Clinical implications of combination therapy with quizartinib and craniospinal irradiation for refractory acute myeloid leukemia positive for FMS-like tyrosine kinase 3-internal tandem duplication with central nervous system involvement

Makiko Suga1 | Kentaro Fukushima1 © | Tomoaki Ueda1 | Yasuyuki Arai2 | Shunsaku Nakagawa3 | Yosuke Minami4 | Jun Toda1 | Akihisa Hino1 | Jiro Fujita1 | TakaFumi Yokota1 | Naoki Hosen1,5,6

1Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Suita, Japan
2Department of Hematology and Oncology, Kyoto University Hospital, Kyoto, Japan
3Department of Clinical Pharmacology and Therapeutics, Kyoto University Hospital, Kyoto, Japan
4Department of Hematology, National Cancer Center Hospital East, Kashiwa, Japan
5Laboratory of Cellular Immunotherapy, World Premier Interenational Immunology Frontier Research Center, Osaka University, Suita, Japan
6Integrated Frontier Research for Medical Science Division, Institute for Open and Transdisciplinary Research Initiatives (OTRI), Osaka University, Suita, Japan

Correspondence
Kentaro Fukushima, Department of Hematology and Oncology, Osaka University Graduate School of Medicine, 2-2-C9, Yamada-Oka, Suita, Osaka, Japan.
Email: kfukushi@bldon.med.osaka-u.ac.jp

Funding information
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Abstract
FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutation-positive acute myeloid leukemia (AML) has a poor prognosis. We report the first case of successful bridge therapy of novel FLT3 inhibitor, quizartinib, to umbilical cord blood stem cell transplantation for FLT3-ITD-positive AML-primary induction failure patients with central nervous system involvement.

KEYWORDS
acute myeloid leukemia, CNS involvement, FLT3, inhibitor, stem cell transplantation

INTRODUCTION
Patients with acute myeloid leukemia (AML)-primary induction failure (PIF) require stem cell transplantation as a curative therapy. On the contrary, quizartinib and gilteritinib have been launched for the treatment of FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutation-positive AML, but there are no coherent reports in the literature on how to treat lesions or use these drugs in the presence of central nervous system involvement.
(CNS) involvement. We report a case of successful bridge therapy of quizartinib to umbilical cord blood stem cell transplantation (uCBT) for FLT3-ITD-positive AML-PIF patients with CNS involvement. A 40-year-old man presented to our hospital with fever. AML with PIF was diagnosed because it was refractory to induction therapy, and salvage chemotherapy was also ineffective. Because next-generation sequencing (NGS) mutational testing revealed FLT3-ITD positivity, oral quizartinib was initiated. Complete hematological remission (CR) was achieved 27 days after administering quizartinib. Intrathecal therapy and craniospinal irradiation were administered for CNS involvement. uCBT was successfully performed, and quizartinib maintenance therapy was administered. The quizartinib concentration was assessed during maintenance, and the concentration in plasma was considered optimal but unmeasurable in the spinal fluid. Hence, quizartinib was ineffective for CNS infiltration because of its penetration incapability across the brain–blood barrier. Therefore, quizartinib administration with craniospinal irradiation is considered a promising option for successful bridging to uCBT to cure AML-PIF patients with CNS infiltration.

2 | CASE REPORT

A 40-year-old man with fever presented to our hospital. Blood test results revealed leukocytosis and thrombocytopenia, and AML was diagnosed. A contrast-enhanced MRI of the head and the number of abnormal cells that led to the diagnosis of CNS involvement are shown in Figure 1.

Induction therapy comprising idarubicin and cytarabine was administered; however, the disease progressed quickly. Therefore, we diagnosed the patient as having AML with PIF (Figure 2). Simultaneously, facial nerve paralysis was observed. Neuropathy, craniospinal fluid (CSF) examination, and enhanced magnetic resonance imaging with gadolinium enhancement revealed central nerve infiltration in AML cells. Combination therapy with mitoxantrone, etoposide, and cytarabine was initiated as re-remission induction therapy. The symptoms were slightly alleviated by the combined use of intrathecal methotrexate (MTX) and cytarabine. Although several intrathecal chemotherapies were administered, residual tumor cells remained in the CSF. Therefore, we conducted craniospinal irradiation (CSI) (14.4-Gy and 9-Gy boosts to the whole brain) for disease control of CNS involvement, which was successful. In contrast, NGS mutational testing revealed FLT3-ITD and nucleophosmin (NPM1) positivity; thus, we initiated oral administration of quizartinib together with CSI. The initial dose was 26.5 mg, which was later increased to 53 mg after confirming that the side effects were tolerable. CR was achieved 27 days after administering quizartinib. Although QTc elongation was observed as an adverse effect of quizartinib, it was acceptable to continue quizartinib. uCBT has been successfully performed in patients with CR under disease control. The patient was administered a myeloablative conditioning regimen comprising cytarabine (4 g/m²), fludarabine (150 mg/m²), busulfan (12.8 mg/kg), and total-body irradiation 4 Gy. The graft-vs.-host disease prophylaxis included tacrolimus and short-term MTX. Stomatitis (Grade III) was observed; however, it improved with blood cell recovery. Neutrophil engraftment was achieved on Day 21. Complete donor chimerism was achieved on Day 27 of remission. On Day 56, quizartinib (17.7 mg) was resumed, and CR was maintained for 1 year. In the steady state of quizartinib administration, the serum trough level was 163.3 ng/ml and the CSF trough level was below the detection limit of 10 ng/ml. At the time of quizartinib administration, NPM1 gene mutation, a surrogate marker, was measured using polymerase chain reaction (PCR). Although NPM1 in bone marrow was 2357.43 (NPM1 mut/ABL, %) at the time of PIF, it was below detection sensitivity after uCBT. These results suggested that molecular genetic remission was achieved.

