Blood Concentrations of Osimertinib and Its Active Metabolite: Impact on Treatment Efficacy and Safety

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Running Title: Osimertinib Blood Levels: Correlation With Efficacy and Safety

Abstract

Background/Aim: Osimertinib (OSI) is a third-generation epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) and the standard treatment for non-small cell lung cancer with *EGFR* mutation. OSI contains active metabolites such as AZ5104 and AZ7550. The correlation between the blood concentrations of OSI, AZ5104, and AZ7550 with their efficacy and safety is not clear; hence, we examined it. *Patients and Methods:* This was a single-center, retrospective study. We measured the blood concentrations of OSI, AZ5104, and AZ7550 in 46 patients who received OSI between March 2016 and December 2022 and examined their relationships with progression-free survival (PFS), overall survival (OS), and adverse events. *Results:* The high OSI group had a longer PFS than the low OSI group (26.5 *vs.* 17.9 months, p=0.058); however, blood levels of AZ7550 and AZ5104 were not correlated with PFS. In the multivariate analysis, blood OSI concentration was an independent prognostic factor for PFS. Overall survival was not different between patients with high or low blood levels of OSI, AZ5104, or AZ7550. In terms of safety, AZ7550 blood levels were higher in patients with higher grades of neutropenia, lymphopenia and anemia, whereas AZ5104 blood levels were higher in patients with higher grades of lymphopenia. *Conclusion:* Higher OSI blood concentrations were associated with a longer PFS, while higher blood concentrations of AZ5104 and AZ7550 were observed in patients with higher hematological toxicity grades. Further research is needed to determine the optimal blood levels of OSI that improve prognosis and minimize

toxicity.

Osimertinib (OSI), a third-generation epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI), is the standard first-line treatment for *EGFR* mutation-positive non-small cell lung cancer (NSCLC). It is also used as a second-line treatment for *EGFR* T790M mutation-positive NSCLC that is resistant to first- or second-generation EGFR-TKIs.

In the FLAURA trial, OSI administration demonstrated significantly longer median progression-free survival (PFS) (18.9 *vs.* 10.2 months) (1) and median overall survival (OS) (38.6 *vs.* 31.8 months) (2) than first-generation EGFR-TKIs such as gefitinib and erlotinib, as first-line therapy in patients with *EGFR*-positive NSCLC. In the AURA3 study, the median PFS was significantly longer with OSI administration than that with platinum plus pemetrexed in patients with NSCLC who were resistant to first- and second-generation EGFR-TKIs and harbored the *EGFR* T790M mutation (10.1 *vs.* 4.4 months) (3). In real-world clinical practice, first-line OSI has been shown to significantly prolong the duration of treatment and improve overall survival compared to standard TKIs (4). As mentioned above, while OSI is a highly effective drug, in Asian populations, the administration of OSI may not lead to an improvement in OS compared to that with first-generation EGFR-TKIs (2). This racial difference may be attributed to variations in drug metabolism. Furthermore, single nucleotide polymorphisms (SNPs) in specific cytochrome P450 enzymes (CYP450) and drug efflux transporters have been

associated with an increased incidence of adverse effects of OSI and a reduced progression-free survival (PFS) (5, 6). It is possible that specific CYP450 variants and SNPs influence OSI blood concentrations, resulting in differences in both PFS and side effects.

OSI is demethylated by CYP3A4 in the liver, producing active metabolites, such as AZ5104 and AZ7550 (Figure 1). AZ5104 exhibits stronger inhibitory activity against both mutant and wild-type EGFR tyrosine kinases than OSI (Table I) (7), which may influence both efficacy and toxicity. Additionally, the plasma half-life of AZ7550 is longer than that of OSI (72 h *vs.* 59 h) (8), which potentially contributes to the accumulation of toxicity. The levels of these active metabolites could affect both the therapeutic effects and safety profile of the drug. However, only a few studies have investigated the relationship of blood concentrations of OSI, AZ5104, and AZ7550 with their efficacy and safety. Therefore, we aimed to investigate this relationship in this study.

