ANTICANCER RESEARCH *43*: 725-732 (2023)
doi:10.21873/anticanres.16211

Relationship Between Osimertinib Concentration and Clinical Response in Japanese Patients With Non-small Cell Lung Cancer

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Abstract. *Background/Aim: Osimertinib is the first-line treatment for patients with advanced epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer (NSCLC). The present study aimed to determine the previously unclarified association of osimertinib plasma trough concentrations with efficacy, adverse events, and genetic polymorphisms in Japanese patients with NSCLC harboring EGFR mutations. Patients and Methods: In this prospective study, blood samples of 25 patients who received osimertinib were collected to measure plasma osimertinib concentrations and to genotypically characterize ATP-binding cassette subfamily B member 1 and ATP-binding cassette subfamily G member 2 polymorphisms. Plasma osimertinib concentrations were analyzed using validated multiple reaction monitoring mode-based liquid chromatography-tandem mass spectrometry. Osimertinib concentration necessary to achieve optimal median progression-free survival (PFS) was determined using receiver operating characteristic curve analysis. PFS and overall survival were analyzed using the Kaplan–Meier method, and between-group differences were compared using the log-rank test. Plasma osimertinib concentrations between different patient groups were compared using the Mann–Whitney U-test. Results: Patients were divided into high and low concentration groups based on a plasma osimertinib cut-off concentration of 211 ng/ml. Median PFS was longer in the high trough concentration group than that in the low trough concentration group (46.3 vs. 16.8 months, p=0.029). Plasma osimertinib concentrations adjusted for dose and body weight did not differ between the patients with and without variant polymorphisms. Conclusion: Monitoring plasma trough concentrations during maintenance might improve osimertinib treatment efficacy in patients with NSCLC harboring EGFR mutations.*

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Key Words: Genetic polymorphism, osimertinib, non-small cell lung cancer, pharmacokinetics.

Lung cancer is the most common cause of cancer-related deaths in Japan, and the prevalence and deaths from lung cancer have been increasing in recent years (1). Non-small cell lung cancer (NSCLC) is the definitive diagnosis in approximately 85% of patients with lung cancer (2, 3). Molecularly targeted therapies have emerged as an essential treatment approach in various cancers. Molecular testing is recommended in all patients with metastatic non-squamous NSCLC. Likewise, squamous NSCLC requires molecular evaluation.

Epidermal growth factor receptor (*EGFR*) is the most frequently mutated gene across Asian populations, and *EGFR* mutations are more common in Asia than in the West (4). EGFR tyrosine kinase inhibitors (TKIs) have higher clinical efficacy than best supportive care or standard chemotherapy in patients with advanced NSCLC harboring *EGFR* activating mutations (5-8).

Despite the demonstrated efficacy of the EGFR-TKIs gefitinib and erlotinib in lung cancer, patients eventually

become resistant to treatment (9-11). Increased adenosine triphosphate (ATP) affinity for EGFR was considered as a mechanism mediating resistance acquisition, and afatinib and dacomitinib were developed as covalent, irreversible EGFR-TKIs. Compared to gefitinib, both afatinib and dacomitinib were reported to be associated with prolonged progression-free survival (PFS) in patients with *EGFR* mutation-positive NSCLC (12, 13). However, these treatments are associated with severe adverse events, such as skin rash, and diarrhea, which are associated with the inhibition of wild-type EGFR in skin and small intestine (14). Plasma trough concentrations of afatinib are associated with adverse events, suggesting the utility of determining plasma concentrations (15, 16).

Osimertinib is a third-generation EGFR-TKI that selectively inhibits *EGFR* activating mutations as well as the *EGFR* T790M resistance mutation. In the phase 3 FLAURA trial evaluating first-line treatment, osimertinib significantly improved PFS compared to erlotinib or gefitinib [18.9 *vs.* 10.2 months; hazard ratio (HR)=0.46; 95% confidence interval (CI)=0.37-0.57; $p<0.001$]. The frequency of adverse events was also lower with osimertinib than with standard EGFR-TKIs (34% *vs.* 45%) (17). In addition, many cases have been reported showing the efficacy of osimertinib in NSCLC patients with the T790M mutation (18-20).