| Spinal tap test |
|-----------------|
| Abnormal cells  | 6 /μL |
| Lymphocyte      | 1 /μL |
| Monocyte        | 5 /μL |
| Protein         | 41 mg/dL |

**FIGURE 1** CNS involvement. Contrast-enhanced MRI of the head showed contrast effects in the bilateral facial nerves (red arrows) and an increase in the number of abnormal cells, which led to the diagnosis of CNS involvement.
2.1 | Measurement of quizartinib concentration

Quizartinib concentrations in the serum and spinal fluid were measured using liquid chromatography-tandem mass spectrometry (MS) (LCMS-8040, Shimadzu). MS/MS analysis was performed using an electron spray ionization source in the positive mode. Detection was performed by monitoring the ion transition of gilteritinib from m/z 561.2 to 421.0. A calibration curve was constructed using the external calibration curve method. The calibrator levels were 10, 30, 100, 300, 1000, and 3000 ng/ml.

2.2 | Genome profiling assay

We performed a comprehensive genome profiling assay for patients with relapsed and refractory and newly diagnosed unfit AML using the Foundation One Heme panel, as a part of Hematologic Malignancies (HM)-SCREEN-Japan 01 (UMIN000035233), a multicenter study.

3 | DISCUSSION

*FLT3* is a type 3 receptor tyrosine kinase similar to KIT, FMS, and platelet-derived growth factor receptors.\(^3\) The FLT3 mutation is one of the typical genetic abnormalities observed in approximately 20%–30% of AML cases.\(^4\) In particular, internal tandem duplication (ITD) mutations are poor prognostic factors. However, not only ITD mutations but also tyrosine kinase domain (TKD) mutations are reported to constitutively activate the downstream RAS, PI3K, and STAT5 pathways and contribute to the promotion of leukemia growth.\(^5,6\) Recently, two FLT3 inhibitors, gilteritinib and quizartinib, have become clinically available in Japan. Therefore, the treatment strategy for FLT3 mutation-positive AML has changed significantly. Gilteritinib is a type 1 inhibitor of ITD and TKD mutations.\(^7\) Although quizartinib is a type 2 inhibitor effective against ITD mutations but not TKD mutations, there have been less reports of severe adverse effects (myelosuppression and QT elongation) following quizartinib administration.\(^8\) It is crucial to select a drug considering its characteristics and the profile of side effects. In this case, we had confirmed that...
the patient did not have a TKD mutation. Allogeneic transplantation was performed as soon as possible after disease control with FLT3 inhibitors, and quizartinib was selected to induce an early response. In addition, quizartinib is highly selective, and we believed that the advantage of avoiding complications such as adverse reactions to off-targets might be an important issue in pretransplant patients.

QUANTUM-R, a randomized, double-blind, phase III trial of quizartinib, demonstrated the effectiveness of quizartinib over conventional chemotherapy in FLT3-ITD-positive AML patients. Moreover, many cases underwent hematopoietic cell transplantation in the quizartinib group compared to those in the chemotherapy group in a sub-analysis.

The optimal dose was not determined after transplantation because CYP3A4 inhibitors such as calcineurin inhibitors and triazole antifungal agents were frequently used in combination with the post-transplantation phase. In this case, we administered quizartinib at 17.7 mg daily, which was one-third of the standard dose (53.3 mg qd [once daily]) in combination with fluconazole and tacrolimus. A phase I study reported that 60 mg of quizartinib hydrochloride was the limiting dose in post-transplant maintenance therapy. Moreover, Usuki et al. reported regarding a phase I Japanese subset in which the serum trough level of quizartinib was 40.9 ng/ml at 20 mg qd, 37.2 ng/ml at 30 mg qd, and 137 ng/ml at 60 mg qd. In this case, the administration of 17.7 mg qd quizartinib (quizartinib hydrochloride 20 mg) could be continued without adverse events such as QT prolongation and cytopenia while achieving optimal quizartinib concentrations in the serum.

