ORIGINAL ARTICLE

BJCP   BRITISH PHARMACOLOGICAL SOCIETY

Clopidogrel, a CYP2C8 inhibitor, causes a clinically relevant increase in the systemic exposure to the active metabolite of selexipag in healthy subjects

Lene Nygaard Axelsen[1] | Italo Poggesi[1] | Freya Rasschaert[2] |
Juan Jose Perez Ruixo[1] | Shirin Bruderer[1]

[1]Department of Clinical Pharmacology, Actelion Pharmaceuticals Ltd, Allschwil, Switzerland

[2]Clinical Pharmacology Unit, Janssen Pharmaceutica NV, Merksem, Belgium

Correspondence
Lene Nygaard Axelsen, Department of Clinical Pharmacology, Actelion Pharmaceuticals Ltd, Allschwil, Switzerland.
Email: laxelsen@its.jnj.com

Funding information
Actelion Pharmaceuticals

Aims: Selexipag is a prostacyclin receptor agonist approved for the treatment of pulmonary arterial hypertension. Cytochrome P450 (CYP) 2C8 is involved in the metabolism of selexipag and its active metabolite, ACT-333679. This study evaluated the interaction of selexipag and clopidogrel, a CYP2C8 inhibitor.

Methods: The study had a 2-treatment, 1-sequence, crossover design. Pharmacokinetics (PK) and CYP2C8 genotype were assessed in healthy male subjects administered selexipag (200 μg twice daily [b.i.d.]) alone or with clopidogrel (300 mg single dose or 75 mg once daily [o.d.]). PK modelling and simulation were conducted to support dosing recommendations.

Results: Clopidogrel had a comparatively small effect on selexipag (<1.5-fold difference in any PK variable). For ACT-333679, the major contributor to the drug effect, the area under the plasma concentration–time curve during a dose interval and the maximum plasma concentration increased 2.25-fold (90% confidence interval [CI] 2.06, 2.46) and 1.69-fold (90% CI 1.55, 1.84), respectively with clopidogrel 300 mg and 2.70-fold (90% CI 2.45, 2.96) and 1.90-fold (90% CI 1.72, 2.11), respectively with clopidogrel 75 mg. The effect of clopidogrel on selexipag and ACT-333679 exposure was comparable for all identified CYP2C8 genotypes. PK simulations predicted comparable exposure to ACT-333679 following selexipag 400 μg b.i.d., 400 μg o.d. in combination with clopidogrel 75 mg o.d and 200 μg b.i.d. with clopidogrel 75 mg o.d.

Conclusion: Results suggest that ACT-333679 exposure can be maintained within the therapeutic range by reducing selexipag dosing frequency to o.d. or dose to half, when selexipag is coadministered with clopidogrel.

KEYWORDS

clopidogrel, CYP2C8, drug interactions, pharmacokinetics, selexipag

1 | INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive disease of the lungs, in which vasoconstriction and enhanced smooth muscle proliferation lead to increased pulmonary arterial pressure, ultimately leading to right heart failure and death.[1] Prostacyclin, a potent vasodilator and inhibitor of smooth muscle cell proliferation, is markedly decreased in the lungs of patients with PAH.[2,3] The prostanoids epoprostenol, treprostinil, iloprost and beraprost, have been approved mainly based on short-term benefits (efficacy).[4] However, their administration is associated with substantial burden and/or potential serious adverse events due to their short half-life, chemical instability and the disadvantages associated with their delivery systems.[5]

Selexipag is the first orally active, selective, nonprostanoid, prostacyclin receptor agonist approved for the long-term treatment of PAH.[6] In a randomized, placebo-controlled study in 1156 patients with PAH, selexipag significantly reduced the risk of the composite endpoint of morbidity/mortality.[7] The most commonly reported adverse events in this study were those typically observed with prostacyclin therapies, including headache, diarrhoea, nausea and jaw pain.[7]

Selexipag is hydrolysed by carboxylesterases to its active metabolite, ACT-333679.[8,9] The structures of both selexipag and ACT-333679 have previously been published.[8] In vitro, ACT-333679 is 37-fold more potent than selexipag in activating the prostacyclin receptor[10] and exposure at steady state to ACT-333679 is approximately 4-fold higher than the parent drug following oral administration in healthy subjects.[11] ACT-333679 is therefore considered to be the major contributor to the pharmacological effect. Both selexipag and ACT-333679 are mainly excreated via hepatic clearance and metabolized by the cytochrome P450 (CYP) enzyme, CYP2C8, and to a lesser extent by CYP3A4.[8,10,12] In addition, ACT-333679 is also metabolised by the uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes, UGT1A3 and UGT2B7.[8] In vitro, organic anion-transporting polypeptide (OATP) 1B1 and OATP1B3 are involved in the disposition of selexipag and ACT-333679.[8,10] The breast cancer resistance protein is also involved in the disposition of ACT-333679, whereas only selexipag is a substrate of P-glycoprotein.[10] In a previous drug–drug interaction (DDI) study with gemfibrozil, a strong CYP2C8 and moderate OATP1B1 inhibitor, an approximately 11-fold increase in ACT-333679 exposure was observed.[13] Concomitant treatment with selexipag and strong CYP2C8 inhibitors are therefore contraindicated. Based on these data, other CYP2C8 inhibitors such as clopidogrel[14] are also expected to cause a clinically relevant increase the exposure to ACT-333679 and clinical data are needed to guide appropriate dosing of selexipag when coadministered with clopidogrel. In contrast, it has previously been shown that there is no clinically relevant pharmacokinetic interaction between selexipag and warfarin, a CYP2C9 substrate, nor does selexipag impact the exposure to the CYP3A4 substrate midazolam.[15,16]

The present study investigates the effect of the CYP2C8 inhibitor, clopidogrel,[14] on the pharmacokinetics (PK) of selexipag and

What is already known about this subject

- Selexipag and its active metabolite, ACT-333679, are selective, nonprostanoid, prostacyclin receptor agonists. ACT-333679 is 37-fold more potent than selexipag and considered to be the major contributor to the pharmacological effect.
- Based on in vitro data, both selexipag and ACT-333679 are substrates of the cytochrome P450 enzyme, CYP2C8.
- Concomitant administration of selexipag and gemfibrozil, a strong CYP2C8 inhibitor, resulted in a marked increase in ACT-333679 exposure. Concomitant treatment with selexipag and strong CYP2C8 inhibitors are therefore contraindicated.

What this study adds

- Concomitant administration of selexipag and clopidogrel resulted in a clinically relevant increase in ACT-333679 exposure.
- There were no unexpected safety findings following administration of selexipag in combination with clopidogrel.
- Reducing the dosing frequency of selexipag to once daily or reducing the dose to half when administered concomitantly with clopidogrel is expected to maintain ACT-333679 exposure within the therapeutic range and should therefore be considered.

ACT-333679 in healthy male subjects. In addition, the effect of the CYP2C8 genotype on the interaction between selexipag and clopidogrel is explored. Finally, the generated data are used to provide a model-based recommendation for an appropriate selexipag dosing with concomitant administration of moderate CYP2C8 inhibitors.

