1 Title Page

NCT03818672

Clinical Study Protocol
Protocol RFPK4045
A PHASE 4, OPEN LABEL STUDY TO EVALUATE THE STEADY-STATE PHARMACOKINETICS OF RIFAXIMIN 550 MG TABLETS IN HEALTHY SUBJECTS AND SUBJECTS WITH SEVERE HEPATIC IMPAIRMENT

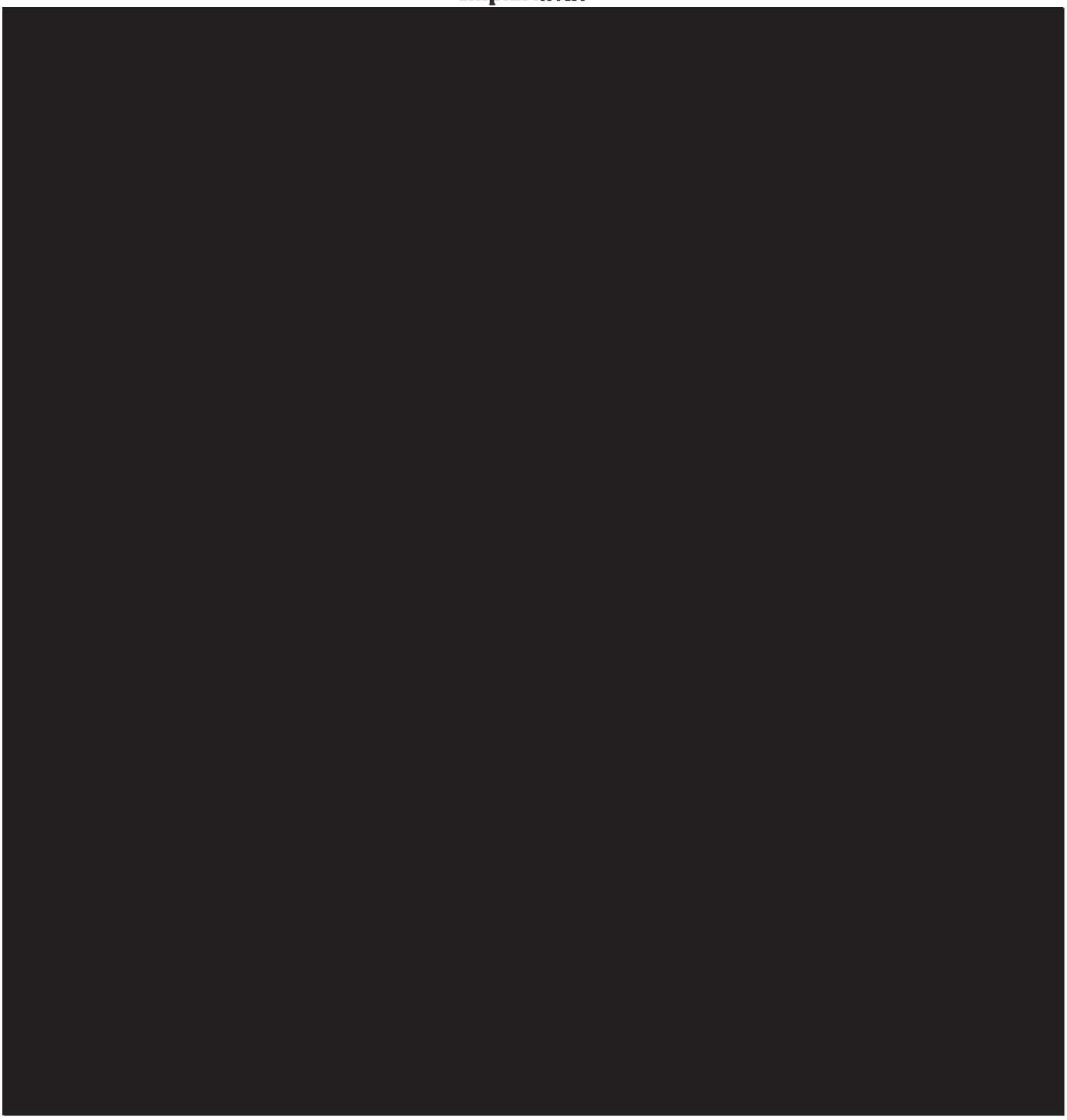
Developmental phase of study:	Phase IV
Study design	Open Label, Repeat Dose, Parallel Design
Date:	21 Nov 2017 08 Oct 2018 (Amendment 1)
Sponsor representative	[redacted]
Sponsor	Salix Pharmaceuticals, Inc., a division of Valeant Pharmaceuticals North America LLC (VPNA) 400 Somerset Corporate Boulevard Bridgewater, NJ 08807 [redacted]

Statement of Confidentiality

Protocol Review and Approvals

Protocol RFPK4045

A Phase 4, Open-Label Study to Evaluate the Steady-State Pharmacokinetics of Rifaximin 550mg Tablets in Healthy Subjects and Subjects with Severe Hepatic Impairment



Personnel Responsible for Conducting the Study
Protocol RFPK4045
A Phase 4, Open-Label Study to Evaluate the Steady-State Pharmacokinetics of Rifaximin 550mg Tablets in Healthy Subjects and Subjects with Severe Hepatic Impairment

[redacted]

Valeant Pharmaceuticals North
America, LLC
400 Somerset Corporate Boulevard
Bridgewater, NJ 08807

Contract Research Organization

[redacted]

MedTrials, Inc.
2626 Cole Avenue, Suite 400
Dallas, TX 75204

Principal Investigator Protocol Agreement Page

I agree:

- To assume responsibility for the proper conduct of this clinical study at this site and to conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by the sponsor.
- That I am aware of, and will comply with, the internationally recognized code of Good Clinical Practices (GCP) and all other applicable regulatory requirements to obtain written and dated approval from the Institutional or Central Review Board (IRB) or Independent Ethics Committee (IEC) for the study protocol, written informed consent, consent-form updates, subject-recruitment procedures (eg, advertisements), and any other written information to be provided to the subjects, before initiating this clinical study.
- Not to implement any changes to, or deviations from the protocol without prior agreement from the sponsor and review and documented approval from the IRB/IEC, except to eliminate an immediate hazard to the study subjects, or when change(s) involves only logistical or administrative aspects of the clinical study.
- To permit direct monitoring and auditing by the sponsor or sponsor's representatives and inspection by the appropriate regulatory authority(ies).
- That I am thoroughly familiar with the appropriate use of the investigational products(s), as described in this protocol, and any other information provided by the sponsor or designee, including, but not limited to, the current investigator Brochure or equivalent document and approved product label (if applicable).
- To provide sufficient time, and adequate numbers of qualified staff and facilities for the foreseen duration of the clinical study to conduct the study properly, ethically, and safely.
- To ensure that all persons assisting in this study are adequately informed about the protocol, investigational product(s), and their clinical study-related duties and functions.

Principal Investigator (print name): _____

Affiliation:_____

_____ _____

Principal Investigator (signature) Date

2 Synopsis

Name of Sponsor/Company: Salix Pharmaceuticals, Inc., a division of VPNA, LLC
Name of Investigational Product: Rifaximin, 550 mg tablets
Name of Active Ingredient: Rifaximin
Title of Study: A Phase 4, Open-Label Study to Evaluate the Steady-State Pharmacokinetics of Rifaximin 550mg Tablets in Healthy Subjects and Subjects with Severe Hepatic Impairment
Number of clinical centers: 1 to 3 clinical centers in the US
Objectives: **Primary**: To characterize the steady-state plasma pharmacokinetics (PK) of rifaximin (550 mg BID) in subjects with severe hepatic impairment (Model for End Stage Liver Disease [MELD] 19 to 25 and MELD >25), as well as healthy subjects with normal hepatic function. **Exploratory:** To assess the effect of rifaximin administration on the intestinal microbiome in subjects with severe hepatic impairment (MELD 19 to 25 and MELD >25).
Methodology: This is a Phase 4, open-label, repeat-dose, parallel-design study in 12 subjects with severe hepatic impairment (MELD 19 to 25 and MELD >25) and 6 healthy subjects with normal hepatic function. At least 6 of the hepatically impaired subjects will have MELD score >25. Efforts will be made to match subjects across groups in terms of age, weight, and sex. Eligible subjects will complete a Screening Period of up to 21 days prior to dosing (Day 1), a 7-day Treatment Period, and Follow-Up by phone 3 (+1) days later. Subjects will be admitted to the Clinical Research Unit (CRU) on the day prior to initiation of dosing (Day -1; CRU admission). A plasma sample for PK analysis will be collected on Day 1 prior to the first dose. Subjects will receive 550 mg rifaximin BID approximately 12 hours apart for 6 days beginning on Day 1 (Baseline). On Day 7 (End of Treatment; EOT), the final dose of study drug will be administered in the morning following an overnight fast of 10 hours. Plasma samples for PK analysis will be collected pre-dose (within 1 hour of the dose) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours post-dose. Additionally, a trough blood sample will be collected on Days 2-6, prior to administration of the first daily dose of rifaximin. Subjects will reside in the CRU for 9 days and will be discharged following the final PK sample collection if safety parameters are acceptable to the Investigator. Each subject will be contacted for a follow-up phone call on 3 (+1) Days following end of treatment.
Number of patients planned: N = 18, 12 subjects with liver impairment and 6 matched healthy volunteer subjects. At least 6 of the hepatic impaired subjects will have a MELD score of >25.

Diagnosis and main criteria for inclusion:

Hepatically impaired subjects will be ≥18 years of age, have a diagnosis of liver cirrhosis and a MELD score of ≥19 at Screening. Note: At least 6 of the hepatically impaired subjects will have a MELD score of >25.

Key exclusion criteria:

Subject has known allergy to rifaximin, rifampin, or other rifamycins, excipients and/or vehicles used in the formulation, or any other clinically significant allergies.

Subject has participated in an investigational drug or device study within 30 days prior to Day 1 (Baseline).

Subject has any concurrent illness (other than liver cirrhosis), disability or circumstance that may affect the interpretation of clinical data, could cause noncompliance with treatment or visits or otherwise contraindicates participation in this study in the opinion of the investigator.

Investigational product, dosage and mode of administration: Rifaximin 550 mg tablets taken orally two times a day (BID).

Duration of treatment: The duration of treatment is for 7 days.

Criteria for evaluation:

Efficacy:

- No efficacy analyses are planned.

Safety:

- Incidence of treatment-emergent adverse events (AEs) and serious adverse events (SAEs).
- Changes from baseline in clinical laboratory parameters.
- Changes from baseline in vital signs.

Pharmacokinetics:

- Pharmacokinetic parameters to be estimated for rifaximin and 25-desacetyl rifaximin, if measurable, will include: maximum observed plasma concentration (C_{max}), time of the maximum concentration (T_{max}), and area under the plasma concentration versus time curve (AUC) during the 12-hour dose interval tau (τ), $AUC_{0-24,}$ $AUC_{last.}$

Exploratory:

- Gastrointestinal microbiota from stool samples and antibiotic resistance from bacteria cultured from stool samples will be analyzed before and after treatment with Rifaximin 550 mg BID for one week**.**

Statistical Methods: The Intent-to-Treat (ITT) population will be used for safety analyses. The ITT population will include all subjects who ingested at least one dose of study drug. Safety evaluations will be based on the incidence of AEs, changes in vital signs, and clinical laboratory results. Exposure to study medication and reasons for discontinuation of study treatment will be tabulated. The PK population will

include subjects from whom sufficient data are obtained for calculation of rifaximin plasma PK parameters.

The effect of severe hepatic impairment will be compared to the matched control group of healthy subjects. An analysis of variance will be performed, and data for Cmax, $AUC_{0-\tau}$, and CL/F will be natural log transformed prior to analysis. The 90% confidence intervals (CIs) of the hepatic impairment group mean relative to the matched control group mean will be obtained. The effect of hepatic impairment will be assessed by examining the 90% CIs for the mean ratio of the hepatic impairment group relative to the matched healthy group.