Plasma concentrations above certain levels have been reported to be associated with adverse events in patients treated with standard EGFR-TKIs; a similar association might be present for osimertinib as well. However, to our knowledge, no study to date has examined the association of plasma trough concentrations of osimertinib with efficacy or adverse events. We previously reported that genetic polymorphisms in ATP-binding cassette (ABC) subfamily B member 1 and 2 (*ABCB1* and *ABCB2*, respectively), which are associated with afatinib pharmacokinetics, contributed to individual variations in adverse events (21). Similar to afatinib, osimertinib is a substrate for P-glycoprotein, encoded by *ABCB1*, and breast cancer resistance protein, encoded by ATP-binding cassette super-family G member 2 (*ABCG2*), raising the possibility that individual variations in plasma osimertinib concentrations may occur due to genetic polymorphisms. Although Yokota *et al.* reported that *ABC* transporter polymorphisms did not contribute to individual variability in osimertinib pharmacokinetics, further studies are needed to resolve this question due to a modest number of enrolled patients (22). Therefore, the present study aimed to clarify the relationship of plasma trough concentrations of osimertinib with efficacy, safety, and associated genetic polymorphisms in Japanese patients with NSCLC harboring *EGFR* mutations.

Patients and Methods

Study design and patients. This prospective study enrolled 25 Japanese patients with NSCLC who were administered osimertinib in the Department of Respiratory Medicine at Gifu University Hospital in Japan between August 2016 and October 2020. The data cut-off date was June 30, 2022. Adverse events during the first three months after the first administration of osimertinib were included in the study. All patients were provided information on study aims and risks involved and written informed consent for study participation was obtained prior to enrolment for all patients.

All patients were diagnosed with *EGFR* mutation-positive advanced NSCLC based on the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. Patient demographics, including age, body weight, height, and sex, and clinical parameters, including osimertinib dose, severity of adverse events, and additional medical issues, were retrieved from the electronic medical records. Treating physicians periodically assessed adverse events associated with osimertinib according to the Common Terminology Criteria for Adverse Events (version 5.0) using a predefined format (23). In all patients, blood samples were collected to measure plasma osimertinib concentration and to perform genotyping for *ABCB1* and *ABCG2* polymorphisms.

The study protocol was approved by the Research Ethics Committees of Gifu Pharmaceutical University (approval no. 30-44) and the Gifu University School of Medicine (approval no. 27-509) and was conducted in full accordance with the tenets of the Declaration of Helsinki, the Ethical Guidelines for Human Genome/Gene Analysis Research in Japan, and Japanese laws.

Genotyping. Whole venous blood samples were collected into Venoject II vacuum tubes containing 4.5 mM ethylenediaminetetraacetic acid-2Na (Terumo, Tokyo, Japan). Genomic DNA of leukocytes from whole-blood specimens were extracted using the QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) and stored at –80˚C until analysis. Genotyping was performed using polymerase chain reaction (PCR) with restriction enzyme digestion, analysis of PCR–fragment length polymorphisms, and allele-specific PCR assays. Table I shows primer sequences, PCR conditions, and restriction enzymes used for these analyses. PCR and restriction enzyme digestion products were separated on 2%-4% agarose gels using electrophoresis, and the products were stained with ethidium bromide and viewed under ultraviolet light.

Determination of plasma osimertinib concentrations. Plasma trough concentrations of osimertinib were determined after the stabilization of prescription dose for more than one month. Blood samples were collected immediately prior to drug administration, and plasma osimertinib concentrations were determined using validated multiple reaction monitoring mode-based liquid chromatography-tandem mass spectrometry, as described in a previous study, with minor modifications (24). Briefly, the internal standard deuterated gefitinib was added to each plasma sample. After mixing, tert-butyl methyl ether was added to the tube, which was vigorously mixed, and centrifuged. The top organic layer was transferred to a polypropylene tube and dried under a stream of nitrogen gas. The sample was reconstituted with the mobile phase and injected to the liquid chromatography instrument for quantitative analysis. The liquid chromatography-tandem mass spectrometry system included a Nexera X2 ultra high-performance liquid chromatograph device (Shimadzu, Kyoto, Japan) and an LCMS-8040 triple quadrupole mass spectrometer equipped with an electrospray ion source (Shimadzu). Osimertinib concentrations were determined using the multiple reaction monitoring transition mode with ion transitions from 500.1 to 72.2 *m/z* with 30 eV collision energy. The