2 | METHODS

2.1 | Subjects

Subjects were eligible to participate in the study if they were male, aged ≥18 to ≤45 years with a body mass index of ≥18 to ≤28 kg m^{-2}. All subjects were healthy based on a physical examination performed at the screening visit. They had normal systolic and diastolic blood pressure and a normal 12-lead electrocardiogram. None of the subjects received any concomitant treatments at enrolment. Exclusion criteria included, but were not limited to, known allergic reactions to the study treatment; previous exposure to the study treatment 3 months prior to screening; and a history of any disease or condition

that could interfere with the absorption, distribution, metabolism or excretion of the study treatment. Subjects with an increased risk of bleeding or a history of bleeding disorders were also excluded. Prior to any study-related procedures, all subjects signed the informed consent form after receiving a full explanation of the study. During the study, safety was assessed as described in the supplementary information.

2.2 | Study design

The study was an open-label, 2-treatment, 1-sequence, crossover study, conducted at Janssen Clinical Pharmacology Unit, Merksem, Belgium from 5 March 2018 to 19 May 2018. An independent ethics committee (Ethics Committee, Universitair Ziekenhuis Antwerpen) and the national health authority of Belgium approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki principles, International Council for Harmonisation and Good Clinical Practice guidelines and applicable regulations and laws.

Subjects received treatment with selexipag alone (treatment A) followed by coadministration of selexipag and clopidogrel (treatment B), as shown in Figure 1. During treatment A, subjects received selexipag 200 μg twice daily (b.i.d.; selexipag, 1 × 200 μg film-coated tablet, Actelion Pharmaceuticals Ltd.), administered in the morning and evening of Days 1 to 3. During treatment B1, subjects received a single loading dose of clopidogrel 300 mg (PLAVIX, 4 × 75 mg film-coated tablets, Sanofi S.A.) and selexipag 200 μg in the morning of Day 4 and selexipag 200 μg in the evening of Day 4. During treatment B2, subjects received a maintenance dose of clopidogrel 75 mg (PLAVIX, 1 × 75 mg film-coated tablet, Sanofi S.A.) and selexipag 200 μg in the morning of Days 5–10 and selexipag 200 μg in the evening of Days 5–9. Study treatment was administered with water between 07:00 and 09:30 after a light breakfast in the morning and between 19:00 and 21:30 after a light meal in the evening. Clopidogrel 300 mg or 75 mg was always administered 1 hour before the morning selexipag administration. All drug intakes were confirmed by a mouth check.

A sample size of 22 healthy subjects was chosen based on empirical considerations. However, a precision estimate was done based on intrasubject coefficient of variations for the area under the plasma concentration–time curve during a dose interval (AUC_τ) and the maximum plasma concentration (C_{max}) from a previous study[17] and assuming a sample size of 16 and a geometric mean ratio of 1.00 (i.e. the assumption that no drug–drug interaction would occur). It was estimated that the 90% confidence interval (CI) of the geometric mean ratio (treatment B/treatment A) would be 0.86–1.16 and 0.83–1.21 for AUC_τ and C_{max}, respectively, of selexipag and 0.89–1.12 and 0.87–1.15 for the AUC_τ and C_{max}, respectively, of ACT-333679.

2.3 | Blood sampling for PK analysis

Serial blood samples were collected for selexipag and ACT-333679 PK analysis for 12 hours (predose, 30 min, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours) after selexipag administration on Day 3 (treatment A), Day 4 (treatment B1) and Day 10 (treatment B2). Blood samples were collected for selexipag and ACT-333679 trough plasma concentration (C_{trough}) analysis on each day of the study, prior to selexipag morning and evening doses. Plasma exposure to light was minimized and sample preparation was conducted under yellow light in order to prevent degradation of selexipag and ACT-333679.

2.4 | Analysis of selexipag and ACT-333679 concentrations in plasma

The concentrations of selexipag and ACT-333679 were measured in plasma using a validated liquid chromatography method with tandem mass spectrometry (LC–MS/MS). To 50 μL of plasma, 50 μL of an internal standard solution (stable isotope-labelled selexipag and ACT-333679) and 150 μL acetonitrile were added. After vortex mixing and centrifugation, 75 μL of the supernatant was transferred into a new tube containing 75 μL of water and vortex mixed. 20 μL of the supernatant was injected onto the LC–MS/MS. The chromatographic system consisted of a pump, autosampler, degasser and thermostat (50°C) analytical column (Acquity UPLC BEH C18, 50 × 2.1 mm ID, 1.7 μm; Waters, Zellik, Belgium). The mobile phase consisted of a gradient of water containing 0.01% ammonium carbonate (volume/volume) and acetonitrile (from 25 to 43% in 2.3 minutes) at a
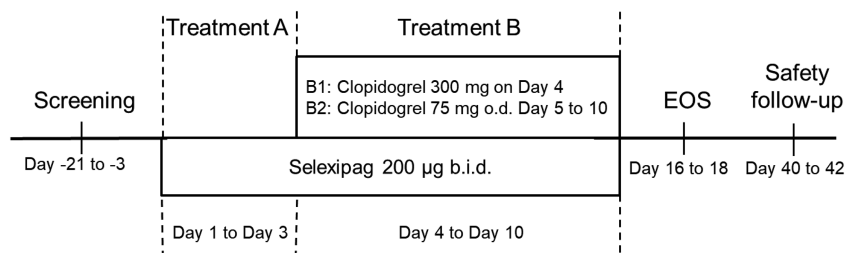

FIGURE 1 Study design. During treatment A, subjects received selexipag 200 μg b.i.d. administered in the morning and evening of Days 1–3. During treatment B1, subjects received a single loading dose of clopidogrel 300 mg and selexipag 200 μg in the morning of Day 4 and selexipag 200 μg in the evening of Day 4. During treatment B2, subjects received a maintenance dose of clopidogrel 75 mg and selexipag 200 μg in the morning of Days 5–10 and selexipag 200 μg in the evening of Days 5–9. b.i.d. = twice daily; EOS = end of study; o.d. = once daily

flow rate of 0.6 mL min^{-1}. Mass spectrometric detection (API 5500, AB Sciex, Nieuwerkerk a/d Ijssel, the Netherlands) was performed with a turbo ion spray operating in positive-ion mode at 650°C. For both analytes, the lower limit of quantification was 0.01 ng mL^{-1} and the method was linear from 0.01 to 20.0 ng mL^{-1}. The concentrations of the analytes were calculated by the internal standardization method, i.e. using the peak area ratio of analyte to internal standard. The performance of the method was monitored using quality control samples of known concentrations. Precision (% coefficient of variation) of the assay was ≤4.6% for selexipag and ≤6.7% for ACT-333679. The interassay bias ranged from 1.3 to 4.0% for selexipag and from 1.0 to 3.7% for ACT-333679.