A continuous analysis using linear regression will be performed to evaluate the relationship between estimated creatinine clearance and rifaximin CL/F.

Gastrointestinal microbiota data will be summarized descriptively by total counts (per gram of stool sample); by species of aerobic and anaerobic bacteria; and by bacterial resistance to antibiotics (including rifaximin and rifampin).

Sample size calculations:

A formal sample size estimate was not calculated. The number of subjects planned for this study is considered sufficient to compare the pharmacokinetics of rifaximin in patients with severe hepatic impairment to healthy subjects.

3 Table of Contents

3.1 List of Figures

3.2 List of Abbreviations and Definitions of Terms

Abbreviation or specialist term	Definition or Explanation
AE	adverse event
AUC	area under the plasma concentration-time curve
BID	two times a day
CI	confidence interval
CLD	Chronic Liver Disease
CRF	Case Report Form
CRU	Clinical Research Unit
DNA	Deoxyribonucleic Acid
ECG	electrocardiogram
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practices
Hb	hemoglobin
Hct	hematocrit
HE	Hepatic encephalopathy
HIPAA	Health Insurance Portability and Accountability Act (of 1996)
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	intent-to-treat
IUD	intrauterine device

Abbreviation or specialist term	Definition or Explanation
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-stage Liver Disease
MIC	Minimum inhibitory concentration
PK	pharmacokinetics
PPACA	Physician Payment Sunshine Provision of the Patient Protection and Affordable Care Act
PP	per protocol
RNA	Ribonucleic Acid
SAE	serious adverse event
SAP	statistical analysis plan
SBP	Spontaneous bacterial peritonitis
SD	standard deviation
SOP	standard operating procedures
US	United States
USPI	US Prescribing Information
WHO	World Health Organization

4 Introduction

4.1 Disease Background

The magnitude of the health care burden caused by liver disease in the United States (U.S.) is significant from both a societal and health economic perspective. Approximately 2.6 million people have liver disease in the U.S. Cirrhosis is a major cause of much of chronic liver disease (CLD) in the U.S., and is the 12th leading cause of death (Pleis, 2007). Hepatic encephalopathy (HE) is a complication of CLD that strongly impacts survival (Stewart, 2009; Bustamante, 1999), results in frequent hospitalizations, (Leevy, 2007; Poordad, 2007; Bajaj, 2010) increases dependence on care providers (Miyazaki, 2010) and greatly contributes to the clinical and socio-economic burden of CLD. Rifaximin, a minimally absorbed, gut-selective antibiotic, has been shown to significantly reduce the risk of overt HE recurrence and HE-related hospitalizations, in a double blind, placebo-controlled, 6-month study enrolling patients with a recent history of recurrent, overt HE, and a Model for End Stage Liver Disease (MELD) score of ≤25 (Bass, 2010), with a safety profile similar to placebo. One of the limitations of this study was that only few patients (<9%) with more severe liver disease (MELD ≥19) were enrolled and adequate pharmacokinetic (PK) and safety data for treatment with rifaximin in this more severe population are not currently available. Therefore, the current study will be conducted to provide rifaximin PK data for patients with MELD scores ≥19.

FDA requires that the sponsor completes post-marketing requirement (PMR) 1615-3 entitled, "A pharmacokinetic trial in patients with severe hepatic impairment (MELD 19 – 25 and MELD > 25). The present trial protocol is designed to meet the PMR objective and is subject to FDA review and approval, under IND 059133.

4.2 Rifaximin Background

Details regarding the safety and efficacy of rifaximin are found in the US prescribing information (XIFAXAN® US Package Insert 2016). Rifaximin is a nonaminoglycoside, semisynthetic antibiotic of the rifamycin class. It is a non-systemic, poorly-absorbed, broad-spectrum, oral antibiotic specific for enteric pathogens of the GI tract (Salix, 2014; Gerard, 2005; Gillis, 1995; Hoover, 1993). Rifaximin has important advantages in treatment of HE relative to previously used antibiotics. For example, rifaximin has minimal systemic exposure regardless of food intake or presence of GI disease. The lack of systemic exposure makes rifaximin safe and well tolerated. Rifaximin's antibacterial activity is due to its binding to the beta-subunit of the bacterial deoxyribonucleic acid (DNA)-dependent

ribonucleic acid (RNA) polymerase, resulting in inhibition of bacterial RNA synthesis. It is active against numerous Gram-positive and Gram-negative bacteria, both aerobic and anaerobic. In vitro data indicate rifaximin is active against species of Staphylococcus, Streptococcus, Enterococcus, and members of the Enterobacteriaceae family. Bacterial reduction or an increase in antimicrobial resistance in the colonic flora does not occur to a clinically significant extent.

4.2.1 Clinical Experience with Rifaximin

Rifaximin has been investigated in numerous clinical trials of subjects with HE at daily doses ranging from 400 to 1200 mg at a treatment duration of 3 to 30 days, or at 1200 mg/day for 14 days/month for 3 to 6 months. Studies conducted have demonstrated short- and long-term efficacy following treatment regimens of ≤21 days (i.e., acute treatment) or longer treatment durations of 3 months and 6 months. Results of these studies demonstrated efficacy and a favorable safety profile for rifaximin in patients with HE. A randomized double-blind placebo controlled multi-center trial of 299 subjects with cirrhosis (Study RFHE3001) (Bass, 2010) was conducted to support registration. This 6-month study enrolled subjects in remission from recurrent HE, (history of ≥2 HE episodes with severity of Conn 2 or greater) and demonstrated that rifaximin significantly reduced the risk of an episode of HE as compared with placebo, (hazard ratio with rifaximin, 0.42; 95% confidence interval [CI], 0.28 to 0.64; P <0.001). Furthermore, significantly fewer subjects in the rifaximin group had a hospitalization involving HE, as compared with placebo group; hazard ratio of 0.50 (95% CI, 0.29 to 0.87; P = 0.01). Rifaximin maintained remission from hepatic encephalopathy more effectively than placebo. Rifaximin 550 mg two times a day (BID) was approved by the US Food and Drug Administration (FDA) in March 2010 for the reduction in risk of recurrent overt HE.

At the time of the NDA submission, 348 patients with HE were exposed to rifaximin, including 265 exposed for 6 months and 202 exposed for more than a year (mean exposure was 364 days). The safety of rifaximin 550 mg taken two times a day for reducing the risk of overt HE recurrence in adult patients was evaluated in a 6-month placebo-controlled clinical trial (n = 140) and in a long-term follow-up study (n = 280). The population studied had a mean age of 56 (range: 21 to 82) years; approximately 20% of the patients were ≥65 years old, 61% were male, 86% were White, and 4% were Black. Ninety-one percent of patients in the trial were taking lactulose concomitantly. The most common adverse reactions that occurred at an incidence ≥5% and at a higher incidence in XIFAXAN-treated subjects than in the placebo group in the 6-month trial were peripheral edema, nausea, dizziness, fatigue,

ascites, muscle spasms, pruritus, abdominal pain, anemia, depression, nasopharyngitis, abdominal pain upper, arthralgia, dyspnea, pyrexia, and rash.

In a study to characterize the pharmacokinetics of rifaximin in healthy subjects, administration of a single 550 mg oral dose to fasted and fed subjects resulted in mean AUC values of 11.1 ng.h/mL and 22.5 ng.h/mL, respectively. Multiple-dose twice daily (BID) or 3 times daily (TID) regimens in healthy subjects resulted in mean AUC values of 12.3 ng.h/mL (AUCtau, steady-state), and 9.3 ng.h/mL (AUCtau, steady-state), respectively (RFPK1007). Subjects with non-C IBS had mean AUCtau values following a single dose and multiple TID doses of 9.69 ng.h/mL and 16.0 ng.h/mL, respectively, reflecting an accumulation ratio (Rc; multiple-dose AUCtau/single-dose AUCtau) of 1.77 (RFPK1010). In subjects with hepatic impairment, systemic exposure is higher than that observed in healthy subjects. Following repeat dosing of a 550 mg BID regimen in hepatic impaired subjects in RFHE3002PK, mean steady-state AUCtau values of 118 ng.h/mL, 161 ng.h/mL, and 246 ng.h/mL were observed in Child-Pugh A, Child Pugh B, and Child-Pugh C subjects, respectively. In a study to determine the effect of P-glycoprotein inhibition on the pharmacokinetics of rifaximin, an in vitro substrate of P-glycoprotein, healthy subjects received a single oral dose of rifaximin 550 mg with or without a single oral 600 mg dose of cyclosporine, a potent P-glycoprotein inhibitor (RFDI1045). Systemic rifaximin exposure (as measured by plasma Cmax and AUC0-∞) was increased following co-administration of rifaximin with cyclosporine in this study; mean Cmax was 40.0 ng/mL and mean AUC0-∞ was 314 ng.hr/mL following single doses of rifaximin plus cyclosporine compared with mean Cmax of 0.48 ng/mL and mean AUC0-∞ of 2.53 ng.hr/mL following single-dose rifaximin alone. While the effect of the combination of hepatic impairment and P-glycoprotein on rifaximin pharmacokinetics has not been examined, a nonclinical toxicology study in dogs (BQG0004) provides greater than 30-fold and greater than 24-fold safety exposure margins over the mean exposures for the P-glycoprotein and hepatic impairment effects, respectively.

4.3 Rationale for the Study

A pharmacokinetic investigation of hepatically impaired subjects (RFHE3002PK) was conducted as a sub-study to Phase 3 open-label study RFHE3002 using a 550 mg BID rifaximin dosing regimen. Systemic exposure to rifaximin was significantly higher in patients with hepatic impairment compared to healthy subjects. Patients with hepatic impairment had mean apparent oral clearance reduced by 88% and the half-life was increased by 2-fold compared to healthy subjects. The mean C_{max} and AUC_{tau} were 6-fold and 11-fold higher, respectively, than in healthy subjects. When the PK parameters were analyzed by

liver function, the mean C_{max} and AUC_{tau} in patients in moderate (Child-Pugh B) hepatic impairment were 28% and 36% higher than in patients with mild (Child-Pugh A) hepatic impairment. The mean C_{max} and AUC_{tau} in patients increased as Model for End Stage Liver Diseases (MELD) score increased as well. Mean steady-state AUC_{tau} values of 118 ng.h/mL, 161 ng.h/mL, and 246 ng.h/mL were observed in Child-Pugh A, Child Pugh B, and Child-Pugh C subjects, respectively.

The clinical trials submitted as part of the approved new drug application (NDA) for rifaximin did not include patients with the most severe hepatic impairment (Model for End-Stage Liver Disease [MELD] score >25) and only limited numbers of patients with Child-Pugh C subjects. Therefore, as part of Phase 4 post marketing requirements requested by FDA, this study is being conducted to characterize the pharmacokinetics of rifaximin in patients with severe hepatic impairment.

5 Study Objectives and Purpose

5.1 Primary Objective

The primary objective of this study is to characterize the steady state plasma PK of rifaximin (550 mg BID) in subjects with severe hepatic impairment (MELD 19 to 25 and MELD >25), as well as healthy subjects with normal hepatic function.

5.2 Exploratory Objectives

The exploratory objective of this study is to assess the effect of rifaximin administration on the intestinal microbiome in subjects with severe hepatic impairment (MELD 19 to 25 and MELD >25).

5.3 Pharmacokinetic Endpoints

Non-compartmental analysis of the plasma PK data for rifaximin and 25-desacetyl rifaximin, if measurable, will be performed using Phoenix WinNonlin version 6.3 or higher (Pharsight Corporation).

