**Pharmacokinetics of edoxaban in EGFR-mutated non-small cell lung cancer patients with venous thromboembolism**

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**Abstract**

**Background:** The risk of venous thromboembolism (VTE) is increased 7-fold in patients with cancer than in those without. Low-molecular-weight heparin is the standard treatment for cancer-associated VTE. Direct oral anticoagulants (DOACs) are not inferior to low-molecular-weight heparin with respect to the general outcome of recurrent VTE. Warfarin is associated with a risk of bleeding when used in combination with gefitinib or erlotinib which are epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs). It is unclear, however, whether combination treatments with EGFR-TKIs and DOACs pose the same risk. We aimed to identify anticancer drugs and anticoagulants that can be used safely in combination, as accompanying research to an observational study on VTE incidence rates in lung cancer patients (Rising-VTE/NEJ037 study).

**Methods:** Twelve patients receiving EFGR-TKI monotherapy and VTE treatment were enrolled. Blood samples were collected in time series after the first dose of edoxaban, and further samples were collected within 8–15 days after administering EGFR-TKIs. The pharmacokinetics (PK) of edoxaban were analyzed using a non-compartmental model.

**Results:** Edoxaban concentrations (30 mg once daily) were measured in eight patients. PK analyses showed no significant differences before and after co-administration of EGFR-TKIs (gefitinib, erlotinib, and afatinib).

**Conclusions:** Our findings indicate that the PK of edoxaban was not considerably affected by co-administration of EGFR-TKIs (gefitinib, erlotinib, and afatinib).

**Keywords:** edoxaban; venous thromboembolism; EGFR-TKIs; direct oral anticoagulant; pharmacokinetics

## 1. Introduction

Deep vein thromboses (DVT) can cause fatal pulmonary thromboembolism (PTE). Acute pulmonary arterial dysfunction caused by PTE is associated with 30% mortality [1] which decreases to 2%–8% with treatment [2], and early diagnosis, appropriate treatment, and prevention are considered to improve prognoses. As most PTEs are caused by DVT, and approximately 50% of proximal DVTs develop into PTE [3], the two afflictions are collectively referred to as venous thromboembolism (VTE) [4].

VTE is a common complication of cancer and cancer therapy. The prognosis for cancer patients with VTE is poor, with a one-year survival rate of 12% and a 2-fold increased mortality rate compared to cancer patients without VTE [5]. In hospitalized patients with cancer, VTE incidence is increased 2-fold compared to patients without cancer [6]. In a population-based case–control study, cancer without chemotherapy was associated with a 4-fold increased risk of thrombosis, whereas cancer with chemotherapy produced a 6-fold increase in thrombosis risk [7]. Prophylactic anticoagulant therapy is recommended for hospitalized cancer patients and patients receiving chemotherapy who have a high risk of developing VTE [5,8,9]. Direct oral anticoagulants (DOACs) can effectively counteract cancer-associated thrombosis [10-12]. VTE in cancer patients has been treated with various blood-thinning agents including rivaroxaban [10], apixaban [11], and edoxaban

*Abbreviations:* AUC0-∞, area under the concentration-time curve; AUMC, area under the first moment curve; CYP, cytochrome P450; DOACs, direct oral anticoagulants; DVT, deep vein thromboses; EGFR, epidermal growth factor receptor; Ke, elimination rate constant; LC, liquid chromatography; MS/MS, tandem mass spectrometry; MRT, mean residence time; NSCLC, non-small cell lung cancer; PK, pharmacokinetics; PTE, pulmonary thromboembolism; TKIs, tyrosine kinase inhibitors; t1/2, half-life; CL, clearance; Vdss, volume of distribution at steady state; VTE, venous thromboembolism.

[12]. Edoxaban is considered not inferior to low-molecular-weight heparin (dalteparin) in preventing recurrent VTE or major bleeding [12].

Currently available studies on drug-drug interactions associated with DOACs focus on medications (e.g., cytochrome P450 (CYP)3A4 inhibitors and P-glycoprotein competitors) that share a common metabolic pathway with DOACs [13-15]. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are key drugs for the treatment of EGFR mutation-positive non-small cell lung cancer (NSCLC). Some EGFR-TKIs can affect the metabolism of DOACs via CYP3A4 inhibition, resulting in reduced or enhanced effect of DOACs, and *in vitro* studies on human liver microsomes showed that gefitinib only exerts weak inhibitory effects on CYP3A4 activities [16]. Furthermore, erlotinib treatment induced CYP3A4 activity in patients with NSCLC [17]. We conducted an observational study on the pharmacokinetics (PK) of edoxaban with EGFR-TKI in NSCLC patients.

## 2. Materials and methods

### 2.1 Study population

Patients with untreated EGFR-mutated NSCLC and cancer-associated VTE were enrolled. All patients were aged at least 20 years, with a performance status (Eastern Cooperative Oncology Group) of 0–2, NSCLC at an advanced stage at which radical treatment was not possible, and an expected survival period of at least six months from the time consent was obtained. Planned treatments included gefitinib, erlotinib, or afatinib monotherapy with edoxaban as an anticoagulant therapy. Patients with a history of edoxaban allergy, reduced renal function (creatinine clearance below 30 mL/min), liver disease with abnormal blood coagulation, or interstitial lung disease were excluded. Patients were further excluded if they had already been treated for VTE, showed alanine transaminase concentrations of more than 2-fold the facility standard value or total bilirubin values

exceeding the facility standard value by more than 1.5-fold, or had active bleeding or uncontrolled hypertension as defined by systolic blood pressure exceeding 170 mmHg or diastolic blood pressure exceeding 100 mmHg despite administration of antihypertensive agents. Pregnant or breastfeeding women, patients taking aspirin at more than 100 mg/day, patients taking other antiplatelet drugs, patients taking drugs that affect kinetics of edoxaban via P-glycoprotein inhibition, and patients with acute bacterial endocarditis were also excluded.