2.5 | CYP2C8 genotyping

Genotyping of CYP2C8 alleles *1, *2, *3, *4, *5, *6, *7 and *8 was performed by Eurofins Medigenomix GmbH, Ebersberg, Germany, by sequencing the relevant regions of the CYP2C8 gene. The single nucleotide variants and their respective rs number are provided in the supplementary information (Table S1). Briefly, 10 mL of blood was collected from each subject using BD vacutainer K$_2$EDTA tubes and genomic DNA was prepared according to standard protocols. Using the extracted genomic DNA as a template, locus specific DNA fragments were amplified by polymerase chain reaction and the purified products used as templates for sequencing reactions. DNA sequence analysis was performed as previously described.[18] The resulting dye-labelled sequence products were detected on an automated sequencing platform. The sequencing data were generated using ABI Software and analysed using SEQPATIENT (JSI Medical Systems).[19]

2.6 | PK analysis

Noncompartmental analysis of selexipag and ACT-333679 plasma concentration–time profiles was performed using WinNonlin version 6.4 (Certara, Princeton, NJ, USA). The individual plasma concentrations of selexipag and ACT-333679 were used to directly obtain C$_{max}$ and the time to reach C$_{max}$ (t$_{max}$) of selexipag and ACT-333679. The AUC$_\tau$ was calculated according to the linear trapezoidal rule using the measured concentration–time values above the lower limit of quantification during 1 dosing interval. The C$_{trough}$ values of selexipag and ACT-333679 were used to determine the attainment of steady state conditions.

2.7 | Statistical analysis

The geometric means and the corresponding 95% CI were calculated for the AUC$_\tau$ and C$_{max}$ noncompartmental analysis of selexipag and ACT-333679 and the median and range were determined for t$_{max}$. The effect of clopidogrel on the AUC$_\tau$ and C$_{max}$ of selexipag and ACT-333679 was determined by using the ratio of the geometric means and the 90% CI of the test treatment (treatment B1 or B2) vs

the reference treatment (treatment A). The log-transformed values were analysed by mixed-effect models with treatment as a fixed effect and subject as a random effect. The difference in t$_{max}$ between treatments was determined by calculating the median differences and corresponding 90% CIs.

The effect of the CYP2C8 genotype on the extent of the interaction between clopidogrel and selexipag was determined by comparing the ratio of geometric means of the test treatments (treatment B1 or B2) vs the reference treatment (treatment A) for each identified genotype with the same mixed-effect approach as described above using genotype as a fixed effect.

2.8 | Modelling and simulation analysis

Open 2-compartment disposition models, with first-order absorption, lag time and first-order eliminations, were developed to describe the plasma concentrations of ACT-333679 following administration of selexipag 200 μg b.i.d. alone (treatment A) and in combination with clopidogrel 75 mg once daily (o.d.; treatment B2) using NONMEM (version 7.3) first-order conditional estimation with interaction algorithm (additional details are provided in the supplementary information).

The modelling data of treatment A and treatment B2 in 22 subjects were used to simulate the steady state plasma concentration–time profiles of ACT-333679 for the following dosing regimens as an example: selexipag 400 μg b.i.d. alone (regimen A), selexipag 400 μg o.d. with clopidogrel 75 mg o.d. (regimen B) and selexipag 200 μg b.i.d. with clopidogrel 75 mg o.d. (regimen C). From the simulated profiles, the maximum concentration at steady state (C$_{max,ss}$) was estimated as the maximal simulated concentration within the 24 hour period on Day 7 administration. The area under the plasma concentration–time curve from 0 to 24 hours after dosing at steady state (AUC$_{0-24h,ss}$) was estimated using the linear trapezoidal rule on the Day 7 data.

The effect of the 3 simulated dosing regimens on the C$_{max,ss}$ and AUC$_{0-24h,ss}$ of ACT-333679 was determined by calculating the ratio of the geometric means and the 5th AND 95th percentiles of the simulated values for regimen A vs regimens B and C.

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.[20]

3 | RESULTS

3.1 | Subjects

Twenty-two male subjects were enrolled, all of whom received at least 1 dose of study treatment. The mean (standard deviation) age

was 28.7 (6.8) years and body mass index was 24.5 (1.9) kg m^{-2}. Twenty-one subjects were Caucasian and 1 was Asian. Two subjects, who developed haematoma (see safety section), discontinued the study treatment on Day 2 and Day 4 as a precautionary measure due to the increased risk of bleeding associated with clopidogrel. One of these subjects was excluded from the PK analysis for missing all planned 12-hour PK assessments, leaving 21 subjects for PK analysis in treatments A and B1 and 20 subjects in treatment B2. Twenty subjects received all study treatments and all 22 subjects attended the end of study visit. No unexpected safety findings were observed during the study (details for reported adverse events are presented in the supplementary information).

3.2 | Effect of clopidogrel on selexipag and ACT-333679 PK

Concomitant administration with clopidogrel increased selexipag exposure slightly (Figure 2). A 1.44-fold (90% CI 1.32, 1.56) and 1.14-fold (90% CI 1.04, 1.26) increase in AUC$_\tau$ was observed in the presence of a single loading dose of clopidogrel 300 mg and 75 mg o.d., respectively. C$_{max}$ increased by 1.35-fold (90% CI 1.22, 1.50) in the presence of clopidogrel 300 mg; clopidogrel 75 mg o.d. had no effect on C$_{max}$. The t$_{max}$ was also unchanged in the presence of clopidogrel 300 mg single dose and 75 mg o.d. (Table 1).

Concomitant administration with clopidogrel resulted in a more pronounced increase in ACT-333679 exposure, when compared with selexipag (Figure 2). A 2.25-fold (90% CI 2.06, 2.46) and 2.70-fold (90% CI 2.45, 2.96) increase in AUC$_\tau$ was observed in the presence of clopidogrel 300 mg single dose and 75 mg o.d.,

respectively. C$_{max}$ increased by 1.69-fold (90% CI 1.55, 1.84) and 1.90-fold (90% CI 1.72, 2.11) in the presence of clopidogrel 300 mg single dose and 75 mg o.d., respectively. Coadministration of a single loading dose of clopidogrel 300 mg delayed attainment of C$_{max}$ by 0.51 hours, whereas clopidogrel 75 mg o.d. had no effect on t$_{max}$ (Table 1).

Graphical assessment of the C$_{trough}$ values showed that selexipag and ACT-333679 concentrations were at steady state on Day 3 (treatment A) and Day 10 (treatment B; Figure S5). There was no relevant difference between the morning C$_{trough}$ of selexipag at steady state in the presence (mean 0.07 ng mL^{-1} [95% CI 0.01, 0.13]) or absence (mean 0.02 ng mL^{-1} [95% CI 0.02, 0.03]) of clopidogrel (treatment B compared to A). The morning C$_{trough}$ of ACT-333679 at steady state was 3-fold higher in the presence of clopidogrel (mean 2.02 ng mL^{-1} [95% CI 1.64, 2.40]) than in the absence (mean 0.66 ng mL^{-1} [95% CI 0.50, 0.82]) of clopidogrel.