Pharmacokinetic parameters to be estimated for rifaximin and 25-desacetyl rifaximin, if measurable, will include:

- Maximum observed plasma concentration (C_{max}).
- Time of the maximum concentration (T_{max}).

- Area under the plasma concentration versus time curve (AUC) during the 12-hour dose interval tau (τ).
- $AUC_{0-24.}$
- $AUC_{last.}$

Other parameters, including apparent oral clearance (CL/F), volume of distribution (V/F), and half-life (t½) will be estimated as permitted by the data.

5.4 Safety Endpoints

The safety endpoints will be the following:

- Incidence of treatment-emergent adverse events (AEs) and serious adverse events (SAEs).
- Changes from baseline in clinical laboratory parameters.
- Changes from baseline in vital signs.

5.5 Exploratory Endpoints

Gastrointestinal microbiota from stool samples and antibiotic resistance from bacteria cultured from stool samples will be analyzed before and after treatment with Rifaximin 550 mg BID for one week.

6 Investigational Plan

6.1 Overview of Study Design

This is a Phase 4, open-label, repeat-dose, parallel-design study in 12 subjects with severe hepatic impairment (MELD 19 to 25 and MELD >25) and 6 healthy subjects with normal hepatic function. At least 6 hepatically impaired subjects will have MELD score >25. Efforts will be made to match subjects across groups in terms of age, weight, and sex. Eligible subjects will complete a Screening Period of up to 21 days prior to dosing (Day 1), a 7-day Treatment Period, and Follow-Up by phone 3 (+1) days later. Subjects will be admitted to the Clinical Research Unit (CRU) on the day prior to the initiation of dosing (Day -1; CRU admission). A plasma sample for PK analysis will be collected on Day 1 prior the first dose. Subjects will receive 550 mg rifaximin BID approximately 12 hour apart for 6 days beginning on Day 1 (Baseline). On Day 7 (End of Treatment; EOT), the final dose of study drug will be administered in the morning following an overnight fast of 10 hours and plasma samples for PK analysis will be collected pre-dose (within 1 hour of the dose) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours post-dose. Additionally, a trough blood sample will be collected on Days 2-6, prior to administration of the first daily

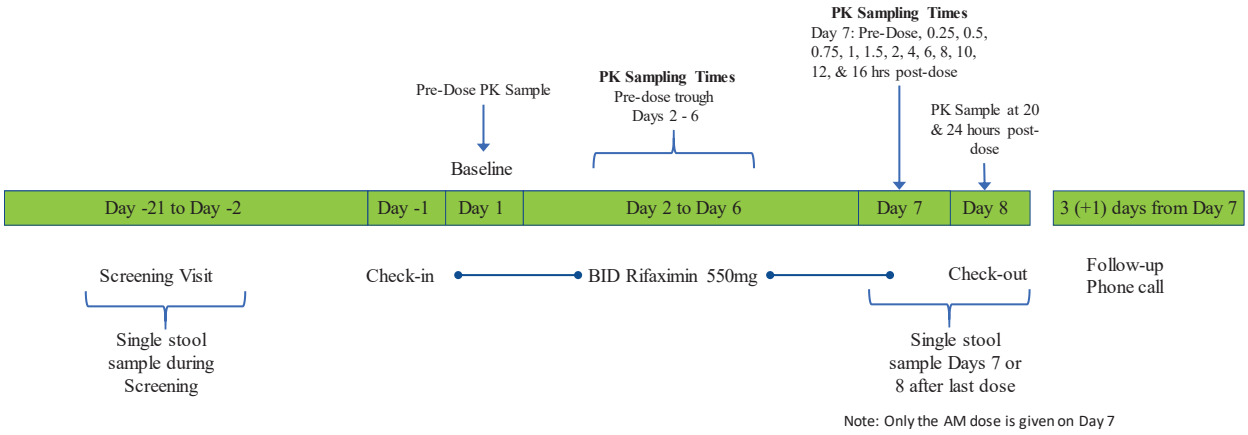
dose of rifaximin. Subjects will reside in the CRU for 9 days and will be discharged following the final PK sample collection if safety parameters are acceptable to the Investigator. Each subject will be contacted for a follow-up phone call 3 + 1 days following end of treatment. The study schematic is presented in Figure 1.

Each dose will be administered with a total of 240 mL of water. During the first 4 hours after dose administration, subjects should not lie flat; they should be allowed to sit or stand. Subjects will receive standardized meals while in the CRU.

Subjects will discontinue non-study rifaximin on Day -1 and will only use rifaximin provided as study drug by the sponsor during the Treatment Period. Subjects may resume their prescription rifaximin use following CRU discharge.

Stool samples for microbiome analysis will be collected in the Screening Period and on Day 7 or 8.

Figure 1: Study Design Schema



7 Selection and Withdrawal of Subjects

7.1 Inclusion/Exclusion for Subjects with Severe Hepatic Impairment

7.1.1 Hepatic Impairment Subject Inclusion Criteria

A subject will be eligible for inclusion in this study if all of the following criteria are met:

1. Subject provides written informed consent as documented on the Institutional Review Board (IRB) approved informed consent document.

2. Subject is ≥18 years of age at Screening.

3. Male or non-pregnant, non-breast feeding female.

4. Females of childbearing (reproductive) potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test at Screening and Day 1, and agree to use an acceptable method of contraception throughout their participation in the study. Examples of acceptable methods of contraception include double barrier methods (condom with spermicide jelly or diaphragm with spermicide), hormonal methods (oral contraceptives, patches or medroxyprogesterone acetate), or an intrauterine device with a documented failure rate of less than 1% per year. Abstinence may be considered an acceptable method of contraception at the discretion of the investigator.

   NOTE: Females who have been surgically sterilized (e.g., hysterectomy or bilateral tubal ligation) or who are postmenopausal (total cessation of menses for >1 year) will not be considered "females of childbearing potential."

5. Subject has a diagnosis of liver cirrhosis.

6. Subject has a MELD score of ≥19 at Screening. Note: At least 6 subjects will have a MELD score of >25.

7. Subject is capable of understanding the requirements of the study, is willing to comply with all the study procedures, and is willing to attend all study visits.

7.1.2 Hepatic Impairment Subject Exclusion Criteria

A subject will **not** be eligible for inclusion in this study if any of the following criteria are met:

1. Subject has known allergy to rifaximin, rifampin, or other rifamycins, or to excipients and/or vehicles used in the formulation or other clinically significant allergies.

2. Subject has participated in an investigational drug or device study within the 30 days prior to Day 1 (Baseline).

3. Subject is an employee of the site that is directly involved in the management, administration, or support of this study or is an immediate family member of the same.

4. Subject has any concurrent illness (other than liver cirrhosis), disability or circumstance that may affect the interpretation of clinical data, could cause noncompliance with treatment or visits or otherwise contraindicates participation in this study in the opinion of the investigator.

5. Subject is pregnant or at risk of pregnancy, or is lactating.

6. Subject has a history of tuberculosis infection or has received treatment for a tuberculosis infection. To rule out tuberculosis infection, any one of the following criteria must apply: a) negative tuberculin skin test or negative tuberculosis blood test or b) if a subject has had a previous positive test, they must have a current negative chest X-ray (within 6 months of enrollment), or a current negative blood tuberculosis test.

7. Subject is positive for human immunodeficiency virus (HIV), hepatitis B and/or hepatitis C on Screening assessments.

8. Subject has been diagnosed with chronic respiratory insufficiency that in the opinion of the investigator would prevent completion of the study, interfere with analysis of study results, or negatively impact the subject's participation in the study.

9. Subject has renal insufficiency requiring routine dialysis.

10. Subject has an active spontaneous bacterial peritonitis infection or subject requires daily prophylactic antibiotic therapy.

11. Subject shows presence of intestinal obstruction or has inflammatory bowel disease.

12. Subject has an active malignancy within the last 5 years (exceptions basal cell carcinomas of the skin, or if female, in situ cervical carcinoma that has been surgically excised).

13. Subject has current GI bleeding or has had a GI hemorrhage of sufficient severity to require hospitalization and a transfusion of ≥ 2 units of blood within 3 months prior to Screening.

14. Subject is anemic, as defined by hemoglobin of ≤8 g/dL.

15. Subject has significant hypovolemia, or any electrolyte abnormality that can affect mental function (e.g., serum sodium <125 mmol/L, serum calcium >10 mg/dL).

16. Subject has severe hypokalemia as defined by a serum potassium level <2.5 mmol/L.

17. Subject's current, required medications are prohibited concurrent medications per protocol. Subjects will discontinue non-study rifaximin on Day -1 and will only use rifaximin provided as study drug by the sponsor during the Treatment Period through CRU discharge.

18. Subject is positive for *Clostridium difficile* toxin via stool examination at Screening. NOTE: Results of stool tests must be confirmed as negative for Toxins A and B prior

to Day -1. A stool sample must be collected during the Screening Period. Testing will be performed by enzyme immunoassay (EIA).

19. Subject has an acute illness within 1 week of CRU admission.

20. Subject has donated plasma within 7 days of drug administration.

21. Subject has donated 1 or more pints of blood (or equivalent blood loss) within 30 days prior to drug administration.

22. The subject has known or suspected illicit drug use within the past year, or positive findings on urine drug screen (except for allowed prescription medications), or has used any tobacco or nicotine products during the 6 months prior to screening and does not agree to refrain from using tobacco or nicotine products during the study.

23. The subject has received any type of vaccination within 7 days of drug administration.

24. Subject reports consumption of more than 2 alcoholic drinks daily, more than 14 alcoholic drinks within 14 days before the screening evaluation (an alcoholic drink is defined as 1.5 oz liquor, one 12 oz can of beer, or 5 oz of wine), or consumption of any alcohol within 24 hours of first drug administration.

25. The subject has consumed grapefruit juice, grapefruits, or Seville oranges within 24 hours of first drug administration and does not agree to refrain from consuming these foods during the study.

7.2 Inclusion/Exclusion for Healthy Subjects

7.2.1 Healthy Subjects Inclusion Criteria

A healthy volunteer subject will be eligible for inclusion in this study if all of the following criteria are met:

1. Subject provides written informed consent as documented on the Institutional Review Board (IRB) approved informed consent document.

2. Subject is ≥18 years of age at Screening.

3. Male or non-pregnant, non-breast feeding female.

4. Females of childbearing (reproductive) potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test at Screening and Day 1, and agree to use an acceptable method of contraception throughout their participation in the study. Examples of acceptable methods of contraception include double barrier

methods (condom with spermicide jelly or diaphragm with spermicide), hormonal methods (oral contraceptives, patches or medroxyprogesterone acetate), or an intrauterine device with a documented failure rate of less than 1% per year. Abstinence may be considered an acceptable method of contraception at the discretion of the investigator.

NOTE: Females who have been surgically sterilized (e.g., hysterectomy or bilateral tubal ligation) or who are postmenopausal (total cessation of menses for >1 year) will not be considered "females of childbearing potential."

5. Subject has normal (or abnormal and clinically insignificant) laboratory values at screening.

6. Subject is medically normal with no significant abnormal findings at the Baseline physical examination.

7. Subject is capable of understanding the requirements of the study, is willing to comply with all the study procedures, and is willing to attend all study visits.

8. Subject has not consumed and agrees to abstain from taking any dietary supplements or non-prescription drugs (except as authorized by the Investigator and Medical Monitor) for 3 days prior to CRU admission through Follow-Up.

9. Subject has not consumed and agrees to abstain from taking any prescription drugs (except as authorized by the Investigator and Medical Monitor) during the 14 days prior to CRU admission through Follow-Up.

7.2.2 Healthy Subjects Exclusion Criteria

A healthy volunteer will **not** be eligible for inclusion in this study if any of the following criteria are met:

1. Subject has known allergy to rifaximin, rifampin, or other rifamycins, or to excipients and/or vehicles used in the formulation or other clinically significant allergies.

