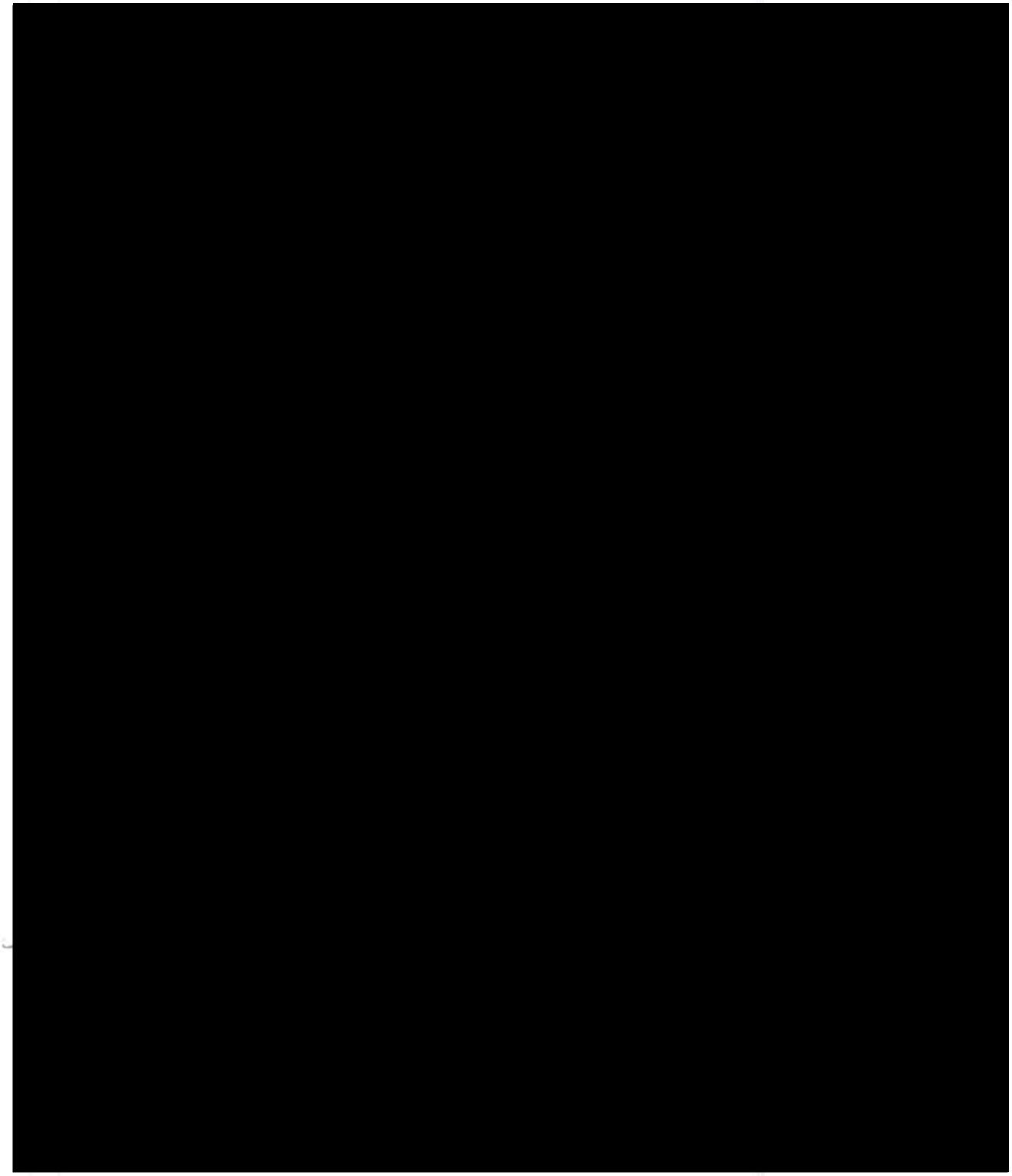
NCT05098028

CLINICAL RESEARCH PROTOCOL

A Phase 2a Randomized, Double-Blind, Placebo-Controlled Study to Characterize the Pharmacokinetics and Pharmacodynamics of Rifaximin Novel Formulations in Patients with Sickle Cell Disease

Protocol ID:	RBSC2161
IND Number:	150048
Clinical Phase:	2a
Treatment Regimen:	Rifaximin Extended Release (ER) or Delayed Extended Release (DER) 40 or 80 mg or Placebo BID for 4 weeks
Sponsor:	Salix Pharmaceuticals, Inc. a division of Bausch Health US, LLC 400 Somerset Corporate Blvd. Bridgewater, NJ, USA 08807 Main Office: +1.908.927.1400
Medical Monitor:	[redacted]
Principal Investigator:	Multicenter
Protocol Date:	12 April 2022, Version 6.0 (Amendment 5) 30 March 2022, Version 5.0 (Amendment 4) 12 January 2022, Version 4.0 (Amendment 3) 22 November, 2021Version 3.0 (Amendment 2) 16 AUGUST 2021, Version 2.0 (Amendment 1) 06 MAY 2021, Version 1.0 (Original)



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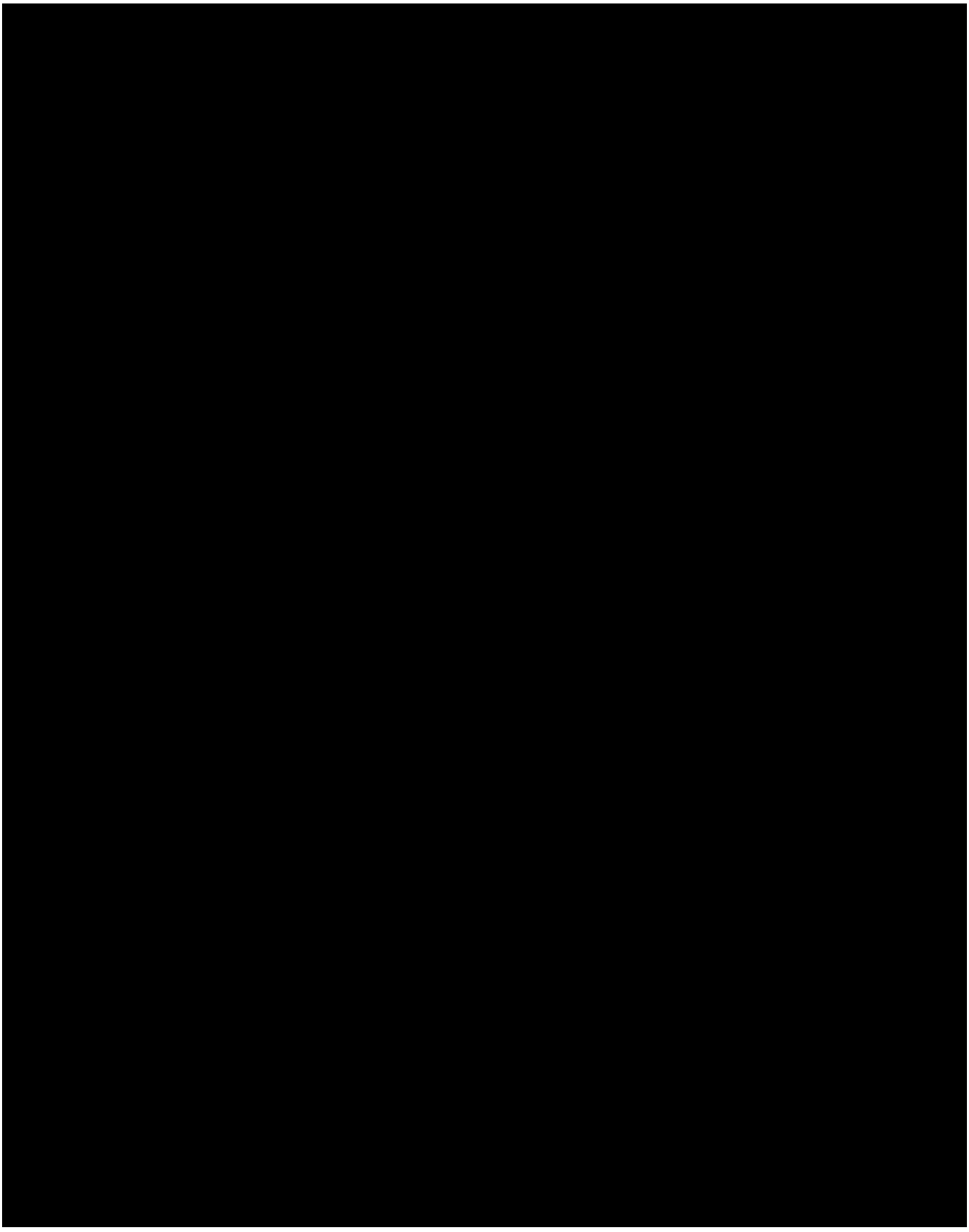

INVESTIGATOR PROTOCOL AGREEMENT

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, with any future amendments, and with any other study conduct procedures provided by the Sponsor, Salix Pharmaceuticals, Inc. a division of Bausch Health US, LLC (Salix).
- Not to implement any deviations from or changes to the protocol without agreement from the Sponsor and prior review and written approval from the Institutional Review Board/Ethics Committee (IRB/EC), except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- That I am thoroughly familiar with the appropriate use of the investigational drug(s), as described in this protocol and any other information provided by the Sponsor including, but not limited to the following: the current Investigator's Brochure or equivalent document provided by Salix, and the approved product label, if applicable.
- That I am aware of, and will comply with, "good clinical practices" (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the investigational drug(s) and of their study-related duties and functions as described in the protocol.
- To periodic on-site monitoring of the electronic case report forms (eCRFs) and source documents by Salix or designee and to on-site inspection of eCRFs and source documents by appropriate regulatory authorities, including but not limited to, the US Food and Drug Administration (FDA), local governing regulatory bodies, and IRB/EC inspectors.
- That I have been informed that certain regulatory authorities require the Sponsor to obtain and supply details about the Investigator's ownership interest in the Sponsor or study drug, and more generally about his/her financial ties with the Sponsor. Salix will use and disclose the information solely for the purpose of complying with regulatory requirements.

  Hence, I:
  - Agree to supply Salix with any information regarding ownership interest and financial ties (including those of my spouse and dependent children);
  - Agree to promptly update this information if any relevant changes occur during the course of the study and for 1 year following completion of the study; and
  - Agree that Salix may disclose this information about such ownership interests and financial ties to regulatory authorities.

Investigator Name [Print]	**Investigator Signature**	**Date**

SYNOPSIS

PROTOCOL TITLE	A Phase 2a Randomized, Double-Blind, Placebo-Controlled Study to Characterize the Pharmacokinetics and Pharmacodynamics of Rifaximin Novel Formulations in Patients with Sickle Cell Disease
PROTOCOL NUMBER	RBSC2161
SPONSOR	Salix Pharmaceuticals, Inc., a division of Bausch Health US, LLC
INVESTIGATIONAL PRODUCT	Rifaximin Extended Release (ER) 20 mg Capsules, Delayed Extended Release (DER) 20 mg Capsules, and placebo capsules
OBJECTIVES	The objectives of this study are: • To characterize the pharmacokinetics/pharmacodynamics (PK/PD) of rifaximin ER and DER in subjects with sickle cell disease (SCD) • To assess the safety and tolerability of rifaximin ER and DER
TREATMENT	Subjects will be enrolled and randomized 2:2:1:2:2:1 to one of 6 parallel arms to receive oral treatment twice daily (BID) for approximately 29 days: • Group 1: 40 mg rifaximin ER, BID • Group 2: 40 mg rifaximin DER, BID • Group 3: Placebo for 40 mg rifaximin, BID • Group 4: 80 mg rifaximin ER, BID • Group 5: 80 mg rifaximin DER, BID • Group 6: Placebo for 80 mg rifaximin, BID
STUDY DESIGN	This is a randomized, double-blind, placebo-controlled study in SCD subjects with a history of Vaso-occlusive Crises (VOCs). Approximately 60 subjects with SCD will be enrolled and randomized: 12 subjects in each of four active rifaximin groups and 6 subjects in each of 2 placebo groups. After a Screening period of up to 21 days, eligible subjects will visit the study site on Day 1. After all Baseline assessments (Day 1 pre-dose) are completed, the study staff will administer subjects their AM dose of assigned treatment. The subject will remain at

	the study site for 8 hours after dosing and safety parameters and samples for PK (rifaximin and 25-desacetyl rifaximin) will be collected for 8 hours post-dose. After completing all 8-hour assessments, and the safety parameters are acceptable to the Investigator, the subjects can go home. Subjects will administer their Day 1 PM dose at home. Subjects will return to the study site for outpatient visits on Days 8, 15, and 29 (±1 day each) for pre-dose PK, PD, and safety assessments. At the final visit (Day 29 [±1 day]), subjects will administer only the AM dose of treatment. Subjects will remain at the clinical site through 8 hours post-dose for collection of PK samples. Subjects will return to the site on the following days for post-final dose PK assessments as follows: Day 30: • 24 hours post-dose • 30-hours post-dose Day 31: 48 hours post-dose Day 32: 72 hours post- dose Two weeks after the last dose subjects will return to the study site for a final follow-up visit on Day 43 (±3 days). Subjects will be discharged from the study after completing all assessments on the Day 43 visit. At every study visit, subjects will also report opioid use since their previous visit. Site staff will record opioid use. Safety, tolerability, PK, and PD data will be collected during each visit at the timepoints listed in the Schedule of Assessments.
STUDY DURATION	Subject participation will be up to 9 weeks, including a Screening Period of up to 21-days, a 4-week Treatment Period, and 2-week Follow-up.
SUBJECT POPULATION	Male and non-pregnant, non-nursing females ≥18 and ≤70 years with SCD and at least 2 VOCs in the previous 12 months.
NUMBER OF SUBJECTS	Up to 60; 12 subjects in each of 4 active rifaximin groups and 6 subjects in each of 2 placebo groups.

