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**A study of the effect of cyclosporine on fevipiprant pharmacokinetics and its absolute bioavailability using an intravenous microdose approach<sup>†</sup>**

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**Cyclosporine effect on fevipirant oral and IV PK**

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**Nonstandard abbreviations (in alphabetical order)**

ADME: absorption, distribution, metabolism and excretion

AE: adverse events

SAE: serious adverse event

$Ae_{0-24h}$ : Amount of drug (or metabolite) excreted into the urine from time zero to 24h]

AG: acyl-glucuronide

ALT: alanine aminotransferase

$AUC_{inf}$ : area under the plasma concentration–time curve from time zero to infinity

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AUC<sub>last</sub>: area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration

BMI: body mass index

CI: confidence interval

C<sub>max</sub>: maximum plasma concentration

CL: clearance

CL<sub>r</sub>: renal clearance

CRTh2: chemoattractant receptor-homologous molecule expressed on Th2 cells

DP<sub>2</sub>: prostaglandin D<sub>2</sub> receptor 2

ECG: electrocardiogram

F: absolute bioavailability

IV: intravenous

LC-MS/MS: liquid chromatography–tandem mass spectrometry

LLOQ: lower limits of quantification

MDR1: multidrug resistance protein 1

M/P: metabolite-to-parent

OAT: organic anion transporter

OATP: Organic anion transporting polypeptide

PGD<sub>2</sub>: prostaglandin D<sub>2</sub>

P-gp: P-glycoprotein

PK: pharmacokinetics

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po: *per os* (oral administration)

R<sup>2</sup>: coefficient of determination in regression analysis

T<sub>1/2</sub>: terminal half-life

T<sub>max</sub>: time to reach peak or maximum concentration following drug administration

UGT: uridine 5'-diphospho-glucuronosyltransferase

V: volume of distribution

V<sub>z</sub>/F: the apparent volume of distribution during the terminal phase following extravascular administration

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## Abstract

This drug-drug interaction (DDI) study determined the effect of cyclosporine, an inhibitor of OATP1B3 and P-gp, on the pharmacokinetics (PK) of fevipiprant, an oral, highly selective, competitive antagonist of the prostaglandin D<sub>2</sub> receptor 2 and a substrate of the two transporters. The concomitant administration of an intravenous (IV) microdose of stable isotope-labeled fevipiprant provided the absolute bioavailability of fevipiprant, as well as mechanistic insights in its PK and sensitivity to drug interactions. Liquid chromatography–mass spectrometry/mass spectrometry was used to measure plasma and urine concentrations. Geometric mean ratios (90% CI) for oral fevipiprant with or without cyclosporine were: for C<sub>max</sub>, 3.02 (2.38, 3.82); for AUC<sub>last</sub>, 2.50 (2.17, 2.88); and for AUC<sub>inf</sub>, 2.35 (1.99, 2.77). The geometric mean ratios (90% CI) for fevipiprant IV microdose with or without cyclosporine were: for C<sub>max</sub>, 1.04 (0.86, 1.25); for AUC<sub>last</sub>, 2.04 (1.83, 2.28) and for AUC<sub>inf</sub>, 1.95 (1.76, 2.16). The absolute bioavailability for fevipiprant was approximately 0.3–0.4 in the absence and 0.5 in the presence of cyclosporine. The IV microdose allowed differentiation between systemic and pre-systemic effects of cyclosporine on fevipiprant, demonstrating a small (approximately 1.2-fold) pre-systemic effect of cyclosporine and a larger (approximately 2-fold) effect on systemic elimination of fevipiprant. Uptake by OATP1B3 appears to be the rate-limiting step in the hepatic elimination of fevipiprant while P-gp does not have a relevant effect on oral absorption.

**Significance statement:** The drug interaction investigated here with cyclosporine, an inhibitor of several drug transporters, provides a refined quantitative understanding of the role of active transport processes in liver and intestine for the absorption and elimination of fevipiprant, as well as the basis to assess the need for dose adjustment in the presence of transporter inhibitors. The applied IV microdose approach presents a strategy to maximize learnings from a trial, limit the number and duration of clinical trials, and enhance mechanistic DDI understanding.

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## Introduction

Fevipirant (QAW039) is an oral, competitive antagonist of the prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) receptor 2 (DP<sub>2</sub>, previously called chemoattractant receptor-homologous molecule expressed on TH2 cells [CRTh2]) that dissociates slowly from this receptor (Sykes et al., 2016). In Phase II trials, fevipirant significantly decreased sputum eosinophil counts, and reduced airway smooth muscle mass in patients with asthma (Erpenbeck et al., 2016a; Gonem et al., 2016; Bateman et al., 2017; Saunders et al., 2019) but Phase III results [ClinicalTrials.gov numbers: NCT02555683; NCT02563067; NCT03215758; NCT03226392] did not support submission in this indication.

One aspect of drug development is to build a detailed understanding of the processes underlying the pharmacokinetics (PK) of the drug in order to determine if, and to what extent, co-medications or other factors can affect its PK. In this context, regulatory authorities encourage obtaining intravenous (IV) PK data of drugs in development (European Medicines Agency, 1987). PK parameters requiring IV data (such as absolute bioavailability and systemic clearance), can increase the predictability of physiologically-based modeling of PK and are generally important for any quantitative estimation of exposure change with, for example, change in formulation, age, partial inhibition of a clearance pathway by DDIs, or organ impairment.

Renal clearance and hepatic elimination via glucuronidation and/or biliary secretion contribute to the elimination of fevipirant; IV PK data can help to estimate the contributions of these clearance pathways more quantitatively. In a human absorption, distribution, metabolism and excretion (ADME) study of fevipirant the fraction of the oral dose absorbed from the intestine was estimated to be at least 43.5% (42.1% of the total radioactive dose recovered from urine and 1.4% as metabolites in feces) (Pearson et al., 2017). However, the absolute bioavailability,

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i.e. the fraction of the oral dose reaching the systemic circulation unchanged, could be higher or lower than this minimal estimate of absorption. *In vitro* data indicate that fevipiprant is taken up via organic anion transporting polypeptide 1B3 (OATP1B3) into the liver, followed by formation of an acyl glucuronide (AG) metabolite by several uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes. Of note, the AG metabolite is the only major circulating metabolite of fevipiprant and is not pharmacologically active (Pearson et al., 2017). Organic anion transporter 3 (OAT3) is responsible for the active renal excretion of fevipiprant (Pearson et al., 2017). Because fevipiprant is a substrate of P-glycoprotein (P-gp or multidrug resistance protein 1; MDR1) and UGT enzymes, intestinal efflux and metabolism could have an impact on its absorption and first-pass metabolism, respectively (Pearson et al., 2017). In addition, the hepatic first-pass extraction by OATP1B3-mediated uptake may influence the bioavailability of fevipiprant.

The aim of this study was to determine the effect of oral cyclosporine, an inhibitor of OATP1B3 (Shitara et al., 2012) and P-gp (Kovarik and Koelle, 1999), on the PK of fevipiprant, and also to increase the overall understanding of fevipiprant PK. Cyclosporine has no inhibitory effects on the other elimination pathways of fevipiprant, that is, UGTs [no inhibition was reported at time of survey in the Metabolism and Transport Drug Interaction Database (DIDB®)] and OAT3 (El-Sheikh et al., 2013). An IV microdose of stable (i.e. non-radioactive) isotope-labeled fevipiprant ( $[^{13}\text{C}_2^{15}\text{N}_2]$ fevipiprant) (Figure 1) was co-administered with unlabeled oral fevipiprant to determine its absolute bioavailability and systemic clearance, both in the absence and presence of cyclosporine. Labeled and unlabeled fevipiprant are identical biologically, i.e. their interaction with e.g. the target or drug transporters is not expected to differ, but they can be distinguished analytically by mass spectrometry. This allows IV and oral data to be obtained on the same drug, from the same study participant, at the same time without the risk of a bias of the IV-PK data due to the microdose in case of PK non-linearity (Lappin et al., 2006). In the context of the DDI assessment this allowed determination of systemic (from fevipiprant IV administration) as

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well as 'pre-systemic plus systemic' (from fevipirant oral administration) effects of cyclosporine, adding mechanistic granularity.

## **Materials and methods**

### *Study objectives*

The primary objective was to determine the effect of cyclosporine on the three key PK measures of orally administered fevipirant in healthy volunteers: (i) the area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration ( $AUC_{last}$ ); (ii) the area under the plasma concentration–time curve from time zero to infinity ( $AUC_{inf}$ ); and (iii) the maximum plasma concentration ( $C_{max}$ ).