2. Subject has participated in an investigational drug or device study within the 30 days prior to Baseline (Day 1).

3. Subject is an employee of the site that is directly involved in the management, administration, or support of this study or is an immediate family member of the same.

4. Subject has any concurrent illness, disability or circumstance that may affect the interpretation of clinical data, could cause noncompliance with treatment or visits or otherwise contraindicates participation in this study in the opinion of the investigator.

5. Subject is pregnant or at risk of pregnancy, or is lactating.

6. Subject has a history of tuberculosis infection or has received treatment for a tuberculosis infection. To rule out tuberculosis infection, any one of the following criteria must apply: a) negative tuberculin skin test or negative tuberculosis blood test or b) if a subject has had a previous positive test, they must have a current negative chest X-ray (within 6 months of enrollment), or a current negative blood tuberculosis test.

7. Subject is positive for human immunodeficiency virus (HIV), hepatitis B and/or hepatitis C on Screening assessments.

8. Subject has an acute illness within 1 week of CRU admission.

9. Subject has donated plasma within 7 days of drug administration.

10. Subject has donated 1 or more pints of blood (or equivalent blood loss) within 30 days prior to drug administration.

11. Subject is positive for *Clostridium difficile* toxin via stool examination at Screening. NOTE: Results of stool tests must be confirmed as negative for Toxins A and B prior to Day -1. A stool sample must be collected during the Screening Period. Testing will be performed by enzyme immunoassay (EIA).

12. Subject has any of the following laboratory abnormalities:

    a. Serum creatinine ≥ 1.5 × the upper limit of normal;

    b. Alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, or total bilirubin ≥ 2 × the upper limit of normal;

    c. Hemoglobin < 10.0 g/dL;

    d. Absolute neutrophil count, or lymphocyte count ≤ 500 cells/μL.

13. Subject has known or suspected illicit drug use within the past year, or positive findings on urine drug screen, or has used any tobacco or nicotine products during the 6 months prior to screening and does not agree to refrain from using tobacco or nicotine products during the study.

14. Subject has received any type of vaccination within 7 days of drug administration.

15. Subject reports consumption of more than 2 alcoholic drinks daily, more than 14 alcoholic drinks within 14 days before the screening evaluation (an alcoholic drink is

defined as 1.5 oz liquor, one 12 oz can of beer, or 5 oz of wine), or consumption of any alcohol within 24 hours of first drug administration.

16. Subject has consumed grapefruit juice, grapefruits, or Seville oranges within 24 hours of first drug administration and does not agree to refrain from consuming these foods during the study.

17. Subject has consumed foods and substances that are known to inhibit and/or induce cytochrome P450 (CYP) or P-glycoprotein within 24 hours of first drug administration and does not agree to refrain from consuming these foods during the study, and/or has received treatment with any drugs known to be P-glycoprotein or CYP enzyme inducers or inhibitors within 30 days prior to first drug administration (see Appendix 2 or http://medicine.iupui.edu/flockhart/table.htm for a complete listing of excluded potential CYP inducers/inhibitors).

18. Subject has a history of Gilbert's disease or cholecystectomy.

7.3 Screen Failures

Screening evaluations will be used to determine eligibility of each subject for enrollment. Subjects who fail to meet eligibility criteria during the Screening period will be considered screen failures.

7.4 Premature Subject Discontinuation from Study Treatment or Study

Subjects will be encouraged to complete the study; however, they may voluntarily withdraw at any time. Subjects withdrawn from the study may be replaced.

In the event that a subject prematurely withdraws consent from further participation in the study for any reason other than an AE/SAE, the subject will be encouraged to complete the End of Treatment (Day 7) and all applicable assessments (except that plasma for PK will not be collected from subjects who early terminate the study) in order to obtain full data capture for that subject.

If a subject prematurely withdraws from the study at any time due to an AE or SAE, applicable End of Treatment (Day 7) assessments will be completed, and the subject will be followed for at least 14 days, or until resolution or stabilization of the condition resulting in withdrawal has been confirmed.

For all cases of premature withdrawal from the study, the investigator must provide the reason for discontinuation on the appropriate CRF page.

A decision by the investigator to discontinue a subject from study treatment and/or study participation should be discussed with the Sponsor.

8 Treatment Plan

8.1 Methods of Assigning Subjects to Treatment Groups

Not applicable

8.2 Randomization and Blinding

This is an open-label, single arm study. Study drug will not be blinded.

8.3 Concomitant Medications and Therapies

Subjects are not to take any medications (prescription or over the counter), alternative therapy(ies), or herbal supplement(s) during this study (14 days prior to dosing through end-of study), unless the investigator, after discussion with the medical monitor (if feasible), determines use of medication is required; contraceptives are allowed during this study as defined in inclusion criterion. All medications, alternative therapy(ies), and/or herbal supplement(s) used during Screening and any ongoing or new medications / therapies taken while the subject is participating in the study will be recorded in the source documents (drug name, start and stop dates, and indication) and on the appropriate page of the electronic case report form (CRF).

For hepatically impaired subjects, stable doses of the following medications will be allowed during the study:

- Stable dose of antidepressants
- Stable opioid regimen. Stable regimen includes dose, pattern of use (e.g., as needed [PRN] or chronic dosing) combinations of opioids, and route of administration (e.g., oral or transdermal administration; parenteral administration is prohibited) for at least 4 weeks prior to Visit 2 (Baseline). The dose and regimen of opioid(s) should remain unchanged (i.e., < 20% change in dose and no change in opioid[s]) throughout the trial).
- Subjects who require concomitant antibiotic therapy with a once per week dosing regimen (e.g., ciprofloxacin 500-750 mg dose/week) for spontaneous bacterial

peritonitis (SBP) may participate in the study if they started their therapy at least 1 month prior to Visit 1 (Screening).

- Neurontin (gabapentin) and Lyrica (pregabalin) if a stable dose has been taken for at least 2 months prior to Visit 1 (Screening) and the dosage is expected to remain unchanged throughout the trial.

- Laxatives or stool softeners will be allowed to treat constipation at the discretion of the investigator but should be used sparingly to avoid diarrhea.

8.3.1 Prohibited Therapy

Use of the following medications is prohibited within the inclusion/exclusion specified pre-study timeframes and throughout the study unless otherwise noted:

- Milk thistle within 30 days of Day 1 (Baseline).
- Investigational drugs within 30 days of Day 1 (Baseline).
- Daily prophylactic antibiotics within 30 days of Day 1 (Baseline).

The following concomitant medications are prohibited after Screening and throughout the study:

- Non-study rifaximin (200 mg or 550 mg tablets) is prohibited beginning Day 1 and throughout the Treatment Period. Subjects may resume their prescription rifaximin use following CRU discharge.
- Psychoactive, neuroactive agents (except as allowed per protocol) and psychotropic drugs.
- Warfarin-type anticoagulants.
- Rifampin or other rifamycin derivatives (except study drug).
- Alternative, herbal or complementary therapies for liver cirrhosis other than those required to manage fluid and electrolyte homeostasis.

Consumption of certain foods and substances that are known to inhibit and/or induce CYP are not permitted during this study as specified in exclusion criteria. (See Appendix 16.2 or http://medicine.iupui.edu/clinpharm/ddis/main-table for a complete listing of excluded potential CYP inducers/inhibitors).

All over the counter and prescription medications are prohibited for healthy subjects. If any medication is required during the study for healthy subjects, the subject may be discontinued from the study at the discretion of the Investigator or the Sponsor.

8.4 Treatment Compliance

All doses of study drug will be administered in the CRU under study staff supervision to monitor and document treatment compliance.

8.5 Protocol Deviations and Violations

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the sponsor and the IRB/IEC and agreed to by the investigator. Deviations usually have an impact on individual patients or a small group of patients and do not involve inclusion/exclusion or primary endpoint criteria.

A protocol violation occurs when there is non-adherence to the protocol that results in a significant, additional risk to the patient, when the patient or investigator has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the patient was enrolled without prior sponsor approval, or when there is non-adherence to FDA regulations and/or ICH GCP guideline.

The investigator or designee must document and explain in the patients' source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study patients without prior IRB/IEC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendment(s) should be submitted to the IRB/IEC for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

9 Study Drug Materials and Management

All study medication will be dispensed by the pharmacy or an appropriately qualified member of the study staff assigned by the investigator to this task.

9.1 Formulation and Supply

Rifaximin will be supplied as pink-colored, coated, oval, biconvex tablets each containing 550 mg rifaximin. Inactive ingredients include: colloidal silicon dioxide, disodium edetate,

glycerol palmitostearate, hypromellose, microcrystalline cellulose, propylene glycol, red iron oxide, sodium starch glycolate, talc, and titanium dioxide.

9.1.1 Packaging and Labeling

The rifaximin 550 mg tablets will be supplied in appropriate containers and labeled for clinical trial material according to applicable FDA and ICH guidelines.

9.1.2 Storage, Handling, and Disposal of Study Drug

The investigator has overall responsibility for ensuring that study medication is stored in a secure, limited-access location under the specified appropriate storage conditions until it is assigned and handed over to the study subject. The locked area will be maintained under controlled temperature conditions at 20-25°C (68-77°F); excursions are permitted to 15 to 30°C (59 to 86° F). Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented. Study medication will be distributed by the pharmacy or assigned member of the study team. Temperature monitoring is required at the storage location to ensure that the investigational product is maintained within the required temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the total duration of the trial and that records are maintained. No study medication stock or returned inventory may be removed from the investigational site without prior consent by the Sponsor except as required to be dispensed to subjects participating in the study. All returned and unused study medication shall be returned or destroyed as instructed by the Sponsor.

9.1.3 Dosing (including Dosing Schedule)

Each subject will receive twice daily doses of rifaximin (approximately 12 hours apart) for 6 days followed by the morning dose only on Day 7.

9.1.4 Rationale for Dose Selection

The dose regimen for rifaximin used in this study (550 mg BID) is approved by the FDA for the reduction of risk in overt HE in patients with liver cirrhosis.

9.2 Study Drug Accountability

Only authorized site personnel may dispense study drug. Investigational drug orders, records of study drug receipt, dispensing, and running inventory will be examined and reconciled throughout the study. Only subjects enrolled in the study may receive study drug in accordance with all applicable regulatory requirements.

Study drug reconciliation will be conducted periodically during monitoring visits to ensure appropriate receipt, storage, dispensing and documentation of returned study drug. Upon completion of the study, this material will be subjected to final inspection and reconciliation.

The following information will be collected regarding study drug and recorded in the subject's source document:

- Subject dosing compliance (%) for study drug.

10 Study Procedures and Evaluations

A schedule of assessments to be performed during the study is provided in Overall Time and Events Schedule, at Section 16.1.

The subject should rest in the seated position for approximately 5 minutes prior to collection of any assessments.

10.1 Schedule of Evaluations and Procedures

10.1.1 Screening (Day –21 to Day -2)

After signing the informed consent, patients will undergo the screening procedures to confirm eligibility to participate in the study.

During Screening, the following procedures/assessments will be performed:

- Obtain written informed consent prior to performing any study evaluations.
- Assign subject identification number.
- Record medical history and demographic information (date of birth, sex, ethnicity, and race), including verification of tuberculosis history. Note: Efforts will be made to match subjects across groups for age, weight and sex.
- Review subject eligibility.
- Assessment of MELD score and Child-Pugh classification (A, B, or C).
- Perform physical examination.
- Measure height and weight. Height and weight will be used to calculate body mass index (BMI).

