Regulatory guidelines do not accurately predict tolvaptan and metabolite interactions at BCRP, OATP1B1, and OAT3 transporters

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Abstract
Tolvaptan (TLV) was US Food and Drug Administration (FDA)-approved for the indication to slow kidney function decline in adults at risk of rapidly progressing autosomal dominant polycystic kidney disease in 2018. In vitro, TLV was a breast cancer resistance protein (BCRP) inhibitor, whereas the oxobutyric acid metabolite of TLV (DM-4013) was an inhibitor of organic anion transport polypeptide (OATP)1B1 and organic anion transporter (OAT)3. Based on the 2017 FDA guidance, potential for clinically relevant inhibition at these transporters was indicated for the highest TLV regimen. Consequently, two postmarketing clinical trials in healthy subjects were required. In trial 1, 5 mg rosuvastatin calcium (BCRP and OATP1B1 substrate) was administered alone, with 90 mg TLV or 48 h following 7 days of once daily 300 mg TLV (i.e., in the presence of DM-4103). In trial 2, 40 mg furosemide (OAT3 substrate) was administered alone and in presence of DM-4103. For BCRP, rosuvastatin geometric mean ratios (90% confidence intervals [CIs]) for maximum plasma concentration (Cmax) were 1.54 (90% CI 1.26–1.88) and for area under the concentration-time curve from time 0 to the time of the last measurable concentration (AUCt) were 1.69 (90% CI 1.34–2.14), indicating no clinically significant interaction. DM-4103 produced no clinically meaningful changes in rosuvastatin or furosemide concentrations, indicating no inhibition at OATP1B1 or OAT3. The BCRP prediction assumed the drug dose is completely soluble in 250 ml; TLV has solubility of ~0.01 g/250 ml. For OATP1B1/OAT3, if fraction unbound for plasma protein binding (PPB) is less than 1%, then 1% is assumed. DM-4103 has PPB greater than 99.8%. Use of actual drug substance solubility and unbound fraction in plasma would have produced predictions consistent with the clinical results.

Study Highlights
WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
The US Food and Drug Administration (FDA) created a guidance for estimating the potential for clinically relevant drug-drug interactions (DDIs) at BCRP, OATP1B1,
INTRODUCTION

Tolvaptan (TLV) is a selective vasopressin receptor (V₂) antagonist that has been shown to be effective for the treatment of rapidly progressing autosomal dominant polycystic kidney disease (ADPKD). The FDA required postmarketing clinical trials following the approval of TLV for the treatment of ADPKD to assess the potential for interaction at breast cancer resistance protein (BCRP), organic anion transport polypeptide (OATP)₁B₁, and organic anion transporter (OAT)₃ transporters.

The highest approved dose regimen is 90 mg in the morning followed by a 30 mg dose given 8–9 h later. The half-life of TLV is about 3.5 h so there is no accumulation and steady-state concentrations are achieved on day 1. TLV has pH independent solubility of about 0.01 g/250 ml. However, TLV tablets contain TLV formulated using a spray-dried technique, which may increase TLV in vitro solubility up to sevenfold. The oxobutyric acid metabolite of TLV (DM-4103) is formed by CYP3A4-mediated metabolism of another TLV metabolite; consequently, plasma concentrations are less than 100 ng/ml for the first 2 h following a 90 mg dose (data on file). The elimination half-life is ~180 h so steady-state concentrations are achieved ~8 weeks after the start of TLV dosing. In vitro studies indicated that TLV is a BCRP inhibitor, whereas the oxobutyric acid metabolite (DM-4013) is an inhibitor of OATP₁B₁ and OAT3.

In 2020, the FDA finalized a guidance document intended to help drug developers determine the in vivo drug-drug interaction (DDI) potential of an investigational drug product. The earlier draft version released in 2017 contained almost identical language with regard to the prediction of the potential for in vivo inhibition at BCRP, OATP₁B₁, and OAT3 transporters. The FDA guidance is also consistent with guidelines published by the European Medicines Agency in 2013 and the Ministry of Labor and Welfare in Japan in 2018.

