

Official Protocol Title:	A Study to Assess the Pharmacokinetics of Midazolam, Dabigatran, Pitavastatin, Atorvastatin, and Rosuvastatin Administered as Microdoses in Subjects With Varying Degrees of Renal Insufficiency in the Presence and Absence of Rifampin
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A Study to Assess the Pharmacokinetics of Midazolam, Dabigatran, Pitavastatin, Atorvastatin, and Rosuvastatin Administered as Microdoses in Subjects With Varying Degrees of Renal Insufficiency in the Presence and Absence of Rifampin

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1 PROTOCOL REVISION HISTORY

Date/Name	Description
26Feb2018 PPD	<p>Amendment 2, Final Protocol for review</p> <p>This protocol amendment is being written because changes or corrections are required to the biomarker and pharmacokinetic blood sampling and processing and urine collection as described in the protocol.</p> <p>As a result of the amendment, the following sections of the protocol were updated (in bold for addition and striketrough for deletion):</p> <ol style="list-style-type: none"> Appendix 6, Sample Handling Instructions for Midazolam and Dabigatran Samples: Updated instructions. Appendix 9: Specimen Collection Procedures – Plasma and Serum Biomarkers: Added or updated procedures. Appendix 10: Sample Handling Instructions for Urine Collection (Biomarkers): Added or updated instructions.
19Dec2017 PPD	<p>Amendment 1, Final Protocol for review</p> <p>This protocol amendment is being written because changes or corrections are required to the biomarker and pharmacokinetic blood sampling and processing and urine collection as described in protocol.</p> <p>In addition, a few other clarifications to the study conduct were incorporated into the protocol.</p> <p>As a result of the amendment, the following sections of the protocol were updated (in bold for addition and striketrough for deletion):</p> <ol style="list-style-type: none"> Section 6, Flow Chart, the row “Blood for Plasma Total and Direct Bilirubin (P1 & P2)” changed to: “Blood for Serum Total and Direct Bilirubin (P1 & P2) (Central Lab).” Section 6, Flow Chart, the row “Blood for Serum Creatinine (P1 & P2)” changed to: “Blood for Serum Creatinine (P1 & P2) (Central Lab).” Section 6, Flow Chart, footer g changed to: “To be performed in Period 1 only. Note: For protein binding, it will be analysed

	<p>as part of the biomarker sample.”</p> <ol style="list-style-type: none"> 4. Section 8.1.3, Exploratory Objectives, Section 8.2, Analysis Endpoints, Biomarkers, and corresponding sections in the synopsis (Exploratory Objectives and Key Assessments, Biomarkers): In these sections, reference to “plasma biomarkers” or “plasma and urinary biomarkers” was changed to include reference to plasma, serum and urine biomarkers, and Appendix 9: Specimen Collection Procedure – “Plasma Biomarkers” changed to: “...Plasma and Serum Biomarkers.” 5. Section 5, Synopsis, Biomarkers, Section 6, Study Events Flow Chart, Footer ‘t’, Section 8.2, Analysis Endpoints, Biomarkers, and Section 10.2.2, Urine Collection: Urine creatinine as a biomarker has been removed from the urine sample analysis, thus these sections changed to remove urine creatinine: “Urine creatinine will also be measured [central lab] in addition to coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, and CMPF.” or “urine creatinine (clinical laboratory)” or “creatinine”, as appropriate. 6. Section 9.2.1.1, Inclusion Criteria, Subjects with Renal Impairment, Inclusion Criterion #2: updated to include note: “A female must be non-pregnant, non-breast feeding and if she is of reproductive potential: must demonstrate a serum β-human chorionic gonadotropin (β-hCG) level consistent with the nongravid state at screening (Note: if a subject has a β-hCG level that is not consistent with the non-gravid state due to disease or medication, the investigators may consult with the sponsor to obtain approval to enroll the subject in the study) and...” 7. Section 9.2.2.2, Exclusion Criteria, Subjects with Renal Impairment, Exclusion Criterion #7: updated to allow cholecystectomy: “Subject with a history of significant endocrine (other than T2DM), gastrointestinal, cardiovascular, hematological, immunological, respiratory, or genitourinary diseases whose current condition is considered clinically unstable in the opinion of the investigator or designee. Subjects who have had a cholecystectomy may be allowed.” 8. Section 9.4.1, Treatments Administered: Added records: “Treatment A will be supplied as microdose cocktail. See Table 2. Oral solutions for Treatment A will be prepared and stored according to the Method of Preparation Manual and Master Batch Records provided separately,” and clarified the water given with dosing: “Subjects will drink one container of approximately 240 mL of water after each complete dosing
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	<p>completion.”</p> <p>9. Section 10.5, Blood Volume Drawn for Study Assessments, Table 4: The following changes were made:</p> <ol style="list-style-type: none"> 1. corrected the number of on-study hematology and serum chemistry from “6” to “4” timepoints during the study, 2. biomarker blood samples for total and direct bilirubin and creatinine will be processed for serum (instructions added/updated in revised Appendix 9), 3. blood samples for other plasma biomarkers and uremic toxins are now detailed in the table (instructions added/updated in revised Appendix 9), 4. a separate sample will now be collected for biomarker plasma coproporphyrin I and III (instructions added/updated in revised Appendix 9), 5. the blood collected for most pharmacokinetic samples will be reduced, 6. a separate sample will now be collected for protein binding (and footer was updated; instructions added in revised Appendix 9), 7. an additional column was added to the table to identify the “Handling Instructions” and relevant appendix, and 8. the volume per timepoint and sample volume over the course of the study were updated. <p>10. Appendix 3, Sample Handling Instructions for Midazolam and Dabigatran Samples: Updated.</p> <p>11. Appendix 4, Sample Handling Instructions for Pitavastatin, Atorvastatin (and metabolites), and Rosuvastatin Samples: Updated.</p> <p>12. Appendix 5, Sample Handling Instructions for Rifampin Samples: Updated.</p> <p>13. Appendix 6, Sample Handling Instructions for Midazolam and Dabigatran Samples: Updated.</p> <p>14. Appendix 8: Medications allowed as per protocol and Section 9.3.1, Prohibitions and Concomitant Therapy: Added: “Proton Pump inhibitors: Note: Proton pump inhibitors must be withheld 1 week prior to each dosing and 4 hours post dosing.” Corrected to be consistent with medications contraindicated for this study to remove “glyburide” from the medications allowed as per protocol: “Medications for treatment of diabetes such as insulin, sulfonylureas (e.g., tolbutamide, glipizide, glyburide, glimepiride, metformin,</p>
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	<p>saxagliptin, sitagliptin), meglitinides (e.g., repaglinide, nateglinide), thiazolidinediones, dipeptidyl peptidase-4 inhibitors, and glucagon-like peptide-1 analogs.”</p> <p>15. Appendix 9: Specimen Collection Procedures – Plasma and Serum Biomarkers: Added or updated instructions for serum total and direct bilirubin, serum creatinine, other biomarkers and uremic toxins, and for plasma coproporphyrin I and III.</p> <p>16. Appendix 10: Sample Handling Instructions for Urine Collection (Biomarkers): Added clarification for urine collection for pharmacokinetic and biomarker samples and updated instructions.</p>
08Sep2017 PPD [REDACTED]	Final Protocol

2 PRINCIPAL INVESTIGATOR AND SPONSOR – SIGNATORIES

A Study to Assess the Pharmacokinetics of Midazolam, Dabigatran, Pitavastatin, Atorvastatin, and Rosuvastatin Administered as Microdoses in Subjects With Varying Degrees of Renal Insufficiency in the Presence and Absence of Rifampin

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5 SYNOPSIS

Compound:	Midazolam, dabigatran, pitavastatin, atorvastatin, rosuvastatin, and rifampin
Clinical Indication:	N/A
Study Phase and Type:	Phase I – Other Clinical Study Reports
Study Objectives and Estimation:	<p>Primary (Period 1):</p> <p>Objective 1: To characterize the plasma pharmacokinetic profiles of midazolam, dabigatran, pitavastatin (and its metabolite, pitavastatin lactone), atorvastatin (and its metabolites, ortho-hydroxyatorvastatin [2-OH atorvastatin] and para-hydroxyatorvastatin [4-OH atorvastatin]), and rosuvastatin following a single oral dose administration of a microdose cocktail in healthy subjects, in subjects with mild, moderate, severe (not on dialysis) renal impairment, and in subjects with end-stage renal disease (ESRD; on dialysis).</p> <p>Estimation 1: The plasma pharmacokinetic parameters (e.g., AUC₀₋₂₄, AUC_{0-last}, AUC_{0-∞}, C_{max}, C₂₄, T_{max}, and apparent terminal t_{1/2}) of midazolam, dabigatran, pitavastatin (and its metabolite, pitavastatin lactone), atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin), and rosuvastatin following a single oral dose administration of a microdose cocktail will be estimated in healthy subjects, in subjects with mild, moderate, severe (not on dialysis) renal impairment, and in subjects with ESRD (on dialysis).</p> <p>Primary Objective for Midazolam:</p> <p>Objective 2: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of midazolam administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Estimation 2: Plasma AUC_{0-∞} of midazolam following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of midazolam following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).</p>

	<p>Primary Objective for Dabigatran:</p> <p>Objective 3: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of dabigatran administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Estimation 3: Plasma AUC_{0-∞} of dabigatran following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of dabigatran following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).</p> <p>Primary Objective for Pitavastatin:</p> <p>Objective 4: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of pitavastatin (and its metabolite, pitavastatin lactone) administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Estimation 4: Plasma AUC_{0-∞} of pitavastatin (and its metabolite pitavastatin lactone) following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of pitavastatin (and its metabolite, pitavastatin lactone) following a single dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).</p> <p>Primary Objective for Atorvastatin:</p> <p>Objective 5: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin) administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Estimation 5: Plasma AUC_{0-∞} of atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin) following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the</p>
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	<p>plasma AUC_{0-∞} of atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin) following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).</p> <p>Primary Objective for Rosuvastatin:</p> <p>Objective 6: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of rosuvastatin administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Estimation 6: Plasma AUC_{0-∞} of rosuvastatin following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of rosuvastatin following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).</p> <p>Secondary Objectives:</p> <p>Objective 1: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of midazolam administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Objective 2: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of dabigatran administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Objective 3: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of pitavastatin (and its metabolite, pitavastatin lactone) when pitavastatin is administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Objective 4: To estimate the effect of a single oral dose of rifampin on the single dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of atorvastatin (and its metabolites, ortho-hydroxyatorvastatin [2-OH atorvastatin] and para-hydroxyatorvastatin [4-OH atorvastatin]) when</p>
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	<p>atorvastatin is administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Objective 5: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC0-24, AUC0-last, Cmax, C24, Tmax, apparent terminal $t_{1/2}$, CL/F, and Vz/F) of rosuvastatin administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Objective 6: To compare the magnitude of the drug-drug interaction (DDI) between rifampin and each of the 5 substrates in the microdose cocktail between healthy subjects and each category of RI subjects (mild, moderate, severe RI).</p> <p>Objective 7: To estimate the plasma pharmacokinetic parameters (e.g., AUC0-24, AUC0-last, AUC0-∞, Cmax, C24, Tmax, and apparent terminal $t_{1/2}$) of dabigatran, atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin), pitavastatin (and its metabolite, pitavastatin lactone), rosuvastatin, and midazolam following a single oral dose administration of a microdose cocktail in ESRD subjects on dialysis when the microdose cocktail is administered at different times relative to dialysis.</p> <p>Objective 8: To estimate the plasma pharmacokinetic parameters (e.g., AUC0-24, AUC0-last, AUC0-∞, Cmax, C24, Tmax, and apparent terminal $t_{1/2}$) of rifampin following a single oral dose administration with a single oral dose of a microdose cocktail in each subject population separately (except for ESRD subjects).</p> <p>Exploratory Objectives:</p> <p>Objective 1: To evaluate plasma, serum, and urinary biomarkers to assess the impact of renal impairment on functions such as organic anion transporting polypeptide (OATP)1B inhibition.</p> <p>Objective 2: To evaluate the urinary pharmacokinetics (e.g., CLr, Ae0-24, and fe) of dabigatran and rosuvastatin in each population.</p> <p>Planned Exploratory Biomarker:</p> <p>Objective: To explore the relationship between genetic variation and response to the treatment(s) administered, and mechanisms of disease. Variation across the human genome may be analyzed for association with clinical data collected in this study.</p>
Summary of Study Design:	<p>This is an open label, 2-period, fixed sequence study in 24 RI subjects (6 subjects with mild renal impairment; 6 subjects with moderate renal impairment; 6 subjects with severe renal impairment who are not on dialysis; 6 ESRD subjects), and 6 healthy control subjects.</p>

	<p>Conduct of the mild RI cohort may be performed, as determined by the Sponsor, following conduct completion and review of data from the moderate and severe RI cohorts and the healthy cohort.</p> <p>Screening of subjects will occur within 28 days prior to the first dosing.</p> <p>Assignment to a renal function group will be as follows:</p> <table><tr><th>Group</th><th>N</th><th>eGFR (mL/min/1.73m²) or CLcr (mL/min)*</th></tr><tr><td>ESRD requiring hemodialysis</td><td>6</td><td>Requiring hemodialysis</td></tr><tr><td>Severe impairment</td><td>6</td><td>< 30 not on hemodialysis**</td></tr><tr><td>Moderate impairment</td><td>6</td><td>30 – < 60 ***</td></tr><tr><td>Mild impairment</td><td>6</td><td>60 - < 90</td></tr><tr><td>Healthy control</td><td>6</td><td>≥ 90 ****</td></tr></table> <p>* Estimated glomerular filtration rate (eGFR) based on Modification of Diet in Renal Disease (MDRD) equation at screening. Baseline eGFR will be obtained twice during the screening period, and the mean of the two values will be used for group assignment. The second baseline eGFR sample may be obtained at the time of check-in in Period 1. Creatinine clearance (CLcr) by Cockcroft-Gault equation will be used for healthy control assignment (normal renal function).</p> <p>** Reasonable efforts will be made to enroll at least 2 subjects with eGFR values of < 20 mL/min/1.73m².</p> <p>*** Reasonable efforts will be made to ensure a broad representation across the range of eGFR values of 30 - < 60 mL/min/1.73m².</p> <p>**** For healthy subjects a creatinine clearance computed over a 24 hour urine collection for subjects that do not qualify with ≥ 90 mL/min CLcr may be done for confirmation purposes.</p>	Group	N	eGFR (mL/min/1.73m ²) or CLcr (mL/min)*	ESRD requiring hemodialysis	6	Requiring hemodialysis	Severe impairment	6	< 30 not on hemodialysis**	Moderate impairment	6	30 – < 60 ***	Mild impairment	6	60 - < 90	Healthy control	6	≥ 90 ****
Group	N	eGFR (mL/min/1.73m ²) or CLcr (mL/min)*																	
ESRD requiring hemodialysis	6	Requiring hemodialysis																	
Severe impairment	6	< 30 not on hemodialysis**																	
Moderate impairment	6	30 – < 60 ***																	
Mild impairment	6	60 - < 90																	
Healthy control	6	≥ 90 ****																	
	<p>In Period 1, subjects will receive Treatment A, a single oral dose of the microdose cocktail (i.e., midazolam oral solution, dabigatran etexilate and pitavastatin oral solution, atorvastatin and rosuvastatin oral solution) on Day 1 followed by pharmacokinetic sampling for 72 hours. The microdose cocktail will be dosed approximately</p>																		

	<p>24 hours prior to the start of dialysis for ESRD subjects.</p> <p>In Period 2, subjects (except ESRD subjects) will receive Treatment B, a single oral dose of rifampin capsules with a single oral dose of the microdose cocktail, coadministered on Day 1.</p> <p>In Period 2, ESRD subjects will receive only Treatment A, a single oral dose of the microdose cocktail on Day 1. The microdose cocktail will be dosed as soon as possible within 24 hours after the completion of dialysis.</p> <p>In each period, pharmacokinetic samples for plasma midazolam, dabigatran (as free dabigatran), pitavastatin, pitavastatin lactone, atorvastatin, 2-OH atorvastatin, 4-OH atorvastatin, and rosuvastatin will be collected for up to 72 hours following dosing. Pharmacokinetic samples for plasma rifampin will be collected for up to 24 hours following dosing in Period 2 only from all subjects except for ESRD subjects.</p> <p>In each period, urine samples will be collected for 24 hours postdose, if possible, to assess dabigatran and rosuvastatin pharmacokinetics and blood and urine samples will be collected for 24 hours postdose for measurement of biomarker concentrations. For subjects with renal impairment, urine samples will be collected whenever possible, as they may not be able to produce urine at each interval. For subjects who are anuric, urine samples will not be collected.</p> <p>There will be at least 14 days washout between dosing in Period 1 and dosing in Period 2.</p> <p>The clinic will attempt to contact subjects (including subjects who terminate the study early) using their standard procedures approximately 14 days after the last study drug administration to determine if any adverse events have occurred since the last visit.</p>
Blinding:	This is an open-label study.
Number of Subjects:	Twenty-four (24) RI subjects between 18 and 78 years of age (inclusive) will be enrolled; 6 subjects with mild renal impairment; 6 subjects with moderate renal impairment; 6 subjects with severe renal impairment who are not on dialysis; and 6 subjects with severe renal impairment who are on dialysis or with ESRD on dialysis. A cohort of 6 healthy control subjects will also be enrolled.

<p>Dosage, Dosage Form, Route, and Dose Regimen:</p>	<p>Subjects will receive the following treatments in a fixed-sequence:</p> <p><u>Treatment A (Period 1):</u> Single oral dose of the microdose cocktail at Hour 0 on Day 1 following an overnight fast. The microdose cocktail will contain:</p> <ul style="list-style-type: none"> • 10 µg midazolam (1 mL of 10 µg/mL oral solution), administered orally as midazolam hydrochloride (HCl); • 375/10 µg dabigatran etexilate and pitavastatin (1 mL of 375/10 µg/mL oral solution), administered as dabigatran etexilate mesylate and pitavastatin calcium; and • 100/50 µg atorvastatin and rosuvastatin (2 mL of 50/25 µg/mL oral solution, dosed as 2 x 1 mL dosing syringes), administered as atorvastatin calcium trihydrate and rosuvastatin calcium. <p>Each oral solution will be administered using 1 mL amber dosing syringes.</p> <p><u>Treatment B (Period 2):</u> 600 mg (2 x 300 mg capsules) single oral dose rifampin coadministered with a single oral dose microdose cocktail (as described in Treatment A) at Hour 0 on Day 1 following an overnight fast.</p> <p>NOTE: Subjects with ESRD will not receive rifampin in Period 2; they will receive Treatment A in Period 2, instead of Treatment B.</p>
<p>Key Assessments:</p>	<p>Pharmacokinetics:</p> <p>The following plasma pharmacokinetic parameters will be calculated for all plasma analytes (rifampin [Period 2 only], midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 4-OH atorvastatin, 2-OH atorvastatin, and rosuvastatin) on Day 1 of each treatment period, as appropriate: AUC₀₋₂₄, AUC_{0-last}, AUC_{0-∞}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F (parent only), and V_z/F (parent only).</p> <p>The following urine pharmacokinetic parameters may also be calculated for rosuvastatin and dabigatran: CL_r, Ae₀₋₂₄, and fe.</p> <p>Protein binding (plasma) assays will be performed on predose samples from Period 1.</p> <p>Non-model based descriptive statistics of pharmacokinetic parameters will be provided by subject group for all plasma analytes (midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 4-OH atorvastatin, 2-OH atorvastatin, rosuvastatin, and rifampin,) and urine analytes (dabigatran and rosuvastatin only). Separate ANOVA models will be constructed for each substrate in the microdose cocktail. AUC_{0-∞} will be compared</p>

	<p>between the different groups (except subjects with ESRD) by constructing the appropriate contrasts from the model and computing the GMRs and corresponding 95% confidence intervals. Similar analyses will be conducted for the following pharmacokinetic parameters: AUC0-24, AUC0-last, Cmax, and C24.</p> <p>Similar statistical analyses describe above will be used to compare Period 1 and Period 2 pharmacokinetic parameters of subjects with ESRD.</p> <p>Safety:</p> <p>Safety will be monitored through physical examination, vital signs, pulse oximetry, 12-lead electrocardiograms (ECGs), adverse events and clinical laboratory tests. Summary statistics for the laboratory safety tests, 12-lead ECGs, and/or vital signs may be computed and provided, as deemed clinically appropriate.</p> <p>Biomarkers:</p> <p>The following biomarkers and also uremic toxins will be measured to assess OATP1B inhibition: serum total/direct bilirubin, plasma coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), glycodeoxycholate-sulfate (GDCA-S), glycochenodeoxycholate-sulfate (GCDCA-S), deoxycholate-sulfate (DCA-S), Taurochenodeoxycholate-sulfate (TCDC-S), and Taurodeoxycholate-sulfate (TCDA-S) concentrations and urine coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, and CMPF concentrations.</p> <p>To evaluate the concentration-time effects of perpetrator-mediated inhibition of OATP1B, the following parameters will be calculated for the biomarkers and uremic toxins on Day 1 following Treatments A and B: AUC0-last, Cmax, Tmax for plasma parameters, and Ae0-24 and fe for urine parameters. Measurements to assess renal clearance will be performed after a 24-hour interval urine collection.</p> <p>A predose sample will also include measurement of serum creatinine (central lab) and may also be used to measure uremic toxins/biomarkers (exploratory).</p> <p>The exploratory objectives will be evaluated using the same statistical models that were used to address the primary objectives. Although no formal hypotheses will be tested, these models will be used to estimate the between-group differences amongst each substrate/perpetrator combination within a substrate.</p>
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6 STUDY EVENTS FLOW CHART

Study Procedures ^a	Days → Hours →	Screening ^b	Study Days in Periods 1 (Treatment A) and 2 (Treatment B) ^c																	FU ^d		
			-2 or -1	1														2		3	4	
			(C-I) ^e	P	0	0.25	0.5	1	1.5	2	3	4	6	8	12	16 ^f	24	32	48	72		
Administrative Procedures																						
Informed Consent		X																				
Informed Consent for Future Biomedical Research		X																				
Inclusion/Exclusion Criteria		X	X ^g																			
Medical History		X																				
Safety Evaluations																						
Full Physical Examination ^h		X																		X ⁱ		
Height		X																				
Weight		X	X ^g																			
Assessment of Renal Function ^j		X																				
12-Lead Electrocardiogram		X		X ^k																X ^l		
Vital Signs (heart rate, blood pressure, & respiratory rate)		X		X ^k					X											X ^l		
Vital Signs (temperature only)		X																				
Pulse Oximetry ^l		X		X		X	X		X		X	X										
Hematology, Serum Chemistry ^m , and Urinalysis		X	X																	X ^l		
Serum Pregnancy Test (female subjects only)		X	X																			
Serum FSH (postmenopausal females only)		X																				
Urine or Saliva Drug Screen		X	X																			
Urine or Breath Alcohol Screen		X	X																			
HIV/Hepatitis Screen		X																				
Adverse Events Monitoring		X	X																			
Concomitant Medication Monitoring		X																				
Study Drug Administration / Pharmacokinetics																						
Microdose Cocktail Administration (P1 & P2)				X																		
Rifampin Administration (P2) ⁿ				X																		
Blood for Microdose Pharmacokinetics (P1 & P2) ^o			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood for Protein Binding (P1)			X ^g																			
Blood for Rifampin Pharmacokinetics (P2) ⁿ			X		X	X	X	X	X	X	X		X		X		X					
Blood for Serum Total and Direct Bilirubin (P1 & P2) (Central Lab)			X		X	X		X		X	X		X		X		X					

Study Procedures ^a	Days → Hours →	Screening ^b	Study Days in Periods 1 (Treatment A) and 2 (Treatment B) ^c																FU ^d	
		-2 or -1	1												2	3	4			
		(C-I) ^e	P	0	0.25	0.5	1	1.5	2	3	4	6	8	12	16 ^f	24	32	48	72	
Blood for Serum Creatinine (P1 & P2) (Central Lab)			X			X	X		X		X	X		X		X				
Blood for other Biomarkers (P1 & P2) ^p			X ^q			X	X		X		X	X		X		X				
Urine Collection for Pharmacokinetics ^{r, s}			X	X																
Urine Collection for Biomarkers ^{s, t}			X	X																
Other Procedures																				
Blood for Genetic Analysis ^u				X																
Confinement in the CRU				X																
Visit		X																		

- For details on Procedures, refer to [Section 10](#) and/or corresponding appendices.
- Within 28 days prior to the first study drug administration.
- Each dose will be separated by a washout of at least 14 days.
- The clinic will attempt to contact subjects (including subjects who terminate the study early) using their standard procedures approximately 14 days after the last study drug administration to determine if any adverse events have occurred since the last visit.
- Subjects will be admitted to the CRU on Day -2 (Orlando clinical site) or Day -1 (Miami clinical site), at the time indicated by the CRU.
- The 16-hour time point following dosing on Day 1 will be either on Day 1 or Day 2, depending on the time of dosing on Day 1.
- To be performed in Period 1 only.
- A symptom-driven physical examination may be performed at other times, at the Investigator's discretion.
- To be performed on Day 4 or prior to early termination from the study.
- Assessment of renal function will be performed using MDRD equation; prior to Period 1, baseline eGFR will be obtained twice (at least 48 hours apart as part of subject screening) and averaged. The second baseline eGFR sample may be obtained at the time of check-in in Period 1. CLcr by Cockcroft-Gault equation will be used for healthy control assignment (normal renal function).
- To be performed within 24 hours prior to dosing.
- Pulse oximetry: oxygen levels and heart rate readings will be performed at screening and prior to dosing (baseline) and will be monitored continuously with a pulse oximeter for approximately 6 hours postdose (with the exception of restroom use), with readings taken at approximately the scheduled time points.
- Samples for serum chemistry will be obtained following a fast of at least 8 hours; however, in case of dropouts or rechecks, subjects may not have fasted for

8 hours before the serum chemistry sample is taken.