3.3 | Effect of CYP2C8 genotype on the interaction between selexipag and clopidogrel

Of the 21 subjects included in the PK evaluation of the study, 13 subjects were identified with the *1/*1 with-type, 6 subjects with the *1/*3 and 2 with *1/*4 genotype (Table 2). Both the selexipag exposure in the absence of clopidogrel and the increase in selexipag exposure, in the presence of clopidogrel (300 mg single dose or 75 mg o.d.), were comparable in all identified genotypes (Table 2, Figure 3). For ACT-333679, the exposure in the absence of clopidogrel was slightly higher in the wild-type subjects as compared to *1/*3 carriers and the variability in AUC$_{\tau,ss}$ also seemed slightly higher in the wild-type

FIGURE 2 Arithmetic mean (+ standard deviation) plasma concentration–time profile of selexipag and ACT-333679 after administration with selexipag 200 μg b.i.d. (treatment A, n = 21), a single loading dose of clopidogrel 300 mg and selexipag 200 μg b.i.d. (treatment B1, n = 21) and clopidogrel 75 mg o.d. and selexipag 200 μg b.i.d. (treatment B2, n = 20). Data are presented on both linear (upper graphs) and semilogarithmic (lower graphs) scales. b.i.d. = twice daily; o.d. = once daily; SD = standard deviation
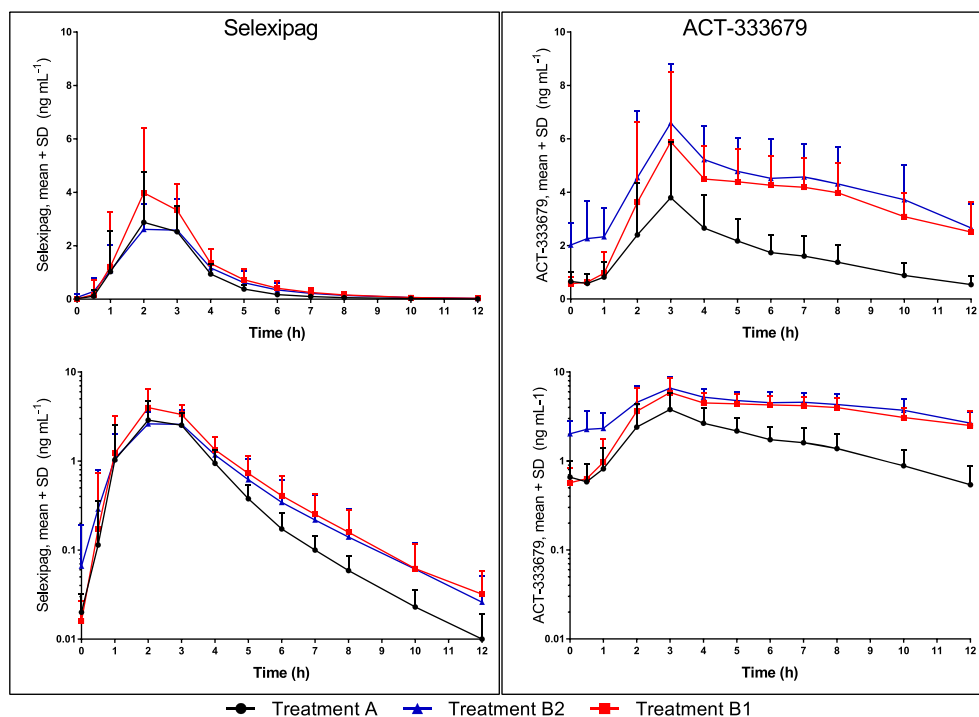
TABLE 1 Summary of the pharmacokinetic variables of selexipag and its active metabolite, ACT-333679, after administration of selexipag 200 μg b.i.d. alone (treatment A) and with a single loading dose of clopidogrel 300 mg (treatment B1) or clopidogrel 75 mg o.d. (treatment B2), and the geometric mean ratios for the comparison of treatments

	Arithmetic mean (SD) Geometric mean (95% CI)[a]			Geometric mean ratio (90% CI)[b] [% CV]	
	Treatment A[c] (n = 21)	Treatment B1[d] (n = 21)	Treatment B2[e] (n = 20)	Treatment B1/A (n = 21)	Treatment B2/A (n = 20)
Selexipag					
C_{max} [ng mL^{-1}]	3.35 (1.60) 3.08 (2.56, 3.70)	4.50 (2.04) 4.16 (3.48, 4.97)	3.18 (1.00) 3.03 (2.60, 3.53)	1.35 (1.22, 1.50) [35.63]	0.98 (0.89, 1.08) [32.82]
AUC_τ [h*ng mL^{-1}]	7.98 (3.37) 7.47 (6.35, 8.79)	11.45 (4.49) 10.73 (9.11, 12.65)	8.87 (2.60) 8.48 (7.33, 9.82)	1.44 (1.32, 1.56) [32.96]	1.14 (1.04, 1.26) [29.03]
t_{max} [h]	2.00 (1.0, 3.0)	2.01 (2.0, 3.0)	2.01 (2.0, 3.0)	0.00 (0.0, 0.0)	0.00 (−0.5, 0.0)
ACT-333679					
C_{max} [ng mL^{-1}]	3.88 (2.03) 3.51 (2.87, 4.28)	6.28 (2.42) 5.92 (5.06, 6.92)	6.91 (2.16) 6.62 (5.76, 7.61)	1.69 (1.55, 1.84) [37.03]	1.90 (1.72, 2.11) [33.83]
AUC_τ [h*ng mL^{-1}]	19.81 (9.45) 18.18 (15.11, 21.88)	42.64 (13.59) 40.87 (35.80, 46.67)	50.19 (13.60) 48.48 (42.69, 55.06)	2.25 (2.06, 2.46) [31.96]	2.70 (2.45, 2.96) [30.91]
t_{max} [h]	3.00 (2.0, 5.0)	3.01 (2.0, 8.0)	3.00 (2.0, 7.0)	0.51 (0.0, 1.0)	0.00 (−0.5, 1.0)

[a]For t_{max}, median and range is shown.
[b]For t_{max}, median difference and 90% CI is shown.
[c]Treatment A: selexipag 200 μg b.i.d.
[d]Treatment B1: selexipag 200 μg b.i.d. and clopidogrel 300 mg single dose.
[e]Treatment B2: selexipag 200 μg b.i.d. and clopidogrel 75 mg o.d.
AUCτ = area under the plasma concentration–time curve during a dose interval; b.i.d. = twice daily; CI = confidence interval; C_{max} = maximum plasma concentration; CV = intersubject coefficient of variation; o.d. = once daily; SD = standard deviation; t_{max} = time to reach maximum plasma concentration.