Table I. *Details of genotyping methods used to determine genetic polymorphisms in ABCB1 and ABCG2.*

Gene	Polymorphism	Method	Primers	Annealing temperature	Restriction enzyme	Digestion temperature
ABCB1	1236C>T	PCR-RFLP	F: 5'-TTC ACT TCA GTT ACC CAT C-3' R: 5'-CAT AGA GCC TCT GCA TCA-3'	56˚C	HaeIII	37˚C
	3435C>T	PCR-RFLP	F: 5'-TGT TTT CAG CTG CTT GAT GG-3' R: 5'-AAG GCA TGT ATG TTG GCC TC-3'	60˚C	Sau3AI	37˚C
	2677 G>T/A	PCR-RFLP	F:5'-TAC CCA TCA TTG CAA TAG CAG-3' (for G allele) R: 5'-TTT AGT TTG ACT CAC CTT GCT AG-3' (for G allele)	52˚C	NheI	37˚C
			F:5'-GCA CTG AAA GAT AAG AAA GAA CTA GAA GCT-3' (for T allele) R: 5'-GAG CAT AGT AAG CAG TAG GGA G-3' (for T allele)	56˚C	HindIII	37˚C
ABCG2	34 G>A	PCR-RFLP	F: 5'-GAT AAA AAC TCT CCA GAT GTC TTGC-3' R: 5'-AGC CAA AAC CTG TGA GGT TCAC-3'	60˚C	BsrI	68˚C
	421C>A	AS-PCR	F: 5'-TGA CGG TGA GAG AAA ACT TGC-3' (for C allele) F: 5'-TGA CGG TGA GAG AAA ACT TGA-3' (for A allele) R: 5'-CAA GCC ACT TTT CTC ATT GTT-3'	60˚C		

ABCB1: ATP-binding cassette subfamily B member 1; *ABCG2*: ATP-binding cassette super-family G member 2; AS-PCR: allele-specific PCR assay; F: forward primer; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; R: reverse primer.

Table II. *Patient characteristics.*

	All patients	Low trough concentration group	High trough concentration group
Male/female, n	7/18	6/12	1/6
Age, years	72 (69-89)	69.5 (60.3-77.3)	71 (67-73)
Body weight, kg	63.2 (51.0-82.5)	55.1 (43.8-65.9)	45.3 (41.7-62.7)
Albumin, g/dl	4.5 (4.3-4.8)	4.3 (4.2-4.5)	4.3 (3.7-4.6)
Aspartate aminotransferase (U/l)	25 (21-36)	21.5 (17.8-25)	20 (17-25)
Alanine aminotransferase (U/l)	21 (13-44)	13 (9.5-21.3)	13 (10-24)
Serum creatinine (mg/dl)	0.88 (0.72-1.28)	0.73 (0.59-0.85)	0.73 (0.59-0.85)
EGFR mutations, n (%)			
Exon 21 L858R	14 (56.0)	9 (50.0)	5 (71.4)
Exon 19 deletions	9 (36.0)	7 (38.9)	2 (28.6)
Exon 21 L861Q	1 (4.0)	1 (5.6)	0
Exon 18 G719X	1 (4.0)	1 (5.6)	0
Initial osimertinib dose, n (%)			
80 mg	23 (92.0)	16 (88.9)	7 (100)
40 mg	2 (8.0)	2 (11.1)	0
Treatment line, n (%)			
First-line	17 (68.0)	13 (72.2)	4 (57.1)
Second-line	4 (16.0)	3 (16.7)	1 (14.3)
After third-line	4 (16.0)	2 (11.1)	2 (28.6)
EGFR-TKIs before osimertinib*, n (%)			
None	19 (76.0)	15 (83.3)	4 (57.1)
Gefitinib	3 (12.0)	1 (5.6)	1 (14.3)
Erlotinib	2 (8.0)	0	2 (28.6)
Afatinib	4 (16.0)	3 (16.7)	1 (14.3)

EGFR: Epidermal growth factor receptor; TKI: tyrosine kinase inhibitor. Each value represents medians and quantiles unless otherwise specified. *Values do not add up to 100%, as there are multiple counts in various subcategories.