- n. Rifampin administration and pharmacokinetic collection to be performed in Period 2 only. Subjects with ESRD will not receive rifampin.
- o. Samples collected for pharmacokinetic measurements of plasma analytes (midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 4-OH atorvastatin, 2-OH atorvastatin, and rosuvastatin).
- p. Samples collected for the measurement of the following plasma biomarkers and uremic toxins: coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, CMPF, GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S. Additional biomarkers/uremic toxins may also be measured.
- q. Predose samples may also be used to measure uremic toxins/biomarkers (exploratory).
- r. Urine collection for pharmacokinetic measurements of dabigatran and rosuvastatin.
- s. Urine collection intervals are: predose (spot collection), 0 - 4 hours, 4 - 8 hours, 8 - 12 hours, and 12 - 24 hours post-dose. For subjects with renal impairment, urine samples will be collected whenever possible, as they may not be able to produce urine at each interval. Note: For subjects who are anuric, urine samples will not be collected.
- t. Samples collected for the measurement of the following biomarkers and uremic toxins in urine: coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, and CMPF. Additional biomarkers/uremic toxins may also be measured.
- u. This sample should be drawn for planned analysis of the association between genetic variants in DNA and drug response. This sample will not be collected at the site if there is either a local law or regulation prohibiting collection, or if the IRB/IEC does not approve the collection of the sample for these purposes. If the sample is collected, leftover extracted DNA will be stored for future biomedical research if the subject signs the FBR consent. If the planned genetic analysis is not approved, but FBR is approved and consent is given, this sample will be collected for the purpose of FBR.

Abbreviations: C-I = Check-in, CLcr = Creatinine clearance, CMPF = 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, CRU = Clinical research unit, DCA-S = Deoxycholate-sulfate, DNA = Deoxyribonucleic acid, eGFR = Estimated glomerular filtration rate, ESRD = End-stage renal disease, FBR = Future biomarker research, FSH = Follicle-stimulating hormone, FU = Follow-Up, GCDCA-S = Glycochenodeoxycholate-sulfate, GDCA-S = Glycodeoxycholate-sulfate, HIV = Human immunodeficiency virus, IRB/IEC = Institutional Research Board/Independent Ethics Committee, MDRD = Modification of Diet in Renal Disease, OH = Hydroxy, P = Predose, P1 = Period 1, P2 = Period 2, TCDA-S = Taurodeoxycholate-sulfate, TCDC-S = Taurochenodeoxycholate-sulfate.

7 BACKGROUND AND RATIONALE

7.1 Background

Renal impairment is a well-recognized influence on drug clearance. Both chronic renal failure (CRF) and ESRD have been shown to not only reduce the systemic clearance of drugs but also impact protein and tissue binding.¹ While this is to be anticipated with drugs typically eliminated renally, it is also appreciated that CRF can modify non-renal disposition, including those typically ‘handled’ hepatically. Over the years, data has emerged suggesting the influence of renal failure on the cytochrome P450 (CYP450) family, but also on transporter function, including hepatic and renal uptake transporters. Alterations in these transporters as well as drug metabolism pathways may have a meaningful impact on predictions and decision-making in drug development. The underlying mechanisms responsible for alterations in drug disposition (transporter and enzyme activity) in the setting of renal failure remain poorly characterized. While plausible through direct inhibition or indirect mediation, tighter predictions remain critical in patients with CRF and ESRD. Current predictive physiologically based pharmacokinetic (PBPK) modeling in this setting with an array of pathways and substrates remains rudimentary and will be strengthened through clinical investigation and subsequent iterative modeling.

The current study takes advantage of a Merck-characterized cocktail² of substrates and now applying them with greater finesse in disease populations. While the initial intent may be to strengthen and guide predictions in the setting of renal impairment, it is the ultimate desire to reduce the conduct of dedicated studies in this more at-risk and potentially frail population. The present study will probe three pathways of disposition: CYP3A using midazolam, P-glycoprotein (P-gp) using dabigatran, OATP1B1/1B3 using rosuvastatin, pitavastatin and atorvastatin, and breast cancer resistance protein (BCRP) using rosuvastatin. The relative contribution of renal impairment to substrate disposition will be examined across the spectrum of renal impairment (mild, moderate, severe, and ESRD). This will provide a holistic framework to better appreciate the influencing of renal clearance of such pathways with comparison to normal healthy kidney function (glomerular filtration rate [GFR] ≥ 90 mL/min). Rifampin, a designated probe inhibitor of OATP1B1/1B3 will be included to examine hepatic uptake in the setting of renal impairment across the spectrum of renal function.

Recently, there is an increased interest in the identification of selective endogenous biomarkers which are substrates of OATP1B and could be used early in clinical development to assess the potential of drug candidates to inhibit these transporters. In the current study, bilirubin, conjugated bilirubins, and coproporphyrins I and III will be measured as candidate biomarkers in pharmacokinetic samples from patients and healthy subjects treated with rifampin.

7.1.1 Midazolam

Midazolam, a short acting benzodiazepine, is a reversible, non-selective agonist of γ -aminobutyric acid (GABA)-A receptors in the central nervous system. Midazolam is

routinely used in clinical medicine as a sedative and is specifically indicated for pediatric pre-procedural sedation. It has been used in the United States as a parenteral sedative and as an oral syrup formulation for pediatric use. Midazolam provides a safe and effective sedation and anxiolysis prior to surgical procedures that require anesthesia as well as before other procedures that require sedation but may not require anesthesia. The sedative effects of midazolam are dependent on the dose administered, the route of administration, and the presence or absence of other medications. Onset time of sedative effects after oral administration is approximately 10-20 minutes depending on the dose administered.

Sedation after intravenous injection is more rapid and is usually achieved within 5 minutes. Rare side effects (occurring in less than 1% of subjects) have been reported following administration of midazolam and include partial or complete impairment of recall, episodes of oxygen desaturation, respiratory depression, apnea, and airway obstruction.³ After oral administration, midazolam is almost completely absorbed with 90% of a radiolabeled dose excreted in the urine within 24 hours; less than 10% is excreted in the feces within 5 days. The mean T_{max} values across dose groups (0.25, 0.5, and 1.0 mg/kg) range from 0.17 to 2.65 hours. Midazolam exhibits linear pharmacokinetics between oral doses of 0.25 to 1.0 mg/kg (up to a maximum dose of 40 mg).³

Midazolam undergoes extensive first-pass metabolism by hepatic and gastrointestinal CYP3A4 and is rapidly converted to its major metabolite, 1'-hydroxymidazolam, which subsequently undergoes conjugation. Because midazolam is primarily metabolized by CYP3A4, it is subject to pharmacokinetic interactions with inhibitors and inducers of this isozyme. Decreases in plasma midazolam concentrations after administration of known inducers of CYP3A4-mediated metabolism all occur to a similar extent: carbamazepine (94%), phenytoin (94%) and rifampin (96%).³

7.1.2 Dabigatran

Dabigatran etexilate is a low-molecular-weight prodrug, without pharmacological activity, that is converted to its active form, dabigatran, which is also subject to conjugation forming pharmacologically active acyl glucuronides. Dabigatran and its acyl glucuronides are potent, competitive, reversible direct thrombin inhibitors indicated to reduce the risk of stroke and systemic embolism in patients with non-valvular atrial fibrillation. Because thrombin (serine protease) enables the conversion of fibrinogen into fibrin during the coagulation cascade, its inhibition prevents the development of a thrombus. Both free and clot-bound thrombin, and thrombin-induced platelet aggregation are inhibited by the active moieties.^{4,5}

Following oral administration, dabigatran etexilate is rapidly absorbed and quickly and completely converted by esterase-catalyzed hydrolysis to dabigatran in the gut, plasma and liver. Peak plasma concentrations of dabigatran occur 1-2 hours after drug administration. Bioconversion of dabigatran etexilate is completed in the liver, and approximately 20% is conjugated with glucuronic acid to pharmacologically active acyl glucuronides and excreted in the bile. Due to its isomerisation, several positional dabigatran glucuronides isomers occur in plasma. The dabigatran glucuronide isomers represent about 20% of the total dabigatran after oral dosing (comparison of the AUC values). The pro-drug, dabigatran etexilate, is not

metabolized by the CYP enzymes but is a substrate of the efflux transporter P-gp. Dabigatran is not a substrate of P-gp.^{4, 5, 6}

The major route of dabigatran elimination is via kidneys. Following intravenous administration of [14C]-labeled dabigatran, a mean of 85% of the administered dose was recovered in urine and 6% in feces over 168 h postdose. Following oral administration of [14C]-labeled dabigatran etexilate, a mean of 7% of the administered dose was recovered in urine and 86% in feces over 168 h postdose, most likely due to unabsorbed dabigatran etexilate. Renal clearance represented 80% of total dabigatran clearance.⁶

No dose adjustment of dabigatran is recommended in patients with mild or moderate renal impairment. A reduced dose of dabigatran (75 mg twice a day [BID] vs. 150 mg BID) is recommended in patients with severe renal impairment (CrCl 15-30 mL/min). Dosing recommendations for patients with CrCl <15 mL/min or on dialysis are not provided in the label.

7.1.3 Atorvastatin

Atorvastatin is a synthetic lipid-lowering agent that inhibits 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. This enzyme catalyzes the conversion of HMG-CoA to mevalonic acid, a precursor of sterols, including cholesterol.

Atorvastatin calcium is indicated as an adjunct to diet to:

- Reduce the risk of myocardial infarction (MI), stroke, revascularization procedures, and angina in patients without coronary heart disease (CHD), but with multiple risk factors;
- Reduce the risk of MI and stroke in patients with type 2 diabetes without CHD, but with multiple risk factors.
- Reduce the risk of non-fatal MI, fatal and non-fatal stroke, revascularization procedures, hospitalization for coronary heart failure (CHF), and angina in patients with CHD.
- Reduce elevated total cholesterol (total-C), low-density cholesterol (LDL-C), apolipoprotein B (apo B), and triglyceride (TG) levels and increase high-density cholesterol (HDL-C) in adult patients with primary hyperlipidemia (heterozygous familial and nonfamilial) and mixed dyslipidemia.
- Reduce elevated TG in patients with hypertriglyceridemia and primary dysbetalipoproteinemia.
- Reduce total-C and LDL-C in patients with homozygous familial hypercholesterolemia.

- Reduce elevated total-C, LDL-C, and apo B levels in boys and postmenarchal girls, 10 to 17 years of age, with heterozygous familial hypercholesterolemia after failing an adequate trial of diet therapy.

Dosing is usually initiated with 10 mg administered daily and may be increased up to 80 mg per day, if necessary. Prior to beginning the treatment, patients should be placed on a standard cholesterol-lowering diet.⁷

Atorvastatin is absorbed from the gastrointestinal tract, within 1 hour. Atorvastatin has an absolute bioavailability of approximately 14% and is approximately 98% bound to plasma protein. Atorvastatin is extensively metabolized in the gut wall and liver with 2 main metabolites: ortho-hydroxyatorvastatin and para-hydroxyatorvastatin. These metabolites have HMG-CoA reductase inhibitory activity equal to that of atorvastatin; and approximately 70% of atorvastatin's pharmacological activity is attributed to active metabolites. Atorvastatin is metabolized in part by CYP3A4 enzymes and is transported into the liver via OATP1B1 transporters.^{7,8}

Atorvastatin's bioavailability is reduced when the drug is administered with food, or when it is given in the evening rather than the morning. However, as LDL-C reduction is similar under fed and fasted conditions, and after morning or evening administration, atorvastatin may be administered without regard to meals or time of day. Atorvastatin and its metabolites are excreted mainly in the bile. Less than 2% of an oral dose of atorvastatin is excreted in the urine. The pharmacokinetic half-life of atorvastatin is approximately 14 hours while the pharmacodynamic half-life of HMG-CoA reductase inhibition is reported to be about 20 to 30 hours. This pharmacodynamic effect persists for longer than the half-life of atorvastatin in plasma, due to the longer half-life of the active metabolites.⁷

Renal disease has no influence on the plasma concentrations or LDL-C reduction of atorvastatin; thus, dose adjustment in patients with renal dysfunction is not necessary. While studies have not been conducted in patients with ESRD, hemodialysis is not expected to significantly enhance clearance of atorvastatin since the drug is extensively bound to plasma proteins.

7.1.4 Pitavastatin

Pitavastatin is a synthetic lipid-lowering agent which competitively inhibits HMG-CoA reductase. Pitavastatin is indicated as an adjunct therapy to diet to reduce elevated total-C, LDL-C, apo B, and TG concentrations and to increase HDL-C levels in patients with primary hyperlipidemia or mixed dyslipidemia. The dose ranges from 1 to 4 mg daily without regards to meal. Following 4 weeks of initiation of therapy, lipids levels should be evaluated in order to adjust if needed the dosage.⁹

Pitavastatin is absorbed at about 1 hour in the small intestine and has an absolute bioavailability of an oral solution is approximately 51%. Pitavastatin is approximately 99% bound to plasma protein, mainly albumin and alpha 1-glycoprotein. Pitavastatin is transported in the liver mainly by OATP1B1 transporters where it is metabolized by

glucuronidation via UGTs (UGT1A3 and UGT2B7) to pitavastatin lactone. Pitavastatin is minimally metabolized by CYP enzymes; CYP2C9 and to a lesser extent CYP2C8.^{8,9}

Administering pitavastatin with a high fat meal decreases C_{max} by 43% but does not affect the extent of exposure. Pitavastatin is mainly excreted unchanged; about 15% is eliminated in urine and about 79% in feces.^{8,9}

In patients with moderate renal impairment (glomerular filtration rate of 30 to <60 mL/min/1.73 m²) and end stage renal disease receiving hemodialysis, pitavastatin AUC_{0-∞} is 79 and 86% higher than those of healthy subjects, respectively, while pitavastatin C_{max} is 60 and 40% higher than those of healthy subjects, respectively. Patients received hemodialysis immediately before pitavastatin dosing and did not undergo hemodialysis during the pharmacokinetic study. Hemodialysis patients have 33 and 36% increases in the mean unbound fraction of pitavastatin as compared to healthy subjects and patients with moderate renal impairment, respectively. The effect of mild and severe renal impairment on pitavastatin exposure is unknown.

7.1.5 Rosuvastatin

Rosuvastatin, a synthetic lipid-lowering agent, is a HMG-CoA reductase inhibitor. As a selective and competitive inhibitor of HMG-CoA reductase, rosuvastatin blocks the formation of mevalonate, a precursor of cholesterol, thus reducing total C, LDL-C and TG and increasing HDL-C concentrations in plasma.^{10,11}

Rosuvastatin is indicated as an adjunct to diet in adult patients:

- with primary hyperlipidemia and mixed dyslipidemia, to reduce elevated total-C, LDL-C, ApoB, non-HDL-C, and TG levels, and to increase HDL-C.
- with hypertriglyceridemia.
- with primary dysbetalipoproteinemia (Type III hyperlipoproteinemia).
- with homozygous familial hypercholesterolemia to reduce LDL-C, total-C, and ApoB.
- with atherosclerosis as part of a treatment strategy to slow the progression of the disease by lowering total-C and LDL-C.
- without clinically evident coronary heart disease, but with multiple risk factors for myocardial infarction, stroke, and arterial revascularization procedures.

Rosuvastatin is also indicated in pediatric patients, 10 to 17 years of age, with heterozygous familial hypercholesterolemia to reduce elevated total-C, LDL-C and ApoB after failing an adequate trial of diet therapy.¹⁰

Peak plasma concentrations of rosuvastatin are reached between 3 to 5 hours following oral administration under both fed and fasting conditions. Both C_{max} and AUC increased in approximate proportion to rosuvastatin dose and the absolute bioavailability of rosuvastatin

is approximately 20%. Administration of rosuvastatin with food did not affect the extent of absorption. BCRP plays a key role in the absorption and hepatic clearance of rosuvastatin. Rosuvastatin is primarily eliminated via the liver; however it is not extensively metabolized with approximately 10% of a radiolabeled dose recovered as metabolite. CYP2C9 appears to be the principal enzyme involved in the formation of the major metabolite, N-desmethyl rosuvastatin, after rosuvastatin is rapidly and selectively taken from the blood into the liver by OATP1B1. Rosuvastatin is also a substrate for BCRP. Rosuvastatin is mainly excreted via bile and feces (90%).^{10, 12, 13}

Rosuvastatin exposure is not influenced by mild to moderate renal impairment ($\text{eGFR} \geq 30 \text{ mL/min/1.73 m}^2$); however, exposure to rosuvastatin is increased to a clinically significant extent in patients with severe renal impairment who are not receiving hemodialysis. CRESTOR dosing should be adjusted in patients with severe renal impairment ($\text{CLcr} < 30 \text{ mL/min}$).¹⁰

7.1.6 Rifampin

Rifampin is a semi-synthetic antibiotic derivative of rifamycin SV which acts by inhibiting deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase activity in susceptible species of *Mycobacterium tuberculosis*. Its activity does not impede mammalian enzyme RNA polymerase, therefore it is an effective treatment for both tuberculosis and meningococcus infections. Rifampin can be administered by oral route or by an IV infusion of 30 minutes to 3 hours. The IV doses are the same as oral doses.¹⁴

Rifampin is readily absorbed from the gastrointestinal tract and is considered to be a highly variable drug in healthy adults and pediatric populations. A single oral dose of 600 mg of rifampin in healthy adults has an average peak serum concentration of 7 $\mu\text{g/mL}$ ranging from 4 to 32 $\mu\text{g/mL}$ with an average half-life of 3.35 ± 0.66 hours. Gastric absorption with food reduces the bioavailability of rifampin by about 30%. Rifampin is widely distributed throughout the body and can reach effective concentrations in various organs and cerebrospinal fluid. Rifampin is 80% protein bound in the blood while the remaining unbound fraction is not ionized and can readily diffuse into tissues.¹⁴

Following multiple dosing, rifampin is known to be a potent inducer of efflux drug transporters such as hepatic P-gp and drug-metabolizing enzymes, among which are CYP2C9 and CYP3A4. However studies have shown that an acute single dose of rifampin inhibits OATP1B1/1B3 as well as P-gp transport enzymes.

Rifampin is rapidly eliminated in the bile and undergoes enterohepatic recirculation; during this process, gradual deacetylation of the drug occurs so that nearly all the drug in the bile is in this form 6 hours postdose. The deacetylated form of rifampin is active and exhibits antibacterial properties. Moreover, intestinal reabsorption is reduced by deacetylation thus promoting drug elimination.

While label data for rifampicin in patients in renal failure is relatively sparse, data exists in the clinical literature. While there may be a reduction in urine excretion of rifampicin and

deacetyl rifampicin in patients with renal failure when compared to healthy controls, there does not appear to be an effect on plasma concentrations of rifampicin.¹⁵ Rifampicin is principally eliminated through hepato-biliary excretion¹⁶ with renal elimination playing a relatively minor role.¹⁷ The general recommendation is not to reduce rifampicin dose for tuberculous patients with renal failure¹⁵ and to also administer at the usual dose of 600 mg in patients on hemodialysis.¹⁸

7.2 Rationale

7.2.1 Rationale for this Study and Study Design

This study is intended to evaluate the pharmacokinetic performance of a cocktail of five probe substrates (i.e., dabigatran etexilate, atorvastatin, pitavastatin, rosuvastatin, and midazolam) in the setting of renal impairment and healthy subjects orally as microdoses. A probe inhibitor, rifampin will be included to assess the influence of transporter function in this setting.

The effect of timing of dosing relative to dialysis will be explored by assessing the pharmacokinetic profile of the different analytes following dosing before and after dialysis in ESRD patients.

7.2.2 Rationale for the Dose Selection

Midazolam:

A midazolam dose of 10 µg has been chosen for administration in the microdose cocktail. The dose of midazolam that is typically required for effective sedation is 0.5 mg/kg (to a maximum of 20 mg). A 2 mg midazolam dose is frequently selected for evaluation of midazolam as a probe substrate in DDI studies. The 10 µg dose selected for use in this study is therefore 1/200th of the standard dose used in DDI studies and is not expected to generate any clinically apparent pharmacodynamic effects. The plasma pharmacokinetic profile of midazolam arising from administration of this microdose is expected to be measurable using the bioanalytical procedures specified for this study.