TABLE 2 Effect of CYP2C8 genotype on the geometric mean ratio of AUC$_\tau$ for selexipag and its active metabolite, ACT-333679

	AUC$_\tau$ geometric mean ratio (90% CI)		
	CYP2C8 genotype *1/*1	CYP2C8 genotype *1/*3	CYP2C8 genotype *1/*4
Treatment B1/A [a]	(n = 13)	(n = 6)	(n = 2)
Selexipag	1.41 (1.29, 1.54)	1.68 (1.47, 1.92)	1.02 (0.81, 1.29)
ACT-333679	2.06 (1.89, 2.25)	2.88 (2.54, 3.27)	1.86 (1.49, 2.32)
Treatment B2/A [b]	(n = 12)	(n = 6)	(n = 2)
Selexipag	1.08 (0.95, 1.23)	1.27 (1.06, 1.53)	1.19 (0.86, 1.63)
ACT-333679	2.42 (2.19, 2.68)	3.36 (2.91, 3.89)	2.70 (2.11, 3.47)

[a]Treatment B1/A: clopidogrel 300 mg single dose and selexipag 200 μg b.i.d./selexipag 200 μg b.i.d.
[b]Treatment B2/A: clopidogrel 75 mg o.d. and selexipag 200 μg b.i.d./selexipag 200 μg b.i.d.
AUCτ = area under the plasma concentration–time curve during a dose interval; b.i.d. = twice daily; CI = confidence interval; CYP = cytochrome P450 enzyme; o.d. = once daily.

subjects. The increase in ACT-333679 exposure, in the presence of clopidogrel, was more pronounced compared with selexipag in all identified genotypes but the observed increase in exposure appeared higher in subjects with the *1/*3 genotype (3.4-fold) compared to carriers of the wild-type (2.4-fold) and at the same time the intersubject variability in the DDI effect seems slightly higher in the *1/*3 genotype subjects as compared to the other genotypes. The mean ACT-333679 exposure in the presence of 75 mg clopidogrel is, however, comparable between all identified CYP2C8 genotypes (Table 2, Figure 3).

3.4 | Selexipag dosing regimen in the presence of clopidogrel

Model-based simulation found that the geometric means of AUC$_{0-24h,ss}$ and C$_{max,ss}$ for ACT-333679 were comparable for selexipag administered as b.i.d. alone, at the same dose o.d. with clopidogrel 75 mg o.d. or at half a dose b.i.d. with clopidogrel 75 mg o.d. (Table 3). The estimated concentration–time profiles of ACT-333679 for the 3 dosing regimens spanned a comparable concentration range during the day (Figure 4).
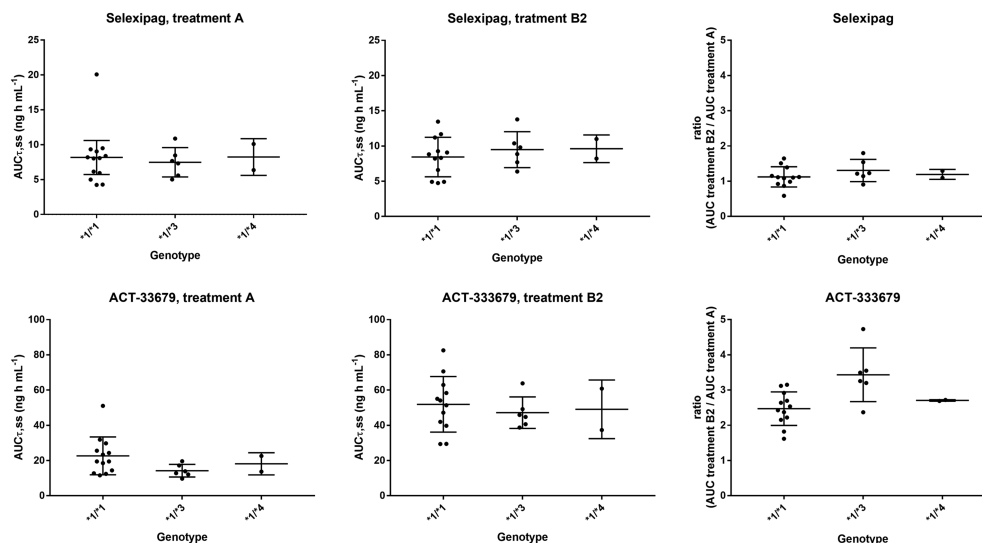
FIGURE 3 Individual and mean ± standard deviation of area under the plasma concentration–time curve during a dose interval at steady state (AUC_τ,ss) of selexipag and ACT-333679 after administration with selexipag 200 µg b.i.d. (treatment A, $n = 21$), and clopidogrel 75 mg o.d. and selexipag 200 µg b.i.d. (treatment B2, $n = 20$) presented by CYP2C8 genotype (*1/*1 $n = 13$, *1/*3 $n = 6$, *1/*4 $n = 2$). The $AUC_{\tau,ss}$ ratio between treatment B2 (clopidogrel 75 mg o.d. and selexipag 200 µg b.i.d.) and treatment a (selexipag 200 µg b.i.d.), as well as geometric mean ratio with 95% confidence interval per CYP2C8 genotype (*1/*1 $n = 12$, *1/*3 $n = 6$, *1/*4 $n = 2$). Individual values are included as black circles. b.i.d. = twice daily; o.d. = once daily

TABLE 3 Metrics of the simulated ACT-333679 systemic exposure for the dosing regimens selexipag 400 µg b.i.d. alone (regimen A), selexipag 400 µg o.d. with clopidogrel 75 mg o.d. (regimen B) and selexipag 200 µg b.i.d. with clopidogrel 75 mg o.d. (regimen C)

	Geometric mean (5th–95th percentile)			Geometric mean ratio (5th–95th percentile)	
ACT-333679	Regimen A [a]	Regimen B [b]	Regimen C [c]	Regimen B/A	Regimen C/A
$C_{max,ss}$ [ng mL^{-1}]	7.66 (3.84– 15.74)	10.08 (5.40– 17.08)	6.30 (3.78– 9.88)	1.31 (0.87– 1.96)	0.82 (0.52– 1.23)
$AUC_{0-24h,ss}$ [h*ng mL^{-1}]	72.2 (39.6– 131.2)	88.6 (59.2– 132.2)	88.6 (59.2– 132.2)	1.23 (1.01– 1.48)	1.23 (1.01– 1.48)

[a]Regimen A: selexipag 400 µg b.i.d.
[b]Regimen B: selexipag 400 µg o.d. with clopidogrel 75 mg o.d.
[c]Regimen C: selexipag 200 µg b.i.d. with clopidogrel 75 mg o.d.
$AUC_{0-24h,ss}$ = area under the plasma concentration–time curve from 0 to 24 hours after dosing at steady state; b.i.d. = twice daily; CI = confidence interval; $C_{max,ss}$ = maximum plasma concentration at steady state; o.d. = once daily.