XBridge Shield RP18 Column (3.5 μm, 2.1×50 mm; Waters Corporation, Milford, MA, USA) was used for chromatographic separation with the Nexera X2 ultra high-performance liquid chromatograph system; the mobile phase was an 80:20 (v/v) combination of 1 mM ammonium hydroxide in methanol and 10 mM aqueous ammonium hydroxide (pH 10.5) at a flow rate of 0.2 ml/min.

Statistical analysis. Receiver operating characteristic curve analysis was used to determine the optimal cut-off osimertinib concentration to achieve the best median PFS. Cut-off values were established using Youden index. Patients were divided into high and low trough concentration groups based on the cut-off value.

Response Evaluation Criteria in Solid Tumors version 1.1 was used in analyses of PFS and overall survival (OS), which were estimated using the Kaplan–Meier method, and between-group differences were compared using the log-rank test. HRs for PFS and OS were determined using univariate Cox proportional hazards models. Genotyping data were evaluated for deviation from the Hardy–Weinberg equilibrium using Fisher's exact test. Plasma osimertinib concentrations between patient groups were compared using the Mann–Whitney *U*-test. A two-sided *p*-value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed using SPSS version 26 (SPSS, Chicago, IL, USA).

Results

Patient characteristics and genotypes. Table II shows the demographics of all 25 patients enrolled in the study. Briefly, the median age was 72 years (range=69-89 years) and the male/female ratio was 7/18. Genotyping was successfully completed in all patients. Table III shows genotype polymorphisms in osimertinib pharmacokinetic-related enzymes and transporters. None of the examined genotype distributions deviated from the Hardy–Weinberg equilibrium.

Relationship of plasma osimertinib concentrations with efficacy. According to the Cox proportional hazards model, the area under the receiver operating characteristic curve was 0.564 (95%CI=0.327-0.801). The optimal cut-off for plasma osimertinib concentration was 211 ng/ml, which was used to categorize patients into high and low trough concentration groups. Figure 1A and B shows the OS and PFS of all 25 patients who underwent blood sampling during the maintenance period. The median OS, which was not calculated for patients in the high trough concentration group due to the low number of events, was 50.1 months in the low trough concentration group; there was no significant difference between the two groups (HR for death=0.71, 95%CI=0.14-3.58, *p*=0.68). The median PFS was 46.3 months in the high trough concentration group and 16.8 months in the low trough concentration group, with a significant difference between the two groups (HR for disease progression or death=0.14, 95%CI=0.02-1.1, *p*=0.14).

Relationship of plasma osimertinib concentrations with adverse events and dose reduction. Table IV shows adverse events observed in the study cohort. The most common adverse event was decreased platelet count of any grade, which was detected in 13 patients (72%) in the low trough concentration group and in 4 patients (57%) in the high trough concentration group. The rate of paronychia was significantly higher in the high trough concentration group than that in the low concentration group [4 (57%) *vs*. 3 (17%) patients; *p*=0.04]. Grade 3 or higher adverse events were not reported in either group. Five patients (27.8%) required dose reduction from the standard prescription dose of 80 mg osimertinib in the low trough concentration group, and two patients (28.6%) in the high trough concentration group required a similar adjustment.

Table III. *Genotype distributions*.

Polymorphism	Genotype	n (%)	Allele	n (%)
ABCB1				
C1236T	C/C	3 (12.0)	C	19 (28.0)
	C/T	13 (52.0)	T	31 (62.0)
	T/T	9 (36.0)		
C3435T	C/C	9 (36.0)	C	29 (58.0)
	C/T	11 (44.0)	T	21 (42.0)
	T/T	5 (20.0)		
G2677T/A	G/G	7 (14.0)	G	20 (40.0)
	G/T	5 (10.0)	T	22 (44.0)
	T/T	6 (12.0)	A	8 (16.0)
	T/A	5 (10.0)		
	G/A	1 (2.0)		
	A/A	1 (2.0)		
ABCG2				
G34A	G/G	12 (48.0)	G	37 (74.0)
	G/A	13 (52.0)	A	13 (26.0)
	A/A	0		
C421A	C/C	12 (48.0)	C	35 (70.0)
	C/A	11 (44.0)	A	15 (30.0)
	A/A	2 (8.0)		

ABCB1: ATP-binding cassette subfamily B member 1; *ABCG2*: ATP-binding cassette super-family G member 2.

