Supplemental Materials

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Supplemental Experimental Methodology

1. Overview of PBPK Modeling Strategy
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Table S1: Summary of baseline demographics for OM-Rosuvastatin clinical drug interaction study

| Parameter                          | Rosuvastatin Study |
|------------------------------------|--------------------|
| Age, mean (SD), y                  | 34.1 (9.71)        |
| Male, n (%)                        | 6 (42.9%)          |
| Female, n (%)                      | 8 (57.1%)          |
| BMI, mean (SD), kg/m²              | 25.1 (2.75)        |
| Height, mean (SD), cm              | 167 (9.49)         |
| Weight, mean (SD), kg              | 70.4 (13.1)        |
| Ethnicity, n (%)                   |                    |
| Hispanic or Latino                 | 10 (71.4%)         |
| Not Hispanic or Latino             | 4 (28.6%)          |
| Race, n (%)                        |                    |
| Black                              | 3 (21.4%)          |
| White                              | 10 (71.4%)         |
| Multiple                           | 1 (7.1%)           |

BMI body mass index, SD standard deviation.
**Table S2.** Predicted effect of BCRP inhibition on rosuvastatin exposure upon administration of 50 mg OM (BID)

|                | BCRP Kᵢ 0.05 µM |
|----------------|-----------------|
| AUC ratio<sup>a</sup> | 1.18 (1.16, 1.20)<sup>c</sup> |
| C<sub>max</sub> ratio<sup>b</sup> | 2.04 (1.99, 2.10)<sup>c</sup> |

Rosuvastatin administered on day 10 with or without 50 mg OM administered orally bid for 14 days;  
<sup>a</sup>Ratio of rosuvastatin AUC in the presence of 50 mg BID OM to that in the absence of OM;  
<sup>b</sup>Ratio of rosuvastatin C<sub>max</sub> in the presence of 50 mg BID OM to that in the absence of OM;  
<sup>c</sup>Geometric mean (90% confidence interval)
**Table S3. Parameters for the Rosuvastatin Compound File**

| Parameter                                      | Value          | Method/ Reference                                                                 |
|------------------------------------------------|----------------|-----------------------------------------------------------------------------------|
| Molecular Weight                               | 481.54         | PubChem                                                                           |
| LogP                                           | 2.4            | (Ahmad et al, 2008)                                                               |
| pKa                                            | 4.27           | "Avdeef, A. Absorption and Drug Development, Second Edition. Wiley-Interscience, Hoboken, 2012." as cited in Wiki-pKa. [http://www.inadme.com/](http://www.inadme.com/) (Jones et al, 2012) |
| Fraction unbound (fu) in plasma                 | 0.625          | (Jones et al, 2012)                                                               |
| Red blood cell partitioning                     | 0.107          | (Jones et al, 2012)                                                               |
| fu,gut                                         | 1              | Assumed                                                                           |
| P_{et,man} (10^{-4} cm/s)                       | 0.85           | Predicted                                                                         |
| Permeability assay                             | Caco2          | (Li et al, 2012)                                                                  |
| Apical: Basolateral pH                          | 7.4:7.4 (Passive) |                                                                                   |
| PappA-B (10^{-6} cm/s)                          | 3.395          |                                                                                   |
| Reference compound                             | Propranolol    |                                                                                   |
| Reference compound                             | 20             |                                                                                   |
| Scalar                                         | 2.15           |                                                                                   |
| Transporter                                    | BCRP           |                                                                                   |
| CL_{int} (µL/min/million hepatocytes)            | 24             | Fitted to recover the t_{max} for the 10mg dose (Cooper et al, 2003)               |
| Distribution                                   | Minimal PBPK model |                                                                                   |
| V_{ss} (L/kg)                                   | 0.81           | Predicted by Rodger’s and Rowland’s Method with predicted lipid binding scalar 2337 |
| CL_{int}, HLM (µL/min/mg protein)               | 17             | Liver CL_{int} has been estimated from CLiv and CLrenal using the retrograde model knowing a 10% metabolic contribution (Martin et al, 2003; Martin et al, 2000) |
| CL,renal (L/h)                                  | 17             | Meta analysis (Keskitalo J et al, 2009; Keskitalo JE et al, 2009; Martin et al, 2003) |
| Permeability limited liver model                |                |                                                                                   |
| CL_{H0} (mL/min/million hepatocytes)             | 0.0025         | (Kotani et al, 2011)                                                             |
| fu_IW                                          | 0.967          | Predicted                                                                         |
| fu_NEW                                         | 0.187          | Predicted                                                                         |
| Transporter                                    | Liver sinusoidal uptake |                                                                                   |
| CL_{H0,T} (µL/min/million hepatocytes)           | 139            | Optimized, See text for details                                                  |
| Transporter                                    | BCRP (biliary efflux) |                                                                                   |
| CL_{H0,T} (µL/min/million hepatocytes)           | 1.23           | Biliary CL_{int} determined in sandwich-cultured human hepatocytes (Abe et al, 2009) was scaled using a liver weight (25g/kg body weight) and an HPGL of 107 million cells/g liver |
Table S4. Validation of Rosuvastatin PBPK Model