4 | DISCUSSION

Both gemfibrozil and clopidogrel are mechanism-based, model CYP2C8 inhibitors, recommended for DDI studies with CYP2C8 substrates.[21] Both compounds have also been shown to inhibit OATP1B1 in vitro but when the clinical effect of clopidogrel was tested on simvastatin (an OATP1B1 and CYP3A4 substrate) and pitavastatin (an OATP1B1 substrate), no clinically relevant interaction was observed.[22,23] In contrast, gemfibrozil has been found to cause an increase in the AUC of simvastatin and simvastatin acid of 1.35-fold and 2.85-fold, respectively.[24] Selexipag and its active metabolite, ACT-333679, are substrates of CYP2C8[8] and OATP1B1/OATP1B3[10] in vitro. In 1 of our previous DDI studies, gemfibrozil increased the AUC of selexipag and ACT-333679 by 2-fold and 11-fold, respectively.[13] Based on these data, concomitant treatment with selexipag and strong inhibitors of CYP2C8 is contraindicated in patients with PAH. By contrast, the DDI study with selexipag and lopinavir/ritonavir, a strong inhibitor of OATP1B1 and OATP1B3 showed no

clinically significant effect on the exposure to selexipag and ACT-333679.[25]

The aim of the present study was to investigate the effect of clopidogrel on the PK of selexipag and ACT-333679 and to provide guidance to prescribers on selexipag dosing. Clopidogrel is an inhibitor of platelet aggregation approved for the treatment of acute coronary syndrome, recent myocardial infarction, recent stroke and peripheral arterial disease.[26] A subgroup of PAH patients may therefore receive concomitant treatment with clopidogrel and selexipag. For patients with non-ST segment elevation acute coronary syndrome, the approved dosing regimen for clopidogrel is a single 300mg loading dose followed by a 75mg maintenance dose o.d.,[26] corresponding to the dosing regimen applied in this study. The results showed that the AUC_τ and C_{max} of ACT-333679, the active metabolite responsible for the majority of the pharmacological effect, increased by 2.25- and 1.69-fold, respectively, after the loading dose of clopidogrel and the effect reached its maximum of 2.70- and 1.90-fold increase at steady state, after the maintenance dose of clopidogrel. Clopidogrel had a
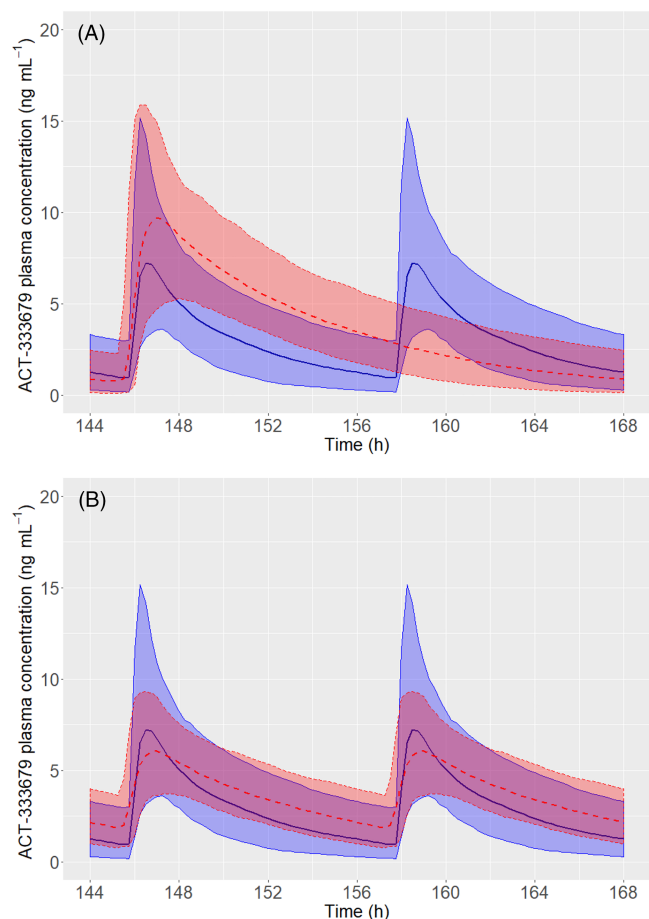
FIGURE 4 The steady-state pharmacokinetic profiles of ACT-333679 following simulated dosing. (A) Selexipag 400 μg o.d. with clopidogrel 75 mg o.d. (regimen B, dashed red lines) and selexipag 400 μg b.i.d. (regimen A (reference dose), solid blue lines). (B) Selexipag 200 μg b.i.d. with clopidogrel 75 mg o.d. (regimen C, dashed red lines) and selexipag 400 μg b.i.d. (regimen A [reference dose], solid blue lines). Thick and thin lines represent the median and 5th–95th percentiles of 500 model-based predictions, respectively. b.i.d. = twice daily; o.d. = once daily

comparatively small effect on the AUC_τ and C_{max} of selexipag (1.44- and 1.35-fold increase, respectively, after the loading dose and only 1.14- and 0.98-fold increase, respectively, at steady state after the maintenance dose). The observed, rather small and not clinically relevant, increase in AUC_τ and C_{max} of selexipag after the loading dose may be explained mainly by the inhibitory effect of clopidogrel on OATP1B1 and this is consistent with previous data.[13,25] In contrast, the more pronounced inhibitory effect of clopidogrel on the AUC_τ of ACT-333679, especially at steady state of maintenance dose, suggests that the effect is mainly due to the inhibitory effect of clopidogrel on CYP2C8 and that CYP2C8 is mainly responsible for the metabolism of ACT-333679.

Taking our gemfibrozil and clopidogrel DDI studies together, the inhibitory effect of clopidogrel is markedly lower than that of gemfibrozil (2.7-fold and 11-fold increase in the AUC of ACT-333679 with clopidogrel and gemfibrozil, respectively).[13] A similar observation was made in a recent study comparing the CYP2C8 inhibitory strength of

clopidogrel with gemfibrozil using the CYP2C8 probe substrate, desloratadine.[14] However, the relative increases in the AUC of desloratadine with clopidogrel vs gemfibrozil were 2.8- vs 4.6-fold, respectively. The mechanism behind the somewhat stronger effect of gemfibrozil on the AUC of ACT-333679 as compared to most other CYP2C8 substrates is unknown and remains to be further elucidated with more data on gemfibrozil and new substrates of CYP2C8 becoming available in the future. The clinically relevant, moderate inhibitory effect of clopidogrel on ACT-333679 identified in the present study (2.7-fold increase in AUC following its maintenance dose) is comparable to what has previously been published for the CYP2C8 substrates montelukast and pioglitazone (2.0 and 2.1-fold increase in AUC, respectively).[27,28] The loading dose of clopidogrel has, however, previously been found to cause a 5.1-fold increase in the exposure to repaglinide, a substrate of CYP2C8, CYP3A4 and OATP1B1, whereas the increase following continued administration of the maintenance dose was 3.9-fold.[29]

