CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210910Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA/BLA Multi-disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	210910
Priority or Standard	Priority
Submit Date(s)	March 16, 2018
Received Date(s)	March 16, 2018
PDUFA Goal Date	November 16, 2018
Division/Office	Division of Anti-Infective Products/Office of Antimicrobial Products
Review Completion Date	November 16, 2018
Established Name	Rifamycin
(Proposed) Trade Name	AEMCOLO
Pharmacologic Class	Ansamycin antibacterial
Code name	CB-01-11
Applicant	Cosmo Technologies, Ltd. / Conventus BioMedical Solutions, Inc.
Formulation(s)	194 mg Tablets
Dosing Regimen	388 mg (2 tablets) BID x 3 days
Applicant Proposed Indication(s)/Population(s)	*Treatment of Travelers' Diarrhea in adults* (b) (4)
Regulatory Action	Approval
Indication(s)/Population(s)	Treatment of travelers' diarrhea in adults caused by non-invasive strains of *Escherichia coli*. Limitations of Use: Do not use in patients with diarrhea complicated by fever or blood in the stool or due to pathogens other than *Escherichia coli*.

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OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
DRISK=Division of Risk Management

Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
ADR	adverse drug reaction
AE	adverse event
AESI	adverse events of special interest
AIDS	acquired immunodeficiency syndrome
BMD	Bone mineral density
BPCA	Best Pharmaceuticals for Children Act
CDC	Center for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CI	confidence interval
CMC	chemistry, manufacturing, and controls
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
DAIDS	Division of AIDS
DAIP	Division of Anti-infective Products
DMC	data monitoring committee
ECG	electrocardiogram
ETEC	Enterotoxigenic *Escherichia coli*
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GLP	good laboratory practice
ID	infectious diarrhea
ICH	International Conference on Harmonization
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent-to-treat
mITT-E	modified intent-to-treat
micro-ITT	microbiological intent-to-treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NDA	new drug application
NME	new molecular entity

OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	protease inhibitor
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
TD	travelers' diarrhea
TEAE	treatment emergent adverse event
TEADR	treatment emergent adverse reaction
USPI	US prescribing information
WHO	World Health Organization

1 Executive Summary Office Level Concurrence

1.1. Product Introduction

Rifamycin SV-MMX® (hereafter referred to as rifamycin or rifamycin delayed-release tablets), proposed trade name AEMCOLO, a new molecular entity (NME) is an antibacterial drug in the class of ansamycins. Rifamycins were first isolated in 1957 from a fermentation culture of *Streptomyces mediterranei*, later renamed *Amycolatopsis rifamycinica.* The first chemical modifications led to the development of Rifamycin SV which was introduced in Europe in 1962 for parenteral and topical treatment of infections caused by gram positive bacteria and biliary tract infections due to gram negative bacteria. (Bergamini and Fowst 1965, Sensi 1983) Rifamycin acts by binding to an inhibiting DNA-dependent RNA polymerase, interfering with bacterial protein synthesis. (b) (4) rifamycin is formulated and administered as its monosodium salt, rifamycin sodium, and is minimally absorbed following oral administration. This product incorporates a pH-dependent protective polymer coating and a multi-matrix structure (MMX) intended to release of the drug in the colon.

The Applicant's proposed indication is the treatment of travelers' diarrhea in adults with the following limitation: (b) (4)

The recommended dose is 400 mg taken orally twice a day for three days, with or without food.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness and adequate safety data to support the approval of rifamycin delayed-release tablets for adults with travelers' diarrhea caused by non-invasive strains of *Escherichia coli (E. coli),* not complicated by fever or blood in the stool.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

In NDA 210910, the Applicant is seeking an indication for rifamycin (AEMCOLO) for the treatment of travelers' diarrhea in adults with a limitation to not use in patients with diarrhea complicated by fever or blood in the stool. To support the safety and efficacy of rifamycin for this indication, the Applicant has provided substantial evidence of effectiveness from a placebo-controlled superiority trial and supportive evidence from an active controlled trial. Rifamycin is an antibacterial drug in the ansamycin class. It is intended to be administered twice a day for three days with or without food.

Acute diarrhea is one of the most common illness affecting travelers, afflicting an estimated 10 to 40% of travelers during a two-week trip, depending on the season and the destination. Preventative measures such hand-washing, hand-sanitizers, and care with food and beverages can reduce but not eliminate the risk. While travelers' diarrhea rarely leads to death, morbidity can be significant, prompting medical care in about 10% of patients.

Rifamycin demonstrated superiority to placebo for the treatment of travelers' diarrhea due to non-invasive *E. coli* in a randomized, double-blind multi-center, placebo-controlled trial in adult travelers (Study C2009-0201). A second active-controlled trial comparing rifamycin to ciprofloxacin provided supportive evidence of efficacy (Study RIT-1/AID). The primary efficacy endpoint in Study C2009-0201 was time to last unformed stool (TLUS) in the intent to treat (ITT) population; in RIT-1/AID the primary efficacy endpoint was TLUS in the full analysis set (FAS) population. In C2009-0201, the median TLUS was significantly shorter in the rifamycin group, 46.0 hours compared with 68.0 hours in the placebo group ($p< 0.0008$, due to the distribution of placebo TLUS values, it was not possible to compute a 95% confidence interval for this difference). In addition, 81.4% of subjects in the rifamycin group achieved clinical cure compared with 56.9% of subjects in the placebo arm ($p<0.0001$). The median TLUS in the rifamycin group was 44.3 hours compared to 40.3 hours in the ciprofloxacin group.

Diarrheagenic *E. coli* species were the most common sole pathogens isolated in C2009-0201 and RIT-1/AID (57.5% and 39.2% of subjects, respectively). Findings of efficacy with regard to TLUS and clinical cure were consistent in patients who were infected only with *E. coli* at baseline. There were very few subjects enrolled with infection due to other invasive pathogens. While subjects with baseline fever or blood in stool were to be excluded from both phase 3 trials according to study protocols, in RIT-1/AID 18 subjects (4.3%) receiving rifamycin had blood and/or fever at baseline. These subjects had longer TLUS (57.0 hours) than subjects without blood in stools and/or fever (42.0 hours).

Overall, the results from C2009-0201 provide substantial evidence of rifamycin efficacy as a treatment of adults with travelers' diarrhea caused

by non-invasive *E. coli,* not complicated by fever or blood in stools. The robust finding of superiority for the primary endpoint of TLUS (p< 0.0008) and a key secondary endpoint of clinical cure in this single adequate and well-controlled trial is supported by the findings from RIT-1/AID.

Rifamycin has an acceptable safety profile. The safety database includes approximately 619 subjects with travelers' diarrhea receiving rifamycin at a dose of 800 mg per day for 3 days. When administered orally, rifamycin is poorly absorbed with approximately 83% excreted unchanged in the stool. In phase 3 studies, headache and constipation were the only reported treatment-emergent adverse events (TEAEs) that occurred with rifamycin at a rate greater or equal to 2% and higher than the comparator. Safety information and the limitation regarding use in patients with fever or blood in the stool or for infection due to pathogens other than *E. coli* are communicated in labeling and no additional risk mitigation strategies are needed at this time.

Table 1: Benefit-Risk Assessment of Rifamycin

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Travelers' diarrhea occurs in 15 to 20 million individuals each year, approximately 10 to 40% of travelers to tropical or subtropical locations during a two-week trip. • Left untreated, the average duration of travelers' diarrhea is 4 to 5 days. Chronic complications, such as post-infectious irritable bowel syndrome, may develop in 3 to 17% of travelers' diarrhea patients.	Given the significant morbidity associated with this condition, therapies that can decrease symptoms, shorten the course of illness, and minimize disruptions to travelers' activities would be of significant benefit to patients and clinicians.
Current Treatment Options	• For mild acute travelers' diarrhea that does not interfere with planned activities, supportive care including hydration is recommended; loperamide or bismuth subsalicylate may be considered to mitigate enteric symptoms. • For moderate or severe diarrhea that is distressing or interferes with planned activities, the Centers for Disease Control and Prevention (CDC) recommendations include use of antibacterial drugs such as fluoroquinolones (FQs), e.g. ciprofloxacin and levofloxacin, azithromycin, and rifaximin (limited to non-invasive strains of *E. coli);* antimotility agents such as loperamide; and bismuth subsalicylate. (Centers for Disease Control and Prevention, 2018) • Drugs with an approved indication for treatment of travelers' diarrhea include rifaximin and trimethoprim-sulfamethoxazole; ciprofloxacin is approved for infectious diarrhea. Due to resistance to FQs and trimethoprim-sulfamethoxazole, azithromycin is currently the most commonly recommended antibacterial drug for empiric treatment of travelers' diarrhea.	For moderate to severe travelers' diarrhea, treatment with effective antibacterial drugs has been demonstrated to shorten the average duration by one day, with further benefit afforded by adding an antimotility agent to the treatment regimen. Therapies that are poorly absorbed from the gastrointestinal tract and that act selectively in the distal ileum or colon have the theoretical advantage of targeting specific colonic pathogens, such as diarrheagenic *E. coli,* while minimizing systemic toxicity.
Benefit	• The clinical efficacy of rifamycin for the treatment of travelers' diarrhea caused by non-invasive *E. coli* was demonstrated in a phase 3 clinical trial C2009-0201, with supportive evidence provided by a second phase 3 trial, RIT-1/AID, in a total of 619 patients. • The primary efficacy endpoint for C2009-0201 was TLUS. The median TLUS	The phase 3 placebo-controlled trial, C2009-0201, provided substantial evidence of rifamycin's effectiveness for the treatment of travelers' diarrhea caused by non-invasive *E. coli.* The active comparator trial RIT-1/AID

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	was significantly shorter in the rifamycin group (46.0 hours) compared with placebo (68.0 hours) (p< 0.0008). In addition, 81.4% of subjects in the rifamycin group achieved clinical cure compared with 56.9% of subjects in the placebo arm (p<0.0001). The findings of rifamycin effectiveness in travelers' diarrhea in the C2009-0201 trial were supported by the results of the RIT-1/AID trial. • Diarrheagenic *E. coli* species were the most common pathogens isolated alone or with other pathogens, with *E. coli* alone found in 57.5% and 39.2% of study subjects in C2009-0201 and RIT-1/AID, respectively. Findings of efficacy with regard to TLUS and clinical cure were consistent in patients who were infected only with *E. coli* at baseline. • Rifamycin appears to act locally in the gastrointestinal tract with limited systemic absorption (bioavailability <1%). Microbial eradication following treatment with rifamycin was not significantly different from placebo. • Overall, the data support the effectiveness of rifamycin in reducing TLUS and in the clinical cure of subjects with travelers' diarrhea due to non-invasive *E. coli* who are not experiencing fever or blood in stools.	provided supportive evidence for efficacy. Rifamycin adds another option to armamentarium for empiric treatment of travelers' diarrhea, with benefits and limitations similar to rifaximin. Specifically, rifamycin has a similar spectrum of activity (i.e. limited to non-invasive *E. coli*), low systemic absorption, and duration of therapy (three-day course of treatment). Like rifaximin, rifamycin is well-tolerated by patients and shortens the course of travelers' diarrhea by about one day.
Risk	• In phase 3 studies, rifamycin was well tolerated. In C2009-0201, constipation was the only adverse event that occurred more often in the rifamycin-treated patients and at rate of ≥ 2% (3.5% vs. 1.5% of rifamycin and placebo recipients, respectively). In RIT-1/AID, for events that occurred ≥ 2% headache was most common, occurring in 3.3% rifamycin subjects compared with 1.9% of those receiving ciprofloxacin. • There were no deaths in the rifamycin development program. Only three subjects (0.5%) receiving rifamycin in the pooled phase 2-3 trials experienced serious adverse events, with only one (abdominal pain)	• No significant safety signals were identified during the clinical development of rifamycin. The adverse event profile for rifamycin in adults with travelers' diarrhea is similar to that observed in clinical trials of rifaximin. The current labeling with changes recommended by FDA provides adequate information on the benefits and risks of rifamycin. • In addition to the finding of longer TLUS in

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	possibly related to the study medication. Only 6 (1.0 %) of subjects in phase 3 studies discontinued treatment due to adverse events. • While no subjects in C2009-0201 had bloody stool or fever, in RIT-1/AID 18 (4.3%) subjects receiving rifamycin had bloody stools and/or temperatures ≥ 38°C at baseline. These subjects had longer TLUS (57.0 hours), compared to those without bloody stools or fever TLUS (42.0 hours).	these patients, there is also concern that an intraluminal drug like rifamycin will have limited efficacy in diarrheal disease due to invasive pathogens. • Product labeling includes a limitation of use statement for patients with diarrhea complicated by fever and/or bloody stool or due to pathogens other than noninvasive strains of *E. coli*.
Risk Management	• The efficacy of rifamycin has not been demonstrated in infectious diarrhea or in travelers' diarrhea due to the pathogens other than *E. coli* or complicated by fever and hematochezia. • The safety and efficacy of rifamycin has not been studied in pediatric patients. • The Applicant plans to use a "lock and key" packaging to satisfy requirements for dispensing of AEMCOLO in child-resistant containers.	Data on the safety and efficacy of rifamycin for the treatment of travelers' diarrhea are only available for adults and will be reflected in product labeling. The Applicant has submitted an Agreed iPSP to evaluate safety and efficacy of rifamycin in pediatric patients. The Applicant will conduct a Human Factors Study as a reportable postmarketing commitment to further evaluate whether the intended population for AEMCOLO can effectively use the packaging to self-administer recommended doses and maintain the child-resistant features.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

□	The patient experience data that was submitted as part of the application include:	Patient experience data not submitted for this application.
	□ Clinical outcome assessment (COA) data, such as	NA
	□ Patient reported outcome (PRO)	NA
	□ Observer reported outcome (ObsRO)	NA
	□ Clinician reported outcome (ClinRO)	NA
	□ Performance outcome (PerfO)	NA
	□ Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	NA
	□ Patient-focused drug development or other stakeholder meeting summary reports	NA
	□ Observational survey studies designed to capture patient experience data	NA

2 Therapeutic Context

2.1. Analysis of Condition

Acute diarrhea is an important cause of morbidity in travelers, including members of the military, business travelers, tourists, students, and expatriates. (Steffen, Hill et al. 2015) By one estimate, 15-20 million travelers to developing countries experience diarrhea annually, or 40,000 travelers daily. (Steffen 2005) While the overall incidence of travelers' diarrhea during a two week trip is decreasing, from over 65% two decades ago to 10-40% more recently (Pitzurra, Steffen et al. 2010), morbidity remains significant for those affected. Diarrheal disease in travelers can have a large impact on military operations and for other populations can exact a cost on productivity, finances, and general well-being. (Riddle, Sanders et al. 2006) (Soonawala, Vlot et al. 2011) As a large number of individuals experiencing symptoms self-treat, the actual magnitude of the disease burden is uncertain.

Travelers' diarrhea is caused by ingestion of contaminated food and/or liquids. Up to 80% of infectious diarrheal illnesses in travelers are caused by bacterial pathogens. (Steffen, Sack et al. 2003) While health experts routinely advise tourists and business travelers on ways to avoid potentially contaminated liquids and food, studies demonstrate that the incidence of diarrhea is more closely linked to the level of sanitation at the destination than interventions by travelers. (Centers for Disease Control and Prevention 2018) More than half of travelers self-treat their symptoms, most commonly with antimotility agents, bismuth subsalicylate, oral rehydration solutions, and/or antibacterial drugs provided prior to travel. (Hill 2000)

Travelers' diarrhea is usually described from the vantage point of an individual residing in a high-income country traveling to a lower income country and is defined at $\geq$ 3 unformed stools during a 24-hour period during the first 2 weeks of travel, accompanied by at least one other symptom such as abdominal cramps, tenesmus, nausea, vomiting, fever, blood in stools, or fecal urgency. (Steffen, Hill et al. 2015)

When a complete microbiologic assessment is conducted, pathogens can be identified in 50 to 90% of patients with travelers' diarrhea. Pathogens most commonly isolated (in declining order) include: ETEC (heat labile and heat-stable toxin producing), enteroaggregative *E coli* (EAEC), diffusely adherent *E coli* (DAEC), which collectively comprise about 35 to 45% of traveler diarrhea cases. Other pathogens that have been identified include *Salmonella* species, *Campylobacter jejuni*, *Shigella* species, *Aeromonas* species, *Plesiomonas shigelloides*, enterotoxigenic *Bacteroides fragilis*, and *Vibrio* species. Certain regions have a higher incidence of travelers' diarrhea caused by parasites including *Giardia duodenalis*, *Cryptosporidium* species, *Entamoeba histolytica*, and *Microsporidium* species. *Cyclospora* is a seasonal cause of travelers' diarrhea with highest risks observed in Nepal, Peru, Haiti, and Guatemala. (Centers for Disease Control and Prevention 2018) Travelers' diarrhea may be caused by rotavirus and noroviruses, with noroviruses identified as a cause of outbreaks on cruise ships. Pathogen identification may be increased with the use of multiplex quantitative PCR tests; in one study of

travelers' diarrhea this technology identified a pathogen in 76% of stool samples. (Antikainen, Kantele et al. 2013)

Whereas ETEC is the most common enteric pathogen for most of the developing world, *Campylobacter* and *Aeromonas* are more frequent in Southeast Asia. (Steffen, Hill et al. 2015) The types of *E. coli* vary in their sites of infection from the small intestine to the colon while *Shigella* spp., *Campylobacter* spp. and non-typhoid *Salmonella* primarily affect the colon. This differential site of pathogenesis has led to the development of targeted therapies, including rifamycin.

Risk factors for travelers' diarrhea include the specific destination, length of exposure, and travel style including the type of meal, e.g. street food, favored by the traveler. Travelers' diarrhea is equally common in males and females and has a higher incidence in young adults rather than older travelers. For short term travelers, one episode of travelers' diarrhea does not protect against additional episodes. Temperate regions exhibit seasonal variations in travelers' diarrhea, for example, with higher rates observed in hot months preceding the monsoon. Increased exposure to fecal pathogens causing travelers' diarrhea can occur in warmer climates with reduced access to plumbing and latrines, inadequate refrigeration, and unsafe or diminished water supplies. Use of strategies to prevent contamination of food during production in hotels and restaurants can reduce the incidence of diarrheal illnesses by up to 72%. (Ashley, Walters et al. 2004) High-risk locations for travelers' diarrhea include most of Asia, the Middle East, Africa, Mexico, and Central and South America. Intermediate-risk countries include Eastern Europe, South Africa, and some Caribbean islands. (Centers for Disease Control and Prevention 2018)

While travelers' diarrhea is generally a self-limited illness and is rarely life-threatening, it is frequently a cause of incapacitation during visits to developing countries. Left untreated, bacterial diarrhea generally resolves within 3-7 days and viral diarrhea within 2-3 days. (Soonawala, Vlot et al. 2011) While the mean length of incapacitation is generally less than one day, nearly 25% experienced nausea and vomiting and more than a third require alteration in their activities. (Steffen, Hill and DuPont; Hill) Travelers' diarrhea prompts medical care in about 10% of patients with up to 3% requiring hospitalization. (von Sonnenburg, Tornieporth et al. 2000) Treatment of travelers' diarrhea with an effective antibacterial drug has been demonstrated to shorten the average duration by one day with further benefit afforded by adding an antimotility agent. (Centers for Disease Control and Prevention 2018). The occurrence of travelers' diarrhea has also been linked to long term sequelae such as post infectious irritable bowel syndrome (PI-IBS) in 3-17% of patients. Factors associated with PI-IBS include the severity of diarrhea, number of episodes, and infection with heat-labile toxin-producing ETEC. Travelers' diarrhea has also been associated with other chronic conditions including reactive arthritis, often linked to HLA-B27, and Guillain-Barré syndrome. (Okhuysen, Jiang et al. 2004) Preventative measures to avoid contaminated food and water often have limited effectiveness, and use of prophylactic antibacterial drugs is not recommended for most travelers due to their effect on the protective microflora of the gut as well as their impact on the development of antimicrobial resistance. (Centers for Disease Control and Prevention 2018)

2.2. Analysis of Treatment Options

Pretravel consultation aims to identify travelers at increased risk for travelers' diarrhea and provide counseling on prevention and empiric treatment if symptoms occur. Travelers' diarrhea can occur despite proper hygiene. (Shlim 2005, Soonawala, Vlot et al. 2011) There are no vaccines available in the U.S. to prevent travelers' diarrhea. Vaccines against cholera (Vaxchora, live oral cholera vaccine) and typhoid (Vivotif, live oral Ty21a); typhoid vaccine (inactivated injectable) are available in the US and recommended in limited circumstances. (Shane, Mody et al. 2017) Limitations in prevention strategies point to the importance of identifying optimum treatment regimens for travelers' diarrhea.

In the analysis of treatment options, six sources of clinical recommendations and/or practice guidelines are referenced: CDC recommendations on treatment of travelers' diarrhea (Centers for Disease Control and Prevention 2018), International Society of Travel Medicine (ISTM) guidelines for the prevention and treatment of travelers' diarrhea (Riddle, Connor et al. 2017), Infectious Disease Society of America (IDSA) practice guidelines for the diagnosis and management of infectious diarrhea (Shane, Mody et al. 2017), IDSA guidelines for the Practice of Travel Medicine (Hill, Ericsson et al. 2006), and American College of Gastroenterology (ASG) clinical guidelines on the diagnosis, treatment, and prevention of acute diarrheal infections in adults. (Riddle, DuPont et al. 2016) These guidelines are summarized in the Table 2 below. Empiric treatment options for travelers' diarrhea overlap with those for infectious diarrhea, and the case definitions are similar but without the requisite travel history.

Although bismuth subsalicylate has been demonstrated to reduce the incidence of travelers' diarrhea by approximately 50% (Centers for Disease Control and Prevention 2018), its palatability, the need for QID dosing, and interactions with doxycycline which may be used concurrently for malaria prophylaxis, can limit its use. Antimicrobial chemoprophylaxis has been shown to be effective in the prevention of travelers' diarrhea (NIH 1985), although no antibacterial drugs have been approved by FDA for this indication. Practice guidelines have generally recommended against prophylaxis due to the occurrence of adverse events particularly those involving fluoroquinolones, the effect of antimicrobial use on the development of drug resistance, and the availability of empiric antibiotic treatment. (Riddle, DuPont et al. 2016) There is limited evidence on the effectiveness of probiotics and prebiotics and use is either not recommended (Riddle, DuPont et al. 2016, Riddle, Connor et al. 2017), or recommended with qualifications. (Shane, Mody et al. 2017)

For mild acute travelers' diarrhea (defined as tolerable and does not interfere with planned activities), antibiotic therapy is not recommended, but loperamide or bismuth subsalicylate (BSS) may be considered. (Riddle, Connor et al. 2017, Centers for Disease Control and Prevention 2018) Loperamide may be considered as monotherapy for moderate to severe travelers' diarrhea; its use as adjunctive therapy has been shown to decrease the duration of symptoms and increase the probability of cure. (Riddle, Arnold et al. 2008)

Per CDC, antibacterial drugs including fluoroquinolones, azithromycin, and rifaximin may be

used to treat cases of acute travelers' diarrhea with moderate symptoms, i.e. diarrhea that is distressing or interferes with planned activities. ASG does not recommend antimicrobial therapy for routine acute diarrheal infection, except in travelers' diarrhea where the higher likelihood of bacterial infections outweighs the potential risks. CDC recommends antibiotics for severe travelers' diarrhea, defined as diarrhea that is incapacitating or completely prevents planned activities; all dysentery is considered severe. Due to increasing antimicrobial resistance to fluoroquinolones, especially by *Campylobacter* isolates in South and Southeast Asia, CDC notes a preference for azithromycin for treatment of severe acute travelers' diarrhea. Fluoroquinolones and rifaximin are acceptable alternatives for nondysenteric travelers' diarrhea. Rifaximin's approved indication is for travelers' diarrhea caused by *E. coli*, the most common bacterial pathogen isolated from travelers in Western Hemisphere. Single dose antibacterial drug regimens of fluoroquinolones can be employed which may decrease the occurrence of adverse events. (Centers for Disease Control and Prevention 2018)

Some caveats exist with the use of empiric treatment of travelers' diarrhea, beyond the usual adverse events associated antimicrobial therapy, including *C. difficile* infection. Antibacterial drugs should be avoided in diarrhea caused by Shiga-like toxin producing *E. coli* due to the increased risk of hemolytic uremic syndrome. (Freedman, Xie et al. 2016) Empiric use of antimicrobial therapy for travelers' diarrhea has been shown to alter the gut microflora and has been associated with colonization by extended spectrum β-lactamases- and carbapenemase-producing Enterobacteriaceae, raising questions about the risks of empiric therapy to individuals and the community. (Kantele, Laaveri et al. 2015) In patients with diarrhea accompanied by fever, bloody or mucoid stools, severe abdominal cramping or tenderness, or signs of sepsis, IDSA recommends specific pathogen testing of stool for *Salmonella*, *Shigella, Campylobacter*, *Yersinia*, *C. difficile*, and STEC, with antimicrobial therapy adjusted accordingly. (Shane, Mody et al. 2017)

Table 2: Summary of Treatment Armamentarium Relevant for Empiric Treatment of Non-Dysenteric Travelers' Diarrhea in Adults

Product (s) Name/Year of Approval	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Source of Recommendation
Antibacterial therapies[1]					
Fluoroquinolones					

Product (s) Name/Year of Approval	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Source of Recommendation
CIPRO®(cipro-floxacin) Oral Suspension, Tablet 1990	Approved for **Infectious Diarrhea (ID)[2]** caused by *E. coli* (Enterotoxigenic strains), *Campylobacter jejeuni, Shigella boydii, Shigella flexneri, Shigella sonnei,* when antibacterial therapy is indicated; Approved for typhoid fever (enteric fever) caused by *Salmonella typhi*	Product label: Mild/Moderate /Severe 500 mg po every 12h for 5 to 7 days ASG[3]: 750 mg po as single dose or 500 mg po for 3 days	ASG: 9 randomized clinical trials found overall reduction in diarrhea compared with placebo with no serious adverse events in short term use.	Boxed Warning on increased risk of tendinitis and tenon rupture on fluoroquinolones, including ciprofloxacin; risk of *C. difficile* infection	Product Labeling Recommended by CDC[4] and ISTM[5] for non-dysenteric TD[6]; Recommended by ACG and IDSA[7] for treatment of ID or TD
Levofloxacin	**No indication in label for TD or ID**	ACG: 500 mg po single dose or 3 day course	See cipro	See cipro	See cipro
Ofloxacin	**No indication in label for TD or ID**	ACG: 400 mg po single dose or 3 day course	See cipro	See cipro	See cipro
Other antibiotics					
Zithromax® (azithromycin tablets and oral suspension)	**No indication in label for TD or ID** ASG: use empirically as first line in Southeast Asia and India to cover fluoroquinolone-resistant *Campylobacter* or in other geographical areas if *Campylobacter* or resistant ETEC are suspected	ASG and ISTM: 1000 mg po as single dose or 500 mg po for 3 days	ASG: No significant differences in efficacy observed in four randomized controlled trials comparing azithromycin to fluoroquinolones for TD; active against *Campylobacter*, *Shigella* and non-invasive *E. coli.*	Hepatotoxicity, C. difficile-associated diarrhea, QT prolongation.	ISTM ACG IDSA (ID and TD) CDC

Product (s) Name/Year of Approval	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Source of Recommendation
XIFAXAN® (Rifaximin) 2004	Approved for treatment of patients (> 12 years of age) with **TD** caused by noninvasive strains of *E. coli*	200 mg TID for 3 days	Demonstrated clinical effectiveness in two randomized clinical studies against *E. coli.* In both studies, the microbiological eradication rates in rifaximin-treated subjects were no different than those receiving placebo.	Should not be used in patients with diarrhea complicated by fever or blood in stool or diarrhea due to pathogens other than *E. coli.*	Product labeling CDC: due to traveler difficulty in distinguishing between invasive and noninvasive diarrhea, overall utility of rifaximin for empiric therapy remains uncertain. ISTM ASG IDSA-TD
BACTRIM™ (800 mg sulfamethoxazole and 160 mg trimethoprim DS tablets; 400 mg SMX and 80 mg TMP tablets)	Approved for **TD in Adults** due to susceptible strains of enterotoxigenic *E. coli*	1 BACTRIM DS tablet or 2 BACTRIM Tablets q 12 h for 5 days	Use supplanted by other agents due to widespread resistance.	The most common adverse effects are gastrointestinal disturbances and allergic skin reactions such as rash and urticaria. Fatalities have been associated with sulfonamides, including Stevens-Johnson Syndrome, Toxic Epidermal Necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia and other blood dyscrasias.	Not recommended for TD/ID by CDC, ISTM, IDSA, or ACG.
SEPTRA®DS (800 mg sulfamethoxazole and 160 mg trimethoprim DS tablets; 400 mg SMX and 80 mg TMP tablets)	Approved for **TD in Adults** due to susceptible strains of enterotoxigenic *E. coli*	Same as BACTRIM	Use supplanted by other agents due to widespread resistance.	Same as BACTRIM	Not recommended for TD/ID by CDC, ISTM, IDSA, or ACG.

Product (s) Name/Year of Approval	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Source of Recommendation
Non-antibacterial drug therapies					
Imodium (loperamide) (OTC)	Label: Control and symptomatic relief of **acute nonspecific diarrhea** CDC: useful for symptomatic relief of **TD** ISTM: May be considered in the treatment of mild **TD** ASG: In patients receiving antibacterial drugs, loperamide can be administered to decrease duration of diarrhea and increase chance of cure.	ASG: Antibacterial drug regimens may be combined with loperamide 4 mg po first dose, then 2 mg after each loose stool, not to exceed 8 mg in 24h period, discontinue after 48 h.	Shortens course of diarrhea in both children (Kaplan, Prior et al. 1999) and adults (Hanauer, DuPont et al. 2007)	Label: contraindicated in patients < 2 years, pts with bacterial enterocolitis caused by invasive organisms including *Salmonella, Shigella*, and *Campylobacter.* FDA limits packaging for safe use.[8] CDC: not recommended in patients with diarrhea and fever or alone in patients with bloody diarrhea. IDSA-ID: Should not be given to children <18 years with acute diarrhea; should not be used in any age in suspected or proven cases where toxic megacolon may result in inflammatory diarrhea or diarrhea with fever. ASG: commonly associated with constipation	CDC ISTM IDSA (ID and TD) ACG

Product (s) Name/Year of Approval	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Source of Recommendation
Bismuth subsalicylate (OTC)	**2004 Amended OTC Monograph: control or relief of TD** ISTM: treatment of mild TD	ASG: 30 ml (525 mg) or 2 tablets po, not to exceed eight doses in 24 hrs Prevention: 2 tablets po four times per day for up to 2 weeks	Treatment: May reduce TD by 40% (DuPont, Sullivan et al. 1977) CDC: For prevention, reduces the incidence of TD by 50%	Turns stool and tongue black. Can decrease absorption of doxycycline. Excessive absorption can cause bismuth encephalopathy in IBD or HIV; avoid in patients with aspirin allergy, renal insufficiency and gout, and those on anticoagulants, probenecid, or methotrexate	FDA OTC Monograph CDC IDSA (ID and TD) ISTM ACG
Other therapies					
Low-osmolality oral rehydration solution (ORS)	**IDSA-ID: infectious diarrhea with mild to moderate dehydration; isotonic crystalloid IV recommended for severe dehydration** **ASG: severe diarrhea in the elderly or any traveler with cholera-like diarrhea**	IDSA-ID: Low-osmolality ORS <250 mmol/L ASG: Balanced ORS with sodium 60-75 mEq/L and glucose 75-90 mmol/L	ORS not needed in most individuals with acute diarrhea	Generally safe	CDC IDSA (ID and TD) ACG

1. Antibacterial drug regimens may be combined with loperamide
2. ID: Infectious Diarrhea
3. ASG: American College of Gastroenterology Clinical Guideline: Diagnosis, Treatment, and Prevention of Acute Diarrheal Infections in Adults (Riddle, DuPont et al. 2016)
4. CDC: Centers for Disease Control and Prevention (CDC) Yellow Book – Travelers' Health (Centers for Disease Control and Prevention 2018)
5. International Society of Travel Medicine
6. TD: Travelers' Diarrhea
7. IDSA: Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea (Abbreviated IDSA-ID) (Shane, Mody et al. 2017) and The Practice of Travel Medicine (Abbreviated IDSA-TD) (Hill, Ericsson et al. 2006)
8. See https://www.fda.gov/Drugs/DrugSafety/ucm594232.htm

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Rifamycin delayed-release tablets, an oral formulation of rifamycin sodium, is a new molecular entity (NME) and is not approved for marketing in any country. Parenteral and topical formulations of rifamycin SV have been used in Europe (Bergamini and Fowst 1965, Sensi 1983). At the time of submission, the Applicant noted that no solid oral dosage forms of rifamycin SV are currently on the market in any region worldwide.[1] The Applicant states that the controlled-release (b) (4) "MMX technology" can be found in FDA approved products, LIALDA® (mesalamine) and UCERIS® (budesonide) for treatment of ulcerative colitis.

3.2. Summary of Presubmission/Submission Regulatory Activity

Drug development for rifamycin was conducted under IND 104034. A summary of key regulatory activity is found in the Table 3 below.

Table 3: Key Regulatory Interactions Regarding Rifamycin Development

Date	Type of Interaction	Issues for Discussion
1/13/2009	Pre-IND Meeting to discuss development plan	• Regulatory pathway • Scope of phase 3 development program
2/11/2009	Teleconference between Sponsor and FDA to follow up on PIND meeting of 1/13/2009 and determine if information in summary product characteristics document was sufficient to support phase 3 studies	• Need for nonclinical reproductive studies prior to phase 3 trials
12/28/2009	Submission of IND	• Study C2009-0201 protocol
1/26/2010	Change of Sponsor from Cosmos Technologies, Ltd. to Santarus, Inc.	
2/27/2014	Change of Sponsor from Salix Pharmaceuticals, Inc. (Salix acquired Santarus in January 2014) to Cosmo Technologies, Ltd.	
8/7/2017	Proposed proprietary name conditional approval	
10/12/2017	Issuance of Agreed Initial Pediatric Study Plan Agreement (iPSP)	
10/20/2017	QIDP designation	
10/22/2017	Fast Track Designation	
11/13/2017	Telecon meeting to discussed planned NDA submission	• Agreement on the trials to support efficacy • Need for NI margin justification for the supportive non-IND trial RIT-1/AID

[1] NDA 210910 Section 1.9.6 – Other correspondence regarding pediatric exclusivity or study plans; Agreed Pediatric Study Plan p. 17. Applicant states that the (b) (4)

Date	Type of Interaction	Issues for Discussion
		• Size of safety database • Pooling strategy • Request for efficacy analysis in patients with fever and bloody diarrhea • Agreement for the submission timing for the final nonclinical study reports (prior to filing date for the NDA)
3/16/2018	NDA submission	

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Five clinical investigator sites were chosen, with three sites selected based on the statistical reviewer's analyses demonstrating the sites' contributions to the primary efficacy endpoint, i.e. the median Time to Last Unformed Stool (TLUS): C2009-0201, sites 14 and 4; and RIT-1/AID, site 116. Two sites were chosen due to high enrollment: C2009-0201, site 6 (102 of 264 [38.6%] enrolled subjects); and RIT-1/AID, site 115 (164 of 835 [19.6%] enrolled subjects).

Due to logistical challenges in scheduling inspections for C2009-0201 in Guatemala and Mexico, certified copies of study records were sent to the U.S. Agent in San Diego, CA for inspection.

The Clinical Inspection Summary (Aisha Johnson, M.D., M.P.H., M.B.A. on 10/18/2018) included the following results:

Table 4: Clinical Investigator Site Inspections

Name of CI, Site #, Address, Country if non-U.S. or City, State if U.S.	Protocol # and # of Subjects	Inspection Date	Classification
Dr. José Flores Figueroa Paseo del Conquistado #611, Colonia Lomas de Cortes. Cuernavaca, Morelos, Mexico 62240 Site #6	Protocol C2009-0201 102 subjects	September 10-13, 2018	NAI
Dr. Manuel Rodolfo Sanchez 13 Avenida 6-20, Zona 3, Of. 213 Edifico Medico San Lucas Quetzaltenango, Guatemala 09001 Site #4	Protocol C2009-0201 27 subjects	September 10-13, 2018	NAI
Dr. Eddy Dagoberto Motta Roldan Private Clinic 2a, Calle Poniente 19 B Antigua, Guatemala Site #14	Protocol C2009-0201 62 subjects	September 10-13, 2018	NAI
Dr. Sanjay Gupta Pushkar Raj Hospital, Ground Floor Hotel Goyel Inn Complex, Near Gurudwara, Pushkar, Rajasthan-305022, India Site #115	Protocol RIT-1/AID 164 subjects	August 20-24, 2018	OAI
Dr. Sandeep Kumar Gupta M.V. Hospital and Research Center, 314/30 Mirza Mandi, Chowk, Lucknow, Utter Pradesh-226003, India Site #116	Protocol RIT-1/AID 81 subjects	August 27-31, 2018	VAI

Key to Compliance Classifications
NAI = No deviation from regulations.
VAI = Deviation(s) from regulations.
OAI = Significant deviations from regulations. Data unreliable.
Pending = Preliminary classification based on information in 483 or preliminary communication with the field; EIR has not been received from the field, and complete review of EIR is pending. Final classification occurs when the post-inspectional letter has been sent to the inspected entity.