Materials and Methods

Study design and patients: This single-center retrospective study was conducted at Shimane University Hospital. Patients with incurable or relapsed EGFR mutation-positive NSCLC who received OSI between May 2016 and December 2022 and were at least 20 years old, with available blood samples and written informed consent, were included. The primary endpoint was the relationship between PFS and blood concentrations of OSI, AZ5104, and AZ7550.

The secondary endpoint was the relationship between OS and safety and concentrations of OSI, AZ5104, and AZ7550.

Data on age, sex, stage, histologic type, genetic mutation, OSI dose, time to disease progression, survival, adverse events, and disease severity were retrieved from the electronic medical records. Adverse events related to OSI were retrospectively evaluated according to the Common Terminology Criteria for Adverse Events v4.0 for predefined items including neutropenia, anemia, thrombocytopenia, aspartate transaminase (AST) elevation, alanine transaminase (ALT) elevation, QTc interval prolongation, interstitial lung disease, paronychia, rash, and diarrhea.

The study was approved by the Medical Ethics Committee of Shimane University School of Medicine (approval number 3013). Written informed consent was obtained from all the participants. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Data collection: Serum samples were collected after day 22 of OSI initiation when the blood levels reached a steady state. Patients received OSI after breakfast, and approximately 10 mL of blood was collected during an outpatient visit on the same day (3–4 h after taking the drug). Blood samples were obtained from the surplus samples collected during routine medical care. The collected serum samples were stored frozen at -80°C and analyzed collectively using liquid chromatograph-tandem mass spectrometry (LC-MS/MS).

Chemical and reagents: Acetonitrile (LC/MS grade), methanol (LC/MS grade), distilled water (HPLC grade), formic acid (GR grade), OSI (free base, Selleck), AZ5105 (free base, Selleck), AZ7550 (mesylate, MedChemExpress), clozapine (internal standard, Sigma), and drug-free plasma (Cosmo Bio) were utilized.

Sample preparation: To 200 µL of plasma, 100 µL of clozapine (10 ng/mL in acetonitrile) was added as an internal standard. After adding 300 µL acetonitrile, the mixture was vortexed and centrifuged at 12,000 rpm for 5 min. The supernatant (200 µL) was used for analysis, and 2 µL was injected into the LC-MS/MS system.

LC-MS/MS conditions: Quantitative analysis was performed using a Nexera X2 system coupled with an LCMS-8030 triple quadrupole mass spectrometer (Shimadzu). Chromatographic separation was achieved on a XBridge C18 column (2.1 × 100 mm, 3.5 µm, Waters) maintained at 40°C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a flow rate of 0.3 mL/min. The gradient program was as follows: 0–2 min, 5% B; 2–7.5 min, 5–45% B; 7.5–8 min, 45–90% B; 8–10.5 min, 90% B; 10.5–14 min, and reconditioning to 5% B. MS detection was performed in positive electrospray ionization mode with multiple reaction monitoring for OSI, AZ7550, AZ5104, and the internal standard.

Data analysis: Data were analyzed using LabSolutions LC-MS software (Shimadzu), with calibration curves generated using GraphPad Prism 9.2.0.

Statistics: The PFS and OS were determined using the Kaplan–Meier method using IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA). The level of significance was set at $p < 0.05$. The Cox proportional hazards model was used for both univariate and multivariate analyses to assess the impact of individual covariates on PFS. Variables included in the multivariate analysis were those with $p < 0.05$ in the univariate analysis.

Results

Patient characteristics. A total of 46 patients were enrolled (Table II). The median age was 69 years (range=30–88 years), with a predominance of women (60.9%). Patients with adenocarcinomas comprised 95.7% of the population. Stage IV disease was present in 76.1% of the patients, and the genetic mutation distribution showed a slightly higher prevalence of del19 (60.9%) than that of L858R. Most patients had a good performance status (84.8%). OSI was used as the first-line treatment in 54.3% of the patients. At the time of blood concentration measurement, 93.5% of patients received a daily dose of 80 mg. The proportion of patients with brain metastases was 56.5%. Blood concentrations of OSI, AZ5104, and AZ7550 were categorized into high and low groups in the second

quartile. The patient characteristics did not significantly differ between the groups.