Based on the FDA website “Drug Development and Drug Interactions; Table of Substrates, Inhibitors and Inducers,” rosvuastatin is considered to be a suitable substrate for use in clinical DDI studies for determining interactions at BCRP and OATP₁B₁. Other statins are also known to be substrates of OATP₁B₁. Furosemide is considered to be a suitable substrate for use in clinical DDI studies for determining interactions at OAT3.

In the phase III trials for ADPKD, statin use was unrestricted and no differences in statin use (duration, dose change, statin change, and permanent discontinuation) or incidences of statin-related adverse events were observed for subjects taking TLV alone or taking TLV plus statins (atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, and simvastatin/ezetimibe). In phase II and III trials for congestive heart failure, although tested daily TLV doses may have been lower and treatment durations shorter than those used in ADPKD trials, DM-4103 concentrations at the minimum level predicted to be inhibitory at OAT3 were achieved (data on file). Furosemide was used by greater than 80% of congestive heart failure subjects enrolled in TLV clinical trials (data on file) and the incidence of adverse events was similar for subjects taking TLV + furosemide compared with placebo + furosemide. Despite the lack of an interaction based on clinical data, the FDA required postmarketing clinical trials.

The primary objective of trial 1 was to determine the potential inhibitory effect of TLV on the BCRP transporter substrate (i.e., rosvuastatin), and the potential inhibitory effect of the TLV DM-4103 metabolite on an OATP₁B₁ transporter substrate (i.e., rosvuastatin). The primary objective of trial 2

OAT3, and other transporters. The predictions use various assumptions. For example, for BCRP, it is assumed that inhibitory drug is completely soluble in 250 ml. For OATP₁B₁ and OAT3, if plasma protein binding for inhibitor is greater than 99%, then fraction unbound is set to 1%.

WHAT QUESTION DID THIS STUDY ADDRESS?

These studies addressed if the predictions for clinically relevant DDIs at BCRP for tolvaptan and at OATP₁B₁ and OAT3 for the oxobutyric acid metabolite were correct and interactions were observed.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

As there were no clinically relevant interactions, the results support the consideration of drug solubility for BCRP and actual plasma protein binding for OATP₁B₁ and OAT3 in the predictions of clinically relevant DDIs.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Assumptions used in the prediction of clinically relevant DDIs would be revised.
was to determine the potential inhibitory effect of DM-4103 on an OAT3 substrate (i.e., furosemide).

**METHODS**

**Transporter assays**

BCRP: Prazosin transport was evaluated in MDCKII BCRP expressing cells (SOLVO Biotechnology) precultured for 6–8 days. Mannitol transport was unaffected by TLV at 10 µM. Ko143 hydrate, a BCRP inhibitor, decreased prazosin transport by 96.8%. TLV concentrations of 0.10, 0.30, 1.0, 3.0, and 10 µM were tested.

OAT3: Estrone 3-sulfate transport was evaluated in HEK293 cells stably expressing human OAT3 (kit with ready to use 24-well plates were obtained from GenoMembrane). Probenecid, 30 µM, inhibited estrone 3-sulfate transport by 91.1%. DM-4103 concentrations of 0.064, 0.32, 1.6, 8.0, 40, and 200 µM were tested.

OATP1B1: Estradiol 17β-D-glucuronide transport was evaluated in HEK293 cells stably expressing human OATP1B1 (kit with ready to use 24-well plates were obtained from GenoMembrane). Rifampicin, 10 µM, inhibited estradiol 17β-D-glucuronide transport by 91.1%. DM-4103 concentrations of 0.064, 0.32, 1.6, 8.0, 40, and 200 µM were tested.

**Informed consent and ethics**

These trials were conducted in compliance with FDA regulations, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use—Good Clinical Practice (ICH GCP) Guideline (E6), and international ethical principles derived from the Declaration of Helsinki and Council for International Organizations of Medical Science (CIOMS) guidelines.

The protocols for both trials were approved by the Advarra Institutional Review Board (Columbia, MD); subjects were treated at Pharmaceutical Research Associates, Inc. (Salt Lake City, UT).

**Trial designs**

Figure 1 highlights the treatments administered in each trial and the comparisons that were made.