Dr. Sanjay Gupta's site, which enrolled 19.6% subjects in RIT-1/AID, received a field classification of Official Action Indicated (OAI). While there were numerous violations including enrollment of subjects who did not meet eligibility criteria, inaccurate records, and subjects without 30-day follow up, OSI concluded that the safety and efficacy results can be used in support of the proposed application.

4.2. Product Quality

Novel excipients: No
Any impurity of concern: Yes (See Section 5.6)
Sufficient controls to insure safety and efficacy of the commercial product: Yes.

Table 5: Composition of Rifamycin SV MMX Tablets, 200 mg

Components	Amount/Tablet (mg)	Function	Reference to Standards
(b) (4)			
Rifamycin Sodium[1]	200.0	Active pharmaceutical ingredient	P-h. Eur.
Ascorbic Acid	(b) (4)		USP
Lecithin			NF
Glyceryl Distearate			NF
Ammonio Methacrylate Copolymer (Type B)			NF
Mannitol[2]			USP
Colloidal Silicon Dioxide			NF
Magnesium Stearate			NF
(b) (4)			(b) (4)
Methacrylic Acid and Methyl Methacrylate copolymer (1:2)			NF
Titanium Dioxide			USP
Talc			USP
Yellow Ferric Oxide			NF
Triethylcitrate			NF
Polyethylene Glycol 6000			NF
(b) (4)			USP
			USP
Tablet Weight	**961**		
			(b) (4)

USP = United States Pharmacopoeia; NF = United States National Formulary; Ph. Eur. = European Pharmacopoeia

Source: NDA 210910; SDN 1

The drug product is a delayed-release oral tablet that contains 194 mg rifamycin (equivalent to 200 mg rifamycin sodium). It is a yellow brown, ellipsoidal enteric film-coated tablet debossed with "SV2" on one side. The enteric coating is a pH-resistant polymer film which breaks down above pH 7. (b) (4) a multimatrix system comprised of (b) (4) designed to release rifamycin in the colon (b) (4) Information provided on the components and composition of the tablet is adequate. The excipients in the formulation are common compendial grades widely used in the pharmaceutical industry. Although the quantities of the excipients were above the Inactive Ingredient Database (IID) limits based on a maximum daily dose of 776 mg of rifamycin (equivalent to 800 mg of rifamycin sodium), their levels were considered qualified (See 5.6 Excipients and Impurities). The drug product specifications include tests and acceptable limits for appearance, identification, assay, uniformity of dosage units, related substances, and dissolution. Several of the specified impurities above the qualification threshold were considered qualified. (See 5.6 Excipients and Impurities)
The drug product specifications are acceptable.

The tablets are packaged in blister packaging system composed of a (b) (4) aluminum foil, containing 12 tablets per blister pack. The secondary packaging system is a carton. The proposed 36-months expiry dating when packaged into the commercial packaging configuration and stored at 20°C to 25°C is acceptable based on the satisfactory stability data of the registration batches on 36-months of the long-term stability studies.

The proposed manufacturing process consists of (b) (4) is the key process used to manufacture Rifamycin Tablets. In-process controls have been established for critical manufacturing steps, which include (b) (4)

The proposed dissolution method and acceptance criteria were revised per FDA's recommendations. The results of the in-vitro alcohol studies indicate alcohol induced dose dumping at the first 1 h time point with concentrations of >10% alcohol, at pH 7.2. Labeling recommendations were made accordingly.

The formulation and manufacturing site proposed for the commercial drug product are the same as those used for the phase 3 clinical batches eliminating the need for the bridging. Finally, the proposed product is considered a delayed-release tablet.

The strength of the product should be expressed in terms of the USP salt policy: 194 mg of

rifamycin (equivalent to 200 mg rifamycin sodium). OPQ recommends the non-proprietary name for the product be rifamycin delayed-release tablets. The USAN is rifamycin, not the proposed rifamycin SV.

Adequate CMC information to assure the identity, strength, purity, and quality of the proposed drug product, rifamycin delayed release tablets, was provided in the NDA. All information requests and review issues have been addressed and there are no pending approvability issues. The manufacturing and testing facilities for this NDA are deemed acceptable to the Office of Process and Facilities (OPF). Therefore, the Office of Pharmaceutical Quality (OPQ) recommends approval for this NDA.

4.3. Devices and Companion Diagnostic Issues

Not applicable.

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Rifamycin SV MMX is formulated as a delayed intra-colonic release oral formulation designed to deliver the antibiotic directly into the lumen of the colon. In clinical trials conducted by the Applicant, rifamycin level in serum after oral administration of rifamycin SV (400 mg given twice daily for three days was 8.72 ng/ml, slightly above LLOQ (See Section 6.3.1). Additionally, published literature (Bergamini 1965)[2] shows that following oral administration, rifamycin SV (the active pharmaceutical ingredient) does not result in quantifiable levels in serum.

In rat toxicokinetic (TK) analysis conducted by the Applicant, rifamycin SV sodium salt, administered orally as an aqueous suspension, was poorly absorbed, and the bioavailability of the compound was calculated at approximately 0.09% to 0.46% of the administered doses. This is consistent with reports of low systemic exposure to rifamycin SV MMX tablets after oral administration to mice, rats, dogs and humans referenced by the Applicant in studies published by Bergamini et al.[1] In in vitro PK studies conducted by the Applicant, rifamycin SV was not a substrate for cytochrome P450 metabolism. However, the drug did inhibit, to varying extent, all of the P450 enzyme isoforms, which suggests likely drug interactions.

Published literature cited by the Applicant shows that cardiovascular and pulmonary safety of rifamycin SV was studied in several animal species, including dog, rabbit, mouse and guinea pig (Bergamini 1965)[1]. These studies did not show any major safety findings with rifamycin SV in the systems studied. Further, rifamycin SV MMX did not have any effect on respiratory functions in rats, as seen in the only stand-alone safety pharmacology study conducted by the Applicant. A Functional Observational Battery was incorporated in the14-day oral repeat-dose

[2] Bergamini N, Fowst G., Rifamycin SV. A Review. Arzneimittel Forschung 1965; (951-1002.

study of rifamycin SV in rats in which the drug was not found to be neurotoxic at doses up to 500 mg/kg/day which is approximately 6 times the recommended human dose based on body surface area comparison. The Applicant also reported that cardiovascular assessments were included in the 28-day oral repeat dose study with rifamycin in rats, which showed no abnormalities. However, no statistical analysis was done on these data and related tables or graphs were not included in the submission. Given that published literature shows no cardiovascular findings, there is no Pharmacology/Toxicology concern regarding this.

The Applicant conducted two repeat dose toxicology studies with TK analysis in rats and dogs, in which there were no major findings reported. No quantifiable levels of rifamycin SV were detected in the plasma following 28-day oral administration of rifamycin SV MMX in dogs. In the 2-week rat study, low systemic levels of rifamycin SV were observed in rats, but no significant accumulation of the drug was seen on day 14 compared to day 1 of dosing. TK comparisons between animal and human exposures were challenging due to the large animal variability and low systemic levels present with oral treatment. The no-observed-adverse-effect-levels (NOAELs) in rats and dogs were determined to be the highest doses of 500 and 180 mg/kg/day, which are 6 and 7 times higher respectively, than the clinical dose of 400 mg twice daily (800 mg/day or 13.3 mg/kg/day assuming a 60-kg individual) based on a body surface area comparison. Published literature on repeat-dose toxicity studies show slight to moderate liver damage in dogs via intravenous or intramuscular administration, whereas there were no adverse findings in rats via oral, intraperitoneal or subcutaneous administration of rifamycin SV (Table 8).

Rifamycin SV was found to be non-genotoxic based on the full battery of genetic toxicology studies performed by the Applicant.

As part of the reproductive toxicology assessment, the Applicant conducted two embryo-fetal Development (EFD) studies, but did not conduct fertility and early embryonic development (FEED) or prenatal and postnatal development studies (PPND) (See Section 5.5.3 for details). In the EFD study in rats, there were no major findings at any of the doses tested. At the high dose, a higher number of fetuses were found having reduced body weight and thin tendinous diaphragm, associated with reduced maternal food consumption and no change in maternal body weight. The fetal NOAEL was determined to be 214 mg/kg/day, which is more than 50 times the maximum human plasma concentration (C_{max}) measured after oral administration of 800 mg/day to patients. In the rabbit EFD study, rifamycin SV caused a slight delay in bone ossification and enlarged fontanelle/skull suture bone associated with reduced fetal weight at the highest dose of 34.2 mg/kg/day, which is equivalent to the maximum levels achieved in the clinic after oral administration of 800 mg/day. However, the highest dose was slightly maternotoxic causing reduced maternal body weight in rabbits, which may have a greater effect on fetal outcomes than direct toxicity by the drug.

No novel excipients were used in the formulation. All excipient and impurity levels in the drug product or drug substance were qualified by the nonclinical repeat-dose toxicology study in dogs (Study no. 20070215TCB), that was conducted using the clinical batch (5938) of rifamycin

SV MMX oral tablets. In this study, animals were exposed to 73 times the level of excipients and 72-75 times the amount of impurities present in the proposed clinical dose.

RECOMMENDATION

There are no Pharmacology/Toxicology objections to the approval of rifamycin SV MMX.

5.2. Referenced INDs, NDAs, BLAs, DMFs

IND 104034, DMF (b) (4) (rifamycin sodium)

5.3. Pharmacology

Primary pharmacology

Rifamycin SV is a semi-synthetic macrocyclic antibiotic belonging to the ansamycin class of antibiotics originating from *Streptomyces mediterranei*. The rifamycin class of compounds selectively inhibits DNA-dependent RNA polymerase in bacterial cells, preventing prokaryote RNA transcription and subsequent protein translation. Rifamycin SV appears to exhibit broad antimicrobial activity against enterobacteria commonly associated with TD including several *E. coli* strains, and against *Mycobacterium tuberculosis, C. difficile,* and *S. aureus.*

Details of the pharmacology studies conducted with Rifamycin SV MMX can be found in Section 8.1 Nonclinical Microbiology.

Secondary Pharmacology

No secondary pharmacology studies were conducted with rifamycin SV MMX.

Safety Pharmacology

The Applicant conducted a safety pharmacology study to assess the effect of rifamycin SV on the respiratory function of the conscious rat (whole body plethysmography) summarized in Table 6.

A Functional Observational Battery was conducted in the 2-week repeat-dose Toxicity study followed by 2-week recovery period in rats (Study no. A3070), that included hindlimb landing foot splay, sensory reactivity to stimuli including grip strength and motor activity as part of neurotoxicity assessments during the last week of treatment and at the end of the recovery period. No treatment-related differences of toxicological relevance were noted in the motor activity measurements at the end of treatment and recovery period in rats up to 500 mg/kg/day, approximately 6 times the recommended human dose based on body surface area comparison.

The Applicant cited published literature (Bergamini 1965)[3] for other Safety Pharmacology studies, including studies evaluating the effects of rifamycin SV on pulmonary, gastrointestinal and cardiovascular systems in several animal species as shown in Table 7.

Table 6: Safety Pharmacology Assessments of Rifamycin SV conducted by the Sponsor

Name of Study and number	Species	Route and Dose	Result(s)
Rifamycin sodium: Effects on respiratory function of the conscious rat (whole body plethysmography) following oral administration (Study A3072)	Rat, conscious	20, 100 and 500 mg/kg, by oral gavage	No changes of biological significance in respiratory parameters (by whole body plethysmography in freely moving rats) up to 4 hours after dosing up to 500 mg/kg.

Source: Applicant's table from Pharmacology Written Summary (SDN 4; 5/10/2018; 2.6.2)

Table 7: Published Safety Pharmacology Assessments of Rifamycin SV

Type of Study	Species	Route and Dose	Results
Pulmonary	Dog, Anesthetized	50 mg/kg, IV	No effect on respiration at 50 mg/kg IV
	Rabbit Anesthetized	20-30 mg/kg, IV	No effect on respiration at 20 mg/kg, increased respiration rate at 30 mg/kg
	Rabbit Non-anesthetized	0.1 – 0.3 mg/kg cisternal injection	No effect on respiration up to 0.3 mg/kg
Gastrointestinal	Rabbit, rat, guinea pig	100 mg/mL, *in vitro*	No effect on response of isolated intestines to acetylcholine, histamine or barium chloride
Cardiovascular	Dog, Anesthetized	50 mg/kg, IV	No effect on ECG at up to 50 mg/kg IV
	Dog, Anesthetized	50, 100 mg/kg, IV	Transient decrease in arterial pressure at
			>50 mg/kg; more prolonged decrease in pressure (60-90 minutes) with 100 mg/kg IV dosing
	Dog, Anesthetized	25-50 mg/kg, IV	No alterations in pressure induced by occlusion of common carotid artery at any dose tested.
	Dog, Anesthetized	250-500 mg, intra-arterial	Direct injection of up to 500 mg/kg into the femoral artery did not affect blood flow to the leg
	Rabbit, Anesthetized	20-30 mg/kg, IV	Arterial pressure not affected by doses of 20 mg/kg; transient decreases in pressure observed at 30 mg/kg
	Rabbit, Nonanesthetized	0.1 – 0.3 mg/kg cisternal injection	No effect on arterial pressure up to 0.3 mg/kg
	Rabbit,	10-1000 mg/mL	Increased permeability of rabbit cutaneous blood vessels to trypan blue at all concentrations tested
	In vitro	*In vitro*	

Source: NDA 210910; SDN 4

[3] Bergamini N, Fowst G., Rifamycin SV. A Review. Arzneimittel Forschung 1965; (951-1002.

5.4. ADME/PK

<table>
<tr><td>Type of Study</td><td>Major Findings</td></tr>
<tr><td>Absorption</td><td></td></tr>
<tr><td>Rifamycin SV Sodium: Evaluation of pharmacokinetics of rifamycin SV sodium after single IV and oral administration to male rats (non-GLP). Study number: CB-01-11-08</td><td>
<table>
<tr><td>Method of administration/Dose</td><td>IV (20 mg/kg)</td><td>Oral gavage (20 mg/kg)</td><td>Oral gavage (200 mg/kg)</td></tr>
<tr><td>PK Parameters</td><td></td><td></td><td></td></tr>
<tr><td>T_{max} [h]</td><td>N/A</td><td>0.367±0.115</td><td>0.583±0.382</td></tr>
<tr><td>C_{max} [ng/mL]</td><td>81033±2902</td><td>33.1±4.64</td><td>814±444</td></tr>
<tr><td>$AUC_{(0-24)}$ [mcg.h/mL]</td><td>27400±4020</td><td>23.0±6.13</td><td>1110±609</td></tr>
<tr><td>$T_{1/2}$</td><td>0.288±.077</td><td>0.451±0.132</td><td>0.46</td></tr>
</table>
Source: NDA 210910; SDN 4
</td></tr>
<tr><td>Distribution: The Applicant did not perform any tissue distribution studies because published literature contains tissue distribution studies that have been performed in various animal species and by different routes of administration. The table presented here is taken from the cited reference. (Bergamini et al., 1965)[4]</td><td>
<table>
<tr><td>Tissue</td><td colspan="6">Animal Species</td></tr>
<tr><td></td><td>Dog</td><td>Rat</td><td>Mouse</td><td>Guinea Pig</td><td>Rabbit</td><td>Cow/Ewes[5]</td></tr>
<tr><td>Liver</td><td>Present (oral, IV)</td><td>Present (IM, IV)</td><td>NR</td><td>Present (SC and Endobrachial)</td><td>NR</td><td>NR</td></tr>
<tr><td>Kidney</td><td>Present (IV)</td><td>Present (IM, IV)</td><td>NR</td><td>Present (SC and Endobrachial)</td><td>NR</td><td>NR</td></tr>
<tr><td>Spleen</td><td>Present (oral)</td><td>Present (IM)</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>Lung</td><td>Present (IV)</td><td>Present (IM, IV)</td><td>NR</td><td>Present (SC and Endobronchial)</td><td>NR</td><td>NR</td></tr>
<tr><td>Heart</td><td>Present (IV)</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>Skeletal Muscle</td><td>Present (oral, IV)</td><td>Present (IM, IV)</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>Adipose Tissue</td><td>Present (IV)</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>Red Bone Marrow</td><td></td><td>NR</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
<tr><td>Brain</td><td>Present (IV)</td><td>Present (IM)</td><td>NR</td><td>NR</td><td>NR</td><td>NR</td></tr>
</table>
</td></tr>
</table>

[4] Bergamini N, Fowst G., Rifamycin SV. A Review. Arzneimittel Forschung 1965; (951-1002)

[5] Ziv G, Sulman FG., Evaluation of rifamycin SV and rifampin kinetics in lactating ewes. Antimicrob Agents Chemother. 1974 Feb;5(2):139-42.

Type of Study	Major Findings						
	Placenta and Fetus	Present in fetus at maternal dose of 50 mg/kg (IM)	Absent at doses of 25 and 50 mg/kg (IM)	Present in fetus at maternal dose of 50 mg/kg (IM)	NR	Absent at doses of 25 and 50 mg/kg/ (IM)	NR
	Bile	Present (IV and IM)	Present (IV)	NR	Present (IV)	NR	NR
	Gastro-enteric contents	Present (IV)	Present (IV)	NR	NR	NR	NR
	Urine	Present (IM, IV)	Present (IV)	NR	Present (IV)	NR	NR
	Cerebrospinal Fluid	Present (IV)	NR	NR	NR	NR	NR
	Aqueous Humor	NR	NR	NR	NR	Present (subconj. Inj)	NR
	Milk	NR	NR	NR	NR	NR	Present in cows via IM and ewes via IM & IV
	Granuloma Pouch Exudate	NR	Present (IV)	NR	NR	NR	NR
	IM=intramuscular; IV=intravenous; SC=subcutaneous; NR=Not Reported Source: NDA 210910; SDN 4						
Metabolism							
Study of Rifamycin Sodium EP Study number: 16108 Rifamycin SV: Potential induction of activity levels of the main CYP450 enzymes in cryopreserved human hepatocytes (Study number: 0547-2008-R)	The Applicant has not evaluated in vivo metabolism, GI/hepatic first-pass effects, chemical structures, quantities of metabolites and possible metabolic pathways of Rifamycin SV MMX because of unquantifiable systemic levels of the drug in dogs and humans after dosing with Rifamycin SV MMX tablets. In vitro metabolism studies examining P450 metabolic induction and enzyme inhibition revealed rifamycin SV not to be a substrate of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5 with > 88% of parent detected following incubation (Study No. 16108). An in vitro study showed that rifamycin SV could significantly induce the enzymatic activity of CYP3A4 (7.9, 12.1 and 20.8-fold respectively at 1, 10 and 50 mcM rifamycin sodium) and, to a lesser extent, CYP2B6 (2.1 and 1.9-fold at 10 and 50 mcM respectively); rifamycin SV, instead, did not increase CYP1A2 activity at all the concentrations tested. (Study No. 0547-2008-R) Less than 6% of CYP1A, CYP2B6, CYP2C8, CYP2D6, and CYP2E1 activities were inhibited when incubated at a concentration of 10 mcM of rifamycin sodium, suggesting that rifamycin sodium is not an inhibitor of those CYPs. Rifamycin sodium moderately inhibited CYP2C9, CYP2C19, and CYP3A, with inhibition ranging from 35% to 47% (Study No. 16108).						

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td></td><td></td></tr>
<tr><td>Excretion</td><td>No excretion studies were conducted by the Applicant and published literature containing data on urinary and biliary excretion as well as enterohepatic circulation of rifamycin SV was referenced (Bergamini 1965)[6]. Studies referenced in this review show that rifamycin SV is primarily cleared and excreted via the biliary system. 5-15% of oral rifamycin SV was measured in the cystic bile in dogs after 50 mg/kg oral administration. Within 7 hours of IV administration of 5-10 mg/kg in dogs, nearly 60% to 90% of the 5 mg/kg administered dose was eliminated into the bile. In the rat, biliary elimination was equally rapid, and biliary concentrations of the compound reached saturation at just 45 minutes following a 5-10 mg/kg IV dose. Similar results have been achieved following IM administration. Urinary elimination was minor in rats, dogs and guinea pigs.</td></tr>
<tr><td>Toxicokinetic (TK) data from general toxicology studies
Rifamycin SV MMX 200-mg Tablets Repeat-Dose 28-Day Toxicity and Toxicokinetic Study in the Dog by the Oral Route
Study number: 20070215TCB

Rifamycin SV: 2-Week Oral Toxicity Study in Rats Followed by a 2-Week Recovery Period
Study number: A3070</td><td><u>Dog (Study number: 20070215TCB)</u>
Blood was collected at predose, and at 1, 2, 6, and 16 hours after dosing on Day 1 and Day 28. No Rifamycin SV was found in plasma of dogs whatever the dose and hence, no TK parameters were assessed.

<u>Rat (Study number: A3070)</u>
Day 1
<table>
<tr><th>Dose (mg/kg)</th><th>20</th><th>100</th><th>500</th><th>20</th><th>100</th><th>500</th></tr>
<tr><th>Gender</th><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><td>C_{max} (ng/ml)</td><td>29.8</td><td>358.6</td><td>48136.7</td><td>8</td><td>2223</td><td>34546.7</td></tr>
<tr><td>T_{max} (h)</td><td>0.25</td><td>0.25</td><td>0.5</td><td>0.5</td><td>0.25</td><td>0.25</td></tr>
<tr><td>$AUC_{0\text{-}tlast}$ (ng*ml/h)</td><td>135.1</td><td>470.1</td><td>73906</td><td>48.6</td><td>993.1</td><td>48279.3</td></tr>
</table>
Day 14
<table>
<tr><th>Dose (mg/kg)</th><th>20</th><th>100</th><th>500</th><th>20</th><th>100</th><th>500</th></tr>
<tr><th>Gender</th><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><td>C_{max} (ng/ml)</td><td>79.5</td><td>431.3</td><td>64900</td><td>14.9</td><td>115.4</td><td>31200</td></tr>
<tr><td>T_{max} (h)</td><td>0.25</td><td>0.25</td><td>0.5</td><td>0.25</td><td>0.5</td><td>0.5</td></tr>
<tr><td>$AUC_{0\text{-}tlast}$ (ng*ml/h)</td><td>58.0</td><td>375.2</td><td>98070</td><td>18.1</td><td>312.9</td><td>49590.3</td></tr>
</table>
Source: NDA 210910; SDN 4</td></tr>
<tr><td>TK data from reproductive toxicology studies
Rifamycin SV Sodium Salt: Study for Effects on Embryo-Fetal Development in the Han Wistar Rat.
Study no.: CB-01-11/25</td><td><u>Rat (Study no.: CB-01-11/25):</u>

<table>
<tr><th>Dose (mg/kg)</th><th>42.7</th><th>214</th><th>855</th><th>42.7</th><th>214</th><th>855</th></tr>
<tr><th>Post-coitum</th><th colspan="3">Day 6</th><th colspan="3">Day 17</th></tr>
<tr><td>C_{max} (ng/ml)</td><td>15.7</td><td>416</td><td>19500</td><td>16.6</td><td>455</td><td>8890</td></tr>
<tr><td>T_{max} (h)</td><td>1.0</td><td>1.0</td><td>1.0</td><td>1.0</td><td>1.0</td><td>2.0</td></tr>
<tr><td>AUC (ng*ml/h)</td><td>61.1</td><td>748</td><td>58800</td><td>52.0</td><td>1130</td><td>25800</td></tr>
<tr><td>$T_{1/2}$ (h)</td><td>4.0</td><td>12</td><td>2.4</td><td>4.6</td><td>13</td><td>2.9</td></tr>
</table>
Source: NDA 210910; SDN 4

<u>Rabbit (Study No. CB-01-11/24):</u></td></tr>
</table>

[6] Bergamini N, Fowst G., Rifamycin SV. A Review. Arzneimittel Forschung 1965; (951-1002)

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>Study for Effects on Embryo-Fetal Development in the Himalayan Rabbit
Study No. CB-01-11/24</td><td>
<table>
<tr><th>Dose (mg/kg)</th><th>12.8</th><th>34.2</th><th>85.5</th><th>12.8</th><th>34.2</th><th>85.5</th></tr>
<tr><th>Post-coitum</th><th colspan="3">Day 6</th><th colspan="3">Day 18</th></tr>
<tr><td>C_{max} (ng/ml)</td><td>8.30 ± 0.57</td><td>26.6 ± 33.1</td><td>33.1 ± 12.2</td><td>34.3 ± 47.7</td><td>10.2 ± 2.0</td><td>81.9 ± 52.8</td></tr>
<tr><td>T_{max} (h)</td><td>0.17</td><td>0.17 - 1</td><td>0.17</td><td>0.17</td><td>0.17</td><td>0.17</td></tr>
<tr><td>AUC (ng*ml/h)</td><td>-</td><td>68.3 ± 70.3</td><td>177 ± 114</td><td>27.1 ± 9.6</td><td>7.45 ± 0.75</td><td>471 ± 477</td></tr>
</table>
Source: NDA 210910; SDN 4
</td></tr>
</table>

5.5. Toxicology

5.5.1. General Toxicology

Study title: 2 Week Oral Toxicity Study In Rats Followed By A 2 Week Recovery Period; Study number: A3070

Key Study Findings:

- There were minor findings in the Clinical Chemistry evaluations in the high dose group compared to controls. In sum, there were no toxicologically relevant findings in any of the dose groups compared to the controls.
- The NOAEL in the study was determined to be the high dose of 500 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 20, 100, 500 mg/kg/day once daily for 2 wks
Route of administration:	Oral gavage
Formulation/Vehicle:	Ascorbic acid in water for injection
Species/Strain:	Sprague Dawley Rats
Number/Sex/Group:	10/sex/group; Recovery: 5/sex/group
Age/Weight:	27-29 days old/ 75-99 gm
Satellite groups/ unique design:	TK - 9/sex/treatment and 3/sex in control group
Deviation from study protocol affecting interpretation of results:	No

Observations and Results:

Parameters	Major findings
Mortality	1 male (MD) in Day 8

Parameters	Major findings
Clinical Signs	HD: Piloerection (all males), hunched posture from Day 9 for a few days (3/15 males, 4/15 females). Soft feces and orange stained feces from week 2. LD: Decrease in the frequency of defecation were seen (Day 10 of treatment period) in male animals (-50%).
Body Weights	Unremarkable
Ophthalmoscopy	Unremarkable
Hematology	HD: +19% and +32% leucocytes in males and females respectively
Clinical Chemistry	Dosing LD & HD (males): -21% and -19% Alkaline Phosphatase (ALP) and HD: +37% Alanine aminotransferase; MD & HD (females): -26% and -22% Glucose Recovery males HD: -23% (ALP)
Urinalysis	Unremarkable
Gross Pathology	Unremarkable
Organ Weights	Unremarkable
Neurotoxicity	Unremarkable
Histopathology **Adequate battery:** Yes	Unremarkable

LD: low dose; MD: mid dose; HD: high dose.
-: indicates reduction in parameters compared to control.

Study title: Rifamycin SV MMX 200 mg tablets: Repeated dose 28-day toxicity and toxicokinetic study in the dog by the oral route.
Study Number: 20070215TCB

Key Study Findings:

- There were no toxicologically relevant findings in any of the dose groups compared to the controls.
- The NOAEL in the study was determined to be the high dose of 180 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 600, 1200, 1800 mg/day (1, 2, or 3 rifamycin SV MMX™ 200 mg tablets, t.i.d.) or 0, 60, 120 and 180 mg/kg/day once daily for 28 days
Route of administration:	Oral tablets
Formulation/Vehicle:	200 mg Placebo tablets
Species/Strain:	Beagle Dogs
Number/Sex/Group:	3/sex/group
Age/Weight:	6-11 months/Mean weight ~10 kg

Satellite groups/ unique design: TK - 3/sex/group
Deviation from study protocol affecting interpretation of results: No

Observations and Results:

Parameters	Major findings
Mortality	None
Clinical Signs	All doses caused soft orange/red feces from Day 2 to the end of the study in a dose-dependent manner.
Body Weights	Unremarkable
Ophthalmoscopy	Unremarkable
Hematology	Unremarkable
Clinical Chemistry	Unremarkable
Urinalysis	Unremarkable
Gross Pathology	Unremarkable
Organ Weights	Unremarkable
ECG	Unremarkable
Histopathology **Adequate battery:** Yes	Unremarkable
Special Evaluation (bacterial count in the gut)	No relevant change in intestinal bacteria count in the treated animals compared with the placebo control group.

Table 8: Published Repeat-Dose Toxicity Assessments of Rifamycin SV

Rats				Dogs			
	IP	**Oral**	**SC**		**IV**	**IV**	**IM**
Dose (mg/kg/day)	50, 100, 200	250, 500	50-300 mg/kg	**Dose (mg/kg/day)**	40	20	20, 40
Duration	30 days	168 days	1-6 months	**Duration**	30 days	6 months	6 months
Adverse findings	None	None	None	**Adverse findings**	Moderate liver damage	Moderate liver damage	Slight liver damage

IP: Intraperitoneal; SC: Subcutaneous; IV: Intravenous; IM: Intramuscular
Source: Bergamini N, Fowst G., Rifamycin SV. A Review. Arzneimittel Forschung 1965; 951-1002)

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Salmonella Typhimurium and Escherichia coli reverse mutation assay with rifamycin SV sodium salt
Study number: 1257001

Key Study Findings:

- Cytotoxicity was seen starting at 100 ug/plate (-S9) and 333 ug/plate (+S9) was determined as the maximal concentration.

- There was no mutagenicity observed in any of the strains after treatment with the drug at any dose level tested in the absence or presence of the S9 metabolic mix.

GLP compliance: Yes
Test system: Salmonella typhimurium strains (TA 1535, TA 1537, TA 98, and TA 100) and Escherichia coli strain WP2 uvrA
Study is valid: Yes

In Vitro Assays in Mammalian Cells

Study title: Cell mutation assay at the thymidine kinase locus (TK+/-) in mouse lymphoma L5178Y cells with rifamycin SV sodium salt
Study number: CB-01-11/13 or 1257002
Key Study Findings:

- No mutations were observed in L5178Y/TK+/- cells treated with rifamycin in the absence and presence of S9 metabolic activation.

GLP compliance: Yes
Test system: L5178Y/TK+/- cells
Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus assay in bone marrow cells of the mouse with rifamycin SV sodium salt
Study number: 1257003
Key Study Findings:

- The drug was not cytotoxic at any of the doses tested as it did not decrease the number of polychromatic erythrocytes (PCE).
- There was no increase in frequency of micronuclei at dose level of intravenous rifamycin evaluated up to 300 mg/kg.

GLP compliance: Yes
Test system: Bone marrow cells of NMRI Mice, 8-12 weeks old, body weight: 39.7 g
Study is valid: Yes

5.5.3. Reproductive and Developmental Toxicology

The Applicant was advised during the Pre-NDA meeting to submit a complete reproductive toxicology package in the NDA as part of the nonclinical toxicology package. However, the Applicant did not perform or submit any FEED or PPND studies to their NDA. The Applicant did submit EFD studies in rats and rabbits that are reviewed below. As discussed in the Pre-NDA meeting, the Applicant presented their justification for the absence of FEED and PPND studies by stating that rifamycin SV MMX is intended for a short treatment duration of 3 days and has shown limited absorption in humans (<1% bioavailability in clinical studies). The Applicant stated that no systemic absorption was detected following administration of rifamycin SV MMX tablets to dogs; all plasma samples were below the Level of Quantification (LOQ) of the assay (2 ng/ml). In addition, histopathological examination of reproductive organs in the 28-day toxicity

study in dogs and the 14-day study in rats did not reveal any treatment-related changes in the male or female reproductive organs. There are no reports on the effects of rifamycin SV or derivatives of rifamycin SV (i.e., rifampin) on male or female fertility in the scientific or clinical literature. No adverse effects on pre- or post-natal development have been reported in the scientific or clinical literature. Rifamycin SV is not excreted in human milk (Bergamini 1965). This justification is acceptable from a Pharmacology/Toxicology perspective.

Embryo-Fetal Development

Study title: Rifamycin SV Sodium Salt: Study for Effects on Embryo-Fetal Development in the Han Wistar Rat

Study number: CB-01-11/25

Key Study Findings:

1 At the mid and high dose, rifamycin SV caused a slight reduction in food consumption at post-coitum days 6- 18 only with no corresponding loss of body weight gain and hence, is of low toxicological relevance. By the end of the dosing period, the body weights of treatment groups were comparable to control animals.
2 Rifamycin SV treatment did not affect any measured reproductive parameter in the dams or result in any external, visceral, or skeletal abnormalities or variations in fetuses at any dose.
3 At 855 mg/kg/day, fetal weight was reduced when calculated on individual basis and slightly when calculated on litter basis. During visceral examination, there were a higher number of fetuses with thin tendinous region of the diaphragm in the high dose group.
4 Maternal NOAEL was considered to be 42.7 mg/kg/day and fetal NOAEL was 214 mg/kg/day.
5 At 214 mg/kg/day, maternal C_{max} was 455 ng/ml at the end of the dosing period, which is more than 50 times the C_{max} in healthy human subjects achieved after oral administration of 800 mg/day (13.3 mg/kg/day) for three days. Maternal AUC in the rat was 25800 ng*ml/hr whereas it was below LLOQ in the clinical trials.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 42.7, 214, 855 mg/kg/day; once daily
Route of administration:	Oral gavage
Formulation/Vehicle:	Anhydrous Powder / Purified Water containing 10% ascorbic acid equivalent to 10% of the weight of the highest dose of rifamycin SV administered.
Species/Strain:	Han Wistar Rat (11 weeks old; 183-247 g)
Number/Sex/Group:	22 female rats/dose group

Satellite groups:	TK: 9 female rats/dose
Study design:	Rifamcyin SV was administered from post coital implantation (day 7) to closure of the hard palate (day 17). All animals were euthanized on day 21 post coitum and the fetuses removed for examination. For TK analysis, blood samples were collected on days 6 and 17 post coitum.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	**Major findings**
Mortality	None
Clinical Signs	Unremarkable other than orange-red soft feces starting from second day of dosing.
Food consumption and Body Weights	MD & HD: -12.6% and -11.7% respectively on days 6-18 post coitum. However, body weight of treatment animals was not different than controls throughout the dosing period.
Necropsy findings Cesarean Section Data *[Implantation Sites, Pre- and Post-Implantation Loss, etc.]*	Unremarkable
Necropsy findings Offspring *[malformations, variations, etc]*	HD: 15/139 or 11% fetuses from 8/21 or 38% litters of the high dose group were found with thin tendinous region in their diaphragm compared to controls (5/145 or 3% fetuses from 4/21 or 19% litters)

Embryo-Fetal Development

Study title: Rifamycin SV Sodium Salt: Study for Effects on Embryo-Fetal Development in the Himalayan Rabbit
Study number: CB-01-11/24

Key Study Findings:

- At the high dose, rifamycin SV caused a reduction in food consumption and bodyweight gain in pregnant dams from treatment start until termination of study.
- Reduced fetal weights corresponded to slight delay in ossification at the high dose including slightly higher incidences of fetuses with skull suture bone variations, enlarged skull fontanelle and incompletely ossified digit 5 medial phalanx of both forelimbs.
- The maternal and fetal NOAEL was 34.2 mg/kg/day. At 34.2 mg/kg/day, rabbit maternal C_{max} was 10.2 ng/ml at the end of the dosing period, which is equivalent to the levels achieved in healthy human subjects after oral administration of 800 mg/day (13.3

mg/kg/day) for three days. Maternal AUC in the rabbit was 7.45 ng*ml/hr whereas it was below LLOQ in the clinical trials.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 12.8, 34.2, 85.5 mg/kg/day; once daily
Route of administration:	Oral gavage
Formulation/Vehicle:	Anhydrous Powder / Purified Water containing 10% ascorbic acid equivalent to 10% of the weight of the highest dose of rifamycin SV administered.
Species/Strain:	Himalayan Rabbit (17 weeks old; 2272-3499 g)
Number/Sex/Group:	20 females/dose group
Satellite groups:	TK; 3 females/dose group
Study design:	Rifamycin SV was administered to pregnant rabbits from post coital (pc) implantation (day 6) to closure of the hard palate (day 18). For TK analysis, blood samples were collected <10 min, 1 h , 3 h, and 24 h, after dosing on days 6 and 18 post coitum (pc)
Deviation from study protocol affecting interpretation of results:	Yes/No

Observations and Results

Parameters	**Major findings**
Mortality	None
Clinical Signs	Unremarkable
Food Consumption	HD: -77% between days 6 to 9 pc and -51% by the end of day 18 pc and continued to be lower after dosing. MD & LD: -66% and -77% respectively between days 6 to 9 pc only and recovered afterwards.
Body Weights	Slight (<10%) decrease in all dose groups compared to controls.
Necropsy findings Cesarean Section Data *[Implantation Sites, Pre- and Post-Implantation Loss, etc.]*	Unremarkable
Necropsy findings Offspring	HD: Low fetal body weights (29% compared to 33% controls); skull suture bone variations (15% versus 7% in controls), enlarged skull

[malformations, variations, etc]	fontanelle (12% versus 0 in controls) and incompletely ossified digit 5 medial phalanx of both forelimbs (37 to 42% versus 6% in controls)

LD: low dose; MD: mid dose; HD: high dose

5.6. **Excipients and Impurities**

Excipients:

No novel excipients are included in the drug product to be marketed. The amount of individual excipients present in rifamycin SV MMX 200 mg tablet formulation (see Section 4.2) is equal or below the amount used in other FDA approved products as per the FDA Inactive Ingredient Database (IID). However, the amount of excipient that the patient will be exposed through the daily proposed dose of 800 mg rifamycin SV MMX oral tablet, exceeds the IID limits. The Applicant conducted their nonclinical repeat-dose toxicology study in dogs (Study no. 20070215TCB) using the current clinical formulation of rifamycin SV MMX (Batch 5938). In this study, animals exposed to 73 times the amount of excipient present in the daily clinical dose of rifamycin SV MMX showed no adverse effects, which adequately qualifies the amount of excipients that the patient will be exposed on a daily basis in the clinic. From a Pharmacology/Toxicology perspective the excipients are qualified.