2.2 Study design and treatments

The Rising-VTE/NEJ037 study is an observational study of the incidence of thromboembolism in patients with lung cancer and includes an efficacy and safety evaluation of edoxaban in patients with active cancer and VTE. Out of the patients enrolled in the Rising-VTE/NEJ037 study, those with indications for both EGFR-TKIs and edoxaban were enrolled in the present study (Figure 1). This study was approved by the institutional ethics committee (November 30, 2015; jRCTs No. 061180026) and was performed in accordance with the 2013 Declaration of Helsinki. All patients signed informed consent before enrollment.

This clinical trial was a multicenter, collaborative, open-label PK study conducted at 26 participating facilities in Japan. VTE screening was performed by contrast-enhanced computed tomography or lower limb ultrasound. Treatment with edoxaban was recommended if VTE was diagnosed during such screenings. Patients with VTE at the time of diagnosis were initially treated with unfractionated heparin by continuous intravenous infusion or subcutaneous injection and were subsequently treated with daily administration of oral edoxaban at 30 mg (at a body weight below 60 kg) or 60 mg (patients weighing at least 60 kg); an edoxaban dosage of 30 mg was also administered in patients who were concomitantly using quinidine sulphate hydrate, verapamil

hydrochloride, erythromycin, or cyclosporine, and in patients who showed creatinine clearance of 30 mL/min or less.

Blood samples were collected using heparin-treated vacuum tubes 0.5, 1, 2, 4, 8, and 24 h after the first edoxaban administration. Samples were gently mixed by inversion and were then placed on ice after which they were centrifuged at 1400 × *g* and 4 °C for 10 min. Plasma was collected and stored at -20 °C. The patients were subsequently treated with different EGFR-TKI anticancer therapies (gefitinib, erlotinib, or afatinib). Blood samples were then collected 0.5, 1, 2, 4, 8, and 24 h within 15 days from day 8 after starting EGFR-TKI therapy, when EGFR-TKIs were expected to have reached a steady state [18-20]. VTE screening was repeated 6 months after the start of oral edoxaban administration. All adverse events were recorded (Figure 2).

2.3 PK analyses

Plasma concentrations of edoxaban were determined according to the area under the concentration-time curve (AUC0-∞) and the mean residence time (MRT) as follows:

$$\mathrm{AUC} = \int_0^{\infty} \mathrm{C}\ \mathrm{dt}$$

$$\mathrm{MRT} = \int_0^{\infty} \mathrm{t} \cdot \mathrm{C}\ \mathrm{dt} / \int_0^{\infty} \mathrm{C}\ \mathrm{dt}$$

where C dt is the plasma concentration of edoxaban at time point t. The MRT and area under the first moment curve (AUMC) were defined as the first moment of the concentration-time curves of edoxaban. These moment parameters were calculated by trapezoidal integration using the Numeric Analysis Program for Pharmacokinetics (Napp ver. 2.01, Hisaka et al) [21]. Analysis of nonlinear and non-steady state hepatic extraction was performed by fitting a dispersion model using the finite difference method. The elimination rate constant (Ke) for edoxaban and first-order kinetics of the

concentration-time profile of edoxaban up to infinite time after the administration were calculated based on C∞.

2.4.1 Liquid chromatography-tandem mass spectrometry (LC-MS/MS) of edoxaban

The standard sample for producing a calibration curve using human plasma (1, 2, 5, 25, 50, 250, and 500 ng/mL) and edoxaban sample (2, 25, and 400 ng/mL; N = 2 per concentration tier) with the plasma sample (50 μL) were pretreated using a solid-phase extraction method. Plasma edoxaban concentrations were measured using LC-MS/MS. All blood concentration measurements of edoxaban were performed at Shin Nippon Biomedical Laboratories.

2.4.2 LC-MS/MS of EGFR-TKIs

Plasma concentrations of gefitinib and erlotinib were determined using LC-MS/MS [22,23]. A TSQ Quantum™ Access MAX Triple Quadrupole Mass device (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used with electrospray ionization in the positive ion mode at 450 °C and at 3000 V. EGFR-TKI concentrations were quantified using the ion peak area ratio (ratio of gefitinib or erlotinib and clozapine) of the sample and were calculated by selected reaction monitoring. The LC-MS/MS conditions for gefitinib, erlotinib, and clozapine were optimized using the conditions shown in Table 1. Plasma samples (200 μL) were spiked with 25 μL clozapine and 1.25 mL acetonitrile and were vortexed vigorously. Samples were then centrifuged at 1850 × *g* and 4 °C for 5 min, and 1.2 mL supernatant was evaporated under a gentle stream of nitrogen at 40 °C. Residues were treated with 250 μL 50% acetonitrile, were dissolved for 10 min, and were then centrifuged at 2050 × *g* for 5 min. Five microliters of the supernatant was injected for quantitative analysis by LC-MS/MS. Chromatographic analysis was performed using a high-performance liquid chromatographic system (Accela; Thermo Fisher Scientific). The mobile phase consisted of a

combination of phase A (4 mM ammonium formate, pH 3.2), and phase B (90% acetonitrile/10% phase A). Phase B was initiated as 5% of the mobile phase for 1 min, increased to 35% over 0.83 min, further increased linearly to 37% over 4.5 min, increased linearly up to 90% over 4 min, continued at 90% for 2.9 min, then decreased linearly to 0% over 0.1 min, and increased back to 5%. Separations were performed at a flow rate of 150 μL/min and 30 °C using an X bridge C18 HPLC column (3.5 μm, 2.1 mm × 100 mm; Waters, Milford, MA, USA).