Dabigatran:

A dabigatran etexilate dose of 375 µg has been chosen for administration in the microdose cocktail. The clinically approved doses of dabigatran etexilate are 75 mg and 150 mg, administered twice daily. The dose selected for this study is therefore 1/200th of the lowest clinically approved dose, and is not expected to generate any clinically apparent pharmacodynamic effects. The plasma pharmacokinetic profile of dabigatran arising from administration of this microdose is expected to be measurable using the bioanalytical procedures specified for this study.

Pitavastatin:

A pitavastatin dose of 10 µg has been chosen for administration in the microdose cocktail. The clinically approved dose range of pitavastatin is 1-4 mg, administered once daily. The usual recommended starting dose is 2 mg. The dose selected for this study is therefore 1/200th of the usual clinical starting dose, and is not expected to generate any clinically apparent pharmacodynamic effects. The plasma pharmacokinetic profile of pitavastatin arising from administration of this microdose is expected to be measurable using the bioanalytical procedures specified for this study.

Atorvastatin:

An atorvastatin dose of 100 µg has been chosen for administration in the microdose cocktail. The clinically approved dose range of atorvastatin is 10-80 mg, administered once daily. The usual recommended starting dose is 10-20 mg. The dose selected for this study is therefore 1/100th of the lowest clinically approved dose, and is not expected to generate any clinically apparent pharmacodynamic effects. The plasma pharmacokinetic profile of atorvastatin arising from administration of this microdose is expected to be measurable using the bioanalytical procedures specified for this study.

Rosuvastatin:

A rosuvastatin dose of 50 µg has been chosen for administration in the microdose cocktail. The clinically approved dose range of rosuvastatin is 5-40 mg, administered once daily. The dose selected for this study is therefore 1/100th of the lowest clinically approved dose, and is not expected to generate any clinically apparent pharmacodynamic effects. The plasma pharmacokinetic profile of rosuvastatin arising from administration of this microdose is expected to be measurable using the bioanalytical procedures specified for this study.

Rifampin:

The single 600 mg rifampin dose has been selected based on the use of this dose in previous DDI studies, and the intent to use this dose in subsequent DDI studies. While 600 mg per day is the maximum daily dose used in the treatment of tuberculosis (typically for several months), higher daily doses are used in other settings (e.g., 600 mg BID for meningococcal prophylaxis and 300 mg three times a day for prosthetic valve endocarditis), and single doses of 600 mg are expected to be well-tolerated.

Table 1: Study Microdose Cocktail Component Doses:

Probe Compound	Cocktail Microdose	Therapeutic Dose
Midazolam	10 µg	1 – 5 mg/dose
Dabigatran	375 µg	75 – 150 mg BID
Pitavastatin	10 µg	1 – 4 mg once a day [QD]
Atorvastatin	100 µg	10 – 80 mg QD
Rosuvastatin	50 µg	5 – 40 mg QD

7.2.3 Rationale for Endpoints

The most representative pharmacokinetic parameter for the assessment of inhibition of metabolic elimination of single oral dose of dabigatran etexilate, atorvastatin, pitavastatin, rosuvastatin, and midazolam is AUC_{0-∞}. Thus, the primary pharmacokinetic measure of the study is the true GMR of AUC_{0-∞} of each substrate with and without probe DDI perpetrators.

7.2.4 Rationale for Planned Exploratory Biomarker Research

Planned Genetic Analysis

Understanding genetic determinants of drug response and the molecular basis of disease is an important endeavor during medical research. This research will evaluate whether genetic variation within a clinical trial population correlates with response to the treatment(s) under evaluation and/or disease. If genetic variation is found to predict efficacy or adverse events, the data might inform optimal use of therapies in the patient population. Knowledge of the molecular basis of disease contributes to the development of novel biomarkers and the identification of new drug targets. This research contributes to understanding molecular basis of disease and the genetic determinants of efficacy and safety associated with the treatments in this study.

7.2.5 Rationale for Future Biomedical Research

The Sponsor will conduct Future Biomedical Research on DNA specimens consented for future biomedical research during this clinical trial.

Such research is for biomarker testing to address emergent questions not described elsewhere in the protocol (as part of the main trial) and will only be conducted on specimens from appropriately consented subjects. The objective of collecting/retaining specimens for Future Biomedical Research is to explore and identify biomarkers that inform the scientific understanding of diseases and/or their therapeutic treatments. The overarching goal is to use such information to develop safer, more effective drugs/vaccines, and/or to ensure that subjects receive the correct dose of the correct drug/vaccine at the correct time. The details of this Future Biomedical Research sub-trial are presented in [Appendix 2](#) - Collection and Management of Specimens for Future Biomedical Research.

8 STUDY OBJECTIVES, ESTIMATIONS, AND ENDPOINTS

8.1 Objectives and Estimations

8.1.1 Primary

Primary (Period 1):

Objective 1: To characterize the plasma pharmacokinetic profiles of midazolam, dabigatran, pitavastatin (and its metabolite, pitavastatin lactone), atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin), and rosuvastatin following a single oral dose administration of a microdose cocktail in healthy subjects, in subjects with mild, moderate, severe (not on dialysis) renal impairment, and in subjects with ESRD (on dialysis).

Estimation 1: The plasma pharmacokinetic parameters (e.g., AUC₀₋₂₄, AUC_{0-last}, AUC_{0-∞}, C_{max}, C₂₄, T_{max}, and apparent terminal t_{1/2}) of midazolam, dabigatran, pitavastatin (and its metabolite, pitavastatin lactone), atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin), and rosuvastatin following a single oral dose administration of a microdose cocktail will be estimated in healthy subjects, in subjects with mild, moderate, severe (not on dialysis) renal impairment, and in subjects with ESRD (on dialysis).

Primary Objective for Midazolam:

Objective 2: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of midazolam administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Estimation 2: Plasma AUC_{0-∞} of midazolam following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of midazolam following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).

Primary Objective for Dabigatran:

Objective 3: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of dabigatran administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Estimation 3: Plasma AUC_{0-∞} of dabigatran following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of dabigatran following a single oral dose administration of the microdose

cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).

Primary Objective for Pitavastatin:

Objective 4: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of pitavastatin (and its metabolite, pitavastatin lactone) administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Estimation 4: Plasma AUC_{0-∞} of pitavastatin (and its metabolite pitavastatin lactone) following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of pitavastatin (and its metabolite, pitavastatin lactone) following a single dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).

Primary Objective for Atorvastatin:

Objective 5: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin) administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Estimation 5: Plasma AUC_{0-∞} of atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin) following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin) following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).

Primary Objective for Rosuvastatin:

Objective 6: To evaluate the effect of a single oral dose of rifampin on the single-dose pharmacokinetics of rosuvastatin administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Estimation 6: Plasma AUC_{0-∞} of rosuvastatin following a single oral dose administration of a microdose cocktail containing midazolam, dabigatran etexilate, pitavastatin, atorvastatin, and rosuvastatin with a single oral dose of rifampin will be estimated and compared to the plasma AUC_{0-∞} of rosuvastatin following a single oral dose administration of the microdose cocktail alone, in each subject population separately (except for ESRD subjects; i.e., healthy subjects and subjects with mild, moderate, or severe renal impairment).

8.1.2 Secondary Objectives

Objective 1: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of **midazolam** administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Objective 2: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of **dabigatran** administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Objective 3: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of **pitavastatin** (and its metabolite, pitavastatin lactone) when pitavastatin is administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Objective 4: To estimate the effect of a single oral dose of rifampin on the single dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of **atorvastatin** (and its metabolites, ortho-hydroxyatorvastatin [2-OH atorvastatin] and para-hydroxyatorvastatin [4-OH atorvastatin]) when atorvastatin is administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Objective 5: To estimate the effect of a single oral dose of rifampin on the single oral dose pharmacokinetics (AUC₀₋₂₄, AUC_{0-last}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F, and V_z/F) of **rosuvastatin** administered as part of a microdose cocktail in each subject population separately (except for ESRD subjects).

Objective 6: To compare the magnitude of the drug-drug interaction (DDI) between rifampin and each of the 5 substrates in the microdose cocktail between healthy subjects and each category of RI subjects (mild, moderate, severe RI).

Objective 7: To estimate the plasma pharmacokinetic parameters (e.g., AUC₀₋₂₄, AUC_{0-last}, AUC_{0-∞}, C_{max}, C₂₄, T_{max}, and apparent terminal t_{1/2}) of dabigatran, atorvastatin (and its metabolites, 2-OH atorvastatin and 4-OH atorvastatin), pitavastatin (and its metabolite, pitavastatin lactone), rosuvastatin, and midazolam following a single oral dose administration of a microdose cocktail in ESRD subjects on dialysis when the microdose cocktail is administered at different times relative to dialysis.

Objective 8: To estimate the plasma pharmacokinetic parameters (e.g., AUC₀₋₂₄, AUC_{0-last}, AUC_{0-∞}, C_{max}, C₂₄, T_{max}, and apparent terminal t_{1/2}) of **rifampin** following a single oral dose administration with a single oral dose of a microdose cocktail in each subject population separately (except for ESRD subjects).

8.1.3 Exploratory Objectives

Objective 1: To evaluate plasma, serum, and urinary biomarkers to assess the impact of renal impairment on functions such as organic anion transporting polypeptide (OATP)1B inhibition.

Objective 2: To evaluate the urinary pharmacokinetics (e.g., CL_r, Ae₀₋₂₄, and fe) of dabigatran and rosuvastatin in each population.

8.1.4 Planned Exploratory Biomarker

Objective: To explore the relationship between genetic variation and response to the treatment(s) administered, and mechanisms of disease. Variation across the human genome may be analyzed for association with clinical data collected in this study.

8.2 Analysis Endpoints

Pharmacokinetics:

The following plasma pharmacokinetic parameters will be calculated for all plasma analytes (rifampin [Period 2 only], midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 2-OH atorvastatin, 4-OH atorvastatin, and rosuvastatin) on Day 1 of each treatment period, as appropriate: AUC₀₋₂₄, AUC_{0-last}, AUC_{0-∞}, C_{max}, C₂₄, T_{max}, apparent terminal t_{1/2}, CL/F (parent only), and V_z/F (parent only).

The following urine pharmacokinetic parameters may also be calculated for dabigatran and rosuvastatin: CL_r, Ae₀₋₂₄, and fe.

Protein binding (plasma) assays will be performed on predose samples from Period 1.

Safety:

Safety endpoints will include adverse events, physical examinations, vital signs, 12-lead ECGs, pulse oximetry, and clinical laboratory tests.

Biomarkers:

The following biomarkers and also uremic toxins will be measured to assess OATP1B inhibition: serum total/direct bilirubin, plasma coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, CMPF, GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S concentrations, and urine coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, and CMPF concentrations.

To evaluate the concentration-time effects of perpetrator-mediated inhibition of OATP1B, the following parameters will be calculated for the biomarkers and uremic toxins on Day 1 following Treatments A and B: AUC_{0-last}, C_{max}, T_{max} for plasma parameters, and Ae₀₋₂₄ and fe for urine parameters. Measurements to assess renal clearance will be performed after a

24-hour interval urine collection (urine will be collected in intervals 0-4 hr, 4-8 hr, 8-12 hr, and 12-24 hr).

A predose sample will also include measurement of serum creatinine (central lab) and may also be used to measure uremic toxins/biomarkers (exploratory).

9 INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This is an open label, 2 period, fixed sequence study in 24 renal impairment subjects and 6 healthy control subjects.

Twenty-four (24) RI subjects between 18 and 78 years of age (inclusive) will be enrolled; 6 subjects with mild renal impairment; 6 subjects with moderate renal impairment; 6 subjects with severe renal impairment who are not on dialysis; and 6 subjects with ESRD on dialysis. A cohort of 6 healthy control subjects will also be enrolled.

Conduct of the mild RI cohort may be performed, as determined by the Sponsor, following conduct completion and review of data from the moderate and severe RI cohorts and the healthy cohort.

Screening of subjects will occur within 28 days prior to the first dosing.

Assignment to a renal function group will be as follows:

Group	N	eGFR (mL/min/1.73m ²) or CLcr (mL/min)*
ESRD requiring hemodialysis	6	Requiring hemodialysis
Severe impairment	6	< 30 not on hemodialysis **
Moderate impairment	6	30 – < 60 ***
Mild impairment	6	60 - < 90
Healthy control	6	≥ 90 ****

* eGFR based on MDRD equation at screening. Baseline eGFR will be obtained twice during the screening period, and the mean of the two values will be used for group assignment. The second baseline eGFR sample may be obtained at the time of check-in in Period 1. CLcr by Cockcroft-Gault equation will be used for healthy control assignment (normal renal function).

** Reasonable efforts will be made to enroll at least 2 subjects with eGFR values of < 20 mL/min/1.73m²

*** Reasonable efforts will be made to ensure a broad representation across the range of eGFR values of 30 - < 60 mL/min/1.73m²

****. For healthy subjects a creatinine clearance computed over a 24 hour urine collection for subjects that do not qualify with >90 mL/min CLcr may be done for confirmation purposes.

In Period 1, subjects will receive Treatment A, a single oral dose of the microdose cocktail (i.e., midazolam oral solution, dabigatran etexilate and pitavastatin oral solution, atorvastatin and rosuvastatin oral solution) on Day 1 followed by pharmacokinetic sampling for 72 hours.

The microdose cocktail will be dosed approximately 24 hours prior to the start of dialysis for ESRD subjects.

In Period 2, subjects (except ESRD subjects) will receive Treatment B, a single oral dose of rifampin capsules with a single oral dose of the microdose cocktail, coadministered on Day 1.

In Period 2, ESRD subjects will receive only Treatment A, a single oral dose of the microdose cocktail on Day 1. The microdose cocktail will be dosed as soon as possible within 24 hours after the completion of dialysis.

In each period, pharmacokinetic samples for plasma midazolam, dabigatran (as free dabigatran), pitavastatin, pitavastatin lactone, atorvastatin, 2-OH atorvastatin, 4-OH atorvastatin, pitavastatin, and rosuvastatin will be collected for up to 72 hours following dosing. Pharmacokinetic samples for plasma rifampin will be collected for up to 24 hours following dosing in Period 2 only from all subjects except for ESRD subjects.

In each period, urine samples will be collected for 24 hours postdose, if possible, to assess dabigatran and rosuvastatin pharmacokinetics and blood and urine samples will be collected for 24 hours postdose for measurement of biomarker concentrations. For subjects with renal impairment, urine samples will be collected whenever possible, as they may not be able to produce urine at each interval. For subjects who are anuric, urine samples will not be collected.

Safety will be monitored throughout the study by repeated clinical and laboratory evaluations.

There will be at least 14 days washout between dosing in Period 1 and dosing in Period 2.

Subjects may be replaced at the discretion of the Sponsor.

9.1.1 Confinement, Return Visit, and Follow-up

Subjects will be housed from Day -2 (Orlando clinical site) or Day – 1 (Miami clinical site) at the time indicated by the Clinical Research Unit (CRU), until after the 72-hour blood draw. At all times, a subject may be required to remain at the CRU for longer at the discretion of the Investigator.

The clinic will attempt to contact subjects (including subjects who terminate the study early) using their standard procedures approximately 14 days after the last study drug administration to determine if any adverse events have occurred since the last visit.

9.1.2 Study Duration

The duration of the study from screening to Day 4 of Period 2 is approximately 6.5 weeks. The duration of the study from screening to follow-up is approximately 8 weeks.

9.2 Selection of Study Population

9.2.1 Inclusion Criteria

9.2.1.1 Subjects with Renal Impairment

Subjects must fulfill all of the following inclusion criteria to be eligible for participation in the study, unless otherwise specified:

1. Adult male or female subject, 18-78 years of age, inclusive, at screening.
2. A female must be non-pregnant, non-breast feeding and if she is of reproductive potential: must demonstrate a serum β -human chorionic gonadotropin (β -hCG) level consistent with the nongravid state at screening (Note: if a subject has a β -hCG level that is not consistent with the non-gravid state due to disease or medication, the investigators may consult with the sponsor to obtain approval to enroll the subject in the study) and agree to use (and/or have their partner use) two (2) acceptable methods of birth control beginning at screening, throughout the study and until 2 weeks after the last dosing of study drug:
 - diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide and contraceptive sponge)
 - cervical cap with spermicide (nulliparous women only)
 - contraceptive sponge with spermicide (nulliparous women only)
 - male condom or female condom with spermicide (cannot be used together)
3. A female of non-childbearing potential: must have undergone one of the following sterilization procedures at least 6 months prior to the first dose:
 - hysteroscopic sterilization;
 - bilateral tubal ligation or bilateral salpingectomy;
 - hysterectomy;
 - bilateral oophorectomy.or be postmenopausal with amenorrhea for at least 1 year prior to the first dose and have FSH serum levels consistent with postmenopausal status.
4. A non-vasectomized male subject must agree to use a condom with spermicide or abstain from sexual intercourse from the first dose until 90 days after the last dose. No restrictions are required for a vasectomized male provided his vasectomy has been performed 4 months or more prior to study start. A male subject who has been vasectomized less than 4 months prior to study start must follow the same restrictions as a non-vasectomized male.

5. A male subject must agree not to donate sperm from dosing until 90 days after dosing/the last dose of study drug.
6. Subject has a body mass index (BMI) $\leq 40.0 \text{ kg/m}^2$, at screening.
7. With the exception of renal impairment, subject is judged to be in good health based on medical history, physical examination, vital signs, pulse oximetry, and laboratory safety tests. Subject has no clinically significant ECG abnormality, as deemed by the Investigator or designee. Subjects who do not qualify based on a reversible condition or mild intercurrent illness may be re-screened after the underlying condition is resolved.
8. Subject is a non-smoker or moderate smoker (≤ 20 cigarettes/day or the equivalent). Subject must agree to consume no more than 10 cigarettes or equivalent/day from the time of screening and throughout the period of sample collection.
9. Subject has a clinical diagnosis of renal impairment and meets the renal impairment function qualifications listed below at the prestudy visit (screening):

Group	eGFR (mL/min/1.73m ²)*
ESRD requiring hemodialysis	Requiring hemodialysis
Severe impairment	< 30 not on hemodialysis **
Moderate impairment	30 – < 60 ***
Mild impairment	60 - < 90
<p>* eGFR based on MDRD equation at screening. Baseline eGFR will be obtained twice as part of subject screening and the mean of the two values will be used for renal impairment assignment. The second baseline eGFR sample may be obtained at the time of check-in in Period 1.</p> <p>** Reasonable efforts will be made to enroll at least 2 subjects with eGFR values of < 20 mL/min/1.73m²</p> <p>*** Reasonable efforts will be made to ensure a broad representation across the range of eGFR values of 30 - < 60 mL/min/1.73m²</p>	

Based on eGFR from the MDRD equation at screening as defined as follows (for females multiply result by 0.742, if African American multiply result by 1.212):

The MDRD equation is:

$$\text{eGFR} = 175 \times (\text{S}_{\text{cr, std}})^{-1.154} \times (\text{Age})^{-0.203}$$

$\text{S}_{\text{cr, std}}$: serum creatinine (mg/dL) measured with a standardized assay.

10. For mild, moderate, and severe RI subjects: Has a stable renal function with no clinically significant change in renal status at least 1 month prior to the first study drug administration and is not currently or has not previously been on hemodialysis.
11. For subjects with ESRD: Is maintained on a stable regimen of HD within 3 months prior to the first dose.
12. Subjects understands the study procedures in the informed consent forms (ICFs), is willing and able to comply with the protocol, and provides written informed consent for the trial, including for Future Biomedical Research. Future Biomedical Research will be conducted as a voluntary part of the trial.

9.2.1.2 Healthy Subjects

Subjects must fulfill all of the following inclusion criteria to be eligible for participation in the study, unless otherwise specified:

1. Adult male or female subject, 18-78 years of age, inclusive, at screening.
2. A female must be non-pregnant, non-breast feeding and if she is of reproductive potential: must demonstrate a serum β -human chorionic gonadotropin (β -hCG) level consistent with the nongravid state at screening and agree to use (and/or have their partner use) two (2) acceptable methods of birth control beginning at screening, throughout the study and until 2 weeks after the last dosing of study drug:
 - diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide and contraceptive sponge)
 - cervical cap with spermicide (nulliparous women only)
 - contraceptive sponge with spermicide (nulliparous women only)
 - male condom or female condom with spermicide (cannot be used together)
3. A female of non-childbearing potential: must have undergone one of the following sterilization procedures at least 6 months prior to the first dose:
 - hysteroscopic sterilization;
 - bilateral tubal ligation or bilateral salpingectomy;
 - hysterectomy;
 - bilateral oophorectomy.

or be postmenopausal with amenorrhea for at least 1 year prior to the first dose and have FSH serum levels consistent with postmenopausal status.

4. A non-vasectomized male subject must agree to use a condom with spermicide or abstain from sexual intercourse from the first dose until 90 days after the last dose. No

restrictions are required for a vasectomized male provided his vasectomy has been performed 4 months or more prior to study start. A male subject who has been vasectomized less than 4 months prior to study start must follow the same restrictions as a non-vasectomized male.

5. Subject has a BMI $\leq 40.0 \text{ kg/m}^2$, at screening.
6. Subject is judged to be in good health based on medical history, physical examination, vital signs, pulse oximetry, and laboratory safety tests. Subject has no clinically significant ECG abnormality, as deemed by the Investigator or designee. Subjects who do not qualify based on a reversible condition or mild intercurrent illness may be re-screened after the underlying condition is resolved.
7. Subject is a non-smoker or moderate smoker (≤ 20 cigarettes/day or the equivalent). Subject must agree to consume no more than 10 cigarettes or equivalent/day from the time of screening and throughout the period of sample collection.
9. Subject has baseline CLcr $\geq 90 \text{ mL/min}$ based on Cockcroft-Gault equation at screening defined as follows (for females multiply result by 0.85)*:

The Cockcroft-Gault equation is:

$$\text{CLcr} = [140 - \text{age (years)}] + \text{weight (kg)} / 72 S_{\text{cr, std}}$$

$S_{\text{cr, std}}$: serum creatinine (mg/dL) measured with a standardized assay.

*For healthy subjects a creatinine clearance computed over a 24 hour urine collection for subjects that do not qualify with $\geq 90 \text{ mL/min}$ CLcr may be done for confirmation purposes.

8. Subject understands the study procedures in the ICFs, is willing and able to comply with the protocol, and provides written informed consent for the trial, including for Future Biomedical Research. Future Biomedical Research will be conducted as a voluntary part of the trial.

9.2.2 Exclusion Criteria

9.2.2.1 Subjects with Renal Impairment

Subjects must not be enrolled in the study if they meet any of the following criteria:

1. Subject is mentally or legally incapacitated or has significant emotional problems at the time of the screening visit or expected during the conduct of the study.
2. Subject has a history or presence of clinically significant medical or psychiatric condition or disease in the opinion of the Investigator or designee.