Progression-free survival and overall survival: Figure 2 shows the Kaplan–Meier curves for PFS according to blood concentrations of OSI, AZ5104, and AZ7550. Patients with higher OSI blood levels tended to have longer PFS than those with lower levels (26.5 vs. 17.9 months), with the difference approaching statistical significance (p=0.058). For AZ5104, the median PFS was 23.4 months in the high-concentration group and 17.9 months in the low-concentration group (p=0.574). In the AZ7550 group, patients with lower blood concentrations tended to have longer PFS than those with higher concentrations (25.5 vs. 16.7 months), with a trend toward statistical significance (p=0.078). Overall, PFS did not differ significantly between the high and low concentration groups for any of the three compounds. Similarly, OS did not significantly differ between high and low blood level groups: 38.9 vs. 47.9 months for OSI (p=0.815), 38.8 vs. 46.2 months for AZ5104 (p=0.944), and 29.2 vs. 50.7 months for AZ7550 (p=0.227).

Table III shows the results of the multivariate analysis with *EGFR* mutation, T790M, CNS metastasis, PS, and OSI concentrations as variables. A low blood concentration of OSI was an independent prognostic factor for shortened PFS. However, a low blood concentration of OSI was not an independent prognostic factor for OS (Table IV).

Safety: Of 46 enrolled patients, adverse events could not be confirmed in 2 cases

because the patients were transferred to another hospital. After excluding these two cases, safety was evaluated in 44 patients (Table V). The relationship between adverse events and the blood concentrations of OSI, AZ5104 and AZ7550 was also investigated. Neutropenia, lymphopenia, anemia, hepatic dysfunction (elevated AST and ALT), pneumonia, QT prolongation, rash, paronychia, and diarrhea were prespecified as adverse events, and the correlation with blood concentrations was analyzed for all of these. For OSI, AZ5104 and AZ7550, although there was no significant difference in the incidence of adverse events between the high-dose and low-dose groups, there was a tendency for more grade 3 or higher adverse events to occur in the high-concentration group.

Figure 3 shows the average blood concentrations of the active metabolites according to the severity of adverse events (CTCAE v4.0). Among the prespecified adverse events, neutropenia, lymphopenia and anemia showed higher blood concentrations in the high-grade group. Specifically, for neutropenia, lymphopenia and anemia, the average blood concentration of AZ7550 was significantly higher in the grade 2–4 group than that in the grade 0 and 1 groups. Additionally, in patients with grade 2–4 lymphopenia, the average blood concentration of AZ5104 was significantly higher than that in the grade 0 and 1 groups. In our study, four patients discontinued OSI usage due to adverse events. The adverse events leading to discontinuation were drug-induced lung injury in three cases and heart failure in one case. Notably, for both OSI and AZ5104, two

of these patients were included in the high-dose group and the other two in the low-dose group, indicating an equal distribution between dose groups. In contrast, all four patients were classified in the high-dose group for AZ7550.

Discussion

We examined the correlation between the blood levels of OSI, AZ5104 and AZ7550 with PFS and OS. Additionally, their relationship with adverse events was investigated. This is the first study to simultaneously measure these three compounds in the same patients and investigate their efficacy and safety.

Firstly, our study indicated that higher blood levels of OSI prolonged PFS more compared to lower levels. Cross *et al.* reported that OSI has a dose-dependent antitumor effect *in vivo* (7). When OSI was administered once daily for 14 days to H1975 (L858R/T790M) xenograft-bearing mice, tumor shrinkage was observed at all doses (2.5, 5, and 10 mg/kg/day). Regarding the long-term effects, the 25 mg/kg/day group achieved a complete response by day 20, which was maintained until the end of the study on day 200. Although the 5 mg/kg/day group did not achieve a complete response, tumor shrinkage was sustained through day 200. By contrast, tumors in the 1 mg/kg/day group initially shrank but began to regrow around day 50. However, when the dose was increased to 25 mg/kg/day, a complete response was achieved, and tumor shrinkage was maintained until day 200. These findings suggest that, although short-term tumor shrinkage can be observed even at low doses, the long-term effect is dose dependent.

