Effects of CYP3A4/5 and ABC transporter polymorphisms on osimertinib plasma concentrations in Japanese patients with non-small cell lung cancer

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Summary
The effects of polymorphisms in CYP3A4 (20230G > A), CYP3A5 (6986A > G), ABCB1 (1236C > T, 2677G > T/A, 3435C > T), ABCG2 (421C > A), and ABCC2 (-24C > T) on the area under the concentration–time curve (AUC) of osimertinib in 23 patients with non-small cell lung cancer were investigated. Blood sampling was performed just prior to and at 1, 2, 4, 6, 8, 12, and 24 h after osimertinib administration at the steady-state on day 15 after beginning therapy. The osimertinib AUC0–24 was significantly correlated with age (P = 0.038), serum albumin (P = 0.002), and serum creatinine (P = 0.012). Additionally, there were significant differences in the AUC0–24 of osimertinib among the groups administered vonoprazan, histamine 2-receptor antagonists or esomeprazole, and no acid suppressants (P = 0.021). By contrast, there were no significant differences in the AUC0–24 of osimertinib between genotypes of CYP3A4/5 or ABC transporters. Furthermore, there were no significant differences in the AUC0–24 of osimertinib between patients with diarrhea, skin rash, or hepatotoxicity and those without these conditions. In multivariate analysis, only serum albumin value was an independent factor predicting the AUC0–24 of osimertinib. Analysis of CYP3A4/5 and ABC transporter polymorphisms before osimertinib therapy may not predict the efficacy or side effects of osimertinib. The lower serum albumin values were associated with an increase in the AUC0–24 of osimertinib; however, further studies are needed to assess the factors contributing to the interindividual variability of osimertinib pharmacokinetics.

Keywords Area under the plasma concentration–time curve · Epidermal growth factor receptor · Serum albumin · Tyrosine kinase inhibitor · Variant

Abbreviations
ABC ATP-binding cassette
AUC Area under the plasma concentration–time curve
BCRP Breast cancer resistance protein
Cmax Maximal plasma concentration
C0 Trough plasma concentration
CYP Cytochrome P450

Introduction
Osimertinib, an oral third-generation epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), is now widely used as first-line therapy for patients with EGFR mutation-positive advanced non-small cell lung cancer (NSCLC) [1]. The most common adverse events associated with osimertinib are diarrhea, rash, dry skin, reduced appetite, stomatitis, fatigue, and hematologic abnormalities [2], and the incidence rates of rash or diarrhea among these adverse events have been reported to be related to the total area under the observed plasma concentration–time curve
(AUC) of osimertinib [3]. Osimertinib is predominantly metabolized in the liver by cytochrome P450 (CYP3A4/5) and is a substrate of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (encoded by ABCB1) and breast cancer resistance protein (BCRP, encoded by ABCG2) [4–6]. Although the intervariability in the osimertinib AUC in patients with NSCLC taking osimertinib is high [7], individual differences in osimertinib AUCs may be attributable to variations in the activity of CYP3A4/5, P-glycoprotein, and BCRP in the intestines and liver. In vitro studies on the clearance of osimertinib in patients with CYP3A4 variants have reported significantly reduced drug clearance compared with that in patients with wild-type CYP3A4*1 [8]; however, the influence of polymorphisms in CYP3A4/5 and ABC transporters on the osimertinib AUC in patients with NSCLC has not been reported. Among the identified single nucleotide polymorphisms (SNPs) in the CYP3A4 and CYP3A5 genes, CYP3A4*1G (20230G > A, rs2242480) and CYP3A5*3 (6986A > G, rs776746) variants appear particularly important owing to their relatively high frequency in the Japanese population [9, 10]. According to previous studies, in the ABC superfamily, the SNPs ABCB1 (1236C > T, 2677G > T/A, 3435C > T), ABCG2 (421C > A), and ABCC2 (-24C > T) have high allelic frequencies in the Japanese population [11–13].

In the current study, we investigated the effects of polymorphisms in CYP3A4 (20230G > A), CYP3A5 (6986A > G), ABCB1 (1236C > T), ABCB1 (2677G > T/A), ABCB1 (3435C > T), ABCG2 (421C > A), and ABCC2 (-24C > T) on the plasma concentration of osimertinib in 23 patients with NSCLC.