Genetic variants of CYP2C8 may significantly alter the metabolism of CYP2C8 substrate drugs in vitro.[30] For example losartan has a greater inhibitory effect on the CYP2C8 substrate, paclitaxel, in human liver microsomes containing the CYP2C8*3 heterozygote genotype compared with the CYP2C8*1 homozygote genotype[31] and CYP2C8*3 is associated with decreased pioglitazone plasma exposure and significantly influences the PK magnitude of the gemfibrozil–pioglitazone DDI in humans.[32] Therefore, genotyping was conducted to explore the potential impact on the clopidogrel–selexipag DDI. Our study population was reflective of the general population with 3 of the most common CYP2C8 genotypes (*1/*1, *1/*3 and *1/*4) identified.[33] Based on our exploratory analysis, the inhibitory effect of clopidogrel on the metabolism of ACT-333679 was slightly higher in heterozygous *3 carriers compared with the wild-type CYP2C8*1 homozygote genotype following the maintenance dose of clopidogrel (3.36- vs 2.42-fold, respectively). However, the mean exposure to ACT-333679 in combination with clopidogrel was comparable between all the identified genotypes and at the same time, a higher variability in the DDI effect was indicated in the *1/*3 genotype compared to the wild-type CYP2C8*1 homozygote genotype (Figure 3). Overall, the observed differences were considered not to be of clinical relevance. In accordance with our exploratory observation, no apparent genotype differences were observed in the recent study comparing the inhibitory strength of clopidogrel and gemfibrozil,[14] although both observations are based on very small sample sizes. Finally, no data are available on the magnitude of interaction between selexipag and CYP2C8 inhibitors in patients with a homozygote *3/*3 genotype. The relatively low frequency of the CYP2C8*3 allele in the population (ranging from 0.095 to 0.17 in Caucasians), however, makes the evaluation in a clinical setting very challenging.[34]

Because of the observed DDI between clopidogrel and selexipag, a dose adjustment is required for patients taking selexipag in combination with clopidogrel or other moderate CYP2C8 inhibitors. Based on the 2.70-fold increase in AUC_τ of ACT-333679 at steady state, in the presence of clopidogrel,

reducing the dose to half or reducing the dosing frequency to o.d. should keep the accumulation of ACT-333679 following multiple-dose administration of selexipag to a minimum. To further support the suggested changes in dosing regimen, a modelling and simulation exercise was performed for ACT-333679, the major contributor to the pharmacological effect. Based on the simulations, $AUC_{0-24h,ss}$ and $C_{max,ss}$ for ACT-333679 are expected to increase by approximately 23 and 31%, respectively, when selexipag is administered o.d. in combination with the maintenance dose of clopidogrel compared with selexipag administered b.i.d. alone. In comparison, $AUC_{0-24h,ss}$ is also expected to increase by approximately 23% but $C_{max,ss}$ is expected to decrease by 18%, when selexipag is administered at half the dose b.i.d. in combination with the maintenance dose of clopidogrel compared with selexipag administered b.i.d. alone. For both scenarios, the simulated changes in $AUC_{0-24h,ss}$ and $C_{max,ss}$ are lower than the intersubject variabilities (31 and 34%, respectively) observed in the clinical study and are considered not to be clinically relevant. This supports reducing the dosing frequency of selexipag to o.d. or reducing the dose to half when it is given in combination with clopidogrel. With the available dose strengths of selexipag (200, 400, 600, 800, 1000, 1200, 1400 and 1600 μg),[10] reducing the dosing frequency to o.d. is considered the best option in order to support a uniform recommendation for all dose levels when concomitant treatment with clopidogrel is initiated. The limited data available from the genotypes identified in the clinical study further indicate that no genotype specific dosing recommendations are needed.

As the observed 2.7-fold increase in selexipag exposure following coadministration of clopidogrel is comparable to what is seen for other known moderate CYP2C8 inhibitors (deferasirox and teriflunomide) when coadministered with the CYP2C8 substrate repaglinide (2.3 and 2.4-fold increase in exposure, respectively),[35,36] the provided dosing recommendation for coadministration of selexipag and clopidogrel may also be applied when coadministering selexipag with other moderate CYP2C8 inhibitors.

In conclusion, there was no relevant change in selexipag exposure following concomitant administration with the CYP2C8 inhibitor, clopidogrel. Concomitant administration of selexipag and clopidogrel resulted in a moderate increase in ACT-333679 exposure, compared to the much larger effect seen with gemfibrozil. There were no unexpected safety findings observed after exposure to selexipag in combination with clopidogrel. Based on these findings and supported by the outcome of the modelling and simulation exercises, dosing adjustment of selexipag when administered in combination with moderate CYP2C8 inhibitors can be done by either reducing the dosing frequency of selexipag to o.d. or reducing the dose to half.

COMPETING INTERESTS

All authors are employees of Actelion Pharmaceuticals Ltd. or Janssen Pharmaceutica NV and may own company stocks.

CONTRIBUTORS

All authors have critically reviewed the manuscript, agreed to be accountable for all aspects of the work and given final approval of the version to be published. In addition, L.N.A. made substantial contributions to the data acquisition, analysis and interpretation. I.P. made substantial contributions to conception, design and execution of the modelling and simulation work. F.R. made substantial contributions to clinical data acquisition and had direct clinical responsibility for subjects included in the study. J.J.P.R. made substantial contributions to the interpretation of data and S.B. made substantial contributions to the conception and design of the clinical study, as well as analysis and interpretation of the data.

DATA ACCESSIBILITY STATEMENT

The data sharing policy of the Sponsor is available at https://www.janssen.com/clinical-trials/transparency. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access (YODA) Project site at http://yoda.yale.edu.

ORCID

Lene Nygaard Axelsen ◎ https://orcid.org/0000-0002-9844-6216

Italo Poggesi ◎ https://orcid.org/0000-0003-3079-6954

Freya Rasschaert ◎ https://orcid.org/0000-0003-1666-4533

Juan Jose Perez Ruixo ◎ https://orcid.org/0000-0001-9890-745X

REFERENCES

1. Lai YC, Potoka KC, Champion HC, Mora AL, Gladwin MT. Pulmonary arterial hypertension: the clinical syndrome. *Circ Res*. 2014;115(1):115-130.

2. Del Pozo R, Hernandez Gonzalez I, Escribano-Subias P. The prostacyclin pathway in pulmonary arterial hypertension: a clinical review. *Expert Rev Respir Med*. 2017;11(6):491-503.

3. Tuder RM, Marecki JC, Richter A, Fijalkowska I, Flores S. Pathology of pulmonary hypertension. *Clin Chest Med*. 2007;28(1):23-42. vii

4. Badesch DB, McLaughlin VV, Delcroix M, et al. Prostanoid therapy for pulmonary arterial hypertension. *J Am Coll Cardiol*. 2004;43(12):56S-61S.

5. Gomberg-Maitland M, Preston IR. Prostacyclin therapy for pulmonary arterial hypertension: new directions. *Semin Respir Crit Care Med*. 2005;26(04):394-401.

6. Bruderer S, Hurst N, Remenova T, Dingemanse J. Clinical pharmacology, efficacy, and safety of selexipag for the treatment of pulmonary arterial hypertension. *Expert Opin Drug Saf*. 2017;16(6):743-751.

7. Sitbon O, Channick R, Chin KM, et al. Selexipag for the treatment of pulmonary arterial hypertension. *N Engl J Med*. 2015;373(26):2522-2533.