However, several clinical trials have demonstrated that the antitumor effect is independent of the OSI concentration in clinical settings. In a phase I study of OSI in patients with EGFR T790M-positive NSCLC conducted by Jänne *et al.*, similar objective response rates (50–70%) were observed across all dose groups (20, 40, 80, 160, and 240 mg/d) (9). However, PFS and OS were not evaluated in this study; therefore, it remains unclear whether the prognosis is consistent across doses. Additionally, Ramalingam *et al.* reported no significant differences in ORR (67% *vs.* 87%) or PFS (22.1 *vs.* 19.3 months) between the 80 mg/day and 160 mg/day OSI dose groups (10). Nonetheless, it is still uncertain whether ORR and PFS are comparable at doses lower than 80 mg. Fukuhara *et al.* compared PFS among the lowest quartile (Q1), middle quartiles (Q2 and Q3), and highest quartile (Q4) of OSI trough concentrations. The PFS in the Q2+Q3 group was longer than that in the Q1 and Q4 groups (22.8 *vs.* 4.6 *vs.* 5.1 months). The Q4 group had more grade 3 or higher adverse events, with 30% of the patients discontinuing the study treatment due to adverse events, likely contributing to the shorter PFS in the highest quartile group (11). Rodier *et al.* showed that patients in the Q4 group had a higher risk of death than those in the Q1, Q2, and Q3 groups. The reason for the shorter prognosis in the OSI-high blood concentration group is considered to be the development of EGFR-TKI resistance through off-target mechanisms, including MET and HER2 amplification (12). In Hashino *et al.*'s study, patients with lower OSI blood concentrations had significantly longer PFS compared to those with higher concentrations (18.7 *vs.*

31.2 months). Multivariate analysis suggested that hypoalbuminemia may be associated with disease progression in non-small cell lung cancer (NSCLC). Since osimertinib irreversibly binds to serum albumin, patients with hypoalbuminemia may have higher free plasma concentrations of osimertinib, potentially worsening prognosis due to off-target resistance (13).

Building on these findings, and incorporating our own research, it can be concluded that the relationship between OSI blood concentration and PFS improvement is not linear. While PFS may improve within a certain concentration range, levels beyond this threshold could worsen prognosis due to increased side effects and off-target resistance. In our study, PFS was prolonged in the high-concentration group, which aligns with some of the findings from the aforementioned studies. However, as we categorized patients into only two groups based on OSI concentration, further analysis within the high-concentration group was not possible. However, because we categorized patients into only two groups based on OSI concentration, we were unable to further analyze PFS within the high-concentration group.

The approved dose of 80 mg/day reflects a balance between efficacy and side effects. However, because not all patients achieved the same blood concentration at this dose, we believe that the precise optimal blood concentration should be clarified in future studies. Furthermore, we hypothesized that the blood concentration of AZ5104, which exhibits stronger EGFR-TKI activity than OSI, would correlate with its antitumor efficacy, but no such relationship was observed

in this study. The area under the plasma concentration-time curve (AUC) and maximum plasma concentration (Cmax) of AZ5104 were 10.2% and 5.2% of those of OSI (8), respectively. These levels were considered insufficient to significantly prolong PFS and OS. In AZ7550, progression-free survival (PFS) tended to be longer in the low-dose group than the high-dose group. Notably, all four patients who discontinued treatment due to adverse events were in the high-dose group, suggesting that early discontinuation may have contributed to the shorter PFS observed in this group. The impact of these active metabolites on prognosis remains largely unclear and warrants further investigation.

Secondly, our research showed that AZ7550 and AZ5104 concentrations were associated with hematological toxicity. First, we hypothesized that AZ5104, which has a stronger inhibitory effect on wild-type EGFR than OSI or AZ7550, would be more strongly correlated with adverse events. However, the blood concentration of AZ5104 was correlated only with lymphocytopenia, whereas that of AZ7550 was correlated with neutropenia, lymphocytopenia, and anemia. There have been several reports on the correlation between OSI concentration, active metabolite concentration, and safety. Fukuhara *et al.* reported that patients with anorexia and hematological toxicity had higher trough OSI concentrations (12). Agema *et al.* found a significant correlation between OSI trough concentration and severe toxicity and determined the optimal toxicity threshold to be 259 ng/ml (14). Ishikawa *et al.* reported that the areas under the blood concentration–time curve from 0 to 24 hours (AUC 0-24 h) for both OSI and AZ5104 were significantly