2.5 Statistical analyses

The PK set for analysis comprised data from 12 enrolled subjects who had received edoxaban. The PK of edoxaban was statistically analyzed in eight cases. Two cases were excluded because their edoxaban dosage was 60 mg which was thus not comparable with the 30 mg treatments; in two other cases, the PK parameters could not be estimated from the blood concentration–time profile of the patients by moments analysis. The plasma trough concentration of EGFR-TKI was analyzed in 10 cases as two samples were defective. Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). Qualitative variables are reported as frequencies and percentages, and quantitative variables are shown as medians or means and standard deviation. Differences in PK parameters were analyzed using a Wilcoxson signed-rank test. Statistical significance is reported at $P \leq 0.05$.

**3. Results**

3.1 Patient characteristics

Demographic data of the 12 enrolled patients are shown in Table 2. Edoxaban was administered at 30 mg to 10 patients weighing less than 60 kg. Three types of EGFR-TKIs were used with four patients receiving gefitinib, seven patients receiving erlotinib, and one patient receiving afatinib. No

changes to EGFR-TKI treatments were made within the 6-month period. No patient took any drugs that would affect edoxaban kinetics through P-glycoprotein inhibition from study enrollment to blood sampling for PK.

### 3.2 PK

Table 3 shows the PK parameters of only edoxaban and of edoxaban combined with EGFR-TKIs. The AUC0-∞, MRT, AUMC, Ke, half-life (t1/2), clearance (CL), and volume of distribution at a steady state (Vdss) of edoxaban did not change before and after administration of EFGR-TKIs. Similar results were observed when EGFR-TKI was limited to erlotinib (Table 3). No significant difference in edoxaban plasma concentrations was observed at any time point (Figure 3A). Similar results were observed in each EGFR-TKI treatment (Figure 3B, 3C, 3D). After 24 h, plasma edoxaban concentrations were 0.0439 μg/mL when only edoxaban was administered, and they were 0.0259 μg/mL when edoxaban was co-administered with EGFR-TKIs. Simultaneous administration of edoxaban and EGFR-TKIs did not affect plasma edoxaban concentrations.
Table 4 shows EGFR-TKI plasma trough concentrations. Trough level measurement was not possible in patients who received afatinib and erlotinib 150 mg, but it was possible in the other 10 cases. Of these, edoxaban PK were assessed in seven cases.

### 3.3 Adverse events

Epistaxis occurred in three patients. None required special treatment, and each patient experienced this event only once. The prothrombin time-international standard ratio (INR), measured 6 months after introduction of edoxaban, did not indicate hyperprolongation (INR: 0.99–1.58). No serious adverse events (grade 3 or 4) were reported.

## 4. Discussion

### 4.1 Drug interactions

This is the first study investigating edoxaban PK in combination with EGFR-TKI in cancer patients. There was no increase in edoxaban plasma levels even after concomitant EGFR-TKI administration. Edoxaban plasma concentrations measured 24 h after administration of 30 mg edoxaban were 0.0259–0.0439 μg/mL, which is within the effective concentration range. Previous plasma concentration data indicate that at edoxaban dosages of 15–60 mg, the mean trough blood concentration is 0.016–0.0485 μg/mL [24]. The concentrations observed in the present study were thus within the same blood concentration range as previously reported.

Warfarin dosages differed significantly among patients; individual and varying degrees of tolerance were observed besides interactions with various drugs and foods. Prothrombin time-INR prolongation was previously reported when warfarin was used in combination with EGFR-TKIs [25-27]. Compared to warfarin, DOACs are less susceptible to dietary effects, and only few studies on the respective drug-drug interactions are available. However, CYP3A4 metabolism must be considered. Administration of dual inhibitors of P-glycoprotein and CYP3A4 increased edoxaban exposure. This effect was mainly due to P-glycoprotein inhibition, and the influence of CYP3A4 inhibition was not substantial [14]. CYP3A4 is involved in the edoxaban metabolic process; however, the involvement of CYP3A4 is below 10% [28]. Therefore, we aimed to confirm safety of edoxaban administration in combination with EGFR-TKIs.

No serious bleeding events were reported, and only one study patient was unable to complete the study owing to cancer progression and death. There were no incidents of DVT relapse, and the therapeutic objective was achieved safely.

4.2 Effects of EGFR-TKIs

In a previous study on 87 patients (median body surface area [BSA] 1.66 $m^2$), the average trough concentration after administration of 250 mg gefitinib was 173.9 ng/mL [29]. Patients receiving 250 mg gefitinib (N = 3) showed average trough levels of 323.4 ng/mL (range: 288.5–357.8 ng/mL) in our study in the combination therapy with edoxaban. A different study (N = 26; median BSA 1.79 $m^2$) reported average trough concentrations of 720 ng/mL after administration of 150 mg erlotinib [30]. For erlotinib 100 mg, a study on 10 patients (median BSA 1.69 $m^2$) reported average trough concentrations of 722 ng/mL [30]. Similarly, in the current study, when edoxaban was used as a combination treatment, average trough concentrations of erlotinib 150 mg (N = 2) and erlotinib 100 mg (N = 5) were 1927.1 ng/mL (range: 1721.1–2133.2 ng/mL) and 1425 ng/mL (range: 954.9–1965.1 ng/mL), respectively. The plasma trough concentrations of EGFR-TKI in the present study did not decrease under combination with edoxaban. Similarly, no decrease in EGFR-TKI concentrations was observed when comparing only cases in which edoxaban PK was analyzed. By contrast, in the current study, EGFR-TKIs concentrations were higher than reported previously, which may be explained by the low BSA rather than by potential drug interactions. A study on 26 patients (median BSA 1.57 $m^2$) using 150 mg erlotinib reported trough concentrations of 1380 ng/mL [31]. We examined a group of patients with lower BSA compared to those used in a previous study [30]. The median BSA of our patients was 1.43 $m^2$, which was also lower than that of the patients enrolled in a previous study [31]. Similarly, regarding the gefitinib 250 mg and erlotinib 100 mg treatments, the BSA was lower than that of patients enrolled in previous studies ([29] and [31], respectively), and consequently, the trough level of EGFR-TKIs was increased.