Impurities:

Quantitative Structure-Activity Relationship (QSAR) prediction methodologies, (i.e. a statistical-based method and an expert rule-based method) were applied to predict the genotoxic potential of the drug substance impurity (b) (4) (RRT (b) (4) and RRT (b) (4)). However, the two different QSAR methodologies provided conflicting results. Specifically, the QSAR statistical-based approach led to a negative prediction, while the QSAR expert rule-based approach led to a positive prediction due to the identification of a structural alert for mutagenicity (i.e., (b) (4)). Because the impurity, (b) (4) (b) (4) physicochemical properties, and mechanism of action, a read-across study was employed to provide additional supportive evidence on the predictions. The read-across study led to the conclusion that the target (b) (4) is predicted as negative for the Ames test given that the API Rifamycin was negative in the Ames test.

The nonclinical repeat-dose Toxicology study in dogs was conducted with the clinical batch (5938) of rifamycin SV MMX oral tablets (Study no. 20070215TCB) that was shown to contain the same amount of impurities as the proposed drug product. At the NOAEL dose of this study, the animals were exposed to 72-75 times the amount of impurities present in the proposed clinical dose.

In sum, all impurity specifications and limits set by the Applicant (Table 9) in the drug product have been qualified from a Pharmacology/Toxicology perspective. This decision is in concurrence with the CMC reviewers Drs. Erika Englund and Thomas Wong.

Table 9: Impurity Specifications in Drug Product and Substance

	(b) (4)
Drug Product	
Drug Substance	

Source: Source: NDA 210910; SDN 1,4

6 Clinical Pharmacology

6.1. Executive Summary

The Office of Clinical Pharmacology (Division of Clinical Pharmacology IV) reviewed the information contained in NDA 210910. The clinical pharmacology information submitted in the application supports the approval of rifamycin SV MMX for treatment of non-infectious travelers' diarrhea.

Table 10: Summary of OCP's Recommendations & Comments on Key Review Issues

Review Issue	Recommendations and Comments
Pivotal or Supportive Evidence of Effectiveness	The primary evidence of effectiveness of rifamycin SV MMX was supported by one Phase 3 trial with supportive evidence from a second Phase 3 trial [C2009-0201 and RIT-1/AID]. Supportive evidence of safety was provided by two Phase 2 clinical studies [CB-01-11/02 and CB-01-11/03].
General Dosing Instructions	Administer two 200 mg tablets (expressed as rifamycin SV sodium; equivalent to 194 mg rifamycin SV) orally twice daily for 3 days. Take with a glass of water with or without food.
Dosing in Patient Sub-Groups	No adjustments are recommended for patients with renal impairment or hepatic impairment.
Labeling	Applicant's proposed labeling is generally acceptable. Specific comments regarding transporter substrate properties (rifamycin SV is a substrate of P-gp) and potential inhibitory effect of rifamycin SV on intestinal P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) transporters were incorporated in the label.
Bridge between the to-be-marketed and clinical trial formulations	The to-be marketed formulation was used in the Phase 3 and supportive Phase 2 trials.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

See Section 6.3.1 for information on the ADME properties of rifamycin SV MMX. Following 6 doses of 400 mg rifamycin SV (as sodium salt)-MMX twice daily, individual plasma rifamycin SV

concentrations above the LLOQ were few (n=10/240; 4.2%) and random (Figure 1), hence an extensive PK analysis was not conducted.

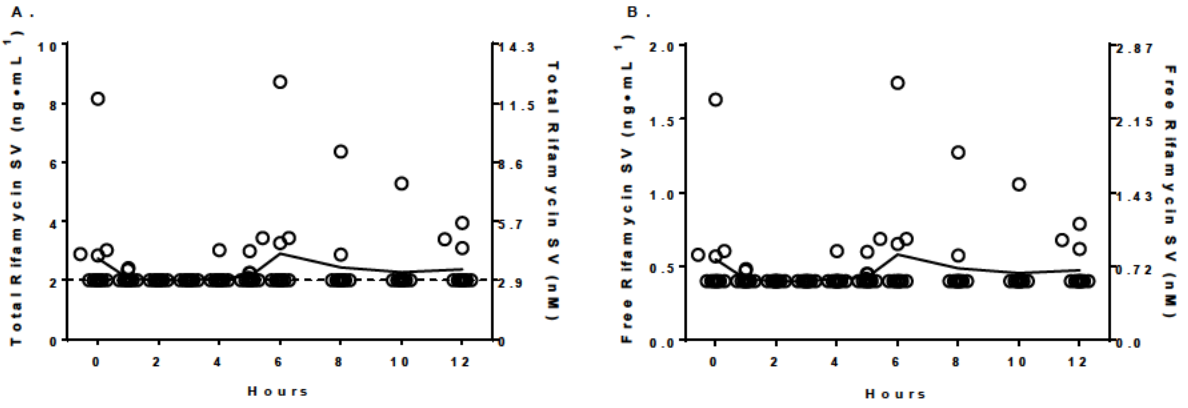


Figure 1: Total (A) and free (B) concentrations of rifamycin SV in plasma after 400 mg rifamycin SV MMX twice daily for three days (Applicant's proposed dosing regimen). Individual data are represented as open circles and the line represents the mean trend line. Fraction bound to serum proteins is 0.8. Doses administered with 150 mL water and irrespective of food. Source: Applicants Study CB-01-11/11, Table 16.2.5.2; pg 11.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The Applicant's proposed dosage regimen of rifamycin SV MMX for adults is 400mg (2 x 200 mg; expressed as the sodium salt) administered twice daily for 3-days and taken orally with water with or without food.

Therapeutic Individualization

No dose adjustment is recommended based on intrinsic (e.g., age, sex, renal/hepatic impairment) and extrinsic factors (e.g., food, concomitant medicines).

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

PHARMACOLOGY	
Mechanism of action	An ansamycin, inhibits bacterial DNA-dependent RNA synthesis; blocking RNA chain initiation via RNA polymerase interactions.
QT Prolongation	Not studied. Majority of concentrations are below lower limit of quantification (LLOQ <2 ng/mL; maximum observed 8.7 ng/mL)

Active Moieties	Rifamycin SV.
GENERAL INFORMATION	
Proposed Dosage Regimen	Two tablets (expressed as 400 mg rifamycin SV sodium; equivalent to 388 mg rifamycin SV) twice-daily for three days
Bioanalysis	Validated LC-MS/MS method. Rifamycin SV LLOQ in plasma and urine was 2 $ng \cdot mL^{-1}$ and in feces 10 $ng \cdot mL^{-1}$.
Healthy vs. Patients PK	Not studied. Previous studies with a similar drug (rifaximin) did not find differences in PK between healthy subjects and infectious colitis patients[7].
Plasma drug exposure at steady state following the therapeutic dosing regimen	Overall, only 11% of plasma rifamycin SV samples from an intensive PK study were quantifiable following the proposed dosage regimen (400 mg given twice daily for three days) [CB-01-11/11]. The highest detectable proportions (33%) were observed for the 5th and 6th hr post-dose. Only slightly above the LLOQ, the maximum observed level was 8.72 $ng \cdot mL^{-1}$ at 6hr. No further PK assessments could be made due to the sparse and random nature of the available plasma data.
Maximally tolerated dose (MTD) or exposure	The maximally tolerated daily dose of 1200-mg (2 tablets thrice daily) for three days was well tolerated in the exploratory phase 2 study CB-01-11/03 (n=12). Doses greater than the proposed 400 mg twice daily for three days have not been explored in Phase 3 studies.
Dose Proportionality	Could not be determined as majority of PK data was below assay limits.
Accumulation	Could not be computed as majority of PK data was below assay limits.
Variability (%CV)	Plasma – Because of limited systemic absorption, there was insufficient PK data available to compute variability.
	Variability in fraction of dose excreted in urine was 48% [CB-01-11/11; 400 mg twice daily for 3 days]
	Variability in fraction of dose excreted in feces was 21% [CB-01-11/10; 400 mg single dose]
ABSORPTION	
Bioavailability	Using total urinary excretion data bioavailability was estimated to be 0.04 ± 0.062 %
T_{max}	Around 6-hr based on urine excretion data [CB-01-11/10]
Food Effects	Food appears to decrease systemic exposure of rifamycin SV based on urine PK (percent of dose excreted 0.001 [under fasting conditions] vs 0.0004 [under fed conditions[a]]. Considering that the pharmacological effect of rifamycin SV occurs prior to its systemic absorption, the effect of food on the pharmacokinetics of rifamycin SV is not clinically relevant.
DISTRIBUTION	
Volume of Distribution	Could not be determined due to minimal systemic absorption
Plasma Protein Binding	Ca. 80%
Blood-to-Plasma Ratio	Not experimentally determined.
Substrate of transporters (*in vitro*)	Substrate of P-gp transporters.
ELIMINATION	
Half-life	Could not be determined due to majority of PK data below assay limits.
Fraction metabolized	83% of the dose was recovered unchanged in feces [Study CB-01-11/10].

[7] Taylor DN, Mckenzie R, Durbin A, Carpenter C, Haake R, Bourgeois AL. Systemic pharmacokinetics of rifaximin in volunteers with shigellosis. Antimicrob Agents Chemother. 2008;52(3):1179-81.

(% dose)	
Primary metabolic pathway(s)	Not fully elucidated. Of note, no CYPP450 enzymes were found to metabolize rifamycin SV [Study 16108].
Primary excretion pathways (% dose) ±SD	After IV administration of rifamycin SV, > 90% of the dose is excreted as unchanged drug in feces via biliary secretion[8] After a single oral dose of rifamycin SV MMX®, ca. 83% of the dose is excreted unchanged in the feces. [Study CB-01-11/10]
Inhibition/Induction of metabolizing enzymes by rifamycin SV	Systemic: No clinically relevant drug-drug interactions (DDI) based on observed drug exposures (and model based *in vivo* DDI prediction) [Studies 16108, CPS/11].
	Intestinal: Based on the cut off criteria outlined in the FDA draft guidance, no clinically relevant inhibition of intestinal CYP3A4/5 is predicted [Study CPS/11]. Furthermore, no clinically relevant induction of CYP3A4/5, CYP2B6, and CYP2C9 is predicted [Study 16108].
Inhibition/Induction of transporters by rifamycin SV	Systemic: No clinically relevant DDIs based on observed rifamycin SV systemic exposures (and model based *in vivo* DDI prediction) [Study CPS/10]
	Intestinal: Based on the cut off criterial outlined in the FDA draft guidance inhibition of P-gp and BCRP by rifamycin SV is anticipated [Study CPS/10].

[a]fed state: High fat, high-calorie (ca. 1000 calories) with 50% calories from fat, 35% calories from carbohydrates, and 15% calories from protein.
[b]Study drug was given at breakfast and dinner time with a glass of liquid in Phase 3 studies C2009-0201 and RIT-1/AID.

6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

Rifamycin SV exerts its actions locally in the intestinal lumen prior to systemic absorption. In a dose-ranging study (total daily dose: 400-, 800-, and 1200-mg; n=12-13/group), no significant changes in time to last unformed stool [TLUS] were observed with increasing dose; however, the total daily dose of 800 mg [400 mg (2 tablets) twice daily] dose was deemed most effective by the Applicant.

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen of 400 mg (rifamycin SV sodium salt) twice daily is effective and appears to have an acceptable safety profile. Because of minimal systemic absorption (bioavailability <0.1%) and limits of bioanalytical methods (LLOQ 2 ng/mL), extensive pharmacokinetic characterization could not be performed (see Section 1.2.1 for additional information on the amount of available PK data).

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

[8] Fueresz S, Acocella G, Scotti R. EXPERIMENTAL DATA FOR THE USE OF RIFAMYCIN SV IN BILIARY TRACT INFECTIONS: IN VITRO ACTIVITY AGAINST VARIOUS PATHOGENIC BACTERIA AND BILE CONCENTRATIONS IN MAN. Chemotherapia (Basel). 1963;12:365-73. Note: No bioanalytical or individual data was provided for review from this manuscript.

No. Rifamycin SV appears to be limited to the gastrointestinal tract where it exerts its pharmacological actions. While not empirically evaluated, the limited oral bioavailability (<0.1%) of rifamycin SV suggests that various intrinsic factors such as age, body weight, sex, or race would not significantly affect the systemic exposure of rifamycin SV. Furthermore, for the above reason (i.e., oral bioavailability <0.1%), organ impairment (hepatic or renal) would not be expected to significantly influence systemic exposure of rifamycin SV; hence, rifamycin SV MMX can be administered to subjects with renal impairment or hepatic impairment without any dosage adjustments.

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

FOOD-DRUG INTERACTION
No clinically relevant food effect is expected with rifamycin SV MMX as low systemic absorption in the fasted and fed state were observed. Bioavailability in the fasted state was 0.041% and fed state 0.014% based on urine data since plasma rifamycin SV concentrations were not quantifiable. In addition, patients in Phase 3 trials demonstrating clinical efficacy received rifamycin SV MMX tablets at meal times with a glass of liquid. Therefore, rifamycin SV MMX can be administered irrespective of food.

ALCOHOL-DRUG INTERACTION
Considering that rifamycin SV MMX tablets are developed to delay the release of its active substance until the colon (pH approx. 7.2), consumption of alcoholic beverages may affect the delayed release characteristics and thus alter drug disposition and kinetics in the body. Based on the totality of information, the review team recommended adding “Do NOT take AEMCOLO concomitantly with alcohol” to Section 2.1 of the prescribing information of AEMCOLO to avoid disruption of the delayed release characteristics of rifamycin MMX due to alcohol.

In vitro dissolution studies were conducted to characterize the effect of alcohol (0-, 10-, 20-, and 40 %) on the delayed release characteristics of rifamycin MMX at two different pH (1 and 7.2). The results of this study indicated that alcohol altered the delayed release characteristics of rifamycin SV release starting at 1 hour with alcohol concentrations >10%, at pH 7.2 (*Table 11*). At a pH of 1, the delayed release characteristics of rifamycin MMX were not altered. Based on this data, it was unclear if alcohol may impact the delayed release characteristics of rifamycin MMX prior to reaching the colon (intended site of action). Hence, in order to assess whether rifamycin MMX maintains its delayed release characteristics until it reaches the colon, the review team requested the Applicant conduct additional dissolution studies in the presence of alcohol at a pH of 6 (approximate pH in the small intestine). The results indicated that alcohol disrupted the delayed release characteristics of rifamycin MMX at concentrations of 40% at pH 6 (Table 12).

Table 11: Mean Dissolution Values at Different Ethanol Concentrations at pH 1 and pH 7.2.

Samples	Rifamycin dissolved amount (%)						
	Stage I: 0.1M HCl	*Stage II: pH 7.2 phosphate buffer*					
	2h	*1h*	*2h*	*3h*	*4h*	*5h*	*6h*
	NMT 10%	NMT 20%	---	---	NLT 75%		
Reference (without ethanol)							
Average	**<3**	**<3**	**41**	**100**	**100**	**101**	**101**
SD	---	---	19.0	1.5	1.8	1.8	1.9
Maximum value	<3	<3	62	102	103	104	104
Minimum value	<3	<3	14	97	97	98	98
Test 1 (5% of ethanol)							
Average	**<3**	**<3**	**67**	**98**	**98**	**99**	**99**
SD	---	---	18.2	2.1	2.0	1.9	2.0
Maximum value	<3	<3	92.4	102.5	101.6	102.1	102.6
Minimum value	<3	<3	23.3	95.7	95.0	95.8	95.7
Test 2 (10% of ethanol)							
Average	**<3**	**9**	**94**	**101**	**101**	**101**	**101**
SD	---	5.4	5.7	1.7	1.5	1.7	1.6
Maximum value	<3	18.4	99.9	104.4	104.1	104.8	105.0
Minimum value	<3	3.0	85.3	98.9	99.4	99.7	99.7
Test 3 (20% of ethanol)							
Average	**<3**	**35**	**94**	**96**	**97**	**97**	**97**
SD	---	12.7	2.4	2.2	2.2	2.1	2.1
Maximum value	<3	71.0	99.0	99.8	99.9	99.5	100.0
Minimum value	<3	20.7	90.7	91.6	91.7	91.9	92.2
Test 4 (40% of ethanol)							
Average	**<3**	**42**	**92**	**96**	**96**	**96**	**96**
SD	---	3.7	2.0	2.2	2.2	2.3	2.1
Maximum value	<3	48.7	95.0	98.1	98.5	98.6	98.6
Minimum value	<3	34.6	88.5	91.9	91.7	91.9	92.2

Source: Sponsor's Response to Quality Information Request (07/16/2018). Test 18-1-07 Report. Page.5, Table 1.

Table 12: Mean Dissolution Values at Different Ethanol Concentration at pH 6.0 and pH 7.2.

Samples	Rifamycin dissolved amount (%)							
	Stage I: pH 6.0 phosphate buffer		*Stage II: pH 7.2 phosphate buffer*					
	1h	*2h*	*1h*	*2h*	*3h*	*4h*	*5h*	*6h*
Reference (without ethanol)								
Average	**<3**	**<3**	**<3**	**40**	**97**	**100**	**100**	**100**
SD	---	---	---	27.0	3.4	1.9	1.6	1.8
Maximum value	<3	<3	<3	85	101	104	103	103
Minimum value	<3	<3	<3	4	88	97	97	97
Test 1 (5% of ethanol)								
Average	**<3**	**<3**	**<3**	**46**	**92**	**98**	**98**	**99**
SD	---	---	---	7.6	3.4	1.2	1.1	1.2
Maximum value	<3	<3	<3	55	96	100	100	100
Minimum value	<3	<3	<3	31	84	96	97	97
Test 2 (10% of ethanol)								
Average	**<3**	**<3**	**3**	**73**	**98**	**99**	**99**	**99**
SD	---	---	1.4	6.6	1.5	1.6	1.6	1.4
Maximum value	<3	<3	5	83	102	102	101	101
Minimum value	<3	<3	1	62	96	96	96	96
Test 3 (20% of ethanol)								
Average	**<3**	**<3**	**52**	**96**	**100**	**100**	**100**	**100**
SD	---	---	4.7	2.2	1.8	1.8	1.9	1.6
Maximum value	<3	<3	63	101	102	102	102	102
Minimum value	<3	<3	46	93	96	96	96	96
Test 4 (40% of ethanol)								
Average	**39**	**100**	*	*	*	*	*	*
SD	5.6	1.6	---	---	---	---	---	---
Maximum value	46	102	---	---	---	---	---	---
Minimum value	27	96	---	---	---	---	---	---

*stage II not performed because 100% of dissolution already reached at 2h timepoint in stage I.

Source: Sponsor's Response to Quality Information Request (09/17/2018). Test 18-1-08 Report. Page.5, Table 1.

DRUG-DRUG INTERACTION

Considering that rifamycin SV shows minimal systemic absorption, the Applicant did not conduct *in vivo* drug-drug interaction trials. Based on the *in vitro* data and available human pharmacokinetic data, the potential for *in vivo* enzyme and transporter mediated DDIs (both at the systemic level and at the level of the GI tract) were assessed as recommended in the draft 2017 FDA DDI guidance[9].

[9] The US FDA. Draft guidance for industry: In vitro metabolism-and transporter-mediated drug-drug interaction studies. 2017. [Cited 2018 July 26]. Available: https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf

Available *in vitro* data suggest the potential for rifamycin SV to inhibit intestinal P-gp or BCRP *in vivo*. Because of the pH triggered, delayed release, formulation of rifamycin SV MMX, its limited 3-day treatment duration, and regional expression of CYP enzymes (majority of the enzymes are expressed in the small intestine whereas the rifamycin SV MMX formulation is designed to release rifamycin SV in the colon), intestinal CYP mediated DDIs were not judged as clinically relevant.

Potential for Transporter-Mediated Drug-Drug Interactions

Co-administration of rifamycin SV MMX with a P-gp inhibitor

In vitro data suggest rifamycin SV is a substrate of P-gp but not BCRP transporters based on a >2-fold increase in efflux ratio[9]. Co-administration of rifamycin SV MMX with P-gp inhibitors is not expected to result in a clinically relevant increase in the systemic exposure of rifamycin SV based on the following: **i)** poor drug solubility; **ii)** wide safety margin in relation to systemic exposures observed with *i.v.* rifamycin SV, **iii)** limited 3-day treatment duration; and **iv)** colonic release (P-gp levels lower in colon[10] and distal to site of most oral drugs' absorption (i.e., duodenum and jejunum).

Co-administration of rifamycin SV MMX with a P-gp inducer:

Concomitant use is not expected to compromise the efficacy of rifamycin SV considering that the site of action is the colon and increased P-gp mediated efflux in the presence of a P-gp inducer would be expected to result in increased drug availability in the gastrointestinal tract. Further, the available safety data at the 1200 mg rifamycin SV MMX total daily dose [Study CB-01-11/03) along with the limited treatment duration (3 days) does not suggest any safety concerns associated with the potential increase in rifamycin SV exposures in the gastrointestinal tract when rifamycin SV MMX tablets are co-administered with a P-gp inducer.

Rifamycin SV effects as an inducer of transporters:

No definitive conclusions regarding the potential for rifamycin SV MMX to affect the pharmacokinetics of substrates of various transporters due to transporter induction can be drawn because the Applicant did not conduct *in vivo* DDI trial(s) and *in vitro* methods to evaluate the induction of drug transporters are not well established at this time (per guidance[9]). Nevertheless, the review team does not expect rifamycin SV to induce drug transporters to a clinically relevant extent considering the limited 3-day treatment duration[10].

Rifamycin SV as an inhibitor of P-gp and BCRP transporters:

In vitro data suggest rifamycin SV is an inhibitor of P-gp (IC_{50} 6.5 µM) and BCRP (IC_{50} 34.4 µM). As recommended[9] the following cut off criteria to assess the potential for a drug to inhibit P-gp or BCRP: I_{gut}/IC_{50}> 10 was used. Where I_{gut} is the dose (400 mg) in molar units for rifamycin SV base (573.25 µmol) over a theoretical intestinal volume of 250 mL and represents a worst-case scenario (2,293 µM). Based on the review team's calculations, an I_{gut}/IC_{50} of ca. 351 and 67.7 were estimated for P-gp and BCRP respectively. Based on the aforementioned predictions,

[10] Englund G, Rorsman F, Rönnblom A, et al. Regional levels of drug transporters along the human intestinal tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells. Eur J Pharm Sci. 2006;29(3-4):269-77.

rifamycin SV may be an inhibitor of P-gp and BCRP transporters in the gut however, the anticipated *in vivo* inhibition of P-gp transporters by rifamycin is not expected to be clinically relevant; majority of rifamycin SV is anticipated to be present in the colon where the expression of P-gp transporters is lower compared with the small intestine (absorption site of majority of orally administered drugs)

Rifamycin SV as an inhibitor of OATP1B1 transporters:
In vitro data from literature describe rifamycin SV as an inhibitor of OATP1B1 (K_i 2µM)[11]. To predict whether rifamycin SV may inhibit OATP1B1 transporter *in vivo*, the review team used a basic static physiological model as recommended[9]. Briefly, incorporation of the estimated maximum hepatic inlet free concentration (0.005 µM) and IC_{50} of 2 µM[11], an "R" value of 1 is obtained which is lower than the recommended cut-off value of 1.1[9] suggesting that oral administration of rifamycin SV is not expected to inhibit OATP1B1 transporters *in vivo*. Importantly, the review team used the same basic static physiological model to successfully predict the potential of rifamycin SV to inhibit OATP1B1 transporters after intravenous administration ("R" value 6.2; carrier-mediated organic anion transporter hyperbilirubinemia[12])].

Rifamycin SV as Inhibitor of other (other than P-gp, BCRP, and OATP1B1) Transporters:
In vitro data do not suggest that rifamycin SV is an inhibitor of OAT1, OAT3, OCT2, MATE1, or MATE2-K.

Potential for metabolism-mediated drug-drug interactions
The Applicant assessed the potential for CYP mediated metabolism of rifamycin SV and the inhibition/induction effect of rifamycin SV using *in vitro* assays and mechanistic analysis as recommended in the 2017 draft FDA guidance.

In vitro data do not suggest CYP mediated metabolism of rifamycin SV (Applicant did not assess the potential for rifamycin SV to be metabolized by non-CYP enzymes).

At the systemic level, rifamycin SV is not anticipated to induce CYP3A and CYP2B6 enzymes (R3 values > 0.8) based on *in vitro* data and subsequent mechanistic analysis conducted by the Applicant. Furthermore, regional distribution of CYP3A4/5 and CYP2B6 (minimal expression in the colon where drug is likely released[13]) and limited 3-day treatment duration do not suggest a potential for clinically significant induction of CYP3A and CYP2B6 by rifamycin SV. Because CYP3A4/5 and CYP2C9 induction is mediated by activation of the pregnane X receptor and CYP3A4 findings do not suggest potential for CYP3A4 induction, the review team does not expect rifamycin SV to induce CYP2C9 *in vivo*.

[11] Vavricka SR, Van montfoort J, Ha HR, Meier PJ, Fattinger K. Interactions of rifamycin SV and rifampicin with organic anion uptake systems of human liver. Hepatology. 2002;36(1):164-72.
[12] Acocella G, Nicolis FB, Tenconi LT. The effect of an intravenous infusion of rifamycin SV on the excretion of bilirubin, bromsulphalein, and indocyanine green in man. Gastroenterology. 1965;49(5):521-5.
[13] Xu J, Lin Y, Boulas P, Peterson ML. Low colonic absorption drugs: risks and opportunities in the development of oral extended release products. Expert Opin Drug Deliv. 2018;15(2):197-211.

Rifamycin SV is not anticipated to inhibit CYP enzymes systemically based on the Applicant's analysis of *in vitro* enzyme inhibition data (using basic model of reversible inhibition, all R_1 values were less than 1.02) and >4000 -fold difference between the lowest IC_{50} value 8.5 µM [for CYP3A4]) and plasma concentration of rifamycin SV observed *in vivo (*maximum unbound plasma concentration 0.0025 µM).

At the pre-systemic level, rifamycin SV is not anticipated to either induce or inhibit CYP enzymes based on the Applicant's analysis using a static mechanistic model to account for effects on CYP3A4/5 in both the intestine and liver (for induction, AUCR > 0.8 and for inhibition, AUCR < 1.25).

7 Statistical and Clinical Evaluation

7.1 Sources of Clinical Data and Review Strategy

7.1.1. Sources of Clinical Data

Two multi-center, randomized, double-blind, controlled trials of rifamycin were submitted in support of this NDA, one with placebo control (C2009-0201), and the second with active comparator ciprofloxacin (RIT-1/AID). Both trials were conducted entirely outside the US. Study C2009-0201 was conducted under the IND; Study RIT-1/AID and other clinical studies were not conducted under the IND.

Table 13: Clinical Studies for Rifamycin

Study No. Phase Study Period	Trial Design	Regimen/ schedule/ route[1]	Study Endpoints	Treatment Duration/ Follow Up	No. of subjects enrolled	Study Population	No. of Centers and Countries
Phase 3 Studies							
C2009-0201 NCT01142089 2010-2012	Randomized, double-blind, placebo-controlled; randomized 3:1 to receive rifamycin or placebo	400 mg po BID or placebo	Superiority; 1° Effic. End Pt: Time to Last Unformed Stool (TLUS)	3 days	264 enrolled/ 264 treated (199 rifamycin, 65 placebo)	Subjects with **travelers' diarrhea** ≥ 18 years old	8 sites in Mexico (6) and Guatemala (2)
RIT-1/AID NCT01208922 2010-2016	Randomized, double blind, active comparator-controlled (ciprofloxacin)	rifamycin 400 mg po BID for 3 days or ciprofloxacin po 500 mg for 3 days (double-dummy to preserve blinding)	Non-inferiority; 1° Effic. End Pt: TLUS	3 days	843 enrolled/ 835 treated (420 rifamycin, 415 ciprofloxacin	Subjects with **travelers' diarrhea** 18-85 years old	19 sites in India (17), Ecuador, Guatemala
Phase 2 Studies							
CB-01-11/02	Randomized, double-blind, active comparator-	rifamycin 400 mg po BID for 3 days or	Non-inferiority; 1° Effic. End Pt: TLUS	3 days/120 hours	115 enrolled/ 115 treated (57 rifamycin,	Subjects with **infectious diarrhea** 18-65 years old	8 sites in South Africa

Study No. Phase Study Period	Trial Design	Regimen/ schedule/ route[1]	Study Endpoints	Treatment Duration/ Follow Up	No. of subjects enrolled	Study Population	No. of Centers and Countries
2006-2007	controlled (rifaximin)	rifaximin 200 mg po QID for 3 days			58 rifaximin)		
CB-01-11/03 NCT03447821 2008	Pilot, dose-finding, double-blind, randomized	rifamycin 200 mg BID or rifamycin 400 mg BID or rifamycin 400 mg TID	1° Effic. End Pt: TLUS	Dosing up to 7 consecutive days	40 enrolled/ 40 treated	Subjects with **infectious diarrhea** 18-65 years old	11 sites in Mexico (7) and Turkey (4)
Other Studies pertinent to the review of efficacy or safety (e.g. clinical pharmacological studies)							
CB-01-11 Phase 1 2005	Open-label, single and multiple doses, oral bioavailability study	Rifamycin 200 mg po on Day 1, 2, and 6, and 200 mg TID on Day 3, 4, and 5 (12 doses [2400 mg] total)	PK analysis not performed since rifamycin not detected in plasma or urine	6 days	12 enrolled/ 12 treated	Healthy volunteers 18-45 years old	Switzerland
CB-01-11/10 Phase 1 2009	Open-label, randomized single dose cross-over to assess absolute bioavailability and food effects	rifamycin 400 mg po fasting compared with 250 mg IV; 400 mg rifamycin po fasting vs 400 mg po fed	Absolute bioavailability of rifamycin po (fasting) vs rifamycin IV; assessment of food effect on absorption	Single dose oral and single dose IV	24 enrolled/ 24 treated	Healthy volunteers 18-55 years old	Switzerland
CB-01-11/11 Phase 2 2009	Open-label, randomized, multiple-dose bioavailability study	200 mg po QID for 3 days or 400 mg BID for 2 days	Descriptive plasma and urine pharmaco-kinetics	3 days	24 enrolled/ 24 treated	Healthy volunteers 18-55 years old	Switzerland

Source: Adapted from M5 5.2 Tabular Listing of All Clinical Studies

[1]The delayed-release oral tablet contains 194 mg rifamycin, equivalent to 200 mg rifamycin sodium. For ease of reference in this section, dose will be represented as the sodium salt.

7.1.2 Review Strategy

This review focuses on two phase 3 multi-center, randomized, double-blind, controlled trials, C2009-0201 and RIT-1/AID, for the proposed indication of travelers' diarrhea. Study C2009-0201, a placebo-controlled study, is the primary basis for the assessment of efficacy. Study RIT-1/AID which used ciprofloxacin as an active comparator, was considered supportive for efficacy. Data sources include Applicant study reports and references and Pubmed literature review. Efficacy results will be presented as analyzed by the Applicant and by FDA. Clinical microbiological assessment will be discussed in Section 8. Safety results are presented in Section 9 using descriptive statistics, using JMP Clinical and the Mercado OND Data Analytics tools.

Phase 2 study CB-01-11/02 with active comparator rifaximin will be briefly reviewed. While this study evaluated a different indication (infectious diarrhea) and lacked a placebo control, CB-01-11/02 included ECG assessments which were not performed in phase 3 trials. CB-01-11/03, a phase 2 dose-ranging study of infectious diarrhea is mentioned briefly given the Applicant's reliance on it for the proposed dose and inclusion in the aggregate safety database.

The application was submitted in eCTD format and was entirely electronic, including protocols, statistical analysis plans (SAPs), clinical study reports, and datasets in Study Data Tabulation Model (SDTM) and Analysis Data Model (ADaM) format.

Data and Analysis Quality

With regard to evaluating efficacy, the data for both phase 3 trials were of adequate quality. For Study C2009-0201, the Applicant's analysis of the primary efficacy endpoint was accurate but the analyses for a number of secondary endpoints were inaccurate. For Study RIT-1/AID, the Applicant's endpoint analyses were systematically inaccurate, due to the incorrect computation of a non-inferiority margin and the lack of adjustment for an adaptive group sequential trial design. The statistical reviewer's comments below provide details.

7.2 Review of Relevant Individual Trials Used to Support Efficacy

7.2.1 Study C2009-0201

7.2.1.1 Study Design

Study C2009-0201 was a randomized, double-blind, placebo-controlled superiority study to evaluate the safety and efficacy of rifamycin for travelers' diarrhea in adults ($\geq$ 18 years). Subjects were randomized 3:1 to receive rifamycin 400 mg BID orally (199 subjects enrolled and treated) or placebo (65 subjects enrolled and treated) for 3 days. Eight centers total, two sites in Guatemala and six sites in Mexico, enrolled at least one subject. Randomization was stratified by site.

Eligibility was based on travel from an industrialized country within 30 days of randomization, and symptoms highly indicative of acute enteric bacterial infection with no fever or other symptoms of systemic infection. Treatment was initiated on the day of screening, within 72 hours of the onset of diarrhea. Subjects completed diary cards covering 120 hours post initial dose of study medication that recorded data and time of each stool, its consistency as either formed or unformed (i.e., watery or soft), the presence of blood or systemic symptoms, the times of study drug administration, adverse events, and concomitant medications. Safety and efficacy were evaluated on day 2 (Visit 2) either at the clinic or by phone, day 3 or 4 (Visit 3), and day 6-10 (Visit 4). A follow up safety assessment occurred 30 days after the last dose. Stool and urine samples were collected on Visits 1 (baseline) and 3. Rescue therapy, based on local standard of care or identified pathogen, was given by a study physician if enteric symptoms worsened during treatment or failed to improve after 24 hours or more of treatment.

The **primary efficacy endpoint** was time to last unformed stool (TLUS), defined as the interval in hours between the first dose of the study drug and the passage of the last unformed stool prior to achieving clinical cure[14], up to 120 hours.

Clinical cure was defined as either:

- Passage of 2 or fewer soft stools and no fever, watery stools or signs or symptoms of enteric infection during a 24-hour period after the first dose of study drug, or
- Passage of no stools or only formed stools, without the presence of fever, during a 48-hour interval in the 120-hour data collection period after the first dose of study drug, with or without other signs or symptoms of enteric infection.

Use of rescue medication prior to achievement of clinical cure implied clinical cure was not achieved. Clinical cure was considered achieved at the start of the 24-hour or 48-hour qualifying period.