Relationship of plasma osimertinib concentrations with pharmacokinetic-related genetic polymorphisms. There was no significant difference in plasma osimertinib concentrations adjusted for dose and body weight between the patients with and without genetic polymorphisms of *ABCB1* and *ABCG2* included in the present study (Figure 2).

Discussion

To our knowledge, this is the first report examining the relationship between plasma osimertinib concentrations and treatment efficacy in patients with NSCLC. In the present

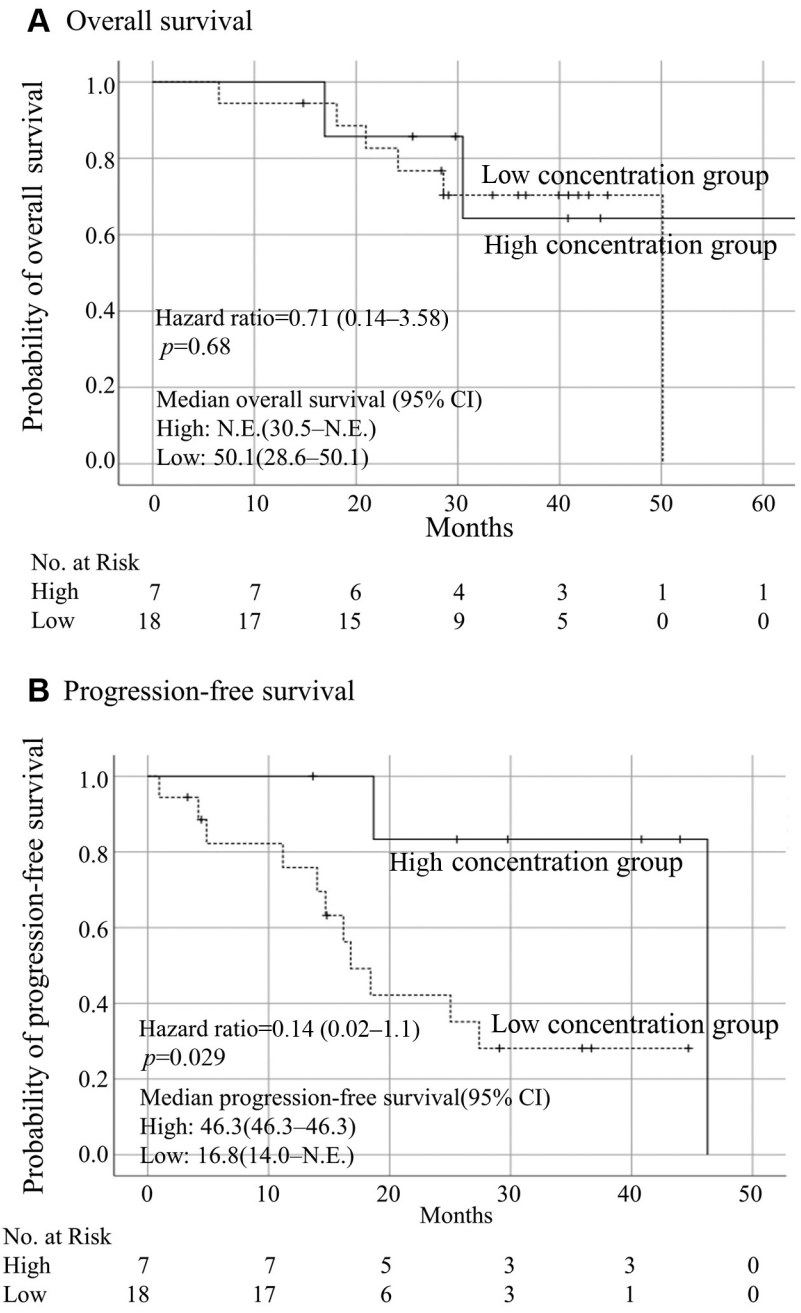

Figure 1. *Kaplan–Meier estimates of overall survival (A) and progression-free survival (B). Censored data are indicated with tick marks. Time to treatment failure was compared between the patients with high and low plasma trough concentrations of osimertinib, and data were analyzed using the Mantel–Cox log-rank test. CI: Confidence interval; N.E.: not evaluable.*