4.3 Study limitations

Our study was limited by the small sample size, with useable trough concentration data from only ten patients. Therefore, further studies with larger sample sizes are needed to confirm our results. However, although more than 1000 cases have been registered in the main Rising-VTE/NEJ037 study, increasing the number of patients used for the current study was difficult. In addition, the timing of PK blood sample collection for edoxaban alone was not uniform. For example, there were cases in which PK blood sampling was performed on the day edoxaban was introduced and cases in which PK blood sampling was performed 4 weeks later. However, limiting the days of edoxaban administration delays the initiation of EGFR-TKIs. This was not observed owing to the urgency of treating NSCLC patients.

## 5. Conclusions

Edoxaban PK parameters were not affected by concomitant EGFR-TKI treatment. EGFR-TKIs can improve the control of coagulation owing to interactions with warfarin. Edoxaban possibly facilitates stable VTE treatments without increasing the risk of bleeding, even when used in combination with EGFR-TKI.

**Conflict of interest**

The Rising-VTE PK study includes investigator-initiated clinical trials led by Shimane University and is funded by Daiichi Sankyo Co., Ltd. TH, KH, AT, MK, AN, JS, MH, NI, NI, MY, KF, TK, and NN have no conflicts of interest to declare. YT received personal fees from AstraZeneca, Daiichi Sankyo Co., Ltd., and Chugai Pharmaceutical Co., Ltd. outside this work. TI received personal fees from Boehringer-Ingelheim, AstraZeneca, and Pfizer outside this work.

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References

[1] Nakamura M, Fujioka H, Yamada N, M Sakuma, O Okada, N Nakanishi, et al. Clinical characteristics of acute pulmonary thromboembolism in Japan: results of a multicenter registry in the Japanese Society of Pulmonary Embolism Research. Clin Cardiol 2001;24:132-8.

[2] Goldhaber SZ, Morpurgo M. Diagnosis, treatment and prevention of pulmonary embolism: Report of the WHO/International Society and Federation of Cardiology Task Force. JAMA 1992;268:1727-33.

[3] Moser KM, Fedullo PF, LitteJohn JK, Crawford R. Frequent asymptomatic pulmonary embolism in patients with deep venous thrombosis. JAMA 1994;271:223-5.

[4] Sorensen HT, Mellemkjaer L, Olsen JH, Baron JA. Prognosis of cancers associated with venous thromboembolism. N Engl J Med 2000;343:1846-50.

[5] Lyman GH, Bohlke K, Khorana AA, Kuderer NM, Lee AY, Arcelus JL, et al. Venous Thromboembolism Prophylaxis and Treatment in Patients with Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update 2014. J Clin Oncol 2015;33:654-56.

[6] Stein PD, Beemath A, Meyers FA, Skaf E, Sanchez J, Olson RE. Incidence of venous thromboembolism in patients hospitalized with cancer. Am J Med. 2006;119:60-8.

[7] Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. Arch Intern Med 2000;160:809-15.

[8] Streiff MB, Bockenstedt PL, Cataland SR, Chesney C, Eby C, Fanikos J, et al. Venous thromboembolic disease. J Natl Compr Canc Netw 2013;11:1402-29.

[9] Mandalà M, Falanga A, Roila F. Management of venous thromboembolism (VTE) in cancer patients: ESMO Clinical Practice Guidelines. Ann Oncol 2011;22:85-92.

[10] Prins MH, Lensing AW, Brighton TA, Lyons RM, Rehm J, Trajanovic M, et al. Oral rivaroxaban versus enoxaparin with vitamin K antagonist for the treatment of symptomatic venous thromboembolism in patients with cancer (EINSTEIN-DVT and EINSTEIN-PE): a pooled subgroup analysis of two randomised controlled trials. Lancet Haematol 2014;1:37-46.

[11] Agnelli G, Buller HR, Cohen A, Gallus AS, Lee TC, Pak R, et al. Oral apixaban for the treatment of venous thromboembolism in cancer patients: results from the AMPLIFY trial. J Thromb Haemost 2015;13: 2187-91.

[12] Raskob GE, van Es N, Verhamme P, Carrier M, Di Niso M, Garcia D, et al. Edoxaban for the treatment of cancer-associated venous thromboembolism. N Engl J Med 2018;378:615-24.

[13] Delavenne X, Ollier E, Basset T, Bertoletti L, Accassat S, Garcin A, et al. A semi-mechanistic absorption model to evaluate drug-drug interaction with dabigatran: application with clarithromycin. Br J Clin Pharmacol 2013;76:107-13.

**[**14**]** Parasrampuria DA, Mendell J, Shi M, Matsushima N, Zahir H, Truitt K. Edoxaban drug-drug interactions with ketoconazole, erythromycin, and cyclosporine. Br J Clin Pharmacol 2016;82:1591-600.

[15] Chang SH, Chou IJ, Yeh YH, Chiou MJ, Wen MS, Kuo CT, et al. Association between use of non-vitamin K oral anticoagulants with and without concurrent medications and risk of major bleeding in nonvalvular atrial fibrillation. JAMA 2017;318:1250-59.

[16] Cohen MH, Williams GA, Sridhara R, Chen G, Pazdur R. FDA drug approval summary: gefitinib (ZD1839; Iressa) tablets. Oncologist 2003;8:303-6.