Multiple **secondary endpoints** were identified:

- Treatment failure, defined as either:
  - Worsening diarrhea and/or signs or symptoms of enteric infection or failure to improve 24 hours or more after the first dose of study drug that results in the administration of rescue therapy, or
  - Not achieving clinical cure in the 120-hour data collection period after the first dose of study drug.
- Microbiologic cure relative to each pathogen (absence in the post-treatment (Visit 3) stool sample of the pathogen when it was identified in the baseline stool sample), relative to participants with a single pathogen identified at baseline (absence at Visit

[14] Per C2009-0201 CSR, the original protocol defined clinical cure as a secondary endpoint, but the sponsor removed this as secondary endpoint prior to database lock and unblinding.

3 of the identified pathogen), and relative to participants with multiple pathogens identified at baseline (absence at Visit 3 of all identified pathogens).

Administration of the Investigational Products:

The medication kit contained blister cards containing the investigational product. Each kit contained 6 doses of 2 tablets each plus 2 extra doses of 2 tablets each for a total of 16 tablets. The containers were labeled with the dosing time (breakfast and dinner) and the number of tablets to be taken at each dose. (see discussion of Protocol Violations, below.)

Study Procedures are described in Table 14 below.

Following screening and randomization, the subject was to return to the clinic or contacted via telephone on Day 2 (Visit 2) and return to the clinic on the day after the last dose of the study drug on Day 4 or 5 (Visit 3).

The subject was also to return to the clinic for Visit 4 on Day 6. Per the protocol, this visit may occur on Day 7, 8, 9 or 10 if the patient has an "appropriate reason" for delaying the visit.

The investigator was to contact the subject via email, fax, or phone to perform a follow-up safety evaluation 30 ± 2 days after the last dose of study drug.

Subjects who prematurely discontinued study participation were asked to perform Visit 3 procedures and to undergo follow up 30 days after the last dose of study drug.

Table 14: Study Procedures for C2009-0201

Procedures	VISIT 1	VISIT 2		VISIT 3	VISIT 4	Follow-up
	Screening and Randomization Day 1	Day 2	Day 3	Day 4 or 5	Final Visit Day 6 + 4 Days	30 ± 2 Days After Last Dose
Informed Consent	X					
Demographics	X					
Inclusion/Exclusion	X					
Medical History	X					
Medication History	X					
Concomitant Meds		X		X	X	
Vital Signs	X			X		
Physical Exam	X			X		
Assess for Enteric Infection	X	X		X	X	
Record Stool Consistency and Freq	24 h pre-dose to 0 hr	> 0-24 hr	> 24-48 hr	>48 -72 hr, >72-96 hr	>96-120 hr	
Review diary		X		X	X	
Chemistry, Hematology, and UA	X			X		
Stool Sample for Micro Assessment	X			X		

Procedures	VISIT 1 Screening and Randomization Day 1	VISIT 2 Day 2	Day 3	VISIT 3 Day 4 or 5	VISIT 4 Final Visit Day 6 + 4 Days	Follow-up 30 ± 2 Days After Last Dose
Dispense Study Drug	X					
Administer Study Drug	X	X	X			
Dispense Rescue Therapy		As needed	As needed	As needed	As needed	
Patient Diary	X	X	X	X	X	
Record AEs/SAEs		X	X	X	X	SAEs
Assess Treatment Compliance		X	X	X	X	

Source: C2009-0201 CSR Table 1

Key Inclusion and Exclusion Criteria

Inclusion:

- Male and female patients 18 years of age or older agreed to use an effective method of birth control during the treatment and follow up study period; female patients must have had a negative pregnancy test in the 72 hours before randomization.
- Recent travel within 30 days of randomization from an industrialized country
- Experienced signs or symptoms indicative of acute bacterial diarrhea (i.e. travelers' diarrhea), defined as at least three unformed, watery, or soft stools within the 24 hours preceding randomization and a duration of illness ≤ 72 hours before randomization, and were able to provide an unformed stool sample during screening.
- Experiencing one or more signs or symptoms of enteric infection (moderate to severe gas/flatulence, nausea, vomiting, abdominal cramps or pain, tenesmus, or defecation urgency.

Exclusion:

- Fever (> 100.4 ºF or 38 ºC) or presence of systemic infection
- Known or suspected infection with a non-bacterial pathogen before randomization
- Presence of diarrhea for > 72 hours duration
- Presence of grossly bloody stool
- Presence of moderate to severe dehydration
- Receiving more than two doses of an antidiarrheal medication (e.g., antimotility, absorbent, adsorbent, anti-secretory, or probiotics) within 24 hours before randomization
- Receiving one or more of the following within 7 days before randomization and during treatment (visits 1-4): antibacterial drugs: trimethoprim-sulfamethoxazole, fluoroquinolone, azithromycin or rifaximin; or antidiarrheal medications, laxatives, stool softeners, antispasmodics, or antacids.

Criteria for Subject Discontinuation From Treatment

- Withdrawal of consent
- Physician's decision based on subject's well-being
- Intolerable AE
- Intact of prohibited concomitant medication
- Significant non-compliance with protocol
- Clinical or administrative reasons consistent with protocol requirements
- Worsening of travelers' diarrhea requiring medical treatment

For subjects withdrawn from the study, evaluations for Visit 3 were to be performed as soon as possible. Subjects withdrawn due to AEs were to be followed until resolution of the AE or investigator discretion.

Rescue Therapy

Rescue therapy was prescribed by the clinical investigator using local standard empiric therapy and/or guided by pathogen identification for any clinical reason.

All patients who received rescue therapy were to be withdrawn from the study and the Visit 3 study procedures performed. If subject received rescue therapy in the 120 hours (5 days) following the first dose of the study drug they were considered treatment failures.

Protocol Amendments

Per the CSR, the SAP dated 6 April 2010 was finalized prior to the database lock and unblinding, and provided the following changes to the protocol:

- Clinical cure was removed as a secondary endpoint

The following changes to the planned analyses were made subsequent to finalization of the SAP but prior to database lock and unblinding:

- Data were analyzed without being stratified by race as the majority of subjects were Caucasian.
- Patients were not considered as a treatment failure if they achieved clinical cure and subsequently received rescue medication.
- Treatment emergent AEs and Travelers' Diarrhea-related AEs from patient diaries were presented separately.

The Applicant reported that slower than expected study enrollment was observed due to decreased tourism resulting from travel alerts and restriction in Mexico. To compensate, additional study sites were added in Mexico (Puerto Vallarta, San Jose del Cabo, Oaxaca, and Puerto Escondido.)

Statistical Reviewer Comments:

It should be noted that "Time to last unformed stool" is a misnomer. For example, if a participant had a watery stool at 12 hours, soft stools at 30 and 35 hours, with no additional unformed stools, fever, or enteric symptoms, then the participant achieved clinical cure prior to the unformed stools at 30 and 35 hours. Hence his/her TLUS value is 12 hours, even though there were two subsequent unformed stools.

Also, the definition of clinical cure seems inadequate, as it accounts for rescue medication administered by study physicians but ignores prohibited medications self-administered (e.g., loperamide) or prescribed or administered by non-study physicians (e.g., antibacterial drugs). Since use of such prohibited medications prior to the achievement of clinical cure (as defined by the Applicant) could have contributed to that achievement, ignoring the use of prohibited medications when assessing clinical cure confounds the attribution of cure to the study medication per se. Similarly, some participants used the extra study medication they were provided with (recall all participants were supplied with an extra day's dose of study medication) prior to the achievement of clinical cure. The Applicant ignored this in assessing clinical cure, again confounding causal attribution to the prescribed dose of study medication. This issue is addressed below in sensitivity analyses of TLUS and clinical cure.

Further, note that, as the Applicant indicated in its response to the FDA information request of May 18, 2018, treatment failure is the "inverse" of clinical cure: a participant is a treatment failure if and only if he/she has not achieved clinical cure. This means that p-values obtained from analyses of treatment failure are identical to the p-values that would be obtained from the corresponding analyses of clinical cure.

Finally, note the plethora of microbiological cure endpoints that the Applicant categorized as secondary endpoints. For example, there are 16 versions of microbiological cure, assessed on a by pathogen basis. As the Applicant did not implement a method for multiplicity adjustment (discussed below), the Applicant's microbiological cure endpoints are treated as exploratory in this review. See the summary of efficacy analysis results for further discussion.

Statistical Analysis Plan

Analysis Populations

- The Intent-to-Treat (ITT) sample includes all participants who receive at least one dose of study drug.
- The Per Protocol (PP) sample includes all participants who complete the study and have complete diary card information. Additionally, participants must meet protocol criteria for treatment compliance and have no major protocol violations.
- The Safety sample includes all participants who receive at least one dose of study drug.

Testing

All hypothesis tests were conducted at a 2-side alpha of .05. All analyses were conducted for each analysis population.

Analysis of Primary Efficacy Endpoint

Participants were considered censored under the following conditions:

- Participants who received rescue therapy prior to clinical cure, who terminated early due to treatment failure, or who completed the study without achieving clinical cure were assigned a censored TLUS of 120 hours.
- Participants who terminated early for reasons other than treatment failure were censored for TLUS at the time of the last available information on unformed stools.

The ITT sample was used for the primary analysis of the primary endpoint. Letting τ denote the largest time at which both arms had at least one participant with an uncensored $TLUS \geq \tau$, the logrank test was used to test the null hypothesis that, at all time points less than or equal to τ, the hazard for the Rifamycin arm equaled the hazard for the Placebo arm.

Additionally,

- Each arm's survival curve was estimated using the Kaplan-Meier product limit method, which allowed estimation of each arm's median.
- A Cox proportional hazards analysis of TLUS was conducted. The initial Cox model used arm, analysis center, and the arm by center interaction terms as predictors. Analysis centers were created by combining sites with five or fewer participants in either arm with the geographically nearest site. If the arm by center interaction was significant, then
    i. Primary and secondary endpoints were analyzed for each center separately.
    ii. A Cox analysis using country as a predictor was conducted.

Analysis of Secondary Efficacy Endpoints

Each secondary endpoint was a binary variable and was tested using the Chi-square test. The null hypotheses were that the secondary endpoints were independent of arm.

Adjustment for Multiplicity

The Applicant did not specify a method for multiplicity adjustment for the multiple secondary endpoints.

Handling Missing Data

The handling of censoring for the primary efficacy endpoint is described above. Per the SAP, when secondary endpoints (treatment failure, microbiological cure) could not be assessed due

to missing data, the Applicant used the "Worst Case Imputation Method," in which the secondary endpoints were assigned the value of "failure."

Statistical Reviewer Comments:

First, it should be noted that the log-rank test tests the null hypothesis that the TLUS distributions of the rifamycin and placebo arms are identical, and it does not test the null hypothesis that the two distributions share a common median. In theory, it is possible that the two distributions differ but share the same median. This is not a criticism of the SAP; rather, this is a note about the accurate interpretation of log-rank test results.

Second, the CSR presents results of analyses of microbiological cure by pathogen; that is, if a participant was infected by two or more pathogens at baseline, then he/she would have two or more corresponding eradication endpoint values, each indicating if the specific baseline pathogen had been eradicated. The Applicant's chi square tests here are of questionable accuracy, as these tests presuppose independent observations, but in fact multiple endpoint values from a given participant may be associated. This review will only consider microbiological cure endpoints assessed on a participant by participant basis (see next comment).

Third, when examining microbiological eradication of a type or class of pathogen by participant (e.g., of the participants infected with Giardia at baseline, what proportion experienced eradication of Giardia by Visit 3?), the Applicant inappropriately used chi square tests when the number of participants with baseline infection was small. The validity of the chi square test presupposes a reasonably sized sample; with small samples, use of Fisher's exact test would be better.

Fourth, the Applicant did not examine microbiological cure across all participants, as it instead separately examined participants with a single pathogen and participants with multiple pathogens identified at baseline. An examination across all participants would be informative.

Fifth, the use of "Worst Case Imputation" for missing data for secondary endpoints privileges the arm with less missing data, as this method is overly "pessimistic" in filling in missing endpoint values. Regarding microbiological cure, the placebo arm usually had more missing data (as detailed below), and this could inflate estimates of the rifamycin vs. placebo treatment effect. Also, as with any single imputation method, the obtained p-values are not exact, as they do not account for uncertainty due to the presence of missing data. Further, the Applicant appears to have intended to apply "Worst Case Imputation" when baseline microbiological data are missing (if a participant's baseline data are missing for a given pathogen, then it is not possible to determine whether or not he/she was infected at baseline and then cured by Visit 3). If blinding was maintained by the technicians who performed the baseline stool analysis, then using the subsample of participants with no missing baseline microbiological data in microbiological cure data analyses respects the randomization to treatment, while using "Worst Case Imputation" at baseline may not. Thus, it may limit the impact of missing data if the

subsample of participants is used in all microbiological cure data analyses. The SAP does not specify sensitivity analyses to examine other possible missing data mechanisms.

Sixth, it is difficult to determine the extent of missing clinical cure data. The clinical cure variable in Applicant data file adtte.xpt has no missing data, as all 264 participants in the ITT sample had meaningful values (Y or N). The Applicant's response to the FDA information request of May 18, 2018 provided the algorithm used for computing clinical cure, and it necessarily assigns Y or N to the clinical cure variable, with no possibility of assigning a missing value. This algorithm cannot be correct, as there were 4 participants who were censored at less than 120 hours due to premature stopping of diary completion; all 4 were assigned a clinical cure value of N, whereas it is in fact unknown whether they achieved clinical cure. Review of the Applicant's adeffmid.xpt data file, which records participants' daily signs and symptoms of traveler's diarrhea, also has no missing data, except for a single missing datum for tenesmus (recall that the definition of clinical cure is, in part, based on the presence or absence of signs and symptoms). In the Applicant's response to the same FDA information request, it noted that "no data fields that are related to secondary endpoints contain the imputed worst case values." At the very least, this is incorrect with regard to the clinical cure values of the 4 just-discussed participants.

Seventh, the Applicant did not correctly handle missing TLUS observations due to incomplete diaries. For example, one participant maintained the daily diary for only 24 hours and recorded no stools during that period. The Applicant assigned the participant a censored TLUS of 24 hours, meaning that the true (but unobserved) TLUS value is larger than 24 hours. However, it is possible that the participant's true TLUS value is 0. This would be the case if the participant also had no unformed stools during hours 24-48, as then hours 0-48 would constitute a 48-hour qualifying period for clinical cure and clinical cure would be achieved at hour 0. Hence, this participant's TLUS value should be censored at 0 hours rather than 24 hours. Note that instances of censoring due to incomplete diaries could be cases of informative censoring. This issue is addressed below in TLUS sensitivity analyses.

7.2.1.2 Study Results

Compliance with Good Clinical Practices

According to the Applicant, the study was conducted in full conformance with ethical principles of Good Clinical Practice (GCP), respecting the guidelines from ICH E6, US Code of Federal Regulations 21 CFR Parts 50, 54, 56, 312, 314, and 511, and the Declaration of Helsinki.

Financial Disclosure

All investigators listed on Form FDA 1572 were required to provide a Financial Disclosure statement to the sponsor at the start of the study and 1 year following completion.

Subject Disposition

Table 15 provides subject disposition, outlining the reasons for withdrawal from study.

Table 15: Subject Disposition in Study C2009-0201

Disposition	Rifamycin N=199 n (%)	Placebo N=65 n (%)	Total N=264 n (%)
Randomized Patients	199 (100.0)	65 (100.0)	264 (100.0)
Completed Study			
Yes	178 (89.4)	53 (81.5)	231 (87.5)
No	21 (10.6)	12 (18.5)	33 (12.5)
Reason for Withdrawal			
Patient required rescue medication	17 (8.5)	8 (12.3)	25 (9.5)
Withdrawal of consent	2 (1.0)	1 (1.5)	3 (1.1)
Patient noncompliance with study procedure	1 (0.5)	2 (3.1)	3 (1.1)
Use of prohibited medications	1 (0.5)	0 (0)	1 (0.4)
Discretion of the investigator	0 (0)	1 (1.5)	1 (0.4)

Source: Adapted from C2009-0201 CSR Table 5

Protocol Violations/Deviations

Overall compliance with study treatment (ITT) population, defined as receiving 2 tablets for each of the first four doses and 10-14 tablets total, was 176 (88.4%) in subjects receiving rifamycin compared with 55 (89.2%) receiving placebo.

A total of 27 protocol violations/deviations were reported in 24 (9.1%) subjects, with the subjects proportionally distributed between those receiving rifamycin, 18 (9.0%), and those receiving placebo, 6 (9.2%).

The following 11 subjects (C2009-0201 CSR Appendix 16.2.11.4) were listed as "protocol deviations" for taking a total of 16 tablets (8 doses), or an extra 4 tablets (2 doses or 1 day), compared with the total prescribed by investigational plan of 12 tablets (6 doses). Of the subjects who received a total of 16 tablets, 10 received rifamycin, 1 received placebo. For all subjects at the Puerta Vallarta site, the Applicant reported that the PI prescribed an extra medication day as the subjects were "still presenting diarrhea symptoms"; subjects at other sites did not have an explanation for the extra doses.

- Guadalajara, # (b) (6) (Rifamycin) – Subject did not receive rescue medication
- Cuernavaca, # (b) (6) (Rifamycin), (b) (6) (Rifamycin), (b) (6) (Rifamycin), (b) (6) (Rifamycin)
- Puerta Vallarta, # (b) (6) (Rifamycin), (b) (6) (Rifamycin), (b) (6) (Rifamycin), (b) (6) (Rifamycin)
- San Jose del Cabo, # (b) (6) (Rifamycin), (b) (6) (Placebo)

The subjects listed above were evaluated as to whether they received rescue medications, per CSR Appendix 16.2.11.2. In addition to the extra doses of study medication, one subject, (b) (6) (Puerto Vallarta) also received rescue medication (ofloxacin) for diarrhea.

Table 16: C2009-0201 - Summary of Protocol Violations (Intent-to-Treat Population)

Category	Rifamycin N=199 n (%)	Placebo N=65 n (%)
Individual subjects with any protocol violations	18 (9.0)	6 (9.2)
Dosing	13 (6.5)	3 (4.6)
Did not take 2 pills per dose	3 (1.5)	2 (3.1)
Took 8 doses (16 pills)	10 (5.0)	1 (1.5)
Inclusion/exclusion criteria	2 (1.0)	3 (4.6)
Prohibited medications[1]	4 (2.0)	0
Protocol procedures	2 (1.0)	0

Source: Adapted from C2009-0201 CSR addendum Tables 4 and 6; Denominator for calculating percentages is number of subjects in each treatment group. Numbers do not add up to total protocol violations as some subjects had violations in more than one category.

[1]Table 4 (Analysis Populations) lists 3 rifamycin subjects with prohibited medications, while Table 6 (Protocol Violations) lists 4; this discrepancy was not explained.

***Clinical Reviewer Comments:** Overall 4.2% of subjects in this study took an extra 4 tablets with a slight imbalance of subjects taking rifamycin (5.0%) compared with placebo (1.5%). In the IR response date 7/20/2018, the Applicant explained that extra doses were provided as a contingency reserve in case of loss or mishap. At least one site recorded that extra doses were prescribed by the clinical investigator due to continued symptoms. See statistical reanalysis below for primary efficacy endpoint (TLUS), in Table 17 below, Sensitivity Version C, that considers the possible effect of extra doses. While this analysis showed a slightly longer TLUS in the rifamycin group, the difference compared to placebo was still statistically significant.*

Demographic Characteristics

Table 17 compares the rifamycin and placebo arms on baseline demographic characteristics.

Table 17: C2009-0201 - Comparing Rifamycin and Placebo Arms on Demographic Characteristics (ITT sample)

Demographic Characteristic	Rifamycin Arm (N=199) % (n)	Placebo Arm (N=65) % (n)	Standardized Difference[1]
Sex			
Male	49.7% (99)	49.2% (32)	0.01
Female	50.3% (100)	50.8% (33)	-0.01
Age			
Mean years (SD)	28.0 (11.4)	28.9 (12.7)	-0.08
Median (years)	24	24	NA
Min, max (years)	18, 87	18, 72	NA
Age Group			
< 65 years	98.5% (196)	96.9% (63)	0.10
≥ 65 years	1.5% (3)	3.1% (2)	-0.10
Race			
White	87.9% (175)	86.2% (56)	0.05
Black or African American	5.0% (10)	7.7% (5)	-0.11
Asian	4.5% (9)	1.5% (1)	0.17
American Indian or Alaska Native	2.0% (4)	1.5% (1)	0.04

Demographic Characteristic	Rifamycin Arm (N=199) % (n)	Placebo Arm (N=65) % (n)	Standardized Difference[1]
Native Hawaiian or Other Pacific Islander	0%	0%	0
Other	0.5% (1)	3.1% (2)	-0.19
Ethnicity			
Hispanic or Latino	12.6% (25)	9.2% (6)	0.11
Not Hispanic or Latino	87.4% (174)	90.8% (59)	-0.11
Country Visited			
Mexico	65.8% (131)	67.7% (44)	-0.04
Guatemala	34.2% (68)	32.3% (21)	0.04

Source: Statistical Reviewer.

Notes. The ITT sample includes 264 participants, with 199 in the rifamycin arm and 65 in the placebo arm.

[1] The standardized difference is the difference in the means of the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled variance term. It gives the effect size difference between the two arms.

The largest standardized differences between the arms involve race; specifically, regarding Asian (0.17) and Other (-0.19). Neither is substantively important, given these racial subgroups comprise a very small proportion of the sample.

Other Baseline Characteristics: Microbiological Status

The following table compares the rifamycin and placebo arms on microbiological status at baseline. It is based on the 252 participants in the ITT sample with completely observed baseline microbiological data; i.e., complete baseline data on the 16 pathogens examined in stool sample analysis. As noted in reviewer comments above, if the staff performing the baseline analysis were (as intended) blinded to treatment assignment, then this subsample of 252 participants respects the treatment randomization.

Table 18: C2009-0201 - Comparing Rifamycin and Placebo Arms on Baseline Microbiological Characteristics in the ITT Subsample with Complete Baseline Data for 16 Pathogens

Microbiological Characteristic	Rifamycin Arm (N= 191) % (n)	Placebo Arm (N=61) % (n)	Standardized Difference[1]
Infected with at least one pathogen	68.1% (130)	73.8% (45)	-0.13
Infected with no pathogens	31.9% (61)	26.2% (16)	0.13
Infected with *E. coli* only	57.6% (110)	57.4% (35)	< 0.01

Source: Statistical Reviewer.

Notes. The ITT sample includes 264 participants, with 199 in the rifamycin arm and 65 in the placebo arm. However, the table is based on the 252 ITT participants (191 rifamycin, 61 placebo) with completely observed values for 16 pathogen-specific baseline variables. These variables indicate whether or not there was a positive culture for the specific pathogen.

[1] The standardized difference is the difference in the means of the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled variance term. It gives the effect size difference between the two arms.

Table 18 indicates that the rifamycin arm had a somewhat smaller proportion of participants infected with at least one pathogen at baseline. No participant in either arm experienced fever at baseline or had blood in a final pre-study-drug-administration stool sample.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Compliance with study treatment regimen was assessed by the clinical investigator at Visits 2 and 3 through interview with the subject, review of the subject diary and counting the tablets remaining. Compliance was assessed as taking at least two tablets for the first four doses, and between 10 and 14 tablets total. The emphasis was on the first four doses as the Applicant asserts this had the greatest impact on efficacy.

Clinical Reviewer Comments: *See comments above under "Protocol Violations/Deviations" for treatment compliance observations regarding extra doses.*

Table 19: C2009-0201 - Study Drug Exposure and Treatment Compliance (ITT)

	Rifamycin N=199 n (%)	Placebo N=65 n (%)
Duration of Exposure (h)		
Mean (SD)	56.71 (10.90)	56.02 (12.61)
Median (Min-Max)	57 (11.3-107.0)	58.17 (0-83.0)
Overall Compliance (n (%))	176 (88.4)	58 (89.2)
2 tablets for each of the first 4 doses	197 (99.0)	63 (96.9)
10-14 tablets total	177 (88.9)	58 (89.2)

Source: C2009-0201 CSR 5.3.5.1, Table 9
Note: Duration of exposure=time of first dose to time of last dose of study drug
Subjects who received only one dose of a study drug were considered to have duration = 0 hrs

Clinical Reviewer Comments: *Overall compliance with study drug medication for the first 4 doses was 98.5%. The possible effects of missing data, concomitant medication use and extra doses is evaluated below in Sensitivity Analyses Versions A, B, C and D.*

Efficacy Results – Primary Endpoint

Table 20 below presents the results of analyses of five versions of TLUS, namely, the Applicant's version and four versions that alter the Applicant's version in different ways. The analyses of these four additional versions are considered sensitivity analyses.

TLUS version A corrects the Applicant's assignment of TLUS values for the 4 participants who did not complete their diaries and had censored TLUS values less than 120 hours (this is discussed in statistical reviewer comments above). *TLUS version B* again alters the values of these 4 participants. The 2 participants in the rifamycin arm are assigned TLUS values of 120, while the 2 participants in the placebo arm retain their version A values but now they are treated as observed rather than censored values. This is a "worst case scenario" for handling these 4 participants' missing diary data, as it makes it hardest for the rifamycin arm to demonstrate superiority over the placebo arm. This can be thought of as an extreme version of informative censoring. *TLUS version C* alters the Applicant's TLUS by assigning censored TLUS

values of 120 to any participants who used prohibited medication or extra study medication prior to the achievement of clinical cure (as defined by the Applicant). 12 participants' TLUS values were altered here. Finally, *TLUS version D* combines the "worst case scenario" of version B with the accounting for prohibited medication or extra study medication of version C.

Table 20: C2009-0201 Rifamycin vs. Placebo TLUS for the ITT Sample

TLUS Endpoint	Rifamycin Median	Placebo Median	Difference in Medians	p-value
Applicant's Version	46.0 (42.8, 50.5)	68.0 (48.7, NA)	-22.0	0.0008
Sensitivity Version A	46.0 (41.9, 50.5)	67.6 (48.7, NA)	-21.6	0.0009
Sensitivity Version B	46.0 (42.8, 50.5)	65.7 (48.7, NA)	-19.7	0.0027
Sensitivity Version C	47.0 (44.2, 56.0)	68.2 (48.7, NA)	-21.2	0.0062
Sensitivity Version D	47.0 (44.2, 56.0)	67.6 (48.7, NA)	-20.6	0.0145

Source: Statistical Reviewer.

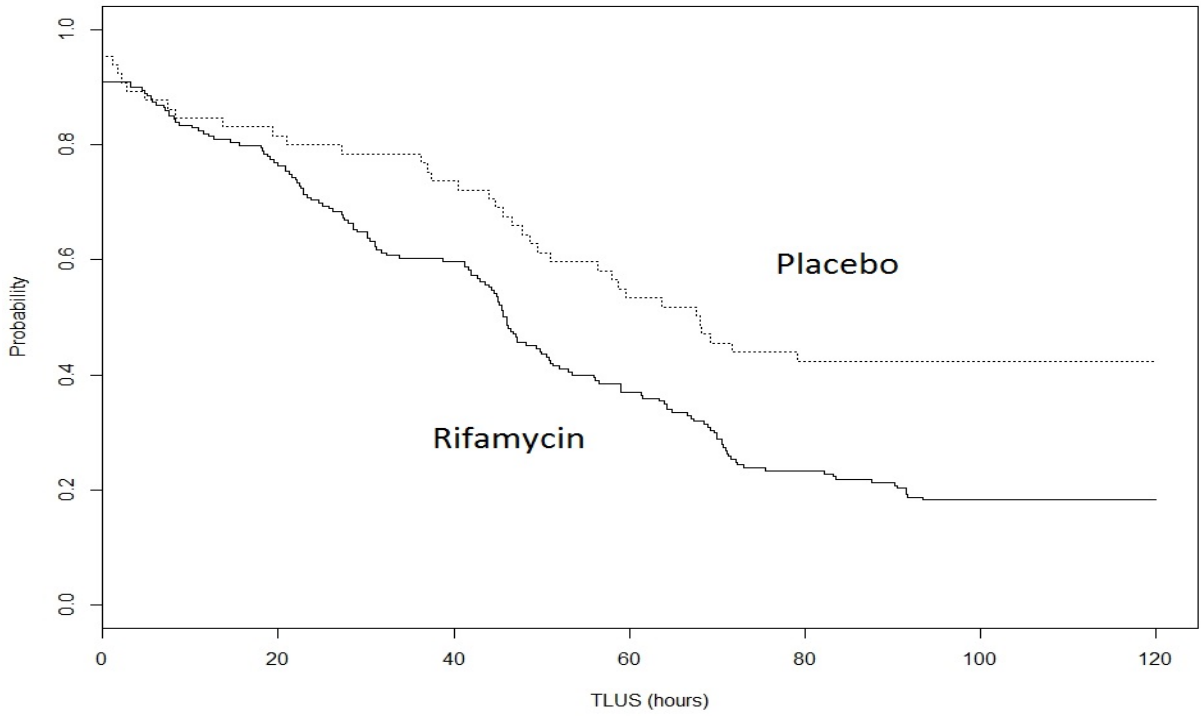
Notes. Median values are in hours. P-values are obtained from log-rank tests and estimated medians from Kaplan-Meier curves. The log-rank tests test the null hypothesis that the rifamycin and placebo TLUS survival curves are identical over the period during which both arms had at least one participant with an uncensored TLUS value. Full 95% confidence intervals cannot be obtained for the placebo median TLUS and for the difference in medians due to the amount of censoring in the placebo arm, but are reported for the rifamycin arm.

All analyses yield p-values < 0.02, with an estimated difference in medians of at least -19.7 hours.

Using a Cox regression model with arm as the sole predictor, a rifamycin vs. placebo hazard ratio estimate of 1.825 was obtained, with a 95% confidence interval of (1.276, 2.611).

The Kaplan-Meier curves for the rifamycin and placebo arms are given in the figure below. The Applicant's version of TLUS was used to generate the curves.

Figure 2: C2009-0201 - Kaplan-Meier Curves for TLUS (ITT)



Source: Statistical Reviewer.

Efficacy Results – Secondary and other relevant endpoints

Clinical Cure and Treatment Failure

The table below presents the results of analyses of clinical cure. Though the Applicant designated treatment failure rather than clinical cure as a secondary endpoint, hypothesis testing for one yields the same results as hypothesis testing for the other, as discussed in reviewer comments above. Because of this, plus our belief that results for treatment success rather than failure are more readily understood, and the fact that the Applicant's proposed label reports clinical cure results, we present results for clinical cure rather than for treatment failure.

Three versions of clinical cure were examined. The first is the Applicant's version. The second, version A, is an alteration of the Applicant's version that accounts for the use of prohibited medications and extra study medication doses, as discussed above. The third, version B, in addition to accounting for prohibited and extra medication, applies a "worst-case" scenario to the four participants with missing clinical cure data (these are the four participants with censored TLUS values less than 120 hours; see the sixth statistical reviewer comment above). "Worst-case" here means that participants with missing data in the placebo arm were

considered cured, while participants with missing data in the rifamycin arm were considered treatment failures. The analyses of the second and third versions (A and B) are considered sensitivity analyses.

Table 21: C2009-0201 - Results for Clinical Cure for the ITT Sample

Version of Clinical Cure	Proportion of Clinical Cure in Rifamycin Arm	Proportion of Clinical Cure in Placebo Arm	Difference in Proportions
Applicant's Version	81.4% (76.0, 86.8)	56.9% (44.9, 60.0)	24.5% (11.3, 37.7) $p<.0002$
Sensitivity Version A	76.4% (70.5, 82.3)	55.4% (43.3, 67.5)	21.0% (7.5, 34.4) $p=.002$
Sensitivity Version B	76.4% (70.5, 82.3)	58.5% (46.5, 70.4)	17.9% (4.6, 31.3) $p=.008$

Source: Statistical Reviewer.

Notes. Results based on 65 participants in placebo arm and 199 participants in rifamycin arm (264 participants total). Sensitivity version of clinical cure accounts for use of prohibited medications or extra study medication prior to achievement of clinical cure (as defined by Applicant). Each table cell reports a point estimate and a 95% confidence interval based on the normal approximation to the binomial distribution. P-values are obtained from chi square tests.

All three analyses yielded p-values less than 0.009, with the estimated difference in proportions of at least 17.9%.

Microbiological Cure

The following table presents the proportion of missing data for four pairs of binary microbiological variables. The first pair consists of the baseline variable *Any Pathogen at Baseline* and the endpoint *Any Pathogen at Visit 3*. The variable *Any Pathogen at Baseline* indicates whether or not a participant had a positive culture for at least one of the 16 pathogens examined in baseline stool analyses. Specifically, *Any Pathogen at Baseline* is assigned

- A missing value if there was at least one missing culture result from the 16 pathogens.
- "Positive" if all 16 pathogen-specific culture results were observed and at least one was a positive result.
- "Negative" if all 16 pathogen-specific culture results were observed and all were negative.

The variable *Any Pathogen at Visit 3* indicates whether or not a participant has a positive culture at Visit 3 for any pathogen that had a positive culture at baseline (per the protocol, at the Visit 3 stool analysis only pathogens with positive cultures at baseline were to be examined). *Any Pathogen at Visit 3* is set to not applicable if the participant's *Any Pathogen at Baseline* value equals negative and is set to missing if Visit 3 culture results are missing for any pathogens with positive baseline cultures. For example, suppose a participant has a positive baseline culture for *Aeromonas* and the remaining 15 baseline cultures are negative. Then *Any Pathogen at Baseline* is set to positive. If, at Visit 3, the culture result for *Aeromonas* is missing,

then *Any Pathogen at Visit 3* is set to missing; if the culture result is positive, then *Any Pathogen at Visit 3* is set to positive; and if the culture result is negative, then *Any Pathogen at Visit 3* is set to negative. The last case represents microbiological cure, as the participant was infected with *Aeromonas* at baseline but was no longer infected at Visit 3; the next-to-last-case represents microbiological treatment failure, as the participant was infected with *Aeromonas* at baseline and was still infected at Visit 3. Note that microbiological cure cannot be ascertained if *Any Pathogen at Baseline* and/or *Any Pathogen at Visit 3* have missing values. The total missing rate represents cases where *Any Pathogen at Baseline* and/or *Any Pathogen at Visit 3* have missing values.

The baseline-Visit-3 variable pair for *Any E. coli Detected* is defined with respect to a group of five *E. coli* bacteria (*Enteroaggregative E. coli, Diffusely adherent E. coli, ETEC Heat labile toxin, ETEC Heat stabile/Heat labile toxin, ETEC Heat stabile toxin*) rather than with regard to all 16 pathogens. The variable pair for *Any Invasive Bacteria Detected* is defined with respect to a group of seven invasive bacteria (*Aeromonas, Campylobacter jejuni, Shigella group, Salmonella group, Plesiomonas group, Vibrio group, Yersinia enterocolitica*). The variable pair for *Any Other Pathogens Detected* is defined with respect to a group of four pathogens (*Giardia lamblia, E. histolytica, Cryptosporidium parvum, Norovirus*). The union of the *E. coli*, invasive, and other pathogen groups gives the 16 pathogens with regard to which the *Any Pathogens* variables are defined. Missing data at baseline, at Visit 3, and in total for the E. coli, invasive, and other variable pairs are defined analogously to missingness for the *Any Pathogen Detected* variable pair, but with respect to the particular pathogen group they are defined in terms of.

Table 22: C2009-0201 - Percent Missing Data in Microbiological Variables for the ITT Sample

	Rifamycin % Missing Data			Placebo % Missing Data		
Microbiological Variable Pair	**Baseline**	**Visit 3**	**Total**	**Baseline**	**Visit 3**	**Total**
Any Pathogen	4.0%	5.0%	8.5%	6.2%	7.7%	13.8%
Any *E. coli*	2.0%	5.0%	7.0%	0%	7.7%	7.7%
Any Invasive Bacteria	1.0%	0%	1.0%	0%	0%	0%
Any Other Pathogens	2.5%	0%	2.5%	6.2%	0%	6.2%

Source: Statistical Reviewer.

Notes. Results based on 65 participants in placebo arm and 199 participants in rifamycin arm (264 participants total). Baseline data are considered missing if the baseline culture results for any pathogen contributing to the endpoint are missing. Visit 3 data are considered missing if Visit 3 culture results are missing for any pathogens contributing to the endpoint that had a positive culture at baseline. Total missing rates reflect missing data at baseline or Visit 3.

We see that, in general but not universally, the rifamycin arm had lower missing data rates than the placebo arm.