NUMBER OF CENTERS	Approximately 20
LOCATION	Multicenter study in North America, Middle East, and Africa
CRITERIA FOR INCLUSION	A subject will be eligible for inclusion in this study if he/she meets all the following criteria: 1. Subject must have the ability and willingness to sign a written informed consent form. 2. Subject is between the ages of 18 to 70 years old (inclusive) at the time of consent. 3. Subject has SCD of any genotype (HbSS, HbSC, HbS β-thalassemia). If the subject's genotype has not been previously documented, genotyping will be performed during Screening using high-performance liquid chromatography (HPLC)/electrophoresis. 4. Subject must have experienced at least 2 VOCs within the 12 months prior to Screening. A VOC is defined as: a. The occurrence of appropriate symptoms consistent with a painful crisis, acute chest syndrome (ACS), or priapism (Section 2.2.3) , and b. Requires a visit to a medical facility and/or healthcare professional, and c. Receipt of either a parenteral or oral opioid or NSAID analgesia. 5. If subject is receiving hydroxyurea (HU)/hydroxycarbamide (HC), subject must have been receiving the treatment for at least 6 months prior to Screening and must agree to maintain the same dose and schedule for the duration of the study. 6. Subjects must have laboratory values at Screening as follows: a. Absolute Neutrophil Count (ANC) $\geq 1.0 \times 10^9$/L b. Platelets $\geq 75 \times 10^9$/L c. Hemoglobin (Hgb) ≥ 6.0 g/dL d. Glomerular filtration rate (GFR) ≥ 45 mL/min/1.73 m^2 using the CKD-EPI formula e. Total bilirubin ≤ 15 mg/dL

	f. Alanine transaminase (ALT) ≤ 3.0 x ULN g. International Normalized Ratio (INR) ≤ 2.0 7. Eastern Cooperate Oncology Group (ECOG) performance status ≤ 2 8. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must have a negative serum pregnancy test at Screening and agree to use standard prevention methods for the duration of the study.
CRITERIA FOR EXCLUSION	A subject <u>will not</u> be eligible for inclusion in this study if any of the following criteria apply: 1. Subject is receiving concomitant treatment with voxelotor, crizanlizumab, or L-glutamine. 2. Subject has any history of stem cell transplant, is planning to begin or has received in past 30 days. 3. Subject experiences an acute VOC, requiring a visit to a medical facility and/or healthcare professional, ending within 7 days prior to Day 1 dosing. 4. Subject has received any blood products within 30 days prior to Day 1 dosing. 5. Subject has uncontrolled liver disease or renal impairment, ulcerative colitis, Crohn's disease, or other chronic GI disorder. 6. Subject has received active treatment in another investigational trial within 30 days or 5 half-lives of the last dose of the investigational agent, whichever is greater, prior to Screening. 7. Subject has received penicillin prophylaxis or antibiotics for treatment of infection within 30 days or 5 half-lives of the treatment, whichever is greater, prior to Screening. 8. Subject has a significant medical condition that required hospitalization (other than for a VOC) within 2 months prior to Screening. 9. Subject is planning on undergoing an exchange transfusion during the duration of the study or has completed one within 4 weeks prior to Day 1 dosing.

	10. Subject has a hypersensitivity to rifaximin, rifampin, rifamycin antimicrobial agents, or any components of rifaximin ER and DER. 11. Subject is pregnant or a nursing woman. 12. Subject has a history of illicit drug use or abuse, either documented or in the opinion of the Investigator. 13. Subject is using any medication that is known to inhibit or induce CYP3A4, P-gp and OATP1B1/B3 within 30 days or 5 half-lives, whichever is longer, prior to Day 1 dosing, or in the opinion of the Investigator, may affect the evaluation of the study product or place the subject at undue risk. 14. Subject has had any prior gastrointestinal surgery which has altered the anatomy of the esophagus, stomach, or small/large intestine (with the exception of appendectomy, cholecystectomy, and fundoplication). 15. Subject has had a colonoscopy or sigmoidoscopy within 30 days prior to Day 1 or plans to undergo such a procedure during the duration of the study. 16. Subject has used bowel prep, laxative, or enema within 30 days prior to Day 1. 17. Subject has a bleeding disorder including, but not limited to, acquired or congenital platelet function defects, disseminated intravascular coagulation (DIC), bleeding factor deficiencies, hemophilia, idiopathic thrombocytopenia purpura (ITP), or von Willebrand's disease. 18. Subject is planning to undergo a major surgical procedure during the duration of the study. 19. Subject has a positive test for human immunodeficiency virus (HIV)1 or HIV2. 20. Subject has an active Hepatitis B infection (HBsAg positive). Prior infection that is not active (i.e., HBsAg negative, HBcAb positive, and HBsAb positive) is permitted. 21. Subject has a positive test for Hepatitis C (HCV RNA). Prior infection with spontaneous resolution or sustained resolution for ≥ 24 weeks after cessation of antivirals is permitted.

	22. Subject has an active COVID-19 infection or complication(s) related to COVID-19 infection that are unresolved or, in the opinion of the Investigator, may affect evaluation of the study drug or place the subject at undue risk. 23. Subjects has received a vaccine (including COVID-19 vaccine) within 2 weeks prior to Screening. If subject has received their first of two COVID-19 vaccination doses, as applicable, they must wait for at least 2 weeks after receiving the second dose, and be symptom-free, prior to beginning Screening. Subject must not be planning for COVID-19 or other vaccinations while on study. 24. Subject has a malignant disease. Exceptions include malignancies that were treated curatively and have not recurred within 2 years prior to study treatment, completely resected basal cell and squamous cell skin cancers, and any completely resected carcinoma in situ. 25. Subjects has prolonged QT interval as assessed by ECG history within the past 3 months. For subjects with no historical ECG information, subject has a resting QTcF $\geq$ 460 msec for males and $\geq$ 470 msec for females at Screening. 26. Subject has any unstable cardiac condition that, in the opinion of the Investigator, may worsen during the study or interfere with successful evaluation of the study treatment. 27. Subject has a serious mental or physical illness which, in the opinion of the Investigator, would compromise participation in the study. 28. Subject has any condition which, in the opinion of the Investigator, is likely to interfere with the successful collection of the measurements required for the study. 29. Subject is unable to understand or comply with study instructions and requirements.
CRITERIA FOR EVALUATION	<u>Pharmacokinetics and Pharmacodynamics</u>: Full rifaximin and 25-desacetyl rifaximin PK profiles following administration on Days 1 and 29 and truncated profiles on Days 8 and 15 will be evaluated.

	Blood samples for PK analysis will be collected at the following timepoints: • Day 1: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-dose • Day 8 and Day 15: pre-dose and 0.5, 1, and 2-hours post-dose. • Day 29: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-final dose. • Day 30: 24 and 30 hours post-final dose • Day 31: 48 hours post-final dose • Day 32: 72 hours post-final dose • During medical facility visit for VOC with estimated time of last dose, when possible. PD biomarkers (absolute neutrophil count [ANC], circulating aged neutrophils [CANs], serum CD62L, high sensitivity C-reactive protein [hsCRP], the gut permeability biomarkers intestinal fatty-acid binding protein [iFABP], lipopolysaccharide [LPS], and the gut bacteria biomarker urine 3-indoxyl sulfate [3-IS Urine]) will be collected at the following timepoints: • Day 1: pre-dose • Day 8, Day 15, and Day 29: during each visit – pre dose • Day 31 • Day 43 • During medical facility visit for VOC, when possible. <u>Safety</u>: Safety will be assessed throughout the duration of the study by collection of adverse events (AEs), vital signs (systolic and diastolic blood pressure, heart rate, oral body temperature, and oxygen saturation), clinical safety laboratory tests (hematology, chemistry, urinalysis, and C-reactive protein), physical exams (PEs), and electrocardiograms (ECGs). Exploratory Assessments: At every study visit, subjects will report, and the site staff will record, opioid use since their previous visit. Information on any potential VOCs experienced during the study will be collected as follows:

	• Dates and duration of each episode. A VOC is defined as: o The occurrence of appropriate symptoms consistent with a painful crisis, acute chest syndrome (ACS), or priapism, and o Requires visit to a medical facility, and o Receipt of either a parenteral or oral opioid or NSAID analgesia. • Categorization of VOCs. Subcategories are uncomplicated pain crises, acute chest syndrome (ACS), hepatic sequestration, splenic sequestration, and priapism (requiring healthcare visit). • Intravenous opioid analgesia (IOA) and oral opioid use during VOCs, including date/time of each start and stop of treatment, dose, drug, inpatient/outpatient setting, and date/time of readiness-to-discharge.
SAMPLE SIZE	The total of 60 subjects is not based on formal statistical considerations. Twelve (12) subjects per arm receiving active rifaximin is considered adequate to evaluate the PK/PD of rifaximin ER and DER.
STATISTICAL METHODOLOGY	Pharmacokinetics and Pharmacodynamics: PK will be evaluated by noncompartmental analysis following the first dose on Day 1 and the final dose on Day 29. Steady state will be assessed for all subjects based on C_{trough} on Day 8, Day 15, and Day 29. Following the first dose on Day 1, C_{max}, T_{max}, AUC_{0-8h}, AUC_{last}, and MR_AUC_{0-8h} will be estimated. Following the final dose on Day 29, C_{max}, T_{max}, C_{min}, C_{avg}, AUC_{0-8h}, CL/Fss, Vz/Fss, R_{Cmax}, R_{AUC}, AUC_{last}, AUC_{inf}, λz, $t_{1/2}$, and MR_AUC_{0-8h} will be estimated. Additional PK parameters maybe calculated if it deemed necessary. The placebo arms will be combined for all descriptive summaries of subject disposition, demography and Baseline characteristics, PD, VOCs, and safety. ANC, CANs (count and % neutrophils), and serum CD62L levels will be summarized by treatment with quantity and change from

	Baseline (pre-dose at Day 1) at each visit for which they are collected. iFABP, LPS, hsCRP, and urine 3-indoxyl sulfate levels will be summarized by treatment with descriptive statistics and change from Baseline (pre-dose at Day 1) at each visit. A dose-response analysis may be conducted to determine the relationship between daily rifaximin ER and DER dosing and reduction of CANs. Dose-response relationships following the final dose on Day 29 may also be assessed for other PD endpoints, if warranted. The relationship between rifaximin PK parameters (C_{trough}, $C_{max,ss}$, $C_{ss,avg}$) and each PD endpoint (ANC, CANs, serum CD62L levels, serum iFABP, serum LPS, serum hsCRP, and urine 3-inoxyl sulfate) may be evaluated following the final dose on Day 29. Additionally, a population PK/PD model may be developed as a separate analysis to explore exposure-response. If conducted, this analysis will be reported separately from the Clinical Study Report. <u>Safety</u>: All AEs occurring during the study will be recorded and classified based on MedDRA terminology. Adverse events that occur after the start of treatment (treatment-emergent adverse events [TEAEs]), will be summarized; all AEs will be included in by-subject listings. TEAEs will be summarized for the entire population, by treatment group, and by severity and relationship to study medication. Each subject will be counted only once within a system organ class or a preferred term by using the adverse event with the highest severity or greatest relationship, respectively, within each category. All SAEs, AEs leading to premature withdrawal from the study, and all deaths will be listed. Vital signs will be summarized at Baseline and for each visit. Changes from Baseline in vital sign measurements will also be summarized. Clinical safety laboratory data will be summarized at Baseline, at each visit, and as changes from Baseline in laboratory parameters. Abnormal findings in ECGs and physical examinations will be summarized and listed. Data permitting, the number and duration of VOCs (overall and by subcategory) and SCD-associated medical facility visits that do

	not meet the definition of a VOC may be summarized by treatment with descriptive statistics. Use of opioids, both intravenous and oral, may be summarized by treatment group. By-subject listings will include the opioid, route of administration, dose, and duration of treatment. Summaries will include descriptive statistics for oral and intravenous use, as well as morphine milligram equivalents (MME).

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ABX	Antibiotics
ANC	Absolute neutrophil count
ACS	Acute chest syndrome
ADL	Activities of daily living
AE	Adverse event
ALT	Alanine aminotransferase
BID	Twice daily
BP	Blood pressure
CBC	Complete blood count
CAN	Circulating aged neutrophils
CDAD	Clostridium difficile-associated diarrhea
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COVID-19	Corona virus disease 2019
CTCAE	Common Terminology Criteria for Adverse Events
DER	Delayed extended release
DIC	Disseminated intravascular coagulation
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
EOS	End of study
EOT	End of treatment
ER	Extended release
ET	Early termination
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GFR	Glomerular filtration rate
Hb	Hemoglobin
HBcAb	Hepatitis B core antibody
HBsAg	Hepatitis B serum antigen
HC	Hydroxycarbamide
HCV	Hepatitis C-virus
HR	Heart rate

Abbreviation	Definition
hsCRP	High sensitivity C-reactive protein
HU	Hydroxyurea
ICF	Informed Consent Form
ICH	International Conference on Harmonization
iFABP	Intestinal fatty-acid binding protein
INR	International normalized ratio
IOA	Intravenous opioid analgesic
IRB	Institutional Review Board
ITP	Idiopathic thrombocytopenia purpura
LPS	Lipopolysaccharide
LTFU	Lost to follow up
MedDRA	Medical Dictionary for Regulatory Activities
MME	Morphine milligram equivalents
NCA	Non-compartmental analysis
NSAID	Non-steroidal anti-inflammatory drug
PD	Pharmacodynamics
PHI	Protected health information
PK	Pharmacokinetics
PPI	Proton pump inhibitor
RBC	Red blood cell
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SCA	Sickle cell anemia
SCD	Sickle cell disease
SCT	Stem cell transplant
SD	Standard deviation
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
US	United States
3-IS Urine	Urine 3-indoxyl sulfate
VOC	Vaso-occlusive crisis
WOCBP	Women of child-bearing potential

1.0 INTRODUCTION

1.1 Disease Background

Sickle cell disease (SCD) is a related group of common and rare hemoglobin (Hb) genotypes in which all affected individuals are either homozygotes for the sickle Hb (HbS) mutation of the β-globin subunit or compound heterozygotes for HbS and another globin gene mutation [Steinberg 2012]. The various forms of SCD are based on inheritance patterns from an individual's parents. The most common type of SCD, HbSS features two sickle cell genes ("S"); one "S" gene is inherited from each parent. HbSS genotype presents as sickle cell anemia (SCA) and is the most severe form of SCD. Another common yet milder type of SCD is HbSC. A single sickle cell gene ("S") is inherited from one parent, and an abnormal Hb gene "C" is inherited from the other parent. A third type of SCD is HbS β-thalassemia. In this genotype, a single sickle cell gene ("S") is inherited from one parent and another gene for β-thalassemia is inherited from the other parent. HbS β-thalassemia can be further divided into two subtypes: subtype HbS β0-thalassemia, which is a more severe form of SCD, and subtype HbS β+-thalassemia, which is a milder form of SCD. Individuals with the sickle cell trait (HbAS) generally do not exhibit signs and symptoms of SCD. In this genotype, a single sickle cell gene is inherited from one parent. However, a normal gene "A" is inherited from the other parent. Nevertheless, there is the risk of passing the sickle cell gene to offspring [CDC 2020; da Guarda 2020; Steinberg 2012].