| Study                         | Dose (mg) | t_{max} (hours) | C_{max} (ng/mL) | AUC_{inf} (ng/mL.h) |
|-------------------------------|-----------|----------------|-----------------|---------------------|
|                               |           | Observed       | Simulated       | Observed            | Simulated           | Simulated/   | Observed            | Simulated           | Simulated/   |
|                               |           |               | Simulated/Observed |            |          | Observed      | Simulated/Observed |            | Observed      | Simulated/Observed |
| Rosuvastatin PK               |           |               |                 |                     |                     |              |                     |                     |              |                     |
| Pasanen et al 2007            | 10        | 5 (1 to 5)    | 4.2 (2.2 - 7.9) | 0.84               | 4.2 ± 2.41          | 4.3 ± 1.4     | 1.02               | 35 ± 18.1          | 51.6 ± 15.6  | 1.47               |
| Allred et al 2011\(^1\)       | 10        | Not available | -               | -                  | 3.7                 | 4.1           | 1.11               | 41.6              | 49.3         | 1.19               |
| Polli et al 2003\(^1\)        | 10        | 4 (1 to 6)    | 4.2 (2.2 - 7.9) | 1.05               | 2.6                 | 4.1           | 1.58               | 35.3              | 49.3         | 1.40               |
| Cooper et al 2003             | 10        | 5 (3 to 5)    | 4.2 (2.2 - 7.9) | 0.84               | 8.7 ± 4.5           | 4.3 ± 1.4     | 0.49               | 84.7 ± 20.2       | 51.6 ± 15.6  | 0.61               |
| Lee et al 2005\(^1,2\)        | 40        | 4.14 1.51     | 4.2 (2.2 - 7.9) | 1.01               | 25                  | 16.3          | 0.65               | 216               | 196.7        | 0.91               |
| Schneck et al 2004\(^2,3\)    | 80        | 4 (0.5 to 5)  | 4.2 (2.2 - 7.9) | 1.05               | 49.5                | 34.2          | 0.69               | 410               | 380.5        | 0.93               |
| Cooper et al 2002\(^1,2\)     | 80        | 5 (2 - 6)     | 4.2 (2.2 - 7.9) | 0.84               | 41.4                | 34.2          | 0.83               | 325               | 393.5        | 1.21               |
| Keskimino et al (c.421AA genotype) | 20      | 4 (2 - 5)    | 1.8 (1.0 - 2.8) | 0.45               | 16.3                | 18.6          | 1.14               | 152.2             | 121.1        | 0.80               |

\(^1\) Geometric mean C_{max} and AUC

\(^2\) AUC 72h

\(^3\) AUC 30h
**Figure S1:** Study design of OM-Rosuvastatin clinical drug interaction study

- **Screening Period:** Days -21 to -2
- **Day 1:** Rosuvastatin 10 mg
- **Day 6:** Rosuvastatin 10 mg + Omecamtiv Mecarbil 50 mg
- **Day 10:** End of Study (EOS)
Figure S2. Graphical presentation of the validation of the PBPK model

1. Develop PBPK model for OM
   - Step 1: Estimate distribution related parameters from iv study (35 mg)

2. Verify OM PBPK model using studies 3 and 4
   - Step 2: Estimate $\text{Cl}_{\text{pGp}}, \text{Ka}$ from studies 1 (50 mg single dose) and 2 (25 mg BID)

3. Use clinical data from rosuvastatin-OM single dosing DDI study to optimize OM BCRP $K_i$

4. Predict BCRP DDI upon 50 mg BID OM dosing
Figure S3. Model-predicted (solid lines) and observed (solid circles) OM plasma concentration-time profiles in healthy subjects (shown in linear and log-linear scale) (A). 50 mg single dose (Study 1, control group; n = 14) (B). 25 mg BID dose for 7 days (Study 2; n =13). The dotted and dashed lines denote upper and lower extremes of the 95% confidence interval; error bars represent standard deviation.
Figure S4. Model-predicted (solid lines) and observed (solid circles) OM plasma concentration-time profiles in healthy subjects (shown in linear and log-linear scale); a. 25 mg BID (Study 4, control group; n = 14) b. 50 mg BID (Study 3; n = 13); The dotted and dashed lines denote 95th and 5th percentiles, respectively; error bars represent standard deviation.
**Figure S5.** Simulated And Observed Rosuvastatin Plasma Concentration-Time Profiles (Validation)

**A.**

![Graph for 10 mg dosing](image)