The following table compares the rifamycin and placebo arms on the four Visit 3 microbiological endpoints described above. A participant was included in the analysis of a given Visit 3 endpoint

if (i) he/she had non-missing endpoint values at baseline and at Visit 3, and (ii) he/she had a positive culture at baseline for at least one of the pathogens associated with the endpoint. Thus, the analyses are complete-case analyses, in that only participants with observed data at baseline and Visit 3 are included. These analyses are statistically valid under the assumption that missingness at baseline and Visit 3 is unrelated to microbiological status at either timepoint and unrelated to any other participant characteristic or outcome.[15]

For *Any Pathogen Detected*, 238 participants satisfied (i), and 161 of them (67.6%) also satisfied (ii). For *Any E. Coli Detected*, 245 participants satisfied (i), and 151 of them (61.6%) also satisfied (ii). For *Any Invasive Bacteria Detected*, the analogous numbers are 262 and 15 (5.7%), respectively. For *Any Other Pathogens*, the analogous numbers are 255 and 18 (7.1%), respectively.

Table 23: C2009-0201 - Results for Microbiological Cure by Visit 3 for the ITT Sample

Microbiological Endpoint	Number of Participants Infected at Baseline	Proportion of Eradication by Visit 3 In Rifamycin Arm	Proportion of Eradication by Visit 3 in Placebo Arm	Difference in Proportions
Any Pathogen	161 (121R/40P)	54.5% (45.7, 63.4)	47.5% (32.0, 63.0)	7.0% (-10.8, 24.9) p=0.554
Any *E. coli*	151 (115R/36P)	56.5% (47.5, 65.6)	47.2% (30.9, 63.5)	9.3% (-9.4, 28.0) p=0.432
Any Invasive Bacteria	15 (10R/5P)	90.0%	60.0%	30.0% p=0.242
Any Other Pathogens	18 12R/6P	41.7%	16.7%	25% p=0.6

Source: Statistical Reviewer.

Notes. In the "any pathogen" and "any *E. coli*" rows, cells in the last 3 columns present point estimates and 95% confidence intervals based on the normal approximation to the binomial distribution, with p-values obtained from chi square tests. In the "any invasive" and "any other" rows, due to small sample sizes, p-values are obtained from Fisher's exact tests and confidence intervals are not provided.

None of the analyses yield statistically significant results, with p-values greater than 0.24. Note that all of these analyses had limited statistical power, given that they included considerably fewer than the 264 participants in the full ITT sample.

The Applicant did not analyze any of these microbiological endpoints.[16] It instead compared the two arms on the endpoints *Only One Pathogen Detected at Baseline* and *Multiple Pathogens Detected at Baseline*, using the "Worst Case Imputation" missing data method described above (see CSR, Table 15). Neither analysis yielded statistically significant results, with p-values of 0.88 and 0.12, respectively. The Applicant also compared the two arms on *Only One Pathogen Detected at Baseline – An E. coli bacterium*, and this yielded the nonsignificant p-value 0.95. In addition, the analyses reported in the preceding table were repeated using the Applicant's

[15] Technical note: This is the assumption of missingness completely at random (MCAR). As discussed above, this likely holds for missingness at baseline, but may not hold at Visit 3.

[16] The endpoints represented in the table above were chosen after consultation with the clinical and microbiological reviewers.

"Worst Case imputation" approach and yielded similar non-statistically significant results (minimum p-value was .20).

Findings in Special/Subgroup Populations or Additional Analyses Conducted on the Individual Trial

Table 24 below presents the rifamycin and placebo TLUS medians and the difference in medians within demographic subgroups.

Table 24: C2009-0201 - Median TLUS and Clinical Cure within Subgroups of ITT Sample

		Median TLUS				Clinical Cure Rates			
Variable	**Subgroup**	**Rifamycin**	**Placebo**	**Diff**	**p**	**Rifamycin**	**Placebo**	**Diff**	**p**
Gender	Female (N=133: 100R/33P)	46.0	68.0	-22.0	0.025	80.0%	57.6%	22.4%	0.020
	Male (N=131: 99R/32P)	46.0	64.0	-18.0	0.014	82.8%	56.2%	26.6%	0.004
Race	White (N=231: 175R/56P)	46.0	68.3	-22.3	0.002	81.1%	55.4%	25.8%	<0.001
	Non-white[b] (N=33: 24R/9P)	45.4	56.4	-11.0	NA	83.3%	66.7%	16.7%	0.358
Ethnicity	Hispanic/ Latino[b] (N=31: 25R/6P)	47.3	74.2	-26.9	NA	80.0%	66.7%	13.3%	0.596
	Non-Hispanic/ Latino (N=233: 174R/59P)	45.5	67.6	-22.1	0.002	81.6%	55.9%	25.7%	<0.001
Country Visited	Guatemala[a] (N=89: 68R/21P)	54.8	NA	NA	0.002	83.8%	47.6%	36.2%	0.003
	Mexico (N=175: 131R/44P)	42.9	48.7	-5.8	0.039	80.2%	61.4%	18.8%	0.016

Source: Statistical Reviewer.

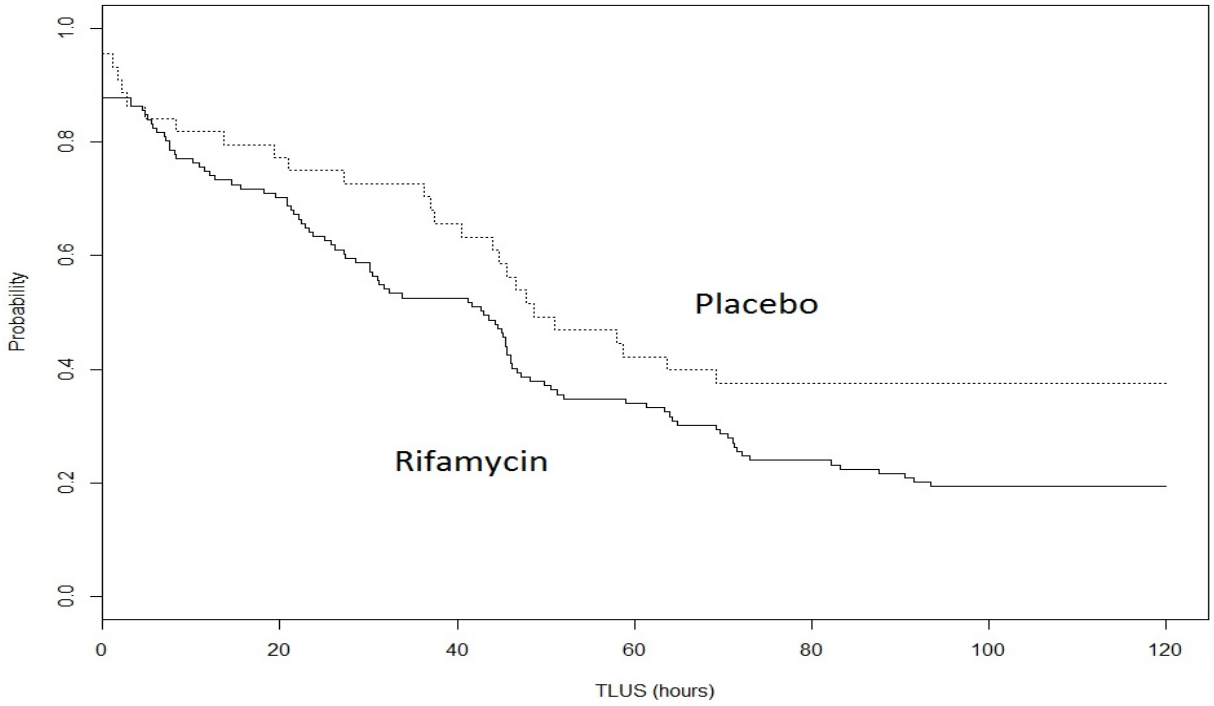
Notes. N = 264 (199 rifamycin, 65 placebo). Median TLUS and Difference in Medians are in hours. Race was dichotomized due to the small proportion of non-white participants. TLUS p values are based on log-rank tests, and median estimates are based on Kaplan-Meier curves. Clinical cure p values are based on Fisher's exact test.

[a] Due to the nature of the TLUS distribution for participants from Guatemala, it was not possible to estimate the placebo median nor the difference in medians.

[b] Due to the small sample size, the log-rank test could not validly be performed.

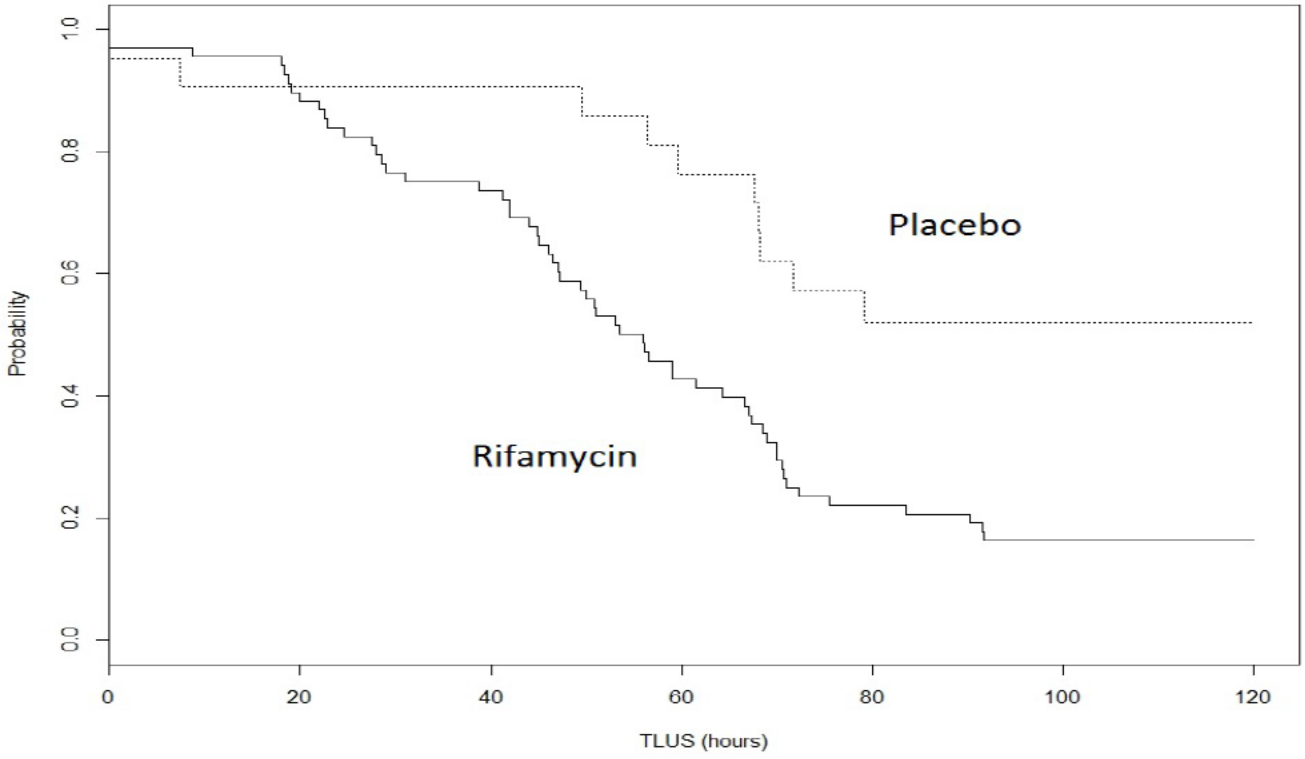
The most dramatic subgroup differences are for Country Visited. The following figures present the Kaplan-Meier curves for Mexico and for Guatemala.

Figure 3: C2009-0201 - Kaplan-Meier for Travelers to Mexico (ITT Sample)



Source: Statistical Reviewer.

Figure 4: C2009-0201 - Kaplan-Meier Curves for Travelers to Guatemala (ITT Sample)



Source: Statistical Reviewer.

In Guatemala, over half of the participants in the placebo arm did not have an observed TLUS value. Hence, the placebo median cannot be estimated. Further comparisons between Mexico and Guatemala are given later.

Table 25 below presents TLUS and clinical cure results for subgroups defined in terms of baseline microbiological status.[17]

Table 25: C2009-0201: Analysis of TLUS and Clinical Cure by Microbiological Subgroups in the ITT Sample

	Median TLUS				Clinical Cure Rates			
Subgroup	**Rifamycin**	**Placebo**	**Diff**	**p**	**Rifamycin**	**Placebo**	**Diff**	**p**
Only *E. coli* at baseline (N=145: 110R/35P)	48.8	68.3	-19.5	0.006	80.9%	54.3%	26.6%	0.003
E. coli plus another pathogen at baseline (N=17: 13R/4P)	22.9	82.4	-59.5	NA	69.2%	50.0%	19.2%	0.584
No *E. coli* but another pathogen at baseline	22.6	94.6	-72.0	NA	100.0%	50.0%	50.0%	0.070

[17] The subgroups represented in the table above were chosen after consultation with the clinical and microbiological reviewers.

	Median TLUS				Clinical Cure Rates			
Subgroup	Rifamycin	Placebo	Diff	p	Rifamycin	Placebo	Diff	p
(N=13: 7R/6P)								
No pathogens at baseline (N=77: 61R/16P)	45.0	33.9	11.1	0.906	82.0%	75.0%	7.0%	0.500
Invasive pathogens at baseline (N=14: 10R/4P)	18.5	57.0	-38.4	NA	70.0%	75.0%	-5.0%	1.00

Source: Statistical Reviewer.

Notes. Table based on the 252 ITT participants who had complete baseline microbiological data. 12 ITT participants (4.5%) had incomplete baseline microbiological data and were excluded. TLUS p-values are based on log-rank tests, and median estimates are based on Kaplan-Meier curves. NA indicates that the subsample was too small to validly perform a log-rank test. Clinical cure p-values are based on Fisher's exact test. *Only E. coli* includes the 5 pathogens *enteroaggregative E. coli, diffusely adherent E. coli, ETEC heat labile toxin, ETEC heat stabile/heat labile toxin, ETEC heat stabile toxin*. *Another pathogen* includes the remaining 11 pathogens that were examined. *Invasive pathogens* include the 7 pathogens *Aeromonas, Campylobacter jejuni, Shigella group, Salmonella group, Plesiomonas group, Vibrio group, Yersinia enterocolitica*; members of this subgroup may additionally be infected with some of the other 9 pathogens examined. The first four subgroups are non-overlapping and partition the entire sample of 252 participants.

With regard to TLUS and clinical cure, rifamycin was significantly superior to placebo for participants who were only infected with *E. coli* at baseline but was not significantly superior to placebo for participants who were not positive for any pathogens at baseline. Log-rank tests, whose validity relies on samples of a reasonable size, were not performed with the small subsamples of participants with *E. coli* plus another pathogen at baseline or who had no *E. coli* at baseline but were infected with another pathogen. For both small subsamples, superiority of rifamycin to placebo was not demonstrated with regard to clinical cure. Note that statistical power is limited for all subgroup analyses, given modest sample sizes, and no multiplicity correction is made.

Returning to the comparison of participants from Mexico and Guatemala, Table 26 examines differences in baseline microbiological subgroups.

Table 26: C2009-0201 - Comparing Mexican and Guatemalan Participants by Baseline Microbiological Status (ITT subsample with complete baseline data on 16 pathogens)

Demographic Characteristic	Mexico (N=168) % (n)	Guatemala (N=84) % (n)	Standardized Difference[1]
Only *E. coli* at baseline	57.7% (97)	57.1% (48)	0.01
E. coli plus another pathogen at baseline	6.5% (11)	7.1% (6)	-0.02
No *E. coli* but another pathogen at baseline	4.8% (8)	6.0% (5)	-0.05
No pathogens at baseline	31.0% (52)	29.8% (25)	0.03

Source: Statistical Reviewer.

Notes. Table based on the 252 ITT participants who had complete baseline microbiological data. 12 ITT participants (4.5%) had incomplete baseline microbiological data and were excluded.

[1] The standardized difference is the difference in proportions divided by the square root of a pooled variance term. It gives the effect size difference between the two countries.

All of the standardized effect sizes are negligible. It appears unlikely that baseline microbiological status accounts for Mexico vs. Guatemala differences in TLUS values.

Statistical Reviewer's Comments:

We summarize the results of efficacy analyses of trial C2009-0201:

- *With regard to the primary efficacy endpoint, TLUS, the rifamycin arm showed statistically significantly better outcomes than the placebo arm. This held for the Applicant's version of TLUS (p = .0008) as well as for the multiple sensitivity versions of TLUS defined by the statistical reviewer (all p values less than .015). Collectively, these results constitute strong evidence of the superiority of rifamycin to placebo with respect to the primary efficacy endpoint.*
- *With regard to the secondary efficacy endpoints, the Applicant did not specify a method for multiplicity adjustment, so one approach to examining the secondary endpoints is to treat all of them as exploratory endpoints. However, after consultation with the clinical reviewer, the statistical reviewer believes that the two most important secondary endpoints are clinical cure and microbiological cure with regard to E. coli. If the conservative Bonferroni multiplicity adjustment is used, then the alpha for testing each of these two endpoints is .025 = .05/2. Per the Bonferroni adjustment, the rifamycin arm showed statistically significantly better clinical cure outcomes than the placebo arm; this held for the Applicant's version of clinical cure as well as for the several sensitivity versions defined by the statistical reviewer. On the other hand, the microbiological cure endpoint did not exhibit significant results, whether or not using the Bonferroni adjustment. Additionally, none of the other microbiological cure endpoints exhibited significant differences between arms.*
- *Regarding the exploratory subgroup analyses:*
    - *All analyses of TLUS and clinical cure for demographic subgroups of size at least 89 yielded p values less than .04 in support of the superiority of rifamycin to placebo.*
    - *In addition, while rifamycin appeared effective in both countries visited, there seem to be systematic differences in the TLUS outcomes of participants visiting Mexico vs. participants visiting Guatemala. Specifically, fewer than half of the placebo participants in Guatemala had observed TLUS values, while more than half of the placebo participants in Mexico had observed TLUS values. It does not appear that these inter-country differences are due to differences in the nature of the pathogens detected at baseline.*
    - *TLUS and clinical cure analyses for the subgroup of individuals only infected with E. coli at baseline yielded statistically significant results (p ≤ .006) in support of the superiority of rifamycin to placebo. Corresponding analyses for other subgroups defined in terms of baseline microbiological status yielded non-*

significant results.

7.2.2 Study RIT-1/AID

7.2.2.1 Study Design and Endpoints

This study was conducted by a different sponsor, Dr. Falk Pharma GmbH, and not performed under U.S. IND. FDA was not consulted on the study design, including choice of ciprofloxacin as control. Dr. Falk Pharma remained the sponsor through the active phase of the trial, with sponsorship transferred to Cosmo following completion of the study.

Study RIT-1/AID was a randomized, double-blind, multicenter non-inferiority trial comparing rifamycin (200 mg po BID for 3 days) versus ciprofloxacin (500 mg po BID for 3 days). The study was conducted in 19 total sites, 17 in India and 1 site each in Ecuador and Guatemala. A 1:1 randomization ratio was used. The trial employed an adaptive group sequential design, with two interim analyses, each with the possibility of early stopping as well as the possibility of increasing the planned sample size (more on this below). The originally planned sample size was 776, but, in fact, a total of 843 subjects were enrolled. Of these, 835 were randomized and received at least one dose, 420 received rifamycin and 415 received ciprofloxacin.

Clinical cure was defined as in Study C2009-0201, except that the 24-hour or 48-hour qualifying periods must occur prior to Visit 4 (scheduled for day 6), rather than within 120 hours of the first study medication dose. TLUS, the primary endpoint, was defined as in C2009-0201, but with respect to the Visit-4 version of clinical cure. Microbiological status variables were defined with respect to a 14-pathogen subset of the 16 pathogens examined in C2009-0201. In addition to treatment failure and microbiological cure, several other secondary endpoints were specified.

Statistical Reviewer Comment:

It is unfortunate that the original sponsor chose to utilize a 2-arm non-inferiority design. Such designs rely on the use of historical information, which necessarily means that their results need to be interpreted with caution. If the original sponsor believed it was clinically important to demonstrate that rifamycin was non-inferior to ciprofloxacin, rather than merely showing it superior to placebo, then the trial should have included a placebo arm as well, to avoid the need for reliance on historical information to conclude efficacy of rifamycin. Given the results of Study C2009-0201, the placebo arm would not have needed to be large.

Choice of control

The sponsor references EMA guidelines for the evaluation of medicinal products for treatment of bacterial infections in its rationale for the trial design. (CPMP 2011, CPMP 2013) The EMA guidelines outline a preference for demonstrating non-inferiority of an antibacterial under evaluation with respect to an active comparative treatment.

Key Inclusion and Exclusion Criteria

The key inclusion and exclusion criteria were very similar to those of Study C2009-0201 with some additional exclusion criteria, including the following:

- Fever, defined as an oral body temperature of >100.4^{o}F or 38.0^{o} C; *no antipyretics in the 6 hours prior to this assessment*.
- History of inflammatory bowel disease or celiac disease.
- Treatment with tizanidine
- History of tendon disease or disorder related to quinolone treatment
- According to protocol amendment #2, the subjects from the US, Canada, and Australia were excluded due to "insurance reasons", although this was not specified in the Sponsor's Integrated Clinical Summary Report

Statistical Analysis Plan

As noted above, the trial was conducted by the original Sponsor using an adaptive 3-stage group sequential test design. The first interim analysis was planned for when 50% of the original planned sample of 776 had completed participation and the second was planned for when 75% of the original planned sample had completed participation. At each of the interim analyses, it was possible for the trial to be stopped if, using the data collected to date, an appropriate test rejected the 1-sided null hypothesis that rifamycin-to-ciprofloxacin hazard ratio is less than or equal to .764 (the rationale for this is provided below). The critical values used for these interim analyses tests were obtained via an O'Brien-Fleming α-spending method, and equaled 2.96 for the first interim analysis and 2.36 for the second interim analysis (if the trial wasn't stopped at the first analysis). If the trial was not stopped at either interim analysis, then the critical value of 2.01 was used for tests that utilized all data. In addition, at each interim analysis, the sample size could be increased from the original planned size.

Analysis Populations

The *full analysis set* includes all randomized participants who received at least one dose of study medication.

The *per protocol set* includes all members of the full analysis set who additionally

- Satisfied all major inclusion criteria and did not satisfy any major exclusion criteria that had an impact on the primary endpoint.
- Had adequate medication compliance, avoided prohibited medications, and completed the diary for at least 2 days starting at baseline.

The *safety set* included all participants (as treated) who received at least one dose of study medication.

Analysis of Primary Efficacy Endpoint

We first discuss the non-inferiority margin and then how non-inferiority was to be tested.

Non-inferiority margin: The Applicant sought to establish a non-inferiority margin with respect to the hazard ratio; that is, with respect to the rifamycin hazard divided by the ciprofloxacin hazard. As the reviewer has best been able to understand the original Sponsor and the Applicant's documentation, a hazard ratio margin was constructed by first specifying a margin with respect to median TLUS and then, by making certain assumptions, converting this median-based margin to a hazard ratio margin.[18]

Based on a qualitative risk-benefit analysis, an M2 margin of 8.5 hours was established with regard to median TLUS (March 18, 2015 protocol, section 9.4.1).[19] That is, rifamycin would be considered non-inferior to ciprofloxacin if it could be demonstrated that the rifamycin median TLUS was less than 8.5 hours greater than the ciprofloxacin median TLUS. However, this TLUS margin does not directly determine a margin for the hazard ratio. For example, if the rifamycin and ciprofloxacin TLUS values have exponential distributions with respective medians of, say, 28.5 and 20 hours, then the associated hazard ratio is .702, whereas if the respective medians are, instead, 38.5 and 30 hours, then the associated hazard ratio is .779.[20] That is, both examples share a difference in medians of 8.5 hours yet have different hazard ratios. The Applicant assumed that both rifamycin and ciprofloxacin TLUS values are exponentially distributed and based on results from Taylor et al. (2006) and Dupont et al. (2001) assumed that the ciprofloxacin TLUS values have a median of 27.5 hours.[21] The associated hazard ratio when rifamycin has median 36 = 27.5 + 8.5 hours is .764. Therefore, per the Applicant,

[18] Technical note: in order for the hazard ratio to be meaningfully defined in this context, the proportional hazards assumption must hold.

[19] The term *M2 margin* was not used by the original Sponsor or Applicant, and is defined in the November 2016 FDA guidance *Non-Inferiority Clinical Trials to Establish Effectiveness* (https://www.fda.gov/downloads/Drugs/Guidances/UCM202140.pdf). It reflects the largest loss compared to Ciprofloxacin by Rifamycin that would be clinically acceptable in order to be considered non-inferior to Ciprofloxacin.

[20] Technical note: These results follow from the fact that, for the exponential distribution, the hazard is equal to the natural log of 2 divided by the distribution's median.

[21] Taylor et al. (2006) analyzed a randomized three-arm trial comparing Rifaxamin, ciprofloxacin, and placebo for the treatment of traveler's diarrhea. In this trial, the ciprofloxacin median TLUS for the ITT sample was 28.8 hours, and for the ITT subsample that excluded participants with fever and/or bloody stools at baseline, the median TLUS was 27.4 hours. The analogous median TLUS values for the placebo arm were 65.5 hours and 48.1 hours, respectively. Dupont et al. (2001) analyzed a randomized two-arm trial comparing Rifaxamin and ciprofloxacin, and the ciprofloxacin median for the ITT sample (which included participants with fever and/or bloody stools at baseline) was 25.0 hours.

rifamycin is non-inferior to ciprofloxacin if it can be demonstrated that the rifamycin-to-ciprofloxacin hazard ratio is greater than .764.

Testing for non-inferiority: The original Sponsor proposed a testing procedure that respected the adaptive group sequential design of the trial (May 12, 2016 SAP). Given that the trial was not stopped at either interim analysis (discussed below), the full sample can be partitioned into three independent subsamples: the subsample of participants examined at the first interim analysis, the subsample of participants examined at the second but not the first interim analysis, and the subsample of participants who were not examined at either interim analysis. The test of the null hypothesis that the rifamycin-to-ciprofloxacin hazard ratio was less than or equal to .764 (i.e., the null hypothesis that rifamycin is inferior to ciprofloxacin) was to be conducted by performing separate log-rank tests for each of the three independent subsamples and then combining the three, resulting p-values via the inverse normal method. As already noted, the resulting test statistic value would then be compared to the critical value of 2.01 derived from an alpha-spending function of the O'Brien and Fleming type. A corresponding 2-sided 95% repeated confidence interval for the hazard ratio would also be constructed.[22]

The Applicant, however, did not propose a testing procedure consonant with the adaptive group sequential design, arguing that because it did not know the results of the two interim analyses, it was warranted in analyzing the data as if the final sample size had been fixed a priori and no interim analyses had been performed. It proposed to test the null hypothesis that the rifamycin-to-ciprofloxacin hazard ratio was less than or equal to .764 by constructing a 2-sided 95% confidence interval based on an (asymptotically normal) nonparametric estimate of the log hazard ratio (over the entire sample) and then examining whether the hazard ratio confidence interval lower bound was greater than .764 (April 26, 2016 SAP). Non-inferiority was to be tested on the full analysis set and the per protocol set, with the latter analysis considered confirmatory and the former analysis considered a sensitivity analysis.

The December 20, 2017 CSR additionally reported a TLUS analysis using the end-of-study O'Brien and Fleming alpha-spending function critical value of 2.01 rather than the usual critical value of 1.96. This was in response to a November 13, 2017 request from the FDA. This leads to computing a 95.6% confidence interval in place of a 95% confidence interval.

Analysis of Secondary Efficacy Endpoints

The secondary endpoints (e.g., clinical cure, microbiological cure) were to be tested via chi square tests of independence. In addition, 95% confidence intervals were to be constructed for the arm-specific proportions of cure and the difference in proportions, presumably based on the normal approximation of the binomial distribution. These analyses use the full analysis set.

[22] The inverse normal combination method and repeated confidence intervals are described in Wassmer & Brannath (2016). *Group Sequential and Confirmatory Adaptive Designs in Clinical Trials*. They are some of the specialized statistical methods used to analyze data from adaptive trials.

Multiplicity Adjustment

The Applicant did not specify a method for multiplicity adjustment.

Handling Missing Data

TLUS was censored at 120 hours in cases of treatment failure, if no diary was available, and if intolerable adverse events related to study medication were experienced (and all such participants were treated as treatment failures). If a participant terminated prematurely for reasons other than treatment failure, TLUS was censored at the last available information concerning stool consistency.

Regarding microbiological cure, participants lacking assessable stool microbiology at Visit 1 and/or Visit 3 are treated as not cured.

Regarding gastrointestinal symptoms, last value carried forward (LOCF) is used to impute missing values.

Statistical Reviewer Comments:

First, the Applicant's establishment of the non-inferiority margin for the hazard ratio is flawed. In order to determine a hazard ratio corresponding to a median TLUS M2 margin of 8.5 hours, the Applicant makes very strong assumptions about the true value of the ciprofloxacin median TLUS value and about the distribution of the rifamycin and ciprofloxacin TLUS values. It is highly implausible that a hazard ratio of .764 corresponds to a median M2 margin of 8.5 hours, given the true but unknown ciprofloxacin median TLUS and the true but a priori unknown distributions of the rifamycin and ciprofloxacin TLUS values. Perhaps more importantly, margins for hazard ratios are appropriately specified as the <u>proportion</u> of the true but unknown standard-treatment-to-placebo log hazard ratio that must be retained by the experimental treatment vs. placebo in order for it to be considered non-inferior to the standard treatment (ciprofloxacin is the standard treatment here). The Applicant's specification of the hazard ratio of .764 is not the specification of a margin.

Second, the Applicant's proposed TLUS analysis does not respect the adaptive group sequential design. The Applicant's argument that ignorance of interim analysis results warrants treating the sample as if a priori fixed in size is not correct, as the distribution of the test statistic the Applicant proposes depends on the results of the interim analyses, whether or not they were initially known by the Applicant (the Applicant now possesses the results of the two interim analyses). Further, the Applicant's TLUS analysis in response to the FDA request does not respect the adaptive nature of the trial design. To do so, the Applicant would need to use the inverse-normal method or an analogous method for testing and construct repeated confidence intervals or stagewise-ordered confidence intervals.

Third, neither the original Sponsor's testing methods nor the Applicant's testing methods conform to the FDA November 2016 non-inferiority guidance's recommendations on testing for non-inferiority. The guidance recommends two testing approaches: the synthesis method (AKA imputed or putative placebo approach) and the "95-95" method (AKA fixed margin approach).[23] In both approaches, the standard error of or confidence interval for the estimator of the ciprofloxacin vs. placebo treatment effect is used. Since the Applicant does not provide a standard error value or confidence interval for this estimator, it is not possible to apply either FDA-recommended approach.

Fourth, the original Sponsor and the Applicant overlooked an estimate of the ciprofloxacin vs. placebo hazard ratio, with associated 95% confidence interval, from Taylor et al. (2006), which was a 3-arm randomized trial comparing rifaximin, ciprofloxacin, and placebo. This hazard ratio confidence interval would allow implementation of the "95-95" method. When participants who had fever and/or bloody stools at baseline are excluded from the Taylor ITT sample (thus approximating the RIT-1/AID exclusion criteria), the estimated ciprofloxacin vs. placebo hazard ratio is 1.8, with 95% confidence interval of (1.2, 2.8).[24] This confidence interval is based on data from 125 participants (65 ciprofloxacin, 60 placebo), an admittedly modest sized sample.

Fifth, both the Applicant's hazard ratio target of .764 and the Taylor et al. (2006) TLUS results discussed in the preceding comment are based on clinical trials that defined TLUS somewhat differently from RIT-1/AID. In Taylor p. 1061, TLUS is defined as "the interval beginning with the first dose of study medication and ending with the last unformed stool passed... Both soft stools... and watery stools... were considered to be unformed... [D]ata from patients for whom TLUS could not be calculated because of premature withdrawal caused by treatment failure or completion of the study without becoming well [i.e., achieving clinical cure] were censored as having a TLUS of 120 hours. Data from patients who terminated early for reasons other than treatment failure were censored at the time of the last available information on unformed stools." For example, suppose a participant had a watery stool at 12 hours, a soft stool at 40 hours, no other unformed stools, no fever, no enteric symptoms, and the participant completed the study. Per RIT-1/AID, TLUS = 12 hours, while per Taylor's definition TLUS = 40.[25] The reviewer was unable to find any relevant alternative trials that defined TLUS as RIT-1/AID does, so Taylor and Dupont are likely the most relevant trials to use for setting non-inferiority margins and testing non-inferiority.

[23] Also see Fleming (2008). Current issues in non-inferiority trials. Statistics in Medicine, 27, 317-332 and Schumi & Wittes (2011). Through the looking glass: Understanding non-inferiority. Trials, 12:106.

[24] Per Taylor et al. (2006, p. 1061), "Cox proportional hazards models … were used to compare TLUS between … ciprofloxacin and placebo." Such models estimate log hazard ratios, from which estimates of the hazard ratio can be readily computed. Taylor et al. use the term "risk ratio" rather than "hazard ratio," but in the context of discussing TLUS results for comparing ciprofloxacin vs. placebo via Cox models, it is clear that "risk ratio" is used as a synonym for "hazard ratio." Further, Fleming (2008, p. 329) notes that "relative risk" (AKA "risk ratio") is sometimes used as a synonym for "hazard ratio."

[25] See Cosmo Technologies' July 20, 2018 Clinical and Statistical Information Request, pp. 7-8.

Sixth, the reviewer proposes the following alternative approach to primary endpoint testing in trial RIT-1/AID:

i. *Create a new TLUS variable for RIT-1/AID, call it TLUS_T, that defines TLUS in accordance with the Taylor definition. TLUS_T will be used for testing non-inferiority.*
ii. *Given that placebo-controlled trials are the expectation for this indication, it seems appropriate to test for non-inferiority of rifamycin to ciprofloxacin using the M1 non-inferiority margin. The M1 margin represents the treatment effect of placebo vs. ciprofloxacin. When the M1 margin is used, then the null hypothesis that rifamycin is inferior to ciprofloxacin can be shown to be equivalent to the hypothesis that rifamycin is not superior to/not efficacious vs. placebo. Similarly, the alternative hypothesis that rifamycin is non-inferior to ciprofloxacin can be shown to be equivalent to the hypothesis that rifamycin is superior to/efficacious vs. placebo.[26] In the context of this non-inferiority trial, hypothesis testing is conducted by statistically demonstrating that the rifamycin-vs-ciprofloxacin hazard ratio is larger than the placebo-vs-ciprofloxacin hazard ratio. Per the 95-95 testing method, this involves showing that the lower bound from a 95% confidence interval for the former is larger than the upper bound from a 95% confidence interval for the latter. This upper bound is based on the Taylor results reported in the fourth reviewer comment. As noted above, these results are obtained by excluding Taylor ITT participants with fever or bloody stools at baseline. From these results, the estimated placebo-vs-ciprofloxacin hazard ratio is .56 = 1/1.8, and the 95% confidence interval for this hazard ratio is (.36, .83) = (1/2.8, 1/1.2). The 95% confidence interval for the rifamycin-vs-ciprofloxacin hazard ratio will be computed as a 95% repeated confidence interval, as described in Wassmer and Brannath.[27]*

Seventh, the proposed analyses of the secondary efficacy endpoints do not respect the adaptive group sequential trial design. Because these endpoints are likely associated with the primary endpoint rather than being independent of it, the chi square test statistics used to test the secondary endpoints cannot be assumed to have the same (chi square) distribution under the null hypothesis that they would have if the sample size had been fixed a priori. Therefore, the reviewer proposes to analyze them via 95% repeated confidence intervals, as described in Wassmer and Brannath.[28]

Eighth, given the Applicant's ascription of microbiological failure for missing Visit 1 and/or Visit 3 microbiological data, the arm with less missing data can be privileged, as this method is overly "pessimistic" in filling in missing endpoint values. Regarding microbiological cure, the ciprofloxacin arm systematically had more missing data (as detailed below), and this could inflate estimates of the rifamycin vs. ciprofloxacin treatment effect. In addition to performing

[26] In the text that follows, the review uses "rifamycin is/isn't superior/efficacious (to/vs. placebo)" instead of "rifamycin is non-inferior/inferior to ciprofloxacin," as this language is more straightforward for readers who are not statisticians. The two phrasings are semantically equivalent.
[27] See Wassmer & Brannath (2016, p. 227, equation (9.7)).
[28] See Wassmer & Brannath (2016, pp. 204-205).

the microbiological cure analyses using the Applicant's "pessimistic" missing data method, the statistical reviewer performed the analogous analyses using a complete case approach.

Protocol Amendments

The original protocol dated 27 November 2009 was amended 5 times during the study for clarification and changes in analysis. Key revisions to the protocol amendments are outlined in Table 27 below.