The secondary objectives were to determine: (i) the absolute bioavailability (F) and the absolute disposition parameters of fevipirant, i.e. clearance (CL) and volume (V) of distribution, by administering an IV microdose of stable isotope-labeled fevipirant concomitantly with the oral dose; (ii) the effect of cyclosporine on the PK of the IV microdose of fevipirant (V, CL,  $AUC_{last}$ , and  $AUC_{inf}$ ); (iii) the safety and tolerability of fevipirant administered both orally and intravenously, with and without co-administration of cyclosporine; and (iv) the effect of cyclosporine on the PK of the major AG metabolite of oral fevipirant. The study protocol was approved by the Institutional Review Board for the study center and the study was conducted according to the ethical principles of the Declaration of Helsinki.

### *Study design*

This was an open-label, single-sequence, two-period, crossover study with two treatment periods separated by a washout period of 7–14 days (Figure 2). The study was carried out at IQVIA (formerly QuintilesIMS), Overland Park, KS, USA. The investigational drug, fevipirant 150 mg film-coated tablets and fevipirant 100 µg labeled IV microdose ( $[^{13}C_2^{15}N_2]$ fevipirant)



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(Figure 1), were prepared and released by Novartis Technical Research and Development. Cyclosporine 100 mg and 25 mg soft gelatin capsules (Neoral® Novartis Pharma AG, Basel, Switzerland) were commercially available and sourced locally by the site.

In Treatment Period 1, study participants were admitted to the clinic on Day -1 for baseline evaluations at least 12 hours before dosing. On Day 1, they received a single oral dose of fevipiprant 150 mg, followed by an IV microdose of labeled fevipiprant (100 µg) 1 hour (± 5 minutes) later. Participants fasted for 8 hours before the oral dose administration and continued to fast for 2 hours afterwards, and were confined to the clinic for approximately 28 hours following the oral study drug administration during which time blood and urine samples for PK analysis and safety assessments were taken. On Days 3-5, participants returned to the study site for outpatient safety and PK visits. They returned to the clinic for Treatment Period 2 following a washout period; overall 7-14 days separated Day 1 in Treatment Period 1 from nominal Day 16 in Treatment Period 2. Anyone that prematurely discontinued from Treatment Period 1 was required to complete an early termination visit (Day 15 visit).

In Treatment Period 2, participants were admitted to the clinic on Day 15 for baseline evaluations at least 12 hours before dosing. On Days 16 to 19, they received oral doses of cyclosporine 175 mg bid and on Day 20, a single oral dose of cyclosporine 175 mg in the morning. On Day 17, participants received a single oral dose of fevipiprant 150 mg in parallel with the morning cyclosporine dose (± approximately 3 minutes), followed by administration of an IV microdose of labeled fevipiprant (100 µg) 1 hour (± 5 minutes) later. On all dosing days participants fasted for 8 hours before the oral drug administration in the morning and continued to fast for 2 hours thereafter. Samples were taken for PK analysis on Days 16-21. Participants fasted for 2 hours before the evening doses of cyclosporine and continued to fast for 1 hour thereafter and were confined to the clinic until study Day 21. On Day 23, participants returned to

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the study site for safety assessments; end of study safety assessments were completed on Day 25.

The sample size (16 participants so that at least 12 would complete the study) was selected to control the width of the confidence interval for the geometric mean ratio for fevipirant AUC and  $C_{\max}$  when given with and without cyclosporine. Further details may be found in the Online Data Supplement.

#### *Key inclusion and exclusion criteria*

Men and women were admitted as study participants if they were aged 18–55 years and in good health, as determined by past medical history, physical examination, vital signs, electrocardiogram (ECG), and laboratory tests at screening and/or at first baseline visit. Further details on vital sign measurement are provided in the Online Data Supplement. Participants had to weigh between 60 and 90 kg and to have a body mass index (BMI) within the range of 20–30 kg/m<sup>2</sup>. Study participants gave written informed consent before any assessment took place, had to be able to communicate well with the investigator, and to understand and comply with the requirements of the study. Exclusion criteria details are provided in the Online Data Supplement.

#### *Pharmacokinetic analyses*

PK blood and urine samples were taken at prespecified time points from all participants and acidified pending analysis to avoid back-conversion of the AG metabolite to fevipirant (details are provided in the Online Data Supplement). Validated liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) methods were used to measure the plasma concentrations of unlabeled fevipirant (given orally) and labeled fevipirant (given as IV microdose) using the transitions of m/z (mass to charge ratio) 427 to m/z 145 and of m/z 431 to

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m/z 149, respectively. The MS/MS transition used for the acyl glucuronide metabolite of (unlabeled) fevipiprant was m/z 603 to m/z 427. The MS/MS transition used for the measurement of cyclosporine was m/z 1219.9 to m/z 1202.9. Further details of the LC-MS/MS methods may be found in the Online Data Supplement. The plasma concentrations of the AG metabolite of unlabeled fevipiprant and the blood concentrations of cyclosporine were measured by validated LC-MS/MS methods. The concentrations of unlabeled fevipiprant and its major AG metabolite in urine were determined using qualified LC-MS/MS methods. Concentrations below the lower limits of quantification (LLOQ) were reported as “zero” and missing data were labeled as such in the bioanalytical data reports.

The following PK measures of fevipiprant were determined using the actual recorded sampling times and non-compartmental method(s) with Phoenix WinNonlin (Version 6.4):  $C_{max}$ ,  $T_{max}$  (time to reach peak or maximum concentration following drug administration),  $AUC_{last}$ ,  $AUC_{inf}$ ,  $T_{1/2}$  (terminal half-life),  $V_z/F$  (the apparent volume of distribution during the terminal phase following extravascular administration),  $CL$ ,  $F$  and  $CL/F$  from the plasma concentration time data. For the AG metabolite,  $C_{max}$ ,  $T_{max}$ ,  $AUC_{last}$ ,  $AUC_{inf}$  and  $T_{1/2}$  were determined using non-compartmental analysis only after oral administration. The amount excreted into the urine ( $Ae_{0-24h}$ ) of unlabeled fevipiprant and its AG metabolite was determined from the urine concentration and volume–time data. The renal clearance ( $CL_r$ ) of fevipiprant and its AG metabolite was determined as  $Ae/AUC$  from the same time period. The absolute oral bioavailability ( $F$ ) was estimated as a ratio of the dose-normalized AUCs following oral (po) and intravenous administration ( $F = [AUC_{po} * DOSE_{iv}] / [AUC_{iv} * DOSE_{po}]$ ). The linear trapezoidal rule was used for AUC calculation. Regression analysis of the terminal elimination phase for the determination of  $T_{1/2}$  included at least three data points after  $C_{max}$ . If the adjusted  $R^2$  (coefficient of determination) value of the regression analysis of the terminal phase was less than 0.75, no values were reported for  $T_{1/2}$ ,  $AUC_{inf}$ ,  $CL$ ,  $V$  or  $CL/F$ .

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### *Key safety and tolerability assessments*

Safety assessments consisted of collecting all adverse events (AEs), serious AEs (SAEs), with their severity and relationship to study drug. Laboratory evaluations included hematology, biochemistry and urinalyses. Vital signs, physical condition, body weight and standard 12-lead ECG were also assessed.

### *Statistical analyses*

Participants' data were analyzed according to the study treatments received for all analysis sets. The safety analysis set included all participants who received any study drug. The PK analysis set included all participants with at least one available valid PK concentration measurement, who received any study drug and with no protocol deviations that had an impact on PK data. For the primary endpoints, the log-transformed fevipirant PK measures ( $AUC_{last}$ ,  $AUC_{inf}$  and  $C_{max}$ ) were analyzed separately by a mixed effects model, with treatment as a fixed effect and participant as random effect. The estimated mean and 90% confidence interval (CI) for treatment difference (fevipirant plus cyclosporine versus fevipirant alone) were back-transformed to obtain a geometric mean ratio and 90% CI of the ratio. SAS software was used for all statistical analyses. Statistical analysis of the secondary endpoints is provided in the Online Data Supplement.

## **Results**

### *Participants*

Sixteen participants entered the study, of whom 13 (81.3%) completed Treatment Period 1. Fourteen participants were male and two were female; 11 were Caucasian, four were black with one of another race (not specified). Their mean age was 32.5 years (range: 20–52) with a mean weight of 77.7 kg (range: 64.5–88.7) and mean BMI of 25.6 kg/m<sup>2</sup> (range: 21.6–30.0). Three

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participants discontinued for the following reasons: because of an AE (n=1), being lost to follow-up (n=1), or physician decision (positive drug screen on Day 15; n=1). All 13 participants who completed Treatment Period 1 entered Treatment Period 2 and completed the study. All 16 participants were included in the PK and the safety analysis sets.