3. Subject has a history of any illness that, in the opinion of the Investigator or designee, might confound the results of the study or poses an additional risk to the subject by their participation in the study.
4. Subject has rapidly fluctuating renal function as determined by historical measurements; or subject has demonstrated or suspected renal stenosis. Rapid fluctuation in renal function is defined as creatinine clearance that differs by more than 30% within 3 months of the screening eGFR determination. If historical measurements are not available, then the 2 screening measurements will be used to demonstrate stability. Subjects with CrCL ≤ 24 mL/min with renal fluctuation of more than 30% within 3 months of the screening eGFR determination may be allowed in the study following discussion with the Sponsor.
5. Subject has a history of stroke, chronic seizures, or major neurological disorders.
6. Subject has uncontrolled type 2 diabetes mellitus (T2DM), a history of Type 1 diabetes, or ketoacidosis.
7. Subject with a history of significant endocrine (other than T2DM), gastrointestinal, cardiovascular, hematological, immunological, respiratory, or genitourinary diseases whose current condition is considered clinically unstable in the opinion of the investigator or designee. Subjects who have had a cholecystectomy may be allowed.
8. Subject has a history of malignant neoplastic disease. Exceptions: (1) Subjects with adequately treated non-melanomatous skin carcinoma or carcinoma in situ of the cervix may participate in the study; (2) Subjects with other malignancies which have been successfully treated ≥ 10 years prior to screening where, in the judgment of both the Investigator and treating physician, appropriate follow-up has revealed no evidence of recurrence from the time of treatment through the time of the screening; or, (3) Subjects, who, in the opinion of the Investigator or designee, are highly unlikely to sustain a recurrence for the duration of the study.
9. For mild, moderate, and severe RI subjects: Subject has had a renal transplant or has had nephrectomy.
10. For subjects with ESRD: Had a failed renal allograft within the last 2 years prior to the first dose, or a successful renal allograft
11. Subject is unable to refrain from or anticipates the use of any medication or substance (including prescription or over-the-counter, vitamin supplements, natural or herbal supplements) as indicated in [Section 9.3.1](#) for the prohibited time period. Hormone replacement therapy is not excluded.
12. Subject has a history or presence of alcoholism or drug abuse within the past 6 months prior to the first dose.
13. Subject has a history or presence of hypersensitivity or idiosyncratic reaction to the study drug(s) or related compounds.

14. A female subject who is pregnant or lactating.
15. Subject has positive results for the urine or saliva drug and/or urine or breath alcohol screen at screening or check-in, unless the positive drug screen is due to prescription drug use that is approved by the Investigator and Sponsor's Clinical Monitor.
16. Positive results at screening for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C antibodies. Subjects who are hepatitis C antibody positive may be enrolled, if they are confirmed with negative viral load at screening.
17. Subject has been on a diet incompatible with the on-study diet, in the opinion of the Investigator or designee, within the 28 days prior to the first dose, and throughout the study.
18. Subject donated blood or had significant blood loss within 56 days prior to the first dose.
19. Subject donated plasma within 7 days prior to the first dose.
20. Subject is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling or child) who is a member of the investigational site or sponsor staff directly involved with this trial.
21. Subject participated in another clinical trial within 28 days prior to the first dose. The 4-week window will be derived from dosing in the previous study to Day 1 of Period 1 of the current study.

9.2.2.2 Healthy Subjects

Subjects must not be enrolled in the study if they meet any of the following criteria:

1. Subject is mentally or legally incapacitated or has significant emotional problems at the time of the screening visit or expected during the conduct of the study.
2. Subject has a history or presence of clinically significant medical or psychiatric condition or disease in the opinion of the Investigator or designee.
3. Subject has a history of any illness that, in the opinion of the Investigator or designee, might confound the results of the study or poses an additional risk to the subject by their participation in the study.
4. Subject has a history of stroke, chronic seizures, or major neurological disorders.
5. Subject has history of hypoglycemia, glucose intolerance, T2DM, or ketoacidosis.
6. Subject has a history of clinically significant endocrine, gastrointestinal, cardiovascular, hematological, hepatic, immunological, renal, respiratory, or genitourinary abnormalities

or diseases. Subjects with a history of uncomplicated kidney stones or childhood asthma may be enrolled in the study at the discretion of the Investigator or designee.

7. Subject has a history of malignant neoplastic disease. Exceptions: (1) Subjects with adequately treated non-melanomatous skin carcinoma or carcinoma in situ of the cervix may participate in the study; (2) Subjects with other malignancies which have been successfully treated ≥ 10 years prior to screening where, in the judgment of both the investigator and treating physician, appropriate follow-up has revealed no evidence of recurrence from the time of treatment through the time of screening; or, (3) Subjects, who, in the opinion of the Investigator or designee, are highly unlikely to sustain a recurrence for the duration of the study.
8. Subject has had a renal transplant or has had nephrectomy.
9. Subject is unable to refrain from or anticipates the use of any medication or substance (including prescription or over-the-counter, vitamin supplements, natural or herbal supplements) as indicated in [Section 9.3.1](#) for the prohibited time period. Hormone replacement therapy is not excluded.
10. Subject has a history or presence of alcoholism or drug abuse within the past 2 years prior to the first dose.
11. Subject has a history or presence of hypersensitivity or idiosyncratic reaction to the study drug(s) or related compounds.
12. A female subject who is pregnant or lactating.
13. Subject has positive results for the urine or saliva drug and/or urine or breath alcohol screen at screening or check-in of Period 1. Unless the positive drug screen is due to prescription drug use that is approved by the Investigator and Sponsor's Clinical Monitor.
14. Subject has positive results at screening for HIV, HBsAg, or HCV.
15. Subject has been on a diet incompatible with the on-study diet, in the opinion of the Investigator or designee, within the 28 days prior to the first dose, and throughout the study.
16. Subject donated blood or had significant blood loss within 56 days prior to the first dose.
17. Subject donated plasma within 7 days prior to the first dose.
18. Subject is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling or child) who is a member of the investigational site or sponsor staff directly involved with this trial.

19. Subject participated in another clinical trial within 28 days prior to the first dose. The 4-week window will be derived from dosing in the previous study to Day 1 of Period 1 of the current study.

9.2.3 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the Investigator should any untoward effect occur. In addition, a subject may be withdrawn by the Investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal procedures, including specific details regarding withdrawal from Future Biomedical Research, are provided in [Section 9.2.3.1](#).

Discontinuation is “permanent”. Once a subject is discontinued, he/she shall not be allowed to enroll again.

Subjects may be replaced at the discretion of the Sponsor.

A subject must be discontinued from the study for any of the following reasons:

- The subject withdraws consent.
- The subject has a confirmed positive serum pregnancy test.
- The subject has a medical condition or personal circumstance which, in the opinion of the Investigator and/or Sponsor, places the subject at unnecessary risk through continued participation in the trial or does not allow the subject to adhere to the requirements of the protocol.

A subject may be discontinued from the study for any of the following reasons:

- Adverse events.
- Difficulties in blood collection.
- Protocol violation.

9.2.3.1 Withdrawal/Discontinuation

The Investigator or designee must notify the Sponsor when a subject has been discontinued/withdrawn from the study. If a subject discontinues for any reason at any time during the course of the study, the procedures scheduled at early termination (as outlined in [Section 6](#)) will be performed. Furthermore, the subject will be asked to return to the clinic or be contacted, if the Investigator deems necessary, for a follow-up (approximately 14 days after the last dose of study drug), to determine if any adverse events have occurred since the last visit. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in [Section 10.1.8](#).

9.2.3.1.1 Withdrawal from Future Biomedical Research

Subjects may withdraw their consent for Future Biomedical Research. Subjects may withdraw consent at any time by contacting the Principal Investigator for the main trial. If medical records for the main trial are still available, the Investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the subject's consent for Future Biomedical Research will be withdrawn. A letter will be sent from the Sponsor to the Investigator confirming the withdrawal. It is the responsibility of the Investigator to inform the subject of completion of withdrawal. Any analyses in progress at the time of request for withdrawal or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (e.g., if the Investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the subject's personal information and their specimens. In this situation, the request for specimen withdrawal cannot be processed.

9.3 Study Restrictions

9.3.1 Prohibitions and Concomitant Therapy

Consumption of foods and beverages containing the following substances will be prohibited as indicated:

- Xanthines/Caffeine: 24 hours before dosing and throughout the period of pharmacokinetic sample collection in each period;
- Alcohol: 48 hours before dosing and throughout the period of pharmacokinetic sample collection in each period;
- Grapefruit/Seville orange: 14 days before the first dose in Period 1 until the last pharmacokinetic sample collection in Period 2.
- Fruit Juice: 72 hours before dosing until the last pharmacokinetic sample collection in each period;
- Vegetables from the mustard green family (e.g., kale, broccoli, watercress, collard greens, kohlrabi, Brussels sprouts, mustard), and charbroiled meats: 7 days before the first dose in Period 1 until the last pharmacokinetic sample collection in Period 2.

Concurrent therapy with any medication during the course of the protocol including both prescription and non-prescription drugs must first be discussed with the Investigator and Sponsor Clinical Monitor prior to dosing, unless appropriate medical care necessitates that therapy should begin before the Investigator and Sponsor Clinical Monitor can be consulted. If a subject is prescribed prohibited medication, upon discussion between the Sponsor and the Investigator, the Investigator may substitute the previously prescribed medication to an

allowed one for the purpose of this study. During the study, acetaminophen (up to 2 g per 24 hours) may be administered at the discretion of the Investigator.

Appropriate sources will be consulted by the Investigator or designee to confirm lack of pharmacokinetic/pharmacodynamic interaction with drug. If deviations occur, the Investigator will decide on a case-by-case basis whether the subject may continue participation in the study based on the time the study drug was administered and its pharmacology.

Also refer to [Appendix 8](#) for additional details on allowable and prohibited concomitant therapy.

All medications taken by subjects during the course of the study will be recorded.

For Renal Impaired Subjects:

All prescription or non-prescription medications (including St. John's Wort) that are strong or moderate inhibitors or strong or moderate inducers of CYP3A and CYP2C9 enzymes or P-gp transporters will be prohibited. These metabolizing enzyme inhibitors and inducers will not be allowed for at least 14 days and 28 days, respectively, prior to the first dose and throughout the study. Medications of particular concern include, but are not limited to azole antifungals (ketoconazole, itraconazole), macrolide antibiotics (erythromycin, clarithromycin), HIV protease inhibitors, nefazodone, rifampin (except when administered as part of Treatment B), dexamethasone, troglitazone, barbiturates, and any drug or supplement (e.g., St. John's Wort) that inhibits or induces P-gp transporters. Weak P-gp inhibitors or inducers may be deemed acceptable following consultation with the Sponsor Clinical Monitor and the Investigator.

Subjects who are taking certain prescription medications to treat manifestations of renal disease or medications needed to treat stable diseases (e.g., angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, beta-blockers, diuretics) will be allowed to participate in the study at the discretion of the Investigator and following consultation with the Sponsor Clinical Monitor. Subjects must be on a stable regimen for at least 1 month (or 5 half-lives of the study drug, whichever is longer) prior to the first study drug administration and is able to withhold the use within 4 hours prior to administration of the study drug.

Any medication (including over-the-counter) that would significantly alter eGFR, which, by the determination of the Investigator, might interfere with the study (e.g., cimetidine) must be discontinued at least 2 weeks (or 5 half-lives of the compound, whichever is longer) prior to the first dosing of study drug.

Note: Proton pump inhibitors must be withheld 1 week prior to each dosing and 4 hours post dosing.

Diuretics must be withheld 8 hours prior to each dosing and 4 hours post dosing.

Phosphate binders containing aluminum, calcium, or lanthanum salts; iron supplements or other metal cations; H2RAs [except cimetidine]; vitamin D, or multivitamins containing iron or zinc must be withheld 8 hours prior to each dosing and 4 hours post dosing.

For Healthy Subjects:

Any medication or substance (including prescription or over-the-counter, vitamin supplements, natural or herbal supplements) are to be discontinued at least 14 days prior to dosing and throughout the study are prohibited. All prescription or non-prescription medications (including St. John's Wort) that are moderate/strong inhibitors or moderate/strong inducers of CYP3A and CYP2C9 enzymes or P-gp transporters will be prohibited for at least 14 days and 28 days, respectively, prior to the first dose and throughout the study. Certain medications may be deemed acceptable following consultation with the Sponsor Clinical Monitor and the Investigator. Appropriate sources will be consulted by the Investigator or designee to confirm lack of potential pharmacokinetic/pharmacodynamic interaction with study drug.

9.3.2 Meals

Water (except water provided with dosing) will be restricted 1 hour prior to and 1 hour after study drug administration, but will be allowed ad libitum at all other times. Other fluids may be given as part of the standard meals and/or snacks, but will be restricted at all other times throughout the confinement period.

Subjects will fast overnight for at least 10 hours prior to each study drug administration. Subjects will continue the fast for at least 4 hours postdose.

On all days that subjects are confined in the CRU, standard meals will be provided at approximately 4 and 9 hours postdose, and at appropriate times thereafter. Snacks will be offered at appropriate times. When confined in the CRU, subjects will fast from all food and drink except water between meals and snacks.

Each meal and/or snacks served at the CRU will be standardized, and will be similar in caloric content and composition.

9.3.3 Activity

Subjects will remain ambulatory or seated upright for the first 4 hours following study drug administration, except when they are supine or semi-reclined for study procedures.

However, should adverse events occur at any time, subjects may be placed in an appropriate position or will be permitted to lie down on their right side.

Subjects will be instructed to refrain from strenuous physical activity which could cause muscle aches or injury, including contact sports at any time from screening until completion of the study.

Depending on the CRU rules and regulations, subjects may be prohibited from smoking during their confinement or during portions of their confinement.

9.4 Treatments

9.4.1 Treatments Administered

Treatment A will be supplied as microdose cocktail. See [Table 2](#). Oral solutions for Treatment A will be prepared and stored according to the Method of Preparation Manual and Master Batch Records provided separately.

Treatment B will be supplied as microdose cocktail and rifampin as 300 mg oral capsules.

Table 2: Study Microdose Cocktail Component Doses

Probe Compound	Cocktail Microdose	Therapeutic Dose
Midazolam	10 µg	1 – 5 mg/dose
Dabigatran	375 µg	75 – 150 mg BID
Pitavastatin	10 µg	1 – 4 mg QD
Atorvastatin	100 µg	10 – 80 mg QD
Rosuvastatin	50 µg	5 – 40 mg QD

Subjects will receive the following treatments in a fixed-sequence:

Treatment A (Period 1): Single oral dose of the microdose cocktail at Hour 0 on Day 1 following an overnight fast. The microdose cocktail will contain:

- 10 µg midazolam (1 mL of 10 µg/mL oral solution), administered orally as midazolam hydrochloride (HCl);
- 375/10 µg dabigatran etexilate and pitavastatin (1 mL of 375/10 µg/mL oral solution), administered as dabigatran etexilate mesylate and pitavastatin calcium; and
- 100/50 µg atorvastatin and rosuvastatin (2 mL of 50/25 µg/mL oral solution, dosed as 2 x 1 mL dosing syringes), administered as atorvastatin calcium trihydrate and rosuvastatin calcium.

Each oral solution will be administered using 1 mL amber dosing syringes.

Treatment B (Period 2): 600 mg oral single dose rifampin (2 x 300 mg capsules) coadministered with a single oral dose microdose cocktail (as described in Treatment A) at Hour 0 on Day 1 following an overnight fast.

NOTE: Subjects with ESRD will not receive rifampin in Period 2; they will receive Treatment A in Period 2, instead of Treatment B. In Period 1, dosing will occur

approximately 24 hours prior to dialysis. In Period 2, dosing will occur as soon as possible within 24 hours after completion of the dialysis.

Subjects will drink one container of approximately 240 mL of water after each complete dosing completion.

The pharmacy at the CRU will provide each dose in individual syringes (microdose cocktail) or unit dose containers (rifampin) for each subject.

The exact clock time of dosing will be recorded.

9.4.2 Method of Assigning Subjects to Treatment Groups

Each subject will be assigned a unique identification number upon screening. Subjects who complete the study screening assessments and meet all the eligibility criteria will receive Treatment A in Period 1 then Treatment B in Period 2 in a fixed-sequence except for subjects with ESRD who will not receive rifampin in Period 2; they will receive Treatment A in Period 2, instead of Treatment B.

If replacement subjects are used, the replacement subject number will be 100 more than the original (e.g., allocation number 0101 will replace allocation number 0001).

9.4.3 Blinding

This is an open-label study.

9.4.4 Treatment Compliance

A qualified designee will be responsible for monitoring the administration of timed oral dose. A mouth check will be performed by the qualified designee to ensure that the subjects have swallowed the study drug. Once a subject has finished the water, the qualified designee will use a flashlight and a tongue depressor to check the subject's mouth. Subjects' hands will also be verified to ensure that the study drug was ingested.

9.4.5 Study Design or Procedure Modifications Permitted within Protocol Parameters

The dose and administration of the trial study drug to any subject may not be modified. If necessary a subject must be discontinued for the reasons described in [Section 9.2.3](#).

10 STUDY PROCEDURES

The Study Events Flow Chart ([Section 6](#)) summarizes the clinical procedures to be performed at each visit. Individual clinical procedures are described in detail below. Additional evaluations/testing may be deemed necessary by the Investigator and/or the Sponsor for reasons related to subject safety.

For this study, the blood collection for all plasma analytes (midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 2-OH atorvastatin, 4-OH atorvastatin, rosuvastatin, and rifampin) is the critical parameter and needs to be collected as close to the exact time point as possible. All other procedures should be completed as close to the prescribed/scheduled time as possible, but can be performed prior or after the prescribed/scheduled time.

Any nonscheduled procedures required for urgent evaluation of safety concerns take precedence over all routine scheduled procedures.

10.1 Safety Assessment

10.1.1 Screening

Within 28 days prior to the first dosing, medical history and demographic data, including name, sex, age, race, ethnicity, body weight (kg), height (cm), and BMI (kg/m²), will be recorded. Each subject will have a physical examination, vital sign measurements (heart rate, blood pressure, temperature, and respiratory rate), 12-lead ECG, pulse oximetry, and the laboratory tests of hematological, hepatic, and renal function, and additional tests as noted in [Section 10.1.7](#).

10.1.2 Physical Examination

A physical examination will be performed as per Study Events Flow Chart ([Section 6](#)). Symptom-driven physical examinations may be performed at other times, if deemed necessary by the Investigator or designee.

10.1.3 Vital Signs

Single measurements of body temperature, respiratory rate, blood pressure, and heart rate, will be measured as outlined in the Study Events Flow Chart ([Section 6](#)).

Vital signs may be taken at any other times, if deemed necessary. Blood pressure and heart rate measurements will be performed with subjects in a seated position for at least 1 minute, except when they are supine or semi-reclined because of study procedures and/or adverse events (e.g., nausea, dizziness) or if deemed necessary by the Investigator.

Blood pressure, heart rate, and respiratory rate will be measured within 24 hours prior to Day 1 dosing for the predose time point. When scheduled postdose, vital signs will be performed within approximately 10 minutes of the scheduled time point.

10.1.4 ECG Monitoring

Single 12-lead ECGs will be performed as outlined in the Study Events Flow Chart ([Section 6](#)).

ECGs will be performed with subjects in a supine position for at least 5 minutes. All ECG tracings will be reviewed by the Study Physician or his/her designee.

ECGs will be measured within 24 hours prior to Day 1 dosing for the predose time point. When scheduled postdose, ECGs will be performed within approximately 20 minutes of the scheduled time point.

A subject will be withdrawn from the study by the Study Physician or his/her designee if, in their medical judgment, ECG findings are present which make continued study participation not in the subject's best interest.

10.1.5 Pulse Oximetry

Each subject will have a baseline pulse oximetry reading done prior to study drug administration and will be monitored continuously for approximately 6 hours with a pulse oximeter (oxygen levels, as saturation [%], and heart rate) with readings taken at scheduled time points as outlined in the Study Events Flow Chart ([Section 6](#)).

Where the time of monitoring coincides with a blood sampling time point, the reading will be taken approximately 10 minutes before the scheduled time point. Readings may be taken at other times, if deemed necessary by the PI or designee.

Any oxygen saturation reading deemed clinically significant by the Investigator or designee will be documented.

10.1.6 Rescue Medication

Administration of midazolam HCl may result in severe AEs including respiratory depression, airway obstruction, oxygen desaturation, apnea, and rarely, respiratory and/or cardiac arrest. Oxygen and/or specific reversal agents (e.g., flumazenil) will be available as a rescue medication in case of any severe midazolam-related events, at the discretion of the Study Physician or his/her designee.

10.1.7 Clinical Laboratory Tests

All tests listed below will be performed as per Study Events Flow Chart ([Section 6](#)). In addition, laboratory safety tests may be performed at various unscheduled time points, if deemed necessary by the Investigator.

Hematology

- Hemoglobin
- Hematocrit
- Total and differential leukocyte count
- Red blood cell count
- Platelet count

Serum Chemistry*

- Blood urea nitrogen
- Bilirubin (total and direct)**
- Alkaline phosphatase
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Albumin
- Sodium
- Potassium
- Chloride
- Glucose (fasting)
- Creatinine
- Creatine phosphokinase

Urinalysis

- pH
- Specific gravity
- Protein***
- Glucose
- Ketones
- Bilirubin
- Blood***
- Nitrite***
- Urobilinogen
- Leukocyte esterase***

Additional Tests

- HIV test
- HBsAg
- HCV
- Urine or saliva drug screen
 - Opiates
 - Amphetamines
 - Cocaine
 - Cannabinoids
- Urine or breath alcohol screen
- Serum pregnancy test (for females only)
- FSH (for postmenopausal females only)

* Serum chemistry tests will be performed after at least an 8-hour fast; however, in case of dropout or rechecks, subjects may not have fasted for 8 hours before the serum chemistry sample is taken.

** Predose samples for total and direct bilirubin will be obtained following a fast of approximately 8 hours; however, all postdose samples collected for the assessment of OATP 1B inhibition are not required to be performed following a fast of approximately 8 hours.

** If urinalysis is positive for protein, blood, nitrite and/or leukocyte esterase, a microscopic examination (red blood cell, white blood cell, bacteria, casts, and, epithelial cells) will be performed. For subjects who are anuric, urine samples for urinalysis will not be collected.

10.1.8 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the Sponsor's product, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Sponsor's product includes any pharmaceutical product, biological product, device, diagnostic agent, or protocol-specified procedure whether investigational (including placebo or active comparator medication) or marketed, manufactured by, licensed by, provided by or distributed by the Sponsor for human use.

Adverse events may occur during clinical trials, or as prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse, and from withdrawal.

For allocated subjects only, all adverse events that occur after the consent form is signed but before allocation must be reported by Investigator if they are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment, or a procedure. From the time of allocation through 14 days following cessation of treatment, all adverse events must be reported by the Investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in [Section 10.1.8.3.1](#). The Investigator will make every attempt to follow all subjects with non-serious adverse events for outcome. Any unexplained drug-related hematuria or evidence of triphosphate crystals should be referred to a nephrologist.