Clinical trials on quizartinib have excluded cases with CNS infiltration, and there are no data on the transfer of quizartinib to the CNS. In this study, we measured quizartinib concentrations in the serum and spinal fluid and found that the concentration in the peripheral blood was sufficient. However, the concentration in the spinal fluid was undetectable. It is challenging to control CNS lesions with quizartinib alone when CNS involvement is present, as in this case. We considered it essential to control the disease by whole-brain and whole-spinal cord irradiation before transplantation. In contrast, it has been confirmed that venetoclax can be transferred to the CSF in a small number of cases and may be a treatment option for AML with CNS involvement in the future. At present, it has not been investigated whether FLT3 inhibitors migrate to the central nervous system. Only a recent case report has shown that gilteritinib is effective in treating patients with CNS involvement. In the future, it will be important to find out which FLT3 inhibitors can be expected to be transferred to the CNS, and if they are not expected to be transferred to the CNS, it will be important to select them appropriately, such as in combination with radiation therapy or chemotherapy. FLT3-ITD-positive AML has a high risk of relapse, and an accurate evaluation of minimal residual disease (MRD) after transplantation helps predict recurrence. MRD detection by standard quantitative PCR for FLT3-ITD mutation-positive AML is challenging because of PCR bias; therefore, novel MRD detection methods using NGS have been attempted. In this case, NPM1 positivity was confirmed by the NGS comprehensive searching project, HM-SCREEN 01 Japan Study, when induction failure was diagnosed in the patient. Therefore, NPM1 and Wilms tumor gene-1 (WT1) were considered candidates for MRD monitoring of the disease. The NPM1 gene expression gradually decreased after administering quizartinib and was undetectable 6 months after transplantation. However, among the NPM1 mutation-positive cases below the NPM1-MRD threshold, recurrent cases with FLT3-ITD positivity but NPM1 negativity have been reported. Conversely, the WT1 level is known to be a marker of non-specific leukemia disease activity and may help predict disease burden or relapse. In this case, we must pay attention to the discrepancy between NPM1-MRD and WT1 mRNA. Moreover, FLT3 mutations such as F691L and D835V/Y sometimes emerged through the treatment. In such cases, quizartinib may not be effective. We could not measure additional mutations because quizartinib responded rapidly and remained effective during post-transplant maintenance therapy in this case. If the disease recurs or flares up during the disease, additional mutations should be considered, not only NPM1 and WT1 measurement.

4 | CONCLUSION

We report the first case in which quizartinib was significantly effective against AML with PIF, and CSI was simultaneously performed for CNS infiltration to avoid high-dose chemotherapy, which causes severe cytopenia. Quizartinib administration prior to stem cell transplantation is a promising bridging strategy; however, if the leukemic cells have infiltrated the central nervous system, appropriate combination therapy such as chemotherapy and radiation therapy should be considered, taking into account the spinal fluid transferability of FLT3 inhibitors.

ACKNOWLEDGEMENTS

We would like to thank Editage [http://www.editage.com] for editing and reviewing this manuscript for English language.
CONFLICTS OF INTERESTS
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION
MS served as a major contributor in writing the manuscript. KF and TU analyzed and interpreted the patient data regarding hematological disease. YA and SN evaluated the concentration of drugs. YM provided the NGS sequence data. Others read and critically improved and approved the final manuscript.

ETHICAL APPROVAL
This observational study was approved by the institutional review board at the Osaka University Hospital (#13167, 16265) and conducted in accordance with the Declaration of Helsinki.

CONSENT
Written informed consent was obtained from the patient before specimen collection.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are not openly available due to their containing information that could compromise the privacy of the research participants. The data are available from the corresponding author upon reasonable request.