correlated with grade 2 or higher diarrhea. They also found that the AUC 0–24 h for AZ755 was significantly correlated with grade 2 or higher paronychia and anorexia (6). Fujiwara *et al.* reported that the AUC 0–24 h of AZ5104 were proportional to the severity of diarrhea and significantly associated with grade ≥2 of any adverse events (15). Brown *et al.* observed a linear relationship between safety (rash, diarrhea, and QTcF prolongation) and OSI concentration. The correlations of OSI and its active metabolites with gastrointestinal toxicity, skin toxicity, and other adverse effects reported in previous studies were not observed in our study. One possible explanation is that our study was retrospective, making it likely that minor gastrointestinal symptoms and skin disorders were not documented in the medical records.

Additionally, this study identified a correlation between the active metabolites (AZ5104 and AZ7550) and hematological toxicity. OSI is associated with hematological toxicities, such as leukopenia, neutropenia, anemia, thrombocytopenia, and lymphopenia (1, 3, 16, 17). However, the underlying mechanisms remain unclear, especially it needs to be determined whether active metabolites contribute to these toxicities. In clinical practice, combinations of OSI and cytotoxic anticancer drugs have been approved (16). Given that both OSI and cytotoxic agents can cause hematological toxicity, their combination is likely to have an increasingly significant impact on future treatment outcomes, particularly with regard to adverse events. Moreover, patients experiencing severe side effects may have blood concentrations exceeding the optimal

therapeutic range. Therefore, future prospective studies are necessary to determine the optimal blood concentration range that minimizes side effects while maintaining therapeutic efficacy and not worsening the prognosis.

Study limitations. Firstly, the time elapsed between OSI administration and blood sample collection varied. The OSI levels were measured after breakfast, whereas blood samples were collected at varying times upon patient arrival for routine visits, leading to variations across patients. However, since all samples were drawn after the OSI levels had reached a steady state, we assumed that this variability had a minimal impact on the results. The second limitation was the small sample size (n=46). Thirdly, as this was a retrospective study, there may have been inaccuracies in capturing patient-reported adverse effects. Therefore, a prospective, multicenter study with a larger patient cohort should be conducted to standardize blood sampling timing, such as by measuring trough levels at steady state.

Conclusion

In conclusion, we demonstrated that patients with high OSI blood concentrations had longer PFS than those with low concentrations, and that OSI blood levels were positively correlated with efficacy. However, high blood concentrations of the active metabolites are associated with hematological toxicity. Further research is needed to determine the optimal OSI concentration

that minimizes the risk of adverse effects while maintaining clinical efficacy and not compromising the prognosis.

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Figure legends

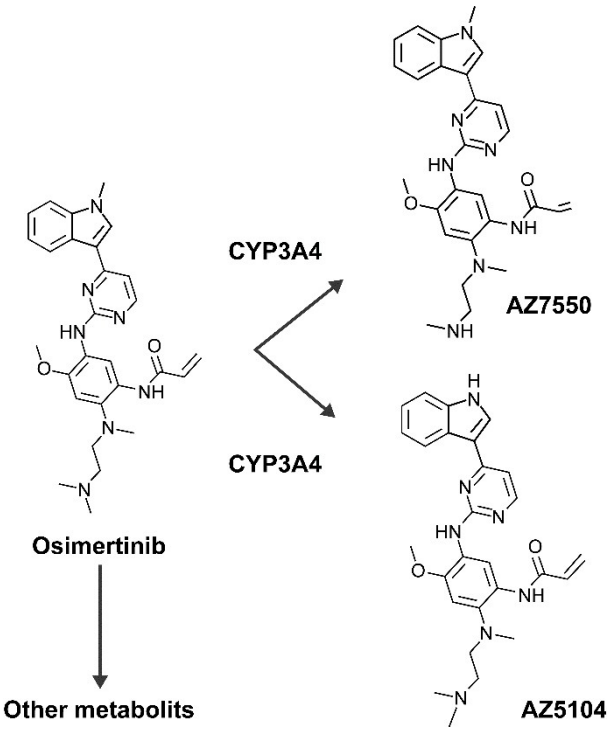

Figure 1. Structure of Osimertinib (OSI) and active metabolites

OSI contains several metabolites, of which AZ5104 and AZ7550 are active metabolites. OSI is demethylated by CYP3A4 to produce AZ5104 and AZ7550.