[17] Svedberg A, Vikingsson S, Vikström A, Hornstra N, Kentson M, Branden E, et al. Erlotinib treatment induces cytochrome P450 3A activity in non-small cell lung cancer patients. Br J Clin Pharmacol 2019;85:1704-9.

[18] Ranson M, Hammond LA, Ferry D, Kris M, Tullo A, Murray PI, et al. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. J Clin Oncol 2002;20:2240-50.

[19] Comis RL. The current situation: erlotinib (Tarceva) and gefitinib (Iressa) in non-small cell lung cancer. Oncologist 2005;10:467-70.

[20] Murakami H, Tamura T, Takahashi T, Nokihara H, Naito T, Nakamura Y, et al. Phase I study of continuous afatinib (BIBW 2992) in patients with advanced non-small cell lung cancer after prior chemotherapy/erlotinib/gefitinib (LUX-Lung 4). Cancer Chemother Pharmacol 2012;69:891-9.

**[**21**]** Hisaka A Sugiyama Y. Analysis of nonlinear and non-steady state hepatic extraction with the dispersion model using the finite difference method. J Pharmacokinet Biopharm 1998;26:495-519.

**[**22**]** Bouchet S, Chauzit E, Ducint D, Castaing N, Canal-Raffin M, Moore N, et al. Simultaneous determination of nine tyrosine kinase inhibitors by 96-well solid-phase extraction and ultra performance LC/MS-MS. Clin Chim Acta 2011;412:1060-67.

[23] Wang LZ, Lim MY, Chin TM, Thuya W-L, Nye P-L, Wong A, et al. Rapid determination of gefitinib and its main metabolite, O-desmethyl gefitinib in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:2155-61.

[24] Ruff CT, Giugliano RP, Braunwald E, Morrow DA, Murphy SA, Kuder JF, et al. Association between edoxaban dose, concentration, anti-factor Xa activity, and outcomes: an analysis of data from the randomised, double-blind ENGAGE AF-TIMI 48 trial. Lancet 2015;385:2288-95.

[25] Hiraide M, Minowa Y, Nakano Y, Suzuki K, Shiga T, Nishio M, et al. Drug interactions between tyrosine kinase inhibitors (gefitinib and erlotinib) and warfarin: Assessment of international normalized ratio elevation characteristics and in vitro CYP2C9 activity. J Oncol Pharm Pract 2019;25:1599-607.

[26] Arai S, Mitsufuji H, Nishii Y, Onoda S, Ryuge S, Mayuko W, et al. Effect of gefitinib on warfarin antithrombotic activity. Int J Clin Oncol 2009;14:332-6.

[27] Thomas KS, Billingsley A, Amarshi N, Nair AB. Elevated International Normalized Ratio associated with concomitant warfarin and erlotinib. Am J Health Syst Pharm 2010;67:1426-29.

[28] DAILYMED. Label: SAVAISA – edoxaban tosylate tablet, film coated. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=e77d3400-56ad-11e3-949a-0800200c9a66 [accessed June 11, 2020].

[29] Xin S, Zhao Y, Wang X, Huang Y, Zhang J, Guo Y, et al. The dissociation of gefitinib trough concentration and clinical outcome in NSCLC patients with EGFR sensitive mutations. Sci Rep 2015;5:12675.

[30] Gruber A, Czejka M, Buchner P, Kitzmueller M, Baroian NK, Dittrich C, Hrgovcic AS. Monitoring of erlotinib in pancreatic cancer patients during long- time administration and comparison to a physiologically based pharmacokinetic model. Cancer Chemother Pharmacol 2018;81:763-71.

[31] Liao D, Yao D, Liu N, Cao L, Xiang D, Yang N, et al. Correlation of plasma erlotinib trough concentration with skin rash in Chinese NSCLC patients harboring exon 19 deletion mutation. Cancer Chemother Pharmacol 2018;82:551-9.

**Figure captions**

**Figure. 1.** Flow diagram of the present study. VTE, venous thromboembolism; EGFR, epidermal growth factor receptor; PK, pharmacokinetics.

**Figure. 2.** Treatment dosages and corresponding blood samples. Blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h (six time points). Blood collection in EGFR-TKI combination treatments was performed within 8–15 days after starting EGFR-TKI treatments (six points). Trough measurements were performed immediately before oral administration of EGFR-TKIs. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

**Figure 3. A:** Time course of mean plasma edoxaban (30 mg once daily; N = 8) concentrations 0.5, 1, 2, 4, 8, and 24 h after the first edoxaban administration. After 24 h, edoxaban plasma concentrations were 0.0439 μg/mL when only edoxaban was administered, and 0.0259 μg/mL when edoxaban was co-administered with EGFR-TKIs. **B:** edoxaban + erlotinib. **C:** edoxaban + gefitinib. **D:** edoxaban + afatinib. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

**Tables**

**Table 1. Operating conditions of tandem mass spectrometry**

| | precursor ion (m/z) | product ion (m/z) | collision energy (eV) |
|---|---|---|---|
| gefitinib | 447.1 | 128 | 35 |
| erlotinib | 393.9 | 278 | 35 |
| clozapine | 327.1 | 270 | 35 |

**Table 2. Patient demographics**

| demographics | patients<br>Total ($N$ = 12) | PK-measurable cases<br>edoxaban 30 mg ($N$ = 8) |
|---|---|---|
| age, median (range), y | 74.5 (59-91) | 81 (63-91) |
| sex, n (%) | | |
| female | 9 (75) | 7 (87.5) |
| male | 3 (25) | 1 (12.5) |
| height, median (range), cm | 150 (135–183) | 143 (138–154) |
| weight, median (range), kg | 49.6 (40.2–85) | 49.6 (43.1–59) |
| body surface, median (range), $m^2$ | 1.42 (1.21–2.07) | 1.40 (1.27–1.54) |
| creatinine clearance (range), mL/min | 62.22 (30.83–116.62) | 57.22 (30.83–72.51) |
| edoxaban dose | | |
| 30 mg | 10 (83) | 8 (100) |
| 60 mg | 2 (17) | — |
| EGFR-TKIs, n (%) | | |
| gefitinib | 4 (33.3) | 2 (25) |
| erlotinib | 7 (58.4) | 5 (62.5) |
| afatinib | 1 (8.3) | 1 (12.5) |