*Effect of cyclosporine on the PK of fevipiprant (oral and IV doses)*

Peak concentrations of oral fevipiprant were seen 1.5 h after the fevipiprant dose and 3 hours after the cyclosporine plus fevipiprant dose (Figure 3; Table 1). The mean concentration-time data for fevipiprant with and without cyclosporine are provided in Supplemental Table 1. For  $C_{max}$ , the geometric mean ratio (90% CI) was 3.02 (2.38, 3.82) (Supplemental Table 2) indicating an approximately three-fold increase in peak exposure of fevipiprant when co-administered with cyclosporine. The geometric mean ratios (90% CI) were 2.50 (2.17, 2.88) for  $AUC_{last}$  and 2.35 (1.99, 2.77) for  $AUC_{inf}$  (Supplemental Table 2), indicating that the effect of cyclosporine on fevipiprant total exposure is smaller than the effect on peak exposure. Oral clearance and volume of distribution of fevipiprant were reduced by approximately 50% upon co-administration with cyclosporine, whereas  $T_{1/2}$  was similar (Table 1).

$C_{max}$  values of the fevipiprant IV microdose were similar in the presence and absence of cyclosporine (Figure 4; Table 2), with a geometric mean ratio for  $C_{max}$  (90% CI) of 1.04 (0.86, 1.25) (Supplemental Table 4). The mean concentration-time data for the fevipiprant IV microdose with and without cyclosporine are provided in Supplemental Table 3.  $T_{max}$  was typically at the first sampling time i.e. 2 min after IV dosing. A terminal half-life and volume of distribution (V) for fevipiprant are not reported, since the terminal phase was not sufficiently covered in the concentration–time data, which is required to derive V. The terminal half-life after IV administration is expected to be identical to that derived from the oral data.  $AUC_{inf}$  and CL were estimated by non-compartmental analysis (Table 2), despite the limitations in describing

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the terminal phase. Since the captured concentration–time profiles cover a 1000-fold range in concentrations, the bias resulting from the incomplete representation of the AUC after concentrations dropped below the LLOQ of 20 pg/mL is considered to be small. Both  $AUC_{last}$  and estimated  $AUC_{inf}$  for fevipirant IV microdose were approximately 2-fold higher in presence of cyclosporine (Table 2), with geometric mean ratios (90% CI) of 2.04 (1.83, 2.28) and 1.95 (1.76, 2.16), respectively (Supplemental Table 4).

#### *Absolute bioavailability of fevipirant*

The combination of oral and IV microdose fevipirant PK data was used to estimate its absolute bioavailability. Because of the limitations to deriving  $AUC_{inf}$  for the IV profiles, the absolute oral bioavailability for fevipirant was based on comparison of dose normalized  $AUC_{last}$  (mean  $\pm$  SD), and was  $0.43 \pm 0.09$  and  $0.53 \pm 0.16$  in the absence and presence of cyclosporine, respectively. These values are expected to be slightly higher than the real values since the covered time interval is longer for the oral data. The absolute bioavailability of fevipirant was also estimated based on comparison of  $AUC_{0-11h}$ , because most IV profiles could be measured up to 11 h post dose. Estimates based on this comparison were (mean  $\pm$  SD)  $0.28 \pm 0.05$  and  $0.48 \pm 0.16$  in the absence and presence of cyclosporine, respectively. Using this method, a larger fraction of the fevipirant oral profile is ignored; therefore, this value is a minimal estimate of the real value, which is expected to be in the range defined by the assessments based on  $AUC_{last}$  and  $AUC_{0-11h}$ , i.e. approximately 0.3–0.4 in the absence and 0.5 in the presence of cyclosporine.

#### *Effect of cyclosporine on the PK of the major AG metabolite of fevipirant*

No relevant change in exposure of the AG metabolite of oral fevipirant was seen in the presence of cyclosporine (Table 3). Consequently, the metabolite-to-fevipirant ratio of  $AUC_{last}$  decreased from 1.5 to 0.59 in presence of cyclosporine. The mean concentration-time data the

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AG metabolite with and without cyclosporine are shown in Supplemental Table 1. Geometric mean ratios for PK measures for the AG metabolite are shown in Supplemental Table 5.

*Effect of cyclosporine on the urinary excretion of fevipiprant and its AG metabolite*

Co-administration with cyclosporine did not result in any relevant changes in the renal clearance of oral fevipiprant (Table 1) and its AG metabolite (Table 3). However, consistent with the higher exposure, the fraction of the dose excreted as unchanged fevipiprant into urine within 24 h increased by 2.5-fold from  $16.7 \pm 3.55\%$  to  $40.9 \pm 7.50\%$  when co-administered with cyclosporine. There was only a slight increase in the amount of AG metabolite excreted into urine from  $31.7 \pm 6.08$  mg to  $37.3 \pm 4.79$  mg in the presence of cyclosporine (corresponding to approximately 15 or 18% of the fevipiprant dose).

*Effect of fevipiprant on the PK of cyclosporine and concentration-effect relationship of cyclosporine*

No relevant change in mean trough blood concentrations of cyclosporine was observed from Day 17 in the morning (before administration of fevipiprant) to any time point after fevipiprant administration (mean trough blood concentrations were in the range of 45–54 ng/mL cyclosporine). This indicates the absence of a major effect of fevipiprant 150 mg on the PK of cyclosporine and that cyclosporine steady state had been achieved at the time of fevipiprant dosing.

The relationship of trough concentrations of cyclosporine to the observed change in oral fevipiprant  $C_{\max}$  was explored graphically (Figure 5). There was no apparent trend for an increasing effect on fevipiprant  $C_{\max}$  with increasing trough concentrations of cyclosporine.

*Safety and tolerability*

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Overall, administration of fevipiprant alone and fevipiprant plus cyclosporine showed no major or novel safety signals. There were 20 AEs reported in nine patients: headache (n=3); nausea (n=2); alanine aminotransferase (ALT) elevation (n=1); back pain (n=1); catheter site hematoma (n=1); contact dermatitis (n=1); dizziness (n=1); dysgeusia (n=1); flushing (n=1); muscle fatigue (n=1); pharyngitis (n=1); upper respiratory tract infection (n=1); chlamydial urethritis (n=1); vessel puncture site pain (n=1); and vomiting (n=1). A breakdown of the safety data by treatment period may be found in the Online Data Supplement (Table S6).

Ten AEs were suspected to be related to study medication: eight were suspected to be related to cyclosporine; one was suspected to be related to fevipiprant (mild postural dizziness); and one was suspected to be related to one or other or both study treatments (mild headache); it was not possible to distinguish which. All reported AEs were of mild intensity, except one AE (pharyngitis) which was of moderate intensity. The participant who experienced increased ALT discontinued the study, this AE was not suspected to be related to study drug. Apart from the elevation in ALT in one participant, no clinically significant changes were seen in laboratory parameters, vital signs or ECG parameters. There were no serious AEs or deaths.

## Discussion

The aim of this study was to assess how cyclosporine, by inhibiting OATP1B3-mediated liver uptake and P-gp-mediated efflux in the intestine and liver, affects the PK of oral fevipiprant. The inclusion of an IV microdose of stable isotope-labeled fevipiprant provided major additional learnings without the need for more participants, or a longer study duration: the absolute bioavailability of fevipiprant was derived, and the mechanistic DDI understanding increased, by the ability to differentiate between systemic and pre-systemic effects of cyclosporine. These learnings would be reflected in a potential future fevipiprant drug label in two sections: 1) the absolute bioavailability and dependence on active transport as part of the PK section and 2) the



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assessment of the need for dose adjustment in presence of OATP1B3 or P-gp inhibitors in the drug interaction section.

In the absence of cyclosporine, the PK properties of fevipiprant including exposure to its major metabolite were consistent with earlier studies (Erpenbeck et al., 2016b; Erpenbeck, 2017; Pearson et al., 2017). Co-administration of cyclosporine increased the  $C_{max}$  of oral fevipiprant 3-fold and the  $AUC_{inf}$  by 2.35-fold. While fevipiprant concentrations shortly after IV-dosing were similar, the AUC of IV administered fevipiprant was 2-fold higher in the presence of cyclosporine, corresponding to a 2-fold decrease in systemic clearance. The approximately 1.2 fold stronger effect on oral as compared to IV exposure ( $2.35/2$  i.e.  $\sim 1.2$ ) can be attributed to the effect of cyclosporine on absorption and/or first-pass elimination of fevipiprant. This combined effect was small and indicates that the inhibition of intestinal P-gp has only a minor or no impact. Early clinical investigations in healthy participants indicated a dose-proportional PK of fevipiprant over a 2-fold dose range (Erpenbeck et al., 2016b), while later more comprehensive data in patients demonstrated dose-proportional PK over a 10-fold dose range (unpublished observations). Therefore, the magnitude of drug interaction observed here is also relevant for other oral fevipiprant dose levels such as 450 mg, which was also tested in patient trials.

Despite the approximate 2-fold reduction in clearance, no increase in the terminal half-life of fevipiprant was observed with co-administration of cyclosporine. The likely explanation is a reduced distribution in the presence of cyclosporine as the terminal half-life depends on the ratio of distribution to clearance. Since cyclosporine reduced  $CL/F$  and  $V_z/F$  to a similar extent, the terminal half-life of fevipiprant remained largely unchanged. Mechanistically, the reduced hepatic uptake due to inhibition of OATP1B3 can explain the lower volume of distribution of fevipiprant in the presence of cyclosporine. In a rat ADME study with radiolabeled fevipiprant the liver showed the highest exposure to drug-related radioactivity (unpublished observations),

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suggesting that liver uptake may be a major determinant of the distribution of fevipiprant. This change in distribution points to inhibition of liver uptake by OATP1B3 as the predominant mechanism for the effect of cyclosporine.