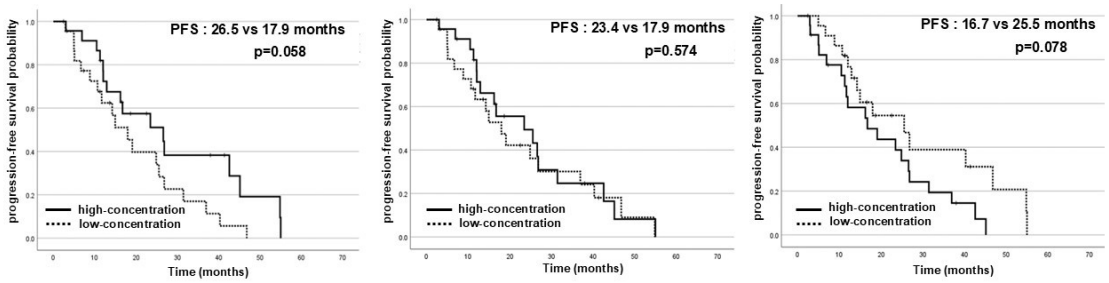

Figure 2. Kaplan–Meier curves for progression-free survival by blood concentrations of OSI and its active metabolites

Although there was no statistically significant correlation between progression-free survival (PFS) and blood levels of osimertinib (OSI) (A), AZ5104 (B), or AZ7550 (C), a trend toward prolonged PFS was observed in patients with high OSI concentrations and low AZ7550 concentrations.