Electronic reporting procedures can be found in the Electronic Data Capture (EDC) data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

10.1.8.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor

The subject has taken (accidentally or intentionally) any drug administered as part of the protocol and exceeding the dose as prescribed by the protocol. It is up to the Investigator or

the reporting physician to decide whether a dose is to be considered an overdose, in consultation with the Sponsor.

If an adverse event(s) is associated with (“results from”) the overdose of Sponsor's product or vaccine, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Sponsor's product or vaccine meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology “accidental or intentional overdose without adverse effect.”

All reports of overdose with or without an adverse event must be reported by the Investigator within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

10.1.8.2 Reporting of Pregnancy and Lactation to the Sponsor

Although pregnancy and lactation are not considered adverse events, it is the responsibility of Investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before allocation must be reported by the Investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure. Pregnancies and lactations that occur from the time of allocation through 14 days following cessation of Sponsor's product must be reported by the Investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Pregnant partners of male subjects will not be followed.

10.1.8.3 Immediate Reporting of Adverse Events

10.1.8.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of Sponsor's product that has the following outcome:

- Death
- Immediately life threatening
- Persistent or significant disability/incapacity
- Inpatient hospitalization or prolongation of hospitalization
- Congenital anomaly/birth defect
- Other important medical event

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor in the same timeframe as serious adverse events to meet certain local requirements. Therefore, these events are considered serious by the Sponsor for collection purposes.

- Cancer
- Overdose

Refer to [Table 3](#) for additional details regarding each of the above criteria.

For the time period beginning when the consent form is signed until treatment allocation, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 14 days following cessation of treatment, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Additionally, any serious adverse event, considered by an Investigator who is a qualified physician to be related to the Sponsor's product that is brought to the attention of the Investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor.

All subjects with serious adverse events must be followed up for outcome.

10.1.8.3.2 Events of Clinical Interest

Selected non-serious and serious adverse events are also known as ECI and must be reported to the Sponsor.

For the time period beginning when the consent form is signed until treatment allocation, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation through 14 days following cessation of treatment, any ECI, or follow up to an ECI, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor, either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Events of clinical interest for this trial include:

- an overdose of Sponsor's product, as defined in [Section 10.1.8.1](#) that is not associated with clinical symptoms or abnormal laboratory results.
- an elevated AST or ALT laboratory value that is greater than or equal to three times (3X) the upper limit of normal (ULN) and an elevated total bilirubin laboratory value that is greater than or equal to 2X the ULN and, at the same time, an alkaline phosphatase laboratory value that is less than 2X the ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder or equivalent.

10.1.8.4 Evaluating Adverse Events

An Investigator who is a qualified physician will evaluate all adverse events with respect to the elements outlined in [Table 3](#). The Investigator's assessment of causality is required for each adverse event. Refer to [Table 3](#) or instructions in evaluating adverse events.

Table 3: Evaluating Adverse Events

Maximum Intensity (Severity)	Mild	awareness of sign or symptom, but easily tolerated (for pediatric trials, awareness of symptom, but easily tolerated)
	Moderate	discomfort enough to cause interference with usual activity (for pediatric trials, definitely acting like something is wrong)
	Severe	incapacitating with inability to work or do usual activity (for pediatric trials, extremely distressed or unable to do usual activities)
Seriousness	A serious adverse event is any adverse event occurring at any dose or during any use of Sponsor's product that:	
	None	
	† Death ; or	
	† Immediately life threatening ; or places the subject, in the view of the Investigator, at immediate risk of death from the event as it occurred [Note: This does not include an adverse event that, had it occurred in a more severe form, might have caused death.]; or	
	† Persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions); or	
	† Inpatient hospitalization or prolongation of hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. (Note: Hospitalization [including hospitalization for an elective procedure] for a preexisting condition which has not worsened does not constitute a serious adverse event.); or	
	† Congenital anomaly/birth defect (in offspring of subject taking the product regardless of time to diagnosis); or	
	Cancer ; or	
	Overdose (whether accidental or intentional). Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.	
	Other important medical event that may not result in death, not be life threatening, or not require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).	
Duration	Record the start and stop dates of the adverse event. If less than 1 day, indicate the appropriate length of time and units.	
Action Taken	The action taken is in reference to the either the Sponsor's Product or the Interacting Drug. Did the adverse event cause the Sponsor's product or the Interacting Drug to be:	
	None	
	Reduced	
	Interrupted	
	Discontinued	
	Increased	
	Not Applicable	
	Unknown	

Relationship to Sponsor's Product	Did the Sponsor's product cause the adverse event? The determination of the likelihood that the Sponsor's product caused the adverse event will be provided by an Investigator who is a qualified physician. The Investigator's signed/dated initials on the source document or worksheet that supports the causality noted on the adverse event form, ensures that a medically qualified assessment of causality was done. This initialled document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the Investigator in assessing the likelihood of a relationship between the test drug and the adverse event based upon the available information.													
Relationship to Sponsor's Product (continued)	<p>The following components are to be used to assess the relationship between the Sponsor's product and the adverse event; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the Sponsor's product caused the adverse event:</p> <table border="1"> <tr> <td>Exposure</td><td>Is there evidence that the subject was actually exposed to the Sponsor's product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?</td></tr> <tr> <td>Time Course</td><td>Did the adverse event follow in a reasonable temporal sequence from administration of the Sponsor's product? Is the time of onset of the adverse event compatible with a drug-induced effect (applies to trials with investigational medicinal product)?</td></tr> <tr> <td>Likely Cause</td><td>Is the adverse event not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors</td></tr> <tr> <td>Dechallenge</td><td>Was the Sponsor's product discontinued or dose/exposure/frequency reduced? If yes, did the adverse event resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the adverse event resulted in death or permanent disability; (2) the adverse event resolved/improved despite continuation of the Sponsor's product; (3) the trial is a single-dose drug trial; or (4) Sponsor's product(s) is/are only used one time.)</td></tr> <tr> <td>Rechallenge</td><td>Was the subject re-exposed to the Sponsor's product in this trial? If yes, did the adverse event recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial adverse event resulted in death or permanent disability, or (2) the trial is a single-dose drug trial; or (3) Sponsor's product(s) is/are used only one time.) NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE SPONSOR'S PRODUCT, OR IF RE-EXPOSURE TO THE SPONSOR'S PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AND THE INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE.</td></tr> <tr> <td>Consistency with Trial Treatment Profile</td><td>Is the clinical/pathological presentation of the adverse event consistent with previous knowledge regarding the Sponsor's product or drug class pharmacology or toxicology?</td></tr> </table>		Exposure	Is there evidence that the subject was actually exposed to the Sponsor's product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?	Time Course	Did the adverse event follow in a reasonable temporal sequence from administration of the Sponsor's product? Is the time of onset of the adverse event compatible with a drug-induced effect (applies to trials with investigational medicinal product)?	Likely Cause	Is the adverse event not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors	Dechallenge	Was the Sponsor's product discontinued or dose/exposure/frequency reduced? If yes, did the adverse event resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the adverse event resulted in death or permanent disability; (2) the adverse event resolved/improved despite continuation of the Sponsor's product; (3) the trial is a single-dose drug trial; or (4) Sponsor's product(s) is/are only used one time.)	Rechallenge	Was the subject re-exposed to the Sponsor's product in this trial? If yes, did the adverse event recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial adverse event resulted in death or permanent disability, or (2) the trial is a single-dose drug trial; or (3) Sponsor's product(s) is/are used only one time.) NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE SPONSOR'S PRODUCT, OR IF RE-EXPOSURE TO THE SPONSOR'S PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AND THE INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE.	Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the adverse event consistent with previous knowledge regarding the Sponsor's product or drug class pharmacology or toxicology?
Exposure	Is there evidence that the subject was actually exposed to the Sponsor's product such as: reliable history, acceptable compliance assessment (pill count, diary, etc.), expected pharmacologic effect, or measurement of drug/metabolite in bodily specimen?													
Time Course	Did the adverse event follow in a reasonable temporal sequence from administration of the Sponsor's product? Is the time of onset of the adverse event compatible with a drug-induced effect (applies to trials with investigational medicinal product)?													
Likely Cause	Is the adverse event not reasonably explained by another etiology such as underlying disease, other drug(s)/vaccine(s), or other host or environmental factors													
Dechallenge	Was the Sponsor's product discontinued or dose/exposure/frequency reduced? If yes, did the adverse event resolve or improve? If yes, this is a positive dechallenge. If no, this is a negative dechallenge. (Note: This criterion is not applicable if: (1) the adverse event resulted in death or permanent disability; (2) the adverse event resolved/improved despite continuation of the Sponsor's product; (3) the trial is a single-dose drug trial; or (4) Sponsor's product(s) is/are only used one time.)													
Rechallenge	Was the subject re-exposed to the Sponsor's product in this trial? If yes, did the adverse event recur or worsen? If yes, this is a positive rechallenge. If no, this is a negative rechallenge. (Note: This criterion is not applicable if: (1) the initial adverse event resulted in death or permanent disability, or (2) the trial is a single-dose drug trial; or (3) Sponsor's product(s) is/are used only one time.) NOTE: IF A RECHALLENGE IS PLANNED FOR AN ADVERSE EVENT WHICH WAS SERIOUS AND WHICH MAY HAVE BEEN CAUSED BY THE SPONSOR'S PRODUCT, OR IF RE-EXPOSURE TO THE SPONSOR'S PRODUCT POSES ADDITIONAL POTENTIAL SIGNIFICANT RISK TO THE SUBJECT THEN THE RECHALLENGE MUST BE APPROVED IN ADVANCE BY THE U.S. CLINICAL MONITOR AND THE INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE.													
Consistency with Trial Treatment Profile	Is the clinical/pathological presentation of the adverse event consistent with previous knowledge regarding the Sponsor's product or drug class pharmacology or toxicology?													
The assessment of relationship will be reported on the case report forms /worksheets by an Investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements.														
Record one of the following:	Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a Sponsor's product relationship).													
Related (there is a reasonable possibility of Sponsor's product relationship)	There is evidence of exposure to the Sponsor's product. The temporal sequence of the adverse event onset relative to the administration of the Sponsor's product is reasonable. The adverse event is more likely explained by the Sponsor's product than by another cause.													

Not Related (there is not a reasonable possibility of Sponsor's product relationship)	Subject did not receive the Sponsor's product OR temporal sequence of the adverse event onset relative to administration of the Sponsor's product is not reasonable OR there is another obvious cause of the adverse event. (Also entered for a subject with overdose without an associated adverse event.)
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10.1.8.5 Sponsor Responsibility for Reporting Adverse Events

All adverse events will be reported to regulatory authorities, IRB or independent ethics committees (IECs), and Investigators in accordance with all applicable global laws and regulations i.e., per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.

10.2 Pharmacokinetic and Biomarker Assessments

10.2.1 Blood Sampling and Processing

For all subjects, blood samples for the determination of all plasma pharmacokinetic analytes (rifampin, midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 2-OH atorvastatin, 4-OH atorvastatin, and rosuvastatin) will be collected and processed according to [Appendix 3](#), [Appendix 4](#), and [Appendix 5](#) at scheduled time points as delineated in the Study Events Flow Chart ([Section 6](#)).

The following windows will be used for pharmacokinetic blood collection and biomarker samples time points:

Sample collection time	Allowed deviation
0.0 – 8.0 hour	$\leq \pm 2$ minutes
>8.0 – 24.0 hour	$\leq \pm 5$ minutes
>24.0 – 72.0 hour	$\leq \pm 10$ minutes

For all subjects, blood samples for the determination of all biomarkers in serum and plasma, as appropriate, (serum total and direct bilirubin, serum creatinine, plasma coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, CMPF, GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S) will be collected and processed according to [Appendix 9](#) at scheduled time points as delineated in the Study Events Flow Chart ([Section 6](#)). Additional biomarkers may be evaluated to measure uremic toxins/biomarkers.

10.2.2 Urine Collection

Prior to the pre-dose sample, each subject will be instructed as to urine collection methods.

Urine samples for determination of dabigatran and rosuvastatin concentrations will be collected and processed according to [Appendix 6](#) and for the determination of all biomarkers in urine (coproporphyrin I and III, p-cresol sulfate, indoxyl sulfate, and CMPF) will be collected and processed according to [Appendix 10](#) at selected intervals as delineated in the

Study Events Flow Chart ([Section 6](#)).

For subjects with renal impairment, urine samples will be collected whenever possible, as they may not be able to produce urine at each interval. For subjects who are anuric, urine samples will not be collected.

On Day 1, a spot collection will be obtained prior to dosing for the pre-dose sample. Subjects will be asked again to empty their bladder within approximately 15 minutes prior to dosing, and no urine will be collected at this time unless it is needed for the pre-dose sample. Only one pre-dose urine sample will be collected on Day 1.

For 24 hours after each dosing, all urine will be collected completely. Urine portions will be pooled per subject within any planned collection interval. Just prior to the end of each sampling interval, subjects will be encouraged to void their bladder again to complete the collection. If they do void at any time during the collection interval, the time should be documented. Should this be the case, subjects need to attempt to void again at the end of the collection period, as scheduled. However, should subjects be unable to void, this will be documented as well.

Urine will be refrigerated during the collection intervals. The weight of an empty urine collection container and total weight of urine collected during each timed interval will be recorded.

10.3 Planned Genetic Analysis Sample Collection

Sample collection, storage and shipment instructions for Planned Genetic Analysis samples will be provided in the [Appendix 7](#).

10.4 Future Biomedical Research Samples

The following samples will be collected for Future Biomedical Research:

- DNA for future research

10.5 Blood Volume Drawn for Study Assessments

Table 4: Blood Volume Drawn During the Study

Sample Type	Number of Time Points	Approximate Volume per Time Point * (mL)	Approximate Sample Volume Over Course of Study (mL)	Handling Instructions
Screening laboratory safety tests (including hematology, serum chemistry, serology), FSH (for postmenopausal female subjects only) and serum pregnancy (for female subjects only).	1	11	11	Reliable (Local Lab)
Blood for Planned Genetic Analysis	1	8.5	8.5	Appendix 7
On-study hematology and serum chemistry (this includes serum pregnancy for female subjects only when scheduled at the same time)	4	9	36	Reliable (Local Lab)
Blood for serum total and direct bilirubin	16	2	32	PPD (Central Lab) (Appendix 9)
Blood for serum creatinine	16	2	32	PPD (Central Lab) (Appendix 9)
Blood for plasma coproporphyrin I and III	16	2	32	PPD (Appendix 9)
Blood for plasma p-cresol sulfate, indoxyl sulfate, and CMPF	16	2	32	TMB/MRL (Appendix 9)
Blood for plasma GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S	16	2	32	Metabolon (Appendix 9)
Blood for free dabigatran and midazolam	32	4	128	Appendix 3
Blood for pitavastatin (and metabolite), atorvastatin (and metabolites), and rosuvastatin	32	3	96	Appendix 4

Sample Type	Number of Time Points	Approximate Volume per Time Point * (mL)	Approximate Sample Volume Over Course of Study (mL)	Handling Instructions
Blood for rifampin	10	3	30	Appendix 5
Blood for protein binding ^	1	3	3	TMB/MRL (Appendix 9)
Total Blood Volume for all Subjects except ESRD (mL)→			472.5 [§]	
Total Blood Volume for Subjects with ESRD (mL)→			442.5 [§]	

* Represents the largest collection tube that may be used for this (a smaller tube may be used).

§ If additional safety, pharmacokinetic, or pharmacodynamic analysis is necessary or if larger collection tubes are required to obtain sufficient plasma/serum for analysis, additional blood may be obtained (up to a maximum of 50 mL).

^ Protein binding (plasma) assays will be performed on predose samples from Period 1.

11 DATA ANALYSIS

11.1 Pharmacokinetic Parameters

11.1.1 Plasma

Pharmacokinetic parameters for all plasma analytes (rifampin, midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 4-OH atorvastatin, 2-OH atorvastatin, and rosuvastatin) will be calculated as follows:

AUC0-last:	Area under the concentration versus time curve, from 0 to the time of the last quantifiable (above LLOQ) sample.
AUC0-∞:	Area under the concentration versus time curve from 0 to infinity after single dosing.
AUC0-24:	Area under the concentration versus time curve, from 0 to 24 hours after dosing (dabigatran and rosuvastatin only).
CL/F:	Apparent clearance after extravascular administration (parent only).
C _{max} :	Maximum observed plasma concentration after the administration of a given dose.
C ₂₄ :	Plasma concentration at 24 hours.
T _{max} :	Time to maximum observed plasma drug concentration.
t _{1/2} :	(Apparent) terminal half-life.
V _z /F:	Apparent volume of distribution during the terminal phase (parent only)

No value for AUC0-∞, CL/F, V_z/F, and apparent terminal t_{1/2}, will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

11.1.2 Urine

Pharmacokinetic parameters for urine dabigatran and rosuvastatin will be calculated as follows:

Ae0-24:	Total amount of drug excreted unchanged in the urine over the period of 24 hours, obtained by adding the amounts excreted over each collection interval.
CL _r :	Renal clearance calculated as $Ae(t'-t'')/AUC(t'-t'')$ where t'-t'' is the longest interval of time during which Ae and plasma AUC are both obtained.

fe: Fraction of drug excretion during each collection interval. Obtained by dividing the amount of drug excreted in each collection interval by the time over which it was collected.

11.2 Statistical Methods

The statistical analysis of the data obtained from this study will be the responsibility of the Data Management and Biometrics department at Celerion.

If, after the study has begun, changes are made to the statistical analysis plan stated below, then these deviations to the plan will be listed, along with an explanation as to why they occurred, in the Clinical Study Report (CSR).

Additional statistical analyses, other than those described in this section, may be performed if deemed appropriate.

11.2.1 Determination of Sample Size

Source of Variance Estimates:

There are no available in-house pharmacokinetic data for most substrates in RI subjects, and the data published in the literature is limited in its usefulness for sample size planning in that relevant parameters are not provided or are not provided on the log scale except for Dabigatran. We assumed that the variances for substrates in RI subjects is the same as those in healthy subjects.

Midazolam:

The estimates of within-subject variability for midazolam AUC_{0-∞} were obtained from prior Merck studies investigating the effects of a therapeutic agent on the single dose pharmacokinetics of midazolam including MK-0633-004, MK-0869-088, MK-1006-003, MK-4305-015, MK-6096-005, MK-7009-010, MK-0974-020, MK-0974-041, MK-0518-016, MK-0524-051, MK-6186-004, MK-0364-010, and MK-0869-155. The pooled estimate for the within-subject standard deviation (SD) for midazolam AUC_{0-∞} is 0.1834. From the previous microdose study conducted by Merck in healthy subjects, the within-subject coefficient of variation (CV) for AUC_{0-∞} was 0.31 when midazolam was given with and without rifampin.

Dabigatran:

In an effort to provide some guidance for the precision and power associated with this study, the published data involving oral dabigatran in RI subjects have been reviewed¹⁸. The CV for severe RI subjects is 0.61 for AUC_{0-∞} in this study. In addition, there is 1.5 (mild RI subject vs healthy subjects) to 6.5 (severe RI subject vs healthy subjects) fold differences in AUC_{0-∞} level based on this study. From the previous microdose study conducted by Merck in healthy subjects, the within-subject CV for AUC_{0-∞} was 0.228 when dabigatran was given with and without rifampin.

Pitavastatin:

An estimate of within-subject variability for pitavastatin is derived from an earlier Merck study, MK-7009-051 in which pitavastatin was given with and without rifampin. The CV for the GMR for $AUC_{0-\infty}$ from this study was 0.214. From the previous microdose study conducted by Merck in healthy subjects, the within-subject CV for $AUC_{0-\infty}$ was 0.10 when pitavastatin was given with and without rifampin. There is no significant exposure difference between RI subject (mild, moderate, and severe) and healthy subjects in related published literature.

Atorvastatin:

An estimate of within-subject variability for atorvastatin is derived from earlier Merck studies, specifically MK-5172-032, MK-0431E-212, and MK-0431E-214. The pooled estimate of the within-subject standard deviation of the log $AUC_{0-\infty}$ values is 0.216 for atorvastatin. From the previous microdose study conducted by Merck in healthy subjects, the within-subject CV for $AUC_{0-\infty}$ was 0.185 when atorvastatin was given with and without rifampin.

Rosuvastatin:

An estimate of within-subject variability for rosuvastatin is derived from an earlier Merck study, MK-7009-051 in which rosuvastatin was given with and without rifampin. The CV for the GMR for $AUC_{0-\infty}$ from this study was 0.32. From the previous microdose study conducted by Merck in healthy subjects, the within-subject CV for $AUC_{0-\infty}$ was 0.25 when rosuvastatin was given with and without rifampin.

The sample size selected for each population to evaluate the effect of renal impairment on the pharmacokinetics of MK-7264 was not chosen to satisfy any a priori statistical requirement. This sample size (N= 6 per group) has historically been shown to be sufficient for studies of this type and should provide adequate data to support the planned analyses. Nevertheless, estimates of the expected precision of the estimates, based on these sample sizes are presented below.

Sample Size and Power:

Six subjects/subjects will be enrolled in each group (i.e., healthy, mild, moderate, severe without dialysis). To evaluate the DDI between each substrate in the microdose cocktail and rifampin, the comparisons will be within subject. The within subject CV varied from 0.1 to 0.6 for the various groups based on internal and external published data. Clinically meaningful true fold increases in $AUC_{0-\infty}$ of 2-, 3-, 4- and 5-fold were assumed for the power calculations. [Table 5](#) below shows the power calculations for the various combinations of assumed values for hypotheses, assuming N=6.

Table 5: Power Calculations for True Fold Increases by Within Subject CV

True Fold	Within subject CV	Power
2	0.1	>0.99
	0.3	0.83
	0.6	0.39
3	0.1	>0.99
	0.3	>0.99
	0.6	0.82
4	0.1	>0.99
	0.3	>0.99
	0.6	>0.99
5	0.1	>0.99
	0.3	>0.99
	0.6	>0.99

11.2.2 Subjects to Analyze

The decision as to which plasma samples collected will be assayed for evaluation of pharmacokinetics/pharmacodynamics will be collaboratively determined by the Departments of Quantitative Pharmacology and Pharmacometrics and the appropriate department within Early-Stage Development. If indicated, these samples may also be assayed and/or pooled for assay in an exploratory manner for metabolites and/or additional pharmacodynamic markers.

The following populations are defined for the analysis and reporting of data. All subjects will be reported, and their data analyzed, according to the treatment(s) they actually received.

All Subjects as Treated: All subjects who received at least one dose of the investigational drug(s). This population will be used for assessments of safety and tolerability.

Per-Protocol: The set of data generated by the subset of subjects who comply with the protocol sufficiently to ensure that these data will be likely to exhibit the effects of treatment, according to the underlying scientific model. Compliance covers such considerations as exposure to treatment, availability of measurements and absence of major protocol violations. Any subjects or data values excluded from analysis will be identified, along with their reason for exclusion, in the CSR. At the end of the study, all subjects who are compliant with the study procedure as aforementioned and have available data will be included in the primary analysis dataset. This population will be used for the pharmacokinetic analyses.