While in the presence of cyclosporine, fevipiprant exposure was increased; little change in exposure to the AG metabolite was observed and consequently, the metabolite:fevipiprant ratio for  $AUC_{last}$  was decreased 2.5-fold from 1.5 to 0.59 by cyclosporine (Table 3). Again, this suggests that reduced uptake into the liver, the major site of fevipiprant glucuronidation, is the primary mechanism of the observed cyclosporine effect. Otherwise, the metabolite:fevipiprant ratio would not decrease so strongly, because glucuronidation, as a high-capacity system, is not easily saturated (Williams et al., 2004).

The renal clearance of oral fevipiprant and its AG metabolite were not affected by cyclosporine. However, because of the higher systemic exposure to fevipiprant in the presence of cyclosporine, renal excretion contributed 2.5-fold more to the elimination of fevipiprant (40.9% vs. 16.7% of the dose excreted into urine).

There was no apparent relationship between the change in oral fevipiprant  $C_{max}$  and trough concentrations of cyclosporine, i.e. within the covered range, higher cyclosporine trough concentrations were not linked to a stronger drug interaction (Figure 5). Therefore, the degree of drug interaction may not be greater at higher cyclosporine oral doses that provide larger exposures than investigated in this study.

The terminal half-life of the IV microdose could not be derived since concentrations dropped below the LLOQ of 20 pg/mL by 23 hours post dose for most profiles. Concentrations shortly after dosing were approximately 1000-fold above LLOQ, suggesting that most of the AUC of the

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IV microdose was captured (extrapolated fraction of  $AUC_{inf}$  based on mean data was  $\leq 6\%$  both with and without cyclosporine).

The disposition of transporter substrates such as fevipiprant is complex, making prospective predictions of exposure changes with inhibitors of transporter activity challenging (Poirier et al., 2009b; Poirier et al., 2009a; Jamei et al., 2014; Taskar et al., 2020). Part of the challenge is that active transport processes influence absorption, clearance and tissue distribution, making it difficult to derive clean PK input parameters for modeling from oral data only. Combined IV and oral data obtained in this study in the presence and absence of a transporter inhibitor allow differentiation between systemic and pre-systemic processes and provide more robust parameters. This supports the development of more reliable physiologically-based PK models. These can be used to predict untested case scenarios, such as transporter DDI effects at steady state, and in case of fevipiprant the impact of other OATP1B3 inhibitors on its PK.

There was no effect of fevipiprant 150 mg on the trough concentrations of cyclosporine, which is consistent with expectations, based on available drug interaction data for fevipiprant as a perpetrator (Poller et al., 2019). Overall, administration of fevipiprant alone and fevipiprant plus cyclosporine was well tolerated with no unexpected or novel AEs. Most AEs suspected to be drug-related were attributed to cyclosporine. These findings are consistent with previous studies showing that fevipiprant was safe and well tolerated at single and multiple oral doses up to 1800 mg/day (Erpenbeck, 2017). The exposure change observed in this study will guide fevipiprant dosing recommendations in presence of OATP1B3 inhibitors in the context of the safety profile of fevipiprant in any potential future drug label.

The addition of a labeled IV microdose in this study allowed investigation of the absolute bioavailability and absolute clearance without the need to conduct an IV toxicology program and

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a separate IV clinical trial. Conventionally, absolute bioavailability studies are crossover studies involving 6–12 participants; the microdose approach negates the need for a separate study with the further advantage that IV and oral dose data from the same participants at the same time avoids variability. Furthermore, use of a microdose simplifies formulation work because of the small amounts involved, and there are fewer potential safety concerns.

Concomitant oral dosing of unlabeled and IV microdosing of labeled compound in humans has previously been used to determine the absolute bioavailability, as well as the oral and IV PK of the HIV protease inhibitor nelfinavir (Sarapa et al., 2005), dabrafenib, a BRAF inhibitor (Denton et al., 2013), saxagliptin, a dipeptidyl peptidase-4 inhibitor, and dapagliflozin, a sodium glucose co-transporter-2 inhibitor (Boulton et al., 2013). In all these studies, the compound given as IV microdose was radiolabeled with  $^{14}\text{C}$  and quantified by accelerator mass spectrometry.

Furthermore, a double tracer technique using oral  $^{14}\text{C}$ -radiolabeled tofogliflozin simultaneously administered with  $^{13}\text{C}$ -stable isotope labeled tofogliflozin has been used successfully in an oral human ADME study to obtain additional information on the IV-PK of the compound (Schwab et al., 2013). IV microdosing for PK analysis has been accepted as a method by major health authorities (FDA Center for Drug Evaluation and Research, 2012; Boulton et al., 2013).

Administering the microdose after the oral dose circumvents the potential problem of PK non-linearity at microdose levels (Lappin et al., 2006) as the (labeled) microdose enters the body while the unlabeled compound given orally is present in the therapeutic drug concentration range. We observed little variability in the IV microdose data suggesting good data quality.

Analytical interferences between the unlabeled 1500-fold higher oral dose and the labeled microdose were avoided by careful selection of the positions of labeling (Figure 1) and the transitions used in the LC-MS/MS analyses. With the MS/MS transitions given in the

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experimental part, the spill-over of the unlabeled compound into the signal of the labeled compound was reduced to only 3 ppm of the unlabeled signal (Gu et al., 2012) which is negligible in the present context. The internal standard used in the LC-MS/MS analyses was fevipiprant labeled with five deuteriums and one  $^{13}\text{C}$  in the 4-methanesulfonyl-2-trifluoromethylbenzyl-part of the molecule; no interference with the IV-dosed labeled fevipiprant is expected.

In this study a single-sequence design without randomization was used. This is common in DDI studies and accepted by health authorities since the risk for bias of PK endpoints is considered small when investigated drugs do not display changes in clearance with time.

In conclusion, co-administration of cyclosporine increased oral fevipiprant  $\text{AUC}_{\text{inf}}$  and  $\text{C}_{\text{max}}$  by 2.35- and 3-fold, respectively. The use of an IV microdose allowed estimation of the absolute bioavailability of fevipiprant (0.3-0.4) and to differentiate between a small (approximately 1.2-fold) pre-systemic effect of cyclosporine and a larger (approximately two-fold) effect on systemic elimination of fevipiprant. The effect seems to be mainly by inhibition of OATP1B3-mediated hepatic uptake of fevipiprant. Additional mechanistic learnings from the IV data did not require any extra study participants, or a separate trial. Although significant DDI was observed, administration of fevipiprant alone and fevipiprant plus cyclosporine was well tolerated with no novel AEs observed.

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### **Data sharing statement**

Novartis will not provide access to patient-level data, if there is a reasonable likelihood that individual patients could be re-identified. Phase 1 studies, by their nature, present a high risk of patient re-identification; therefore, patient individual results for phase 1 studies cannot be shared. In addition, clinical data, in some cases, have been collected subject to contractual or consent provisions that prohibit transfer to third parties. Such restrictions may preclude granting access under these provisions. Where co-development agreements or other legal restrictions prevent companies from sharing particular data, companies will work with qualified requestors to provide summary information where possible.

### **Authorship contributions**

Markus Zollinger, Veit Erpenbeck, Meredith Cain, Janardhana Vemula, Walid Elbast and Markus Weiss participated in the research design. Walid Elbast conducted the experiments. Janardhana Vemula, Ken-Ichi Umehara and Markus Weiss performed data analysis. All authors contributed to the writing of the manuscript.

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## Footnotes

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## Legends for Figures

**Figure 1** Structure of fevipiprant showing the positions of the stable isotope-labeling ( $^{13}\text{C}_2^{15}\text{N}_2$ ) used for the IV microdosing

**Figure 2** Study design.

**Figure 3** Mean (SD) plasma concentration–time profiles of oral fevipiprant 150 mg in the presence (closed squares; n=13) and absence (open circles; n=16) of cyclosporine 175 mg b.i.d., linear view and semi-logarithmic view (inset).

**Figure 4** Mean (SD) plasma concentration–time profiles of labeled fevipiprant after IV dosing of 100  $\mu\text{g}$  in the presence (closed squares; n=13) and absence (open circles; n=16) of cyclosporine 175 mg b.i.d., linear view and semi-logarithmic view (inset).

**Figure 5** Scatter plot of oral fevipiprant ratio (Treatment Period 2/ Treatment Period 1) for  $C_{\text{max}}$  with different cyclosporine concentrations.

On the X-axis is the trough cyclosporine concentration on Day 17, before the co-administration with fevipiprant. Only study participants with data from both study periods are included.