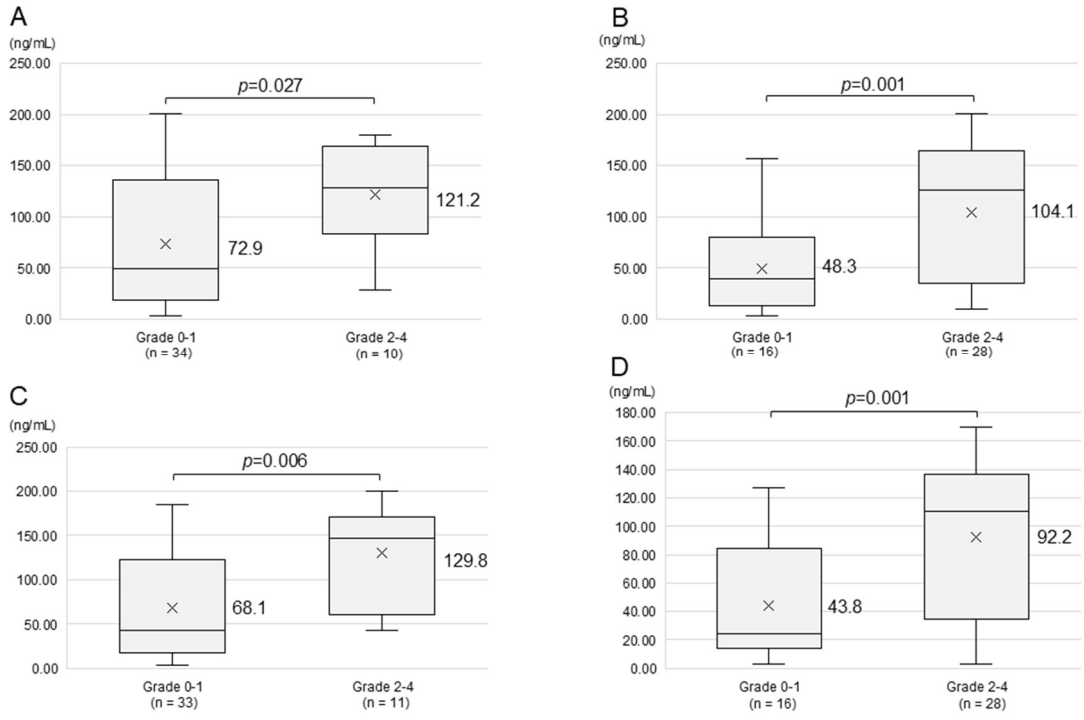

Figure 3. Mean blood concentration of adverse events by CTCAE v 4.0 Grade

There were significant differences in the mean blood levels of AZ7550 between patients with (A) neutropenia (p=0.027), (B) lymphopenia (p=0.001), and (C) anemia (p=0.006) at Grades 0–1 *vs.* 2–4. Additionally, (D) AZ5104 levels differed significantly between lymphopenia Grades 0–1 and 2–4 (p=0.001). p-Values were derived using Welch's t-test.

Compounds	Apparent IC50, nM (95% CI)			
	EGFR (T790M/L858R)	EGFR (L858R)	EGFR (L861Q)	EGFR (wild type)
OSI	1	12	5	184
AZ5104	<1	6	1	25
AZ7550	4	56	29	519

Table I. IC_{50} of OSI, AZ5104, and AZ7550 against the isolated EGFR tyrosine kinases. Effects of OSI, AZ5104, and AZ7550 on the enzymatic activities of wild-type and mutant EGFR were evaluated using a commercial enzyme assay kit. The results are reported as apparent IC_{50} values.

EGFR, epidermal growth factor receptor; OSI, osimertinib

	All	OSI			AZ5104			AZ7550		
	N=46	Low concentration N=23	High concentration N=23	*P*	Low concentration N=23	High concentration N=23	*P*	Low concentration N=23	High concentration N=23	*P*
Age, years Median (range)	69 (30-88)	69 (30-85)	69 (42-88)	0.498	69(30-85)	69(42-88)	0.717	67(30-85)	70(42-88)	0.583
Sex, n (%) Male	18 (39.1%)	11 (47.8%)	7 (30.4%)	0.365	12(52.2%)	6(26.1%)	0.130	13(56.5%)	5(21.7%)	0.033
Histology, n (%) Adeno	44 (95.7%)	22 (95.7%)	22 (95.7%)	1.000	23(100.0%)	22(91.3%)	0.489	22(95.7%)	22(95.7%)	1.000
Stage, n (%) Ⅳ recurrent	35 (76.1%) 11 (23.9%)	16 (69.6%) 7 (30.4%)	19 (82.6%) 4 (17.4%)	0.491	17(73.9%) 6(23.1%)	18(78.3%) 5(21.7%)	1.000	15(65.2%) 8(34.8%)	20(87.0%) 3(13.0%)	0.165
EGFR mutation, n (%) Ex19del L858R Others	28 (60.9%) 16 (34.8%) 2 (4.3%)	16 (72.7%) 6 (26.0%) 1 (4.3%)	12 (52.2%) 10 (43.5%) 1 (4.3%)	0.347	17(73.9%) 5(21.7%) 1(4.3%)	11(47.8%) 11(47.8%) 1(4.3%)	0.116	16(69.6%) 6(26.1%) 1(4.3%)	12(52.2%) 10(43.5%) 1(4.3%)	0.347
T790M	19 (41.3%)	12 (52.1%)	7 (30.4%)	0.231	12(52.2%)	7(30.4%)	0.231	10(43.5%)	9(39.1%)	1.000
Performance Status (%) 0,1	39 (84.8%)	19 (82.6%)	20 (87.0%)	1.000	18(78.3%)	21(91.3%)	0.414	20(87.0%)	19(82.6%)	1.000
Treatment line, n (%) first	25 (54.3%)	10 (43.5%)	15 (65.2%)	0.236	10(43.5%)	15(65.2%)	0.236	12(52.2%)	13(56.5%)	1.000
Dose 80mg/日	43(93.5%)	21(91.3%)	22(95.7%)	1.000	21(91.3%)	22(95.7%)	1.000			1.000
CNS meta, n (%) yes	26 (56.5%)	10 (43.5%)	16 (69.6%)	0.124	11(47.8%)	15(65.2%)	0.358	12(52.2%)	14(60.9%)	0.763

Table II. Patient characteristics of the 46 patients are shown. Patient characteristics are also shown for OSI, AZ5104, and AZ7550, which are divided into high- and low-concentration groups.