**Trial 1**

Trial 1 was designed as an open-label, 3-period, sequential crossover. Screening was conducted in the 28 days prior to dosing. Subjects checked into the clinic on day −1 and remained in-clinic for the duration of the study. On day 1, 5 mg rosuvastatin calcium was administered alone. On day 4, 5 mg rosuvastatin calcium was co-administered with 90 mg TLV. On days 7 to 13, 300 mg TLV was administered q.d. On day 15, 5 mg rosuvastatin calcium was administered alone. Subjects were discharged from the clinic on day 18 and a follow-up telephone call was conducted on day 22.

Doses on pharmacokinetic (PK) sampling days were administered in the fasted state with 240 ml of room temperature still water. No food was allowed from 10 h prior to dosing until lunch was given following the 4-h postdose assessments; water was available ad libitum except for +/− 2 h around dosing. On non-PK sampling days, TLV was administered at least 30 min prior to breakfast. Safety was evaluated by laboratory values, vital signs, and 12-lead electrocardiogram (ECG) at screening, day −1, and discharge (no ECG), and adverse event reports.

The 5 mg dose of rosuvastatin calcium was chosen based on the high predicted value for a BCRP interaction and that increases in rosuvastatin maximal peak plasma concentration

![Diagram of treatments administered and comparisons](image_url)
(C_{\text{max}}) up to 11-fold have been observed.\textsuperscript{16} A 90-mg dose of TLV was chosen as it is the highest dose administered at any given time in the treatment of ADPKD. The FDA required that DM-4103 concentrations tested in this trial be similar to those observed at steady-state in ADPKD trials (~7500 ng/ml), therefore, TLV doses of 300 mg were administered for 7 days. Previously, 300 mg TLV administered for 5 days to healthy subjects was shown to be safe and well-tolerated.\textsuperscript{17} After 48 h, TLV would be completely eliminated due to its short half-life, leaving only DM-4103 concentrations present in the plasma.

**Trial 2**

Trial 2 was designed as an open-label, 2-period, sequential crossover. Screening was conducted in the 28 days prior to dosing. Subjects checked into the clinic on day −1 and remained in-clinic for the duration of the study. On day 1, 40 mg furosemide was administered alone. On days 2 to 8, 300 mg TLV was administered q.d. On day 10, 40 mg furosemide was administered alone. Subjects were discharged from the clinic on day 11 and a follow-up telephone call was conducted on day 17.

Treatments were administered and safety assessed as described for Trial 1.

A 40 mg dose of furosemide was chosen as it was expected that furosemide concentrations would be measurable up to 24 h and that this dose would have at least a 2-fold safety margin, as up to 80-mg doses have been administered safely to healthy subjects.\textsuperscript{18}

**Sample collection**

For trial 1, blood samples for rosuvastatin (4 ml using dipotassium ethylenediaminetetraacetic acid [K\textsubscript{2}EDTA] as anticoagulant) were taken at predose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, 56, and 72 h postdose on days 1, 4, and 15. Samples for TLV (4 ml with sodium heparin as anticoagulant) were taken at 2 and 3 h postdose on day 4. Samples for DM-4103 (4 ml with sodium heparin as anticoagulant) were taken at predose and 24, 48, 72, 96, and 120 h postdose on day 13 (7th day of dosing).

For trial 2, blood samples for furosemide (4 ml using K\textsubscript{2}EDTA as anticoagulant) were taken at predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h on days 1 and 10. Samples for DM-4103 (4 ml with sodium heparin as anticoagulant) were taken at 2 and 3 h postdose on day 4. Samples for TLV (4 ml with sodium heparin as anticoagulant) were taken at predose and 24, 48, 72, 96, and 120 h postdose on day 13 (7th day of dosing).

For trial 1, blood samples for rosuvastatin, TLV, and DM-4103 were stored at −70°C or colder (rosuvastatin, TLV, and DM-4103) or −20°C or colder (furosemide) until analyzed.

**Bioanalytical**

Plasma rosuvastatin concentrations were determined using a specific and validated reversed-phase high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS). Rosuvastatin and the internal standard rosuvastatin-d\textsubscript{6} were extracted from human plasma containing K\textsubscript{2}EDTA as an anticoagulant using solid phase extraction. The method was validated for concentrations ranging from 0.500 to 300 ng/ml for rosuvastatin. All validation work and sample analysis were performed at ICON Laboratory Services, Inc. (Whitesboro, NY).