Hb in SCD has the physical tendency for polymerization in deoxygenated conditions. Among the many changes that result from the damage to the sickle RBC (SS-RBC) membrane is the propensity to adhere to the vascular endothelium. SS-RBCs and activated endothelial cells can produce a proinflammatory environment that is exacerbated during episodes of vaso-occlusive crisis (VOC) [Manwani 2013]. SS-RBCs are rigid and do not easily flow through the microcirculation; they exhibit changes in solubility and molecular stability and can obstruct the vasculature, resulting in the clinical manifestations of SCD [Darbari 2020].

A hallmark symptom of SCD is a painful VOC. An interplay of the various pathologic processes in SCD perpetuates and feeds forward an inflammatory priming that, in the presence of a precipitating event, can lead to a VOC. Precipitating events for a VOC may include hypoxia, infection, dehydration, acidosis, alcohol intoxication, emotional stress, pregnancy, and cold weather (vasospasm). A VOC is initiated and sustained by interactions among sickle cells, endothelial cells, and plasma constituents. There is a cytokine mediated exchange implicated in the development of a VOC between sickled erythrocytes, aged and activated neutrophils, inflammatory messenger molecules, monocytes, platelets, and endothelium. Activated neutrophils play a pivotal role in initiating a VOC and act as central elements during the crisis. Higher neutrophil counts correlate with more severe disease manifestation, including earlier death, increased incidence of silent brain infarcts, hemorrhagic stroke, and acute chest syndrome (ACS) [Darbari 2020, Lim 2016].

VOCs are responsible for a wide variety of clinical complications of SCD, including pain syndromes, acute chest syndrome (ACS), stroke, priapism, leg ulcers, spontaneous abortion, renal

insufficiency, and multiorgan failure leading to death. Frequency of VOCs, along with ACS, is the most common predictor of death in patients with SCD. Thus, early identification, treatment, and prevention of VOCs could significantly impact the natural course of the disease. During a VOC, patients present with acute severe pain that is often described as throbbing and sharp. Common sites of pain include the lower back, joints, and extremities [Darbari 2020, Yale 2000].

1.2 Rationale for the Use of Rifaximin in the Treatment of Vaso-Occlusive Crises in Patients with Sickle Cell Disease

Recent studies have identified various cellular and molecular factors that contribute to the pathophysiology of SCD. These include the complex interaction between erythrocytes, platelets, vascular endothelial surface molecules, and neutrophils. Both activated and aged neutrophils may be immobilized on vascular endothelium and form the nidus for sickled erythrocytes to adhere, eliciting a VOC [Zhang 2016, Lim 2019].

Circulating aged neutrophils (CANs), a subset of neutrophils with high surface expression of CXCR4 and low CD62L (L-selectin), may be significantly elevated during a VOC. CD62L is shed on neutrophil activation and acts as a sensitive marker. The expression of CD62L on neutrophils decreases in patients with SCD experiencing a VOC [Lard 1999].

In several scientific experiments, Zhang, et. al. demonstrated a relationship between the microbiome and neutrophil aging/activation. Treatment of mice with broad-spectrum antibiotics (ABX) led to a highly efficient depletion and dramatic alterations in the composition of the gut microbiota. Microbiota depletion resulted in significant and selective reductions of neutrophil numbers in the circulation. Both the percentages and numbers of aged neutrophils were significantly reduced in ABX-treated mice. To assess the role of neutrophil aging in an applicable model, genetically engineered mice with SCD were studied. SCD mice exhibited expansion of all major white blood cell subsets compared with healthy control mice. ABX-mediated microbiota depletion led to a significant and specific decrease of neutrophils, but not the other leukocyte populations. Importantly, in mice with SCD, aged neutrophils populations were increased by > 10-fold, and their expansion was completely negated by microbiota depletion [Zhang 2015]. Therefore, modulating intestinal microbial composition may be a therapeutic option in SCD to reduce VOC by decreasing both activated and aged neutrophils.

Lim, et. al. hypothesized that an intestinal dysbiosis is associated with VOC episodes characteristic of SCD. Abnormalities in the integrity of the intestinal wall/mucosa, a chronic dysbiosis of the intestinal flora, or both are likely to occur in patients with SCD. The intestinal mucosa and the ability of the gut wall to support a normal microbiome in patients with SCD are impaired, due to periodic or global low-grade intestinal ischemia that occurs as a result of VOCs in the splanchnic vasculature. The compromised intestinal barrier renders patients with SCD more susceptible to recurrent translocation of intestinal bacteria into the submucosa and bloodstream. Although the bacterial inocula are not numerous enough to cause overt infections, they can activate an immune response [Lim 2016, Lim 2018].

Frequent microbial and pathophysiologic changes have been observed in the intestines of patients with SCD, including enterocyte injury, altered microbial composition, increased permeability, and bacterial overgrowth. Dutta, et. al. measured serum intestinal fatty-acid binding protein (iFABP) and lipopolysaccharides (LPS) levels in patients with SCD. Both iFABP and LPS are markers for intestinal injury and impaired bowel wall integrity ("leaky gut"). Levels were then compared to patients with iron deficiency anemia, but without SCD. Individuals with SCD had significantly higher iFABP (P = 0.04) and LPS (P = 0.03), indicating intestinal injury and increased intestinal bacterial translocation into the systemic circulation. In addition, serum soluble CD62L (P = 0.04) was elevated, and soluble CD62L correlated positively with CANs (R = 0.7, P = 0.03) and LPS (R = 0.66, P = 0.027). These results provide evidence of the presence of intestinal compromise and increased gut permeability in patients who have SCD [Dutta 2019]. It has been demonstrated that rifaximin modulation of ileal microbiome communities prevents impairment of intestinal barrier function induced by chronic stress. [Xu 2014].

Lim, et al, also hypothesized that rifaximin is an ideal antimicrobial agent for gut decontamination in patients with SCD and will reduce hospital admissions due to painful VOC through modification of the intestinal microbiome and a reduction of CANs. In August 2019, Lim et al completed a single-arm, open-label study of 13 adult patients with SCD (HbSS, HbSC, HbS β-thalassemia), administered rifaximin 550 mg BID for 6 months (NCT03719729). The primary objective of the study was to determine the feasibility and toxicity profile of long-term rifaximin in patients with SCD [Dutta 2020, Lim 2019].

Results from the Phase 2 study demonstrated that treatment with rifaximin significantly reduced the frequency of painful VOCs and the number of days patients needed IOA compared with the prior 12 months when patients were not on rifaximin therapy. In the 12 patients evaluated, there was a mean decrease from the expected 2.25 VOCs (range 1–6.5) per 6 months to 1 VOC per 6 months (range 0–4) in patients receiving rifaximin (P = 0.003). There was also a significant decrease with rifaximin in days needing IOA over the 6-month study period (median 9 days; range 1–55; P = 0.008). Furthermore, the total number of days that patients with SCD required IOA was reduced from the expected 254 days over 6 months to 98 days during the 6-month rifaximin treatment period.

Serum CD62L was serially measured and found to be elevated in 9 patients at baseline, but then consistently declined to levels observed in non-SCD controls (P < 0.001). In addition, most (75%) patients exhibited > 10% CANs in their venous blood before starting rifaximin (median 12.6; range 6–40) and levels of CANs dropped significantly in all patients after starting rifaximin to below 5% (P = 0.005). Drops in CANs occurred as early as 2 weeks after initiation of rifaximin and remained low throughout the 6-month study period. Likewise, baseline serum iFABP was elevated in all patients and decreased significantly (P = 0.008) with rifaximin in all patients to levels comparable with non-SCD controls.

Overall, rifaximin was well tolerated. Mild nausea was common in the first 1–2 weeks of therapy and occurred in 8 patients. Other adverse events included self-limiting diarrhea (n = 1), which

occurred during the first month of therapy and polydipsia (n = 2). No episodes of *Clostridium difficile* colitis were reported. Laboratory analyses did not show any significant change in Hb, white cell counts, serum lactate dehydrogenase, bilirubin, or haptoglobin due to rifaximin therapy [Lim 2019]. This was the first study in humans showing the potential efficacy of microbial modulation on VOCs in patients with SCD and forms the basis for further investigation.

Rifaximin extended release (ER) and delayed extended release (DER) Capsules are new pharmaceutical formulations designed to have better dissolution properties within the lumen of the gut to target different areas of the GI tract. The ER Capsule is a controlled release solid oral dosage form designed to target delivery of rifaximin to the upper GI tract, and the DER Capsule is a controlled release solid oral dosage form designed to target delivery of rifaximin to the mid-GI tract.

The current study is designed to evaluate the PK of ER and DER Capsules and to assess the response to treatment of PD markers associated with VOCs including ANC, CANs, serum CD62L, hsCRP, the gut permeability biomarkers iFABP and LPS, and the gut bacteria biomarker urine 3-indoxyl sulfate in subjects with SCD. The safety and tolerability of these new formulations will also be evaluated in this patient population.

2.0 OBJECTIVES AND ENDPOINTS

2.1 Objectives

The objectives of this study are:

- To characterize the pharmacokinetics/pharmacodynamics (PK/PD) of rifaximin ER and DER in subjects with sickle cell disease (SCD)
- To assess the safety and tolerability of rifaximin ER and DER

2.2 Endpoints

2.2.1 Pharmacokinetics and Pharmacodynamics

Full rifaximin and 25-desacetyl rifaximin plasma PK profiles following administration on Days 1 and 29 and C_{max} and C_{trough} on Days 8, 15, and 29 will be evaluated.

Blood samples for PK analysis will be collected at the following timepoints:

- Day 1 and Day 29: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-dose.
- Day 8 and Day 15: pre-dose and 0.5, 1, and 2-hours post-dose.
- Day 29: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-final dose
- Day 30: 24 and 30 hours post-final dose
- Day 31: 48 hours post-final dose
- Day 32: 72 hours post-final dose
- During medical facility visit for VOC with estimated time of last dose, when possible.

PD biomarkers (ANC, CANs, serum CD62L, hsCRP, the gut permeability biomarkers serum iFABP and LPS, and the gut bacteria biomarker urine 3-indoxyl sulfate) will be collected at the following timepoints:

- Day 1: pre-dose
- Day 8, Day 15, and Day 29: during each visit – pre dose
- Day 31
- Day 43
- During medical facility visit for VOC, when possible.

2.2.2 Safety

Safety will be assessed throughout the duration of the study by collection of adverse events (AEs), vital signs (systolic and diastolic blood pressure, heart rate, oral body temperature, and oxygen saturation), clinical safety laboratory tests (chemistry, coagulation, hematology, urinalysis, and high sensitivity C-reactive protein (hsCRP), physical examinations, and electrocardiograms (ECGs). The safety endpoints are the following:

- Incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs)
- Clinically significant changes from Baseline in clinical laboratory results
- Changes from Baseline in vital signs

2.2.3 Exploratory Assessments

Information on any potential VOCs experienced during the study will be collected as follows:

- Dates and duration of each episode. A VOC is defined as:
    - The occurrence of appropriate symptoms consistent with a painful crisis, acute chest syndrome (ACS), or priapism, and
    - Requires visit to a medical facility, and
    - Receipt of either a parenteral or oral opioid or NSAID analgesia.
- Categorization of VOCs. Subcategories will consist of:
    - Uncomplicated pain crisis, defined as a painful crisis that is NOT classified as an acute chest syndrome, hepatic sequestration, splenic sequestration, or priapism.
    - Acute Chest Syndrome (ACS), defined as a finding of a new pulmonary infiltrate involving at least one complete lung segment that was consistent with alveolar consolidation, but excluding atelectasis (as indicated by chest X-ray). Must also present with at least one of the following signs or symptoms: patient-reported chest pain, body temperature of more than 38.5 °C, tachypnea, wheezing, or cough.
    - Hepatic sequestration, defined as findings of right upper quadrant pain, an enlarged liver, and an acute decrease in hemoglobin concentration.
    - Splenic sequestration, defined as findings of left upper quadrant pain, an enlarged spleen, and an acute decrease in hemoglobin concentration.
    - Priapism, defined as having a sustained penile erection requiring a visit to a medical facility.

- Intravenous opioid analgesia (IOA) and oral opioid use during VOCs, including date/time of each start and stop of treatment, dose, drug, inpatient/outpatient setting, and date/time of readiness-to-discharge.

3.0 INVESTIGATIONAL PLAN

3.1 Overview of Study Design

Study RBSC2161 is a Phase 2a, randomized, double-blind, placebo-controlled, multicenter study to evaluate the pharmacokinetics (PK), pharmacodynamics (PD) safety and tolerability of rifaximin ER and DER in subjects with SCD. Exploratory assessments of the effect of rifaximin ER and DER on the incidence of VOCs will also be explored.

Approximately 60 subjects who successfully complete the Screening Period will enter the Treatment Period and will be randomized to one of six treatment groups. Approximately 20 study centers are expected to participate in the study.

Objectives and endpoints for the study are provided in Section 2. Assessments of PK/PD and safety, and documentation of VOCs (Appendix 1 and Section 7.3) will be performed during clinic visits at Baseline (Day 1), Day 8, Day 15, and Day 29 (End of Treatment/Early Termination). All subjects will also complete an End of Study (EOS) Visit 2 weeks later (Day 43; End of Study) for final assessments. Assessments will also be conducted when a subject visits a healthcare facility for treatment of a VOC. If a subject prematurely discontinues treatment, every attempt will be made to obtain EOT and EOS assessments and subjects should be followed until the end of the 43-day trial.

Before study subjects can be screened, the laboratory where the CANs assessment will be performed shall undergo a qualification run. This is to ensure that the flow cytometry assessment for CANs can be performed at the local site as outlined in the Laboratory Manual. The qualification run will be performed with blood from healthy, adult, non-pregnant, and capable volunteers of general good health (and non-sickle cell disease subjects). Approximately 25 healthy volunteers (one per site, or per local lab) is expected to participate. Healthy volunteers will visit the study center once for this assessment (single blood draw) and it should take no more than 30 minutes to complete the testing.

3.2 Treatment Assignment/Randomization

Subjects who meet the eligibility criteria and have given written informed consent will be randomized in a 2:2:1:2:2:1 allocation to one of 6 treatment groups. Approximately 12 subjects will be randomized to each active rifaximin treatment and approximately 6 subjects to each placebo group. The treatment groups will be run in parallel.