11.2.3 Descriptive Statistics

Non-model based descriptive statistics will be provided for all plasma analytes (rifampin, midazolam, dabigatran, pitavastatin, pitavastatin lactone, atorvastatin, 2-OH atorvastatin, 4-OH atorvastatin, and rosuvastatin) and urine analytes (rosuvastatin and dabigatran) pharmacokinetic parameters by subject group. The following (non-model-based) descriptive statistics will be provided: N (number of subjects with non-missing data), arithmetic mean, standard deviation, arithmetic percent CV (calculated as $100 \times \text{standard deviation} / \text{arithmetic mean}$), median, minimum, maximum, geometric mean, and geometric percent CV (calculated as $100 \times \sqrt{\exp(s^2) - 1}$, where s^2 is the observed variance on the natural log-scale).

11.2.4 Analysis Overview

11.2.4.1 Primary Analyses

Objective 1:

This analysis will include Period 1 data only. Separate ANOVA models will be constructed for each substrate in the microdose cocktail. Each model will include the natural logarithm of the AUC_{0-∞} data as the dependent variable and a fixed effect term for subject group (healthy subjects, mild, moderate, severe RI subjects, and ESRD subjects). AUC_{0-∞} will be compared between each RI subject group and the healthy subject group by constructing the appropriate contrasts from the model and computing the GMRs and corresponding 95% confidence intervals. Sample SAS code is given below:

```
proc mixed data=dataset;  
  
class group;  
  
model endpoint = group /ddfm=kr;  
  
lsmeans group / CIalpha = 0.05  
  
run;
```

Similar analyses will be conducted for the following pharmacokinetic parameters: AUC₀₋₂₄, AUC_{0-last}, C_{max}, and C₂₄. For the other pharmacokinetic parameters, appropriate summary statistics will be provided.

Objectives 2 to 6:

This analysis will include data from Period 1 and Period 2. This analysis will not include data from the ESRD subjects as they will not have received rifampin in Period 2. A linear mixed-effects model will be used and separate models will be constructed for each substrate in the microdose cocktail. Each model will include the natural logarithm of the AUC_{0-∞} data as the dependent variable, with a fixed effect term for treatment (microdose only or microdose+rifampin), subject group (healthy subjects, mild, moderate, or severe RI subjects

only) and the interaction of the terms. An unstructured covariance matrix will be used to allow for unequal treatment variances and to model the correlation between the treatment measurements within each subject via the REPEATED statement in SAS PROC MIXED. Kenward and Roger's method will be used to calculate the denominator degrees of freedom for the fixed effects (using the DDFM=KR option in SAS). The pharmacokinetic parameters will be compared between the different groups by constructing the appropriate contrasts from the model. Sample SAS code is given below:

```
proc mixed data=dataset;  
class treatment group subject;  
model endpoint = treatment group treatment*group /ddfm=kr;  
repeated treatment / subject=subject type=un;  
run;
```

For each substrate in the microdose cocktail, the GMRs and corresponding 95% confidence intervals for the comparison of microdose cocktail + rifampin vs. microdose cocktail alone in each group of subjects will be computed from the model.

Similar analyses will be conducted for the following pharmacokinetic parameters: AUC0-24, AUC0-last, Cmax, and C24. For the other pharmacokinetic parameters, appropriate summary statistics will be provided.

Plots with the individual ratios, GMR and 95% confidence intervals will be provided for AUC0-24, AUC0-last, AUC0-∞, Cmax, and C24.

11.2.4.2 Secondary Analyses

Secondary objectives will estimate the effect of rifampin on the single oral dose pharmacokinetics (AUC0-last, Cmax, and C24) using the same statistical models that were used to address the primary objectives 2 through 6. These models will be used to estimate the differences with/without rifampin for each substrate. For example, from the model constructed for midazolam, a between treatment comparison of midazolam+rifampin and midazolam will be made for each subject group (except subjects with ESRD). In addition, a comparison of the extent of the DDI between rifampin and each of the 5 substrates in the microdose cocktail between healthy subjects and each category of RI subjects (with the exception of the ESRD) will be conducted by providing the appropriate GMRs and 95% confidence intervals.

A mixed effects model with period as fixed effect and subject as random effect will be used to assess the impact of dialysis on the pharmacokinetic parameters of each of the substrates in the microdose cocktail by comparing the pharmacokinetics parameters in Period 1 verses Period 2 in the ESRD subjects.

11.2.4.3 Exploratory Analyses for Biomarkers

The exploratory objectives will be evaluated using the same statistical models that were used to address the primary and secondary objectives. Although no formal hypotheses will be tested, these models will be used to estimate the between-group differences amongst each substrate/perpetrator combination within a substrate.

11.3 Safety Evaluation

The safety will be evaluated by clinical assessment of adverse events and other safety measurements. Summary statistics for the laboratory safety tests, ECGs, pulse oximetry, and/or vital signs may also be computed and provided, as deemed clinically appropriate.

12 STUDY ADMINISTRATION

12.1 Ethics

12.1.1 Institutional Review Board

This protocol will be reviewed by the Chesapeake Research Review, Inc. IRB, and the study will not start until the IRB has approved the protocol or a modification thereof. The IRB is constituted and operates in accordance with the principles and requirements described in the US Code of Federal Regulations (21 CFR Part 56). The IRB is compliant with the International Conference on Harmonization (ICH).

12.1.2 Ethical Conduct of the Study

This research will be carried out in accordance with the protocol, US Code of Federal Regulations, Good Clinical Practices (GCP), 21 CFR Parts 50, 56, and 312, the ethical principles set forth in the Declaration of Helsinki, and the ICH harmonized tripartite guideline regarding GCP (E6 Consolidated Guidance, April 1996).

12.1.3 Subject Information and Consent

The purpose of the study, the procedures to be carried out and the potential hazards will be described to the subjects in non-technical terms. Subjects will be required to read, sign and date an ICF summarizing the discussion prior to screening, and will be assured that they may withdraw from the study at any time without jeopardizing their medical care.

Subjects will be given a copy of their ICF.

The initial ICF, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB approval/favorable opinion in advance of use. The subject should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature.

The informed consent will adhere to IRB/Ethics Research Committee (ERC) requirements, applicable laws and regulations and Sponsor requirements.

12.1.4 Consent and Collection of Specimens for Future Biomedical Research

The Investigator or qualified designee will explain the Future Biomedical Research consent to the subject, answer all of his/her questions, and obtain written informed consent before performing any procedure related to the Future Biomedical Research sub-trial. A copy of the informed consent will be given to the subject.

12.2 Termination of the Study

Celerion and/or Merck reserve the right to terminate the study in the interest of subject welfare.

12.2.1 Clinical Criteria for Early Trial Termination

There are no pre-specified criteria for terminating the trial early.

12.3 Data Quality Assurance

Standard operating procedures are available for all activities performed at Celerion relevant to the quality of this study. Designated personnel of Celerion will be responsible for implementing and maintaining quality assurance and quality control systems to ensure that the trial is conducted, and that data are generated, documented and reported in compliance with the study protocol, GCP and Good Laboratory Practice requirements as well as applicable regulatory requirements and local laws, rules and regulations relating to the conduct of the clinical study.

The CSR will be audited by the quality assurance (QA) department and the QA audit certificate will be included in the study report.

All clinical data will undergo a 100% quality control check prior to clinical database lock. Edit checks are then performed for appropriate databases as a validation routine using SAS[®] to check for missing data, data inconsistencies, data ranges etc. Corrections are made prior to database lock.

Case Report Forms (CRFs) are printed off directly from the database. Each CRF is reviewed and signed by the Investigator.

12.4 Data Management

The Investigator or qualified designee is responsible for recording and verifying the accuracy of subject data. By signing this protocol, the Investigator acknowledges that his/her electronic signature is the legally binding equivalent of a written signature. By entering his/her electronic signature, the Investigator confirms that all recorded data have been verified as accurate.

Detailed information regarding Data Management procedures will be outlined in Celerion Data Management Plan.

12.5 Direct Access to Source Data/Documents

Celerion will ensure that the Sponsor, IRB and inspection by domestic and foreign regulatory authorities will have direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing (ICH[E6] 5.1.2 & 6.10). In the event that other

trial-related monitoring should be done by other parties, they will be required to sign a confidentiality agreement prior to any monitoring and auditing.

12.6 Drug Supplies, Packaging and Labeling

The Sponsor will supply sufficient quantities of atorvastatin calcium trihydrate, pitavastatin calcium, rosuvastatin calcium, and dabigatran etexilate mesylate reference standard to allow completion of this study. Celerion or sites will purchase midazolam 2 mg/mL syrup and rifampin 300-mg capsules from commercially available supplies. The lot numbers and expiration dates (where available) of the drugs supplied will be recorded in the final report.

Records will be made of the receipt and dispensing of the drugs supplied. At the conclusion of the study, any unused drugs will be returned to the Sponsor unless otherwise specified by the Sponsor. If no supplies remain, this fact will be documented in the pharmacy product accountability records.

12.7 Data Handling and Record Keeping

Celerion's Merck library CRFs will be supplied.

12.8 Report Format

According to the ICH Harmonized Tripartite Guideline (Organization of the Common Technical Document for the Registration of Pharmaceuticals for Human Use M4 and the ICH M2 Expert Working Group), the final report will be written according to the ICH E3 Guideline (Structure and Content of Clinical Study Reports).

12.9 Compliance with Law, Audit, and Debarment

By signing this protocol, the Investigator agrees to conduct the trial in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of GCP (e.g., International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice: Consolidated Guideline and other generally accepted standards of good clinical practice); and all applicable federal, state and local laws, rules and regulations relating to the conduct of the clinical trial.

The Code of Conduct, a collection of goals and considerations that govern the ethical and scientific conduct of clinical investigations sponsored by Merck, is provided in [Appendix 1](#).

The Investigator also agrees to allow monitoring, audits, IRB/ERC review and regulatory authority inspection of trial-related documents and procedures and provide for direct access to all trial-related source data and documents.

The Investigator agrees not to seek reimbursement from subjects, their insurance providers or from government programs for procedures included as part of the trial reimbursed to the Investigator by the Sponsor.

The Investigator shall prepare and maintain complete and accurate trial documentation in compliance with GCP standards and applicable federal, state and local laws, rules and regulations; and, for each subject participating in the trial, provide all data, and, upon completion or termination of the clinical trial, submit any other reports to the Sponsor as required by this protocol or as otherwise required pursuant to any agreement with the Sponsor.

Trial documentation will be promptly and fully disclosed to the Sponsor by the Investigator upon request and also shall be made available at the trial site upon request for inspection, copying, review and audit at reasonable times by representatives of the Sponsor or any regulatory authorities. The Investigator agrees to promptly take any reasonable steps that are requested by the Sponsor as a result of an audit to cure deficiencies in the trial documentation and worksheets/case report forms.

The Investigator must maintain copies of all documentation and records relating to the conduct of the trial in compliance with all applicable legal and regulatory requirements. This documentation includes, but is not limited to, the protocol, worksheets/case report forms, advertising for subject participation, adverse event reports, subject source data, correspondence with regulatory authorities and IRBs/ERCs, consent forms, Investigator's curricula vitae, monitor visit logs, laboratory reference ranges, laboratory certification or quality control procedures and laboratory director curriculum vitae. By signing this protocol, the Investigator agrees that documentation shall be retained until at least 2 years after the last approval of a marketing application in an ICH region or until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Because the clinical development and marketing application process is variable, it is anticipated that the retention period can be up to 15 years or longer after protocol database lock. The Sponsor will determine the minimum retention period and notify the Investigator when documents may be destroyed. The Sponsor will determine the minimum retention period and upon request, will provide guidance to the investigator when documents no longer need to be retained. The sponsor also recognizes that documents may need to be retained for a longer period if required by local regulatory requirements. All trial documents shall be made available if required by relevant regulatory authorities. The Investigator must consult with and obtain written approval by the Sponsor prior to destroying trial and/or subject files.

ICH GCP guidelines recommend that the Investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

The Investigator will promptly inform the Sponsor of any regulatory authority inspection conducted for this trial.

Persons debarred from conducting or working on clinical trials by any court or regulatory authority will not be allowed to conduct or work on this Sponsor's trials. The Investigator will immediately disclose in writing to the Sponsor if any person who is involved in

conducting the trial is debarred or if any proceeding for debarment is pending or, to the best of the Investigator's knowledge, threatened.

In the event the Sponsor prematurely terminates a particular trial site, the Sponsor will promptly notify that trial site's IRB/IEC.

According to European legislation, a Sponsor must designate an overall coordinating Investigator for a multi-center trial (including multinational). When more than one trial site is open in an EU country, Merck, as the Sponsor, will designate, per country, a national principal coordinator (Protocol CI), responsible for coordinating the work of the Principal Investigators at the different trial sites in that Member State, according to national regulations. For a single-center trial, the Protocol CI is the Principal Investigator. In addition, the Sponsor must designate a principal or coordinating Investigator to review the trial report that summarizes the trial results and confirm that, to the best of his/her knowledge, the report accurately describes the conduct and results of the trial [CSR CI]. The Sponsor may consider one or more factors in the selection of the individual to serve as the Protocol CI and or CSR CI (e.g., availability of the Protocol/CSR CI during the anticipated review process, thorough understanding of clinical trial methods, appropriate enrollment of subject cohort, timely achievement of trial milestones). The Protocol CI must be a participating trial Investigator.

12.10 Publication Policy

The Sponsor will provide separate guidance on the criteria for publication of clinical trial data when contacted for permission to publish.

12.11 Privacy Notice

In order to comply with government regulations governing clinical studies, as well as ICH GCP 3.2.1, Merck & Co., Inc., and its corporate affiliates ("Sponsor"), is required to record the name and address of each IRB or IEC member that reviews and approves this study. The Sponsor is also required to document that each IRB or IEC meets regulatory and ICH GCP requirements by requesting and maintaining records of the names and qualifications of the IRB/IEC members and to make these records available for regulatory agency review upon request by those agencies (ICH GCP 8.2.8).

13 REFERENCES

1. Sun H, Frassetto L, Benet LZ. Effects of renal failure on drug transport and metabolism. *Pharmacol Therap.* 2006;109:1–11.
2. Prueksaritanont T, et al. Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. *Clin Pharmacol Ther.* 2017;101(4):519-530.
3. Midazolam hydrochloride syrup, full prescribing information (electronic monograph) published on the Boehringer Ingelheim/Roxane Laboratories, Inc. website (document revised: SEP/2012).
4. Pradaxa® (dabigatran etexilate mesylate) capsules, monograph by Boehringer Ingelheim Pharmaceuticals, Inc, full prescribing information (electronic monograph / revised November 2015) published on the FDA website.
5. Hankey GJ, Eikelboom JW. Dabigatran Etexilate – A New Oral Thrombin Inhibitor. *Circulation* 2011;123:1436-1450.
6. NDA 22-512 for Boehringer Ingelheim GmbH dabigatran etexilate mesylate (Pradaxa®) 110 and 150 mg capsules.
7. Lipitor® (atorvastatin calcium) tablets, monograph by Pfizer, full prescribing information (electronic monograph / revised 2015) published on the FDA website.
8. Neuvonen P.J., Niemi M., and Backman J.T., Drug Interactions with Lipid-Lowering Drugs: Mechanisms and Clinical Relevance. *Clin Pharmacol Ther.* 2006; 80(6):565-581.
9. Livalo® (pitavastatin calcium) tablets, monograph by Kowa Pharmaceuticals America, full prescribing information (electronic monograph / revised October 2013) published on the FDA website.
10. Crestor® (rosuvastatin calcium) tablets, full prescribing information (electronic monograph) published on the Drugs@FDA website (document revised: 07/2014).
11. Martin PD, Mitchell PD, and Schneck DW. Pharmacodynamic effects and pharmacokinetics of a new HMG-CoA reductase inhibitor, rosuvastatin, after morning and evening administration in healthy volunteers. *Br J Clin Pharmacol* 2002; 54:472-477.
12. Zhang W, et al. Role of BCRP 421C. A polymorphism on rosuvastatin pharmacokinetics in healthy chinese males. *Clin Chim Acta* 2006; 373(1-2):99-103.
13. NDA 21-366 for AstraZeneca Rosuvastatin Calcium (Crestor®) 5, 10, 20 and 40 mg tablets.

14. Rifadin® (Rifampin capsules or for injection), monograph by Sanofi-Aventis U.S. LLC, full prescribing information (electronic monograph / revised February 2013) published on the FDA website.
15. Gurumurthy P, Sarina GR, Jayasankar K, Thyagarajan K, Prabhakar R, Muthusetupathi MA, et al. Single dose pharmacokinetic of isoniazid and rifampicin in patients with chronic renal failure. *Ind J Tub* 1992; 39: 221-8.
16. Acocella G. Clinical pharmacokinetics of rifampicin *Clin Pharmacokinet*. 1978; 3(2):108-27.
17. Ellard G.A. Chemotherapy of Tuberculosis for Patients with Renal Impairment. *Nephron* 1993;64: 169–181.
18. Malone RS, Fish DN, Spiegel DM, Childs JM, Peloquin CA. The effect of hemodialysis on isoniazid, rifampin, pyrazinamide, and ethambutol. *Am J Respir Crit Care Med*. 1999 May;159(5 Pt 1): 1580-4.

Appendix 1: Merck* Code of Conduct for Clinical Trials

I. Introduction

A. Purpose

Merck, through its subsidiaries, conducts clinical trials worldwide to evaluate the safety and effectiveness of our products. As such, we are committed to designing, implementing, conducting, analyzing and reporting these trials in compliance with the highest ethical and scientific standards. Protection of subject safety is the overriding concern in the design of clinical trials. In all cases, Merck clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

B. Scope

Such standards shall be endorsed for all clinical interventional investigations sponsored by Merck irrespective of the party (parties) employed for their execution (e.g., contract research organizations, collaborative research efforts). This Code is not intended to apply to trials which are observational in nature, or which are retrospective. Further, this Code does not apply to Investigator-initiated trials which are not under the control of Merck.

II. Scientific Issues

A. Trial Conduct

1. Trial Design

Except for pilot or estimation trials, clinical trial protocols will be hypothesis-driven to assess safety, efficacy and/or pharmacokinetic or pharmacodynamic indices of Merck or comparator products. Alternatively, Merck may conduct outcomes research trials, trials to assess or validate various endpoint measures, or trials to determine subject preferences, etc.

The design (i.e., subject population, duration, statistical power) must be adequate to address the specific purpose of the trial. Research subjects must meet protocol entry criteria to be enrolled in the trial.

2. Site Selection

Merck selects investigative sites based on medical expertise, access to appropriate subjects, adequacy of facilities and staff, previous performance in Merck trials, as well as budgetary considerations. Prior to trial initiation, sites are evaluated by Merck personnel to assess the ability to successfully conduct the trial.

3. Site Monitoring/Scientific Integrity

Trial sites are monitored to assess compliance with the trial protocol and general principles of Good Clinical Practice. Merck reviews clinical data for accuracy, completeness and consistency. Data are verified versus source documentation according to standard operating procedures. Per Merck policies and procedures, if fraud, misconduct or serious GCP-non-Compliance are suspected, the issues are promptly investigated. When necessary, the clinical site will be closed, the responsible regulatory authorities and ethics review committees notified and data disclosed accordingly.

B. Publication and Authorship

To the extent scientifically appropriate, Merck seeks to publish the results of trials it conducts. Some early phase or pilot trials are intended to be hypothesis-generating rather than hypothesis testing. In such cases, publication of results may not be appropriate since the trial may be underpowered and the analyses complicated by statistical issues of multiplicity.

Merck's policy on authorship is consistent with the requirements outlined in the ICH-Good Clinical Practice guidelines. In summary, authorship should reflect significant contribution to the design and conduct of the trial, performance or interpretation of the analysis, and/or writing of the manuscript. All named authors must be able to defend the trial results and conclusions. Merck funding of a trial will be acknowledged in publications.

III. Subject Protection

A. IRB/ERC review

All clinical trials will be reviewed and approved by an independent IRB/ERC before being initiated at each site. Significant changes or revisions to the protocol will be approved by the IRB/ERC prior to implementation, except that changes required urgently to protect subject safety and well-being may be enacted in anticipation of IRB/ERC approval. For each site, the IRB/ERC and Merck will approve the subject informed consent form.

B. Safety

The guiding principle in decision-making in clinical trials is that subject welfare is of primary importance. Potential subjects will be informed of the risks and benefits of, as well as alternatives to, trial participation. At a minimum, trial designs will take into account the local standard of care. Subjects are never denied access to appropriate medical care based on participation in a Merck clinical trial.

All participation in Merck clinical trials is voluntary. Subjects are enrolled only after providing informed consent for participation. Subjects may withdraw from a Merck

trial at any time, without any influence on their access to, or receipt of, medical care that may otherwise be available to them.

C. Confidentiality

Merck is committed to safeguarding subject confidentiality, to the greatest extent possible. Unless required by law, only the Investigator, Sponsor (or representative) and/or regulatory authorities will have access to confidential medical records that might identify the research subject by name.

D. Genomic Research

Genomic Research will only be conducted in accordance with informed consent and/or as specifically authorized by an Ethics Committee.

IV. Financial Considerations

A. Payments to Investigators

Clinical trials are time- and labor-intensive. It is Merck's policy to compensate Investigators (or the sponsoring institution) in a fair manner for the work performed in support of Merck trials. Merck does not pay incentives to enroll subjects in its trials. However, when enrollment is particularly challenging, additional payments may be made to compensate for the time spent in extra recruiting efforts.

Merck does not pay for subject referrals. However, Merck may compensate referring physicians for time spent on chart review to identify potentially eligible subjects.

B. Clinical Research Funding

Informed consent forms will disclose that the trial is sponsored by Merck, and that the Investigator or sponsoring institution is being paid or provided a grant for performing the trial. However, the local IRB/ERC may wish to alter the wording of the disclosure statement to be consistent with financial practices at that institution. As noted above, publications resulting from Merck trials will indicate Merck as a source of funding.

C. Funding for Travel and Other Requests

Funding of travel by Investigators and support staff (e.g., to scientific meetings, Investigator meetings, etc.) will be consistent with local guidelines and practices including, in the U.S., those established by the American Medical Association (AMA).

V. Investigator Commitment

Investigators will be expected to review Merck's Code of Conduct as an appendix to the trial protocol, and in signing the protocol, agree to support these ethical and scientific standards.

* In this document, "Merck" refers to Merck Sharp & Dohme Corp. and Schering Corporation, each of which is a subsidiary of Merck & Co., Inc. Merck is known as MSD outside of the United States and Canada. As warranted by context, Merck also includes affiliates and subsidiaries of Merck & Co., Inc.

Appendix 2: Collection and Management of Specimens for Future Biomedical Research

1. Definitions

- a. Biomarker: A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition.¹
- b. Pharmacogenomics: The investigation of variations of DNA and RNA characteristics as related to drug/vaccine response.²
- c. Pharmacogenetics: A subset of pharmacogenomics, pharmacogenetics is the influence of variations in DNA sequence on drug/vaccine response.²
- d. DNA: Deoxyribonucleic acid.
- e. RNA: Ribonucleic acid.