The assay used to determine plasma TLV concentrations\textsuperscript{19} also included DM-4103 as a standard. The precursor→product ion was m/z 479 → 252. DM-4103 concentrations were linear over the range of 12.5 to 12,500 ng/ml. All validation work and sample analysis were performed at ICON Laboratory Services, Inc. (Whitesboro, NY).

Plasma furosemide concentrations were determined using a specific and validated reversed-phase HPLC-MS/MS method. Furosemide and the internal standard, furosemide-d\textsubscript{5}, were extracted from human plasma containing K\textsubscript{2}EDTA by protein precipitation. The method was validated for concentrations ranging from 5.00 to 5000 ng/ml. All validation work and sample analysis were performed at Altasciences (Quebec, Canada).

**Noncompartmental analysis**

Rosuvastatin and furosemide concentrations were analyzed using noncompartmental methods (SAS version 9.4). For calculation of descriptive statistics and PK parameters, plasma concentration values below the lower limit of quantitation (LLOQ) prior to first measurable concentrations were imputed to 0. Concentration values below the LLOQ following the last sample with a measurable concentration were excluded from the analyses. Actual blood sample times postdose were determined and reported as 2 decimals. Values for C_{\text{max}} and the time of maximal plasma concentration (T_{\text{max}}) were determined directly from the observed data. Values of area under the concentration-time curve from time 0 to the time of the last measurable concentration (AUC\textsubscript{t}) were estimated using the linear trapezoidal rule. The terminal-phase elimination rate constant (λ_z) was estimated by a log-linear regression of at least three non-zero concentrations; regressions with $r^2$ values less than 0.8 were not reported. The terminal-phase elimination half-life (t_{1/2,z}) was determined as (ln2)/λ_z. Values of AUC from time 0 to infinity (AUC∞) were determined as AUC\textsubscript{t} + Clast/λ_z. The value of apparent clearance from plasma following extravascular drug administration (CL/F) was determined as dose/AUC\textsubscript{∞}.
Descriptive statistics were presented by treatment and analyte. A maximum of 3 significant figures were used for table presentations except for $T_{\text{max}}$, which was reported to 2 decimals and $t_{1/2,z}$, which was reported to 1 decimal.

Statistical

The geometric mean ratios with 90% confidence intervals (CIs) of $C_{\text{max}}$, $AUC_{t}$, and $AUC_{\infty}$ were determined for rosuvastatin with TLV and rosuvastatin in the presence of DM-4103 compared with rosuvastatin alone (periods 2 or 3 compared with period 1) and for furosemide in the presence of DM-4103 compared with furosemide alone (period 3 compared with period 1). An analysis of variance with a factor of period and random effect of subjects was performed on the natural-log transformed PK parameters using the MIXED procedure in SAS. From each analysis, the least square means, their difference, and the 90% CI for their difference were derived. Then, the anti-logs of the difference and the confidence limits provided the estimate and 90% CI for the ratio of the geometric means of the test treatment to the reference treatment. Only subjects with values in both treatments were used in the analysis.

RESULTS

TLV inhibition of prazosin transport in BCRP overexpressing MDCKII cells provided a half-maximal inhibitory concentration (IC$_{50}$) estimate of 8.32 µM. The FDA guidance predicts an interaction when “…drug is administered orally, and the unbound fraction (fu,p) should be set to the liver. Considering uncertainties in the protein binding measurements, the unbound fraction (fu,p) should be set to 1% if experimentally determined to be less than 1%. As a metabolite formed slowly by metabolism after absorption of TLV, DM-4013 has no intestinal availability and portal vein concentrations are negligible compared with circulating concentrations at steady-state, so $I_{\text{in,max}}$ was set equal to the circulating concentrations of DM-4013. Following the 90 + 30 mg dose regimen of TLV in subjects with ADPKD, mean DM-4103 concentrations were ~7500 ng/ml or 15.7 µM (data on file). DM-4103 is greater than 99.8% plasma protein bound so $f_{u,p}$ was set to 0.01. Consequently, for DM-4103, $R = 1 + ([0.01 \times 15.7]/0.255) = 1.62$, indicating potential for an interaction.