8. Gnerre C, Segrestaa J, Seeland S, et al. The metabolism and drug-drug interaction potential of the selective prostacyclin receptor agonist selexipag. *Xenobiotica*. 2018;48(7):704-719.

9. Imai S, Ichikawa T, Sugiyama C, Nonaka K, Yamada T. Contribution of human liver and intestinal carboxylesterases to the hydrolysis of Selexipag in vitro. *J Pharm Sci*. 2019;108(2):1027-1034.

10. US Department of Health and Human Services, Food and Drug Administration, US prescribing information: Uptravi (Selexipag). December 2015. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/207947s005lbl.pdf (last accessed 17 March 2020).

11. Bruderer S, Hurst N, Kaufmann P, Dingemanse J. Multiple-dose up-titration study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of selexipag, an orally available selective prostacyclin receptor agonist, in healthy subjects. *Pharmacology*. 2014;94(3-4):148-156.

12. Kaufmann P, Cruz HG, Krause A, Ulč I, Halabi A, Dingemanse J. Pharmacokinetics of the novel oral prostacyclin receptor agonist selexipag in subjects with hepatic or renal impairment. *Br J Clin Pharmacol*. 2016;82(2):369-379.

13. Bruderer S, Petersen-Sylla M, Boehler M, Remeňová T, Halabi A, Dingemanse J. Effect of gemfibrozil and rifampicin on the pharmacokinetics of selexipag and its active metabolite in healthy subjects. *Br J Clin Pharmacol*. 2017;83(12):2778-2788.

14. Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M, Backman JT. Clopidogrel and gemfibrozil strongly inhibit the CYP2C8-dependent formation of 3-hydroxydesloratadine and increase desloratadine exposure in humans. *Drug Metab Dispos*. 2019; 47(4):377-385.

15. Bruderer S, Okubo K, Mukai H, Mant T, Dingemanse J. Investigation of potential Pharmacodynamic and pharmacokinetic interactions between Selexipag and warfarin in healthy male subjects. *Clin Ther*. 2016;38(5):1228-1236.

16. Juif PE, Boehler M, Donazzolo Y, Bruderer S, Dingemanse J. A pharmacokinetic drug-drug interaction study between selexipag and midazolam, a CYP3A4 substrate, in healthy male subjects. *Eur J Clin Pharmacol*. 2017;73(9):1121-1128.

17. Baldoni D, Bruderer S, Muhsen N, Dingemanse J. Bioequivalence of different dose-strength tablets of selexipag, a selective prostacyclin receptor agonist, in a multiple-dose up-titration study. *Int J Clin Pharmacol Ther*. 2015;53(09):788-798.

18. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977;74(12):5463-5467.

19. Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res*. 1998;8(3):186-194.

20. Alexander SP, Kelly E, Marrion NV, et al. The concise guide to pharmacology 2017/18. *Br J Pharmacol*. 2017;174:S272-S359.

21. Backman JT, Filppula AM, Niemi M, Neuvonen PJ. Role of cytochrome P450 2C8 in drug metabolism and interactions. *Pharmacol Rev*. 2016;68(1):168-241.

22. Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M, Backman JT. Clopidogrel has no clinically meaningful effect on the pharmacokinetics of the organic anion transporting polypeptide 1B1 and cytochrome P450 3A4 substrate simvastatin. *Drug Metab Dispos*. 2015;43(11):1655-1660.

23. Kim SJ, Yoshikado T, Ieiri I, et al. Clarification of the mechanism of Clopidogrel-mediated drug-drug interaction in a clinical cassette small-dose study and its prediction based on in vitro information. *Drug Metab Dispos*. 2016;44(10):1622-1632.

24. Backman JT, Kyrklund C, Kivistö KT, Wang JS, Neuvonen PJ. Plasma concentrations of active simvastatin acid are increased by gemfibrozil. *Clin Pharmacol Ther*. 2000;68(2):122-129.

25. Kaufmann P, Niglis S, Bruderer S, et al. Effect of lopinavir/ritonavir on the pharmacokinetics of selexipag an oral prostacyclin receptor agonist and its active metabolite in healthy subjects. *Br J Clin Pharmacol*. 2015;80(4):670-677.

26. US Department of Health and Human Services, Food and Drug Administration, US prescribing information: Plavix (clopidogrel bisulfate tablets). October 2009. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020839s044lbl.pdf (last accessed 17 March 2020).

27. Itkonen MK, Tornio A, Filppula AM, et al. Clopidogrel but not Prasugrel significantly inhibits the CYP2C8-mediated metabolism of Montelukast in humans. *Clin Pharmacol Ther*. 2018;104(3): 495-504.

28. Itkonen MK, Tornio A, Neuvonen M, Neuvonen PJ, Niemi M, Backman JT. Clopidogrel markedly increases plasma concentrations of CYP2C8 substrate pioglitazone. *Drug Metab Dispos*. 2016;44(8): 1364-1371.

29. Tornio A, Filppula AM, Kailari O, et al. Glucuronidation converts clopidogrel to a strong time-dependent inhibitor of CYP2C8: a phase II metabolite as a perpetrator of drug-drug interactions. *Clin Pharmacol Ther*. 2014;96(4):498-507.

30. Soyama A, Hanioka N, Saito Y, et al. Amiodarone N-deethylation by CYP2C8 and its variants, CYP2C8*3 and CYP2C8 P404A. *Pharmacol Toxicol*. 2002;91(4):174-178.

31. Mukai Y, Senda A, Toda T, et al. Drug-drug interaction between losartan and paclitaxel in human liver microsomes with different CYP2C8 genotypes. *Basic Clin Pharmacol Toxicol*. 2015;116(6): 493-498.

32. Aquilante CL, Kosmiski LA, Bourne DW, et al. Impact of the CYP2C8 *3 polymorphism on the drug-drug interaction between gemfibrozil and pioglitazone. *Br J Clin Pharmacol*. 2013;75(1):217-226.

33. Aquilante CL, Niemi M, Gong L, Altman RB, Klein TE. PharmGKB summary: very important pharmacogene information for cytochrome P450, family 2, subfamily C, polypeptide 8. *Pharmacogenet Genomics*. 2013;23(12):721-728.

34. Martínez C, García-Martín E, Blanco G, Gamito FJ, Ladero JM, Agúndez JA. The effect of the cytochrome P450 CYP2C8 polymorphism on the disposition of (R)-ibuprofen enantiomer in healthy subjects. *Br J Clin Pharmacol*. 2005;59(1):62-69.

35. Skerjanec A, Wang J, Maren K, Rojkjaer L. Investigation of the pharmacokinetic interactions of deferasirox, a once-daily oral iron chelator, with midazolam, rifampin, and repaglinide in healthy volunteers. *J Clin Pharmacol*. 2010;50(2):205-213.

36. US Department of Health and Human Services, Food and Drug Administration, US prescribing information: Aubagio (teriflunomide). June 2016. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/202992s003lbl.pdf (last accessed 17 March 2020).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.