**Materials and methods**

**Patients and protocols**

Twenty-three Japanese patients with NSCLC (15 women and 8 men) taking osimertinib (TAGRISSO; AstraZeneca K.K., Osaka, Japan) were treated at Akita University Hospital from August 2016 through February 2022 and were prospectively enrolled in the study. The clinical characteristics of the patients at the time of analysis of osimertinib plasma concentrations are listed in Table 1. The study was conducted according to the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Akita University School of Medicine (approval numbers: 790 and 1140), and all patients provided written informed consent for participation in the study.

Osimertinib (80 mg) was orally administered once daily at 08:00 AM. Blood sampling was performed just prior to (C₀ on day 15) and at 1, 2, 4, 6, 8, 12, and 24 h (day 16) after osimertinib administration at the steady-state on day 15 after beginning osimertinib therapy [14]. DNA was extracted from peripheral blood samples using a QIAamp Blood Mini Kit (Qiagen, Tokyo, Japan) and was stored at -80 °C until analysis. Plasma was isolated by centrifugation from blood samples at 1,900 × g for 15 min and was stored at -80 °C until analysis. The grades of toxicity for diarrhea, skin rash, and hepatotoxicity were determined based on the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The onset of diarrhea and skin rash was evaluated until the last blood sampling on day 16 after beginning osimertinib therapy. The onset of hepatotoxicity was evaluated until discontinuation of osimertinib therapy.

A dose of 10 or 20 mg vonoprazan (Takecab; Takeda Pharmaceutical Co. Ltd., Osaka, Japan; 9 patients), 20 mg esomeprazole (Nexium; AstraZeneca; 6 patients), or 10 mg lufutidine (Protecadin; Taiho Pharmaceutical Co. Ltd., Tokyo, Japan; 1 patient) was taken orally once daily for 16 days for blood sampling. A dose of 20 mg/day famotidine (Gaster; Astellas Co. Ltd., Tokyo, Japan; 1 patient) was taken orally twice daily for 16 days for blood sampling (Table 1).

**Analytical methods**

Plasma concentrations of osimertinib were measured by high-performance liquid chromatography (HPLC) and ultraviolet spectroscopic analysis, as previously reported for quantitative analysis of gefitinib plasma concentrations [15, 16]. Following the addition of gefitinib (10 ng/10 μL methanol) as an internal standard to a 100 μL plasma sample, the plasma sample was diluted with 900 μL water and vortexed for 30 s. This mixture was applied to an Oasis HLB extraction cartridge (Waters, Milford, MA, USA) that had been activated previously with methanol and water (1.0 mL each). The cartridge was then washed with 1.0 mL water and 1.0 mL of 40% methanol in water and eluted with 0.4 mL of 100% methanol and then 1.0 mL of 100% acetonitrile. Eluates were dried by vortex-vacuum evaporation at 70 °C using a rotary evaporator (AS-ONE CVE-2AS; Osaka, Japan). The resulting residue was then dissolved in 20 μL methanol and vortexed for 30 s; 20 μL of the mobile phase was added to the sample, and the sample was vortexed for another 30 s. A 20-μL aliquot of the sample was then processed by HPLC. The HPLC system comprised a PU-2080 plus chromatography pump (JASCO, Tokyo, Japan) equipped with a CAPCELL PAK C18 MG II HPLC column (250 mm × 4.6 mm I.D.; Osaka Soda, Osaka, Japan), a UV-2075 light source, and an ultraviolet detector (JASCO). The mobile phase was 0.5% KH₂PO₄ (pH 3.5)-acetonitrile-methanol (55:25:20, v/v/v), which was degassed in an ultrasonic bath prior to use. The flow rate was 0.5 mL/min at ambient temperature, and sample detection was carried out at 250 nm. Analysis of the calibration curve for osimertinib in human plasma showed...
that the curve was linear for the concentration range from 10 to 1000 ng/mL. The coefficients of variation and accuracies for intra- and interday assays at the concentration range from 10 to 1000 ng/mL were less than 11.4% and within 10.2%, respectively. The limit of quantification of osimertinib was 10 ng/mL.

Pharmacogenomic analysis

Genotypes of CYP3A4 20230G > A (*1G) (rs2242480) and CYP3A5 6986A > G (*3) (rs776746) were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) [10]. Genotyping procedures identifying the C and T alleles in exon 12 (1236C>T, rs1128503), the G and T/A alleles in exon 21 (2677G>T/A, rs2032582), and the C and T alleles in exon 26 (3435C>T, rs1045642) of the ABCB1 gene were performed using PCR–RFLP, as previously reported [17–19].