study, the cut-off plasma osimertinib concentration was 211 ng/ml and plasma osimertinib concentrations above this limit were associated with 29% reduction in the risk of disease progression, suggesting that plasma osimertinib concentrations above this cut-off exhibited enhanced treatment efficacy and prolonged PFS.

In the current study cohort, the median PFS of the low concentration group was consistent with the results of the FLAURA trial (18.9 months) whereas the median PFS of the high concentration group was longer (17). Osimertinib dose-finding trials in patients with NSCLC have shown that an osimertinib dose range of 20-240 mg yields similar tumor

Table IV. *Adverse events*.

	Low-group	High-group	*p*-Value*
Diarrhea, n (%)	3 (17)	2 (29)	0.50
Dry skin, n (%)	2 (11)	1 (14)	0.83
Paronychia, n (%)	3 (17)	4 (57)	0.04
Skin rash, n (%)	3 (17)	2 (29)	0.50
Stomatitis, n (%)	3 (17)	2 (29)	0.50
Elevated aspartate aminotransferase, n (%)	11 (61)	2 (29)	0.14
Elevated alanine aminotransferase, n (%)	5 (28)	2 (29)	0.97
Decreased platelet count, n (%)	13 (72)	4 (57)	0.47
Decreased white blood cell count, n (%)	9 (50)	4 (57)	0.75

*Fisher's exact test.

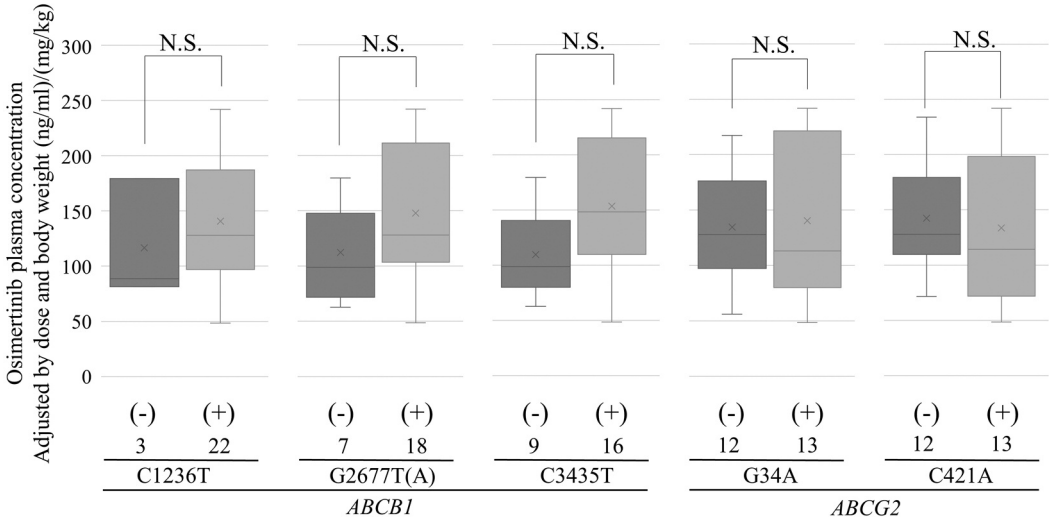

Figure 2. *Relationship between plasma osimertinib concentrations and five genetic single-nucleotide polymorphisms of two genes that affect osimertinib pharmacokinetics. Boxes indicate 25% and 75% quantile ranges, and whiskers indicate 5% and 95% quantiles. The Mann–Whitney U test was used to compare groups. N.S.: Not significant; (–): patients without the allele; (+): patients with the allele.*

growth inhibition rates (25), indicating the absence of a correlation between osimertinib dose and anticancer efficacy. However, the association between plasma osimertinib concentrations and therapeutic effect has been unclear.