- Group 1: 40 mg rifaximin ER, BID
- Group 2: 40 mg rifaximin DER, BID
- Group 3: Placebo for 40 mg rifaximin, BID

- Group 4: 80 mg rifaximin ER, BID
- Group 5: 80 mg rifaximin DER, BID
- Group 6: Placebo for 80 mg rifaximin, BID

3.3 Procedures for Breaking the Blind

Subjects, study staff, and the Sponsor will be aware of the dose groups (2 x 20 mg capsules BID or 4 x 20 mg capsules BID) but will be blinded to active or placebo. Only in the case of an emergency, when knowledge of the study drug is essential for the clinical management or welfare of the subject, will the Investigator be allowed to unblind a subject's treatment assignment. To discuss breaking of the blind, the Investigator should contact the Medical Monitor. The Investigator will unblind via an Interactive Response Technology system.

If the Investigator breaks the blind for an individual subject, the reason must be in the subject's source documents and the subject will be removed from the study.

4.0 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Inclusion Criteria

A subject will be eligible for inclusion in this study if he/she meets all of the following criteria:

1. Subject must have the ability and willingness to sign a written informed consent form.
2. Subject is between the ages of 18 to 70 years old (inclusive) at the time of consent.
3. Subject has SCD of any genotype (HbSS, HbSC, HbS β-thalassemia). If the subject's genotype has not been previously documented, genotyping will be performed during Screening using high-performance liquid chromatography (HPLC)/electrophoresis.
4. Subject must have experienced at least 2 VOCs within the 12 months prior to Screening. A VOC is defined as:
    a. The occurrence of appropriate symptoms consistent with a painful crisis, acute chest syndrome (ACS), or priapism, and
    b. Requires a visit to a medical facility and/or healthcare professional, and
    c. Receipt of either a parenteral or oral opioid or NSAID analgesia.
5. If subject is receiving hydroxyurea (HU)/hydroxycarbamide (HC), subject must have been receiving the treatment for at least 6 months prior to Screening and must agree to maintain the same dose and schedule for the duration of the study.
6. Subjects must have laboratory values at Screening within the following ranges:
    - Absolute Neutrophil Count $\geq 1.0 \times 10^9$/L
    - Platelets $\geq 75 \times 10^9$/L
    - Hemoglobin (Hgb) ≥ 6.0 g/dL
    - Glomerular filtration rate (GFR) $\geq$ 45 mL/min/1.73 m^2 using the CKD-EPI formulaTotal bilirubin ≤ 15 mg/dL
    - Alanine transaminase (ALT) $\leq 3.0 \times$ ULN
    - International Normalized Ratio (INR) ≤ 2.0
7. Eastern Cooperate Oncology Group (ECOG) performance status ≤ 2.
8. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must have a negative serum pregnancy test at Screening and agree to use standard prevention methods for the duration of the study.

4.2 Exclusion Criteria

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

1. Subject is receiving concomitant treatment with voxelotor, crizanlizumab, or L-glutamine.
2. Subject has any history of stem cell transplant is planning to begin or has received in the past 30 days.
3. Subject experiences an acute VOC, requiring a visit to a medical facility and/or healthcare professional, ending within 7 days prior to Day 1 dosing.
4. Subject has received any blood products within 30 days of Day 1 dosing.
5. Subject has uncontrolled liver disease or renal impairment, ulcerative colitis, Crohn's disease, or other chronic GI disorder.
6. Subject has received active treatment in another investigational trial within 30 days or 5 half-lives of the last dose of the investigational agent, whichever is greater, prior to Screening.
7. Subject has received penicillin prophylaxis or antibiotics for treatment of infection within 30 days or 5 half-lives of the treatment, whichever is greater, prior to Screening.
8. Subject has a significant medical condition that required hospitalization (other than for a VOC) within 2 months prior to Screening.
9. Subject is planning on undergoing an exchange transfusion during the duration of the study or has completed one within 4 weeks prior to Day 1 dosing.
10. Subject has a hypersensitivity to rifaximin, rifampin, rifamycin antimicrobial agents, or any components of rifaximin ER and DER.
11. Subject is pregnant or a nursing woman.
12. Subject has a history of illicit drug use or abuse, either documented or in the opinion of the Investigator.
13. Subject is using any medication that is known to inhibit or induce CYP3A4, P-gp, or OATP1B1/B3 (See Appendix 3) within 30 days or 5 half-lives, whichever is longer, prior to Day 1 dosing, or in the opinion of the Investigator, may affect the evaluation of the study product or place the subject at undue risk.
14. Subject has had any prior gastrointestinal surgery which has altered the anatomy of the esophagus, stomach, or small/large intestine (with the exception of appendectomy, cholecystectomy, and fundoplication).
15. Subject has had a colonoscopy or sigmoidoscopy within 30 days prior to Day 1 or plans to undergo such a procedure during the duration of the study.

16. Subject has used bowel prep, laxative, or enema within 30 days prior to Day 1.
17. Subject has a bleeding disorder including, but not limited to, acquired or congenital platelet function defects, disseminated intravascular coagulation (DIC), bleeding factor deficiencies, hemophilia, idiopathic thrombocytopenia purpura (ITP), or von Willebrand's disease.
18. Subject is planning to undergo a major surgical procedure during the duration of the study.
19. Subject has a positive test for human immunodeficiency virus (HIV)1 or HIV2.
20. Subject has an active Hepatitis B infection (HBsAg positive). Prior infection that is not active (i.e., HBsAg negative, HBcAb positive, and HBsAb positive) is permitted.
21. Subject has a positive test for Hepatitis C (HCV RNA). Prior infection with spontaneous resolution or sustained resolution for ≥ 24 weeks after cessation of antivirals is permitted.
22. Subject has an active COVID-19 infection or complication(s) related to COVID-19 infection that are unresolved or, in the opinion of the Investigator, may affect evaluation of the study drug or place the subject at undue risk.
23. Subjects has received a vaccine (including COVID-19 vaccine) within 2 weeks prior to Screening. If subject has received their first of two COVID-19 vaccination doses, as applicable, they must wait for at least 2 weeks after receiving the second dose, and be symptom-free, prior to beginning Screening. Subject must not be planning for COVID-19 or other vaccinations while on study.
24. Subject has a malignant disease. Exceptions include malignancies that were treated curatively and have not recurred within 2 years prior to study treatment, completely resected basal cell and squamous cell skin cancers, and any completely resected carcinoma in situ.
25. Subjects has prolonged QT interval as assessed by ECG history within the past 3 months. For subjects with no historical ECG information, subject has a resting QTcF ≥ 460 msec for males and ≥ 470 msec for females at Screening.
26. Subject has any unstable cardiac condition that, in the opinion of the Investigator, may worsen during the study or interfere with successful evaluation of the study treatment.
27. Subject has a serious mental or physical illness which, in the opinion of the Investigator, would compromise participation in the study.
28. Subject has any condition which, in the opinion of the Investigator, is likely to interfere with the successful collection of the measurements required for the study.
29. Subject is unable to understand or comply with study instructions and requirements.

4.3 Concomitant Therapies

A concomitant medication is any drug or substance administered between the signing of the informed consent and the EOS/ET Visit. The use of concomitant therapies or procedures must be recorded on the subject's eCRF, according to instructions for eCRF completion. Adverse events related to administration of these therapies or procedures must be documented in the appropriate eCRF.

Other than the medications listed in Section 4.3.1, medications that are used as the standard of care for SCD will be allowed during the study.

Use of HU/HC is allowed during the study. However, subjects receiving should maintain a stable dose throughout the study.

A COVID-19 vaccination is to be recorded as concomitant medication.

4.3.1 Prohibited Medications and Therapies

Treatment with the following is not allowed during the study:

- Non-study rifaximin (e.g., commercial rifaximin, XIFAXAN® 550 mg tablets), rifampin or other rifamycin derivatives are prohibited within 30 days of providing consent, and throughout the study.
- Initiation of treatment with HU/HC, voxelotor, crizanlizumab, or L-glutamine.
- COVID-19 vaccination.
- Gene therapy for SCD.
- Initiation of a chronic transfusion program (pre-planned series of transfusions for prophylactic purposes).
- Enrollment in any other drug, biologic, or device clinical study or treatment with an approved therapy for investigational development or unapproved investigational drug under development is not allowed.
- Chronic antibiotic use throughout the study is prohibited. A short-course of antibiotic therapy (≤ 7 days; non-rifamycin and non-quinolone classes only) to treat infections such as sinusitis, urinary tract infections, etc. or for dental therapy prophylaxis is permitted. Longer courses of therapy will require the permission of the Sponsor.
- Treatment with CYP3A4 inhibitors/inducers, P-glycoprotein (P-gp) inhibitors/inducers, and organic anion transporting polypeptide (OATP1B1/B3) inhibitors shown in Appendix 3.
- Use of proton pump inhibitors (PPIs).

4.3.2 Concomitant Procedures

A concomitant procedure is any therapeutic intervention (eg, surgery/biopsy, physical therapy) or diagnostic assessment (eg, blood gas measurement, bacterial cultures) performed between the time the subject is enrolled in the study and the EOS/ET Visit.

Subjects are not to receive a stem cell transplant during the study.

Subjects are not to undergo elective surgeries during the study.

4.4 Females of Reproductive Potential

There are no adequate and well controlled studies in pregnant women. Rifaximin has been shown to be teratogenic in rats and rabbits at doses that caused maternal toxicity.

Women of childbearing (reproductive) potential must have a negative serum pregnancy test at Visit 1 (Screening) and negative urine pregnancy test at Visit 2 prior to randomization on Day 1. These subjects must agree to use an acceptable method of contraception throughout their participation in the study (see details in Section 7.12). If a female subject becomes pregnant while on this study, the study drug will be immediately discontinued, and the subject will be followed until the outcome of the pregnancy is known. The pregnancy will be reported to Salix using the guidelines provided in Section 7.10 and Section 7.11.

4.5 Premature Subject Discontinuation

All subjects are free to withdraw from participating in this study at any time for any reason, specified or unspecified, and without prejudice. No constraints will be placed on ordinary subject management, and subjects, when appropriate, will be placed on other conventional therapy upon request or whenever clinically necessary as determined by their physician.

Withdrawal of the subject from treatment with study drug or from the study will be considered, in consultation with the Sponsor, or designee, for any of the following reasons:

- The subject withdraws consent.
- The subject experiences an intolerable AE or SAE.
- The subject experiences CDAD
- The subject enrolls into another interventional clinical study in which an investigational treatment or approved therapy for investigational use is administered.
- The subject becomes pregnant. Study treatment must be discontinued immediately. Report the pregnancy according to the instructions in Section 7.10.
- The subject is unwilling or unable to comply with the protocol.
- At the discretion of the Investigator or Sponsor.

- Investigator decision that it is not in the best medical interest of the subject to continue participation in the investigation.

Prior to discontinuing a subject, every effort should be made to obtain as much follow-up data as possible. Withdrawn or discontinued subjects will undergo early termination procedures per the scheduled assessments for the Early Termination (ET) Visit. AEs will be followed as described in Section 7.8. Subject withdrawals will be documented clearly on the source documents and applicable eCRFs.

Notification of subject withdrawals will be made to the Sponsor, or designee.

4.6 Subject Replacement

Subjects that are withdrawn, terminate the study early (including for an AE), or are lost to follow-up (LTFU) during the study may be replaced to ensure appropriate number of subjects complete the study. The decision to replace a subject will be made in agreement between the Sponsor and Investigator.

5.0 STUDY DRUG FORMULATION AND SUPPLY

Rifaximin ER and DER are supplied as 20 mg capsules. Subjects will be randomized to receive either 40 mg or 80 mg of ER or DER and matching placebo capsules for the duration of the study.

5.1 Packaging and Labeling

Outer packaging and capsules will be packaged and labeled in a manner consistent with the study design and according to applicable national / regional and local regulations for an investigational drug.

Each unit of rifaximin ER and DER capsules and placebo capsules will bear a label that meets applicable laws for an investigational drug, which includes, but is not limited to, the following information:

- Protocol Number
- Kit Number
- A statement reading: "Caution: New Drug Limited by Federal Law to Investigational Use"
- A statement reading, "Manufactured for Salix Pharmaceuticals, Inc., a division of Bausch Health US, LLC"
- Recommended storage conditions.

5.2 Storage and Handling

All study drug will be kept in a locked area with limited access. All units of study drug must be stored at 20º-25º C (68º-77º F) with excursions of 15º-30º C (59º-86º F) permitted. Study drug will be dispensed by qualified study staff only in accordance with the procedures described in this protocol.

5.3 Dosing and Dosing Schedule

Each subject will be dispensed study drug at each visit with enough capsules to dose through their next visit.

During the Treatment Period, eligible subjects will take study drug BID according to their treatment assignment as outlined in Table 1. Dosing will begin on Study Day 1 (Visit 2) and continue through Study Day 29 (Visit 5). Doses will be taken orally with water, and capsule will be swallowed whole. Study participants should be instructed to take the study drug on an empty stomach. The morning (AM) dose should be taken on an empty stomach, and subjects must avoid food for two hours after taking the study drug. The evening (PM) dose should be taken on an empty stomach (at least two hours after the last meal), and subjects must avoid food for two hours after taking the study drug.

On Days 1, 8, 15, and 29, participants will fast for a minimum of 10 hours prior to, and 2 hours after, the morning study drug administration. Water will be restricted from one hour prior to dosing through one hour following dosing, except for the water that will be consumed at dosing. Study drug should be administered with approximately 240 mL of water.

Table 1 Dosing by Treatment Assignment

Dose Group	Formulation	Study Day	Total Number of Capsules	Capsule (mg)	Daily Dose (mg)	No. of Subjects
1	ER	Daily	4 (2 in AM, 2 in PM)	20	80	12
2	DER	Daily	4 (2 in AM, 2 in PM)	20	80	12
3	Placebo	Daily	4 (2 in AM, 2 in PM)	20	-	6
4	ER	Daily	8 (4 in AM, 4 in PM)	20	160	12
5	DER	Daily	8 (4 in AM, 4 in PM)	20	160	12
6	Placebo	Daily	8 (4 in AM, 4 in PM)	20	-	6

Note: AM and PM refer to approximate 12-hour period between doses

Before any study drug is administered, each subject will be instructed by the Investigator or his/her representative on the proper self-administration of study drug. All unused study drug will be returned to the study site at the next clinic visit.