Genotyping of ABCG2 421C > A (rs2231142) and ABCC2 -24C > T (rs717620) was performed using PCR–RFLP as described by Kobayashi et al. and Naesens et al. respectively [20, 21]. All frequencies for the different loci were at Hardy–Weinberg equilibrium.

Statistical analyses

Pharmacokinetic analysis of osimertinib was carried out using the standard noncompartmental method with WinNonlin (Pharsight Co., Mountain View, CA, USA; version 5.2). The AUC was calculated using the linear trapezoidal rule. The maximum plasma concentration (C_max) and time required to reach the peak concentration

| Characteristics | Number |
|-----------------|--------|
| Women : Men     | 15 : 8 |
| Stage IV : IIIb | 22 : 1 |
| Tumor history, Adenocarcinoma : Other | 23 : 0 |
| Osimertinib therapy, First-line : second-line or later | 7 : 16 |
| EGFR mutation, exon19 deletions : exon21 L858R | 16 : 7 |
| CYP3A4 20230G > A(*1G), *1/*1 : *1G/*1G | 12 : 11 : 0 |
| CYP3A5*1/*1 : *3/*3 | 0 : 8 : 15 |
| ABCB1 1236C>T, C/C : C/T : T/T | 5 : 7 : 11 |
| ABCB1 2677G>T/A, G/G : G/T : T/A : A/A | 3 : 4 : 7 : 4 : 1 |
| ABCB1 3435C>T, C/C : C/T : T/T | 10 : 6 : 7 |
| ABCG2 421C>A, C/C : C/A : A/A | 12 : 11 : 0 |
| ABCC2 -24C>T, C/C : C/T : T/T | 11 : 10 : 2 |
| H2RAs : PPI : P-CAB : none | 2 : 6 : 9 : 6 |
| Diarrhea, Grade 1 : Grade 2 : No diarrhea | 4 : 2 : 17 |
| Skin rash, Grade 1 : Grade 2 : No skin rash | 3 : 2 : 18 |
| Hepatotoxicity, Grade 1 : Normal function | 5 : 18 |

| Characteristics | Mean ± standard deviation |
|-----------------|--------------------------|
| Age, years      | 68.6 ± 8.2 (53 - 82)     |
| Body weight, kg | 55.5 ± 13.8 (33.8 - 89.5) |
| Body surface area, m² | 1.54 ± 0.21 (1.20 - 1.95) |
| Laboratory test values |                  |
| White blood cell, x10³/mm³ | 6.2 ± 2.0 (3.9 - 11.6) |
| Red blood cell, x10³/mm³ | 410 ± 52 (277 - 535)   |
| Hemoglobin, g/dL | 12.4 ± 1.5 (8 - 15)      |
| Platelets, x10³/mm³ | 257 ± 65 (81 - 373)     |
| Aspartate aminotransferase, IU/L | 25.5 ± 6.9 (18 - 47) |
| Alanine aminotransferase, IU/L | 27.1 ± 22.2 (9 - 91)   |
| Serum albumin, g/dL | 3.6 ± 0.6 (2.5 - 4.6) |
| Total bilirubin, mg/dL | 0.6 ± 0.4 (0.2 - 1.7) |
| Serum creatinine, mg/dL | 0.69 ± 0.22 (0.29 - 1.19) |

Data are presented as number or mean ± standard deviation (range).

H2RAs histamine 2-receptor antagonists, PPI proton pump inhibitor, P-CAB potassium-competitive acid blocker.
(t_{max}) were obtained directly from the profile. Kolmogorov–Smirnov tests were used to assess the distribution in each dataset. The clinical characteristics of patients taking osimertinib were expressed as the number or mean value ± standard deviation (range). Kruskal–Wallis tests or Mann–Whitney U tests were used to elucidate differences between groups. The AUC and C_{0} in each group are presented as the number or median (quartile 1 – quartile 3). Correlations in continuous variables between groups were determined using Spearman’s rank correlation coefficient test, with results presented as correlation coefficients (r). The effects of factors in univariate analysis were evaluated using stepwise multiple linear regression analysis. Variables with borderline significance (p < 0.2) on univariate analysis were subjected to multivariate regression analyses. Dummy variables were used to replace the groups (1 and 0 in 2 groups; 1 and 0, 0 and 0, and 0 and 1 in 3 groups). Results with p values of less than 0.05 were considered significant, and SPSS 20.0 for Windows (SPSS IBM Japan Inc., Tokyo, Japan) was used for all statistical analyses.