- Perform vital signs (oral temperature, seated blood pressure and pulse). Subjects should be allowed to rest in the seated position for approximately 5 minutes prior to collection of blood pressure and pulse.
- Obtain 12-lead ECG. Assessments for clinically significant findings may be repeated if necessary.
- Collect samples for laboratory tests:
    - Blood samples for hematology, chemistry, coagulation, and testing for tuberculosis, HIV, hepatitis B surface antigen, and hepatitis C virus.
    - Blood sample for serum pregnancy testing for females of childbearing potential. A negative pregnancy test must be confirmed prior to check-in on Day -1.
    - Urine for a urine pregnancy test for females of child-bearing potential. A negative pregnancy test must be confirmed to continue participation in the study.
    - Urine samples for urinalysis and urine drug/nicotine/alcohol screen.

      NOTE: Blood sample collection for the clinically significant laboratory results may be repeated, if necessary. Results from all laboratory tests must be received and reviewed prior to CRU check-in on Day -1.
- Record any AEs and SAEs since the time of informed consent.
- Record concomitant medications.
- Stool collection and processing for:
    - Presence of enteric infections (this stool sample analysis must be available prior to check-in on Day -1).
    - Microbiota characterization.

NOTE: If the subject cannot produce a stool sample at Screening, dispense a stool sample kit and instruct subjects on collection of the stool sample to be returned to the site at least 7 days prior to Day -1. The results for the stool sample are needed before check-in to the CRU.

10.1.2 CRU Check-in: Day-1

The following are to be completed upon checking into the facility on Day -1 (subjects should check-in the day prior to dosing and will be confined until the completion of the blood sample collection for PK on Day 8):

- Check-in to CRU.
- Review subject eligibility.
- Perform symptom-driven physical exam (general appearance, skin, thorax/lungs, cardiovascular, abdomen, extremities), if necessary.
- Perform vital sign assessments (oral temperature, seated blood pressure and pulse). Subjects should be allowed to rest in the seated position for approximately 5 minutes prior to collection of blood pressure and pulse.
- Assess for AEs and SAEs.
- Assess for changes in concomitant medications.

Note: subjects will have to fast overnight (water provided ad libitum) for approximately 10 hours prior to the AM dosing on Day 1.

10.1.3 Day 1 (Baseline; pre-dose)

The following procedures will be performed on Day 1 pre-dose:

- Review subject eligibility.
- Assessment of MELD score and Child-Pugh classification (A, B, or C).
- Perform symptom-driven physical exam (general appearance, skin, thorax/lungs, cardiovascular, abdomen, extremities), if necessary.
- Perform pre-dose vital sign assessments (oral temperature, seated blood pressure and pulse). Subjects should be allowed to rest in the seated position for approximately 5 minutes prior to collection of blood pressure and pulse.
- Collect samples for laboratory tests:
    - Blood samples for hematology, chemistry, and coagulation.
    - Blood sample for serum cystatin C.
    - Urine samples for urinalysis and urine drug/nicotine/alcohol screen.

- For females of childbearing potential, blood for a serum pregnancy test and urine for a urine pregnancy test. A negative urine pregnancy test must be confirmed prior to dosing.

- Assess for changes in concomitant medications.
- Assess for pre-dose AEs and SAEs.
- Collect a pre-dose PK blood sample (3 mL in a sodium heparin tube) within 1 hour prior to administration of first daily dose of rifaximin 550 mg.

10.1.4 Day 1 (Dosing and Post-dose)

- Administer 550 mg tablet of rifaximin with a total of 240 mL of water. Actual time of dose should be recorded in source documents and eCRF. The fast should continue for at least 1 hour after dosing. Water is permitted *ad libitum*. The subject should remain upright for 4 hours after the dose administration.
- Perform symptom-driven physical exam (general appearance, skin, thorax/lungs, cardiovascular, abdomen, extremities), if necessary.
- Administer the 2nd daily dose of rifaximin 550 mg approximately 12 hours after the first dose. Actual time of the dose should be recorded in source documents and eCRF.
- Perform vital sign assessment 6 hours post-dose (oral temperature, seated blood pressure and pulse). Subjects should be allowed to rest in the seated position for approximately 5 minutes prior to collection of blood pressure and pulse.
- Assess for AEs and SAEs.
- Asses for changes in concomitant medications

10.1.5 Day 2 – Day 6

The following procedures will be performed on Days 2 through 6:

- Perform symptom-driven physical exam (general appearance, skin, thorax/lungs, cardiovascular, abdomen, extremities), if necessary.
- Collect a blood sample for PK every morning prior to administration of the first daily dose of rifaximin 550 mg. Administer rifaximin 550 mg BID. Each dose of rifaximin should be given with a total of 240 mL of water. The two doses should be given approximately 12 hours apart, and the actual time of dosing should be recorded in source documents and eCRF.

- Assess for AEs and SAEs.
- Asses for changes in concomitant medications.

Note: subjects will have to fast overnight (water provided *ad libitum*) for approximately 10 hours prior to the AM dosing on Day 7.

10.1.6 Day 7 (End of Treatment [EOT] or Early Termination)

The following procedures will be performed on Day 7:

- Perform symptom-driven physical exam (general appearance, skin, thorax/lungs, cardiovascular, abdomen, extremities), if necessary.
- Perform pre-dose vital sign assessments (oral temperature, seated blood pressure and pulse). Subjects should be allowed to rest in the seated position for approximately 5 minutes prior to collection of blood pressure and pulse.
- Collect samples for laboratory tests:
    - Blood samples for hematology, chemistry, coagulation and serum pregnancy test (for females of child-bearing potential).
    - Urine samples for urinalysis.
- Collect a pre-dose PK blood sample (3 mL in a sodium heparin tube) within 1 hour prior to administration of first daily dose of rifaximin 550 mg.
- Administer morning 550 mg tablet of rifaximin with a total of 240 mL of water. Actual time of dose should be recorded in source documents and eCRF. The fast should continue for at least 1 hour after dosing. Water is permitted *ad libitum*. The subject should remain upright for 4 hours after the dose administration.
- Collect PK blood samples (3 mL in sodium heparin tube) at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, and 16 hours with a window of ± 5 minutes from the 0.25 hour time point to the 4 hour time point, and ± 15 minutes from the 6 hour time point to the 16 hour time point.
- On Day 7, subjects will be asked to provide a stool sample for microbiota characterization prior to leaving the CRU on Day 8. Subjects can provide a stool sample at any time on Day 7 or 8.
- Assess for AEs and SAEs.
- Asses for changes in concomitant medications.

- Assessment of MELD score and Child-Pugh classification (A, B, or C).

10.1.7 Day 8

- Perform symptom-driven physical exam (general appearance, skin, thorax/lungs, cardiovascular, abdomen, extremities), if necessary.
- Assess for AEs and SAEs.
- Asses for changes in concomitant medications.
- Collect a PK blood samples (3 mL in sodium heparin tube) at 20 and 24 hours (± 15 minutes) post Day 7 dose.
- Discharge from the CRU after the 24 hour PK blood sample collection.

NOTE: Subjects must provide a stool sample on Day 7 or 8 prior to discharge from the CRU.

10.1.8 3 days (+ 1) after EOT: Follow-Up Phone Call

The follow-up phone call will occur 2 (+1) days following discharge from the clinic on Day 8. For early termination, follow-up phone call will occur 3 + 1 days after the last study drug dose.

- Record AEs and SAEs.
- Record concomitant medication.

10.2 Assessment of Pharmacokinetics

Pharmacokinetic endpoints are presented in Section 5.3. Pharmacokinetic analyses to be performed are described in Section 12.6.1.

Blood Samples

Blood samples (3 mL) will be drawn into 3 mL sodium heparin tubes at the following times (actual blood collection times will be recorded in the source documents and eCRF):

- Day 1: pre-dose (within 1 hour before dosing).
- Day 2-6: trough sample collected prior to administration of the first daily dose of rifaximin (AM dose).
- Day 7/8: pre-dose (within 1 hour before dosing), 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours.

Windows for blood samples are ± 5 minutes from the 0.25 hour time point to the 4 hour time point, and ± 15 minutes from the 6 hour time point to the 24 hour time point.

Collecting, Processing, and Shipping PK Samples

Blood samples will be drawn and processed for plasma as described in the Laboratory Manual. The yield of plasma will be subdivided into 2 samples (~0.5 mL). One set (Set 1) of these samples will be shipped to MicroConstants, Inc. (San Diego, California, USA) for analysis upon processing of the final PK sample for each subject. The second set of samples (Set 2) will be shipped to MicroConstants, Inc., as directed by the Sponsor.

Bioanalytical Methods

Bioanalysis of all plasma samples will be performed by MicroConstants, Inc. (San Diego, California, USA). Plasma concentrations of rifaximin and 25-desacetyl rifaximin will be determined using a validated reversed-phase high performance liquid chromatographic method with tandem quadrupole mass spectrometric detection using a validated analytical procedure. The lower limit of quantification, deviation of calibration standards from the theoretical value, and precision will be established using standard methods. Performance of the assay during the analysis of unknown samples will be monitored and reported.

10.3 Stool Testing/Microbiome Assessment

Fresh stool samples will be collected if possible. If the subject cannot produce a sample during the visit, the subject will be issued a collection kit and asked to collect a specimen at home, refrigerate it immediately, and bring it to the clinic as soon as possible. Stool samples will be separated into 2 mL aliquots in polypropylene cryovials, stored at ≤ -20° C at the clinical site, and shipped on dry ice to the central laboratory.

- Screening:
    - Testing will be performed prior to Day -1 (Check-in) to identify the presence of enteric infections. A negative test must be confirmed for all subjects to enroll, including negative *C. difficile* Toxin A & B. Stool samples will also be collected for microbiota testing.
- Treatment:
    - Stool samples will be collected for microbiota testing on Day 7/8.

Microbiota Characterization (Susceptibility Testing): This study will characterize the susceptibility of the stool microbiota (specifically Gram-negative rods and *C. difficile*) to rifaximin, rifampin, and other selected antibiotics of clinical interest. For Gram-negative cultures, colonies will be grown on MacConkey agar and minimum inhibitory concentrations

(MICs) will be determined by the microbroth dilution method (per CLSI document M-7 A8). For *C. difficile* anaerobic chamber cultures, tested antibiotics will include rifaximin, vancomycin, metronidazole, and fidaxomicin. The assessment of bacteria (aerobic and anaerobic) will be characterized by the number of colony forming units/gram of stool for both anaerobic and aerobic bacteria.

Microbiota Characterization (Composition, Diversity, DNA Sequencing): This study will characterize intestinal microbiota in terms of composition and diversity, as well as sequencing for specific organisms of interest. Bacterial DNA will be isolated from fecal samples, and the hyper-variable regions of the 16S rRNA gene will be amplified using two-step PCR with Illumina HiSeq2000 sequencing technology.

Long-term storage for future exploratory analysis. Subjects must be advised of and provide consent to this stool collection for storage.

10.4 Assessment of Safety

10.4.1 Adverse Events

Safety data collection for this study begins at the time of the subject's signing of the informed consent document according to the operating definitions defined in Section 11.1 of the protocol. The investigator is responsible for the detection and documentation of events that meet the definition of an AE or SAE as provided in this protocol. Assessments of occurrences of AEs or SAEs should be conducted during each study visit or as reported by the subject outside of scheduled visits. In order to fulfill safety reporting obligations, the investigator should include in his or her assessment any AEs resulting from study participation regardless of relation to study drug (e.g., complications resulting from the taking of a blood sample)

10.4.2 Physical Examination

A physical examination will be performed at Screening.

A symptom-driven physical exam will be performed at any time during the study as deemed necessary by the investigator.

10.4.3 Vital Signs

Vital signs will include seated blood pressure (mmHg), pulse (beats per minute), and oral temperature (°C) and will be obtained after the subject has been seated for at least 5 minutes. Blood pressure will be measured on the same arm throughout the study. Blood pressure,

pulse, and oral temperature will be obtained at Screening, after CRU Check-in on Day -1, prior to dosing on Day 1, 6 hours post-dose on Day 1, and at Day 7/Early Termination.

Height and weight will be measured at Screening to calculate BMI.