EGFR-TKIs, epidermal growth factor receptor–tyrosine kinase inhibitors; PK, pharmacokinetics

**Table 3. PK parameters of edoxaban after oral administration (30 mg once daily).** Shown are the means ± standard deviation.

| | **all EGFR-TKIs** | | | **erlotinib** | | |
|---|---|---|---|---|---|---|
| PK parameter | edoxaban 30 mg (N = 8) | edoxaban + EGFR-TKIs (N = 8) | *P* | edoxaban 30 mg (N = 5) | edoxaban + Erlotinib (N = 5) | *P* |
| AUC0-∞ (lg/mL) | 2.17 ± 0.60 | 2.51 ± 0.79 | 0.38 | 2.52 ± 0.33 | 2.76 ± 0.94 | >0.99 |
| MRT (h) | 10.98 ± 3.99 | 9.97 ± 4.02 | 0.64 | 13.00 ± 3.75 | 11.04 ± 4.72 | 0.43 |
| AUMC (h) | 24.62 ± 11.61 | 27.70 ± 22.77 | 0.94 | 32.05 ± 6.36 | 34.02 ± 27.61 | 0.62 |
| Ke ($h^{-1}$) | 0.11 ± 0.04 | 0.10 ± 0.02 | 0.74 | 0.10 ± 0.05 | 0.09 ± 0.03 | 0.81 |
| T1/2 (h) | 7.39 ± 3.66 | 7.28 ± 2.23 | 0.74 | 8.29 ± 4.56 | 8.00 ± 2.55 | 0.81 |
| CL (L/h) | 15.19 ± 5.75 | 12.72 ± 2.83 | 0.25 | 12.08 ± 1.70 | 11.72 ± 3.25 | > 0.99 |
| Vdss (L/kg) | 157.51 ± 56.15 | 117.23 ± 16.57 | 0.25 | 160.86 ± 69.16 | 117.41 ± 12.51 | 0.62 |

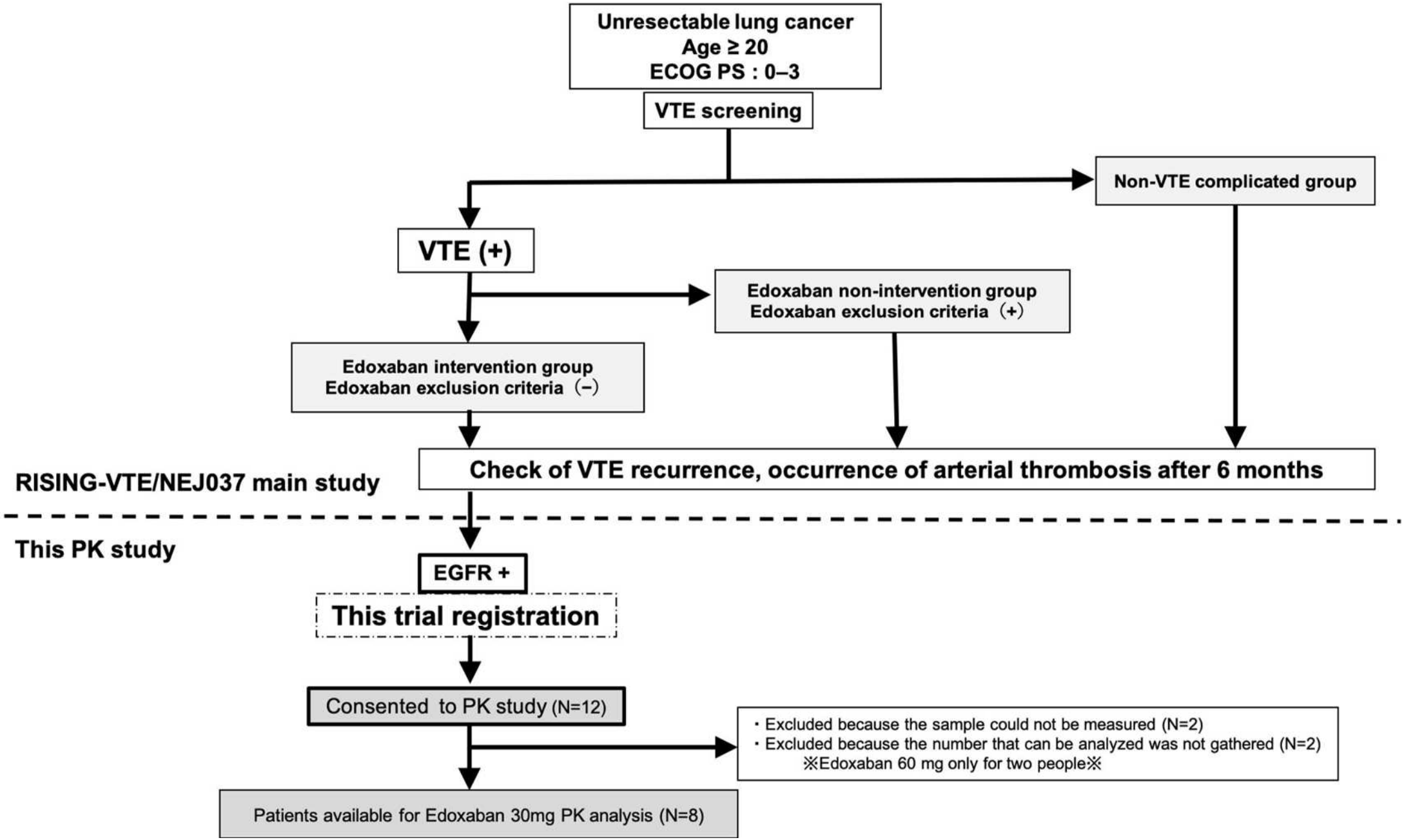
AUC0-∞, area under the curve for the time 0-∞ h; MRT, mean residence time; AUMC, area under the first moment curve; Ke, elimination rate constant; T1/2, half-life; CL, clearance; Vdss, volume of distribution at steady state; PK, pharmacokinetics

