assessed the potency of quizartinib in maintenance treatment. A Phase III clinical trial conducted by Schlenk et al demonstrated that patients receiving quizartinib had a 24% lower risk of death than those receiving salvage chemotherapy. The median total survival time of patients receiving salvage chemotherapy was 6.2 months (95% CI 5.3–7.2 on both sides), and 4.7 months for patients receiving quizartinib (95% CI 4.0–5.5 on both sides). The 1-year survival rate for quizartinib was 27%, compared with 20% for patients receiving salvage chemotherapy.55
Table 2 Summary of clinical trials of quizartinib in combination with chemotherapy

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier</th>
<th>Trial Number (ages eligible for study)</th>
<th>Disease characteristics</th>
<th>Chemotherapy</th>
<th>Clinical response rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT03552029</td>
<td>Phase I 156 (18 years and older)</td>
<td>AML with FLT3-ITD(+)</td>
<td>Quizartinib+milademetan, 20 or 30 mg quizartinib</td>
<td>Recruiting without results</td>
</tr>
<tr>
<td>NCT01390337*</td>
<td>Phase I 19 (18 years to 60 years)</td>
<td>Newly diagnosed AML</td>
<td>Escalating doses of AC220 7+3 cytarabine and daunorubicin</td>
<td>OR: 84% (74% CRc)</td>
</tr>
<tr>
<td>NCT01892371</td>
<td>Phase I/II 72 (18 years and older)</td>
<td>AML and MDS</td>
<td>Quizartinib start concomitantly with azacitidine/cytarabine Phase I: 60 mg daily of a 28 day cycle Phase II: maximum tolerated dose from Phase I. Decitabine: 20 mg/m²/d by vein on days 1–10 Quizartinib: 40 mg/d by mouth every day beginning on day 5 of cycle I</td>
<td>OR: 67% MS: 14.8 months</td>
</tr>
<tr>
<td>NCT03661307</td>
<td>Phase I/II 52 (18 years and older)</td>
<td>AML with FLT3-ITD(+)</td>
<td>Omacetaxine mepesuccinate+quizartinib</td>
<td>Recruiting without results</td>
</tr>
<tr>
<td>NCT03135054</td>
<td>Phase II 40 (18 years and older)</td>
<td>AML carrying FLT3-ITD</td>
<td>Quizartinib 30 mg daily continuously; every 28 days</td>
<td>Recruiting without results</td>
</tr>
<tr>
<td>NCT02668653</td>
<td>Phase III 536 (18–75 Years)</td>
<td>Newly diagnosed FLT3-ITD (+) AML</td>
<td>Induction: 2 cycles cytarabine and daunorubicin/idarubicin Consolidation: 4 cycles of cytarabine Maintenance: 12 cycles experimental quizartinib Quizartinib: 30 mg daily by mouth Venetoclax: 400 mg daily by mouth</td>
<td>Recruiting without results</td>
</tr>
</tbody>
</table>

Notes: These clinical trials are updated as of the time of manuscript preparation. All information comes from the PubMed website.

Abbreviations: AML, acute myeloid leukemia; FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; MDS, myelodysplastic syndrome; OR, overall response; CR, complete remissions; PRs, partial remissions; MS, median survival; OS, overall survival; CRc, composite complete response.
Mechanisms of resistance to quizartinib

Ordinarily, resistance to FLT3 inhibitors gives rise to treatment failure. Data obtained from clinical trials applying FLT3-TKI monotherapy illustrate the existence of primary resistance in almost 30% of FLT3-mutated AML patients. Current data have revealed that high expression of RUNX1 is one inference for emerging quizartinib-resistant FLT3/ITD+ cells. When RUNX1 was silenced, the emergence and proliferation of quizartinib-resistant FLT3/ITD+ cells became ineffective while quizartinib was applied.

Some researchers have shown that re-activation of downstream FGF/Ras/ERK and Wnt signaling as a significant mechanism of resistance to quizartinib. FGF2 promoted resistance by activating FGFR1 and downstream MAPK effectors. p21Cdkn1a (p21) and pre-B cell leukemia transcription factor 1 (Pbx1), which can inhibit the proliferation of FLT3-ITD cells, are also important pathways involved in quizartinib resistance.

The effect of FLT3 kinase domain mutation on drug resistance may be due to the disturbance of protein inactivation, which is necessary for drug binding, resulting in a reduction in drug affinity to mutant. The co-crystal structure of FLT3-quizartinib suggests that the combination of quizartinib depends on the basic aromatic interactions with the gatekeeper F691 residue and F830. Any changes in F691 and F830 may result in a significant loss of binding affinity. Furthermore, in most AML patients, FLT3-ITD mutations are activated when acquiring resistance to the FLT3 inhibitor quizartinib, accompanying enormous clonal diversity. Besides, great ancestral heterogeneity also destroys the response to the targeted therapeutics with quizartinib.

Adverse effect

Quizartinib produces adverse effects, although it is reported to be ordinarily well tolerated by patients. But the adverse effects were considered manageable. Common adverse effects included febrile neutropenia, neutropenia, thrombocytopenia, anemia and QTcF prolongation.

Conclusion and future directions

Quizartinib is an effective therapy for patients with FLT3-ITD AML, continuing to inhibit the activity of FLT3 gene, leading to apoptosis of tumor cells. In clinical trials, quizartinib presents its advantages as a very promising agent in the treatment of AML, though some side effects still happened in patients, and there was quizartinib resistance in clinical treatment. We mentioned that the high expression of RUNX1 leads to the emergence of quizartinib-resistant FLT3/ITD+ cells, so the development of targeted drugs for RUNX1 and the combination with quizartinib are the future considerations. In addition, it is worth noting that 30% of the FLT-WT cases have shown benefit with quizartinib therapy and so identifying a predictive biomarker of response in this population should become next focus. Furthermore, a larger sample of clinical trials is needed to verify its safety and efficacy.
of quizartinib combined with chemotherapy or allogeneic hematopoietic cell transplantation should also be estimated in clinical trials. Meanwhile, for the side effects of quizartinib, further studies are needed to find a way to reduce its toxicity.

Acknowledgment
This work was supported by the National Natural Science Foundation of China (grant numbers 81170492 and 81370673), National High Technology Research and Development Program 863 of People’s Republic of China (grant number 2012AA022703), National Key Basic Research Program 973 of People’s Republic of China (grant number 2010CB732404), Key Medical Projects of Jiangsu Province (grant number BL2014078), Key Medical of Jiangsu Province (grant number ZDKXB2016020), Jiangsu Social Development Project (grant number BE2018711), and Postgraduate Research & Practice Innovation Program of Jiangsu Province (grant number SJCX18-0068).

Disclosure
The authors report no conflicts of interest in this work.

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Drug Design, Development and Therapy 2019:13

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