Black lines denote simulated and the circles, triangles, diamonds and squares denote observed concentrations after an oral dose of A. 10mg (Polli et al, 2013; Allred et al, 2011; Pasanen et al, 2007; Cooper et al, 2003) B. 40mg (Lee et al, 2005) C. 80mg (Schneck et al, 2004; Cooper et al, 2002). The black lines represent mean of individual trials (10 trials x 10 subjects; 18-65 years; 50% female). The dotted and dashed lines represent the 95th and 5th percentile, respectively.
Figure S6. Model-predicted* (solid and dotted lines) and observed (closed and open circles) rosvastatin plasma concentration-time profiles in healthy subjects in the presence and absence of OM (shown in linear and log-linear scale). *10 mg rosvastatin was co-administered with 50 mg (black) OM orally; the dotted and dashed lines denote 95th and 5th percentiles, respectively; error bars represent standard deviation
Figure S7. Impact of BCRP Ki of OM on rosuvastatin AUC ratio and Cmax ratio
Supplemental experimental methodology

1. Overview of PBPK Modeling Strategy

Simcyp Simulator (Simcyp LTD, Version 17.1, Sheffield, UK) was used for PBPK modeling. All simulations. The steps followed are listed below:

1) OM Model development: The distribution of OM was parameterized from clinical PK generated upon 35 mg intravenous administration. With fixed distribution parameters, the apparent oral clearance ($CL_{po}$) and the first order absorption rate constant ($K_a$) were parameterized from clinical PK data generated upon administration of single 50 mg dose of OM and twice daily dosing of 25 mg OM for 7 days.

2) OM model verification: The OM PBPK model was verified by comparison of model predicted PK parameters to the observed PK parameters. The model was considered verified if the predicted $C_{max}$, $t_{max}$ and AUC fell within 2-fold of the observations.

3) Model application: The verified model was used for BCRP inhibition mediated clinical DDI prediction.

2. Development of the OM Compound File in Healthy Population

Clinical PK upon administration of single 50 mg dose of OM (Study 4, control group) along with twice daily dosing of 25 mg OM for 7 days (Study 1) was used in combination to obtain parameter estimates of $CL_{po}$ and $K_a$ that reasonably predict plasma concentration-time profiles upon both single and multiple oral dosing. The ‘maximum likelihood’ objective function was used along with the ‘expectation maximization’ minimization method.

The optimized parameters were used to simulate plasma concentration-time profiles in virtual populations mimicking the respective designs of studies 4 and Study 2 with respect to the administered dose, dosing regimen, age range, number of subjects in the study and the proportion of females in each study. Table 1 summarizes the virtual study design for each clinical study. The simulated $C_{max}$, $t_{max}$ and AUC were compared to the observed values and deemed acceptable if they fell within 2-fold of observations which is the commonly acceptable criteria (Shebley et al, 2018).
3. Development and validation of the rosuvastatin PBPK model

The input parameters for rosuvastatin PBPK model are summarized in Supplemental Table 3. All input parameters were taken from the rosuvastatin Simcyp (V17) library file with the exception of liver sinusoidal uptake intrinsic clearance and intestinal BCRP intrinsic clearance. First, the parameter estimation module in Simcyp was used to obtain the liver sinusoidal uptake intrinsic clearance. For this purpose, the liver metabolic intrinsic clearance and biliary BCRP intrinsic clearance were fixed (HLM CL\text{int} \ 17 \ \mu L/min/mg protein, BCRP CL\text{int,T} \ 1.23 \ \mu L/min/million hepatocytes), and the PK data generated upon iv rosuvastatin administration was used (Martin et al, 2003). Next, the intestinal BCRP CL\text{int,T} was parameterized from PK data generated upon oral administration of 10mg rosuvastatin (Cooper et al, 2003). Although BCRP in the kidney may be responsible for rosuvastatin active tubular secretion, no significant impact of BCRP polymorphism was observed on rosuvastatin renal clearance (Keskitalo J et al, 2009) possibly due to compensatory effect of other efflux transporters like MRP2 since rosuvastatin is also a substrate for MRP2 (Ellis et al, 2013). Hence, explicit transporter mediated renal excretion was not modeled for rosuvastatin.

The ability of the PBPK model to predict rosuvastatin PK from multiple independent studies was evaluated. The results from the validation are summarized in Supplemental Figure 5 and Supplemental Table 4. The C\text{max}, t\text{max} and AUC for rosuvastatin were predicted within 2-fold by the PBPK model.

BCRP polymorphisms are known to result in reduced transport of certain BCRP substrates associated with decreased BCRP expression or decreased ATPase activity of BCRP (Morisaki et al, 2005; Kondo et al, 2004). The most significant impact of BCRP on rosuvastatin PK has been observed in subjects with c.421AA genotype (Keskitalo J et al, 2009). The ability of the model to predict the impact of BCRP on rosuvastatin PK was assessed by simulating the plasma concentration-time profile when BCRP intrinsic clearance in the intestine and liver was set to 0, and comparing the simulated data with PK from subjects with c.421AA genotype (Keskitalo J et al, 2009). The C\text{max} and AUC were predicted within 2-fold by the model. The results from the validation are summarized in Supplemental Table 4.

The rosuvastatin PBPK model was thus considered verified and used for DDI prediction in presence of OM.
Supplemental References

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