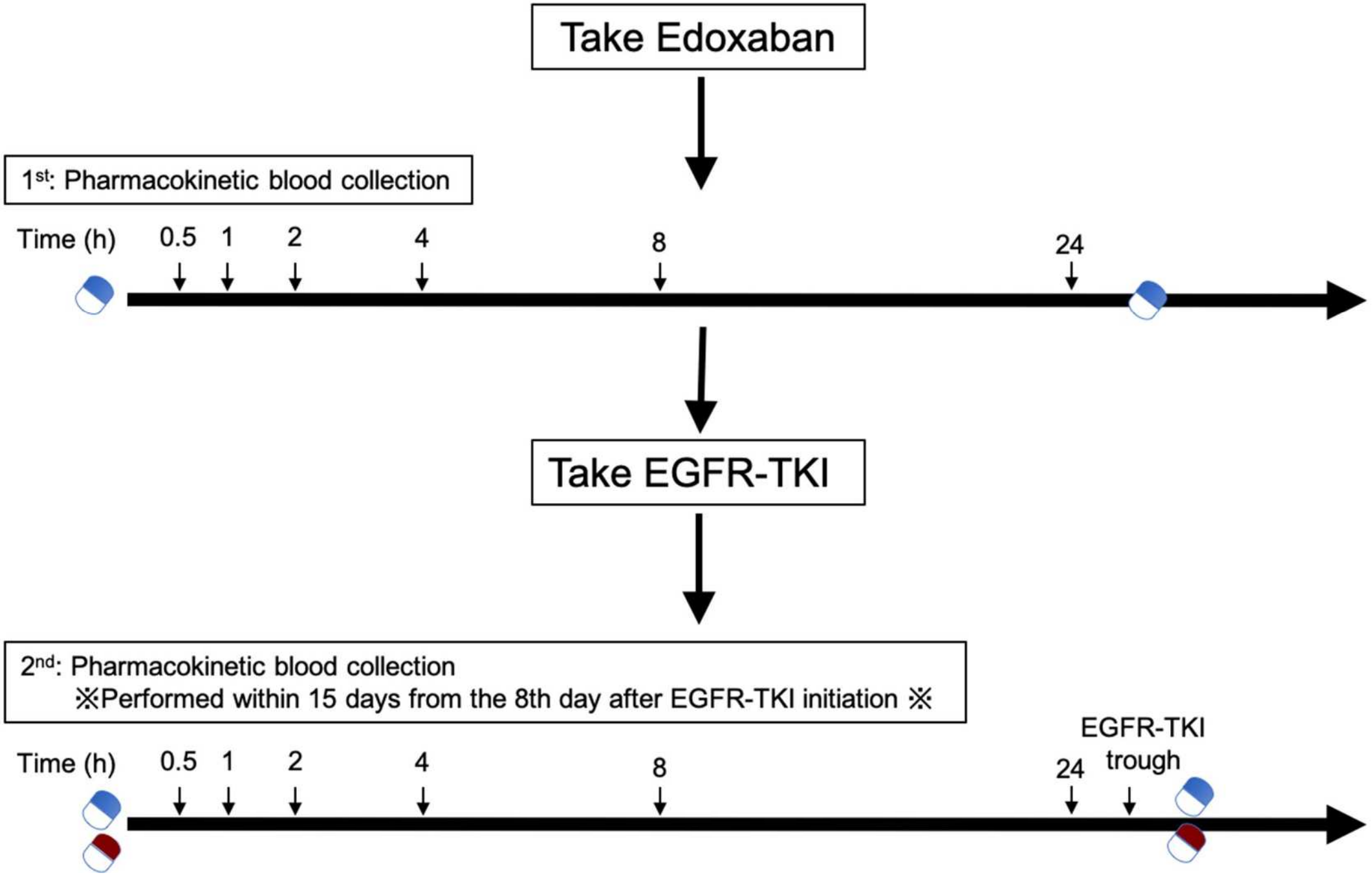
**Table 4. EGFR-TKI plasma trough concentration.**