Table 27: Protocol Amendment Summary for Study RIT-1/AID

Amendment (Final Date)	Major Changes
1 (18 February 2010)	• Justification of non-inferiority margin ▪ According to the Sponsor, the original non-inferiority margin of 10 hours was based on the "Note for Guidance on evaluation of medicinal products indicated for the treatment of bacterial infections" (source not further identified). The Sponsor stated that this non-inferiority margin was deemed too large by the German health authority because a high percentage of total patients enrolled is expected to recover by the end of the 120 hour observation period. The Sponsor chose a narrower non-inferiority margin of 8.5 hours, with a corresponding hazard ratio of 0.764 between the reference and the test, with the justification that rifamycin has a favorable safety profile and the decreased risk of developing antibiotic resistance compared to ciprofloxacin as standard treatment. ▪ The sample size calculation was also adjusted based on the change to the non-inferiority margin.[29] • Microbiological analysis: Elimination of microbiological testing of stool samples for *Yersinia enterocolitica,* based on new data on demonstrating its absence as a pathogen in patients with travelers' diarrhea in India.
2 (15 July 2010)	• Inclusion criteria were changed from a history of traveling from an industrialized country to a history of arriving from their country of residence in the industrialized part of the world within the past 4 weeks. • An additional exclusion criterion was added to exclude subjects from countries with a high rate of travelers' diarrhea (20-90% per 2-week stay) as these patients might possess natural immunity to pathogens that cause travelers' diarrhea; enrollment will be permitted if 6 months have elapsed since leaving a high-risk country.[30]
3 (29 June 2012)	• The Sponsor added a group sequential adaptive design to adapt the sample size based on the second of two prespecified interim analyses. The first interim analysis occurred after completion of 388 patients; no changes were recommended. The second interim analysis, conducted following completion of 582 patients, found a positive but not yet significant trend in favor of non-inferiority and did not identify any safety concerns. The DMC recommended adding 256 subjects to the sample size, in addition to the 776 previously specified, with a revised total sample size of 1032 subjects, which includes a 10% rate of subjects excluded from the per protocol analysis.

[29] The estimated placebo effect for the sample size calculation is derived from a clinical trial comparing rifaximin to ciprofloxacin and placebo Taylor, D. N., A. L. Bourgeois, C. D. Ericsson, R. Steffen, Z. D. Jiang, J. Halpern, R. Haake and H. L. Dupont (2006). "A randomized, double-blind, multicenter study of rifaximin compared with placebo and with ciprofloxacin in the treatment of travelers' diarrhea." Am J Trop Med Hyg **74**(6): 1060-1066., submitted to FDA in support of rifaximin, RFID3001, Study 2 (b) (4).

[30] Of note, the exclusion criteria did not specify which countries were excluded due to a high incidence of travelers' diarrhea.

Amendment (Final Date)	Major Changes
4 (30 April 2014)	• Amendments made to increase recruitment of patients in the study with the addition of clinical trials sites Latin America. • The handling of missing data in subject diaries for the primary endpoint of TLUS was altered as follows: TLUS for patients with drug-related censoring, due to either lack of efficacy or AEs related to study drug (certain, probable or possibly-related) or to underlying disease will not be censored but TLUS will be set to 120 hours in order to prevent an artificial increase in treatment response rate.

Source: RIT-1/AID CSR 5.3.5.1 Protocol Amendments 01-05

7.2.2.2 Study Results

Compliance with Good Clinical Practices

According to the Applicant, the study was conducted in full conformance with ethical principles of Good Clinical Practice (GCP), respecting the guidelines from ICH E6, US Code of Federal Regulations 21 CFR Parts 50, 54, 56, 312, 314, and 511, and the Declaration of Helsinki.

Financial Disclosure

All investigators listed on Form FDA 1572 were required to provide a Financial Disclosure statement to the Sponsor at the start of the study and 1 year following completion.

Subject Disposition

Table 28 presents subject disposition in the Full Analysis Set.

Table 28: Subject Disposition in Study RIT-1/AID (FAS)

Disposition	Rifamycin N=420 n (%)	Ciprofloxacin N=415 n (%)
Randomized Patients	420	415
Completed Study		
Yes	409 (97.4)	405 (97.6)
No	11 (2.6)	10 (2.4)
Reason for Withdrawal		
Lack of efficacy	4 (1.0)	3 (0.7)
Lack of patient cooperation	6 (1.4)	7 (1.7)
Intolerable adverse events	1 (0.2)	0

Source: From RIT-1/AID CSR Table 10 (Section 14, Table 14.1.1.7

Protocol Violations/Deviations

Table 29 displays the Applicant's Per Protocol and Modified Per Protocol populations and the reasons subjects were excluded from each.

Table 29: RIT-1/AID Disposition of Subjects (FAS)

Category	Rifamycin n (%)	Ciprofloxacin n (%)
Full Analysis Set (FAS)	420	415
Per Protocol	384	383
• Major protocol deviation regarding Inclusion/exclusion criteria	19 (4.5)	12 (2.9)
• Major protocol deviation (excluding inclusion/exclusion criteria)	18 (4.3)	21 (5.1)
○ Prohibited medications	8 (1.9)	10 (2.4))
○ Severe trial medication error	0	1 (0.2)
Modified Per Protocol	365 (86.9)	363 (87.5)
Premature withdrawal	11 (2.6)	9 (2.2)
Non-compliance with Patient Diary Card	19 (4.5)	13 (3.1)
Non-compliance with study treatment	13 (3.1)	18 (4.3)

Source: Adapted from RIT-1/AID CSR Table 14.1.1.2 Percentages are based on the number of subjects randomized in each treatment group. (Note: Reasons for exclusion do not add up to number of per protocol subjects in each arm as all categories of protocol deviations not included in the table and some subjects had more than one reason for exclusion).

Results of the Interim Analyses

The original Sponsor's interim analyses used the inverse-normal combination method for testing the null hypothesis that rifamycin is inferior to ciprofloxacin. More specifically, the null hypothesis states that the rifamycin vs. ciprofloxacin hazard ratio is less than or equal to 0.764 (the selection of the value 0.764 is described above). The critical value used for testing at the first interim analysis was 2.96, corresponding to a 1-sided alpha of 0.0015. The critical value used for testing at the second interim analysis was 2.36, corresponding to a 1-sided alpha of 0.0092. 2-sided 95% repeated confidence intervals (RCIs) were computed at each interim analysis.

Data from 388 participants (194 rifamycin, 194 ciprofloxacin) were analyzed in the first interim analysis. The null hypothesis of rifamycin being inferior to ciprofloxacin was not rejected for either the per protocol set or the full analysis set. The associated RCIs were (0.687, 1.406) and (0.704, 1.405), respectively. No adjustment was made to the originally intended sample size.

Data from 582 participants (289 rifamycin, 293 ciprofloxacin) were analyzed in the second interim analysis. The null hypothesis of rifamycin being inferior to ciprofloxacin was not rejected for either the per protocol or full analysis set, with associated RCIs of (0.730, 1.142) and (0.754, 1.165), respectively. The originally intended sample size of 776 was increased to 1032.

The trial was stopped when 843 participants had been recruited. Stoppage was due to difficulties with recruitment.

Demographic Characteristics

Table 30 below compares the rifamycin and ciprofloxacin arms on baseline demographic

characteristics.

Table 30: RIT-1/AID - Comparing Rifamycin and Ciprofloxacin Arms on Demographic Characteristics in the Full Analysis Sample

Demographic Characteristic	Rifamycin Arm (N=420) % (n)	Ciprofloxacin Arm (N=415) % (n)	Standardized Difference[1]
Sex			
Male	48.8% (205)	52.5% (218)	-0.07
Female	51.2% (215)	47.5% (197)	0.07
Age			
Mean years (SD)	40.0 (16.1)	40.4 (16.6)	-0.02
Median (years)	35	34	NA
Min, max (years)	18,84	18,80	NA
Age Group			
< 65 years	90.0% (378)	87.7% (364)	0.07
≥ 65 years	10.0% (42)	12.3% (51)	-0.07
Race			
White	81.4% (342)	82.9% (344)	-0.04
Asian	17.9% (75)	16.4% (68)	0.04
Other	0.7% (3)	0.7% (3)	0.00
Country Visited			
India	96.4% (405)	96.4% (400)	0.00
Guatemala	3.3% (14)	3.6% (15)	-0.02
Ecuador	0.2% (1)	0% (0)	NA

Source: Statistical Reviewer.

Notes. The full analysis sample includes 835 participants, with 420 in the rifamycin arm and 415 in the ciprofloxacin arm.

[1] The standardized difference is the difference in the means of the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled variance term. It gives the effect size difference between the two arms.

All of the standardized differences are of negligible size.

Table 31 presents the type of traveler enrolled in the study. Most of the study subjects originated in Europe.

Table 31: RIT-1/AID: Demographics - Type of Traveler (FAS)

	Rifamycin (N=420) n (%)	Ciprofloxacin (N=415) n (%)
Type of Traveler		
Tourist	354 (84.3%)	360 (86.7%)
Business	24 (5.7%)	18 (4.3%)
Student	26 (6.2%)	21 (5.1%)
Visiting friends/relatives	16 (3.6%)	16 (3.9%)
Previous Stays in Developing Countries		
No	365 (86.9%)	359 (86.5%)
Yes	55 (13.1%)	56 (13.5%)

Source: From RIT-1/AID CSR Table 17

Table 32 presents the country of origin for subjects enrolled in this study.

Table 32: RIT-1/AID Country of Residence[1] (FAS)

Country of Origin	Rifamycin (N=420) n (%)	Ciprofloxacin (N=415) n (%)
England	127 (30.2%)	131 (31.6%)
Japan	35 (8.3%)	37 (8.9%)
Russia	38 (9.0%)	30 (7.2%)
Israel	22 (5.2%)	33 (8.0%)
Germany	23 (5.5%)	31 (7.5%)
France	21 (5.0%)	14 (3.4%)
Republic Korea	17 (4.0%)	18 (4.3%)
Spain	14 (3.3%)	20 (4.8%)
United States	11 (2.6%)	11 (2.7%)
Finland	12 (2.9%)	9 (2.2%)
Italy	13 (3.1%)	4 (1.0%)
Scotland	9 (2.1%)	7 (1.7%)
Argentina	11 (2.6%)	4 (1.0%)

Source: RIT-1/AID CSR Table 18 (Section 14, Table 14.1.1.14)

[1]Includes countries with at least 15 Patients in Entire FAS

Clinical Reviewer Comments: *Most study subjects were from Europe, with about a third from England and Scotland (32.8%). Overall only 2.6% of subject were from the U.S. Note: The Applicant stated that the country of residence was not collected for study subjects enrolled in C2009-0201.*

Other Baseline Characteristics: Microbiological Status, Fever, and Bloody Stools

Table 33 compares rifamycin and ciprofloxacin on baseline microbiological status.

Table 33: RIT-1/AID - Comparing Rifamycin and Ciprofloxacin Arms on Microbiological Status, Fever, and Bloody Stools at Baseline

Baseline Characteristic	Rifamycin Arm (% (n)	Ciprofloxacin Arm % (n)	Standardized Difference[1]
Microbiological Status at Baseline			
Infected with at least one pathogen	64.4% (237)	65.6% (225)	-0.03
No pathogens isolated	35.6% (131)	34.4% (118)	0.03
Infected with *E. coli* only	37.5% (138)	41.1% (141)	-0.07
Infected with invasive pathogen (± additional pathogen)	11.4% (42)	14.3% (49)	-0.09
Bloody Stool and/or Fever Prior to First Administration of Study Drug			
Bloody stool/fever	4.3% (18)	5.3% (22)	-0.05

Source: Statistical Reviewer.

Notes. The full analysis sample includes 835 participants, with 420 in the rifamycin arm and 415 in the ciprofloxacin arm. For the *Microbiological Status at Baseline* characteristics, however, the table entries are based on the 711 full analysis participants (368 rifamycin, 343 ciprofloxacin) with completely observed values for 14

pathogen-specific baseline variables. These variables indicate whether or not there was a positive culture for the specific pathogen. For *Bloody Stool and/or Fever prior to First Administration of Study Drug*, table entries are based on the 835-participant full analysis set. Regarding this characteristic, 1 participant had a grossly bloody stool without fever, 1 participant had fever and a (non-grossly) bloody final pre-treatment stool, and 38 participants had a (non-grossly) bloody final pre-treatment stool without fever.
[1] The standardized difference is the difference in the means of the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled variance term. It gives the effect size difference between the two arms.

All of the standardized differences are negligible.

Treatment Compliance

The Applicant's analysis of medication compliance is found in Table 34.

Table 34: RIT-1/AID- Treatment Compliance (FAS)

	Rifamycin N=420 n (%)	Ciprofloxacin N=425 n (%)
Compliance regarding active drug (%)		
N	418	410
Mean (SD)	100.5 (6.57)	100.9 (7.44)
Median	100	100
Min, Max	50, 133	50, 133
Compliance category (%)		
<66	2 (0.5)	2 (0.5)
66-<80	2 (0.5)	1 (0.2)
80-<100	1 (0.2)	4 (1.0)
100	398 (94.8)	381 (91.8)
>100-134	15 (3.6)	22 (5.3)

Source: Adapted from RIT-1/AID CSR Table 14.1.1.34. Compliance is calculated as the ratio between actual administered medication and the expected amount of medication which was administered in the treatment period.

Efficacy Results – Primary Endpoint

In the statistical reviewer's analyses of the primary and secondary efficacy endpoints, reported below, 95% repeated confidence intervals were constructed to account for the adaptive group sequential trial design. In addition, point estimates computed via treating the sample as if its size were fixed a priori are often reported. These point estimates are termed "exploratory," to denote the fact that they do not adjust for the adaptive group sequential design. Such estimates have adequate statistical properties (e.g., modest Mean Square Error) but may be slightly biased.[31]

Differences between the Applicant's TLUS and Taylor's TLUS_T

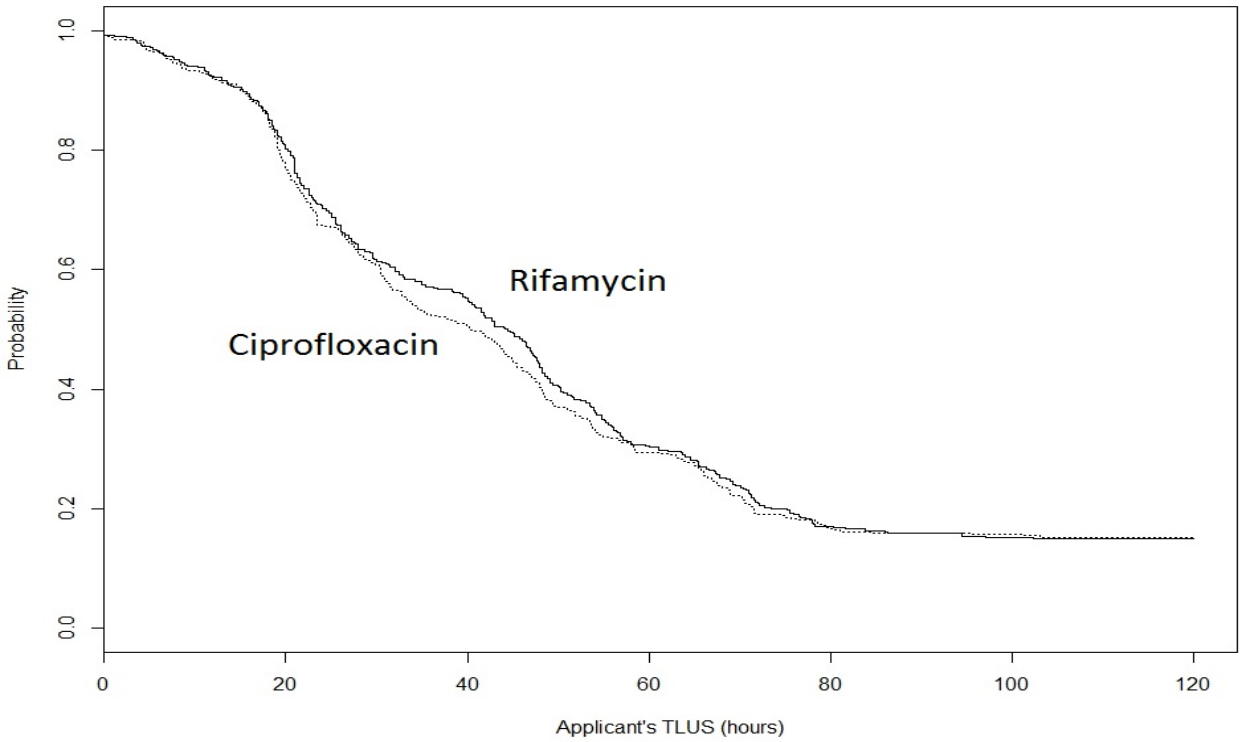
[31] See Wassmer and Brannath (2016, section 8.3).

Recall that TLUS_T is the Taylor et al. (2006) version of TLUS, which was used by the statistical reviewer to derive the upper bound for a 95% confidence interval for the placebo vs. ciprofloxacin hazard ratio (see the statistical reviewer's fourth, fifth, and sixth comments above). Of the 835 participants in the full analysis set, 126 had censored TLUS values and the same 126, plus one additional participant, had censored TLUS_T values. Of the 126 participants with censored TLUS and TLUS_T, 115 had TLUS equal to TLUS_T and 11 had TLUS larger than TLUS_T. Of the 708 participants with observed (i.e., uncensored) TLUS and TLUS_T values, 539 had the same value for TLUS and TLUS_T, and 169 had a larger TLUS_T than TLUS value. None of these 708 participants had a larger TLUS than TLUS_T value.

In estimating the arm-specific medians for TLUS over the full analysis sample (and ignoring the adaptive group sequential structure of the trial), the Kaplan-Meier estimates for the rifamycin and ciprofloxacin arms are 44.3 and 40.3 hours, respectively, with a difference in medians of 4.1 hours. The corresponding estimates for TLUS_T are 50.3 and 48.2 hours, respectively, with a difference of 2.1 hours.

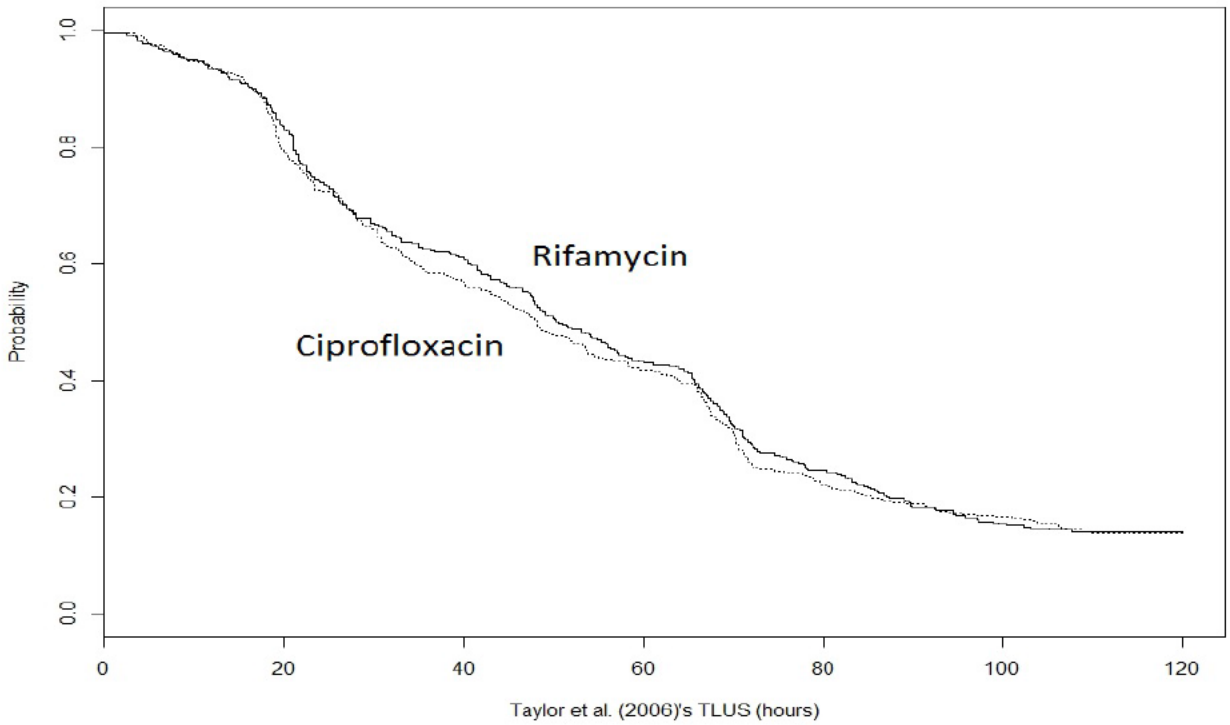
The Kaplan-Meier curves for the two versions of TLUS are given in the figures below.

Figure 5: RIT-1/AID - Kaplan-Meier Curves for Rifamycin and Ciprofloxacin Arms, Using the Applicant's Version of TLUS



Source: Statistical Reviewer.

Figure 6: RIT-1/AID - Kaplan-Meier Curves for Rifamycin and Ciprofloxacin Arms, Using the Taylor et al (2006) Version of TLUS



Source: Statistical Reviewer.

Results of the Analyses of the Primary Endpoint

The table below presents 95% repeated confidence intervals for the rifamycin-vs-ciprofloxacin hazard ratio. Per the 95-95 test, the lower bounds of these confidence intervals should be compared to .83, which is the Taylor et al. (2006) upper bound for the placebo vs ciprofloxacin hazard ratio. If a lower bound is larger than .83, then the null hypothesis that rifamycin is not efficacious is rejected; otherwise, the null hypothesis is not rejected.

Table 35: RIT-1/AID - 2-sided Repeated Confidence Intervals for the Rifamycin-vs-Ciprofloxacin TLUS Hazard Ratio for the Full Analysis and Per Protocol Samples

Version of TLUS	Sample	Hazard Ratio Confidence Interval and Point Estimate
Applicant's TLUS	Full Analysis Set	(0.826, 1.119) 0.962
	Per Protocol Set	(0.804, 1.100) 0.943
Taylor et al. (2006) TLUS_T	Full Analysis Set	(0.826, 1.120) 0.962
	Per Protocol Set	(0.812, 1.111) 0.949

Source: Statistical Reviewer.

Notes. Table cells in column 3 contain 2-sided 95% repeated confidence intervals, which respect the adaptive group sequential trial design. Exploratory hazard ratio point estimates are also reported; these were obtained from Cox regressions using arm as the sole predictor.
The full analysis set included 835 participants (420R, 415C). The per protocol set included 767 participants (384R, 383C).

In the preceding table, we see that the TLUS and the TLUS_T confidence intervals for the full analysis set are very similar, and similarly for the pair of confidence intervals for the per protocol set. There is a complication in comparing the lower bounds of these confidence intervals to the upper bound of the Taylor (2006) confidence interval for the placebo vs. ciprofloxacin hazard ratio related to the fact that Taylor reported confidence intervals bounds to a single decimal place. That is, Taylor reported that the lower bound for the ciprofloxacin vs. placebo hazard ratio is 1.2, which, if exact, implies that the upper bound for the placebo vs. ciprofloxacin hazard ratio is 1/1.2 = .833. However, reporting the ciprofloxacin vs. placebo hazard ratio as 1.2 means that the actual bound, to two decimal places, is between 1.15 and 1.24, as all values in this range round to 1.2. If the ciprofloxacin vs. placebo hazard ratio lower bound were actually 1.15, then the corresponding upper bound for the placebo vs. ciprofloxacin hazard ratio is .870, and if the ciprofloxacin vs. placebo hazard ratio lower bound were actually 1.24, then the corresponding upper bound for the placebo vs. ciprofloxacin hazard ratio is .806. Hence,

- The TLUS_T and TLUS lower bounds for both the full analysis and per protocol sets are smaller than .833.
- However, due to the imprecision of the stated Taylor results, the TLUS_T lower bounds for both the full analysis and per protocol sets and the TLUS lower bound for the full analysis set may be larger than the upper bound for the placebo vs. ciprofloxacin hazard ratio, but it is also possible that only the full analysis set lower bounds are larger, or that none of the lower bounds are larger. It is not possible to know which is the case.
- Whether larger or not, the TLUS_T and TLUS lower bounds for both the full analysis and per protocol sets are very close in value to the imprecisely known Taylor upper bound. This means that, at worst, statistical significant results are barely missed at alpha = .05.

The implications of this are discussed later.

In addition, a sensitivity analysis was performed that excluded participants who had fever or bloody stools at baseline. By excluding these participants, the resulting sample more closely resembles the Taylor subsample from which the non-inferiority margin was derived. Recall that fever and grossly bloody stools at baseline were RIT-1/AID exclusion criteria, and one participant for each criterion was mistakenly enrolled. In addition, there were 38 participants whose last stool prior to first administration of study medication was (non-grossly) bloody. After excluding these 40 individuals, the sensitivity analysis repeated the TLUS_T and TLUS analyses. Regarding TLUS_T, the confidence interval lower bounds were .824 and .813 for the full analysis and per protocol sets, respectively, and the analogous lower bounds for TLUS were

.819 and .798, respectively. Thus, the sensitivity analysis results were very similar to the table's results and did not yield an unambiguous rejection of the null hypothesis of non-efficacy.

Efficacy Results – Secondary and other relevant endpoints

Clinical Cure

Table 36 reports the results of analyzing clinical cure.

Table 36: RIT-1/AID - Results for Clinical Cure of the Full Analysis Sample

Version of Clinical Cure	Percentage of Clinical Cure in Rifamycin Arm	Percentage of Clinical Cure in Ciprofloxacin Arm	Difference in Percentages
Applicant's	(83.5, 89.8) 85.0%	(84.3, 90.4) 84.8%	(-4.8, 4.1) 0.2%
Sensitivity	(83.5, 89.8) 85.0%	(85.5, 91.3) 86.3%	(-5.9, 2.8) -1.3%

Source: Statistical Reviewer.

Notes. Results based on 420 participants in rifamycin arm and 415 participants in ciprofloxacin arm (835 participants total). Each cell reports a 2-sided 95% repeated confidence interval, which accounts for the adaptive group sequential design, and an exploratory point estimate.

Based on Table 4 in Taylor et al. (2006), an M1-based 95-95 test of the superiority of rifamycin to placebo with regard to clinical cure can be performed (note "clinical cure" is referred to as "wellness" in Taylor).[32] In the ITT subsample of the Taylor study that excluded participants with fever or bloody stools at baseline, the 65 participants in the ciprofloxacin arm had a clinical cure rate of 80.0% and the 60 participants in the placebo arm had a clinical cure rate of 61.7%. Using the normal approximation to the binomial distribution, this gives a 95% confidence interval for the placebo vs. ciprofloxacin difference in proportions of (-0.340, -0.026).

Per the 95-95 method of testing, the null hypothesis that rifamycin is not superior to placebo with regard to clinical cure is rejected if the lower bound of a 95% confidence interval for the rifamycin vs. ciprofloxacin difference is larger than the upper bound for the analogous interval for placebo vs. ciprofloxacin. However, using the Applicant's version of clinical cure, the null hypothesis is not rejected because -0.048 is not larger -0.026.

The table above also presents results for a sensitivity analysis version of clinical cure, in which the ten participants with systematically missing diary data are assigned a clinical cure value of "Y" if in the ciprofloxacin arm and a value of "N" if in the rifamycin arm (in the Applicant's version of clinical cure, all ten had values of "N"). Per the 95% confidence interval for this "worst case" sensitivity version, the rifamycin cure rate may be as much as 5.9% lower than the ciprofloxacin cure rate.

Microbiological Cure

[32] Recall that, when using the M1 margin, testing the superiority of rifamycin to placebo is equivalent to testing the non-inferiority of rifamycin to ciprofloxacin.

The following table presents the proportion of missing data for four pairs of binary microbiological variables. As in trial C2009-0201, the first pair consists of the baseline variable *Any Pathogen at Baseline* and the endpoint *Any Pathogen at Visit 3*. The variable *Any Pathogen at Baseline* indicates whether or not a participant had a positive culture for at least one of the 14 pathogens examined in baseline stool analyses. Specifically, *Any Pathogen at Baseline* is assigned

- A missing value if there was at least one missing culture result from the 14 pathogens.
- "Positive" if all 14 pathogen-specific culture results were observed and at least one was a positive result.
- "Negative" if all 14 pathogen-specific culture results were observed and all were negative.

. The variable *Any Pathogen at Visit 3* indicates whether or not a participant has a positive culture at Visit 3 for any pathogen that had a positive culture at baseline (per the protocol, at the Visit 3 stool analysis only pathogens with positive cultures at baseline were to be examined). *Any Pathogen at Visit 3* is set to not applicable if the participant's *Any Pathogen at Baseline* value equals negative, and is set to missing if Visit 3 culture results are missing for any pathogens with positive baseline cultures. For example, suppose a participant has a positive baseline culture for *Aeromonas* and the remaining 13 baseline cultures are negative. Then *Any Pathogen at Baseline* is set to TRUE. If, at Visit 3, the culture result for *Aeromonas* is missing, then *Any Pathogen at Visit 3* is set to missing; if the culture result is positive, then *Any Pathogen at Visit 3* is set to TRUE; and if the culture result is negative, then *Any Pathogen at Visit 3* is set to FALSE. The last case represents microbiological cure, as the participant was infected with *Aeromonas* at baseline but was no longer infected at Visit 3; the next-to-last-case represents microbiological treatment failure, as the participant was infected with *Aeromonas* at baseline and was still infected at Visit 3. Note that microbiological cure cannot be ascertained if *Any Pathogen at Baseline* and/or *Any Pathogen at Visit 3* have missing values. The total missing rate represents cases where *Any Pathogen at Baseline* and/or *Any Pathogen at Visit 3* have missing values.

The baseline-Visit-3 variable pair for *Any E. coli Detected* is defined with respect to a group of four *E. coli* bacteria (*Enteroaggregative E. coli, ETEC heat labile toxin, ETEC heat stabile/heat labile toxin, ETEC heat stabile toxin*). The variable pair for *Any Invasive Bacteria Detected* is defined with respect to a group of six invasive bacteria (*Aeromonas, Campylobacter jejuni, Shigella group, Salmonella group, Plesiomonas group, Vibrio group*). The variable pair for *Any Other Pathogens Detected* is defined with respect to a group of four pathogens (*Giardia lamblia, E. histolytica, Cryptosporidium parvum, Norovirus*). The union of the E. coli, invasive, and other pathogen groups gives the 14 pathogens with regard to which the *Any Pathogens* variables are defined. Missing data at baseline, at Visit 3, and in total for the *E. coli*, invasive, and other variable pairs are defined analogously to missingness for *Any Pathogen Detected*, but with respect to the particular pathogen group they are defined in terms of.

Table 37: RIT-1/AID - Percent Missing Data in Microbiological Variables for the Full Analysis Sample

	Overall % Missing Data			Rifamycin % Missing Data			Ciprofloxacin % Missing Data		
Microbiological Variable Pair	Baseline	Visit 3	Total	Baseline	Visit 3	Total	Baseline	Visit 3	Total
Any Pathogen	14.9%	8.0%	21.8%	12.4%	9.5%	21.0%	17.3%	6.5%	22.7%
Any *E. coli*	7.4%	7.1%	14.5%	5.0%	8.6%	13.6%	9.9%	5.5%	15.4%
Any Invasive Bacteria	4.0%	0.8%	4.8%	2.6%	1.0%	3.6%	5.3%	0.7%	6.0%
Any "Other" Pathogens	8.4%	0.5%	8.9%	8.3%	0.5%	8.8%	8.4%	0.5%	8.9%

Source: Statistical Reviewer.

Notes. Results based on 420 participants in rifamycin arm and 415 participants in ciprofloxacin arm (835 participants total). Baseline data are considered missing if the baseline culture results for any pathogen contributing to the endpoint are missing. Visit 3 data are considered missing if Visit 3 culture results are missing for any pathogens contributing to the endpoint that had a positive culture at baseline. Total missing rates reflect missing data at baseline or Visit 3.

For all four variable pairs, the ciprofloxacin arm had higher missingness rates at baseline and in total than the rifamycin arm. In general, the missingness rates are appreciably higher than in trial C2009-0201.

Table 38 compares the rifamycin and ciprofloxacin arms on the four Visit 3 microbiological endpoints described above. As in trial C2009-0201, a participant was included in the analysis of a given Visit 3 endpoint if (i) he/she had non-missing endpoint values at baseline and at Visit 3, and (ii) he/she had a positive culture at baseline for at least one of the pathogens associated with the endpoint. Thus, the analyses are complete-case analyses, in that only participants with observed data at baseline and Visit 3 are included. These analyses are statistically valid under the assumption that missingness at baseline and Visit 3 is unrelated to microbiological status at either timepoint and unrelated to any other participant characteristic or outcome.[33]

For *Any Pathogen Detected*, 653 participants satisfied (i), and 404 of them (61.9%) also satisfied (ii). For *Any E. coli Detected*, 714 participants satisfied (i), and 364 of them (51.0%) also satisfied (ii). For *Any Invasive Bacteria Detected*, the analogous numbers are 795 and 93 (11.7%), respectively. For *Any Other Pathogens*, the analogous numbers are 761 and 107 (14.1%), respectively.

Table 38: RIT-1/AID: Results for Microbiological Cure by Visit 3 for the Full Analysis Sample

Microbiological Endpoint	Number of Participants Infected at Baseline	Proportion of Eradication by Visit 3 In Rifamycin Arm	Proportion of Eradication by Visit 3 in Ciprofloxacin Arm	Difference in Proportions
Any Pathogen	404 201R/203C	(53.0, 66.9) 59.7%	(48.8, 62.8) 55.2%	(-5.9, 13.8) 4.5%
Any *E. coli*	364	(51.1, 65.7)	(53.4, 67.7)	(-12.7, 7.7)

[33] Technical note: This is the assumption of missingness completely at random (MCAR). As discussed above, this likely holds for missingness at baseline, but may not hold at Visit 3.

Microbiological Endpoint	Number of Participants Infected at Baseline	Proportion of Eradication by Visit 3 In Rifamycin Arm	Proportion of Eradication by Visit 3 in Ciprofloxacin Arm	Difference in Proportions
	181R/183C	57.5%	58.5%	-1.0%
Any Invasive Bacteria	93 42R/51C	85.7%	84.3%	1.4%
Any "Other" Pathogens	107 61R/46C	62.3%	56.5%	5.8%

Source: Statistical Reviewer.

Notes. In the "any pathogen" and "any *E. coli*" rows, cells in the last 3 columns present 2-sided 95% repeated confidence intervals, which respect the adaptive group sequential design. In the "any invasive" and "any other" rows, due to small sample sizes, confidence intervals are not presented. Exploratory point estimates are given in all cells.

The difference in proportions confidence intervals for *Any Pathogen Detected* and *Any E. coli Detected* both contain zero, implying no significant evidence that either drug was superior to the other. The results for the other two endpoints should be understood as descriptive statistics. When these analyses were repeated using the Applicant's method for handling missing data, the exploratory difference in proportions point estimates were similar to those in the table (3.5%, 0.0%, 7.0%, and 6.2%, respectively) and all 95% repeated confidence intervals contained zero.[34]

Findings in Special/Subgroup Populations or Additional Analyses Conducted on the Individual Trial

Table 39: RIT-1/AID - Median TLUS within Subgroups of Full Analysis Sample

Variable	Subgroup	N N_R/N_C	Rifamycin Median	Ciprofloxacin Median	Difference in Medians
Gender	Female	412 (215/197)	46.5	43.5	3.0
	Male	423 (205/218)	40.3	36.4	3.9
Race	White	686 (342/344)	48.4	44.9	3.5
	Non-white	149 (78/71)	21.2	20.3	0.9
Age Group	< 65	742 (378/364)	46.4	42.9	3.5
	>= 65	93 (42/51)	30.3	30.5	-0.3
Country Visited[a]	Guatemala	29 (14/15)	26.3	29.5	-3.1
	India	805 (405/400)	45.0	41.0	4.0
Pre-treatment Presence of Fever/Bloody Stool	Yes	40 (18/22)	57.0	62.2	-5.2
	No	795 (402/393)	42.8	38.0	4.8

Source: Statistical Reviewer.

[34] Table 4 in Taylor et al. (2006) presents results on a 13-pathogen, rather than 14-pathogen, version of *Any Pathogens* (Taylor did not examine norovirus) that permit 95-95 testing of the efficacy of rifamycin with respect to this endpoint. However, Taylor does not provide the corresponding results for the other three microbiological cure endpoints, and hence the corresponding tests of efficacy cannot be performed. The 95-95 test for the 13-pathogen version of *Any Pathogens* yields statistically significant results in support of the efficacy of rifamycin with respect to this endpoint, but this result is not relevant to the focal clinical question: against what groups of pathogens is rifamycin curative? The *Any Pathogens* result does not imply that rifamycin is curative for all pathogen groups, and unfortunately, as noted, the focal question cannot be addressed via 95-95 testing given the limited results reported by Taylor.