5.4 Rationale for Dose Selection

The dose and formulation of rifaximin ER and DER formulations selected for this study are based on the safety of these formulations observed in the Phase 1 healthy volunteer study, RBPK 1001 and on the safety of rifaximin in clinical studies supporting the approval of XIFAXAN®. For additional details on the safety of the ER and DER formulations and on XIFAXAN refer to the Investigators' Brochure.

5.5 Study Drug Accountability

All study drug will be kept in a secure, temperature-controlled area with limited access. Investigational drug orders, records of study drug receipt, dispensing, re-dispensing, returned study drug, and running inventory will be examined and reconciled throughout the study. Upon the completion of the study, all material will be subjected to final inspection and reconciliation. Instructions will be provided by the Sponsor regarding final disposition of all study medication in compliance with applicable regulations.

Study drug reconciliation will be conducted during monitoring visits to ensure appropriate receipt, storage, dispensing, and documentation of returned study drug.

5.6 Assessment of Compliance

After study drug is dispensed, the Investigator or his/her representative will count and record the unused study drug capsules that are returned by the subject at each visit. If the subject forgets to return unused study drug at a visit, then compliance will be confirmed after direct questioning of the subject.

6.0 STUDY PROCEDURES AND ASSESSMENTS

The schedule of assessments to be performed during the study is provided in Appendix 1, Schedule of Assessments, and Appendix 2, Schedule for Acquisition of PK Samples. For this study, Day 1 (Visit 2), which is the day of randomization, shall be defined as the subject's Baseline.

Note: Prior to screening study subjects, the flow cytometry assessment (including healthy volunteers) will need to be certified at the laboratory where the assessment will be completed.

In case of situations that prevent a participant from attending a post-baseline study visit, a home health visit may be scheduled, where possible. The home health visit may include an assessment of changes in health and medications, measurement of vital signs and body weight, and checking of study medication compliance. The home health visit may also include all sample collections outlined in the study protocol (PK, hematology and urinalysis samples, urine sample for urine 3-inoxyl sulfate, and blood samples for CANs (if applicable), CD62L, iFABP and LPS).

All documented assessments and laboratory results will be reviewed by the investigator and documented in relevant eCRFs as appropriate.

6.1 Study Schedule by Treatment Visit

6.1.1 Visit 1; Screening (Day –21 to Day –1)

The initial Screening Visit (Visit 1) should be conducted between Days -21 and -1, inclusive. The following procedures are to be completed at Visit 1:

- Obtain informed Consent signed by the subject prior to performing any study evaluations or procedures including asking subjects to discontinue any prohibited medications.
- Record demographics and medical history information. Detailed history of VOCs occurring over the past 12 months will be obtained. This will include collection of treatments for VOCs including drug, dose, route of administration, duration of treatment, and inpatient/outpatient setting.
- Record all opioid use within the 30 days prior to Screening.
- Review Inclusion/Exclusion criteria to ensure subject qualifies for the study.
- Obtain height and weight.
- Obtain vital sign measurements. Vital signs to be collected are blood pressure (BP) heart rate (HR), body temperature, and O_2 saturation.
- Conduct full physical examination.
- Obtain electrocardiogram (ECG; 12-lead).

- Collect blood samples for hematology, blood chemistry, coagulation, and viral testing, and urine sample for urinalysis.
- Perform hemoglobin genotyping (for subjects with unknown genotype).
- Conduct serum pregnancy test for women of childbearing potential (WOCBP).
- Record concomitant medications (record all medications within 30 days of Visit 1).
- Have subjects discontinue use of prohibited medications immediately after Visit 1 (Screening).
- Record adverse events.
- Schedule Visit 2 (Day 1).

6.1.2 Visit 2; Randomization (Day 1)

Randomization will be performed on Day 1. Participants will be in a fasted state. There is no Day 0 in this protocol. The following assessments are to be completed prior to randomization:

- Review Inclusion/Exclusion Review to ensure subject continues to qualify for the study.
- Collect any adverse events since the Screening Visit.
- Collect concomitant medication changes since the Screening Visit. Specifically collect opioid use since Screening.
- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed.
- Obtain ECG (12-lead).
- Collect blood samples for hematology and blood chemistry and urine for urinalysis.
- Collect blood samples for CANs, CD62L, iFABP and LPS.
- Collect urine sample for 3-indoxyl sulfate.
- Confirm negative serum pregnancy test from Screening and conduct urine pregnancy test for WOCBP.
- Query subject and record VOCs that have occurred since Screening.
- If subject continues to meet eligibility requirement, randomize subject.

After randomization, conduct the following assessments:

- Collect pre-dose PK sample.

- Dispense study drug and instruct subject in administration (i.e., number of capsules to take [2 or 4] and timing of BID dosing).
- Have subject administer first dose of study drug.
- Collect PK blood samples at 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-dose.
- Record any concomitant medications.
- Record any adverse events.
- Schedule Visit 3

6.1.3 Visit 3, (Day 8 ±1 day) and Visit 4, (Day 15 ±1 day)

Participants will be in a fasted state. The following assessments are to be completed at Visit 3 and Visit 4:

- Collect any adverse events since Visit 2 or Visit 3.
- Collect concomitant medication changes since Visit 2 or Visit 3. Specifically collect opioid use since Visit 2 or Visit 3.
- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed.
- Collect blood samples for hematology and blood chemistry and urine for urinalysis.
- Collect blood samples for CANs, CD62L, iFABP and LPS.
- Collect urine sample for 3-indoxyl sulfate.
- Query subject and record VOCs that have occurred since Visit 2 or Visit 3.
- Collect unused study drug and review compliance and accountability.
- Collect pre-dose PK sample.
- Dispense study drug and confirm subject's compliance with administration (i.e., number of capsules to take [2 or 4] and timing of BID dosing).
- Have subject administer study drug, record AM or PM dose.
- Collect PK blood samples at 0.5, 1, and 2-hours post-dose.
- Record any concomitant medications.
- Record any adverse events.
- Schedule next Visit (Visit 4 or Visit 5).

6.1.4 Visit 5, (Day 29 ±1 day) EOT/ET Visit

Participants will be in a fasted state. The following assessments are to be completed at the EOT/ET Visit:

- Collect any adverse events since Visit 4.
- Collect concomitant medication changes since Visit 4. Specifically collect opioid use since Visit 4.
- Obtain subject's weight.
- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed.
- Obtain ECG (12-lead).
- Conduct urine pregnancy test for WOCBP.
- Collect blood samples for hematology and blood chemistry and urine for urinalysis.
- Collect blood samples for CANs, CD62L, iFABP and LPS.
- Collect urine sample for 3-indoxyl sulfate.
- Query subject and record VOCs that have occurred since Visit 4.
- Collect pre-dose PK sample.
- Collect unused study drug and review compliance and accountability.
- Have subject administer final dose of study drug.
- Collect PK blood samples at 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-dose.
- Record any concomitant medications.
- Schedule Visit 6.

6.1.5 Visit 6 (Day 30)

- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed
- Record any concomitant medications.
- Record any adverse events.
- Collect PK blood sample at 24-hours post dose
- Collect PK blood sample at 30-hours post dose

6.1.6 Visit 7 (Day 31)

- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed
- Record any concomitant medications.
- Record any adverse events.
- Collect blood samples for CANs, CD62L, iFABP and LPS at 48-hours post dose.
- Collect blood samples for hematology and blood chemistry
- Collect urine sample for 3-indoxyl sulfate.
- Collect PK blood sample at 48-hours post dose

6.1.7 Visit 8 (Day 32)

- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed
- Record any concomitant medications.
- Record any adverse events.
- Collect PK blood sample at 72-hours post dose

6.1.8 Visit 9 (Day 43 ± 3 days) EOS Visit

The following assessments are to be completed at the EOS Visit:

- Collect any adverse events since Visit 8.
- Collect concomitant medication changes since Visit 8. Specifically collect opioid use since Visit 8.
- Obtain subject's weight.
- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation.)
- Conduct symptom-directed physical exam, as needed.
- Obtain ECG (12-lead) if abnormal findings were observed at Visit 5 (Day 29).
- Conduct urine pregnancy test for WOCBP.

- Collect blood samples for hematology and blood chemistry and urine for urinalysis only if needed for follow-up of AEs or SAEs.
- Collect blood samples for CANs, CD62L, iFABP and LPS.
- Collect urine sample for 3-indoxyl sulfate
- Query subject and record VOCs that have occurred since Visit 5.
- Record any concomitant medications.
- Record any adverse events.
- Discharge subject from study. Follow subject for AEs or SAEs as described in Section 7.7.

6.1.9 Visit to a Medical Facility for a VOC Event

The following assessments are to be completed, when possible, when a subject visits a medical facility for treatment of a VOC:

- Collect any adverse events since previous visit. Do not record VOC events as an AE (See Section 7.3).
- Collect concomitant medication changes since previous visit. Specifically collect opioid use.
- Obtain vital sign measurements (BP, HR, body temperature, and O_2 saturation).
- Conduct symptom-directed physical exam.
- Collect blood samples for hematology and blood chemistry and urine for urinalysis.
- Collect blood samples for CANs, CD62L, iFABP and LPS.
- Collect PK blood samples.
- Collect urine sample for 3-inoxyl sulfate.

6.1.10 Unscheduled Visit

The Investigator may perform an unscheduled visit at any time during the study at his/her discretion. Assessments performed at an unscheduled visit should be symptom directed.

The following procedures must be performed at all unscheduled visits:

- Collect and record hospital admission and death data.
- Safety laboratory testing. Repeat laboratory testing may be needed based on results from the unscheduled laboratory testing.

- Concomitant medications
- Adverse events

If the subject is discontinued from the study at an unscheduled visit, the visit will be considered Visit 9 (EOT) and all of the EOT assessments should be completed.

6.2 Outcome Assessments

Endpoints are presented in Section 2.2. Analyses are described in Section 8.0.

The following assessments will be utilized throughout the study.

6.2.1 Assessment of Pharmacokinetics

Blood samples for analysis of plasma rifaximin and 25-desacetyl rifaximin pharmacokinetics will be collected as described in Appendix 2. Full PK assessments through 8-hour and 72-hours post dose will be performed at Visit 2 (Day 1) and Visits 5 to 8 (Days 29 to 32), respectively. Samples will be obtained pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5, and 8-hours post-dose on Days 1 and 29.

At Visit 3 (Day 8) and Visit 4 (Day 15) samples will be obtained pre-dose and at 0.5, 1, and 2-hours post-dose.

Additionally, post-final dose assessments will be done on:

Visit 6: 24 and 30 hours post-final dose

Visit 7: 48 hours post-final dose

Visit 8: 72 hours post-final dose.

The windows around the collection timepoints are:

- within 15 minutes prior to dose for pre-dose samples,
- ± 5 minutes for samples taken at 0.5 hours through 1.5 hours, and
- ±10 minutes for samples taken 2 hours through 8 hours.
- ±2 hours for samples taken 24 hours through 72 hours.

In addition, when possible, a sample will be obtained for PK assessment during a medical facility visit for a VOC. The time of the last dose of study drug will be recorded.

If the subject is to begin treatment with a P-gp inhibitor, a plasma sample for PK analysis should be collected during treatment with both rifaximin and the P-gp inhibitor. This sample should be collected at the next scheduled visit, if possible. Alternately, an unscheduled PK sampling visit

should occur. The date and time of the last 2 rifaximin doses, as well as the last 2 doses of the P-gp inhibitor should be recorded.

Study participants should be instructed to take the study drug on an empty stomach. The morning (AM) dose should be taken on an empty stomach, and subjects must avoid food for two hours after taking the study drug. The evening (PM) dose should be taken on an empty stomach (at least two hours after the last meal), and subjects must avoid food for two hours after taking the study drug.

On Days 1, 8, 15, and 29, Participants will fast for a minimum of 10 hours prior to, and 2 hours after, the morning study drug administration. Water will be restricted from one hour prior to dosing through one hour following dosing, except for the water that will be consumed at dosing.

6.2.2 Assessments of Pharmacodynamics

The pharmacodynamics of rifaximin ER and DER will be assessed through collection of ANC, CANs, serum CD62L, hsCRP, the gut permeability biomarkers serum iFABP and LPS, and the gut bacteria biomarkers urine 3-indoxyl sulfate according to the schedule in Appendix 1. These assessments will be conducted at a central laboratory. Instructions for collection, preparation, storage, and shipment of samples will be provided in the Laboratory Manual.

6.2.3 Assessment of Safety

Safety endpoints are presented in Section 2.2.2. Safety will be assessed throughout the duration of the study.

Safety assessments will include the following:

- Adverse events (AEs),
- Vital signs (systolic and diastolic blood pressure, heart rate, oral body temperature, and oxygen saturation),
- Clinical safety laboratory tests,
- Physical exams (PEs), and
- Electrocardiograms (ECGs).

6.2.3.1 Clinical Laboratory Tests

Clinical laboratory tests to be performed during the study are outlined in Table 2. The samples will be analyzed by a central clinical laboratory. Exceptions may be made as necessary for local laboratory testing. At the Investigator's discretion, additional blood may be collected for clinical laboratory tests. Results from laboratory specimens collected during Screening must be received by the site prior to Visit 2.

Note: CANs assay examiners will be trained and certified on CANs assessment requirements using flow cytometry prior to screening patients (conducted on separate, healthy volunteers).

It is the responsibility of the Investigator to assess the clinical significance of all abnormal clinical laboratory values as defined by the list of normal values on file for the central laboratory. All clinically significant laboratory value abnormalities are to be recorded as AEs.

A clinically significant laboratory value is any abnormal result that, in the judgment of the Investigator, is an unexpected or unexplained laboratory value or if medical intervention or corrective action (transfusion, hydration, initiation of antibiotics or other concomitant medication) is required. Any abnormal values that persist should be followed at the discretion of the Investigator. Additional and repeat laboratory safety testing for the evaluation of abnormal results and/or AEs during the study may be performed at the discretion of the Investigator or upon request of the Sponsor. Repeat laboratory testing of abnormal potentially clinically significant or clinically significant results for the Screening evaluation of the subject may be repeated once at the discretion of the Investigator.

Tests listed in Table 2 will be performed as outlined in the Schedule of Assessments (Appendix 1). Details on collection, preparation, and shipping of blood samples are provided in the Laboratory Manual.