DM-4103 inhibition of estrone 3-sulfate transport in OAT3 overexpressing HEK293 cells provided an IC$_{50}$ estimate of 0.425 µM. The FDA guidance predicts an interaction if “…the $I_{\text{max,u}}$/IC$_{50}$ value is ≥0.1.” For DM-4103, $15.7 \times 0.01/0.425 = 0.37$, indicating potential for an interaction. The ratio is equal to 0.1 when DM-4103 concentration is ~2034 ng/ml.

For trial 1, 16 subjects were enrolled and 14 completed. One subject was discontinued due to a treatment-emergent adverse event (TEAE) of palpitations at 70 h following rosuvastatin dosing on day 1. One subject withdrew following rosuvastatin dosing on day 1. For trial 2, 14 subjects were enrolled and completed. The demographic characteristics of enrolled subjects are summarized in Table 1.

Plots of mean (SD) rosuvastatin and furosemide plasma concentrations are presented in Figures 2 and 3, respectively. PK parameters for rosuvastatin and furosemide are presented in Tables 2 and 3, respectively. Geometric mean ratios and 90% CIs are presented in Table 4.

TLV administration concomitant with rosuvastatin administration was confirmed as mean (SD) concentrations at 2 and 3 h postdose were 422 (241) and 409 (184) ng/ml, respectively.

Mean (SD) DM-4103 concentrations at 48 h postdose (i.e., predose of the last dosing period) were 7240 (1880) ng/ml and 6430 (2030) ng/ml in trial 1 and trial 2, respectively, well above the predicted inhibitory levels.

| TABLE 1 Demographic characteristics for enrolled subjects |
|-----------------|-----------------|-----------------|
| **Demographics** | **Trial 1** $(N = 16)$ | **Trial 2** $(N = 14)$ |
| **Age, Mean (range)** | 27.3 (20–42) | 28.1 (19–43) |
| **Weight, Mean (range)** | 70.8 (48.5–92.4) | 70.3 (53.7–87.2) |
| **Sex, n (%)** | | |
| Male | 10 (62.5) | 7 (50.0) |
| Female | 6 (37.5) | 7 (50.0) |
| **Race, n (%)** | | |
| White | 13 (81.3) | 12 (85.7) |
| Black | 2 (12.5) | 1 (7.1) |
| American Indian or Alaskan Native | 1 (6.3) | 1 (7.1) |
| **Ethnicity, n (%)** | | |
| Hispanic or Latino | 1 (6.3) | 4 (28.6) |
| Not Hispanic or Latino | 15 (93.8) | 10 (71.4) |
For trial 1, TEAEs reported in at least 2 subjects/period were, in period 1 (rosuvastatin), nausea (12.5%), in period 2 (rosuvastatin + TLV), pollakiuria (35.7%), thirst (50%), dysgeusia (14.3%), and headache (14.3%), in period 3 (TLV alone), pollakiuria (71.4%), thirst (41.9%), nausea (21.4), and constipation and dry mouth (both 14.3%), and in period 3 (rosuvastatin in presence of DM-4103), headache (14.3%).

For trial 2, TEAEs reported in at least 2 subjects/period were, in period 1 (furosemide alone), polyuria (64.3%), in period 2 (TLV alone), polyuria (100%), thirst (92.9), dry mouth (21.4%), constipation, dizziness, headache, and insomnia (all 14.3%) and, in period 3 (furosemide in presence of DM-4103), and headache (21.4%).

All incidences of pollakiuria, polyuria, dry mouth, and thirst were considered to be related to the tolvaptan. No subjects had AEs related to changes in laboratory values or vital signs.

**FIGURE 2** Mean (SD) rosuvastatin plasma concentrations after administration of 5 mg rosuvastatin calcium alone, with 90 mg of tolvaptan or 48 h following 7 days of 300 mg tolvaptan (TLV) q.d. (i.e., in the presence of DM-4103) to 14 healthy adult subjects. No clinically relevant inhibition of BCRP by TLV and no inhibition of OAT1B1 by DM-4103 is observed

**FIGURE 3** Mean (SD) furosemide plasma concentrations after administration of 40 mg furosemide alone or 48 H following 7 days of 300 mg tolvaptan (TLV) q.d. (i.e., in the presence of DM-4103) to 14 healthy subjects. No inhibition of OAT3 by DM-4103 is observed