Notes. N = 835 (420 rifamycin, 415 ciprofloxacin). Median TLUS and Difference in Medians are in hours. Race was dichotomized due to the small proportion of non-white participants. Information on whether participants were Latino was not collected by study staff and hence is not reported in the table. TLUS median estimates are based on Kaplan-Meier curves, and should be considered exploratory, as they do not account for the adaptive group sequential design.
[a] A single participant was recruited in Ecuador and is not included in the table.

Two patterns are notable:

- Rifamycin vs. ciprofloxacin differences in estimated subgroup medians are relatively small, with range 0.3 to 5.2 hours in absolute value.
- Within the subgrouping variables race, age group, country visited, and pre-treatment presence of fever and/or blood in stool, differences in estimated subgroup medians for the rifamycin arm and for the ciprofloxacin arm are at least 11.5 hours. For example, the estimated difference for white vs. non-white for the rifamycin arm is 27.2 hours and for the ciprofloxacin arm is 24.6 hours. However, per the prior comment, the estimated rifamycin vs. ciprofloxacin differences in subgroup medians are relatively small.

Table 40: RIT/1 - AID - Median TLUS within Baseline Microbiological Subgroups of Full Analysis Sample with Complete Baseline Microbiological Data

	Median TLUS			Clinical Cure Rates		
Subgroup	Rifamycin	Ciprofloxacin	Diff	Rifamycin	Ciprofloxacin	Diff
Only *E. coli* at baseline (N=279: 138R/141C)	42.8	40.0	2.8	84.1%	87.2%	-3.2%
E. coli plus another pathogen at baseline (N=97: 52R/45C)	42.6	30.9	11.7	78.8%	84.4%	-5.6%
No *E. coli* but another pathogen at baseline (N=86: 47R/39C)	46.9	40.0	7.0	78.7%	89.7%	-11.0%
No pathogens at baseline (N=249: 131R/118C)	40.8	40.3	0.5	86.3%	80.5%	5.8%
Invasive Pathogens at baseline (N=91: 42R/49C)	55.6	36.1	19.6	66.7%	85.7%	-19.0%

Source: Statistical Reviewer.
Notes. Table based on the 711 (368R, 343C) full analysis set participants who had complete baseline microbiological data for the 14 pathogens examined. TLUS median estimates (in hours), based on Kaplan-Meier curves, and estimated clinical cure rates should be considered exploratory, as they do not account for the adaptive group sequential design. *Only E. coli* includes the 4 pathogens *enteroaggregative E. coli, ETEC heat labile toxin, ETEC heat stabile/heat labile toxin, ETEC heat stabile toxin*. *Another pathogen* includes the remaining 10 pathogens that were examined. *Invasive pathogens* includes the 6 pathogens *Aeromonas, Campylobacter jejuni, Shigella group, Salmonella group, Plesiomonas group, Vibrio group*; members of this subgroup may additionally be infected with some of the other 8 pathogens examined. The first four subgroups are non-overlapping and partition the entire sample of 711 participants.

For the *only E. coli subgroup*, estimated TLUS medians and clinical cure rates are slightly better

for the ciprofloxacin arm than for the rifamycin arm. Other than for the *no pathogens at baseline* subgroup, the ciprofloxacin arm estimates are appreciably superior to the rifamycin arm estimates.

Statistical Reviewer's Comments:

We summarize the results of efficacy analyses of non-inferiority trial RIT-1/AID:

- *With regard to the primary efficacy endpoint, both using the version defined by the Applicant (TLUS) and the version defined by the Taylor study that was used to derive the relevant confidence interval upper bound (TLUS_T), and with regard to both the full analysis and per protocol sets, it was not possible to unambiguously reject the null hypothesis that rifamycin is not efficacious. However, due to the imprecise reporting of results in the Taylor study, it is possible, but not knowable, that the TLUS_T analysis results imply the rejection of this null hypothesis for both sets. At the very least, both versions of the primary endpoint are very close in value to the Taylor-derived upper bound for the placebo vs. ciprofloxacin hazard ratio, meaning that at worst statistical significance is narrowly missed at alpha = .05. In sum, we believe that the analyses lend qualified support for the efficacy of rifamycin .*
- *With regard to the clinical cure secondary endpoint, results from the Taylor study were used to perform a 95-95 test. The null hypothesis of the non-efficacy of rifamycin was not rejected. The 95% repeated confidence interval for the rifamycin vs. ciprofloxacin difference in clinical cure rates of (-4.8%, 4.1%) indicated that the rifamycin cure rate is at most 4.8% smaller than the ciprofloxacin cure rate. This confidence interval contained zero, meaning there wasn't evidence that ciprofloxacin is strictly superior to rifamycin with regard to clinical cure.*
- *With regard to the secondary microbiological cure endpoints, it was not possible to perform 95-95 tests of the efficacy of rifamycin regarding the microbiological cure of pathogen groups E. coli, invasive bacteria, and other pathogens. The 95% repeated confidence interval for the difference in rifamycin vs. ciprofloxacin cure rates included zero.*
- *Regarding exploratory subgroup analyses, among demographic subgroups and the subgroups defined with respect to absence/presence of pre-treatment fever/blood in stool, rifamycin vs. ciprofloxacin differences in estimated subgroup TLUS medians were relatively small. Among subgroups defined by baseline microbiological status, "only E. coli" and "no pathogens" had rifamycin arm median TLUS and clinical cure rate estimates closest to the corresponding ciprofloxacin arm estimates. The rifamycin arm estimates for "invasive pathogens" were markedly inferior to the ciprofloxacin arm estimates.*

7.2.3 Phase 2 Studies: CB-01-11/02 and CB-01-11/03

7.2.3.1 Study CB-01-11/02

Because this study used a proposed indication of infectious diarrhea rather than travelers' diarrhea, utilized a different rifamycin dose as the phase 3 trials, lacked a placebo control, and included different efficacy definitions, this study was not evaluated in detail but was assessed for general points regarding clinical and microbiological efficacy. CB-01-11/02 was considered relevant to the proposed indication by providing additional clinical context given the overlap in clinical presentation, diagnostic tests, microbiology, and treatment options between travelers' diarrhea and infectious diarrhea. In addition, this study contributed to the safety database as discussed in Section 9 – Review of Safety and included ECG data on each subject.

CB-01-11-02 was a phase 2 randomized, double-blind, active-controlled study conducted in 8 sites in South Africa comparing the safety and efficacy of rifamycin at 200 mg QID with rifaximin 200 mg QID for 3 days in subjects with infectious diarrhea. (Note: the labeled dose of rifaximin for travelers' diarrhea is 200 mg TID for 3 days.) Of note, as this study was not conducted under IND, FDA did not have input into the study design or statistical analysis plan.

This study included subjects 18-65 years of age with infectious diarrhea of no more than 72 hours of duration and excluded subjects with fever ($\geq$ 38° C) at baseline or in the preceding 24 hours and visible blood in stool at first visit. Subjects were seen at Day 1 and Day 4 and had telephone follow up on Days 8-11. Stool microscopy and culture, hematology, blood chemistry, and ECGs were performed on Day 1 and Day 4.

A total of 115 subjects were randomized and received at least one dose of study medication, with 57 receiving rifamycin and 58 receiving rifaximin. The primary efficacy endpoint was TLUS.

Table 41 below presents the subject disposition.

Table 41: Subject Disposition in Study CB-01-11/02

Disposition	Rifamycin n (%)	Rifaximin n (%)
Randomized Patients	57 (100)	58 (100)
ITT Patients	46 (80.7)	53 (91.4)
Excluded from Applicant's ITT analysis (missing post-dose efficacy assessments)	**11 (19.3)**	**5 (8.6)**
• Did not produce stool within 4 hrs of first dose	8 (14.0)	3 (5.2)
• Lost to follow up	2 (3.5)	1 (1.7)
• Discontinued due to SAE	--	1 (1.7)
• Withdrawal of consent	1 (1.8)	--
PP Patients	38 (66.7)	40 (69.0)
Excluded from PP analysis[1]	**8 (14.0)**	**13 (22.4)**
• Discontinued due to AE	2 (3.5)	2 (3.4)
• Discontinued due to SAE	1 (1.8)	1 (1.7)
• Withdrawal of consent	--	2 (3.4)

Disposition	Rifamycin n (%)	Rifaximin n (%)
• Lost to follow up	1 (1.8)	--
• Patient noncompliance with study procedure	3 (5.3)	4 (6.9)
• Use of prohibited medications	--	2 (3.4)
• Did not produce final stool sample	--	1 (1.7)
• Blood in stool at baseline	1 (1.8)	--

Denominator used in treatment groups is total subjects randomized in that group.
Source: adapted from CB-01-11/02 CSR (Module 5, 5.3.5.1 Study Body report text Section 11.1 and Table 11.1.1; subject assignment pulled from SDTM dataset.
[1]Per table 11.1.1, total number excluded from per protocol analysis did not include Subject (b) (6) who was included by agreement with Sponsor.

Table 42 presents the demographic characteristics of the study.

Table 42: CB-01-11/02 Demographics (ITT)

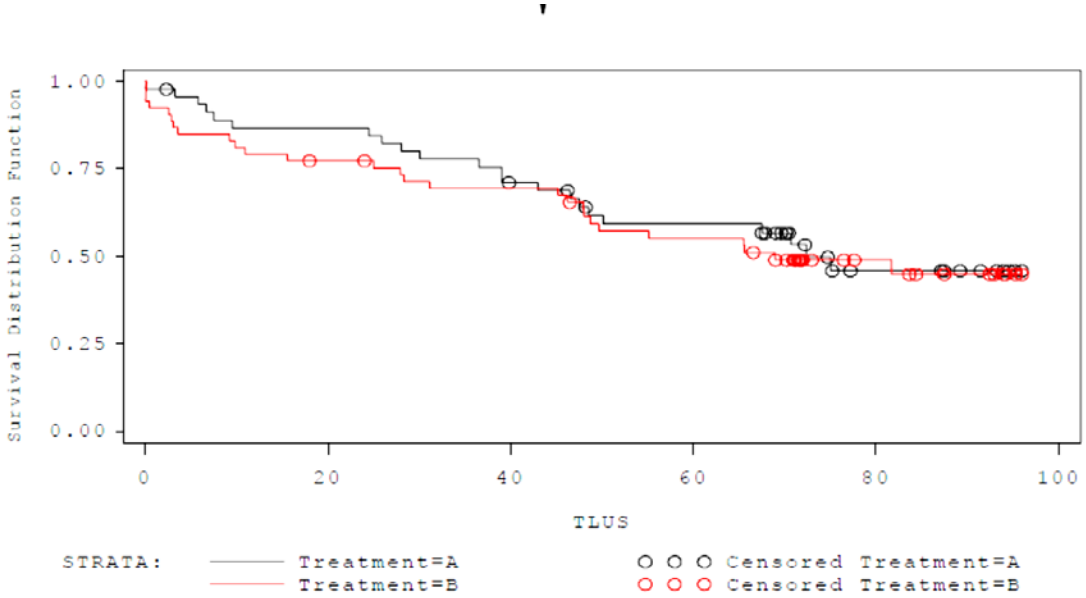
Characteristics	Statistics	Rifamycin N=57 (%)	Rifaximin N=58 (%)
Sex [n (%)]	F	27 (47.4)	28 (48.3)
	M	30 (52.6)	30 (51.7)
Age [n (%)]	<65 years of age	57 (100.0)	58 (100.0)
BMI Category 01 [n (%)]	<25	31 (54.4)	21 (36.2)
	>=25 to <30	15 (26.3)	16 (27.6)
	>=30	10 (17.5)	19 (32.8)
At Least One AE [n (%)]	Adverse Events	29 (50.9)	25 (43.1)
At Least One SAE [n (%)]	Y	1 (1.8)	2 (3.4)
At Least One Non-Fatal SAE [n (%)]	Non-Fatal SAE	1 (1.8)	2 (3.4)
AE resulted in Drug Withdrawn [n (%)]	DRUG WITHDRAWN	3 (5.3)	5 (8.6)
Disposition [n (%)]	ADVERSE EVENT	3 (5.3)	3 (5.2)
	COMPLETED	40 (70.2)	43 (74.1)
	LOST TO FOLLOW-UP	3 (5.3)	1 (1.7)
	OTHER	9 (15.8)	8 (13.8)
	PROTOCOL VIOLATION	1 (1.8)	0
	WITHDRAWAL BY SUBJECT	1 (1.8)	3 (5.2)
	INFORMED CONSENT	57 (100)	58 (100.0)
	RANDOMIZED	57 (100)	58 (100.0)
	TELEPHONE FOLLOW-UP	44 (77.2)	50 (86.2)
Countries [n (%)]	South Africa	57 (100.0)	58 (100)

Source: Clinical Reviewer - J Review Demographics by Actual Arm

Efficacy Results – Primary Endpoint

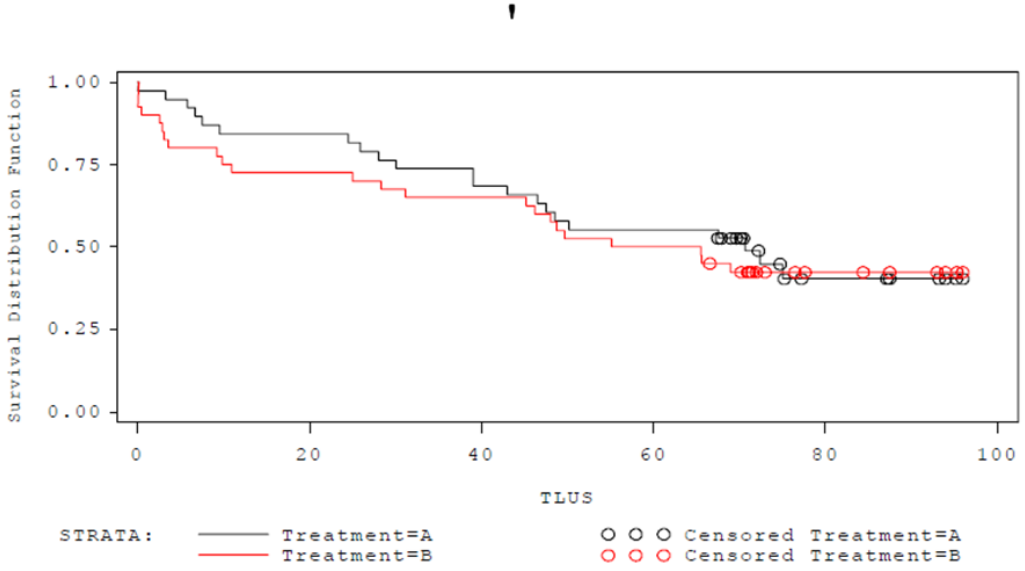
The median TLUS in the ITT population was 72.4 hours for subjects receiving rifamycin and 68.9 hours for subjects receiving rifaximin. The two treatments appeared to have numerically similar rates or frequencies of treatment success.

Figure 7: CB-01-11/02 - Kaplan Meier TLUS (ITT)



Treatment A: Rifamycin; Treatment B: Rifaximin
Source: CB-01-11/02 CSR Figure 14.3.1

Figure 8: CB-01-11/02 – Kaplan Meier TLUS (PP)



Treatment A: Rifamycin; Treatment B: Rifaximin
Source: CB-01-11/02 CSR Figure 14.3.2

Clinical Reviewer Comment: *Although numerically TLUS was similar between two arms, these findings are not directly applicable to the current application given the differences in the indication, patient population, comparator choice (no placebo), endpoints, and lack of*

justification for non-inferiority assessment.

Efficacy Results – Secondary endpoints

Summary data on pathogens identified and microbial eradication was not provided by Applicant. Table 43 presents individuals' results of TLUS by the pathogens identified at baseline according to study arm.

Table 43: CB-01-11-02: Pathogens Identified at Baseline Visit 1 (ITT)

Treatment Group	Subject ID	Pathogen	TLUS (hours)
Rifamycin	(b) (6)	*Salmonella enteritidis*	24.5
		E. coli	39.1
		Anaerobiospirillum sp.	89.3
		Campylobacter lari	39.0
		Shigella flexneri	5.8
		Salmonella enteritidis	46.3
Rifaximin		*Campylobacter lari*	96.0
		Campylobacter jejuni	18.0
		Campylobacter lari	46.5
		Campylobacter jejuni	69.0
		Shigella sonnei	28.4
		Campylobacter jejuni	81.7
		Shigella boydii	65.5

Source: Adapted from CB-01-11-02 CSR Appendix 4 and CSR Section 11.4.1.4 Table 14.3.22, Study Data Reviewer's Guide (SDTM) Appendix 1.

Clinical Reviewer Comments:

The pathogen profile in this study of infectious diarrhea was considerably different than that found in the phase 3 studies C2009-0201 and RIT-1/AID. Only 13% of subjects had pathogens isolated at baseline, and E. coli (not further characterized) was identified as a pathogen in only one subject (0.9%). By contrast, by the Applicant's analyses 68.6% of study subjects in C2009-0201 and 38.0% of study subjects in RIT-1/AID had diarrheagenic E. coli isolated at baseline (ITT/FAS populations).

7.2.3.2 Study CB-01-11/03

Study CB-01-11/03 was a pilot, randomized, double-blind, multicenter, dose-finding study of safety and efficacy conducted in Mexico and Turkey in subjects 18-65 years of age who had infectious diarrhea. Eligible subjects were randomized to receive oral rifamycin at daily doses of 400 mg, 800 mg, or 1200 mg, with or without food. There was no placebo or active control arm in this study. Treatment lasted up to seven consecutive days. Subjects self-administered the study medication and discontinued treatment when they recovered from diarrhea, defined as passing fewer than three unformed stools in the previous 24 hours and had no symptoms of enteric infection.

The primary efficacy endpoint was TLUS with secondary endpoints including the number of subjects with improvement in diarrhea in a 24-hour period, the number of subjects declared to be "well" (defined as having 48 hours with no unformed stools, a maximum of two soft stools and no clinical symptoms of infectious diarrhea), and treatment failure. Microbiological analysis of the stool sample was conducted at the baseline visit, to confirm infectious diarrhea, but no subsequent stool collection or analysis was performed.

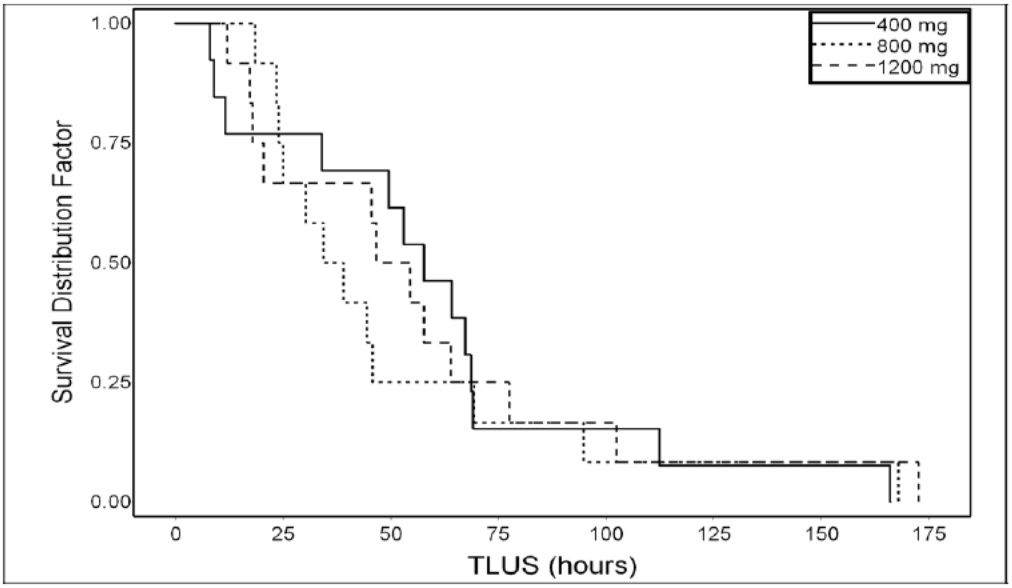
A total of 40 subjects were enrolled and treated. The Applicant's ITT population consisted of 37 subjects: two subjects withdrew consent and one was lost to follow up.

Table 44: CB-01-11/03 - TLUS (ITT)

Dose	N	Mean	SD	Median	Min	Max
400 mg	13	59.3	12.1	57.7	8.0	166.0
800 mg	12	51.4	12.3	36.7	18.5	168.0
1200 mg	12	57.4	13.1	50.6	12.0	172.7
All	37	56.2	7.0	46.8	12.0	102.5

Source: CB-01-11/03 CSR (Module 5, 5.3.5.1, Study Body Report, Table 29

Figure 9: CB-01-11/03: TLUS by Treatment Group



Abbreviations: mg = milligram (total per day); TLUS = time to last unformed stool.
Source: CB-01-11/03 CSR Figure 4; ISE Figure 2

Secondary Endpoints

Baseline culture of stool isolated *E. coli* in 33 of 37 subjects; further characterization was not performed. No other enteric pathogens were identified.

Clinical Reviewer Comments: *It is unclear if stools were cultured for enteric pathogens as the summary culture results provided normal enteric flora, thus limiting the interpretability of microbiological findings.*

Although this study evaluated efficacy in infectious diarrhea, this study served as the basis for the Applicant's dose selection of rifamycin of 800 mg per day (400 mg BID) for three days for travelers' diarrhea. The 800 mg per day dose was associated with the most favorable TLUS curve.

7.3 Integrated Review of Effectiveness

7.3.1 Assessment of Efficacy Across Trials

Demographics

The two tables in this section compare baseline characteristics of the two Phase 3 trials' study populations; this is in contrast to Tables 17 and 30 above, which compared arms' baseline characteristics within trials. Because the interest here is in study population rather than arm, the full C2009-0201 ITT sample is compared to the full RIT-1/AID full analysis sample. Table 45 examines baseline demographic characteristics.

Table 45: Baseline Demographic Characteristics by Phase 3 Study Population

Demographic Characteristic	C2009-0201 ITT Sample (N= 264)	RIT-1/AID Full Analysis Sample (N=835)	Difference
Sex			
Male	49.6% (131)	50.7% (423)	-1.1%
Female	50.4% (133)	49.3% (412)	1.1%
Age			
Mean years (SD)	28.3 (11.7)	40.2 (16.3)	-11.9 years
Median (years)	24	34	-10 years
Min, max (years)	18, 87	18,84	NA
Age Group			
< 65 years	98.1% (259)	88.9% (742)	9.2%
≥ 65 years	1.9% (5)	11.1% (93)	-9.2%
Race			
White	87.5% (231)	82.2% (686)	5.3%
Black or African American	5.7% (15)	0.1% (1)	5.6%
Asian	3.8% (10)	17.1% (143)	-13.3%
American Indian or Alaska Native	1.9% (5)	0%	1.9%
Native Hawaiian or Other Pacific Islander	0%	0%	0%
Other	1.1% (3)	0.6% (5)	0.5%
Ethnicity			
Hispanic or Latino	11.7% (31)	Not assessed	NA
Not Hispanic or Latino	88.3% (233)	Not assessed	NA

Demographic Characteristic	C2009-0201 ITT Sample (N= 264)	RIT-1/AID Full Analysis Sample (N=835)	Difference
Country Visited			
Mexico	66.3% (175)	0%	66.3%
India	0%	96.4% (805)	-96.4%
Guatemala	33.7% (89)	3.5% (29)	30.2%

Source: Statistical Reviewer.

Notes. The C2009-0201 ITT sample includes 264 participants, and the RIT-1/AID full analysis sample includes 835 participants.

Key points regarding baseline demographic characteristics for the rifamycin-treated patients in the two trials:

- The populations were quite similar on gender composition.
- The biggest difference is country visited, as nearly all of the RIT-1/AID participants were visiting India and none were visiting Mexico, while none of the C2009-0201 participants were visiting India and two thirds were visiting Mexico.
- The RIT-1/AID study population was older than the C2009-0201 population, with mean and median age at least 10 years older than the latter population. In addition, the former was comprised of 11% seniors, while the latter contained less than 2% seniors.
- Patients were more than four fifths White. The maindifference in ethnic composition is that 17% of RIT-1/AID participants were Asian, while less than 4% of C2009-0201 participants were Asian.

Table 46 examines baseline microbiological status.

Table 46: Baseline Microbiological Status by Phase 3 Study Population

Microbiological Status	C2009-0201 ITT Subsample (N= 252)	RIT-1/AID Full Analysis Subsample (N=711)	Difference
Only *E. coli* at baseline	57.5% (145)	39.2% (279)	18.3%
E. coli plus another pathogen at baseline	6.7% (17)	13.6% (97)	-6.9%
No *E. coli* but another pathogen at baseline	5.2% (13)	12.1% (86)	-6.9%
No pathogens at baseline	30.6% (77)	35.0% (249)	-4.4%

Source: Statistical Reviewer.

Notes. The table is based on data from subsamples of participants with baseline microbiological data. The C2009-0201 ITT sample included 264 participants, of which 252 had baseline microbiological data. The RIT-1/AID full analysis sample included 835 participants, of which 711 had baseline microbiological data. If both trials maintained study staff blindedness to treatment assignment, as intended, then the percentages reported in the table are unbiased for the respective study populations.

Important caveat: The percentages in the C2009-0201 column are only partly commensurable to the percentages in the RIT-1/AID column, as RIT-1/AID assessed 14 pathogens while C2009-0201 assessed 16. Hence, it is possible that an RIT-1/AID participant categorized as having no pathogens at baseline would have been categorized as having a pathogen if instead included in the other study. "Only E. coli at baseline" was based on 5 pathogens in C2009-0201 and 4 pathogens in RIT-1/AID.

. The proportion of the C2009-0201 study population only infected with *E. coli* is 18% larger than the analogous proportion of the RIT-1/AID population.

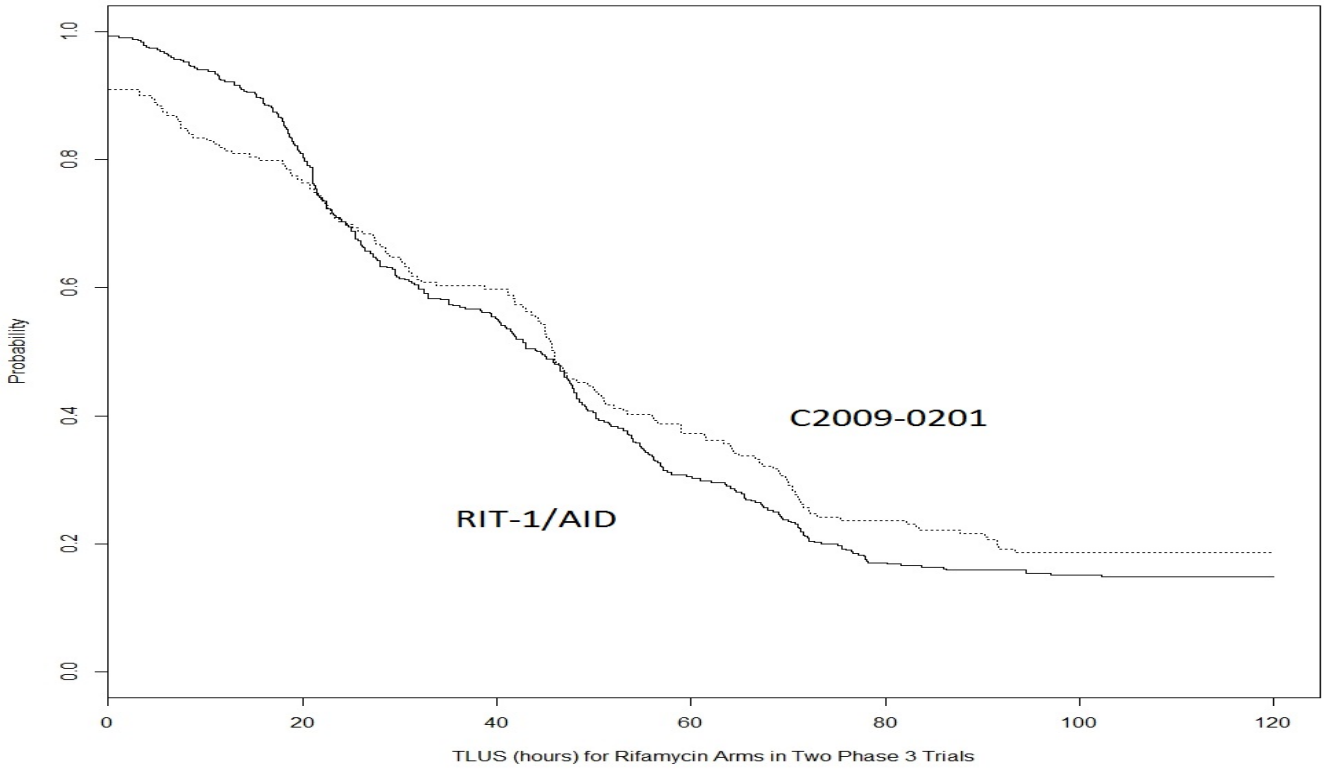
Primary Endpoints

In superiority trial C2009-0201, rifamycin was shown to be statistically significantly superior to placebo with regard to TLUS, the primary endpoint. A log-rank test comparing the TLUS distributions in the two arms rejected the null hypothesis that they were identical, $p = .0008$, and the estimated rifamycin vs. placebo difference in medians was -22.0 hours (due to the distribution of placebo TLUS values, it was not possible to compute a 95% confidence interval for this difference). Several sensitivity analysis versions of TLUS were developed by the statistical reviewer, and for each the log-rank test yielded $p < .015$ and the estimated difference in medians was of comparable size.

Evaluating the possible efficacy of rifamycin was considerably more complex in non-inferiority trial RIT-1/AID. In such trials, testing whether an experimental treatment such as rifamycin is efficacious relies on historical estimates of the benefit of ciprofloxacin relative to placebo. It does not directly test if the experimental treatment is superior to placebo, but does so indirectly via so-called 95-95 tests. In seeking to demonstrate the non-inferiority (i.e., the at-most-modest inferiority) of rifamycin to ciprofloxacin, the Applicant made incorrect use of historical data analytic results from Taylor et al. (2006), and this yielded an invalid test of non-inferiority. However, the statistical reviewer used results from Taylor to develop a valid test of the efficacy of rifamycin This requires adopting the version of TLUS used by Taylor, which differs somewhat from the version defined by the Applicant. The result of this test very narrowly misses statistical significance at alpha = .05 for both the full analysis sample and the per protocol sample. It should be noted that this test is of limited statistical power, given the very modest size of the sample from which the relevant Taylor results were obtained; the modest sample size leads to large variability of the estimate of the ciprofloxacin vs. placebo treatment effect. In sum, we believe that the analyses lend qualified support for the superiority of rifamycin to placebo with regard to the primary endpoint.

The following figure provides Kaplan-Meier plots of the rifamycin arms' TLUS distributions (Applicant's version) from the two Phase 3 trials. It can be used to examine the consistency of the TLUS responses for rifamycin across the two study populations.

Figure 10: TLUS Kaplan-Meier Curves for Rifamycin Arms from C2009-0201 and RIT-1/AID



The C2009-0201 median is 46.0 hours and the RIT-1/AID median is 44.3 hours. By visual inspection, the TLUS response to rifamycin in RIT-1/AID is similar to and generally at least as good as in C2009-0201. Please note that this does not imply that rifamycin is similarly efficacious in both trials, as such a conclusion would only follow if TLUS responses to placebo in both study populations were similarly distributed; given that RIT-1/AID did not include a placebo arm, this cannot be assessed.

Secondary and Other Endpoints

We examined clinical cure and multiple versions of microbiological cure.

Clinical cure: In superiority trial C2009-0201, rifamycin was shown to be statistically significantly superior to placebo with regard to clinical cure. A chi square test rejected the null hypothesis of no difference in arms, $p < .0002$, and the estimated rifamycin vs. placebo difference in clinical cure rates was 24.5%, with 95% confidence interval (11.3%, 37.7%). Two sensitivity analysis versions of clinical cure were developed by the statistical reviewer, and for each the chi square test yielded $p < .01$ and the estimated difference in rates was greater than 17%.

Non-inferiority trial RIT-1/AID was also used to test the efficacy of rifamycin with regard to clinical cure. Again, the Taylor ciprofloxacin vs. placebo results were used to develop a 95-95

test of efficacy. Per the test, there was not significant evidence of efficacy. A 95% confidence interval indicated that the rifamycin cure rate may be as much as 4.8% smaller than the ciprofloxacin cure rate.

Microbiological cure: this was defined for different sets of pathogens and was only meaningful for participants who were infected with a relevant pathogen at baseline. In trial C2009-0201, about two thirds of patients were infected with at least one pathogen at baseline, and the microbiological cure rate was the proportion of those infected at baseline who had no pathogen isolated at Visit 3. There was no statistically significant difference in cure rates between the rifamycin and placebo arms. Slightly more than 60% of the sample was infected with *E. coli* at baseline, and again the difference in microbiological cure rates for *E. coli* infection was not statistically significant. Differences in microbiological cure rates for invasive bacteria infection and other pathogen infection were also not significant.

For trial RIT-1/AID, Taylor did not provide relevant results for microbiological cure for pathogen groups *E. coli*, invasive pathogens, or other pathogens. Hence, it was not possible to test the efficacy of rifamycin for these classes of pathogens.

Subpopulations

We discuss two subpopulations of interest: individuals who were only infected with *E. coli* at baseline, and individuals aged 65 years or older.

Baseline Infection with E. coli Only: With regard to both the primary endpoint and the secondary endpoint clinical cure, per trial C2009-0201, rifamycin was significantly superior to placebo for the subpopulation of individuals who are infected only with *E. coli* prior to treatment. No other subpopulation based on baseline microbiological status exhibited significantly better TLUS or clinical cure outcomes with rifamycin, although this may be largely due to small subsample sizes. Hence, per C2009-0201, evidence of the general superiority of rifamycin regarding TLUS and clinical cure appears to be driven by the superiority of rifamycin within the *E. coli* only subpopulation.

In trial RIT-1/AID, lack of relevant information from Taylor et al. (2006) precluded testing the efficacy of rifamycin within this subpopulation. *E. coli* only estimates of median TLUS and clinical cure rate for the rifamycin arm were slightly inferior to the corresponding estimates for the ciprofloxacin arm, and this slight inferiority does not raise concerns about rifamycin's efficacy here.

Age 65 or Older: Trial C2009-0201 included only 5 participants aged 65 or older (seniors), so it cannot be used to examine the efficacy of rifamycin within this subpopulation. Trial RIT-1/AID, however, included 93 seniors (42 rifamycin, 51 ciprofloxacin). The estimated rifamycin vs. ciprofloxacin difference in TLUS medians for seniors equaled -0.2 hours. In sum, no concerns are raised regarding the use of rifamycin by seniors.

7.3.2 Onset, Duration, and Durability of Efficacy Effects

Time to Onset and Persistence of Treatment Effect: According to the trial C2009-0201 Kaplan-Meier curves given in Figure 2, the superiority of rifamycin to placebo becomes apparent by about 30 hours after initiation of study treatment. This superiority is maintained throughout the 120 hours that participants were followed. Trial RIT-1/AID does not provide information about time to onset or persistence of the Rifamycin treatment effect.

Persistence of Clinical Benefit: Trial C2009-0201 included a 30-day follow-up, but this was solely for the purpose of assessing safety. Neither trial provides information about clinical benefit beyond the 120-hour window.

7.4 Summary and Conclusions

7.4.1 Summary and Conclusions – Statistics

The evaluation of the efficacy of rifamycin for the treatment of travelers' diarrhea is based on data from two Phase 3 trials, superiority trial C2009-0201 and non-inferiority trial RIT-1/AID. The Applicant's two Phase 2 trials do not provide relevant data here.

As detailed above, trial C2009-0201 provides strong evidence of the superiority of rifamycin vs. placebo with regard to the primary endpoint, TLUS, and with regard to the secondary endpoint, clinical cure. Statistically significant results were obtained when analyzing the Applicant's version of these endpoints ($p = .0008$ and $p < .0002$, respectively), as well as when considering a variety of other versions (defined by the statistical reviewer) in sensitivity analyses. In addition, for all demographic subgroups of at least moderate size, rifamycin demonstrated a statistically significant superiority to placebo with regard to TLUS and clinical cure.

As detailed above, trial RIT-1/AID provides some evidence of the efficacy of rifamycin. With regard to TLUS and based on imprecisely reported results from analyses of small-sample historical data, rifamycin narrowly misses exhibiting statistically significant efficacy at alpha = .05.