Table 2. Clinical Laboratory Tests

<table>
<tr>
<td>Hematology
• Hemoglobin
• Hematocrit
• White blood count and differential
• Red blood cell count
• Percent and absolute reticulocyte count
• Platelet count

Coagulation
• Prothrombin time
• INR</td>
<td rowspan="2">Serum Chemistry
• Blood Urea Nitrogen
• Bilirubin (total, direct, and indirect)
• Alkaline phosphatase
• Aspartate aminotransferase
• Alanine aminotransferase
• Albumin
• Sodium
• Potassium
• Magnesium
• Calcium
• Chloride
• Bicarbonate
• Glucose
• Creatine kinase
• Creatinine
• Lactate dehydrogenase
• High sensitivity C-Reactive protein (hs-CRP)
• Total globulin
• Total protein
• Lipid Panel
– Total cholesterol
– HDL
– LDL
– Triglycerides</td>
</tr>
<tr>
<td>Additional Tests
• Hemoglobin genotype (if not previously determined) using HPLC/electrophoresis
• Serum pregnancy test (WOCBP only)*
• Urine pregnancy test (WOCBP only)

* WOCBP, women of child-bearing potential</td>
</tr>
</table>

6.2.3.2 Pregnancy Tests

A pregnancy test will be performed for all women of childbearing potential according to the schedule in Appendix 1. A negative serum pregnancy test performed at Screening must be confirmed along with a negative urine pregnancy test on Day 1 prior to randomization and dispensing/administration of study drug.

6.2.3.3 Adverse Events

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. 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).

6.2.3.4 Vital Signs

Vital signs will include blood pressure (systolic and diastolic in mm Hg), heart rate (beats per minute), body temperature, and O_2 saturation. Vital sign measurements will be collected at every visit. Subjects should be seated for 5 minutes prior to assessment of vital signs. Blood pressure measurements should be measured on the same arm throughout the study.

6.2.3.5 Electrocardiogram (ECG)

A 12-lead ECG (Supine) will be performed on all subjects using a standard ECG machine at Visit 1, (Screening), Visit 2 (Baseline), Visit 5 (Day 29/EOT/ET), and Visit 9 (Day 43/EOS), if applicable.

6.2.3.6 Physical Examination

A full physical examination will be performed at Visit 1 (Screening). Symptom-directed physical examinations will be performed at other visits. Weight will be collected as outlined in Appendix 1. Height will only be collected at Visit 1 (Screening).

6.3 Other Assessments

6.3.1 Assessment of VOCs

VOCs that subjects experience during the study will be collected. A VOC is defined as:

- The occurrence of appropriate symptoms consistent with a painful crisis, acute chest syndrome (ACS), or priapism, and
- Requires visit to a medical facility, and
- Receipt of either a parenteral or oral opioid or NSAID analgesia.

For each VOC, the information outlined in Section 7.3 will be collected.

6.3.2 Medical History

Each subject's complete medical history is to be recorded in source documents and the appropriate eCRF pages. Relevant medical history will be collected for the 3 months prior to the Screening Visit. A detailed history of the subject's VOCs over the past 12 months will be obtained and will include the start and stop dates (if known), the treatment setting, and treatment including drug, dose, route of administration, and duration of treatment for each VOC, when possible.

7.0 SAFETY REPORTING

7.1 Operating Definitions for Assessing Safety

Safety data collection for this study begins at the time of the subject's signing of the informed consent 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 AE (Section 7.1.1), or SAE (Section 7.1.2).

7.1.1 Adverse Event

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a study product and which does not necessarily have to have a causal relationship with this 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).

An AE does include the following:

- Exacerbation or worsening of a pre-existing illness including symptoms associated with SCD.
- Condition detected or diagnosed after study product administration even though it may have been present prior to the start of the study.

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).
- 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 (exception is VOC and presenting symptoms, Section 7.3).

7.1.2 Serious Adverse Event

A serious adverse event is any untoward medical occurrence that, at any dose:

- Results in death,

- Is life-threatening.

  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was, in the view of the Investigator or Sponsor, at risk of death at the time of the event. It 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 for 24 hours or more. It does not include presentation at a casualty or emergency room.

- Results in 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.

- Is 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, and/or do not require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or 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.

Additionally, an adverse event meeting any of the serious outcomes previously defined above that is assessed by the Investigator as resulting from study participation (regardless of relationship to study drug) should be treated as an SAE for this protocol (e.g., complications resulting from the taking of a blood sample or performance of a protocol required procedure).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in case of events that do not meet the serious criteria.

If a subject has an SAE, a plasma sample for PK analysis will be collected as soon as feasible, and the date and time of the last 2 rifaximin doses will be recorded.

7.2 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, the taking of vital signs, 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. Abnormal laboratory findings consistent with pre-existing illness or confirmatory of a reported AE or SAE should not be reported as a separate AE or SAE. If a clinically significant change from Baseline is observed which is indictive of worsening in condition, then the worsening condition should be reported as the AE or SAE.

7.3 Adverse Events That Are VOC Endpoint Events

VOC events that are listed in Table 3 are being captured as endpoints and **SHOULD NOT** be reported as an AE or SAE for purposes of this study. These events will not be considered as SAEs for reporting requirements.

Table 3: VOC Events Not Requiring AE/SAE Reporting

Uncomplicated pain crisis*
Acute chest syndrome
Hepatic sequestration
Splenic sequestration
Priapism requiring a visit to a medical facility

* Defined as the occurrence of appropriate symptoms, results in a visit to a medical facility and or healthcare professional and requires either parenteral or oral opioid or NSAID analgesia, but is NOT classified as an acute chest syndrome, hepatic sequestration, splenic sequestration, or priapism.

All VOCs are to be recorded only once on the "Vaso-Occlusive Event" eCRF page. All VOCs are to be recorded throughout the entire treatment period, up to Visit 5 (Day 29), and should continue until the Follow-Up Visit at Day 43. The Investigator will classify and provide all the following information for each VOC as follows:

- Diagnosis, which will be limited to one of the five pre-defined VOC events as:
    - Uncomplicated pain crisis
    - ACS

  - Hepatic sequestration
  - Splenic sequestration
  - Priapism (requiring a visit to a medical facility)

- Onset date,
- Stop date,
- Action taken (None, Required concomitant medication, Temporarily withheld study drug, Permanent discontinuation of study drug, or Other [explain]),
- Treatment setting,
- Concomitant medications given, and
- Outcome (Recovered without sequelae, Resolved with sequelae, Ongoing, Unknown, Death).

If a subject is hospitalized due to a VOC and during hospitalization develops a non-VOC event that meets the criteria for a SAE, then that event should be reported as a SAE. Any prolongation of a hospitalization due to a non-VOC event (even though they may have initially been hospitalized due to a VOC) is reportable as a SAE in the eCRF.

7.4 Method, Frequency, and Time Period for Detecting AEs and SAEs

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

1. Have there been any (other) medical problems since your last visit/assessment?"
2. Have any new medications been taken, other than those given to you in this study, since your last visit/assessment?"

7.4.1 Time Period for Detecting and Reporting of AEs and SAEs

The time period for detecting and reporting AEs and SAEs is from the time of informed consent through study completion/withdrawal, including the follow-up period.

7.4.2 Post-Study SAEs

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

7.5 Documenting AEs and SAEs

All AEs that occur after the subject has signed the ICF through 28 days after last dose of study medication, regardless of causality or seriousness, will be assessed and recorded in the subject's

source documents and in the eCRF. If a subject is withdrawn during treatment, they must be followed for 28 days after the last dose as well. In addition, SAEs must be documented on the paper 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 or SAE and not the individual signs/symptoms. When this is not possible, the AE should be documented in terms of signs and/or symptoms observed by the Investigator or reported by the subject at each study visit. Each AE which appears to be independent of any prior event will be reported separately.

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 a 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 (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 Sponsor/designee. Investigators should not wait to receive additional information to fully document the event before notifying the Sponsor of an SAE. It is very important that the Investigator (or designee) provide his/her assessment of causality to study product at the time of initial SAE reporting.

7.6 Common Terminology Criteria for Adverse Events

The severity of events will be determined based on the Common Toxicity Criteria for Adverse Events CTCAE), version 5.0. The CTCAE quick reference guide can be found at:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

For events not specifically addressed in the CTCAE, the following definitions should be considered when evaluating the severity of AEs and SAEs:

Severity of Event	
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

Activities of Daily Living (ADL)

* Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

** Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

7.7 Assessment of Causality

The Investigator should assess the relationship of the AE, if any, to the study drug as either "Related" or "Not Related."

- Related: There is at least a reasonable possibility that the AE/SAE is related to the study drug. Reasonable possibility means that there is evidence to suggest a causal relationship between the drug and the AE.
- Not Related: There is little or no reasonable possibility that the AE/SAE is related to the study drug. This assessment implies that the AE/SAE has little or no temporal relationship to the study drug and/or a more likely or certain alternative etiology exists.

The following should be considered when assessing AE/SAE causality:

- Positive temporal relationship to study drug, such as if study drug was withdrawn and the SAE resolved, or the event recurred after re-introduction,
- If there is a reasonable possibility that the AE is associated with an underlying or concomitant illness.

- Possible association with previous or concomitant therapy,
- No temporal relationship to treatment with study drug and/or a more likely alternative etiology exists.
- If the AE is directly related to study procedures.

7.8 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 until the subject is lost to follow-up. The Investigator is responsible for ensuring 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.

Salix 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 follow-up period, the Sponsor/designee should be provided with a copy of any postmortem findings, including histopathology.

If cause of death is not available within the 24-hour reporting period, "death" must be reported as SAE term to meet timelines and the cause of death actively queried and submitted as a follow-up report.

When additional SAE information becomes available, it should be recorded on a new SAE Report Form and should be labeled as a "Follow-up Report". The original SAE Report Form should not be altered. All completed SAE Report Forms must be 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/EC must be notified about SAEs in accordance with the requirements of the governing IRB/EC.

Patients should be monitored for development of Clostridium difficile-associated diarrhea (CDAD). If a patient develops severe, unremitting diarrhea, a diagnosis of Clostridium difficile-associated diarrhea (CDAC) should be considered and investigated. If CDAD is confirmed during the study, the interventional therapy will be stopped, and the subject will be discontinued from the trial. Symptoms of CDAD may be non-specific and indistinguishable from other causes of diarrhea, but if CDAD is suspected, study drug must be held until CDAD can be ruled out. If CDAD is confirmed, then the interventional therapy will be stopped, and the subject will be discontinued from the trial. CDAD must also be reported as an AE or SAE depending on presentation. Appropriate fluid and electrolyte management, protein supplementation, antibiotic treatment of C. difficile, and surgical evaluation should be instituted as clinically indicated.

7.9 Prompt Reporting of SAEs to Salix Pharmaceuticals, Inc.

SAEs must be reported promptly to the Sponsor/designee once the Investigator determines that the event meets the protocol definition of a SAE.

Prompt reporting of an SAE requires:

- Completion and transmission of the SAE Report Form to the Sponsor/designee via email or fax 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.

7.10 Pregnancy Reporting

Pregnancies detected in subjects assigned to study treatment should be promptly reported to the Sponsor/designee via email/fax, using the Pregnancy Report Form, as soon as the Investigator is notified. If a female subject becomes pregnant following assignment to study treatment, the study drug will be immediately discontinued, and the subject will be followed until the outcome of the pregnancy is known. (Refer to the Section 4.4).

The Sponsor/designee should be notified via email/fax of any updates on the status of the pregnancy within 24 hours by completion of a separate follow-up pregnancy notification form. Outcome of pregnancy must be reported.

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 will be recorded as an AE or SAE and followed as such. If a pregnancy is associated with an SAE, an SAE Report Form should be forwarded to the Sponsor/designee within 24 hours of the Investigator's awareness, along with the Pregnancy Report Form.

7.11 Transmission of SAE Report Forms and Pregnancy Report Forms

Completed SAE Report Forms and completed Pregnancy Report Forms should be transmitted to the Sponsor/designee via the email address provided below:

[redacted]

7.12 Contraception Requirements

All women of childbearing (reproductive) potential and all men must practice effective contraception during the study and for 30 days after the last dose.

For the purposes of this study, women who do not meet one of the following criteria listed below are considered physiologically capable of becoming pregnant and are, therefore, defined as women of childbearing potential:

- Postmenopausal
- 24 months of natural (spontaneous) amenorrhea without an alternative medical cause
- 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Post-hysterectomy
- Female surgical sterilization (e.g., bilateral tubal ligation)

For the purposes of the study, effective contraception is defined as use of 2 of the following for females:

- Oral contraceptives taken for at least 30 days prior to the Screening Visit and maintain contraceptive regimen throughout the study.
- Placement of a non-hormonal intrauterine device or intrauterine system.
- Barrier methods of contraception with use of a spermicide: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream suppository. The use of barrier contraceptives should always be supplemented with the use of a spermicide.
- Male surgical sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).

For the purposes of the study, effective contraception is defined as use of 1 of the following for males:

- Effective male contraception includes a vasectomy with negative semen analysis at follow-up.
- The use of condoms with spermicide.
- Confirmation of birth control practiced by female partner with condom use.

True abstinence, when this is consistent with the preferred and usual lifestyle of the subject, can be considered an acceptable method of contraception based on the evaluation of the Investigator, who should also take into consideration the duration of the clinical trial. Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not considered acceptable methods of contraception.

8.0 STATISTICAL CONSIDERATIONS

Detailed methodology for statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP). The SAP will be finalized prior to database lock.

All continuous variables will be summarized using descriptive statistics; N, mean, standard deviation (SD), median, maximum, and minimum. All categorical variables will be summarized using frequency counts and percentages.

Baseline value is defined as last available value prior to the first dose of study drug.

EOT value is defined as last available post-Baseline value during the treatment period.

All data collected in the database will be presented in data listings.

8.1 Randomization

Subjects who meet the eligibility criteria and have given written informed consent will be randomized in a 2:2:1:2:2:1 allocation to one of six treatment groups as noted in Section 3.2.

8.2 Sample Size

The total of 60 subjects is not based on formal statistical considerations. Twelve (12) subjects per group receiving active rifaximin is considered adequate to evaluate the PK/PD of rifaximin ER and DER.

8.3 Analysis Populations

The analysis populations will be defined as follows:

- Safety Population will include all subjects who have taken at least one dose of study drug.
- PK Population will include all subjects who have taken at least one dose of study drug and have sufficient data to calculate PK parameters for rifaximin and 25-desacetyl rifaximin.

Summarization of disposition, demography and Baseline characteristics, PD assessments, VOCs, and safety will be performed using the Safety Population. PK analyses will be conducted using the PK Population.