**TABLE 3** Mean (SD) plasma furosemide pharmacokinetic parameters in 14 subjects

| Parameters | 40 mg Furosemide alone | 40 mg Furosemide in presence of DM-4103 |
|------------|------------------------|-----------------------------------------|
| C<sub>max</sub>, ng/ml | 992 (580) | 873 (475) |
| T<sub>max</sub>, h | 2.00 (1.00–4.00) | 2.00 (1.00–4.00) |
| AUC<sub>t</sub>, ng⋅h/ml | 2920 (1400) | 2900 (1140) |
| AUC<sub>∞</sub>, ng⋅h/ml | 2950 (1390)<sup>b</sup> | 3070 (1150)<sup>b</sup> |
| t<sub>1/2,z</sub>, h | 6.1 (2.4)<sup>b</sup> | 6.7 (1.3)<sup>b</sup> |
| CL/F, ml/min/kg | 3.88 (1.55)<sup>b</sup> | 3.53 (1.12)<sup>b</sup> |

Abbreviations: AUC<sub>t</sub>, area under the concentration-time curve from zero to time of last measurable concentration; AUC<sub>∞</sub>, area under the concentration-time curve from zero to infinity; C<sub>max</sub>, maximal peak plasma concentration; CL/F, apparent clearance from plasma following extravascular administration; t<sub>1/2,z</sub>, terminal phase elimination half-life; T<sub>max</sub>, time of maximal peak concentration.

<sup>a</sup>Values are median (minimum-maximum); <sup>b</sup>n = 13.

**TABLE 2** Mean (SD) ROS plasma pharmacokinetic parameters in 14 healthy adults subjects

| Parameters | 5 mg ROS alone | 5 mg ROS + 90 mg TLV | 5 mg ROS in presence of DM-4103 |
|------------|---------------|---------------------|--------------------------------|
| C<sub>max</sub>, ng/ml | 2.81 (1.27) | 4.01 (1.30) | 3.03 (1.20) |
| T<sub>max</sub>, h | 3.50 (2.00–6.00) | 3.03 (2.00–4.12) | 4.00 (3.00–6.00) |
| AUC<sub>t</sub>, ng⋅h/ml | 18.9 (10.1) | 29.7 (14.6) | 18.4 (8.26) |
| t<sub>1/2,z</sub>, h | 2.4–4.3<sup>b</sup> | 4.5 (1.5)<sup>c</sup> | 3.6 (0.6)<sup>d</sup> |
| AUC<sub>∞</sub>, ng⋅h/ml | 20.3–36.0<sup>b</sup> | 38.0 (14.6)<sup>c</sup> | 27.2 (8.21)<sup>d</sup> |
| CL/F, ml/min/kg | 28.5–68.2<sup>b</sup> | 36.6 (16.4)<sup>c</sup> | 44.3 (16.6)<sup>d</sup> |

Abbreviations: AUC<sub>t</sub>, area under the concentration-time curve from zero to time of last measurable concentration; AUC<sub>∞</sub>, area under the concentration-time curve from zero to infinity; C<sub>max</sub>, maximal peak plasma concentration; CL/F, apparent clearance from plasma following extravascular administration; ROS, rosuvastatin; t<sub>1/2,z</sub>, terminal phase elimination half-life; TLV, tolvaptan; T<sub>max</sub>, time of maximal peak concentration.

<sup>a</sup>Values are median (minimum-maximum); <sup>b</sup>n = 7; <sup>c</sup>n = 11; <sup>d</sup>n = 8.
DISCUSSION

Rosuvastatin $\text{C}_{\text{max}}$ and $\text{AUC}_{\text{i}}$ were increased 1.54- and 1.69-fold, respectively, when administered with 90 mg TLV. Table 4 in the prescribing information for CRESTOR\textsuperscript{19} indicates that interactions are only considered clinically significant if the increases in AUC are at least 1.9-fold and the increases in $\text{C}_{\text{max}}$ are at least 2.2-fold. Therefore, the increases observed in this trial would not be considered clinically significant.

Based on the guidances for prediction of potential for interaction at BCRP, it is assumed the dose is completely soluble in 250 ml; a 90-mg TLV dose (200 µmol) in 250 ml has a predicted concentration of 800 µM. However, TLV has pH independent solubility of about 0.01 mg/250 ml (~0.09 µM). Even if TLV solubility was increased sevenfold, due to spray-drying, TLV would not be predicted to have clinically relevant interactions at BCRP transporters.