Agema *et al*. reported 259 ng/ml as the osimertinib dose limit for toxicity in a prospective observational study of 159 patients with NSCLC in Netherlands (26). Major adverse events such as skin toxicities, creatinine kinase elevation, and pneumonia were significantly higher in patients with plasma osimertinib concentrations of >259 ng/ml; however, the authors did not observe a difference in efficacy possibly because the plasma osimertinib concentrations exceeded 211 ng/ml in most patients. No other studies have investigated the association of plasma osimertinib concentrations with therapeutic effect, and further investigation is warranted to clarify the effective osimertinib concentration range in patients with NSCLC.

Only grade 1 or grade 2 adverse events were reported in the present study. Subjective adverse events, such as diarrhea, dry skin, paronychia, skin rash, and stomatitis, tended to be more frequent in patients with high trough concentrations than in those with low trough concentrations. Only paronychia was significantly more frequent in the high trough concentration group than that in the low trough concentration group. Appropriate, optimized management of adverse events to avoid dose reduction is necessary to maintain plasma osimertinib concentrations above the effective range and to further improve treatment efficacy.

Osimertinib is expected to be more frequently associated with a reduction in the risk of progression of central nervous system metastasis compared with other EGFR-TKIs (27, 28). Additionally, the expression level of *ABCB1*/*ABCG2* affects the delivery of osimertinib to the central nervous system.

Therefore, in the present study, we focused on polymorphisms in *ABCB1* and *ABCG2* in our analyses evaluating the variability of osimertinib concentrations in plasma. We did not find a correlation between these genetic polymorphisms and plasma osimertinib concentrations. This finding is in agreement with a previous study, which reported that genetic polymorphisms did not have an effect on plasma drug concentrations (22). Based on these results, *ABCB1*/*ABCG2* genotyping before osimertinib administration might not be necessary to predict its efficacy and adverse events.

Other factors that may affect plasma osimertinib concentrations include genetic polymorphisms in metabolic pathways. Osimertinib is metabolized by cytochrome P450, family 3, subfamily A (CYP3A), which also exhibits genetic polymorphisms. In Japanese patients, genetic polymorphisms in *CYP3A4**1G and *CYP3A5**3 were reported to have no effect on the area under the receiver operating characteristic curve in reported evaluations (22). Additionally, genetic polymorphisms of *CYP3A4* were shown to affect plasma concentrations *in vitro*, suggesting that further investigation of the relationship between the role of genetic polymorphisms in metabolic pathways and plasma osimertinib concentrations is necessary.

Limitations of this preliminary pilot study include the small sample size and short follow-up period. Additionally, the median OS could not be evaluated at the time of data cut-off date. Thus, further large-scale studies are warranted to confirm these results.

In conclusion, plasma osimertinib concentration above 211 ng/ml was associated with prolonged PFS in the current study, suggesting that monitoring plasma trough concentrations of osimertinib during the maintenance period might ensure treatment efficacy in patients with NSCLC harboring *EGFR* mutations.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

M.Y., N.K., Y.Y., Y.O., A.S., and H.H. conceived the study. M.Y., N.K., Y.Y., S.Y., and K.T. analyzed drug concentrations and genetic polymorphisms. M.Y., N.K., K.Y., and C.H. conducted claim data analyses. M.Y., N.K., and Y.Y. performed statistical analyses. H.I. contributed to the interpretation of data and assisted in the preparation of the manuscript. M.Y. and Y.Y. drafted the initial manuscript. Y.O., A.S., and H.H. conducted the critical evaluation of the manuscript. All Authors reviewed and approved the final version of the manuscript.

Acknowledgements

The Authors would like to thank the physicians and nursing staff of Gifu University Hospital, Gifu, Japan, as well as Yoshihisa Fukuda and Nozomi Hayama from the Gifu Pharmaceutical University, Gifu, Japan, for their technical support.

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Received December 14, 2022
Revised January 10, 2023
Accepted January 11, 2023