8.4 Analysis Methods

8.4.1 Methods of Analysis

All statistical procedures and non-compartmental analyses (NCA) will be completed using SAS® version 9.4 or higher or WinNonlin v8.1, respectively, or more recent versions of either program.

The placebo arms will be combined for all descriptive summaries of subject disposition, demography and Baseline characteristics, PD, VOCs, and safety.

8.4.2 Subject Disposition

Subject disposition will be summarized for all randomized subjects, by dose (40 mg BID or 80 mg BID) within formulation (ER or DER), and combined placebo and will include the number of subjects randomized; number and percentage of subjects who completed or prematurely discontinued the study, classified by reasons for premature discontinuation; the number of subjects randomized at each study site; and the number and percentage of subjects who completed or discontinued the study at each study site.

8.4.3 Demographics and Baseline Characteristics

Age, gender, race, ethnicity, height, and weight will be summarized for the entire population, by dose within formulation and combined placebo. Baseline characteristics will include number of VOCs in the previous year, concurrent treatment with HU, use of IOA and orally administered opioids (in morphine milligram equivalents [MME]), and SCD genotype.

8.4.4 Pharmacokinetics

Concentrations of rifaximin and its metabolite (25-desacetyl rifaximin) will be summarized by formulation and by dose. Concentrations will be summarized for samples taken for 8 and 72 hours after the initial dose and the final dose, respectively.

Dense sampling for initial dose (first dose interval): C_{max}, T_{max}, AUC_{last}, $AUC_{0\text{-}8h}$, and $MR\_AUC_{0\text{-}8h}$, will be summarized.

Dense sampling for final dose: C_{max}, T_{max}, C_{min}, C_{avg}, $AUC_{0\text{-}8h}$, CL/Fss, Vz/Fss, R_{Cmax}, $R_{AUC\tau}$, AUC_{last}, AUC_{inf}, λz, $t_{1/2}$, and $MR\_AUC_{0\text{-}8h}$, will be summarized.

Steady state levels will be summarized for all subjects based on C_{trough} on Day 8, Day 15, and Day 29.

Additional PK parameters maybe calculated if it deemed necessary.

LIST OF PHARMACOKINETIC TERMS

Parameter	Definition
AUC_{last}	Area under the plasma drug concentration-time curve from time 0 to the last quantifiable drug concentration
AUC_{inf}	Area under the plasma drug concentration-time curve from time 0 extrapolated to infinity

$AUC_{0\text{-}8h}$	Area under the plasma drug concentration-time curve from time 0 through 8 hours
C_{avg}	Average plasma concentration at steady-state
C_{max}	Maximum observed plasma concentration
C_{min}	Minimum observed plasma concentration
C_{trough}	Observed plasma concentration at the end of a dosing interval, just prior to the administration of the next dose
T_{max}	Time of maximum observed plasma concentration
$t_{1/2}$	Apparent terminal elimination half-life
λz	Terminal elimination rate constant
CL/Fss	Apparent systemic extravascular clearance at steady-state
Vz/Fss	Apparent extravascular volume of distribution during the terminal elimination phase at steady-state
R_{AUC}	Accumulation ratio of AUC_{τ} at steady-state to AUC_{last} after the first dose
R_{Cmax}	Accumulation ratio of C_{max} at steady-state to C_{max} after the first dose
MR_AUC	Metabolite-to-parent ratio for AUC

8.4.5 Pharmacodynamics

The changes from Baseline in ANC, the number of CANs, and the levels of serum CD62L, serum iFABP, serum LPS, serum hsCRP, and urine 3-indoxyl sulfate will be summarized by dose within formulation and combined placebo.

A dose-response analysis may be conducted to determine the relationship between daily rifaximin ER and DER dosing and reduction of CANs. Dose-response relationships following the final dose on Day 29 may also be assessed for other PD endpoints, if warranted.

The relationship between rifaximin PK parameters (C_{trough}, $C_{max,ss}$, $C_{ss,avg}$) and each PD endpoint (ANC, CANs, serum CD62L levels, serum hsCRP, serum iFABP, serum LPS and urine 3-inoxyl sulfate) may be evaluated following the final dose on Day 29. Additionally, a population PK/PD model may be developed as a separate analysis to explore exposure-response. If conducted, this analysis will be reported separately from the Clinical Study Report.

8.4.6 Analysis of Safety

All adverse events occurring during the study will be recorded and classified based on MedDRA terminology. Only adverse events that occur after the start of study drug, (treatment emergent adverse events [TEAEs]); all AEs will be included in by-subject listings.

TEAEs will be summarized for the entire population, by dose within formulation and combined placebo, and by severity and relationship to study medication. Each subject will be counted only once within a system organ class or a preferred term by using the adverse event with the highest severity or greatest relationship, respectively, within each category.

Treatment-emergent AEs will be summarized as follows:

- Number and percentage of subjects experiencing TEAEs,
- Number and percentage of TEAEs by relationship to study treatment (i.e., related or unrelated),
- Number and percentage of TEAEs by severity,
- Number and percentage of subjects experiencing serious AEs (SAEs),
- Number and percentage of subjects experiencing study drug-related SAEs,
- TEAE leading to permanent treatment discontinuation,
- TEAEs with an outcome of death.

All SAEs, AEs leading to premature withdrawal from the study, and all deaths, will be listed.

Reports of diarrhea due to *C. difficile* will be summarized.

Vital signs will include supine blood pressure (mmHg), heart rate (beats per minute), body temperature, and O_2 saturation. Vital signs will be summarized at Baseline and for each day when vital signs were obtained. Changes from Baseline in vital sign measurements will also be summarized. For each summary the N, mean, median, SD, minimum, and maximum values will be given for all subjects, by dose within formulation and combined placebo. Pretreatment vital signs obtained closest in time to first dose of study drug will be used for summarization and analysis of Baseline and change from Baseline vital signs.

Changes from Baseline in abbreviated physical exam findings of clinical significance will be summarized and listed.

8.4.7 Clinical Safety Laboratory Findings

Clinical safety laboratory data (hematology, chemistry, and urinalysis) will be summarized at Baseline and at each visit per the schedule of assessments, as well as changes from Baseline in laboratory parameters. For each summary the N, mean, median, SD, minimum, and maximum values will be given by dose within formulation and combined placebo. Pretreatment laboratory values obtained closest in time to first dose of study drug will be used for summarization of Baseline and change from Baseline. Pregnancy testing results (urine and serum) will be presented in by-subject listings.

Clinical safety laboratory tests performed during the study will be primarily analyzed by a central clinical laboratory.

8.4.8 ECG Findings

ECGs with abnormal findings will be summarized and listed.

8.4.9 Physical Examinations

Abnormal findings from full and symptom-directed physical examinations will be summarized and listed.

8.4.10 Protocol Deviations

Protocol deviations will be presented in a listing.

8.4.11 Treatment Compliance

Treatment compliance, based upon expected number of capsules to be used and actual number of capsules used, will be calculated and summarized.

8.4.12 Treatment Exposure

The total exposure to study drug will be calculated for each subject.

8.4.13 Missing Data

Only available data will be analyzed. There will be no imputation of missing data.

8.5 Statistical and Analytical Issues

8.5.1 Adjustment for Covariates

Not applicable.

8.5.2 Interim Analyses and Data Monitoring

Not applicable.

8.5.3 Multicenter Study

Statistical analyses of efficacy and safety parameters will be based upon pooled data from all study centers.

8.5.4 Noncompartmental Analysis of Rifaximin and 25-desacetyl rifaximin

NCA will be conducted for subjects using intensive PK samples collected at Visit 2 (Day 1) and Visits 5 to 8 (Days 29 to 32). PK parameters listed in Section 8.4.4 will be summarized using descriptive statistics, including number of observations, mean, median, CV%, SD, minimum, and maximum.

9.0 STUDY ADMINISTRATION

9.1 Investigator Information and Training

The Investigators and essential support staff will be trained by Salix or designee on Good Clinical Practices (GCPs) and all aspects of protocol application and study management. It is the responsibility of the Investigator to ensure training of ancillary study staff. CANs assay examiners will be trained and certified on CANs assessment requirements using flow cytometry prior to screening patients (using healthy volunteers).

9.2 Monitoring

This study will be monitored by Salix or designee, in accordance with GCPs and applicable regulations. By signing this protocol, the Investigator agrees to periodic, on-site monitoring of all appropriate study documentation.

The progress of the study will be monitored by periodic on-site visits and frequent communications between the Sponsor or designee(s) 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 are 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), GCPs, and all applicable regulatory requirements.

9.3 Audits

At its discretion, Salix, or its 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 such an inspection occurs, 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.

10.0 ETHICAL AND LEGAL ASPECTS

The Principal 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.

This trial will be performed in compliance with the protocol and in accordance with Good Clinical Practice (International Conference on Harmonisation [ICH], Guidance E6), principles of human subject protection, and applicable country-specific regulatory requirements.

10.1 Institutional Review Board/Ethics Committee (IRB/EC) Approval

This protocol, the Informed Consent, relevant supporting information and all types of subject recruitment or advertisement information must be approved by the appropriate IRB/EC before the study is initiated. Any amendments to the protocol must also be approved, where necessary, by the IRB/EC 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/EC are as follows:

- Obtaining IRB/EC approval of the protocol, Informed Consent, and any advertisements to recruit subjects prior to their use.
- Obtaining IRB/EC approval for any protocol amendments and Informed Consent revisions before implementing the changes.
- Providing the IRB/EC with any required information before or during the study.
- Submitting progress reports to the IRB/EC, as required, during the conduct of the study; requesting re-review and approval of the study, as needed; providing copies of all IRB/EC re-approvals and relevant communication to the Sponsor.
- Notifying the IRB/EC within 10 days (unless required sooner by IRB/EC) of all serious and unexpected AEs related to the study medications that are reported to you by the Sponsor. The Investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case, at least once a year. The Investigator must also keep the IRB/EC informed of any AEs, according to the IRB/EC policy.
- Notifying Salix within 24 hours of awareness and the IRB/EC within in 10 days (unless required sooner by IRB/EC) 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 the following criteria:

- Unexpected in nature, severity, or frequency (i.e., not described in study-related documents such as the IRB-approved protocol or consent form, the Investigators brochure, etc.).
- 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).

10.2 Subject Information and Informed Consent

Informed consent will be obtained from each subject prior to conducting/obtaining any study-related assessments including the discontinuation of any study prohibited medications. An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. The document must be in a language understandable to the subject and must specify who informed the subject.

The Investigator's draft Informed Consent Form (ICF) must be reviewed by Salix or its designee, prior to IRB/EC submission for approval. An IRB/EC-approved copy of the ICF will be forwarded to Salix or its designee. If during the study, the subject's protected health information (PHI) is to be used or disclosed in a manner that is inconsistent with the informed consent that was provided, the Investigator must obtain a new authorization from the subject or a waiver of authorization from the IRB/EC or privacy board, as applicable. If the ICF is amended during the study, the Investigator must follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the IRB/EC and use of the amended form (including for ongoing subjects).

The ICF documents the study-specific information the Investigator provides to the subject and the subject's agreement to participate. The Investigator will fully explain in layman's terms the nature of the study, along with the aims, methods, anticipated benefits, potential risks, and any discomfort participation may entail. The ICF must be appropriately signed and dated before the subject undergoes any study-related procedure. The decision regarding subject participation in the study that is made either by the subject or the subject's legally acceptable representative is entirely voluntary. The Investigator or his/her designee must emphasize to the subject that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The ICF shall also contain the subject's authorization for the use and disclosure of his/her PHI in connection with the study. The authorization shall include at a minimum a clear description of: 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 re-disclosure of PHI. For purposes of this study, only those subjects participating in the study or the subject's legally acceptable representative(s) will be allowed to sign the ICF. This applies to all amended Informed Consent Forms as well.

A separate healthy volunteer Inform Consent will be provided for flow cytometry assessment (approximately 25 subjects). These healthy volunteers will not be asked to take part in the study.

The original and any amended signed and dated ICFs must be retained in the subject's file at the study site; and a copy must be given to the subject or subject's legally acceptable representative(s).

10.3 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 related data to Salix (or designee)
- Accounting, reconciliation, and final disposition of used and unused study drug and placebo
- Review of site study records for completeness

In addition, Salix 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, Salix will discuss this with the Investigator (including the reasons for taking such action) at that time. Salix will promptly inform all other Investigators and/or institutions conducting the study if the study is suspended or terminated for safety reasons and will also 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/EC promptly and provide the reason for the suspension or termination.

10.3.1 Study Discontinuation

Should the Investigator, Salix, 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 Salix, 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 Salix to alter, suspend, or discontinue the development of the investigational product.

10.3.2 Study Site Discontinuation

The study site can be discontinued at the request of Salix, the Investigator, IRB/EC, or by the FDA or local regulatory authorities. Conditions that may warrant discontinuation of the study site include, but are not limited to, the following:

- The failure of the Investigator to accrue subjects into the study at an acceptable rate.
- The failure of the Investigator to comply with applicable current regulations and/or GCPs.
- The 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, all study data must be returned to Salix or designee. In addition, the site must conduct final disposition of all used and unused study drugs in accordance with Salix 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 Salix.

10.4 Handling and Record Keeping

10.4.1 Case Report Forms and Database Processing

Subject data will be collected in an electronic Case Report Form (eCRF) using Electronic Data Capture (EDC) system. The EDC system will be 21 CFR 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 Salix. The Investigator must sign and date 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 case report form data will be provided to the Investigator at the end of the study and will need to be retained by the Investigator.

10.4.2 Source Documents

Source documents consist of, but are not limited to, in-patient hospital charts, clinic notes, out-patient records, original test results, laboratory data, worksheets, drug accountability records, consent forms, etc. Source documents must be available for review and inspection during on-site monitoring of the study by Salix, their designees, IRB/IEC, and/or regulatory authorities.

10.4.3 Subject Tracking

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

10.4.4 Study Files

The investigational center will maintain a specific study file. This file is subject to inspection as described under Section 9.2 and Section 9.3 of this protocol.

10.4.5 Data Management

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

10.5 Confidentiality

Anonymity of subjects participating in this study will be maintained. Only subject numbers will be on any study documents submitted to the Sponsor. Every effort will be made to maintain the confidentiality of documents that identify the subject by name (e.g., signed informed consent document, laboratory reports, clinic charts), except to the extent necessary to allow auditing by the FDA, or other regulatory authorities. Should the name and/or address of a subject participating in this trial be on a document submitted 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 number.