ORCID
Kentaro Fukushima ✉ https://orcid.org/0000-0002-8003-2584

REFERENCES
1. Weisdorf DJ, Millard HR, Horowitz MM, et al. Allogeneic transplantation for advanced acute myeloid leukemia: the value of complete remission. Cancer. 2017;123:2025-2059. doi:10.1002/cncr.330536
2. Perl AE. Availability of FLT3 inhibitors: How do we use them? Blood. 2019;134(9):741-745. doi:10.1182/blood.2019876821
3. Rosnet O, Mattei MG, Marchetto S, Birnbaum D. Isolation and chromosomal localization of a novel FMS-like tyrosine kinase gene. Genomics. 1991;9(2):380-385. doi:10.1016/0888-8754(91)90027-O
4. Kiyoi H, Naoe T. Biology, clinical relevance, and molecularly targeted therapy in acute leukemia with FLT3 mutation. Int J Hematol. 2006;83(4):301-308. doi:10.1532/IJH97.06071
5. Mizuki M, Fenski R, Haltier H, 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(12):3907-3914. doi:10.1182/blood.v96.12.3907
6. Hayakawa F, Towatari M, Kiyoi 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(5):624-631. doi:10.1038/sj.onc.1203354
7. Mori M, Kaneko N, Ueno Y, et al. Gilteritinib, a FLT3/AXL inhibitor, shows antileukemic activity in mouse models of FLT3 mutated acute myeloid leukemia. Invest New Drugs. 2017;35(5):556-565. doi:10.1007/s10637-017-0470-z
8. Zarrinkar PP, Gunawandane RN, Cramer MD, et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). Blood. 2009;114(14):2984-2992. doi:10.1182/blood-2009-05-222034
9. Cortes JE, Khaled S, Martinelli G, 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(7):984-997. doi:10.1016/S1470-2045(19)30150-0
10. Cortes J, Perl AE, Döhner H, 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(7):889-903. doi:10.1016/S1470-2045(18)30240-7
11. Ganguly S, Cortes JE, Krämer A, et al. Clinical outcomes in patients with FLT3-ITD-mutated relapsed/refractory acute myelogenous leukemia undergoing hematopoietic stem cell transplantation after quizartinib or salvage chemotherapy in the QuANTUM-R trial. Biol Blood Marrow Transplant. 2020;26:153-162. doi:10.1016/j.bbmt.2020.09.036
12. Li J, Kankam M, Trone D, Gammon G. Effects of CYP3A inhibitors on the pharmacokinetics of quizartinib, a potent and selective FLT3 inhibitor, and its active metabolite. Br J Clin Pharmacol. 2019;85(9):2108-2117. doi:10.1111/bcp.14022
13. Cortes JE, Kantarjian H, Foran JM, et al. Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukaemia irrespective of FMS-like tyrosine kinase 3 internal tandem duplication status. J Clin Oncol. 2013;31(29):3681-3687. doi:10.1200/JCO.2013.48.8783
14. Usuki K, Handa H, Choi I, et al. Safety and pharmacokinetics of quizartinib in Japanese patients with relapsed or refractory acute myeloid leukemia in a phase 1 study. Int J Hematol. 2019;110(6):654-664. doi:10.1111/ijh.12859
15. Altman JK, Foran JM, Pratz KW, Trone D, Cortes JE, Tallman MS. Phase I study of quizartinib in combination with induction and consolidation chemotherapy in patients with newly diagnosed acute myeloid leukaemia. Am J Hematol. 2018;93(2):213-221. doi:10.1002/ajh.24974
16. Cortes JE, Tallman MS, Schiller GJ, et al. Phase 2b study of 2 dosing regimens of quizartinib monotherapy in FLT3-ITD-mutated, relapsed or refractory AML. Blood. 2018;132(6):598-607. doi:10.1182/blood-2018-01-821629
17. Reda G, Cassin R, Dovtrelova G, et al. Venetoclax penetrates in cerebrospinal fluid and may be effective in chronic lymphocytic leukemia with central nervous system involvement. Haematologica. 2019;104(5):e222-e223. doi:10.3324/haematol.2018.213157
18. Perrone S, Ortu La Barbera E, Viola F, et al. A relapsing meningeal acute myeloid leukaemia FLT3-ITD+ responding to gilteritinib. Chemotherapy. 2021;66(4):134-138. doi:10.1159/000518356
19. Ball B, Stein EM. Which are the most promising targets for minimal residual disease-directed therapy in acute myeloid leukemia prior to allogeneic stem cell transplant?
SUGA et al. Haematologica. 2019;104(8):1521-1531. doi:10.3324/haematol.2018.208587

20. Krönke J, Bullinger L, Teleanu V, et al. Clonal evolution in relapsed NPM1-mutated acute myeloid leukemia. Blood. 2013;122(1):100-108. doi:10.1182/blood-2013-01-479188

21. Miyamoto K, Minami Y. Precision medicine and novel molecular target therapies in acute myeloid leukemia: the background of hematologic malignancies (HM)-SCREEN-Japan 01. Int J Clin Oncol. 2019;24(8):893-898. doi:10.1007/s10147-019-01467-1

How to cite this article: Suga M, Fukushima K, Ueda T, et al. Clinical implications of combination therapy with quizartinib and craniospinal irradiation for refractory acute myeloid leukemia positive for FMS-like tyrosine kinase 3-internal tandem duplication with central nervous system involvement. Clin Case Rep. 2022;10:e05384. doi:10.1002/ccr3.5384