**Results**

The clinical characteristics of the patients are listed in Table 1. The mean age was 68.6 ± 8.2 years, and the mean body weight was 55.5 ± 13.8 kg. There were no patients with serious hepatic or renal dysfunction. The plasma concentration–time profiles from 0 to 24 h on day 15 after administration of osimertinib 80 mg once daily are shown in Online Resource 1. The median (minimum–maximum) AUC_{0-24}, C_{max}, and C_{0} of osimertinib were 8285 (3250–15,714) ng·h/mL, 396 (205–992) ng/mL, and 247 (88–519) ng/mL, respectively, and the corresponding coefficients of variation (CVs) for interpatient analyses were 41.3%, 42.3%, and 45.5%, respectively. The median (range) t_{max} of osimertinib was 6 (2–12) h.

The AUC_{0-24} of osimertinib was significantly correlated with age (P = 0.038), serum albumin (P = 0.002), and serum creatinine (P = 0.012), and the osimertinib C_{0} was significantly correlated with alanine transaminase (P = 0.017) and serum albumin (P = 0.008; Table 2). A significant negative correlation was observed between the AUC_{0-24} of osimertinib and serum albumin values (r^2 = 0.442, P = 0.001; Fig. 1).

Furthermore, there were significant differences in the AUC_{0-24} and C_{0} values of osimertinib among the group administered potassium-competitive acid blocker (P-CAB, all vonoprazan; n = 9), the group administered histamine 2-receptor antagonists or proton pump inhibitor (all esomeprazole; n = 8), and the group not given acid suppressants (n = 6; P = 0.021 and P = 0.046, respectively; Table 3). By contrast, there were no significant differences in the AUC_{0-24} and C_{0} values of osimertinib between patients with diarrhea, skin rash, or hepatotoxicity and those without these conditions (Table 3). Furthermore, there were no significant differences in the AUC_{0-24} and C_{0} values of osimertinib between genotypes of CYP3A4/5 or ABC transporters (Table 4). In multiple linear regression analysis, only serum albumin value was an independent factor predicting the AUC_{0-24} of osimertinib (slope = -3995.7, standardized regression coefficient = -0.665; P = 0.001), and the multiple correlation coefficient adjusted for the degrees of freedom was 0.415.

**Fig. 1** Correlation between the AUC_{0-24} of osimertinib and serum albumin values

![Correlation graph](image-url)
Discussion

To the best of our knowledge, this is the first study to report the effects of polymorphisms in CYP3A4/5 or ABC transporters on the pharmacokinetics of osimertinib in patients with NSCLC. CYP3A4/5 and ABC transporters have been reported to contribute to the pharmacokinetics of osimertinib [4–6]; however, polymorphisms in CYP3A4*1G, CYP3A5*3, ABCB1 (1236C>T, 2677G>T/A, and 3435C>T), ABCG2 (421C>A), and ABCC2 (-24C>T) did not affect the AUC of osimertinib in Japanese patients with NSCLC in our current analysis.

In multivariate analysis, lower serum albumin value was associated with an increase in the AUC0-24 of osimertinib, consistent with a previous report [3]. Several factors, including body weight and serum albumin concentrations, have been shown to affect variations in the pharmacokinetics of osimertinib in patients [3]. Moreover, osimertinib forms an irreversibly covalent bond with serum albumin [4], and hypoalbuminemia seems to reduce the number of binding sites available for osimertinib, consequently affecting the volume of distribution of osimertinib. However, because the distribution volume of osimertinib was saturated at steady-state on day 15 after beginning therapy, the osimertinib AUC in patients with lower serum albumin concentrations may be high.

In our current study, polymorphisms in CYP3A4/5 did not affect individual differences in osimertinib pharmacokinetics; however, this result was not consistent with the results obtained from a previous in vitro study using human recombinant CYP3A4 variants [8]. In a clinical study, co-administration of osimertinib with itraconazole, a strong CYP3A4 inhibitor, showed no clinically relevant effects on the Cmax and AUC of osimertinib [22]. Osimertinib is predominantly metabolized to N-demethylated metabolites by CYP3A4/CYP3A5 [4, 5], and it is metabolized to hydroxylated metabolites by CYP1A1/CYP1A2 [5]. Therefore, even if the metabolic pathways to N-demethylation of osimertinib via CYP3A4/5 were blocked by gene mutation or drug interaction, other pathways to hydroxylation via CYP1A1/CYP1A2 may be activated, and consequently, the pharmacokinetics of osimertinib may not be changed by CYP3A4/5 polymorphisms or CYP3A4 inhibitors. Therefore, analysis of CYP3A4/5 polymorphisms before osimertinib therapy may not predict the efficacy or side effects of osimertinib.