A potential mechanism behind the observed interaction could be TLV inhibition of the sodium-dependent taurocholate co-transporting polypeptide (NTCP). It has been reported that rosuvastatin is transported by NTCP with human hepatocyte studies suggesting that NTCP alone could account for ~35% of rosuvastatin uptake.\textsuperscript{20} TLV was shown to be an inhibitor of NTCP, although the IC\textsubscript{50} value was higher than $\text{C}_{\text{max}}$.\textsuperscript{21} However, liver concentrations of TLV were predicted to be much higher than blood concentrations (liver:blood ratio 38.76)\textsuperscript{22} and TLV was shown to accumulate at least 10-fold in sandwich-cultured human hepatocytes\textsuperscript{23}. “The total cellular concentration of tolvaptan ranged from ~2 µM to 500 µM and was greater than the respective incubation concentration (0.15–50 µM).” Thus, liver concentrations of TLV may be reaching inhibitory levels.

As shown in Table 4, the lower bounds of the 90% CI for $\text{C}_{\text{max}}$ and $\text{AUC}_{\text{i}}$ are less than 1 for both rosuvastatin in the presence of DM-4103 compared with rosuvastatin alone and furosemide in the presence of DM-4103 versus furosemide alone, therefore we can conclude that DM-4103 is not an inhibitor of OATP1B1 or OAT3 at clinically relevant concentrations.

For OATP1B1 and OAT3, the FDA guidance instructs that fraction unbound for plasma protein binding less than 1% be set to 1% “considering uncertainties in protein binding measurements.” DM-4103 protein binding was determined to be greater than 99.8% using a micropartition device (Centrifree YM-30) followed by HPLC-MS/MS detection of concentrations in the ultrafiltrate. The effect of 6 other highly plasma protein bound compounds on DM-4103 binding was also evaluated; no interactions were detected with DM-4013 binding greater than 99.8% in all samples (data on file). No potential for interaction at either transporter would have been predicted if a free fraction of 0.1% had been used, consistent with the clinical findings.

These results support the use of drug substance solubility and actual unbound fraction in plasma in the predictions for clinically significant drug interactions.

DATA SHARING
To submit inquiries related to Otsuka Clinical Research, or to request access to individual participant data (IPD) associated with any Otsuka clinical trial, please visit https://clinical-trials.otsuka.com/. For all approved IPD access requests, Otsuka will share anonymized IPD on a remotely accessible data sharing platform.

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CONFLICT OF INTEREST
All authors are employees of Otsuka Pharmaceutical Development & Commercialization, Inc. (OPDC).
AUTHOR CONTRIBUTIONS
S.E.S. wrote the manuscript. S.E.S., P.B., and J.R.G. designed the research. P.B. and J.R.G. performed the research. S.E.S. analyzed the data.