10.4.4 Clinical Laboratory Tests

Clinical laboratory evaluations are to be performed by licensed clinical laboratory. Blood samples for clinical laboratory tests are to be drawn at Screening, Day 1, and Day 7/ET, prior to dosing.

Additional laboratory testing may occur as needed for safety assessments during the study.

The clinical laboratory parameters to be evaluated are as follows:

- **Hematology:** hemoglobin, hematocrit, red blood cell count, red cell mass measurements, white blood cell count with differential, and platelet count (Screening, Day 1 pre-dose and Day 7/ET)
- **QuantiFERON®-TB Gold test** (Screening)
- **Serum cystatin C** (Day 1 pre-dose)
- **Blood Chemistry:** Alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, blood urea nitrogen, creatinine, calculated creatinine clearance, uric acid, electrolytes (Na^+, K^+, HCO_3^- and Cl^-), lactate dehydrogenase, calcium, albumin, glucose, cholesterol (total and HDL), and triglycerides (Screening, Day 1 pre-dose and Day 7/ET)
- **Coagulation**: Prothrombin time and international normalized ratio. (Screening, Day 1 pre-dose and Day 7/ET)
- **Urinalysis**: Routine urine analysis (including, but not limited to, white blood cell count, red blood cell count, and protein) as indicated by dipstick. If there are any positive findings microscopic examination should be performed to quantify the results (Screening and Day 7/ET)
- **HIV and hepatitis B & C:** Testing will be performed for HIV, hepatitis B surface antigen, and hepatitis C virus (Screening)

- **Urine alcohol/drug/nicotine screen:** Urine will be collected for a drug screening test from all subjects. At a minimum, the sample will be tested for the presence of cocaine, tetrahydrocannabinol, barbiturates, amphetamines, benzodiazepines, opiates, and cotinine (Screening and Day 1 pre-dose)

10.4.5 Electrocardiogram

At Screening, a 12-lead ECG will be recorded on all subjects (hepatically impaired and healthy subjects) in the supine position after 5 minutes of rest to assess for cardiac abnormalities.

10.4.6 Pregnancy Testing

A urine pregnancy test will be performed for all females of child-bearing potential at Screening and Day -1. A serum pregnancy test will be performed at Screening, Day -1 and Day 7/Early Termination.

10.5 Other Assessments

10.5.1 Medical History

Each subject's complete medical history is to be recorded in source documents and the appropriate eCRF pages during Visit 1 (Screening). Medical history must include the current MELD score, the current Child-Pugh score, etiology and the date of first diagnosis of chronic liver impairment (cirrhosis).

10.5.2 Model for End Stage Liver Disease

MELD is a scoring system used in assessing the severity of CLD.

- The OPTN MELD calculator will be used for MELD calculations in this study; https://optn.transplant.hrsa.gov/resources/allocation-calculators/meld-calculator/ (or most current link available for the OPTN MELD calculator)

10.5.3 Child-Pugh Classification System

The Child-Pugh classification system for the severity of liver disease is listed below. The classification system determines severity of liver disease according to the degree of ascites (via clinical assessment), the serum concentrations of bilirubin and albumin, International Normalized Ratio, and the degree of encephalopathy.

Parameter	Score Assigned		
	1	2	3
Ascites	Absent	Mild	Moderate to Severe
Bilirubin, mg/dL	< 2	2-3	> 3
Albumin, g/dL	> 3.5	2.8-3.5	< 2.8
International Normalized Ratio	< 1.7	1.71 - 2.20	> 2.20
Encephalopathy*	None	Grade 1-2	Grade 3-4

Encephalopathy is classified as Grade 0-4 using the Conn scoring system below:

- Grade 0 = No personality or behavioral abnormality detected.
- Grade 1 = Trivial lack of awareness, euphoria or anxiety; shortened attention span, impairment of addition or subtraction.
- Grade 2 = Lethargy; disorientation for time; obvious personality change; inappropriate behavior.
- Grade 3 = Somnolence to semi-stupor, responsive to stimuli; confused; gross disorientation; bizarre behavior.
- Grade 4 = Coma; unable to test mental state.

Grade	Score
A: well-compensated disease	5 to 6
B: significant functional compromise	7 to 9
C: decompensated disease	10 to 15

A total score of 5 to 6 is considered grade A (well-compensated disease); 7 to 9 is grade B (significant functional compromise); and 10 to 15 is grade C (decompensated disease).

10.5.4 Concomitant Medications

All concomitant medications will be collected and recorded on the source documents and eCRF from Screening through end of study.

11 Safety Reporting

The Sponsor maintains a robust pharmacovigilance system comprised of a governance framework and standard operating procedures supporting a systematic process for review, evaluation, and management of accumulating safety data from clinical trials and other sources to:

- Identify a potential new safety signal;
- Ensure that an investigational product's risks are adequately assessed and communicated to investigators, IRBs/IECs, and regulatory bodies during clinical development.

Findings and/or safety data obtained during this study will provide information for the overall review of safety that is conducted by the Sponsor on a routine basis. The Sponsor will report expeditiously any findings from clinical trials (ongoing or completed), epidemiological studies, pooled analysis of multiple studies, and findings from animal or in vitro testing that suggest a significant risk in humans exposed to the study product.

Safety data collection for this study begins at the time of the subject's signing of the informed consent document according to the operating definitions defined in this section of the protocol. The investigator is responsible for the detection and documentation of events that meet the definition of an unanticipated problem (refer to protocol Section 10.1), AE or SAE.

11.1 Operating Definitions for Assessing Safety

11.1.1 Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a study product and which does not necessarily have a causal relationship with the treatment.

An AE can therefore be any unfavorable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a study product, without any judgment about causality (i.e., whether or not considered related to the study product).

Additionally, an event that is associated with study participation (regardless of administration of or relationship to study drug) should be treated as a reportable adverse event for this protocol (e.g., complications resulting from the taking of a blood sample or performance of a protocol required procedure).

An AE **does** include the following:

- Exacerbation or worsening of a pre-existing illness.

NOTE: If the pre-existing illness is the disease under study, then "exacerbation" refers to an unexpected worsening from the condition at baseline.

- Increase in frequency or intensity of a pre-existing episodic event or condition.
- Condition detected or diagnosed after study product administration even though it may have been present prior to the start of the study.
- Symptom associated with disease not previously reported by the subject.

An AE **does not** include the following:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion) as event terms; the condition that led to the procedure is the AE if it meets the definition of an AE.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for cosmetic elective surgery; and social and/or convenience admissions).
- Overdose of either study product or concurrent medication without any signs or symptoms.
- Symptoms associated with disease, which are consistent with the subject's usual clinical course; unless the subject experiences worsening of their symptom(s) or the symptom(s) meet the criteria for an SAE.

11.1.2 Serious Adverse Event

A SAE is any AE, occurring at any dose, which results in any of the following outcomes ("Occurring at any dose" does not imply that the subject received study product.):

- Results in death.
- Is life threatening.

NOTE: Life-threatening means that the subject was, in the view of the investigator or Sponsor, at immediate risk of death at the time of the event. This definition does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization.

NOTE: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered to be an AE. "In-patient" hospitalization means the subject has been formally admitted to a hospital for medical reasons. This may or may not be overnight. It does not include presentation at a casualty or emergency room.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.

- A congenital anomaly or birth defect in the offspring of a subject who received study product.
- Important medical events that do not result in death, are not life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

NOTE: Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

11.1.3 Assessment of Severity

The severity assigned to an AE should be determined by the maximum severity of the AE. The categories described below should be used to estimate the severity of AEs:

- Mild: Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required
- Moderate: Mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention/therapy required
- Severe: Marked limitation in activity; some assistance usually required; medical intervention/therapy required; hospitalization or prolongation of current hospitalization possible; may be incapacitating or life threatening

11.1.4 Assessment of Causality

The investigator should assess the relationship of the AE, if any, to the study drug using the following definitions:

Related: Event or laboratory test abnormality with reasonable time relationship to drug intake. Cannot be attributed to disease or other drugs. Response to withdrawal is clinically reasonable and re-challenge is not required.

Probable: Event or laboratory test abnormality with reasonable time relationship to drug intake. Unlikely to be attributed to disease or other drugs. Response to withdrawal is clinically reasonable and re-challenge is not required.

Possible: Event or laboratory test abnormality with reasonable time relationship to drug intake. Could also be explained by disease or other drugs. Information on drug withdrawal may be lacking or unclear.

Unlikely: Event or laboratory test abnormality with a time to drug intake that makes a relationship improbable (but not impossible). Disease or other drugs provide plausible explanations.

Not Related: Event or laboratory test abnormality with a time to drug intake that makes a relationship implausible. Disease or other drugs provide plausible explanations.

11.2 Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs

Abnormal laboratory findings (e.g., clinical chemistry, hematology) or other abnormal assessments (e.g., abnormal findings during examinations or ECG monitoring) that are judged by the investigator as **clinically significant** must be recorded as AEs or SAEs if they meet the definition of an AE or SAE. The investigator should exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment (including ECG monitoring) is clinically significant.

11.3 Method and Frequency for Detecting AEs and SAEs

At each visit, after the subject has had an opportunity to spontaneously mention any problems, the investigator should inquire about AEs by asking the following standard questions:

1. "Have you had any (other) medical problems since your last visit/assessment?"

2. "Have you taken any new medicines, other than those given to you in this study, since your last visit/assessment?"

11.3.1 Time period for Detecting and Reporting of AEs and SAEs

From the time of informed consent through study completion/withdrawal, including the follow-up period.

11.3.2 Post-Study SAEs

Investigators are not obligated to actively seek SAE information in former study participants, but investigators are encouraged to notify the Sponsor of any SAEs of which they become aware occurring at any time after a subject has discontinued or completed the study that they judge may be reasonably related to study treatment or study participation.

11.4 Documenting AEs and SAEs

All AEs that occur after the subject has signed the informed consent document and during the course of the study, regardless of causality or seriousness, will be assessed and recorded in the subject's medical records and in the eCRF. In addition, SAEs must be documented on the paper SAE Report Form.

A separate paper SAE Report Form should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present that are temporally and/or clinically related, they may be reported on the same SAE Report Form.

The investigator should attempt to establish a diagnosis for the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and SAE and not the individual signs/symptoms.

For clinically significant abnormal laboratory findings or other abnormal assessments meeting the definition of an AE or SAE, a diagnosis, if known (or clinical signs and symptoms if diagnosis is unknown), should be recorded by the investigator. If a diagnosis is unknown and clinical signs and symptoms are not present, then the abnormal finding should be recorded. When documenting as an SAE on the SAE Report Form, relevant laboratory data should either be recorded in the 'Details of Relevant Assessments' section of the SAE Report Form (including the reference range and units) or copies of the laboratory report (with reference ranges and units) should be sent with the SAE Report Form.

The SAE Report Form should be completed as thoroughly as possible and signed by the investigator or his/her designee before transmittal to the CRO. It is **very important** that the

investigator provide his/her assessment of causality to study product at the time of initial SAE reporting.

11.5 Follow-Up of AEs and SAEs

All AEs, regardless of seriousness, must be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or the subject is lost to follow-up. The investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, relevant hospital records (i.e., discharge summary), or consultation with other health care professionals.

The Sponsor may request that the investigator perform or arrange for the conduct of supplemental measurements and/or evaluations. If a subject dies during participation in the study or during a recognized follow-up period, the Sponsor should be provided with a copy of any postmortem findings, including histopathology.

For SAEs, new or updated information should be recorded on the originally completed paper SAE Report Form and all changes signed and dated by the investigator or designee. By signing the SAE Report Form, the investigator or designee attests to the accuracy and completeness of the data and that he/she has reviewed and approved the report being submitted. The investigational sites IRB must be notified about SAEs in accordance with the requirements of the governing IRB.