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## Tables

**Table 1** Pharmacokinetic measures for oral fevipiprant with and without co-administration of cyclosporine

Measure (unit)	Fevipiprant 150 mg oral n=16	Cyclosporine 175 mg b.i.d. + fevipiprant 150 mg oral n=13
C <sub>max</sub> (ng/mL)	724 ± 207 (28.5) [n=16]	2270 ± 809 (35.7) [n=13]
AUC <sub>last</sub> (h*ng/mL)	3210 ± 601 (18.7) [n=16]	8110 ± 1980 (24.4) [n=13]
AUC <sub>inf</sub> (h*ng/mL)	3330 ± 718 (21.5) [n=15]	7900 ± 1980 (25.1) [n=10]
T <sub>max</sub> (h) <sup>#</sup>	1.50 (0.50–4.50) [n=16]	3.00 (0.92–4.50) [n=13]
CL/F (L/h)	47.0 ± 10.3 (22.0) [n=15]	20.2 ± 5.71 (28.2) [n=10]
Vz/F (L)	1090 ± 585 (53.6) [n=15]	446 ± 256 (57.4) [n=10]
T <sub>1/2</sub> (h)	17.5 ± 13.5 (77.1) [n=15]	14.9 ± 6.46 (43.4) [n=10]
CL <sub>r</sub> (L/h)	9.49 ± 1.25 (13.2) [n=16]	8.45 ± 2.39 (28.3) [n=13]

Data are arithmetic means ± SD (CV%) [n]. CV% = Coefficient of variation (%) = SD/mean\*100.

<sup>#</sup>For T<sub>max</sub>, data are median (min–max) [n].

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**Table 2** Pharmacokinetic measures for labeled IV fevipiprant with and without co-administration of cyclosporine

Measure (unit)	Fevipiprant 100 µg IV n=16	Cyclosporine 175mg b.i.d. + fevipiprant 100 µg IV n=13
C <sub>max</sub> (pg/mL) #	23600 ± 6030 (25.5) [n=16]	25000 ± 8500 (34.1) [n=13]
AUC <sub>last</sub> (h*pg/mL)	5040 ± 774 (15.3) [n=16]	10300 ± 2160 (20.9) [n=13]
AUC <sub>inf</sub> (h*pg/mL)	5360 ± 959 (17.9) [n=15]	10400 ± 2150 (20.7) [n=13]
CL (L/h)	19.2 ± 3.32 (17.3) [n=15]	9.97 ± 1.97 (19.7) [n=13]

Data are arithmetic means ± SD (CV%) [n]. CV% = Coefficient of variation (%) = SD/mean\*100

#T<sub>max</sub> was typically at the first sampling time i.e. 2 min (0.0330 hours) after IV dosing.

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**Table 3** Pharmacokinetic measures for the AG metabolite of fevipiprant (unlabeled, derived from oral fevipiprant) with and without co-administration of cyclosporine

Measure (unit)	Fevipiprant 150 mg oral n=16	Cyclosporine 175 mg BID + fevipiprant 150 mg oral n=13
C <sub>max</sub> (ng/mL)	1180 ± 333 (28.3) [n=16]	1330 ± 369 (27.7) [n=13]
AUC <sub>last</sub> (h*ng/mL)	6650 ± 1450 (21.8) [n=16]	6720 ± 1470 (21.9) [n=13]
M/P*	1.5	0.59
AUC <sub>inf</sub> (h*ng/mL)	6880 ± 1580 (22.9) [n=16]	6800 ± 1510 (22.3) [n=12]**
T <sub>max</sub> (h) <sup>#</sup>	2.0 (0.92–6.0) [n=16]	3.0 (1.5–4.5) [n=13]
T <sub>1/2</sub> (h)	18.9 ± 13.0 (68.9) [n=16]	13.3 ± 6.96 (52.4) [n=12]**
CL <sub>r</sub>	6.07 ± 1.02 L/h (16.7) [19.4]	6.29 ± 1.22 (19.4) [n=13]

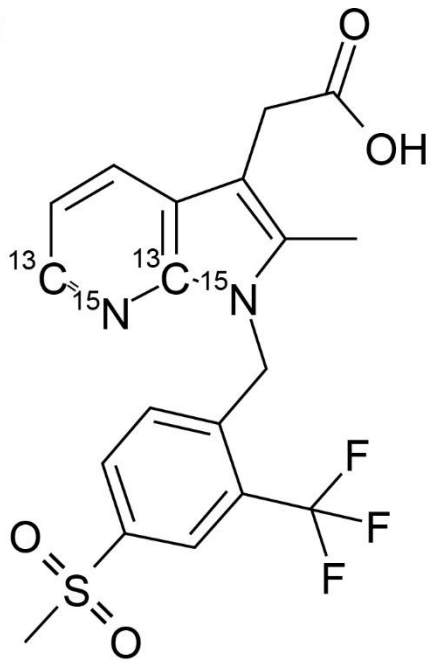
Data are arithmetic means ± SD (CV%) [n]; CV% = Coefficient of variation (%) = SD/mean\*100.

\* Molar metabolite-to-parent (M/P) ratio for mean AUC<sub>last</sub> considering the molecular weight difference (426 g/mol for fevipiprant and 602 g/mol for the AG metabolite). <sup>#</sup>For T<sub>max</sub>, data are median (min-max) [n]. \*\* AUC<sub>inf</sub> and T<sub>1/2</sub> were reported when R<sup>2</sup> (coefficient of determination in the regression analysis) adjusted value of the terminal elimination phase was > 0.75.

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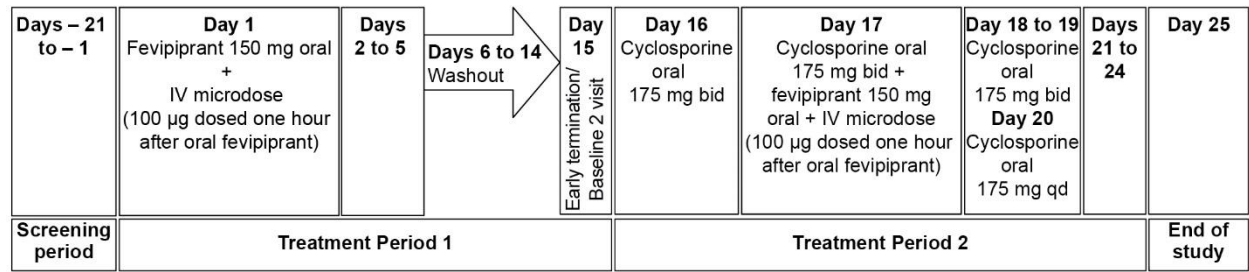
## Figures

Figure 1



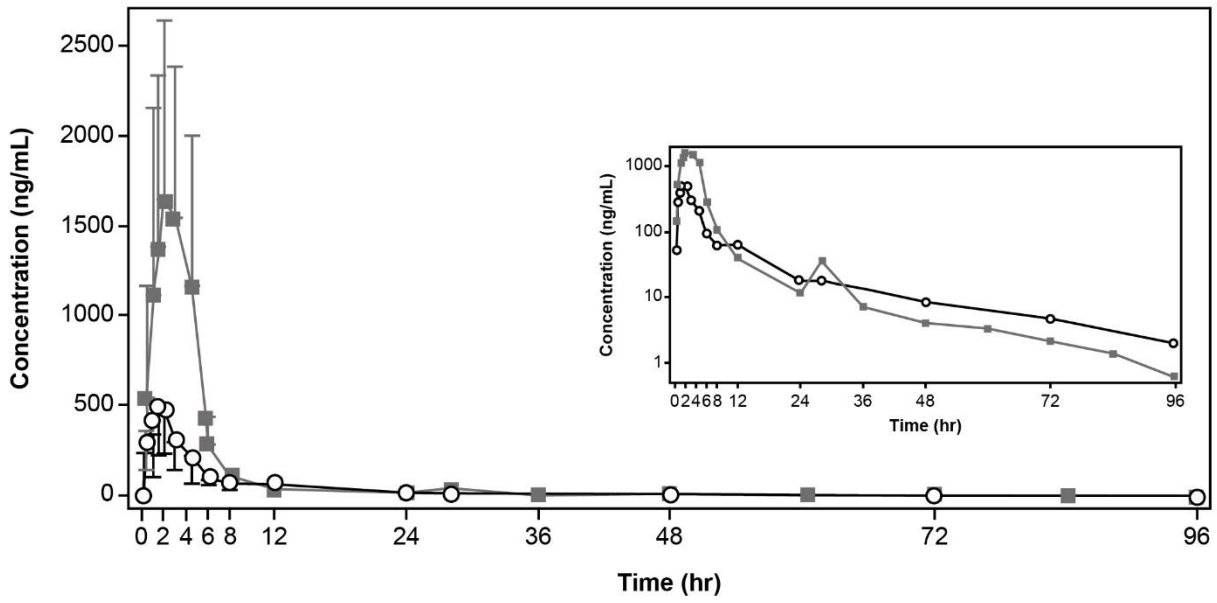
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**Figure 2**