2. Scope of Future Biomedical Research

The specimens consented and/or collected in this trial as outlined in [Section 10.4](#) will be used in various experiments to understand:

- The biology of how drugs/vaccines work
- Biomarkers responsible for how a drug/vaccine enters and is removed by the body
- Other pathways drugs/vaccines may interact with
- The biology of disease

The specimen(s) may be used for future assay development and/or drug/vaccine development.

It is now well recognized that information obtained from studying and testing clinical specimens offers unique opportunities to enhance our understanding of how individuals respond to drugs/vaccines, enhance our understanding of human disease and ultimately improve public health through development of novel treatments targeted to populations with the greatest need. All specimens will be used by the Sponsor or those working for or with the Sponsor.

3. Summary of Procedures for Future Biomedical Research

a. Subjects for Enrollment

All subjects enrolled in the clinical trial will be considered for enrollment in the Future Biomedical Research sub-trial.

b. Informed Consent

Informed consent for specimens (i.e., DNA, RNA, protein, etc.) will be obtained during screening for protocol enrollment from all subjects or legal guardians, at a trial visit by the investigator or his or her designate. Informed consent for Future Biomedical Research should be presented to the subjects on the visit designated in the trial flow chart. If delayed, present consent at next possible Subject Visit. Consent forms signed by the subject will be kept at the clinical trial site under secure storage for regulatory reasons.

A template of each trial site's approved informed consent will be stored in the Sponsor's clinical document repository.

c. CRF Documentation for Future Biomedical Research Specimens

Documentation of subject consent for Future Biomedical Research will be captured in the electronic Case Report Forms (CRFs). Any specimens for which such an informed consent cannot be verified will be destroyed.

d. Future Biomedical Research Specimen(s)

Collection of specimens for Future Biomedical Research will be performed as outlined in the trial flow chart. In general, if additional blood specimens are being collected for Future Biomedical Research, these will usually be obtained at a time when the subject is having blood drawn for other trial purposes.

4. Confidential Subject Information for Future Biomedical Research

In order to optimize the research that can be conducted with Future Biomedical Research specimens, it is critical to link subject's clinical information with future test results. In fact little or no research can be conducted without connecting the clinical trial data to the specimen. The clinical data allow specific analyses to be conducted. Knowing subject characteristics like gender, age, medical history and treatment outcomes are critical to understanding clinical context of analytical results.

To maintain privacy of information collected from specimens obtained for Future Biomedical Research, the Sponsor has developed secure policies and procedures. All specimens will be single-coded per ICH E15 guidelines as described below.

At the clinical trial site, unique codes will be placed on the Future Biomedical Research specimens. This code is a random number which does not contain any personally identifying information embedded within it. The link (or key) between subject identifiers and this unique code will be held at the trial site. No personal identifiers will appear on the specimen tube.

5. Biorepository Specimen Usage

Specimens obtained for the Sponsor will be used for analyses using good scientific practices. Analyses utilizing the Future Biomedical Research specimens may be performed by the Sponsor, or an additional third party (e.g., a university investigator) designated by the Sponsor. The investigator conducting the analysis will follow the Sponsor's privacy and confidentiality requirements. Any contracted third party analyses will conform to the specific scope of analysis outlined in this sub-trial. Future Biomedical Research specimens remaining with the third party after specific analysis is performed will be reported to the Sponsor.

6. Withdrawal From Future Biomedical Research

Subjects may withdraw their consent for Future Biomedical Research and ask that their biospecimens not be used for Future Biomedical Research. Subjects may withdraw consent at any time by contacting the principal investigator for the main trial. If medical records for the main trial are still available, the investigator will contact the Sponsor using the designated mailbox (clinical.specimen.management@merck.com). Subsequently, the subject's specimens will be flagged in the biorepository and restricted to main study use only. If specimens were collected from study participants specifically for Future Biomedical Research, these specimens will be removed from the biorepository and destroyed. Documentation will be sent to the investigator confirming withdrawal and/or destruction, if applicable. It is the responsibility of the investigator to inform the subject of completion of the withdrawal and/or destruction, if applicable. Any analyses in progress at the time of request for withdrawal/destruction or already performed prior to the request being received by the Sponsor will continue to be used as part of the overall research trial data and results. No new analyses would be generated after the request is received.

In the event that the medical records for the main trial are no longer available (e.g., if the investigator is no longer required by regulatory authorities to retain the main trial records) or the specimens have been completely anonymized, there will no longer be a link between the subject's personal information and their specimens. In this situation, the request for withdrawal of consent and/or destruction cannot be processed.

7. Retention of Specimens

Future Biomedical Research specimens will be stored in the biorepository for potential analysis for up to 20 years from the end of the main study. Specimens may be stored for longer if a regulatory or governmental authority has active questions that are being

answered. In this special circumstance, specimens will be stored until these questions have been adequately addressed.

Specimens from the trial site will be shipped to a central laboratory and then shipped to the Sponsor-designated biorepository. If a central laboratory is not utilized in a particular trial, the trial site will ship directly to the Sponsor-designated biorepository. The specimens will be stored under strict supervision in a limited access facility which operates to assure the integrity of the specimens. Specimens will be destroyed according to Sponsor policies and procedures and this destruction will be documented in the biorepository database.

8. Data Security

Databases containing specimen information and test results are accessible only to the authorized Sponsor representatives and the designated trial administrator research personnel and/or collaborators. Database user authentication is highly secure, and is accomplished using network security policies and practices based on international standards to protect against unauthorized access.

9. Reporting of Future Biomedical Research Data to Subjects

No information obtained from exploratory laboratory studies will be reported to the subject, family, or physicians. Principle reasons not to inform or return results to the subject include: Lack of relevance to subject health, limitations of predictive capability, and concerns regarding misinterpretation.

If important research findings are discovered, the Sponsor may publish results, present results in national meetings, and make results accessible on a public website in order to rapidly report this information to doctors and subjects. Subjects will not be identified by name in any published reports about this study or in any other scientific publication or presentation.

10. Future Biomedical Research Study Population

Every effort will be made to recruit all subjects diagnosed and treated on Sponsor clinical trials for Future Biomedical Research.

11. Risks Versus Benefits of Future Biomedical Research

The Sponsor has developed strict security, policies and procedures to address subject data privacy concerns. Data privacy risks are largely limited to rare situations involving possible breach of confidentiality. In this highly unlikely situation there is risk that the information, like all medical information, may be misused.

12. Questions

Any questions related to the future biomedical research should be e-mailed directly to clinical.specimen.management@merck.com.

13. References

1. National Cancer Institute: <http://www.cancer.gov/dictionary/?searchTxt=biomarker>
2. International Conference on Harmonization: DEFINITIONS FOR GENOMIC BIOMARKERS, PHARMACOGENOMICS, PHARMACOGENETICS, GENOMIC DATA AND SAMPLE CODING CATEGORIES - E15; <http://www.ich.org/LOB/media/MEDIA3383.pdf>
3. Industry Pharmacogenomics Working Group. Understanding the Intent, Scope and Public Health Benefits of Exploratory Biomarker Research: A Guide for IRBs/IECs and Investigational Site Staff. Available at <http://i-pwg.org/>
4. Industry Pharmacogenomics Working Group. Pharmacogenomics Informational Brochure for IRBs/IECs and Investigational Site Staff. Available at <http://i-pwg.org/>

Appendix 3: Sample Handling Instructions for Midazolam and Dabigatran Samples

Supplies and Equipment

1. Plastic Lavender Topped Vacutainers containing K₂EDTA as the anticoagulant. Capable of holding 4 - 5 mL of whole blood.
2. 25% o-Phosphoric acid: Ricca Chemical Company (585116 or equivalent)
3. Midazolam tubes: 3.6 mL NUNC Internal Thread Round Bottom Cryotubes (NUNC Part #366524).
4. Dabigatran Tubes: 3.6 mL NUNC Internal Thread Round Bottom Cryotubes (NUNC Part #366524) containing 15 µL of 25% o-phosphoric acid per tube (any brand 25% o-phosphoric acid solution is acceptable).
 - 15 µL of the acid can be pre-pipetted into tubes one day prior to blood collection. Tubes should be stored upright at room temperature.
5. Calibrated Adjustable Volume Pipettes: Suitable for delivering volumes between 10-200 µL and 100 -1000 µL.
6. Disposable Plastic Pipettes: Suitable for delivering volumes between 10-200 µL and 100 -1000 µL.
7. Refrigerated Centrifuge. Capable of rotating between 1000-1300 RCF (x g) at between 4°C to 10°C for 10 minutes. Note that RCF varies according to the centrifuge rotor radius. The formula for computing RCF from rotation speed and centrifuge radius is $RCF = 11.2r(RPM/1000)^2$, where r is rotor radius, in cm, and RPM is the rotations per minute setting of the centrifuge. A typical refrigerated centrifuge (GH-3.8 model from Beckman) yields 1150 RCF at 2500 RPM, for example.
8. -65°C to -85°C Sample Storage Freezer.

Collection of Blood

For specific time points of sample collection, please refer to the Study Flow Chart.

Sample Labeling

1. Whole Blood Samples. Vacutainers containing whole blood should be labeled (non-barcoded) as appropriate.
2. Plasma Samples. NUNC tubes containing plasma samples should be labeled with the pre-printed barcoded labels with the allocation number, day, date and time (hours postdose) provided by the Sponsor. Labels should be placed on the NUNC tubes toward the top 30% of the tube in order for the level of plasma in the tube to be

viewed. Only **one (1)** layer of label should be placed on the tube (not 2). This is critical for the proper functioning of the automated liquid handling station.

Procedure

1. Draw approximately 4 mL whole blood into plastic (PET) vacutainer containing K₂EDTA as the anticoagulant and invert 6 times. The vacutainer should be labeled as appropriate (see above).
2. Immediately after collection, the blood containing tubes should be placed on ice and centrifuged promptly at between 1000-1300 RCF (x g) at between 4°C to 10°C for 10 minutes. Note that RCF varies according to the centrifuge rotor radius. The formula for computing RCF from rotation speed and centrifuge radius is $RCF = 11.2r(RPM/1000)^2$, where r is rotor radius, in cm, and RPM is the rotations per minute setting of the centrifuge. If the samples cannot be centrifuged immediately, the tubes should be kept on ice and centrifuged within 30 minutes of collection.

Note: Be sure to account for rotor size variations by adjusting the revolutions per minute (RPM) for the specific centrifuge to yield between 1000-1300 RCF (xg) as noted in the Supplies and Equipment section.

3. Immediately after separation of the whole blood, carefully transfer the plasma into two tubes with pre-printed barcoded labels. Transfer 0.6 mL plasma into the tube containing 15 µL of 25% O-Phosphoric acid for Dabigatran and the remaining plasma into the Midazolam labeled tube using calibrated pipettes. Store at -65°C to -85°C until transfer to Pharma Medica Research Inc. on DRY ICE.

Note: In the event that the whole blood samples cannot be processed immediately the samples should be kept on ice. No more than 60 minutes should elapse between blood draw and the freezing of plasma samples.

Sample Shipping

It is the responsibility of the Primary Investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens act in conformance with International Air Transport Association (IATA) regulations relating to the handling and shipping of hazardous goods.

1. All shipments will be made in freezer boxes containing sufficient DRY ICE.
2. Please include a sample inventory with each shipment.
3. Samples should be sent at intervals to be determined by the Sponsor and the Investigator. Shipments should be sent on MONDAY or TUESDAY to assure receipt by Friday.

4. Samples should be shipped to:

Analytical Laboratory
Pharma Medica Research Inc.
6100 Belgrave Road
Mississauga, Ontario, Canada
L5R 0B7

Phone

PPD

Fax

PPD

PPD

Manager, Lab Operations Support

PPD

Note: Sample storage for this study is -65°C to -85°C.

Appendix 4: Sample Handling Instructions for Pitavastatin, Atorvastatin (and metabolites), and Rosuvastatin Samples

Supplies and Equipment

1. Plastic Lavender Topped Vacutainers containing K₂EDTA as the anticoagulant. Capable of holding 3- 4 mL of whole blood.
2. 3.6 mL NUNC Internal Thread Round Bottom Cryotubes (NUNC Part #366524).
3. Calibrated Adjustable Volume Pipettes. Suitable for delivering volumes between 10-200 µL, 100 -1000 µL, and 1.0 - 10.0 mL.
4. Disposable Pipette Tips. Suitable for delivering volumes between 10-200 µL; 100-1000 µL, and 1.0 – 10.0 mL.
5. Refrigerated Centrifuge. Capable of rotating between 1000-1300 RCF (x g) at between 4°C to 10°C for 10 minutes. Note that RCF varies according to the centrifuge rotor radius. The formula for computing RCF from rotation speed and centrifuge radius is $RCF = 11.2r(RPM/1000)^2$, where r is rotor radius, in cm, and RPM is the rotations per minute setting of the centrifuge. A typical refrigerated centrifuge (GH-3.8 model from Beckman) yields 1150 RCF at 2500 RPM, for example.
6. -65°C to -85°C Sample Storage Freezer.
7. Corning Pyrex Reusable Media Storage Bottle (250 mL): Fisher Scientific Catalog #06-414-1B. To be used for buffer preparation.
8. Ammonium acetate, certified ≥97%, 500 g; Fisher Scientific Catalog #A637-500 or equivalent.
9. Acetic Acid, Glacial ≥97% (w/w%), 500 mL; Fisher Scientific Catalog #A35-500 or equivalent.
10. Water Optima, 1L; Fisher Scientific Catalog#W6-1 or equivalent.
11. Top loading digital balance capable of weights listed below.
12. Glass cylinder for measuring liquid volume up to 250 mL.

Procedures

Preparation of 1M Ammonium acetate buffer, pH 5

1. Weigh 19.25g of ammonium acetate.

2. Transfer the weighed ammonium acetate to the 250-mL pyrex storage bottle and dissolve with 230 mL of bottled water (Optima). Mix.
3. Pipet 8.25 mL of glacial acetic acid into the mixture. Mix.
4. Store refrigerated (~4°C) when not in use for up to 90 days after preparation. Let it stand at room temperature and mix before use.

Sample Labeling

Whole Blood Samples. Vacutainers containing whole blood should be labeled (non-barcoded) as appropriate.

Plasma Samples. NUNC tubes containing **buffered plasma samples** should be labeled with the pre-printed barcoded labels with the allocation number, day, date and time (hours postdose) provided by the Sponsor. Labels should be placed on the NUNC tubes toward the top 30% of the tube in order for the level of plasma in the tube to be viewed. Only **one (1)** layer of label should be placed on the tube (not 2). This is critical for the proper functioning of the automated liquid handling station.

Collection of Blood

For specific time points of sample collection, please refer to the Study Flow Chart.

1. Prior to blood draw, prepare the Nunc tube for plasma collection by pipetting **50 µL of 1M ammonium acetate buffer (pH5)** into each tube.
2. Draw approximately 3 mL whole blood into plastic (PET) vacutainer containing K₂EDTA as the anticoagulant and invert 6 times. The vacutainer should be labeled as appropriate (see above).
3. Immediately after collection, the blood containing tubes should be placed on ice and centrifuged promptly at between 1000-1300 RCF (x g) at between 4°C to 10°C for 10 minutes. Note that RCF varies according to the centrifuge rotor radius. The formula for computing RCF from rotation speed and centrifuge radius is $RCF = 11.2r(RPM/1000)^2$, where r is rotor radius, in cm, and RPM is the rotations per minute setting of the centrifuge. If the samples cannot be centrifuged immediately, the tubes should be kept on ice and centrifuged within 30 minutes of collection.

Note: Be sure to account for rotor size variations by adjusting the revolutions per minute (RPM) for the specific centrifuge to yield between 1000-1300 RCF (xg) as noted in the Supplies and Equipment section.

4. Immediately after separation of the whole blood, carefully transfer **1 mL** of the plasma using a pipette into a 3.6 mL internally-threaded NUNC cryotube containing **50 µL of 1M ammonium acetate buffer (pH5)** identified with pre-printed barcoded labels (see above).

5. Vortex mix for approximately 10 seconds, and store at -65°C to -85°C until transfer to Pharma Medica Research Inc. on DRY ICE.

***Note:** In the event that the whole blood samples cannot be processed immediately the samples should be kept on ice. No more than 60 minutes should elapse between blood draw and the freezing of plasma samples.*

Sample Shipping

It is the responsibility of the Primary Investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens act in conformance with International Air Transport Association (IATA) regulations relating to the handling and shipping of hazardous goods.

1. All shipments will be made in freezer boxes containing sufficient DRY ICE.
2. Please include a sample inventory with each shipment.
3. Samples should be sent at intervals to be determined by the Sponsor and the Investigator. Shipments should be sent on MONDAY or TUESDAY to assure receipt by Friday.
4. Samples should be shipped to:

Analytical Laboratory
Pharma Medica Research Inc.
6100 Belgrave Road
Mississauga, Ontario, Canada
L5R 0B7
Phone [REDACTED]
Fax [REDACTED]
Manager, Lab Operations Support
[REDACTED]

***Note:** Sample storage for this study is -65°C to -85°C .*

Appendix 5: Sample Handling Instructions for Rifampin Samples

SAMPLE LABELING

K₂EDTA vacutainer tubes and plasma transfer vials (cryotubes) should be labeled with allocation number, period, day, date, and actual time (hours postdose). Bar-coded labels will be provided by Sponsor for plasma and samples.

Place label toward the top 30% of the cryotube in order for the level of plasma in the tube to be viewed. Only one (1) layer of label should be placed on the tube (not 2). This is critical for the proper function of the automatic liquid handling station.

SAMPLE COLLECTION, HANDLING, LABELING, STORAGE, AND SHIPMENT

Blood samples will be collected in labelled 3 mL blood collection tubes containing K₂EDTA as the anticoagulant. The blood-containing tubes should be gently inverted 8 to 10 times and placed immediately in an ice water bath. The samples will be maintained in an ice bath throughout sample collection and until further processing. Within 240 minutes of collection, the blood samples will be centrifuged at 1700 g (approximately 3000 rpm) for approximately 10 minutes and at 4°C (2°C to 8°C). Samples that are interrupted during centrifugation, disturbed during the separation process or exhibit inadequate separation will be re-spun under the same conditions in an attempt to separate the maximum amount of plasma from each sample. The samples will be maintained in an ice bath following centrifugation and until storage in the freezer.

The plasma from each sample will be aliquoted into separately labelled cryotubes (3.6 mL NUNC Internal Thread Round Bottom Cryotubes [NUNC Part #366524]). Cryotubes are immediately frozen or stored temporally on ice and frozen at -15°C to -30°C within 180 minutes from start of centrifugation. Plasma samples are stored in a freezer at -15°C to -30°C pending shipment.

At the completion of the clinical portion of the study, the plasma samples will be packed on dry ice and sent to the analytical facility. The shipment of the plasma samples will be made in freezer boxes containing sufficient DRY ICE (and labeled as HUMAN SAMPLES: NONINFECTIOUS). All shipments will be made on Monday or Tuesday only.

Clinic personnel will notify the analytical laboratory via e-mail prior to shipment with the following information:

- Date of the shipment
- Number and type of samples
- Special handling requirements and
- Number of shipping containers

This information will be e-mailed to:

PPD

In addition, on the day of shipment, please notify inVentiv Health Clinique (at the e-mail coordinates above) of the shipment as well as the name of the shipping carrier and the tracking number. The shipment will be accompanied by an inventory list with appropriate documents and delivered to:

inVentiv Health Clinique
Attention: Sample Controller
2500 rue Einstein
Québec (Québec), Canada G1P 0A2
Phone: ^{PPD} Fax: ^{PPD}

Appendix 6: Sample Handling Instructions for Urine Collection (Dabigatran and Rosuvastatin)

Urine Collection Procedure

Introduction

Buffering urine in the urine collection procedure is required. Buffer must be added to the original urine collection container after each void into the container. Any sample transfers from the original sample container prior to the addition of buffer must be avoided in that loss of drug and metabolite may occur. Urine weights, rather than volumes, are determined in order to eliminate the need to pour the sample into a graduated cylinder before the addition of the buffer.

Supplies and Equipment

1. 24 hour Urine amber collection Jugs (from PPD for the biomarker urine collection) (or Nalgene Series 2105 Wide Mouth Bottles may be used if for PK only). These bottles are to be used for the actual urine collection, and are available in sizes ranging from 250 to 1000 mL. Thus, the bottle size may be varied depending on the length of the collection interval. The bottles are available in the United States from Fisher Scientific. Their catalog numbers are as follows:

30 mL bottle: #02-893AA

60 mL bottle: #02-893BB

250 mL bottle: #02-893B

A list of Nalgene distributors for countries outside of the U.S. may be found on the World Wide Web at <http://nalgene.com/gen/order.html>

2. Set of pipettes suitable for delivering volumes between 0.25 mL and 20 mL.
3. 4.5 mL NUNC cryotube vials (NUNC #363452 or Fisher #12-565-173N)
4. Corning Pyrex Reusable Media Storage Bottle (250 mL): Fisher Scientific Catalog #06-414-1B. To be used for buffer preparation.
5. Ammonium acetate, certified 97%, 500 g; Fisher Scientific Catalog #A637-500 or equivalent.
6. Acetic Acid, Glacial 97% (w/w%), 500 mL; Fisher Scientific Catalog #A35-500 or equivalent.
7. Water Optima, 1L; Fisher Scientific Catalog#W6-1 or equivalent.

8. Top loading digital balance capable of weights listed below.
9. Glass cylinder for measuring liquid volume up to 250 mL.

Preparation of 1M Ammonium acetate buffer, pH 5 (Prepare fresh at the start of the study)

1. Weigh 19.25g of ammonium acetate.
2. Transfer the weighed ammonium acetate to the 250-mL pyrex storage bottle and dissolve with 230 mL of bottled water (Optima). Mix.
3. Pipet 8.25 mL of glacial acetic acid into the mixture. Mix.
4. Store refrigerated ($\sim 4^{\circ}\text{C}$) when not in use for up to 90 days after preparation. Let it stand at room temperature and mix before use.

Procedure

1. Prior to use, the urine collection bottles, together with their caps, should be weighed on a digital balance. The bottle weights should then be recorded.
2. During the sample collection interval, the entire volume should be voided directly into the pre-weighed collection bottle.
3. At times when they are not in use, the collection bottles may be stored at 4°C .
4. At the end of each void, determine and record the weight of the capped collection bottle and remove the biomarker aliquots (see [Appendix 10](#)), then determine and record the weight of the capped collection bottle containing the pharmacokinetic urine specimen.
5. Subtract the weight of the empty bottle from the weight of the bottle containing the specimen in order to determine the weight of the specimen. For additional collections within the interval, record the current weight and subtract the previous weight.
6. Record the weight of the specimen.
7. Calculate the volume of 1M ammonium acetate buffer, pH 5 solution that needs to be added to the specimen. The volume (mL) of buffer solution that needs to be added to the sample is calculated by multiplying the weight of the specimen in grams by 0.05. For example, a specimen weighing 500 grams requires 25 mL of buffer solution to be added to it.
8. Add the calculated volume of buffer solution to the specimen after each void. The volume of buffer solution added to the specimen should also be recorded.