11.6 Prompt Reporting of SAEs to Sponsor

SAEs must be reported promptly to the CRO once the investigator determines that the event meets the protocol definition of a SAE.

Prompt reporting of a SAE requires the following:

- Completion and transmission of the SAE Report Form to the CRO via fax or email within 24 hours of the investigator's knowledge of the event. In parallel, a corresponding AE with the SAE details should be entered into the AE eCRF within 48 hours of submitting the paper SAE Report Form.

Prompt reporting of additional information for previously reported SAEs should follow the same reporting timeframe as initial reports. In addition, the corresponding AE in the AE eCRF (as applicable) should be updated to ensure all data points documented in the AE eCRF are aligned with the matching data points on the paper SAE Report Form.

11.7 Pregnancy Reporting

Pregnancies detected in subjects assigned to study treatment should be reported within 24 hours of investigator's awareness to CRO via fax or email, using the Sponsor's Pregnancy Notification Form. If a female subject becomes pregnant following assignment to study treatment, the study product will be immediately discontinued and the subject will be followed until the outcome of the pregnancy is known.

The CRO should be notified via fax or email of any updates on the status of the pregnancy as soon as the information becomes available by update and/or amendment of the initial pregnancy notification form.

Although pregnancy occurring in a clinical study is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy, for medical reasons, will be recorded as an AE or SAE and followed as such.

11.8 Transmission of SAE Reports and Pregnancy Notification Forms

Completed SAE Report Forms and completed pregnancy notification forms will be transmitted to the CRO via the fax number provided below:

[redacted]

11.9 Regulatory Reporting Requirements for SAEs

The investigator, or responsible person per local requirements, must comply with the applicable local regulatory requirements related to the reporting of SAEs and IND safety reports to regulatory authorities and their IRB.

12 Statistics

A detailed statistical analysis plan (SAP) will be prepared for this study. The plan will contain a discussion of the statistical methods, a description of the computational algorithms and data handling conventions, and specifications for the data summaries and listings. It will be finalized before database lock.

12.1 Randomization

This is an open-label, non-randomized study.

12.2 Determination of Sample Size

Six subjects per group is the minimum recommendation from FDA guidance "Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling." A total of 12 subjects with severe liver impairment will be enrolled to meet the combined objectives of effects of liver impairment on the PK of rifaximin.

12.3 Analysis Populations

The Intent-to-Treat (ITT) population will be used for safety analyses. The ITT population will include all subjects who ingested at least one dose of study drug.

The PK population will include subjects from whom sufficient data are obtained for calculation of rifaximin plasma PK parameters.

12.4 Subject Disposition

Subject disposition will be summarized using descriptive statistics.

12.5 Demographic and Baseline Characteristics

Demographic and other baseline characteristics will be summarized using descriptive statistics.

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Medical history data will be listed in subject data listings.

12.6 Analysis Methods

Pharmacokinetic and safety endpoints are shown in Section 5.3 and Section 5.4.

12.6.1 Pharmacokinetic Analyses

All subjects who receive study medication and have sufficient samples collected for valid estimation of PK parameters during any dosing period will be considered evaluable for the PK analyses. Descriptive PK parameters for rifaximin and 25-desacetyl rifaximin will be determined using Phoenix WinNonlin version 6.3 (Pharsight Corporation) or higher.

At a minimum, the following PK Parameters will be calculated, data permitting:

Cmax,ss	Maximum observed concentration at steady-state, observed by inspection of individual study participant plasma concentration time plots.
Tmax,ss	Time of maximum observed concentration at steady-state, obtained directly from the observed concentration time data
λz	Terminal phase elimination rate constant, estimated by linear regression of logarithmically transformed concentration versus time data. Calculation of λz requires a minimum of 3 data points after (and not including) Cmax. The adjusted R^2 for the linear regression must be at least 0.80.
t½	Terminal phase half-life, estimated using the equation [ln(2)/λz]
AUCτ	At steady state, the area under the plasma concentration time curve from time 0 to the end of the dosing interval (12 hours).
CL/F (Rifaximin only)	Apparent plasma clearance divided by bioavailability, calculated as Dose/AUCτ at steady state.

Rifaximin and 25-desacetyl rifaximin plasma concentrations will be summarized by hepatic function group for each collection time point and collection period with statistics of the number of subjects evaluated (N), arithmetic mean, geometric mean, standard deviation, coefficient of variation, median, minimum, and maximum.

An analysis of variance (ANOVA) will be performed and PK parameters of Cmax and AUCτ for rifaximin and 25-desacetyl rifaximin will be natural log transformed prior to analysis. The 90% confidence intervals (CIs) of the hepatic impairment group mean relative to the matched control group mean will be obtained. The effect of hepatic impairment will be assessed by examining the 90% CIs for the mean ratio of the hepatic impairment group relative to the matched healthy group.

A continuous analysis using linear regression will be performed to evaluate the relationship between estimated creatinine clearance and rifaximin CL/F.

12.6.2 Microbiome Analyses

Gastrointestinal microbiota data will be summarized descriptively by total counts (per gram of stool sample); by species of aerobic and anaerobic bacteria; and by bacterial resistance to antibiotics (including rifaximin and rifampin).

12.6.3 Safety Analyses

Safety analyses will be descriptive and evaluations will be based on the incidence, intensity and types of treatment-emergent AEs, and changes in clinical laboratory results and vital signs. Concomitant medication will be summarized descriptively. Physical examination findings will be listed. Adverse events will be coded using the MedDRA coding dictionary. Concomitant medications will be coded using the World Health Organization Drug dictionary.

12.6.3.1 Extent of Exposure and Treatment Compliance

Exposure to study drug and treatment compliance will be summarized descriptively.

12.6.3.2 Adverse Events

Adverse events will be coded using the Medical Dictionary for Regulatory Affairs (MedDRA) coding dictionary. Treatment-emergent AEs will be defined as any event with a start date occurring on or after treatment on Day 1.

The incidence of treatment-emergent AEs will be summarized by body system and MedDRA preferred term, overall and by treatment group. If a subject reports the same AE more than once, then that subject will only be counted once for the summary of that AE, using the most severe intensity.

Treatment-emergent AEs will be summarized as follows:

- All treatment-emergent AEs.
- All treatment-emergent AEs by intensity.
- All treatment-emergent AEs by relationship to study drug.
- All treatment-emergent SAEs.
- All treatment-emergent AEs that led to premature discontinuation of study drug.

12.6.3.3 Clinical Laboratory Assessments

Hematology, blood chemistry, and urinalysis parameters will be summarized at baseline and at each time point, as well as changes from baseline to end of study. Laboratory values and changes from baseline in laboratory values will be summarized descriptively.

A summary of shifts from baseline to final evaluation will be given for each parameter. The normal range for each parameter will be used to create categories of low, normal, or high. Any result higher than upper limit of normal or lower than lower limit of normal will be categorized as high or low respectively, and any result within the lower and upper limits of normal will be categorized as normal. The number and percentage of subjects in each shift category from baseline to final evaluation will be shown for each parameter.

All potentially clinically significant values for clinical laboratory tests will be assessed by the investigator. Out of range laboratory values that may be potentially clinically significant will be flagged for review.

12.6.3.4 Vital Signs

Vital sign measurements will be summarized at baseline and at each time point. In addition, changes from baseline to last visit in vital sign measurements will be summarized. For each summary, the N, mean, median, SD, minimum, and maximum values will be presented.

12.6.3.5 Concomitant Medications

Concomitant medications taken from Screening and during the study will be categorized by the World Health Organization (WHO) classification for therapeutic class and drug name and summarized by number and percentage of subjects.

12.7 Statistical and Analytical Issues

12.7.1 Adjustment for Covariates

Not Applicable.

12.7.2 Handling of Dropouts or Missing Data

Subjects who discontinue from the study will not be replaced. No imputations will be made for missing data.

12.7.3 Interim Analysis and Data Monitoring

No interim analysis is planned.

13 Quality Control and Quality Assurance

13.1 Investigator Information and Training

The investigators and essential support staff will be trained by the Sponsor (or designee) in regards to Good Clinical Practices (GCPs) and all aspects of protocol execution. It is the responsibility of the investigator to train ancillary study staff.

13.2 Study Monitoring

This study will be monitored by the Sponsor (or designee) in accordance with GCPs and applicable regulations. By signing this protocol, the investigator agrees to periodic, onsite monitoring of all appropriate study documentation.

The progress of the study will be monitored by periodic onsite visits and frequent communications between the Sponsor (or designee) and the investigator (either by phone, fax, email, or post).

During these contacts, the monitor will:

- Check and assess the progress of the study;
- Review study data collected;
- Conduct source document verification; and
- Identify any issues and address their resolution.

The objectives of monitoring procedures are to verify that data is authentic, accurate, and complete; that the safety and rights of subjects are being protected; and that the study is conducted in accordance with the currently approved protocol (and any amendments), GCP and all applicable regulatory requirements.

13.3 Audits and Inspections

At its discretion, the Sponsor (or designee) may conduct a quality assurance audit of this study. If such an audit occurs, the investigator agrees to allow the auditor direct access to all relevant documents and to schedule his/her time and the time of his/her staff to permit meetings with the auditor to discuss findings and any relevant issues.

In addition, regulatory agencies may conduct a regulatory inspection of this study. If the investigator is notified of an inspection by a regulatory authority, the investigator agrees to notify the Sponsor immediately. The investigator agrees to allow the inspector direct access

to all relevant documents and to schedule his/her time and the time of his/her staff to permit meetings with the inspector to discuss findings and any relevant issues.

13.4 Data Quality Assurance

All assessments performed will be accurately documented in the subject's source documents and CRFs. The investigator or designee will enter the information required by the protocol into the source documents and CRFs provided by the Sponsor or designee.

The investigators must read the protocol thoroughly and follow the instructions exactly. Any deviations should be agreed to by prior discussion between the Sponsor and the investigator, with appropriate written protocol amendments made prior to implementing the agreed changes. Any amendment containing major modifications, particularly if it may involve an increased risk to the subjects, will be approved by the IRB before it may be implemented. No change in the conduct of the study may be instituted without written approval from the Sponsor.

14 Ethics and Administrative Issues

The investigator and the study staff are responsible for conducting this study in accordance with the applicable principles which have their origins in the Declaration of Helsinki, ICH, GCPs, and all other applicable laws and regulations.

14.1 Institutional Review Board (IRB) Approval

This protocol, the informed consent document, relevant supporting information, and all types of subject recruitment or advertisement information must be approved by the appropriate IRB before the study is initiated. Any amendments to the protocol also must be approved, where necessary, by the IRB prior to implementing changes in the study. Documentation of these approvals must be provided to the Sponsor prior to the initiation of the amendment.

The investigator's responsibilities regarding the IRB are as follows:

- Obtain IRB approval of the protocol, informed consent document, and any advertisements for subject recruitment prior to their use.
- Obtain IRB approval for any protocol amendments and revisions to the informed consent document before implementing the changes.
- Provide the IRB with any required information before or during the study.

- Submit progress reports to the IRB, as required, during the conduct of the study; request re-review and approval of the study, as needed; provide copies of all IRB re-approvals and relevant communication to the Sponsor.

- Notify the IRB within 10 days (unless required sooner by IRB) of all serious and unexpected AEs related to the study medications that are reported to you by the Sponsor. The investigator is responsible for updating the IRB on the progress of the study and of any changes made to the protocol at least once a year or at regular intervals as deemed appropriate. The investigator must also keep the IRB informed of any AEs, according to the IRB policy.
- Notify the Sponsor within 24 hours of awareness and the IRB within in 10 days (unless required sooner by IRB) of all unanticipated problems involving risk to subjects or others. For the purposes of this study, an Unanticipated Problem is defined as any incident, experience, or outcome that meets all of the following criteria:
    - Unexpected in nature, severity, or frequency (i.e., not described in study-related documents, such as the IRB-approved protocol, informed consent document, US Prescribing Information (USPI)
    - Related or possibly related to participation in the research (i.e., possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
    - Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

14.2 Subject Information and Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation in layman's terms regarding the nature of the study, along with the aims, methods, objectives, and any potential risks. The informed consent document must be appropriately signed and dated by the subject or the subject's legally authorized representative and the person obtaining the consent (if required by the IRB) prior to conducting/obtaining any study-related assessments, including the discontinuation of any medications prohibited for the study.