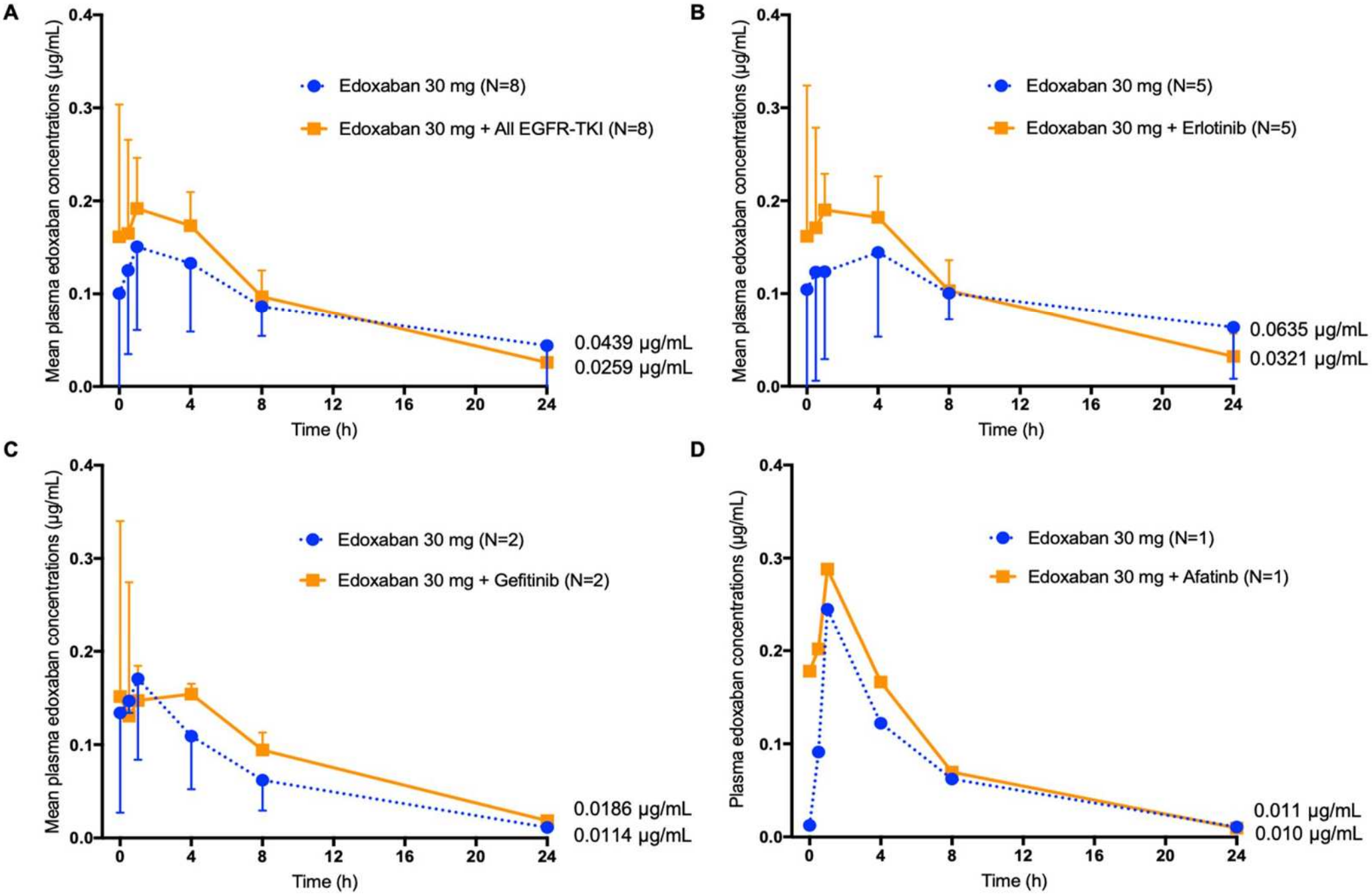
| EGFR-TKI trough measurable cases | Trough concentration (ng/mL) |
|---|---|
| gefitinib 250 mg (N = 3) | 323.4 (288.5–357.8) |
| erlotinib 150 mg (N = 2) | 1927.1 (1721.1–2133.2) |
| erlotinib 100 mg (N = 5) | 1425.0 (954.9–1965.1) |
| EGFR-TKI trough and PK measurable cases | |
| gefitinib 250 mg (N = 2) | 323.2 (288.5–357.8) |
| erlotinib 150 mg (N = 1) | 2133.2 |
| erlotinib 100 mg (N = 4) | 1290 (954.9–1720.9) |

Values are presented as means (range).

EGFR-TKIs, epidermal growth factor receptor–tyrosine kinase inhibitors; PK, pharmacokinetics


Unresectable lung cancer
Age ≥ 20
ECOG PS : 0–3
VTE screening
Non-VTE complicated group
VTE (+)
Edoxaban non-intervention group
Edoxaban exclusion criteria（+）
Edoxaban intervention group
Edoxaban exclusion criteria（−）
Check of VTE recurrence, occurrence of arterial thrombosis after 6 months
RISING-VTE/NEJ037 main study
This PK study
EGFR +
This trial registration
Consented to PK study (N=12)
・Excluded because the sample could not be measured (N=2)
・Excluded because the number that can be analyzed was not gathered (N=2)
※Edoxaban 60 mg only for two people※
Patients available for Edoxaban 30mg PK analysis (N=8)


Take Edoxaban
1st: Pharmacokinetic blood collection
Time (h)
0.5
1
2
4
8
24
Take EGFR-TKI
2nd: Pharmacokinetic blood collection
※Performed within 15 days from the 8th day after EGFR-TKI initiation ※
Time (h)
0.5
1
2
4
8
24
EGFR-TKI
trough


A
Mean plasma edoxaban concentrations (µg/mL)
0.4
0.3
0.2
0.1
0.0
Edoxaban 30 mg (N=8)
Edoxaban 30 mg + All EGFR-TKI (N=8)
0.0439 µg/mL
0.0259 µg/mL
0
4
8
12
16
20
24
Time (h)
B
Mean plasma edoxaban concentrations (µg/mL)
Edoxaban 30 mg (N=5)
Edoxaban 30 mg + Erlotinib (N=5)
0.0635 µg/mL
0.0321 µg/mL
Time (h)
C
Mean plasma edoxaban concentrations (µg/mL)
Edoxaban 30 mg (N=2)
Edoxaban 30 mg + Gefitinib (N=2)
0.0186 µg/mL
0.0114 µg/mL
Time (h)
D
Plasma edoxaban concentrations (µg/mL)
Edoxaban 30 mg (N=1)
Edoxaban 30 mg + Afatinb (N=1)
0.011 µg/mL
0.010 µg/mL
Time (h)