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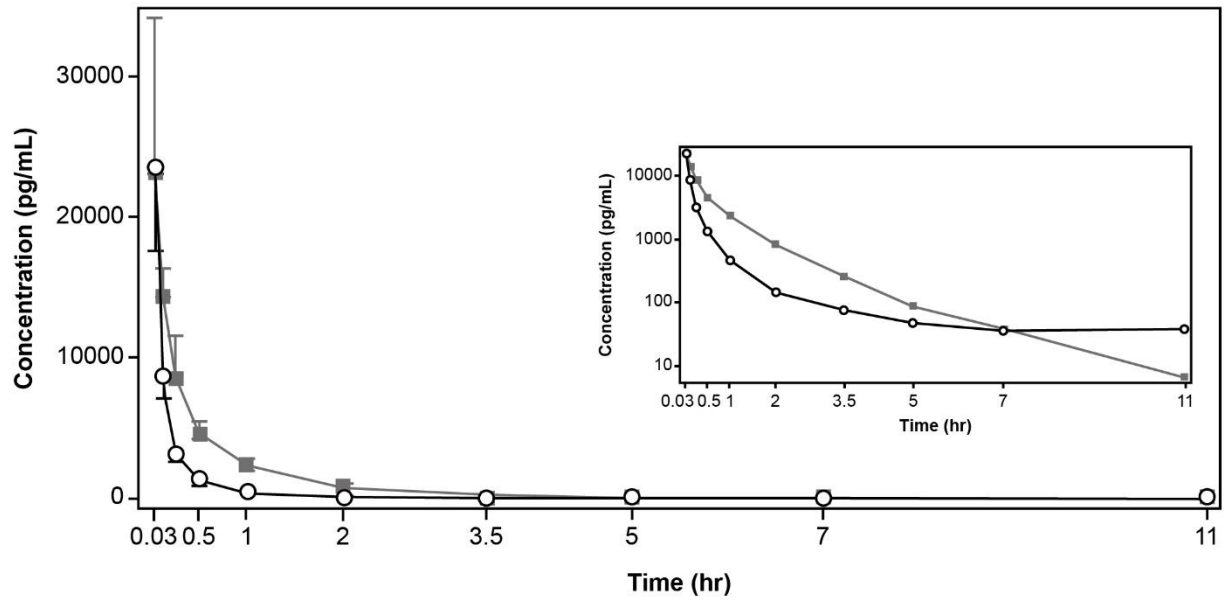
Figure 3





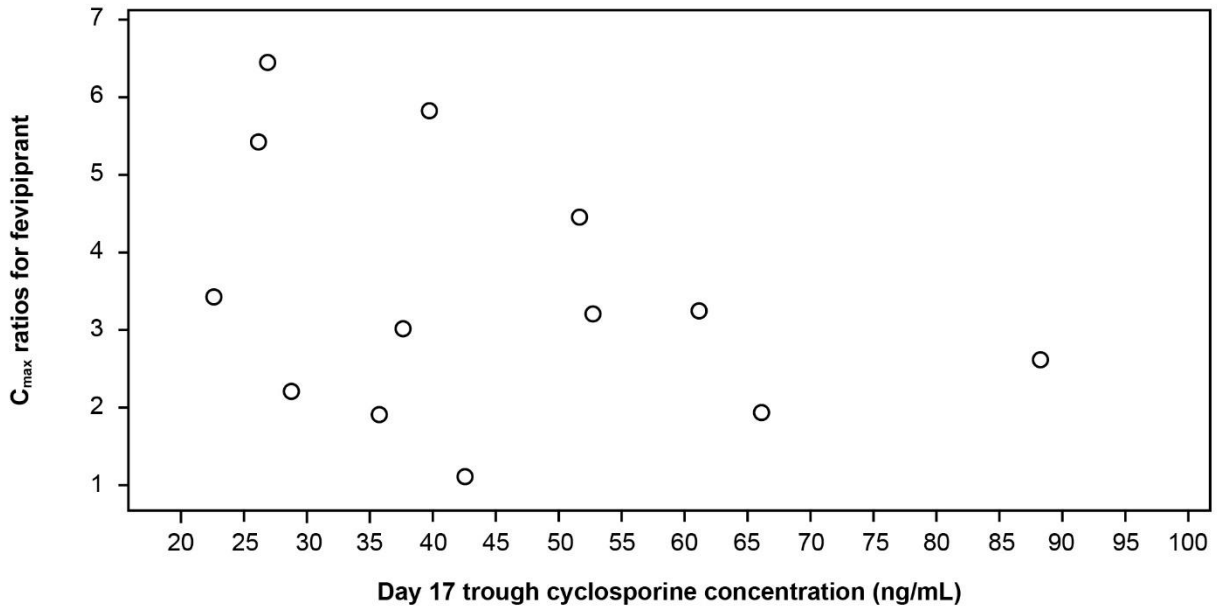
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Figure 4



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Figure 5



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**A study of the effect of cyclosporine on fevipiprant pharmacokinetics and its absolute bioavailability using an intravenous microdose approach**

**Authors:**

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Online data supplement

## Materials and methods

### *Study design*

The sample size (16 participants so that at least 12 would complete the study, allowing for a 20% dropout rate) was selected to control the width of the confidence interval for the geometric mean ratio for fevipirant area under the curve (AUC) and maximum plasma concentration ( $C_{max}$ ) when given with and without cyclosporine. A 2-3 fold increase in AUC was expected. The relative half-width of the confidence interval was the upper limit of the 90% confidence interval for the geometric mean divided by the estimated geometric mean. With the chosen sample size of 12 completers and assuming an intra-subject (coefficient of variation) CV 20% for AUC based on previous clinical experience, the relative half-width is 1.14 (0.133 in log scale). The lower and upper 90% confidence intervals (CI) for the geometric mean ratio was evaluated as geometric mean ratio\*exp(+/-0.133). The 90% CI for different geometric mean ratios for pharmacokinetic (PK) parameters are shown in the table below.

**Table** Expected upper and lower 90% CI for different geometric mean ratios with sample size of 12 completers

<b>Geometric mean ratio</b>	<b>Lower 90% CI</b>	<b>Upper 90% CI</b>
2	1.75	2.28
2.5	2.19	2.86
3	2.63	3.43

### *Inclusion criteria*

Subjects eligible for inclusion in this study had to fulfill all of the following criteria:

- Written informed consent was obtained before any assessment was performed.
- Healthy male and female subjects 18 to 55 years of age (inclusive), and in good health as determined by past medical history, physical examination, vital signs, electrocardiogram, and laboratory tests at screening and/or first baseline visit as indicated.
- Subjects were required to weigh between 60 and 90 kg (inclusive) to participate in the study, and were required to have a body mass index (BMI) within the range of 20 - 30 kg/m<sup>2</sup>. BMI = Body weight (kg) / [Height (m)]<sup>2</sup>.
- Subjects had to be able to communicate well with the investigator, and were able to understand and comply with the requirements of the study.
- At screening and first baseline visit, vital signs (systolic and diastolic blood pressure and pulse rate) were assessed in the sitting position after the participant had rested for at least three minutes, and again after three minutes in the standing position. Sitting vital signs were required to be within the following ranges:
  - oral body temperature between 35.0-37.5 °C
  - systolic blood pressure, 90-139 mm Hg
  - diastolic blood pressure, 50-89 mm Hg
  - pulse rate, 40 - 90 bpm

If vital signs were out-of-range, the Investigator was permitted to obtain two additional readings for the respective parameter(s) so that up to three consecutive assessments were made, with the participant seated quietly for approximately five minutes preceding each repeat assessment. At least the last reading was required to be within the ranges provided above in order for the participant to qualify. Subjects were excluded if their

standing vital signs (relative to sitting) showed findings, which, in the opinion of the Investigator, were associated with clinical manifestation of postural hypotension (i.e. absence of any other cause). The Investigator carefully considered enrolling subjects with either a > 20 mm Hg decrease in systolic or a >10 mm Hg decrease in diastolic blood pressure, accompanied by a > 20 bpm increase in heart-rate (comparing standing to sitting results).

#### *Exclusion criteria*

Subjects fulfilling any of the following criteria were not eligible for inclusion in this study:

- Use of other investigational drugs at the time of enrollment, or within 5 half-lives of initial dosing, or within 30 days of initial dosing, whichever was longer; or longer if required by local regulations
- History of clinically significant electrocardiogram (ECG) abnormalities, or any of the following ECG abnormalities at screening or first baseline visit:
  - PR > 200 msec
  - QRS complex > 120 msec
  - QTcF > 450 msec (males)
  - QTcF > 460 msec (females)
- History or presence of long QT syndrome or other clinically significant ECG abnormalities, e.g. arrhythmia or tachycardia.
- History or presence of malignancy of any organ system, treated or untreated.
- Pregnant or nursing (lactating) women, where pregnancy was defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test.
- Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant. Women were considered post-menopausal and not of child-bearing

potential if they had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least six weeks prior to screening. In the case of oophorectomy alone, only when the reproductive status of the woman was been confirmed by follow up hormone level assessment was she considered not of child-bearing potential.