If the informed consent document is amended during the study, the investigator must follow all applicable regulatory requirements pertaining to approval of the amended informed consent document by the IRB and use of the amended document (including for ongoing subjects, as directed by the IRB).

The informed consent document also shall contain the subject's authorization for the use and disclosure of his/her protected health information (PHI) in connection with the study as defined by Health Insurance Portability and Accountability Act of 1996 (HIPAA) or equivalent local regulations in studies conducted outside the United States. The authorization shall include at a minimum a clear description of the following: the duration of the authorization, the subject's right of access to the PHI (or any suspension thereof during the course of the study), type of information to be used/disclosed in the study, the names or classes of parties that may use or disclose the PHI, the purpose of the use/disclosure of PHI, the extent of the subject's right to revoke the authorization, the extent to which participation in the study is conditioned on signing the authorization, and the potential for redisclosure of PHI or other such information as may be required by local regulations outside of the United States.

The original and any amended signed and dated informed consent documents must be retained at the study site; and a copy must be given to the subject or subject’s legally authorized representative(s).

14.3 Study and Site Closure or Discontinuation

Upon completion of the study, the following activities, when applicable, must be conducted by the monitor in conjunction with the investigator, as appropriate:

- Return of all study data to the Sponsor (or designee).
- Data clarifications and/or resolutions.
- Accounting, reconciliation, and final disposition of used and unused study drug.
- Review of site study records for completeness.

In addition, the Sponsor reserves the right to temporarily suspend or prematurely discontinue this study either at a single site or at all sites at any time and for any reason. If such action is taken, the Sponsor will discuss this with the investigator (including the reasons for taking such action) at that time. The Sponsor will promptly inform all other investigators and/or institutions conducting the study if the study is suspended or terminated for safety reasons, and also will inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IRB promptly and provide the reason for the suspension or termination.

14.3.1 Study Discontinuation

Should the investigator, the Sponsor, the FDA, or local regulatory authorities become aware of conditions arising during the conduct of this study that may warrant the cessation of the

study, such action may be taken. Prior to such action, consultation between the Sponsor, the investigator, and, as appropriate, the FDA and/or local regulatory authorities will take place. Conditions that may result in the termination of the study or parts thereof include, but are not limited to, the following:

- The discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study.
- A decision on the part of the Sponsor to alter, suspend, or discontinue the development of the investigational product.

14.3.2 Study Site Discontinuation

The study site can be discontinued at the request of the Sponsor, the investigator, or by the FDA or local regulatory authorities.

Conditions that may warrant discontinuation of the study site include, but are not limited to, any of the following:

- Failure of the investigator to accrue subjects into the study at an acceptable rate.
- Failure of the investigator to comply with current GCPs and/or applicable regulations.
- Submission of knowingly false information from the research facility to the FDA or other regulatory authorities.
- Insufficient adherence to protocol requirements and procedures.

If the study is prematurely discontinued at a study site, all study data must be returned to the Sponsor or designee. In addition, the site must conduct final disposition of all used and unused study drug in accordance with the Sponsor's procedures for the study. Study termination and follow-up will be performed in compliance with the conditions set forth in GCPs and applicable regulations.

Financial compensation to investigators and/or institutions will be in accordance with the agreement established between the investigator and the Sponsor.

14.4 Data Handling and Record Keeping

14.4.1 Case Report Forms and Database Processing

Subject data will be collected in an eCRF using Electronic Data Capture (EDC). The EDC system will be Part 11 compliant and will have a documented audit trail for all changes made to the eCRF.

The investigator or designee must enter all required subject data using the specified data collection method defined by the Sponsor. The investigator must complete a declaration on the eCRF attesting to his/her responsibility for the quality of all data entered, and that the data represents a complete and accurate record of each subject's participation in the study.

Electronic CRF data will be provided to the investigator at the end of the study and will need to be retained by the investigator.

14.4.2 Source Documents

Source documents consist of, but are not limited to, subject hospital charts, clinic notes, subject medical records, original test results, laboratory data, worksheets, drug accountability records, consent forms, etc. Source documents must be available for review and inspection during onsite monitoring of the study by the Sponsor, their designees, IRB and/or appropriate regulatory authorities.

14.4.3 Subject Tracking

A drug accountability log, subject identification log (to be retained by the investigator only), and subject screening/enrollment log will be used to track subject participation in the study.

14.4.4 Study Files

The investigator study file and all source data will be maintained by the investigator as required by applicable federal and local regulations. These files are subject to inspection as described under Section 13.3 of this protocol. If an investigator moves, withdraws from an investigation or retires, the investigator must notify the Sponsor. The investigator understands and agrees to maintain study files for the required retention period, as defined in applicable federal and local regulations.

14.4.5 Data Management

Data management will be performed in accordance with the standard operating procedures of the Sponsor or its designee.

14.5 Confidentiality

The anonymity of subjects participating in this study will be maintained. The investigator will only provide subject initials and subject number on study documents submitted to the Sponsor. The investigator will make every effort to maintain the confidentiality of documents that identify the subject by name (e.g., signed informed consent documents, laboratory reports, clinic charts), except to the extent necessary to allow inspection by the FDA or other regulatory authorities. Should the name and/or address of a subject participating in this trial be on a document for submission to the FDA or other regulatory

authorities (e.g., laboratory report), the name and/or address will be completely blocked out and replaced with the subject initials and number prior to submission.

The investigator and other study site personnel will keep confidential any information provided by the Sponsor (including this protocol) related to this study and all data and records generated in the course of conducting the study, and will not use the information, data, or records for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the investigator or study site personnel; (2) information which it is necessary to disclose in confidence to an IRB solely for the evaluation of the study; (3) information which it is necessary to disclose in order to provide appropriate medical care to a study subject, or (4) study results which may be published as described in Section 14.8. If a written contract for the conduct of the study which includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply rather than this statement.

14.6 Record Retention

The investigator should properly store and maintain all study records in accordance with Sponsor directives. All records relating to the conduct of this study are to be retained by the investigator until notified by the sponsor in writing that the records may be destroyed.

The investigator will allow representatives of the sponsor's monitoring team, the governing IRB/IEC, the FDA, and other applicable regulatory agencies to inspect all study records, CRFs, and corresponding portions of the subject's clinic and/or hospital medical records at regular intervals throughout the study. These inspections are for the purpose of verifying adherence to the protocol, completeness and accuracy of the data being entered onto the eCRF, and compliance with FDA or other regulatory agency regulations.

14.7 Financing and Insurance

14.7.1 Finance

The study is supported by Salix Pharmaceuticals, Inc., a a division of VPNA.

14.7.2 Insurance and Indemnification

Documentation of product liability insurance is on file at the Sponsor and is available upon request.

14.8 Publication Policy

All data furnished to the investigator and his/her staff and all data obtained through this protocol will be regarded as confidential and proprietary in nature and will not be disclosed to any third party, except for the FDA or other regulatory body, without written consent from the Sponsor.

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16 Appendices

16.1 Overall Time and Events Schedule

	Screening	CRU Admission	Baseline	Treatment				Follow-Up
Study Assessments	**Day -21 to Day -2**	**Day -1**	**Day 1 Pre-dose**	**Day 1 Post-dose**	**Days 2-6**	**Day 7 EOT [b]/ Early Term**	**Day 8**	**3 days (+1) from EOT**
Informed consent	X							
Assign subject number	X							
Medical history/ demographics	X							
Review eligibility criteria	X	X	X					
MELD/Child-Pugh assessment	X		X			X		
Physical Examination [a]	X	X	X	X	X	X	X	
Height	X							
Weight	X							
Vital signs [c]	X	X	X	X		X		
12-lead ECG [d]	X							
Clinical chemistry / hematology /coagulation	X		X			X		
HIV, hepatitis (B & C)	X							
Tuberculosis test	X							
Pregnancy test	X (urine, serum)		X (urine, serum)			X(serum)		
Urinalysis	X					X		
Urine drug/nicotine/alcohol	X		X					
AEs and SAEs	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X
Serum cystatin C			X					
Drug Administration				X	X	X		
PK blood samples [e]			X		X	X	X	
Stool sample for enteric infections	X							
Stool sample for microbiome	X					X [f]	X [f]	
CRU check-in		X						
Discharge from CRU							X	
Follow-up Phone Call								X

a. A full physical examination will be conducted at Screening. A symptom-driven physical exam will be performed at any time during the study as deemed necessary by the investigator.
b. EOT=End of Treatment
c. Seated blood pressure, pulse, and oral temperature will be obtained at Screening, after CRU Check-in on Day -1, prior to dosing on Day 1, 6 hours post-dose on Day 1, and at Day 7/Early Termination.
d. Both hepatically impaired and healthy subjects will undergo an ECG recording.
e. Plasma for PK will not be collected from subjects who terminate early from the study.
f. Subjects must provide a stool sample on Day 7 or 8 prior to discharge from the CRU.

16.2 CYTOCHROME P450 ENZYME INDUCER/INHIBITOR COMPOUNDS*

*Adapted from http://medicine.iupui.edu/clinpharm/ddis/main-table

Inducers					
1A2	**2C19**	**2C9**	**2D6**	**2E1**	**3A4,5,7**
broccoli	carbamazepine	rifampin	dexamethasone	ethanol	efavirenz
brussel sprouts	norethindrone	secobarbital	rifampin	isoniazid	nevirapine
char-grilled meat	prednisone				barbiturates
insulin	rifampin				carbamazepine
Methyl					glucocorticoids
cholanthrene					phenobarbital
nafcillin					phenytoin
beta-naphthoflavone					pioglitazone
omeprazole					rifampin
tobacco					St. John's Wort
					troglitazone
Inhibitors					
1A2	**2C19**	**2C9**	**2D6**	**2E1**	**3A4,5,7**
amiodarone	cimetidine	amiodarone	amiodarone	disulfiram	delaviridine
cimetidine	felbamate	fluconazole	celecoxib	diethyl-dithiocarbamate	indinavir
fluoroquinolones	fluoxetine	fluvastatin	chlorpheniramine		nelfinavir
fluvoxamine	fluvoxamine	fluvoxamine	cimetidine		ritonavir
furafylline	indomethacin	isoniazid	clomipramine		saquinavir
Interferon	ketoconazole	lovastatin	cocaine		amiodarone
methoxsalen	lansoprazole	paroxetine	doxorubicin		cimetidine
mibefradil	omeprazole	phenylbutazone	fluoxetine		ciprofloxacin
ticlopidine	paroxetine	probenicid	halofantrine		clarithromycin
	probenicid	sertraline	haloperidol		diltiazem
	ticlopidine	sulfaphenazole	levomepromazine		erythromycin
	topiramate	teniposide	methadone		fluconazole
		trimethoprim	mibefradil		fluvoxamine
		zafirlukast	moclobemide		gestodene ++
			paroxetine		grapefruit juice
			quinidine		itraconazole
			ranitidine		ketoconazole
			ritonavir		mifepristone
			terbinafine		nefazodone
					norfloxacin

16.2 CYTOCHROME P450 ENZYME INDUCER/INHIBITOR COMPOUNDS* (CONT'D)

Inhibitors					
1A2	**2C19**	**2C9**	**2D6**	**2E1**	**3A4,5,7**
					norfluoxetine
					mibefradil
					troleandomycin
					diethyl-dithiocarbamate