The probability of developing rash or diarrhea is thought to be related to the osimertinib AUC [3]; however, in the current study, there were no significant differences in osimertinib AUC between patients with rash or diarrhea and those without these conditions. In the results obtained from the AURA and AURA2 studies, the mean AUC of osimertinib at steady-state in patients receiving an 80 mg daily dose was reported to be 12,802 nmol·h/L (6396 ng·h/mL) [3]. In our study in Japanese patients, the median and mean AUC0-24 of osimertinib were 8285 and 8153 ng·h/mL, respectively, which were similar to or slightly higher than the AUC values described in a previous report [3]. Although the AUC of osimertinib at steady-state in Japanese patients has been reported to be approximately 1.4-fold higher than those in non-Japanese Asians or non-Asians [14, 23], there were no significant differences between ethnicities; the mean AUC of osimertinib in our current study (8153 ng·h/mL) was also approximately 1.3-fold higher than those in a previous report obtained from various ethnicities, including Japanese individuals (6396 ng·h/mL) [3]. Therefore, the lack of relationship between the osimertinib AUC and rash or diarrhea in the current study may not have been related to differences in osimertinib exposure between our current study and the previous study [3]. Similar to the previous study [3], the CTCAE grades of rash and diarrhea induced by osimertinib in our study were 1 or 2, and the frequencies of these side effects were lower (n = 5 and 6, respectively) than those in studies of other EGFR-TKIs, such as gefitinib and afatinib [15, 24, 25]. Therefore, additional studies with larger sample sizes may be needed to evaluate the target plasma concentration of osimertinib required to avoid the onset of rash or diarrhea.

In univariate analysis, the AUC0-24 and C0 of osimertinib in patients administered vonoprazan were significantly greater than those in patients without acid suppressants. Notably, the AUC of osimertinib is not altered by

Table 2 Comparisons and correlations of clinical characteristics with the AUC0-24 and C0 values of osimertinib

| Clinical characteristics | AUC0-24 | C0  |
|--------------------------|---------|-----|
|                          | Correlation coefficient (r) | P value | Correlation coefficient (r) | P value |
| Age                      | 0.436   | 0.038 | -0.375 | 0.078 |
| Body weight              | -0.065  | 0.768 | -0.036 | 0.872 |
| Body surface area        | 0.000   | 0.998 | 0.058  | 0.791 |
| Laboratory test values   |         |       |        |       |
| White blood cells        | 0.292   | 0.177 | 0.305  | 0.158 |
| Red blood cells          | -0.117  | 0.596 | 0.138  | 0.529 |
| Platelets                | 0.284   | 0.189 | 0.234  | 0.283 |
| Aspartate transaminase   | 0.275   | 0.205 | 0.390  | 0.066 |
| Alanine transaminase     | 0.395   | 0.062 | 0.494  | 0.017 |
| Serum albumin            | -0.622  | 0.002 | -0.536 | 0.008 |
| Total bilirubin          | -0.159  | 0.469 | -0.098 | 0.656 |
| Serum creatinine         | -0.516  | 0.012 | -0.300 | 0.165 |

AUC0-24 area under the plasma concentration-time curve from 0 to 24 h, C0 trough plasma concentration
omeprazole or vonoprazan in rats [26] or by omeprazole in humans [27]. In addition, the side effect of hypalbuminemia induced by vonoprazan has not been reported until now. Therefore, we cannot explain the mechanism through which vonoprazan increased the osimertinib AUC. In the current study, the Cmax of osimertinib in patients administered vonoprazan was significantly greater than that in patients without vonoprazan administration (P = 0.008); however, there were no significant differences in the elimination half-life between these groups. These results suggested that the rate of absorption of osimertinib from the gastrointestinal tract is increased by vonoprazan. Vonoprazan inhibits the transporter activity of P-glycoprotein, a drug efflux transporter expressed in the intestinal lumen, with a half-maximal inhibitory concentration of 50.3 μM [28]. Because the intestinal lumen is exposed following the clinical dose of vonoprazan (10–20 mg once daily), P-glycoprotein in the intestinal lumen may be inhibited by vonoprazan, thereby increasing the plasma concentrations of osimertinib. However, in multivariate analysis, the influence of vonoprazan was excluded as an independent factor predicting the AUC of osimertinib. Therefore, additional studies of drug interactions between osimertinib and vonoprazan are necessary.