- Smokers (use of tobacco products in the previous three months). Smokers were defined as any subject who reported tobacco use and/or who had a urine cotinine  $\geq 500$  ng/ml at screening or first baseline.
- Usage of any prescription drugs and/or herbal supplements within four weeks prior to initial dosing, and/or over-the-counter (OTC) medication, dietary supplements (vitamins included) within two weeks prior to initial dosing. If needed, (i.e. an incidental and limited need) acetaminophen, was acceptable, but was required to be documented in the Concomitant medications / Significant non-drug therapies page of the CRF.
- Donation or loss of 400 mL or more of blood within eight weeks prior to initial dosing, or longer if required by local regulation.
- Plasma donation within four weeks prior to initial dosing.
- Hemoglobin levels below normal ranges of local laboratory for males and females, respectively at screening and first baseline visit.
- Significant illness or infection which was not resolved within two weeks prior to initial dosing.
- Recent (within the last three years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
- History of multiple and recurring allergies or allergies/hypersensitivities or allergy/hypersensitivity to the investigational compounds/compound class being used in



this study (e.g. cyclosporine or any of its ingredients and DP<sub>2</sub> antagonists).

- History of any food allergies.
- Any surgical or medical condition which significantly altered the absorption, distribution, metabolism, or excretion of drugs, or which jeopardized the subject in case of participation in the study. The Investigator was required to make this determination in consideration of the subject's medical history and/or clinical or laboratory evidence of any of the following:
  - Inflammatory bowel disease, peptic ulcers, gastrointestinal including rectal bleeding within 12 months prior to screening;
  - History of major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection;
  - Pancreatic injury or pancreatitis within 12 months prior to screening;
  - Liver disease or liver injury as indicated by abnormal liver function tests. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GT), alkaline phosphatase and serum bilirubin were tested.
  - ALT or AST > 2.0 X the upper limit of normal (ULN) or total bilirubin > 1.3 X ULN at screening or at the first baseline visit
  - γ-GT or alkaline phosphatase >1.5 X ULN at screening or at the first baseline visit
  - Any elevation above ULN of more than one parameter of ALT, AST, γ-GT, alkaline phosphatase or serum bilirubin at screening or at the first baseline visit excluded a subject from participation in the study

If necessary, laboratory testing was to be repeated on one occasion (as soon as possible) prior to randomization, to rule out any laboratory error.

- History or presence of impaired renal function as indicated by clinically significantly abnormal creatinine or blood urea nitrogen (BUN) and/or urea values, or abnormal urinary constituents (e.g., albuminuria)
- Evidence of urinary obstruction or difficulty in voiding at screening or at the first baseline visit
- History of immunodeficiency diseases or active disease, including a positive HIV (e.g., chemiluminescence assay and MultiSpot) test result.
- A positive Hepatitis B surface antigen or Hepatitis C test result.
- Any vaccination with live-attenuated vaccines within two months prior to screening.
- History of drug or alcohol abuse within the 12 months prior to dosing, or evidence of such abuse as indicated by the laboratory assays conducted during screening and first baseline visit.

No additional exclusions were applied by the investigator, in order to ensure that the study population was representative of all eligible subjects.

#### *Pharmacokinetic analyses*

At each collection time 1.5 mL of plasma (from 3 mL of blood collected with K3EDTA as anticoagulant) was transferred into a VACUETTE FC Mixtube for acidification to avoid back-conversion of the AG metabolite to fevipirant. Two aliquots were frozen and stored at  $\leq -70^{\circ}\text{C}$  until analysis. From the 24 h urine pools 1 mL aliquots were transferred into a VACUETTE FC Mixtubes for acidification and then frozen and stored at  $\leq -70^{\circ}\text{C}$  until analysis.

In Treatment Period 1 blood was collected for PK analysis of oral fevipirant on at 0h, 0.25h, 0.5h, 1h, 1.5h 2h 3h 4.5h 6h 8h 12h post dose on Day 1, 24h and 28h post dose (Day 2), 48h post dose (Day 3) and 72h post dose (Day 4) and on Day 5. PK blood collection for the fevipirant IV microdose (dosed 1 h after the oral dose) was collected at 0.03h, 0.12h, 0.5h 1h

2h 3.5h 5h 7h 11h post IV dose on Day 1, 23h and 27h post dose on Day 2, 47h post dose on Day 3 and 71h post dose on Day 4 and 95 h post dose on Day 5. Urine collection for PK analysis took place on Day 1 (24 h pool).

In Treatment Period 2 blood was collected for PK analysis of oral fevipiprant on at 0h, 0.25h, 0.5h, 1h, 1.5h, 2h, 3h, 4.5h, 6h, 8h, 12h post dose on Day 17, 24h, 28h, 36h post dose on Day 18 and at 48h and 60h post dose on Day 19, and 72h and 84h post-dose on Day 20 and at 96h post-dose on Day 21. PK blood collection for the fevipiprant IV microdose (dosed 1 h after the oral dose) was collected at 0.03h, 0.12h, 0.5h 1h 2h 3.5h 5h 7h 11h post dose on Day 17, 23h, 27h, 35h post dose (Day 18) and at 47h and 59h post-dose on Day 19, and 71h and 83h post-dose on Day 20 and at 95h post-dose on Day 21. PK blood collection for cyclosporine took place pre cyclosporine dose on Day 16 (evening dose only), Days 17 and 18 both doses. Urine collection for PK analysis took place on Day 17 (24 h pool).

#### *LC-MS/MS materials and methods for oral fevipiprant and its AG metabolite*

Samples were prepared as follows: 50 µL of plasma sample were added and mixed with 120 µL of water 2% acetic acid. The mixture was loaded on Isolute SLE-96 well plate (200 mg). The plate was then eluted twice with 300 µL of tert-butyl-methyl ether (MTBE). The solvent was evaporated to dryness under nitrogen stream at a temperature of 50°C. The dry residue was reconstituted with 250 µL of 0.2% formic acid in methanol/water (50/50; v/v). The plate was sealed, mixed and then centrifuged for 5 min at a temperature of 4°C. A volume of 10 µL was injected into the LC-MS/MS system.

Lower limit of quantification (LLOQ), and inter- and intra-day variability were: 1.0 ng/mL (LLOQ) and 400 ng/mL (upper limit of quantification; ULOQ) for fevipiprant and 0.480 ng/mL (LLOQ) and 192 (ULOQ) ng/mL for the AG metabolite.

Sample analysis was performed on a LC-MS/MS system consisting of an API 4000 triple quadrupole mass spectrometer equipped with a TurbolonSpray™ interface (Applied Biosystems). The MS system was connected to a LC-20AC XR auto-sampler (Shimadzu) and to a LC-20AD XR pump (Shimadzu). Chromatographic separations were performed at a flow rate of 0.500 mL/min on Zorbax Rapid Resolution HT SB-C<sub>18</sub> column (50 mm × 2.1 mm, 1.8 μm). A binary gradient with a mobile phase consisting of 0.2% formic acid in water (A) and methanol (B) was used for the LC-separation. The elution gradient program was as follows: time (min), (% mobile phase B): (0, 20) (2.5, 70) (2.6, 95) (3.0, 95) (3.1, 20) (4.25, 20). The column temperature was maintained at 60°C using a column heater. The system was operated in electrospray positive ionization using MRM mode. The other MS conditions were as follows: turbo ion spray 5500 V; source temperature 650°C; collision activated dissociation 8; curtain gas 10 psi; Gas1 60 psi; Gas2 40 psi; entrance potential 12 V (compound-1 and [internal standard] ISTD); dwell time 60 ms (compound-1 and ISTD); collision energy 60 eV (fevipirant and ISTD) and 36 eV (AG metabolite and ISTD); declustering potential 94 V, collision cell exit potential 11 V (fevipirant and ISTD) and 25 eV (AG metabolite and ISTD).

#### *Statistical analysis of secondary endpoints*

For fevipirant and AG metabolite PK data and parameters, descriptive summary statistics were provided by treatment and visit/sampling time point. Summary statistics included mean (arithmetic and geometric), SD, CV (arithmetic and geometric), median, minimum and maximum. Concentrations below LLOQ were treated as zero in summary statistics. A geometric mean was not reported if the dataset included zero values.