EGFR, epidermal growth factor receptor; OSI, osimertinib

Table III. Multivariate analysis of PFS.

Covariate	HR (95%CI)	*p*-Value
EGFR mutation, L858R	0.739 (0.327–1.674)	0.469
T790M mutation, Yes	0.680 (0.298–1.551)	0.359
CNS metastases, Yes	1.603 (0.748–3.438)	0.225
PS, 2–4	0.589 (0.183–1.898)	0.375
Blood concentration of OSI, low	2.774 (2.226–6.275)	0.014

Multivariate analysis of PFS was performed using EGFR mutation status, T790M status, CNS metastasis status, PS, and OSI blood concentration as covariates. Lower OSI blood levels were associated with shorter PFS. PFS, Progression-free survival; OS, overall survival; CNS, central nervous system: PS, performance status; EGFR, epidermal growth factor receptor; OSI, osimertinib.

Table IV. Multivariate analysis of overall survival (OS).

Covariate	HR (95%CI)	*p*-Value
EGFR mutation, L858R	1.417 (0.549–3.661)	0.471
T790M mutation, Yes	0.491 (0.178–1.358)	0.171
CNS metastases, Yes	1.683 (0.695–4.073)	0.248

PS, 2–4	1.830 (0.536–6.243)	0.335
Blood concentration of OSI, low	1.697 (0.687–4.191)	0.252

Multivariate analysis of OS was performed with similar covariates; EGFR mutation, T790M status, CNS metastasis status, PS, and blood OSI levels had no effect on OS. PFS, Progression-free survival; OS, overall survival; CNS, central nervous system: PS, performance status; EGFR, epidermal growth factor receptor; OSI, osimertinib.

Table IV. Incidence of adverse events (CTCAE v 4.0)

	Overall(n=44)		OSI				
			Low-concentration(n=22)		High-concentration(n=22)		*P*
	Any grade	Grade≥3	Any grade	Grade≥3	Any grade	Grade≥3	
Neutropenia	23 (52.3%)	2 (4.5%)	11	0	12	2	0.4783
Anemia	43 (97.7%)	2 (4.5%)	21	0	22	2	0.4884
Thrombocytopenia	34 (77.3%)	4 (9.1%)	16	1	18	3	0.6041
Lymphocytes decreased	33 (75.0%)	13 (29.5%)	12	4	21	9	0.7188
AST elevation	24 (54.5%)	1 (2.3%)	12	0	12	1	1.0000
ALT elevation	15 (34.1%)	2 (4.5%)	6	1	9	1	1.0000
QTc interval prolonged	12 (27.3%)	1 (2.3%)	3	0	9	1	1.0000
Interstitial lung disease	5 (11.4%)	1 (2.3%)	3	0	2	1	0.4000
Paronychia/Rash	27 (61.4%)	2 (4.5%)	12	1	15	1	1.0000
Diarrhea	20 (45.5%)	1 (2.3%)	14	0	6	1	0.3000

	AZ 7550					AZ 5104				
	Low-concentration(n=22)		High-concentration(n=22)		*P*	Low-concentration(n=22)		High-concentration(n=22)		*P*
	Any grade	Grade≥3	Any grade	Grade≥3		Any grade	Grade≥3	Any grade	Grade≥3	
Neutropenia	9	1	14	1	1.0000	10	0	13	2	0.4862
Anemia	22	0	21	2	0.2326	21	0	22	2	0.4884
Thrombocytopenia	17	2	17	2	0.3697	16	1	18	3	0.6041
Lymphocytes decreased	14	4	19	9	0.3095	14	4	19	9	0.3095
AST elevation	15	0	9	1	0.3095	13	0	11	1	0.4583
ALT elevation	8	1	7	1	1.0000	6	1	9	1	1.0000
QTc interval prolonged	5	1	7	0	0.4167	4	0	8	1	1.0000
Interstitial lung disease	2	0	3	1	1.0000	3	0	2	1	0.4000
Paronychia/Rash	12	1	15	1	1.0000	10	1	17	1	1.0000
Diarrhea	9	0	11	1	1.0000	11	0	9	1	0.4500

AST, aspartate transaminase; ALT, alanine transaminase