The Investigator and other study site personnel will keep confidential any information provided by Salix (including this protocol) related to this study and all data and records generated while 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/IEC 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 10.8. 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.

10.6 Record Retention

Essential documents as described above should be retained for one of the following time periods:

- At least 2 years after the last marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region.

OR

- At least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

These documents should be retained for a longer period however if required by applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of Salix to inform the Investigator/institution as to when these documents no longer need to be retained.

10.7 Financing and Insurance

10.7.1 Finance

This study is supported by Salix Pharmaceuticals, Inc. a division of Bausch Health US, LLC.

10.7.2 Insurance and Indemnification

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

10.8 Publication Policy

The institution and the Investigator shall have the right, consistent with academic standards, to publish or present the results of the study, provided such publication or presentation does not disclose confidential information or other proprietary trade secrets of Salix. Salix will work with the protocol development team to identify a lead author for manuscript development. The manuscript authors will be determined by the amount of effort and participation each Investigator contributes towards the study design, the conduct of the study and the analysis of the study data. Prior to any publication, presentation or other disclosure, institution will submit the manuscript of any proposed publication (including abstracts, or presentation to a journal, editor, meeting, seminar, or other third party) to Salix at least sixty (60) days before publication, and Salix shall have the right to review and comment upon the proposed publication. Upon the Sponsor's request, publication will be delayed up to ninety (90) additional days to enable Salix to secure adequate intellectual property protection for Salix's property that could be affected by said publication. Salix shall have the right to require the deletion of any trade secret, or proprietary or confidential information of the Sponsor. Any publication or disclosure by the institution or the Investigator shall give appropriate credit to the Sponsor. Where title is not retained by the publisher, title to and the right to determine the disposition of any copyrightable material, first produced or composed in the performance of the study, shall remain with the institution. With respect to such materials, the institution hereby grants to Salix an irrevocable, royalty-free, nonexclusive right to reproduce, translate, and use any such copyrighted material for its own research and commercial purposes. The Investigator acknowledges and agrees that the study data and results may be disclosed by Salix to other clinical Investigators, the FDA, and other regulatory authorities.

10.9 Ownership

All data and records provided by Salix Pharmaceuticals, Inc., or Bausch Health Companies Inc. and its affiliates, or generated during the study (other than a subject's medical records) and all inventions discovered during the course of conducting the study are the property of Salix. If a written agreement is executed by the parties for the conduct of the study, which includes ownership provisions inconsistent with this statement, the ownership provisions contained in the written agreement between the parties shall control.

11.0 REFERENCES

Centers for Disease Control and Prevention (CDC). Incidence of Sickle Cell Trait – United States, 2010, 2014. MMWR. 2014, 63(49);1155-1158. Obtained 24 April 2020 at: https://www.cdc.gov/ncbddd/sicklecell/features/keyfinding-trait.html

da Guarda CC, Yahouédéhou SCMA, Santiago RP, Neres JSdS, Fernandes CFdL, et al. Sickle cell disease: A distinction of two most frequent genotypes (HbSS and HbSC). PLOS ONE. 2020, 15(1)

Darbari, DS, Sheehan, VA, Ballas, SK. The vaso-occlusive pain crisis in sickle cell disease: Definition, pathophysiology, and management. *Eur J Haematol*.; 2020, 105:237–246.

Dutta D, Methe B, Amar S, et al. Intestinal injury and gut permeability in sickle cell disease. *J Transl Med* 2019, 17**:**183

Dutta D, Li K, Methe B, Lim SH. Rifaximin on intestinally-related pathologic changes in sickle cell disease. *Am J Hematol.* 2020 Apr;95(4):E83-E86

Lard LR, Mul FP, de Haas M, Roos D, Duits AJ. Neutrophil activation in sickle cell disease. *J Leukoc Biol.* 1999 Sep;66(3):411-5

Lim SH, Fast L, Morris A. Sickle cell vaso-occlusive crisis: it's a gut feeling. *J Transl Med*. 2016 Dec 1;14(1):334

Lim SH, Morris A, Li K, Fitch AC, Fast L, Goldberg L, Quesenberry M, Sprinz P, Methé B. Intestinal microbiome analysis revealed dysbiosis in sickle cell disease. *Am J Hematol.* 2018 Aug;93(4):E91-E93

Lim SH, Dutta D, Moore J. Rifaximin for sickle cell disease. *Am J Hematol.* 2019 Dec;94(12):E325-E328

Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. *Blood*. 2013 Dec 5;122(24):3892-8

Steinberg, M.H. and Sebastiani, P. (2012), Genetic modifiers of sickle cell disease. *Am. J. Hematol*., 2012, 87: 795-803

Xu D, Gao J, Gillilland M 3rd, Wu X, Song I, Kao JY, Owyang C. Rifaximin alters intestinal bacteria and prevents stress-induced gut inflammation and visceral hyperalgesia in rats. *Gastroenterology*. 2014 Feb;146(2):484-96

Yale SH, Nagib N, Guthrie T. Approach to the vaso-occlusive crisis in adults with sickle cell disease. *Am Fam Physician*. 2000, Mar 1;61(5):1349-56, 1363-4. Erratum in: *Am Fam Physician* 2001 Jul 15;64(2):220

Zhang, D., Chen, G., Manwani, D. *et al.* Neutrophil ageing is regulated by the microbiome. *Nature* 2015, 525**:**528–532

Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. *Blood*. 2016 Feb 18;127(7):801-9

APPENDIX 1: SCHEDULE OF ASSESSMENTS

Study Period	**Screening**	**Treatment**							**Follow-up**	**During Visit to Medical Facility for VOC**
Study Day	**-21 to -1**	**1**	**8 (±1 day)**	**15 (±1 day)**	**29 (±1 day) EOT/ET**	**30**	**31**	**32**	**43 (±3 days) EOS**	
Visit Number [a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
Informed consent	X									
Demographics	X									
Medical history [b] (VOC history)	X									
Eligibility criteria	X	X								
Height and Weight	X				X [j]				X [j]	
Vital signs [c]	X	X	X	X	X	X	X	X	X	X
Physical examination [d]	X	X	X	X	X	X	X	X	X	X
ECG [e]	X	X			X				X [e]	
Hematology and Chemistry	X	X	X	X	X		X		X [j]	X
Hemoglobin genotype [f]	X									
Coagulation Panel	X									
HIV1 and HIV2	X									
HBsAg, HBcAb, HBsAb, and HCV	X									
Urinalysis	X	X	X	X	X				X [j]	X
Pregnancy testing [g]	X	X			X				X	
Randomization		X								
CANs		X	X	X	X		X		X	X
Serum CD62L		X	X	X	X		X		X	X
Serum iFABP		X	X	X	X		X		X	X
Serum -LPS		X	X	X	X		X		X	X
Urine 3-indoxyl sulfate		X	X	X	X		X		X	X
PK Samples	See Appendix 2									

VOCs [h]		X	X	X	X				X	
Dispense Study Drug		X	X	X						
Collect Study Drug			X [j]	X [j]	X					
Assessment of Compliance			X	X	X					
Adverse Events	X	X	X	X	X	X	X	X	X	X
Concomitant	X	X	X	X	X	X	X	X	X	X
Opioid use [i]	X	X	X	X	X				X	X

Abbreviations: CAN = circulating aged neutrophils; ECG = electrocardiogram; EOS = end of study; EOT = end of treatment; ET = early termination; hsCRP = high-sensitivity C-reactive protein; iFABP = intestinal fatty acid binding protein; LPS = Lipopolysaccharide PK = pharmacokinetics, VOC = vaso-oclusive crisis

a. In case of situations that prevent a participant from attending a post-baseline study visit, a home health visit may be scheduled, where possible. The home health visit may include an assessment of changes in health and medications, measurement of vital signs and body weight, and checking of study medication compliance. The home health visit may also include all sample collections outlined in the study protocol (PK, hematology and urinalysis samples, urine sample for urine 3-inoxyl sulfate, and blood samples for CANs (if applicable), CD62L, iFABP and LPS). All documented assessments and laboratory results will be reviewed by the investigator and documented in relevant eCRFs as appropriate.

b. Medical history will be obtained from prior 3 months. Detailed history of VOCs occurring over the past 12 months will be obtained. This will include collection of treatments for VOCs including drug, dose, route of admin, duration of treatment, and inpatient/outpatient setting.

c. Vital signs will be obtained after the subject has been in a supine position for a minimum of 5 minutes. Vital signs include systolic and diastolic blood pressure, heart rate, body temperature, and oxygen saturation.

d. Perform a full physical examination at Screening and symptom-directed physical examination at subsequent visits.

e. A 12-lead ECG (supine) will be performed. An ECG will be performed at the Day 43 (EOS) Visit only if abnormal findings were observed at the Day 29 (EOT/ET) Visit.

f. Hemoglobin genotyping will be recorded if known. If unknown at Screening, hemoglobin genotyping will be performed using HPLC/electrophoresis.

g. Serum pregnancy test is to be performed at Screening for all women of childbearing potential. A negative Screening serum pregnancy test and a negative urine pregnancy test must be confirmed before randomization. Subsequent pregnancy testing will be with urine pregnancy test.

h. Information on whether a VOC requiring a visit to a medical facility occurred will be recorded. Medications received during the VOC visit will be recorded.

i. Only weight will be collected.

j. Hematology, chemistry, and/or urinalysis will only be collected at this visit if needed for follow-up or resolution of AEs/SAEs.

k. Not all treatment groups will return study drug at Visit 3 and Visit 4.

l. During Screening, record all opioid use for the prior 30 days. At each visit, record all opioid use since the previous visit.

APPENDIX 2: SCHEDULE FOR ACQUISITION OF PK SAMPLES

		Time (hours) Post-dose											
	Pre-Dose	0.5	1	1.5	2	3	4	5	8	24	30	48	72
Day 1 (Visit 2)	X	X	X	X	X	X	X	X	X				
Day 8 (Visit 3)	X	X	X		X								
Day 15 (Visit 4)	X	X	X		X								
Day 29[a] (Visit 5)	X	X	X	X	X	X	X	X	X	X	X	X	X
During Visit for VOC	PK sample obtained during visit, when possible, and estimated time from last dose recorded												

Abbreviations: VOC = vaso-occlusive crisis

a. Following the final dose on Day 29, blood samples will be collected pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 5, 8, 24 (Visit 6), 30 (Visit 6), 48 (Visit 7), and 72 (Visit 8) hours post-dose.

The windows around the collection timepoints are:

- within 15 minutes prior to dose for pre-dose samples,
- ± 5 minutes for samples taken at 0.5 hours through 1.5 hours, and
- ±10 minutes for samples taken 2 hours through 8 hours.
- ±2 hours for samples taken 24 hours through 72 hours.

APPENDIX 3: PROHIBITED CYP3A4, P-GP AND OATP1B1/B3 INHIBITORS/INDUCERS

The following table (not exhaustive) provides a list of prohibited drugs, herbal products, and foods. Any questions or concerns should be referred to the Sponsor or designee.

CYP3A4 Inhibitors	Drugs	amprenavir, aprepitant, atazanavir, boceprevir, ciprofloxacin, clarithromycin, cobicistat, conivaptan, crizotinib, cyclosporine, danoprevir, darunavir, diltiazem, dronedarone, elvitegravir, erythromycin, faldaprevir, fluconazole, idelalisib, imatinib, indinavir, isavuconazole, itraconazole, ketoconazole, mibefradil, nefazodone, nelfinavir, netupitant, nilotinib, posaconazole, ribociclib, ritonavir, saquinavir, telaprevir, telithromycin, tofisopam, troleandomycin, verapamil, voriconazole
	Herbals and Foods	grapefruit juice, *Schisandra sphenanthera* extract
CYP3A4 Inducers	Drugs	bosentan, carbamazepine, dabrafenib, efavirenz, enzalutamide, etravirine, genistein, lopinavir, lumacaftor, mitotane, modafinil, nafcillin, phenobarbital, phenytoin, rifabutin, rifampicin[rifampin], telotristat, thoridazine
	Herbals and Foods	St. John's wort
P-gp Inhibitors	Drugs	alogliptin, amiodarone, azithromycin, canaglifozin, captopril, carvedilol, clarithromycin, clopidrogel, cobicistat, conivaptan, cremophor EL and RH40, daclatasvir, diltiazem, dronedarone, eliglustat, erythromycin, felodipine, fluvoxamine, fostamatinib, glecaprevir, isavuconazole, itraconazole, ivacaftor, ketoconazole, lapatinib, lasmiditan, ledipasvir, lopinavir, mibefradil, mirabegron, nifedipine, nitrendipine, paroxetine, pibrentasvir, propafenone, quinidine, quinine, ranolazine, rifampicin[rifampin], ritonavir, rolapitant, saquinavir, sarecycline, simeprevir, suvorexant, talinolol, telaprevir, telithromycin, telmisartan, ticagrelor, tipranavir, tolvaptan, valspodar, vandetanib, velpatasvir, vemurafenib, verapamil, voclosporin, vorapaxar, voxilaprevir
	Herbals and Foods	Ginkgo biloba, milk thistle, quercetin, *Schisandra chinensis* extract, St. John's wort, curcumin
P-gp Inducers	Drugs	carbamazepine, efavirenz, indinavir, nelfinavir, phenytoin, rifampicin[rifampin]
	Herbals and Foods	danshen, genistein
OATP1B1/OATP1B3 Inhibitors		atazanavir, clarithromycin, cyclosporine, erythromycin, gemfibrozil, lopinavir and ritonavir, rifampin (single dose), simeprevir

		Cobicistat, Daclatasvir, Eltrombopag, Glecaprevir, Letermovir, Paritaprevir, Pibrentasvir, Sacubitril, Saquinavir, Telithromycin, Teriflunomide, Tipranavir, Velpatasvir, Voclosporin, Voxilaprevir horny goat weed and milk thistle

Prohibited substances compiled from multiple sources, including:

1. FDA DDI Index Inhibitors and Inducers (https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers)
2. Flockhart Table at Indiana University (https://drug-interactions.medicine.iu.edu/Home.aspx)
3. UW DIDB (https://www.druginteractionsolutions.org/solutions/drug-interaction-database/) Prescriber's Information for FDA approved products
   Ali et al., The involvement of human organic anion transporting polypeptides (OATPs) in drug-herb/food interactions, Chin Med. 2020 Jul 9;15:71. doi: 10.1186/s13020-020-00351-9. eCollection 2020.