REFERENCES
1. Torres VE, Chapman AB, Devuyst O, et al. Tolvaptan in patients with autosomal dominant polycystic kidney disease. N Engl J Med. 2012;367:2407-2418.
2. Torres VE, Chapman AB, Devuyst O, et al. Tolvaptan in later-stage autosomal dominant polycystic kidney disease. N Engl J Med. 2017;377:1930-1942.
3. Shoaf SE, Bricmont P, Mallikarjun S. Absolute bioavailability of tolvaptan and determination of minimally effective concentrations in healthy subjects. Int J Clin Pharmacol Ther. 2012;50:150-156.
4. Australian Government, Dept. of Health, Therapeutic Goods Administration. Australian Public Assessment Report for Tolvaptan. https://www.tga.gov.au/auspar/auspar-tolvaptan-0 (2018). Accessed January 20, 2021.
5. Magar DD, Magar MD, Sakarkar DM, Jaiswal SB. Design and evaluation of solid dispersion based tolvaptan immediate release tablets. Indo Am J Pharm Res. 2014;4:1333-1343.
6. Ramesh K, Shekar BC, Khadgapathi P, Bhikshapathi DVRN. Design and evaluation of tolvaptan solid dispersions using hot-melt extrusion and spray drying technique—a comparative study. Der Pharm Lett. 2015;7:218-231.
7. Jynarque (tolvaptan) [US prescribing information] (Otsuka Pharmaceutical Co., Ltd., Tokyo, 101-8535 Japan, 2020). https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/204441lbl.pdf. Accessed January 20, 2021.
8. US Food and Drug Administration. In vitro drug interaction studies — cytochrome P450 enzyme- and transporter-mediated drug interactions: guidance for industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in vitro-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions (January 2020). Silver Spring, MD. Accessed January 20, 2021.
9. Guideline on the Investigation of Drug Interactions, European Medicines Agency, 21 June 2012, CPMP/EWP/560/95/Rev. 1 Corr. 2**, Committee for Human Medicinal Products (CHMP) – finalized 2013. <https://www.ema.europa.eu/en/documents/scientific-guide line/guideline-investigation-drug-interactions-revision-1_en.pdf>.
10. Ministry of Labor and Welfare. Guideline on drug interaction for drug development and proper provision of information, notification No.0723-4, pharmaceutical evaluation division, pharmaceuticals safety and environmental Health bureau, Japan. July 23, 2018. https://www.pmda.go.jp/files/000228122.pdf. January 20, 2021.
11. US Food and Drug Administration. Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers (Content current as of March 2020). Accessed January 20, 2021.
12. Shoaf SE, Ouyang J, Sergeyeva O, Estile A, Li H, Leung D. A post hoc analysis of statin use in tolvaptan autosomal dominant polycystic kidney disease pivotal trials. Clin J Am Soc Nephrol. 2020;15:643-650.
13. Konstam MA, Gheorghiade M, Burnett JC, et al. Effects of oral tolvaptan in patients hospitalized for worsening heart failure: the EVEREST Outcome Trial. JAMA. 2007;297:1319-1331.
14. Udelson JE, McGrew FA, Flores E, et al. Multicenter, randomized, double-blind, placebo-controlled study on the effect of oral tolvaptan on left ventricular dilation and function in patients with heart failure and systolic dysfunction. J Am Coll Cardiol. 2007;49:2151-2159.
15. Gheorghiade M, Gattis WA, O’Connor CM, et al. Effects of tolvaptan, a vasopressin antagonist, in patients hospitalized with worsening heart failure: a randomized controlled trial. JAMA. 2004;291:1963-1971.
16. AstraZeneca Pharmaceuticals, L.P. Crestor (rosuvastatin calcium) [US prescribing information]. Wilmington, DE: AstraZeneca Pharmaceuticals, L.P.; 2010.
17. Shoaf SE, Ouyang J, Bricmont P, Mallikarjun S. Tolvaptan, a selective V2-receptor antagonist, has no effect on QTcI following multiple oral dosing in healthy men and women. Clin Pharmacol Ther. 2009;85(Suppl 1):S32.
18. Shoaf SE, Bramer SL, Bricmont P, Zimmer CA. Pharmacokinetic and pharmacodynamic interaction between tolvaptan, a non-peptide AVP antagonist, and furosemide or hydrochlorothiazide. J Cardiovasc Pharmacol. 2007;50:213-222.
19. Shoaf SE, Wang Z, Bricmont P, Mallikarjun S. Pharmacokinetics, pharmacodynamics and safety of tolvaptan, a non-peptide AVP antagonist, during ascending single-dose studies in healthy subjects. J Clin Pharmacol. 2007;47:1498-1507.
20. Ho RH, Tirona RG, Leake BF, et al. Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression and pharmacogenetics. Gastroenterology. 2006;130:1793-1806. https://doi.org/10.1053/j.gastro.2006.02.034.
21. Slizgi JR, Lu Y, Brouwer KR, et al. Inhibition of human hepatic bile acid transporters by tolvaptan and metabolites: contributing factors to drug-induced liver injury. Toxicol Lett. 2016;249:237-250.
22. Woodhead JL, Brock WJ, Roth SE, et al. Application of a mechanistic model to evaluate putative mechanisms of tolvaptan drug-induced liver injury and identify patient susceptibility factors. Toxicol Sci. 2017;155:61-74.
23. Lu Y, Slizgi JR, Brouwer KR, et al. Hepatocellular disposition and transporter interactions with tolvaptan and metabolites in sandwich-cultured human hepatocytes. Drug Metab Dispos. 2016;44:867-870.

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