### Table 3 Comparisons of patient background or osimertinib-induced side effects and AUC0–24 or C0 of osimertinib

| Parameters/characteristics | n | AUC0-24 Median (quartile 1 - quartile 3) | P value | C0 Median (quartile 1 - quartile 3) | P value |
|----------------------------|---|----------------------------------------|---------|--------------------------------------|---------|
| Sex                        |   |                                        |         |                                      |         |
| Woman                      | 15 | 8470 (4690-11324)                     | 0.636*  | 235 (157-366)                        | 0.925*  |
| Man                        | 8  | 7432 (7108-8912)                      |         | 263 (188-310)                        |         |
| Age                        |   |                                        |         |                                      |         |
| ≥ 75 years                 | 6  | 4355 (3738-9075)                      | 0.074*  | 145 (114-277)                        | 0.062*  |
| < 75 years                 | 17 | 8470 (7134-10024)                     |         | 272 (208-338)                        |         |
| Osimertinib therapy        |   |                                        |         |                                      |         |
| First-line                 | 7  | 9384 (4020-12295)                     | 0.413*  | 331 (133-422)                        | 0.222*  |
| Second-line or later       | 16 | 7919 (5862-8886)                      |         | 238 (171-292)                        |         |
| EGFR mutation status       |   |                                        |         |                                      |         |
| Exon 19                    | 16 | 7919 (6715-10202)                     | 0.624*  | 263 (188-341)                        | 0.175*  |
| Exon 21                    | 7  | 8470 (3594-9384)                      |         | 190 (99-281)                         |         |
| Smoking                    |   |                                        |         |                                      |         |
| Yes                        | 10 | 8525 (6959-9847)                      | 0.522*  | 254 (185-334)                        | 0.784*  |
| No                         | 13 | 7553 (4355-10354)                     |         | 247 (145-332)                        |         |
| Antacid                    |   |                                        |         |                                      |         |
| None                       | 6  | 6350 (3738-7279)                      | 0.173*  | 173 (114-255)                        |         |
| H2RAs or PPI               | 8  | 8305 (5165-8858)                      | 0.021** | 241 (174-267)                        | 0.046** |
| P-CAB                      | 9  | 9669 (7946-13313)                     |         | 331 (236-404)                        |         |
| Side effects               |   |                                        |         |                                      |         |
| Diarrhea                   |   |                                        |         |                                      |         |
| Yes                        | 6  | 6952 (3824-8590)                      | 0.201*  | 211 (116-260)                        | 0.177*  |
| No                         | 17 | 8470 (6837-10852)                     |         | 272 (180-355)                        |         |
| Skin rash                  |   |                                        |         |                                      |         |
| Yes                        | 5  | 8325 (5604-10982)                     | 0.745*  | 247 (187-383)                        | 0.638*  |
| No                         | 18 | 7919 (5387-9633)                      |         | 244 (167-318)                        |         |
| Hepatotoxicity             |   |                                        |         |                                      |         |
| Yes                        | 5  | 9669 (6465-13047)                     | 0.257*  | 331 (245-432)                        | 0.080*  |
| No                         | 18 | 7919 (4522-9087)                      |         | 238 (151-285)                        |         |

Data are presented as number or median (quartile 1 - quartile 3)

AUC0–24 area under the plasma concentration-time curve from 0 to 24 h, C0 trough plasma concentration, H2RAs histamine 2-receptor antagonists, PPI proton pump inhibitor, P-CAB potassium-competitive acid blocker

* Mann-Whitney U test
** Kruskal-Wallis test
Our study had several limitations. First, the number of patients treated with osimertinib was only 23, which may have prevented the precise evaluation of osimertinib pharmacogenomics. Second, to investigate the causes of larger interindividual differences in osimertinib pharmacokinetics, several factors were analyzed; however, in our analysis of the influence of concomitant drugs, the number of patients taking vonoprazan was only 9. Our data for drug interactions between osimertinib and vonoprazan also may lack accuracy. Therefore, our results should be interpreted within the context of these study limitations. Further studies with larger patient cohorts are needed to confirm our results.