## **Results**

**Table S1** Mean concentration-time data for oral fevipiprant and its AG metabolite without cyclosporine and with cyclosporine

	Sampling Time	Without cyclosporine		With cyclosporine 175 mg	
		Fevipiprant (ng/mL)	AG metabolite (ng/mL)	Fevipiprant (ng/mL)	AG metabolite (ng/mL)
Fevipiprant 150 mg oral + fevipiprant 100µg IV		(n = 16)	(n = 16)	(n = 13)	(n = 13)
	0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.0473 (0.170)
	0.25	52.9 (118)	2.36 (4.29)	146 (218)	8.42 (11.1)
	0.5	289 (324)	90.3 (126)	540 (632)	152 (187)
	1	416 (312)	429 (436)	1120 (1030)	588 (506)
	1.5	492 (272)	814 (540)	1380 (953)	820 (612)
	2	495 (267)	907 (488)	1660 (990)	934 (588)
	3	306 (164)	791 (261)	1550 (838)	1010 (472)
	4.5	214 (153)	580 (354)	1170 (824)	988 (317)
	6	95.2 (38.2)	297 (139)	286 (148)	421 (197)
	8	63.2 (23.2)	159 (54.9)	109 (41.8)	170 (64.2)
	12	64.2 (21.6)	106 (28.1)	39.7 (19.1)	66.7 (27.2)
	24	18.0 (9.12)	43.3 (13.1)	11.6 (3.42)	22.5 (5.76)
	28	18.4 (8.02)	44.4 (19.9)	36.4 (12.7)	30.5 (10.3)
	36	–	–	7.16 (2.68)	12.9 (4.88)

48	8.41 (5.15)	18.2 (10.0)	4.06 (1.67)	7.45 (4.16)
60	–	–	3.33 (2.74)	4.91 (4.31)
72	4.69 (5.26)	9.85 (11.1)	2.14 (1.74)	3.88 (3.58)
84	–	–	1.37 (2.79)	2.46 (4.58)
96	1.96 (2.98)	4.52 (6.18)	0.601 (1.80)	1.52 (3.53)

Data are mean  $\pm$  SD

**Table S2** Geometric mean ratio (test/reference) and 90% confidence intervals for PK parameters of oral fevipiprant

Parameter	Treatment	n*	Adjusted Geometric mean	Comparison	Treatment Comparison#	
					Geometric mean ratio	(90% CI)
C <sub>max</sub> (ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	2100.93	Test vs Reference	3.02	(2.38, 3.82)
	Fevipiprant 150 mg oral and 100 µg IV	16	696.76			
AUC <sub>last</sub> (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	7885.36	Test vs Reference	2.50	(2.17, 2.88)
	Fevipiprant 150 mg oral and 100 µg IV	16	3155.39			
AUC <sub>inf</sub> (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	10	7659.86	Test vs Reference	2.35	(1.99, 2.77)
	Fevipiprant 150 mg oral and 100ug IV	15	3263.71			

Test: Cyclosporine 175mg b.i.d + fevipiprant 150mg oral and 100 µg IV; Reference: Fevipiprant 150mg oral and 100 µg IV.#Geometric mean ratio and 90% CI are back transformed from log scale. \*Number of evaluable subjects.

**Table S3** Mean concentration-time data for fevipirant 100 µg IV without and with cyclosporine

		Fevipirant 100µg IV (pg/mL)	
Scheduled Sampling		Without cyclosporine	With cyclosporine 175
Time (h)			mg b.i.d.
Fevipirant 100µg IV		(n = 16)	(n = 13)
	0.03	23600 (6030)	23200 (11000)
	0.12	8660 (1490)	14400 (1970)
	0.25	3240 (516)	8720 (2890)
	0.5	1350 (348)	4700 (789)
	1	480 (124)	2350 (551)
	2	146 (39.4)	841 (313)
	3.5	77.2 (23.9)	270 (115)
	5	48.2 (11.9)	89.4 (34.8)
	7	36.3 (8.26)	39.3 (12.2)
	11	39.6 (15.9)	6.65 (13.0)
	23	2.01 (8.03)	0.00 (0.00)
	27	4.34 (9.34)	3.99 (9.77)
	35	–	0.00 (0.00)
	47	0.00 (0.00)	0.00 (0.00)
	59	–	0.00 (0.00)
	71	0.00 (0.00)	0.00 (0.00)
	83	–	0.00 (0.00)
	95	0.00 (0.00)	0.00 (0.00)

Data are mean ± SD



**Table S4** Geometric mean ratio (test/reference) and 90 percent confidence intervals for PK parameters of IV administered fevipiprant ( $[^{13}\text{C}_2^{15}\text{N}_2]$ fevipiprant)

Parameter	Treatment	n*	Adjusted Geometric mean	Comparison	Treatment Comparison#	
					Geometric mean ratio	(90% CI)
C <sub>max</sub> (pg/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150mg oral and 100 µg IV	13	23780.39	Test vs Reference	1.04	(0.86, 1.25)
	Fevipiprant 150mg oral and 100 µg IV	16	22908.72			
AUC <sub>last</sub> (h*pg/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	10201.76	Test vs Reference	2.04	(1.83, 2.28)
	Fevipiprant 150 mg oral and 100 µg IV	16	4989.97			
AUC <sub>inf</sub> (h*pg/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150mg oral and 100 µg IV	13	10395.08	Test vs Reference	1.95	(1.76, 2.16)
	Fevipiprant 150mg oral and 100 µg IV	15	5340.58			

Test: Cyclosporine 175mg b.i.d + fevipiprant 150mg oral and 100 µg IV; Reference: Fevipiprant 150mg oral and 100 µg IV. #Geometric mean ratio and 90% CI are back transformed from log scale. \*Number of evaluable subjects.

**Table S5** Geometric mean ratio (test/reference) and 90% confidence intervals for PK parameters for the AG metabolite

Parameter	Treatment	n*	Adjusted Geometric mean	Comparison	Treatment Comparison#	
					Geometric mean ratio	(90% CI)
C <sub>max</sub> (ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	1314.91	Test vs Reference	1.16	(1.01, 1.35)
	Fevipiprant 150 mg oral and 100 µg IV	16	1129.80			
AUC <sub>last</sub> (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	13	6634.48	Test vs Reference	1.02	(0.92, 1.13)
	Fevipiprant 150 mg oral and 100 µg IV	16	6506.48			
AUC <sub>inf</sub> (h*ng/mL)	Cyclosporine 175 mg b.i.d + Fevipiprant 150 mg oral and 100 µg IV	11	6697.04	Test vs Reference	1.00	(0.88, 1.12)
	Fevipiprant 150 mg oral and 100ug IV	16	6713.60			

Test: Cyclosporine 175mg b.i.d. + Fevipiprant 150mg oral and 100 µg IV. Reference: Fevipiprant 150mg oral and 100ug IV. n\* = Number of evaluable subjects data considered.  
 #Geometric mean ratio and 90% CI are back transformed from log scale.

**Table S6** Incidence of adverse events by preferred term (Safety analysis set)

<b>EPOCH</b>	<b>Treatment 1</b>	<b>Treatment 2</b>	<b>All treatments</b>	
	Fevipirant 150mg oral and 100ug IV	Cyclosporine 175mg b.i.d	Cyclosporine 175mg b.i.d + Fevipirant 150mg oral and 100ug IV.	
	N=16	N=13	N=13	N=16
Preferred term	n (%)	n (%)	n (%)	n (%)
Number of subjects with at least one AE	5 (31.3)	1 (7.7)	5 (38.5)	9 (56.3)
Headache	0	0	3 (23.1)	3 (18.8)
Nausea	0	1 (7.7)	1 (7.7)	2 (12.5)
Alanine aminotransferase increased	1 (6.3)	0	0	1 (6.3)
Back pain	0	0	1 (7.7)	1 (6.3)
Catheter site hematoma	1 (6.3)	0	0	1 (6.3)
Dermatitis contact	1 (6.3)	0	0	1 (6.3)
Dizziness postural	1 (6.3)	0	0	1 (6.3)
Dysgeusia	0	0	1 (7.7)	1 (6.3)
Flushing	0	0	1 (7.7)	1 (6.3)

Muscle fatigue	0	0	1 (7.7)	1 (6.3)
Pharyngitis	0	0	1 (7.7)	1 (6.3)
Upper respiratory tract infection	0	0	1 (7.7)	1 (6.3)
Urethritis chlamydial	0	0	1 (7.7)	1 (6.3)
Vessel puncture site pain	1 (6.3)	0	0	1 (6.3)
Vomiting	0	0	1 (7.7)	1 (6.3)

n = number of subjects with at least one AE (preferred term); N = number of subjects studied within treatment. A participant with multiple AEs is counted only once in the “at least one AE” row. A participant with multiple AEs with the same preferred term is counted only once for that preferred term and treatment. Preferred terms are sorted in descending frequency, as reported in the “All treatments” column. An AE starting in one treatment and continuing into the next treatment is counted in the earlier treatment only. In Treatment 2, an AE starting before combination (cyclosporine + fevipirant) treatment is counted under cyclosporine treatment. All other AEs are counted under combination (cyclosporine + fevipirant) treatment.  
 AE: adverse event