Note that if urine collection volumes exceed 1 L for any given interval, separate additions of buffer (to each container within a collection interval) are to be completed as described above, and the separate collections are to be combined after the solutions are mixed thoroughly.

9. Cap the specimen bottle and shake the sample well.
10. During each collection interval, separate collection voids following addition of buffer are to be combined into a pooling container and stored at 4°C.
11. At the end of each collection interval, transfer a 3 mL aliquot of the buffer treated specimen into two pre-labeled 4.5 mL NUNC tubes.
12. The sample aliquots should be frozen at -65°C to -85°C prior to shipment on dry ice to Pharma Medica Research Inc.

Sample Shipping

It is the responsibility of the primary investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens act in conformance with International Air Transport Association (IATA) regulations relating to the handling and shipping of hazardous goods.

1. All shipments will be made in freezer boxes containing sufficient DRY ICE.
2. Please include a sample inventory with each shipment.
3. Samples should be sent at intervals to be determined by the Sponsor and the investigator. Shipments should be sent on MONDAY or TUESDAY to assure receipt by Friday.
4. Samples should be shipped to:

Analytical Laboratory
Pharma Medica Research Inc.
6100 Belgrave Road
Mississauga, Ontario, Canada
L5R 0B7
Phone ^{PPD} [REDACTED]
Fax ^{PPD} [REDACTED]
^{PPD} [REDACTED]
Manager, Lab Operations Support
^{PPD} [REDACTED]

Note: Sample storage for this study is -65°C to -85°C.

Appendix 7: PAXgene™ Blood for DNA Analysis

PAXgene™ BLOOD FOR DNA ANALYSIS SPECIMEN COLLECTION PROCEDURE

SPECIMEN COLLECTION NOTES*

SCP-124-00

***NOTE:** Refer to protocol flow chart or Specimen Collection Overview Chart for scheduled collection time points.

***NOTE:** Collection of specimens from vascular access devices and heparin or saline locks is not recommended due to the potential for specimen contamination. This specimen should be collected as a peripheral blood draw.

Supplies and Materials (per patient, per time point)

Provided to the Institution

- Requisition form/card
- "PAXgene Blood DNA" labels
- One 8.5 mL PAXgene™ Blood DNA collection tube (Cat#761115)

Precautions

*** SAFETY PRECAUTION:** Contents of the PAXgene™ tube are irritating to skin. Wear disposable gloves, safety glasses or goggles and a laboratory coat and follow standard laboratory safety procedures while working with these tubes. If inhaled, supply fresh air; consult doctor in case of complaints. If skin contact, immediately wash with water and soap, and rinse thoroughly. If contents make eye contact, rinse opened eye for 15 minutes under running water, then consult a doctor. If swallowed, immediately call a doctor.

Required Equipment

- Freezer for -20°C for PAXgene™ tube storage (For storage exceptions/monthly batch shipments).

Labeling

1. Place patient-specific label on the PAXgene Blood DNA tube.
2. If required: Fill out the requisition form/card appropriately (ensure that you follow specific processing instructions per the protocol specific Laboratory Procedure Manual).

Preparation

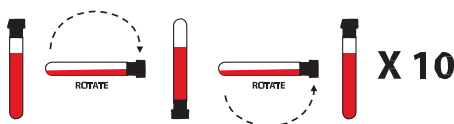
1. Ensure the patient has signed the appropriate IRB/ERC-approved consent for genetic specimen collection prior to collecting the specimen.
2. Ensure the PAXgene™ Blood DNA collection tubes are at room temperature prior to collecting blood.

***NOTE:** Do not use tubes after the expiration date printed on the label.

3. The PAXgene™ Blood DNA collection tubes should not be the first tubes drawn during venipuncture. **It should be the last tube collected.**

Specimen Collection

1. Collect blood into each PAXgene™ Blood DNA collection tube via your institution's recommended standard procedure for venipuncture.
 - Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.
 - Each tube holds approximately 8.5 mL of blood.
 - Under-filling of the tubes could result in an incorrect blood-to-additive ratio and may lead to poor performance (e.g. poor quality or low quantity)
2. Immediately after collection, completely and gently invert the tube 10 times to mix uniformly.



***NOTE:** After each tube is collected, it is **CRITICAL** to gently invert PAXgene™ 10 times to ensure proper mixing of blood & PAXgene™ proprietary reagent.

Specimen Processing & Handling

1. Within 5-10 minutes of the blood draw, place tubes upright in a wire or hard plastic rack at ambient temperature (18-25°C).
2. Tubes **must be shipped within 24 hrs of collection** to the laboratory at ambient temperature.

Storage Exceptions (special circumstances only)

If storing specimens for batch shipment: Specimen tubes MUST be transferred to a -20°C freezer, in a wire or hard plastic rack in the upright position, after collection. **Specimen tubes may be stored frozen upright at -20°C for no longer than 4 weeks at -20°C.** Tubes stored at -20°C must be shipped on dry ice to the Laboratory.

***NOTE:** Any storage time and temperature excursions must be documented and communicated upon specimen shipment within the shipment inventory documents.

***NOTE:** Frozen PAXgene™ Blood DNA collection tubes are subject to breakage on impact. To reduce the risk of breakage during handling and shipment, frozen tubes should be treated in the same manner as glass tubes. If freezing is required, a wire or hard plastic rack should be used (**NO STYROFOAM**) as the tubes may crack.

Packaging and Shipping

1. It is the responsibility of the primary investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens are trained and certified as required by National and International regulations and they ship materials in accordance with all current regulations relating to the handling and shipping of hazardous goods.
2. Follow packing and shipping instructions for **AMBIENT** shipments.
3. Contact shipping courier to obtain any required documentation/forms required for shipment.
4. Ship to the Laboratory **within 24 hr of the blood draw**.
5. **Shipping schedule** – Select overnight or priority delivery and ensure that shipments are received at the destination vendor Monday through Friday, except on U.S. holidays. Shipments can be received on Saturday with advanced notification. **Contact the Vendor if you are uncertain about the shipping or receiving schedule.**

***NOTE:** For storage exceptions where ambient shipment was not possible, and specimens were frozen (-20°C), always ship frozen specimens on DRY ICE.

Shipping Address:

BioProcessing Solutions Alliance

Attn: CommStaff

Nelson Biological Laboratories

604 Allison Road, C120

Piscataway, New Jersey 08854, USA

Tel.: ^{PPD} [REDACTED]

Email: ^{PPD} [REDACTED]

Appendix 8: Prohibited and Allowed Medications for Subjects with RI

Systemically absorbed drugs specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. Listed below are some specific restrictions for concomitant therapy use during the course of the trial. If there is a clinical indication for one of these or other medications specifically prohibited during the trial, discontinuation from trial therapy may be required. The Investigator should discuss any questions regarding this with the Sponsor Clinical Monitor. The final decision on any supportive therapy rests with the Investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy requires the mutual agreement of the Investigator, the Sponsor and the subject.

The following medications/therapies are contraindicated in this study:

Strong and moderate CYP3A/P-gp inhibitors, including but not limited to:

- Antibiotics: clarithromycin, erythromycin, telithromycin
- Antifungals: itraconazole, ketoconazole, voriconazole
- Antihypertensives: nifedipine
- Nefazodone

Strong and moderate CYP3A/P-gp inducers, including but not limited to:

- Anti-infectives: nafcillin, rifampin
- Anticonvulsants: carbamazepine, phenytoin, phenobarbital
- Bosentan
- Modafinil
- St. John's wort

OATP inhibitors, including but not limited to:

- Immunosuppressants: cyclosporine
- Anti-infectives: rifampin, ritonavir, atazanavir, saquinavir, tipranavir, lopinavir
- Diabetes agents: glimeclamide, glyburide
- Lipid lowering agents: gemfibrozil
- Eltrombopag
- Lapatinib

All HMG-CoA reductase inhibitors (statins)

In general, CYP3A4 substrates with narrow therapeutic ranges (e.g., warfarin, amiodarone, flecainide, propafenone, quinidine, fentanyl, sildenafil or tadalafil when used for the treatment of pulmonary arterial hypertension) are not prohibited but their levels have the potential to be increased by approximately 30%. Therefore, subjects taking these medications should be monitored closely or dose adjusted appropriately.

Medications Allowed Per Protocol

Concomitant use of the medications listed below will be allowed during the conduct of the study as long as the subject has not had a clinically significant change 14 days prior to the first study drug administration and is anticipated to remain stable during the study.

- **Proton Pump Inhibitors**

Note: Proton pump inhibitors must be withheld 1 week prior to each dosing and 4 hours post dosing.

- **Anti-hypertensive agents**, including mono- or combination therapy with an angiotensin converting enzyme (ACE) inhibitor, an angiotensin II receptor antagonist, a betablocker, a calcium-channel blocker or a diuretic (e.g., lisinopril, ramipril, candesartan, irbesartan, olmesartan, hydrochlorothiazide, furosemide, or nitrates).

Note: Diuretics must be withheld 8 hours prior to each dosing and 4 hours post dosing.

- **Medications for treatment of diabetes** such as insulin, sulfonylureas (e.g., tolbutamide, glipizide, glimepiride, metformin, saxagliptin, sitagliptin), meglitinides (e.g., repaglinide, nateglinide), thiazolidinediones, dipeptidyl peptidase-4 inhibitors, and glucagon-like peptide-1 analogs.
- **Iron.**
- **Vitamin D**
- **Thyroid hormone replacement.**
- **Acetaminophen (up to 2 g per 24-hour period).**
- **Phosphate binders containing aluminum, calcium or lanthanum salts; iron supplements or other metal cations; antacids; or multivitamins containing iron or zinc.**

Note: These drugs must be withheld 8 hours prior to each dosing and 4 hours post dosing.

Appendix 9: Specimen Collection Procedure – Plasma and Serum Biomarkers

**STANDARD & K₂EDTA PLASMA
SPECIMEN COLLECTION PROCEDURE**

SPECIMEN COLLECTION NOTES*

SCP107-00

***NOTE:** Refer to protocol flow chart for scheduled collection time points.

***NOTE:** Collection of specimens from a vascular access device or heparin / saline locks is not recommended due to the potential for specimen contamination. This specimen should be collected as a peripheral blood draw.

The collection procedures below are for the following collections:

Sample Type (Per Tube)	Approximate Volume per Time Point * (mL)	Shipping Destination
Blood for serum total and direct bilirubin	2	PPD
Blood for serum creatinine	2	PPD
Blood for plasma coproporphyrin I and III	2	PPD
Blood for plasma p-cresol sulfate, indoxyl sulfate, and CMPF	2	TMB/MRL
Blood for plasma GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S	2	Metabolon
Blood for protein binding [^]	3	TMB/MRL

Supplies and Materials (per patient, per time point)

Provided to the Institution for total and direct bilirubin, creatinine, and coproporphyrin I and III (kits from PPD):

- Requisition forms
- Requisition forms for K₂EDTA Plasma
- Shipment notification forms
- Supply re-order forms
- Shipping kit with packing supplies
- K₂EDTA Vacutainer collection tubes

- Transfer pipettes
- 15mL amber polypropylene conical tubes
- 2.0mL amber or regular Eppendorf microcentrifuge tubes
- Preprinted return air bills
- Collection Flow Charts

***NOTE:** Kits and materials provided by the laboratory vendors may differ slightly than details outlined here. Laboratory manuals, instructions, and collection flow charts should be followed.

***NOTE:** For international shipments, Merck approved couriers must be used. The selected courier will provide the air bill and customs documentation at the time your package is picked up.

Institution to procure for (p-cresol sulfate, indoxyl sulfate, and CMPF), (GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S), and protein binding:

- K₂EDTA Vacutainer collection tubes
- Transfer pipettes
- 15mL polypropylene conical tube
- Appropriate number of 2.0mL Eppendorf microcentrifuge tubes
- Other supplies as needed, per site decision

Required Equipment

- Refrigerated centrifuge (4 ° C)

Labeling

- Place patient-specific labels on the 15mL collection tube and the 2mL microcentrifuge tubes
- If applicable, fill out the requisition form/card appropriately (ensure that you follow specific processing instructions per the protocol specific Laboratory Procedure Manual).

Specimen Collection

Total and direct bilirubin, creatinine, (p-cresol sulfate, indoxyl sulfate, and CMPF), (GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S), and protein binding:

For all subjects, blood samples will be collected in 2 mL K₂EDTA Vacutainer tubes blood collection tubes containing no additive at scheduled time points as delineated in the Study Events Flow Chart ([Section 6](#)).

Coproporphyrin I and III:

Patients should sit in a room with low fluorescent lighting for all biomarker and PK blood draws. Lighting should be as low as possible but light enough for the collection to be done safely and effectively.

CPI/CPIII is very light sensitive. Exposure to ambient light should be minimized to the greatest extent possible during sample collection and processing. Precautions will include covering collection and processing tubes with aluminum foil.

- Collect 2mL of whole blood in the pre-labeled K₂EDTA Vacutainer. The pre-labeled K₂EDTA Vacutainer tubes for Coproporphyrin I/III should be covered in aluminum foil. Fill the collection tube completely or until blood flow stops to ensure adequate anticoagulant/blood ratios.

***NOTE:** Allow vacuum to be exhausted prior to removing vacutainer from needle. To prevent backflow of chemical additives from the vacutainer, keep the height of the vacutainer at or below the level of the blood draw site and do not allow the contents of the vacutainer to contact the stopper or end of the needle during procedure.

- After collection, gently invert the K₂EDTA Vacutainer tube 10 times to ensure proper mixing of anti-coagulant additive.

Specimen Processing & Handling

Total and direct bilirubin, creatinine, (p-cresol sulfate, indoxyl sulfate, and CMPF), (GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S), and protein binding:

Following blood collection, each blood sample will be gently inverted 5 times and samples will be allowed to clot at room temperature (for at least 60 minutes).

Within 60-90 minutes of collection, the content of the tubes will then be centrifuged (approximately at 1100-1300 x g for 10 minutes) under refrigeration at a temperature of 4°C to separate the serum.

Aliquot serum in a cryo/transfer tube (or equivalent).

Coproporphyrin I and III:

Processing should occur in a room with low fluorescent lighting for all biomarker and PK samples. Lighting should be as low as possible but light enough for the procedures to be done effectively.

CPI/CPIII is very light sensitive. Exposure to ambient light should be minimized to the greatest extent possible during sample collection and processing. Precautions will include covering collection and processing tubes with aluminum foil.

***NOTE:** The K₂EDTA Vacutainer tubes should be processed immediately, or within 30 minutes of collection. If the specimens cannot be processed immediately, place the tubes on ice until centrifugation.

- Centrifuge the K₂EDTA Vacutainer in a refrigerated at 4°C the K₂EDTA Vacutainer for 10 minutes at 1,300 x g.

***NOTE:** The RCF (Relative centrifugal force) varies according to the centrifuge rotor radius. The formula for computing RCF from rotation speed and centrifuge radius is $RCF = 11.2r (RPM/1000)^2$, where r is rotor radius, in cm, and RPM is the rotations per minute setting of the centrifuge.

- Without disturbing the bottom red blood cell layer, slowly and carefully collect the plasma from the top layer of the tube (approximately 1/2 the original blood volume) using a transfer pipette, and transfer the plasma into a pre-labelled 15mL polypropylene collection tube. Discard the remaining red blood cell layer appropriately.

***NOTE:** Do not try to remove all the possible plasma. Stay about 5 mm away from the red blood layer to avoid contamination of plasma with the red blood cell layer at the bottom of the tube.

- Mix the plasma in the foil covered 15mL collection tube by inversion 5-6 times, and then transfer equal volumes to the **two (2)** pre-labelled 2mL microcentrifuge tubes.

All plasma aliquots **MUST be frozen upright immediately at -80°C or lower** and maintained in the frozen state during shipment. **Please protect from light.**

Specimen Storage (All samples)

- The specimens can either be stored frozen overnight at the site at -80°C or lower and then shipped next day or stored at -80°C or lower to accommodate batch shipment (e.g. weekly or monthly).
- Avoid any freeze-thaw cycles
- Any temperature excursions should be documented and communicated upon specimen shipment within the shipment inventory documents.

Reminder: CPI/CPIII is very light sensitive. Exposure to ambient light should be minimized to the greatest extent possible during sample collection and processing. Precautions will include covering collection and processing tubes with aluminum foil.

Packaging and Shipping (All samples)

1. It is the responsibility of the primary investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens are trained and certified as required by National and International regulations and they ship materials in accordance with all current regulations relating to the handling and shipping of hazardous goods.
2. Contact shipping courier to obtain any required documentation/forms required for shipment.
3. Follow packing and shipping instructions for **DRY ICE** shipments provided by Central Laboratory and/or shipping courier.
4. All shipments should be made in freezer boxes containing sufficient DRY ICE, and labeled as HUMAN SAMPLES: NONINFECTIOUS

***NOTE:** The packaging and shipping instructions provided by the Central Lab and/or shipping courier are not considered nor are they intended to be formal Dangerous Goods training.

5. Include a specimen inventory form with each shipment.
6. Shipments should be sent on a **MONDAY** or **TUESDAY** only to assure receipt by Friday.

7. Ship pre-labelled 2mL microcentrifuge tubes appropriately to all of the following addresses:

Address I (plasma p-cresol sulfate, indoxyl sulfate, and CMPF) and protein binding:

PPD

TMB/ Merck Research Laboratories

KW015C-C302-17

2015 Galloping Hill Road

Kenilworth, NJ 07033

PPD

Address II (plasma coproporphyrin I/III, serum total and direct bilirubin, and serum creatinine):

PPD

Central Labs

Attn:

PPD

2 Tesseneer Drive

Highland Heights, KY 41070

Address III (GDCA-S, GCDCA-S, DCA-S, TCDC-S, and TCDA-S):

PPD

Metabolon

617 Davis Drive, Suite 400

Morrisville, NC 27560

United States

PPD

Appendix 10: Sample Handling Instructions for Urine Collection (Biomarkers)

URINE: SPECIMEN COLLECTION PROCEDURE

SPECIMEN COLLECTION NOTES*

SCP108-00

***NOTE:** Refer to the protocol flow chart for scheduled collection time points.

The collection procedures below are for the following collections:

Urine Biomarker Sample Type	Number of Samples (Aliquots)	Shipping Destination
Urine coproporphyrin I and III	2	PPD
Urine p-cresol sulfate, indoxyl sulfate, and CMPF	1	TMB/MRL

Supplies and Materials (per patient, per time point)

Provided to the Institution:

- 24 hour Urine amber collection jugs (should be also used for PK urine collection if possible)
- 120mL amber Urine collection cups
- 15mL amber polypropylene conical tubes
- 2.0mL amber Eppendorf microcentrifuge tubes (for urine coproporphyrin I/III)
- Boxes for storage of the 2.0 mL microcentrifuge tubes

***NOTE:** Kits and materials provided by the laboratory vendors may differ slightly than details outlined here. Laboratory manuals, instructions, and collection flow charts should be followed.

Required Equipment (provided by the clinical site)

- 2.0mL Eppendorf microcentrifuge tubes (for urine p-cresol sulfate, indoxyl sulfate, and CMPF)
- -70°C or lower freezer for sample storage
- Restroom with a toilet for the donor to have privacy while providing the urine specimen (single toilet restroom preferred)
- Source of water for washing hands

Labeling

1. Complete any appropriate information on the patient-specific Central Laboratory or Merck labels and place one on each of the polypropylene centrifuge tubes, and the 2.0mL microcentrifuge tubes.
2. Fill out the Central Laboratory requisition form/card or Merck Inventory Form appropriately.

Specimen Collection

Patients should sit in a room with low fluorescent lighting for all biomarker and PK urine collections. Lighting should be as low as possible but light enough for the collection to be done safely and effectively.

CPI/CPIII is very light sensitive. Exposure to ambient light should be minimized to the greatest extent possible during sample collection and processing. Precautions will include covering collection and processing tubes with aluminum foil.

The labeled amber urine collection cup will have been provided to patient with verbal instructions on collecting (also see [Appendix 6](#)).

NOTE: The amber collection tube will be used for collection of all urine and further processed and aliquoted for PK and biomarkers.

Specimen Processing & Handling

Processing should occur in a room with low fluorescent lighting for all biomarker and PK processing. Lighting should be as low as possible but light enough for the procedures to be done effectively.

CPI/CPIII is very light sensitive. Exposure to ambient light should be minimized to the greatest extent possible during sample collection and processing. Precautions will include covering collection and processing tubes with aluminum foil if they are not provided as amber.

***NOTE:** Begin processing immediately after collection, or **within maximum of 30 minutes of collection. Keep samples on ice at all times until frozen.** Freeze samples at -70°C or lower immediately after processing.

1. From the urine collected at time points in the flow chart, before PK processing with buffer, transfer 1.0mL of urine to each the pre-labelled 2.0mL microcentrifuge tubes (two amber for PPD and one non-amber for Merck).
2. Transfer all urine samples in upright position to the provided boxes for -80°C or lower freezer storage until shipment on dry ice.
3. Discard any remaining urine per site guidelines.

Specimen Storage

1. Store the specimens in at -80°C or lower until shipment to Merck / Central Laboratory on DRY ICE.
2. Any temperature excursions should be documented and communicated upon specimen shipment within the shipment inventory documents.

***NOTE:** Frozen tubes are subject to breakage on impact. To reduce the risk of breakage during handling and shipment, please package appropriately (i.e. place storage tubes in appropriately sized sample box).

Packaging and Shipping

1. It is the responsibility of the primary investigator to ensure that all staff personnel who will be handling, packaging, and/or shipping clinical specimens are trained and certified as required by National and International regulations and they ship materials in accordance with all current regulations relating to the handling and shipping of hazardous goods.
2. Contact shipping courier to obtain any required documentation/forms required for shipment.
3. Follow packing and shipping instructions for **DRY ICE** shipments provided by Central Laboratory and/or shipping courier.
4. All shipments should be made in freezer boxes containing sufficient DRY ICE, and labeled as HUMAN SAMPLES: NONINFECTIOUS

***NOTE:** The packaging and shipping instructions provided by the Central Lab and/or shipping courier are not considered nor are they intended to be formal Dangerous Goods training.

5. Include a specimen inventory form with each shipment.

6. Shipments should be sent on a **MONDAY** or **TUESDAY** only to assure receipt by Friday.
7. Ship pre-labelled 2.0mL microcentrifuge tubes (one non-amber for TMB/ Merck and two amber for PPD) to both of the following addresses:

Address I (urine p-cresol sulfate, indoxyl sulfate, and CMPF):

PPD

TMB/ Merck Research Laboratories

KW015C-C302-17

2015 Galloping Hill Road

Kenilworth, NJ 07033

PPD

Address II (urine coproporphyrin I/III):

PPD

Central Labs

Attn: PPD

2 Tesseneer Drive

Highland Heights, KY 41070