In conclusion, polymorphisms in CYP3A4*1G, CYP3A5*3, ABCB1 (1236C>T, 2677G>T/A, and 3435C>T), ABCG2 (421C>A), and ABCC2 (-24C>T) did not affect the AUC of osimertinib in Japanese patients with NSCLC. Therefore, analysis of these polymorphisms before osimertinib therapy may not predict the efficacy or side effects of osimertinib. In multivariate analysis, lower serum albumin values in patients increased the AUC_{0-24} of osimertinib, consistent with a previous report. Therefore, after beginning osimertinib therapy, we may need to perform periodic measurement of serum albumin values. However, further studies are required to assess the factors contributing to the interindividual variability of osimertinib pharmacokinetics.

### Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1007/s10637-022-01304-9.

### Author contributions
Hayato Yokota, Kazuhiro Sato, Katsutoshi Nakayama, and Masatomo Miura participated in the design of the study and reviewed the results. Kazuhiro Sato, Sho Sakamoto, Yuji Okuda, Mariko Asano, Masahide Takeda, and Katsutoshi Nakayama were responsible for patient enrollment and were involved in data acquisition. Hayato Yokota carried out genotyping. Hayato Yokota and Natsuki Fukuda investigated the incidence of osimertinib-induced side effects and medication status. Masatomo Miura analyzed plasma concentrations. Hayato Yokota and Masatomo Miura were responsible for the statistical analysis. Hayato Yokota and Masatomo Miura drafted the manuscript.

### Table 4
Comparison of the AUC0-24 or C0 of osimertinib between genotypes of CYP3A4/5 or ABC transporters

| Parameters/characteristics | n | AUC_{0-24} P value | C0 P value |
|----------------------------|---|-------------------|-----------|
|                           |   | Median (quartile 1 - quartile 3) | Median (quartile 1 - quartile 3) |
| **CYP3A4 20230G>A(*1G)**   |   |                  |           |
| *1/*1                      | 12| 7919 (5985-8552) | 0.449* 241 (171-294) 0.347* |
| *1/*1G                     | 11| 8988 (4020-12295) | 272 (133-422) 0.166* |
| **CYP3A5**                 |   |                  |           |
| *1/*3                      | 8 | 6890 (3930-10740) | 0.392* 233 (125-343) 0.466* |
| *3/*3                      | 15| 8325 (7081-9669) | 253 (175-331) 0.466* |
| **ABCB1 1236C>T**         |   |                  |           |
| C/C                        | 5 | 7312 (4295-10310) | 247 (140-377) 0.945** |
| T/T                        | 11| 8285 (7081-9384) | 253 (170-298) 0.945** |
| **ABCB1 2677G>T/A**       |   |                  |           |
| G/G                        | 3 | 10380 (9384-11087) | 314 (281-) 0.168** |
| G/T + G/A                  | 8 | 7250 (4922-8423) | 213 (162-250) 0.168** |
| T/T + T/A + A/A            | 12| 7939 (4663-10910) | 260 (142-361) 0.168** |
| **ABCB1 3435C>T**         |   |                  |           |
| C/C                        | 10| 7798 (5387-10858) | 267 (171-354) 0.728** |
| T/T                        | 7 | 7553 (3594-9669) | 272 (99-344) 0.728** |
| **ABCG2 421C>A**          |   |                  |           |
| C/C                        | 12| 7073 (4187-11087) | 253 (139-353) 0.786* |
| C/A                        | 11| 8325 (7188-8989) | 247 (190-331) 0.786* |
| **ABCC2 -24C>T**          |   |                  |           |
| C/C                        | 11| 8580 (4020-11324) | 247 (133-366) 0.880* |
| C/T + T/T                  | 12| 7432 (5862-8858) | 244 (171-323) 0.880* |

Data are presented as number or median (quartile 1 - quartile 3).

- AUC_{0-24} area under the plasma concentration-time curve from 0 to 24 h, C0 trough plasma concentration
- * Mann-Whitney U test
- ** Kruskal-Wallis test
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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval The study was conducted according to the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Akita University School of Medicine (approval numbers: 790 and 1140).

Informed consent Informed consent was obtained from all participants enrolled in this study.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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