In tandem, the Phase 3 trials provide evidence of the efficacy of rifamycin for the treatment of travelers' diarrhea.

7.4.2 Summary and Conclusions – Clinical

The clinical efficacy of rifamycin for the treatment of travelers' diarrhea caused by non-invasive *E. coli* was established in a phase 3 clinical trial C2009-0201, with supportive evidence provided by a second phase 3 trial, RIT-1/AID, as discussed in the statistical review by Dr. Edward Bein above. A single successful adequate and well-controlled trial, Study C2009-0201 was considered adequate to support effectiveness based on the robust efficacy findings for the primary endpoint (TLUS) and the secondary endpoint of clinical cure. Supportive evidence for a single

trial comes from the findings of Study RIT-1/AID and the known information on the efficacy of rifamycins for the treatment of travelers' diarrhea.

Diarrheagenic *E. coli* species were the most common pathogens isolated in the absence of other pathogens at baseline in 57.5% and 39.2% of subjects in C2009-0201 and RIT-1/AID, respectively. Rifamycin was superior to placebo (p-values < 0.006 and 0.003 for TLUS and clinical cure, respectively) for participants who were infected only with *E. coli* at baseline. Superiority of rifamycin to placebo could not be demonstrated for other invasive pathogens due to the insufficient number of infected subjects in this subgroup.

While no subjects had bloody stool or fever in C2009-0201, 18 (4.3%) subjects receiving rifamycin had bloody stools and/or temperatures $\geq$ 38^{o}C at baseline in RIT-1/AID. These subjects had notably longer TLUS (57.0 hours), compared to those without bloody stools or fever TLUS (42.0 hours).

In summary, the Applicant has provided substantial evidence of effectiveness to support the approval of rifamycin delayed-release tablets for adults with travelers' diarrhea caused by non-invasive strains of *E. coli,* not complicated by fever or blood in the stool.

8 Clinical Microbiology Review

8.4 Nonclinical Microbiology

8.4.2 Mechanism of Action

Rifamycin, belongs to the ansamycin class of antibacterial drugs originating from the fermentation products of the gram-positive bacterium *Streptomyces mediterranei*. (Kohanski, Dwyer et al. 2010). Rifamycin interferes with bacterial DNA transcription process of the genetic information from DNA to bacterial messenger RNA. It is hypothesized that rifamycin SV binds irreversibly to the beta subunit of the bacterial DNA-dependent RNA polymerase which results in inhibition of bacterial protein synthesis and consequently inhibiting the growth of bacteria.

8.4.3 Spectrum of antibacterial activity

Rifamycin exhibits *in vitro* activity against the following bacterial pathogens:

- Against *E. coli* (n=595), rifamycin minimum inhibition concentration (MIC) ranged from 2 to >512 µg/mL with $MIC_{50/90}$ values of 32-64 µg/mL and 64-128 µg/mL, respectively[35,36,37,38] (Farrell, Putnam et al. 2011). Rifamycin SV was 8- to 16-fold less active (MIC_{90}, 128 µg/mL) than rifaximin (MIC_{90}, 16 µg/mL) or rifampin (MIC_{90}, 8 µg/mL). Rifamycin showed similar activity against all groups of pathogenic *E. coli* including EHEC, ETEC, EPEC and EAEC producing isolates, with $MIC_{50/90}$ values ranging from 32 to 64 µg/mL and 64 – 128 µg/mL, respectively.
- Similar MIC values were observed against *Shigella* spp (n = 110) with $MIC_{50/90}$ values of 32 µg/mL and 64 µg/mL[35,38]. (Farrell, Putnam et al. 2011) Rifamycin was less active against *Salmonella* spp (n = 115), with $MIC_{50/90}$ values of 128 µg/mL and 256 µg/mL[35,37,38].
- Among the non-Enterobacteriaceae isolates tested, rifamycin activity against *Campylobacter* spp. (n=137) were > 512 µg /mL, with $MIC_{50/90}$ values of >512 µg/mL and > 512 µg/mL, respectively[35,38]. There were no significant differences between the rifamycin MIC values against *C. coli* isolates (16 – 128 µg/mL) compared to *C. jejuni* isolates (64 - >128 µg/mL). Against *Aeromonas hyrophila* (n = 106), rifamycin MICs

[35]JMI Laboratories Study Report # 09-SAN-01. In vitro susceptibility of rifamycin SC tested against pathogens associated with traveller's diarrhea.
[36]JMI Laboratories Study Report# 08-COS-01. In vitro testing of rifamycin SV against pathogens producing gastroenteritis, including pathogenic *E. coli* and *C. difficile* isolates.
[37]COSMO Pharmaceuticals Study Report# CB-01-11 NAMRU06. In vitro activity of rifamycin SV and comparator agents against bacterial pathogens isolated from patients with traveller's diarrhea in Peru.
[38]COSMO Pharmaceutical Study Report# CB-01-11 AFRIMS. In vitro activity of rifamycin SV and comparator agents against bacterial pathogens isolated from patients with traveller's diarrhea in Thailand.
[39] (b) (4) Study Report# CB-01-11/38. In vitro evaluation of antibacterial activity of rifamycin SV, metronidazole and vancomycin on *Clostridium difficile* strains. [40] Study Report# CB-01-11/39. Evaluation of in vitro antibacterial activity of rifamycin SV and rifaximin on ATCC strains of pathogens frequently involved in intestinal infections.

ranged from 2 to 512 µg/mL with $MIC_{50/90}$ values of 4 µg/mL and 16 µg/mL[35,38]. All *Plesiomonas shigelloides* (n=22) isolates were inhibited by rifamycin at MICs ≤ 8µg/mL ($MIC_{50/90}$, 8/8 µg/mL)[35,38]. The most susceptible isolates among the non-Enterobacteriaceae was *Vibrio parahemolyticus* (n=43), which showed rifamycin MICs ranging from 2 to 4 µg/mL, with $MIC_{50/90}$ values of 2 µg/mL and 2 µg/mL, respectively[35,38].

- Against *C. difficile*, rifamycin MICs ranged from ≤0.015 to > 2 µg/mL with $MIC_{50/90}$ values of 0.03 µg/mL and > 2 µg/mL, respectively. Rifamycin (MIC_{90}, >2 µg/mL) was similar in activity to rifaximin (MIC_{90}, > 2 µg/mL)[36,39].

Rifamycin minimum bactericidal concentrations (MBC) were similar to MICs, suggesting bactericidal activity against Enterobacteriaceae isolates including *E. coli, E. cloacae, P. mirabilis, S. sonnei, S. typhimurium, Y. enterocolitica* and gram-positive cocci (*E. faecium, E. faecalis, S. epidermidis* and *S. aureus*), *N. gonorrhoeae,* and *C. difficile*[40].

8.4.4 Resistance

The development of spontaneous resistance *in vitro* to rifamycin was evaluated against selected *E. coli* strains[41] (Farrell, Putnam et al. 2011) The tested strains included *E. coli* ATCC 25922 (wild type), *E. coli* 012-1222G (EHEC, 0157:H7), *E. coli* 5347 (ETEC), *E. coli* 915J (EPEC) and *E. coli* 5753J (EAEC). Rifamycin mutants were observed among five *E. coli* strains tested when exposed to 4x, 8x or 16x the MIC value. The frequency of spontaneous mutation at 4x or 8x MIC ranged from 1.4×10^{-7} to 5.9×10^{-8}, compared to at 16x rifamycin MIC where it ranged from 1.6×10^{-7} to $< 5.0 \times 10^{-10}$. The rate of spontaneous development of resistance in rifamycin was similar to that of rifaximin.

Serial passage studies of the selected *E. coli* pathogens at concentrations equivalent to 1x MIC evaluated over 7 days, showed that three of the five isolates tested (EHEC, ETEC and EAEC) had a 4- to 32-fold increase in rifamycin MIC values, whereas two (ATCC 25922 and EPEC) showed no significant variation (no more than one-fold change) in the rifamycin MIC. The MIC values were re-assessed after three passages in drug-free MH media; all isolates maintained the MIC value obtained after passaging for seven days and failed to revert to the original MIC values on antibacterial free media[41]. (Farrell, Putnam et al. 2011) This is consistent with other studies which noted that rifamycins, including rifaximin and rifampin, are prone to inducing resistant mutants in *E. coli* at approximate rate of 10^{-8} and these mutants are stable, maintaining increased MIC values even after successive passage on antibacterial-free medium.(Ruiz, Mensa

[39] (b) (4) Study Report# CB-01-11/38. In vitro evaluation of antibacterial activity of rifamycin SV, metronidazole and vancomycin on *Clostridium difficile* strains. [40] Study Report# CB-01-11/39. Evaluation of in vitro antibacterial activity of rifamycin SV and rifaximin on ATCC strains of pathogens frequently involved in intestinal infections.

[40] Study Report# CB-01-11/39. Evaluation of in vitro antibacterial activity of rifamycin SV and rifaximin on ATCC strains of pathogens frequently involved in intestinal infections.

[41] JMI Laboratories Study Report 09-COS-01. Determination of resistance selection rates for rifamycin SV tested against pathogens associated with traveller's diarrhea by passaging and single step mutational analysis.

et al. 2008) In vitro susceptibility testing against the parent and mutant strains showed that isolates remained susceptible to β-lactams, fluoroquinolones, aminoglycosides, tetracyclines, polymyxin B and trimethoprim/sulfamethoxazole.

Pulsed field gel electrophoresis (PFGE) analysis with digestion using the restriction enzyme *Spe*I showed no genetic changes between parent and rifamycin mutants from the serial passage studies. The results suggest that similar to rifaximin, rifamycin resistance is likely due to the presence of chromosomal mutation in the *rpoB* gene or a stable de-regulated efflux pump. Mutations in the *rpoB* gene result in changes in the binding site thus decreasing rifaximin affinity to DNA dependent RNA polymerase. However, no specific molecular analyses were conducted in rifamycin mutants in the study[41] . (Farrell, Putnam et al. 2011)

Reviewer's comments:
Rifamycin showed in vitro activity against clinical enteropathogens associated with travelers' diarrhea. The spontaneous mutation frequency rate ranged from 10^{-7} to 10^{-10} at 4x – 16x MIC. Rifamycin mutants were observed in vitro following exposure to 4x to 16x MIC for enteropathogenic E. coli strains. Isolates showing an increase in rifamycin MIC were observed following at least 2 – 3 passages. These rifamycin mutants were stable, maintaining increased MIC values on antibacterial free medium. However, molecular characterization of the mechanism for rifamycin resistance was not determined.

8.5 Susceptibility Testing Methods

The MICs were determined by the broth microdilution in accordance with CLSI methods M07-A7 [2006][36]. For Studies 08-COS-01 and 09-COS-01, the microdilution panels were produced by JMI laboratories and utilized cation-adjusted Mueller-Hinton broth for testing of *E. coli* strains, whereas *Campylobacter* spp. was tested in CAMHB plus 5% laked horse blood (LHB). For CB-01-11 study, custom Sensititre®microbroth dilution MIC panels were manufactured by ThermoFisher (Westlake, OH, USA). Testing of *C. difficile* isolates was determined by the agar dilution method in accordance with CLSI methods M11-A07 [2012]. Plates containing concentrations of rifamycin were tested on *Brucella* agar supplemented with laked sheep blood. Plates were incubated under anaerobic conditions for 24 hours and results were confirmed after 48 hours[39].

Reviewer's comments: *No in vitro susceptibility testing for rifamycin is proposed by the Applicant. No interpretive criteria for rifamycin are recommended.*

8.6 Pharmacodynamics

Rifamycin a non-absorbable antibacterial drug with limited systemic absorption (*for further details see* **Section 6 Clinical Pharmacology**).

8.7 Clinical Microbiology

This section summarizes analyses of the microbiologic data of the individual study results from the two phase 3 studies (C2009-0201 and RIT-1/AID) and two supportive phase 2 studies (Study# CB-01-11/02 and Study# CB-01-11/03).

8.7.2 Phase 2 study

The two phase 2 studies of rifamycin were conducted in subjects with infectious diarrhea. CB-01-011/02 was a comparative study of rifamycin compared to the marketed drug rifaximin (Normix®) in subjects with infectious diarrhea. CB-01-011/03 was a dose finding study using 3 dosing regimens of rifamycin.

8.7.2.1 CB-01-011/02

Study CB-01-11/02 was a multi-center, double-blinded, double-dummy, randomized, active-comparator study that evaluated the efficacy and safety of rifamycin compared to rifaximin (Normix®) in subjects with infectious diarrhea (for further details on see Section 7.2.3). Rifamycin did not differ from rifaximin in terms of the rate or frequency of therapeutic success. The cure rate in patients receiving rifamycin was 55% compared to 58% in the rifaximin-treated group. Enteropathogens were identified in few patients (Table 47). In the rifamycin-treatment group, 3 of 6 cases had isolates of *S. enteritidis, C. jejuni,* and *Blastocystis hominis* at study entry that were still present after treatment. In the rifaximin treatment group, isolates of *Campylobacter lari* and *Cryptosporidium* oocytes were detected in stools in 2 patients before and after treatment. A general overgrowth of yeasts, was also observed in both treatment groups.

Table 47: Number of subjects with enteropathogens in stools both in pre- and post-treatment

Isolates	Nr. of Patients Who Received Treatment with:							
	Rifamycin SV				Rifaximin			
	N	Findings at the pre-treatment assay	Findings at the post-treatment assay	Findings at both assays in the same subject	N	Findings at the pre-treatment assay	Findings at the post-treatment assay	Findings at both assays in the same subject
	46				49			
Blastocystis hominis		1	1	--		6+1*	2	--
Campylobacter jejuni		1*+1	--	1		1*+2	1	--
Candida albicans		--	2	--		--	--	--
Salmonella enteritidis		1	1	1		--	--	--
Campylobacter lari		1	--	--		1*	--	1
Entamoeba species		--	--	--		--	1	--
Cryptosporidium		--	--	--		--	--	1
Escherichia coli		1	--	--		--	--	--
Anaerobiospirillum		1	--	--		--	--	--
Shigella flexneri		1	--	--		--	--	--
Shigella boydii		--	--	--		1	--	--
Shigella sonnei		--	--	--		1	--	--
Yeasts NOS		2	12	5		1*+4	9	2

*: post-treatment specimen was not available for analysis.

Source: DiStefano et al., Anti-Infective Agents 2013 (Table 3)

Reviewer comments: *The study demonstrated that rifamycin TLUS was numerically similar to rifaximin's when both agents were administered four times daily for 3 days. It is important to note that the rifamycin treatment regimen evaluated in this study is different from the proposed regimen of 400 mg twice daily (BID) for 3 consecutive days. In addition, the rifaximin regimen in the study was not the approved recommended regimen of 200 mg given three times daily (TID) for 3 days. No conclusions can be made on the efficacy of rifamycin compared to rifaximin due to small study size and the limited number of pathogens identified in the study.*

8.7.2.2 CB-01-011/03

Study CB-01-11/03 was a multicenter, double-blind, randomized parallel group study that assessed the efficacy and safety of different doses of rifamycin in patients with infectious diarrhea (for further details see Section 7.2.3). At baseline, 97.3% (36/37) of study subjects stools were culture positive for *E. coli*. Follow-up stool cultures were not obtained at the end of therapy.

Table 48: Presence of bacterial pathogens at baseline across treatment groups (CB-01-011/03)

COPROCULTURE	Item	All (N=37)	400 mg (N=13)	800 mg (N=12)	1200 mg (N=12)	P(test)
BACTERIAL GROWTH	Negative	1*	1*	0	0	1.000
	Positive	36	12	12	12	(Fisher)
ISOLATED BACTERIA§						
Escherichia Coli	Presence	33	11	11	11	
Enterobacter spp	Presence	9	3	2	4	
Citrobacter freudii	Presence	16	6	4	6	
Proteus spp	Presence	11	4	2	5	
Morganella morganii	Presence	4	2	1	1	
Serratia marcescens	Presence	6	1	2	3	
Klebsiella pneumoniae	Presence	2	0	1	1	
Providencia rettgeri	Presence	3	1	1	1	

* The presence of leucocytes confirmed the diagnosis of infectious diarrhoea
§ multiple answers are possible

Source: CB-01-011-03 Table 12

Reviewer comments: *The study supported selection of 400 mg rifamycin dose administered twice daily for 3 consecutive days. This was based on the primary endpoint of TLUS showing the shortest median TLUS for an 800 mg/day regimen. No conclusions can be made on the pathogen-specific efficacy of rifamycin due to small number of pathogens identified in the study. In addition, no-follow up microbiological assessments were performed post-treatment.*

8.7.3 Phase 3 studies

This section of the review will focus on the microbiologic aspects of the two phase 3 studies (*for further details on study design and clinical efficacy see* Section 7 Statistical and Clinical Evaluation). The microbiology results for the two pivotal studies are provided separately.

Stool samples were collected at Day 1 as well as on Day 4/5 for microbiological assessment. Each sample was evaluated for the presence of gross blood and/or mucus. Samples were sent to a central laboratory ((b) (4)) for pathogen identification. Stool samples were evaluated for a range of pathogens including diarrheagenic *E. coli* (enterotoxigenic *E. coli* [ETEC] producing heat labile toxin [ETEC LT] or heat stable toxin [ETEC ST] or both [ETEC LT/ST], enteroaggregative *E. coli* [EAEC], diffusely adherent *E. coli* [DAEC]), other invasive bacteria (*Salmonella* spp., *Shigella* spp., *Campylobcter* spp., *Vibrio* spp., *Aeromonas* spp. and *Plesiomonas* spp.), protozoa (*Entamoeba histolytica, Cryptosporidium* spp., *Giardia* spp.) and viruses (Norovirus). Antimicrobial susceptibility testing was performed at the central laboratory. The MIC values were determined

for pre-treatment (visit 1) and post-treatment (visit 3) bacterial pathogens and the emergence of resistance to study drug was evaluated.

8.7.3.1 Study# C2009-0201

A total of 264 subjects with TD were included in the ITT population. At baseline 68.6% (181/264) of subjects had a pathogen identified in the stools (Table 49). Most subjects (60.8%) had one pathogen isolated and 39.2% (71/181) had $\geq$ 2 pathogens identified. Diarrheagenic *E. coli,* identified in 62.9% of 264 subjects, was the predominant enteric pathogen. Forty-one percent of the subjects had at least an enterotoxigenic *E. coli* that produced heat-stable toxin, 3.4% isolates produced heat-stable toxin and 5.3% produced both heat-labile and heat-stable toxins. Non-diarrheagenic *E. coli* pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Campylobacter* spp., *Plesiomonas* spp. accounted for 6.8% of subjects in the study. In addition, less than 5% of subjects had diarrhea due to protozoan parasites (*Cryptosporidium parvum, Giardia lamblia* and *Entamoeba histolytica*) or viruses (Norovirus). Mixed infections accounted for 39.1% (52 of 133) subjects in the rifamycin treated group and 39.6% (19 of 48) subjects of the placebo-treated group. Consistent with the eligibility criteria where subjects were excluded if they had acute bacterial infection or systemic infection, no subjects had gross blood and few subjects (11.6% in rifamycin versus 16% in placebo groups) had mucus in stools.

Table 49: Baseline Microbial characteristics by subject (Study# C2009-0201, ALL PATIENTS)

Pathogen	Rifamycin MMX (n = 199) n/N (%)	Placebo (n=65) n/N (%)
Pathogen Identification at baseline		
Pathogen Negative	66 (33.2%)	17 (26.2%)
Pathogen Positive	133 (66.8%)	48 (73.8%)
1	81 (40.1%)	29 (44.6%)
2	38 (19.1%)	14 (21.5%)
3	12 (6.0%)	5 (7.7%)
4	1 (0.5%)	0
5	1 (0.5%)	0
Pathogen Identified		
Diarrheagenic *E. coli*	125 (62.8%)	41 (63.1%)
Other bacterial pathogen	10 (5.0%)	5 (7.7%)
Parasite or Virus	12 (6.0%)	6 (9.2%)
Macroscopic Stool Findings	9	
Presence of gross blood	0 (0.0%)	0 (0.0%)
Presence of mucus	23/199 (11.6%)	8/65 (12.3%)
Presence of gross blood and mucus	0 (0.0%)	0 (0.0%)

Source: C2009-0201 CSR (Table 7)

The microbiological eradication rates were numerically higher in the rifamycin treatment group compared with placebo; but the difference did not reach statistical significance. Significant treatment differences were not observed in the patients without a pathogen identified at baseline as the placebo subjects had shorter TLUS; in addition, higher clinical cure rates were observed in the pathogen negative placebo subjects than pathogen positive subjects (Table 50).

Table 50: Summary of TLUS, clinical cure and microbiological endpoints in subjects enrolled in C2009-201 (ITT population)

Endpoint	Rifamycin (n = 199)	Placebo (n =65)
Media TLUS in hours (95% CI)		
All patients	46.0 (42.8, 50.5)	68.0 (48.7, NC)
Pathogen positive	48.2 (41.7, 56.1)	68.3 (56.4, NC)
Pathogen negative	45.3 (31.0, 51.3)	40.5 (1.1, NC)
Clinical Cure (n/N [%])		
All Patients	162/199 (81.4%)	37/65 (56.9%)
Pathogen positive	99/133 (74.4%)	22/48 (45.8%)
Pathogen negative	63/66 (95.0%)	15/17 (88.2%)
Microbial Eradication (n/N; %)		
All pathogens	124/185 (67.0%)	34/62 (54.8%)
Diarrheagenic *Escherichia coli*	108/178 (60.7%)	28/59 (47.5%)
Other Bacterial pathogens	9/11 (81.8%)	5/7 (71.4%)
Protozoa	4/9 (44.4%)	1/6 (16.7%)
Norovirus	2/4 (50.0%)	1/1 (100.0%)

TLUS = time to the last unformed stool; CI = confidence interval; NC= not calculable
Source: C2009-0201 CSR (Table 14-2-1); Table 14, Table 15

Table 51 shows the *E. coli* eradication in the rifamycin group was 67.1% compared to 57.1% in the placebo group..

Table 52 shows the response for subjects with a single pathogen identified at baseline. Against the diarrheagenic *E. coli* as a single pathogen, rifamycin had a microbiological eradication rate of 62.3% subjects and a clinical cure of 71.6%. In the placebo arm, the microbiological eradication rate was 60.7% and clinical cure was 66.7%.

Eradication rates were high for other invasive bacterial pathogens (i.e., *Campylobacter jejuni, Shigella* spp., *Salmonella* spp., *Plesiomonas* spp. and *Vibrio* spp.) in the rifamycin group (90.9%) compared to placebo group (71.4%). Of note, there were no patients that had a single pathogen due to *Plesiomonas* spp., *Salmonella* spp., *Shigella* spp or *Vibrio* spp in stool specimens. Most of these invasive bacterial pathogens were found in combination with a diarrheagenic *E. coli*. There was only 1 patient with *Campylobacter jejuni* infection in the rifamycin treatment arm; this patient had a clinical cure and the pathogen was also eradicated.

Table 51: Microbiological eradication, clinical cure and Median TLUS by patient and pathogen type (MITT Population, C2009-201)

	Microbiological Eradication		Clinical Cure		TLUS in hours	
Pathogen	**Rifamycin MMX (n = 133) n/N (%)**	**Placebo (n=48) n/N (%)**	**Rifamycin MMX (n = 133) n/N (%)**	**Placebo (n=48) n/N (%)**	**Rifamycin MMX (n = 133) Median [range]**	**Placebo (n=48) Median [range]**
E. coli			**99/133 (74.4%)**	**22/48 (45.8%)**	**48.2 [41.7, 56.1]**	**68.3 [56.4, NC]**
ETEC heat stable toxin	45/82 (54.9%)	13/27 (48.1%)	68/82 (82.9%)	13/27 (48.1%)	46.7 [42.0, 53.1]	79.2 [51, NC]
ETEC heat labile toxin	6/7 (85.7%)	1/2 (50.0%)	4/7 (57.1%)	1/2 (50.0%)	90.5 [21.5, NC]	--
ETEC heat stable/labile	8/11 (72.7%)	1/3 (33.3%)	8/11 (72.7%)	0/3 (0.0%)	50.5 [0, NC]	--
EAEC	31/48 (64.6%)	9/16 (56.3%)	36/48 (75.0%)	8/16 (50.0%)	47.2 [42.8, 69.2]	67.6 [37.4, NC]
Diffusely adherent *E. coli*	18/30 (60.0%)	4/11 (36.4%)	25/30 (83.3%)	6/11 (54.5%)	46.4 [24.7, 69.2]	67.6 [36.3, NC]
Invasive Pathogens	**10/11 (90.9%)**	**5/7 (71.4%)**	**8/11 (72.7%)**	**4/7 (57.1%)**	**18.5 [0, NC]**	**69.2 [13.6, NC]**
Campylobacter jejuni	3/4 (75.0%)	3/3 (100.0%)	4/4 (100.0%)	1/3 (33.3%)	13.5 [3.2, 22.2]	--
Plesiomonas spp.	2/2 (100.0%)	1/1 (100.0%)	1/2 (50.0%)	1/1 (100.0%)	--	13.6 [NC, NC]
Salmonella spp.	2/2 (100.0%)	1/3 (33.3%)	1/2 (50.0%)	2/3 (66.7%)	--	44.8 [13.6, NC]
Shigella spp.	2/2 (100.0%)	--	1/2 (50.0%)	--	--	--
Vibrio spp.	1/1 (100.0%)	--	1/1 (100.0%)	--	--	--
Protozoa	**5/9 (55.6%)**	**0/5 (0.0%)**	**8/9 (88.9%)**	**2/5 (40.0%)**	**43.0 [5.2, 64.3]**	**--**
Cryptosporidium parvum	1/4 (25.0%)	0/4 (0.0%)	3/4 (75.0%)	1/4 (25.0%)	--	--
Entamoeba histolytica	3/3 (100.0%)	--	3/3 (100.0%)	--	--	--
Giardia lamblia	0/2 (0.0%)	0/1 (0.0%)	2/2 (100.0%)	1/1 (100.0%)	--	--
Norovirus	**2/4 (50.0%)**	**1/1 (100.0%)**	**4/4 (100.0%)**	**0/1 (0.0%)**	**--**	**--**

Table 52: Microbiological eradication, clinical cure and Median TLUS by patient and pathogen type with a single pathogen (MITT Population, C2009-201)

	Microbiological Eradication		Clinical Cure		TLUS in hours	
Pathogen	**Rifamycin MMX (n = 81) n/N (%)**	**Placebo (n=29) n/N (%)**	**Rifamycin MMX (n = 81) n/N (%)**	**Placebo (n=29) n/N (%)**	**Rifamycin MMX (n = 81) Median [range]**	**Placebo (n=29) Median [range]**
E. coli			**58/74 (71.6%)**	**15/23 (51.7%)**	**49.3 [33.8, 61.4]**	**63.8 [49.5, NC]**
ETEC heat stable toxin	23/42 (59.0%)	9/12 (75.0%)	35/42 (83.3%)	8/12 (66.7%)	50.4 [31.2, 61.4]	68.2 [8.3, NC]
ETEC heat labile toxin	4/4 (100.0%)	0	2/4 (50.0%)	0	--	--
ETEC heat stable/labile	2/3 (33.3%)	0/1 (0.0%)	2/3 (66.7%)	0/1 (0.0%)	41.2 [0, NC]	--
EAEC	11/19 (57.9%)	4/7 (57.1%)	14/19 (73.7%)	5/7 (71.4%)	45.7 [23.3, 69.7]	49.5 [7.4, NC]
Diffusely adherent *E. coli*	5/6 (83.3%)	1/3 (33.3%)	5/6 (83.3%)	2/3 (66.7%)	39.6 [4.5, NC]	59.7 [58.8, NC]
Invasive Pathogens	**1/1 (100.0%)**	**2/2 (100.0%)**	**1/1 (100.0%)**	**1/2 (50.0%)**	**NC**	**NC**
Campylobacter jejuni	1/1 (100.0%)	2/2 (100.0%)	1/1 (100.0%)	1/2 (50.0%)	NC	NC
Parasites and Viruses			**6/6 (100.0%)**	**2/4 (50.0%)**	**NC**	**NC**
Cryptosporidium parvum	1/2 (50.0%)	0/2 (0.0%)	2/2 (100.0%)	1/2 (50.0%)	NC	NC
Giardia lamblia	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	1/1 (100.0%)	NC	NC
Norovirus	2/3 (66.7%)	1/1 (100.0%)	3/3 (100.0%)	0/1 (0.0%)	NC	NC

Table 53 shows the *in vitro* antimicrobial activity of rifamycin against all bacterial pathogens isolated from study subjects before and after treatment. In the pre-treatment isolates, the rifamycin MIC values were similar for all subtypes of *E. coli* (MIC values ranging from 4 μg/mL to 1024 μg/mL with MIC_{50} values of 16 μg/mL and MIC_{90} values 32 -128 μg/mL). Decreased susceptibility to rifamycin was observed in the *E. coli* isolates still present after rifamycin treatment with MICs increasing at least 8-fold to 64-fold with $MIC_{50/90}$ values ≥ 512 μg/mL and ≥ 1024 μg/mL, respectively. In the post-therapy visit, at least 18.5% (33/178) of *E. coli* isolates had rifamycin MICs ≥ 512 μg/mL. Similar increases were observed for rifaximin against *E. coli* subtypes between pre-treatment (MIC_{90} values 128 μg/mL) and post-treatment (MIC_{90} values > 1024 μg/mL). In contrast, no significant increase in MIC_{90} values were observed between baseline and visit 3 for azithromycin, ciprofloxacin or trimethoprim-sulfamethoxazole against *E. coli* subtypes. For the other invasive bacterial pathogens, the baseline rifamycin $MIC_{50/90}$ ranged from 8 – 64 μg/mL; for a single *Campylobacter jejuni* isolate MIC was >1024 μg/mL.

Table 53. Activity (MIC) of rifamycin in pre-treatment and post-treatment bacterial isolates in the rifamycin treatment group

MICs	Pre-Treatment				Post-Treatment			
	N	MIC_{50}	MIC_{50}	Range	N	MIC_{50}	MIC_{50}	Range
Escherichia coli		**16**	**64**	**4 - ≥1024**				
ETEC heat stable toxin	78	16	32	4 - ≥1024	35	≥1024	>1024	16 - >1024
ETEC heat labile toxin	7	--	--	8 - 128	2	--	--	>1024
ETEC heat stable/labile	11	16	32	8 - 64	5	--	--	512 - >1024
EAEC	48	16	64	8 - >1024	14		≥1024	8 - >1024
Diffusely adherent *E. coli*	29	16	64	8 - 64	13	≥1024	>1024	16 - >1024
Other Bacterial Pathogens		**32**	**64**	**8 - 64**				
Campylobacter jejuni	4	--	--	16 - 64	3	--	--	>1024
Plesiomonas spp.	1	--	--	8	--	--	--	
Salmonella spp.	2	--	--	16 - 64	2	--	--	32 - 64
Shigella spp.	2	--	--	16 - 32	--	--	--	--
Vibrio spp.	1	--	--	64	--	--	--	--

Source: CSR C2009-0201 [Table 21a]

Reviewer comments: *Rifamycin was significantly more efficacious than placebo for the treatment of acute travelers' diarrhea, with median duration of diarrhea being 46.0 hours compared to 68 hours, respectively. The predominant pathogen isolated at baseline was diarrheagenic E. coli enterotoxigenic (producing heat-labile or heat-stable toxins) as well as enteroaggregative strains. Of patients infected with E. coli, the clinical cure rate for rifamycin-treated subjects was 74.4%. There were no patients with a single baseline pathogen such as Plesiomonas spp., Salmonella spp., Shigella spp. or Vibrio spp. in stool. A single patient with Campylobacter jejuni infection was enrolled in the rifamycin treatment arm; this patient had a clinical cure and the pathogen was also eradicated. The rifamycin MIC against diarrheagenic E. coli in the clinical study was 16 μg/mL, similar to surveillance $MIC_{50/90}$ values of 32 -128 μg/mL. Increase in MICs were observed in E. coli isolates still present after rifamycin treatment.*

8.7.3.2 Study# RIT-1/AID

A total of 835 subjects were enrolled in the study, of whom 420 received rifamycin and 415 subjects received ciprofloxacin (Table 54). Macroscopic stool findings were similar among treatment groups. Mucus diarrhea was observed in 26.2% of subjects and bloody diarrhea with fever in 6.7%; 4.8% of subjects had gross blood and 1.5% of subjects had both gross blood and mucus in stools. At baseline, 62.9 % (525/835) of the subjects had a causative pathogen identified in the stools, with 76.7% of the subjects having diarrheagenic *E. coli*. Monomicrobial infections accounted for 58.6% - 64.2% of the infections across the treatment groups. Subjects that had a mixed infection often had a diarrheagenic *E. coli* with another diarrheagenic *E. coli*, other invasive bacterial pathogen, protozoan, or virus.

Table 54: Baseline Microbial characteristics by subject (Study# RIT-1/AID, FAS Dataset)

Pathogen	Rifamycin (n = 420) n/N (%)	Ciprofloxacin (n=415) n/N (%)
Macroscopic Stool Findings		
Presence of gross blood	19 (4.5%)	21 (5.1%)
Presence of mucus	115 (27.4%)	104 (25.1%)
Presence of gross blood and mucus	7 (1.7%)	6 (1.5%)
Presence of bloody diarrhea or fever	31 (7.4%)	25 (6.0%)
Pathogen Identification at baseline		
Pathogen Negative	152 (36.4%)	158 (28.4%)
Pathogen Positive	268 (63.8%)	257 (61.9%)
1	157 (37.6%)	165 (40.1%)
2	81 (19.4%)	68 (16.5%)
3	22 (5.3%)	16 (3.9%)
4	6 (1.4%)	4 (1.0%)
5	0	1 (0.2%)
Pathogen Identified in subjects with:		
Diarrheagenic *E. coli*	206/268 (81.6%)	217/257 (81.1%)
Other bacterial pathogens	47/268 (17.8%)	55/257 (21.7%)
Parasites and Viruses	68/268 (25.6%)	51/257 (20.1%)

Source: RIT-1/AID Study Report Table 14.1.1.20

The median time to last unformed stool was shorter in the ciprofloxacin treatment group (40.3 hours) compared rifamycin group (44.3 hours, Table 55). Clinical symptoms were resolved within 120 hours in 85.0% in the rifamycin treatment group and 84.8% in the ciprofloxacin treatment group. In subjects with a microbiologically confirmed causative pathogen, clinical cure was achieved in 83.5% (222/266) of the subjects treated with rifamycin group and 87.4% (130/161) subjects in the ciprofloxacin group. However, microbiological eradication (defined as absence of all baseline pathogens after treatment) was confirmed in 31.2% of subjects receiving rifamycin and 30.4% of subjects receiving ciprofloxacin.

Table 55: Summary of TLUS, clinical cure and microbiological endpoints in subjects enrolled in RIT-1/AID (ITT population)

Endpoint	Rifamycin (n = 420)	Ciprofloxacin (n =415)
Median TLUS in hours (95% CI)		
All patients	44.3 (40.1, 47.5)	40.3 (33.4, 44.8)
Pathogen positive	45.5 (40.3, 48)	39.1 (31.2, 44.1)
Pathogen negative	42.5 (29.3, 48.5)	43.0 (32.4, 51.3)
Clinical Cure (n [%])		
All Patients	357/420 (85.0%)	352/415 (84.8%)
Pathogen Negative	135/154 (87.7%)	130/161 (80.8%)
Pathogen Positive	222/266 (83.5%)	222/254 (87.4%)
Microbial Eradication (n/N; %)		
All pathogens	221/403 (54.8%)	211/365 (57.8%)
Diarrheagenic *Escherichia coli*	152/287 (53.0%)	144/256 (56.3%)
Other Bacterial pathogens	38/48 (79.2%)	47/58 (81.0%)
Protozoa	23/57 (40.3%)	14/39 (35.9%)
Norovirus	8/11 (72.7%)	6/12 (50.0%)

TLUS = time to the last unformed stool; CI = confidence interval; NC= not calculable
Source: **Reviewer's analysis (ADSL, ADMS, ADTTE, ADEFF2 Datasets); CSR RIT-1/AID Table 14.2.2.34**

A subgroup analysis by pathogen type showed that the median TLUS was shorter in the ciprofloxacin treatment group compared rifamycin group for all pathogen subtypes (Table 56). Overall, the number of subjects achieving clinical cure and/or microbiological eradication were lower for rifamycin than ciprofloxacin for most pathogen subtypes isolated in the study. The presumed eradication rate for diarrheagenic *E. coli*, the predominant causative organism, was 43.3% for rifamycin treated subjects compared to 45.6% for the ciprofloxacin treated subjects. Lower efficacy rates were reported against ETEC heat stable toxin (45.8% v. 50.0%). A similar trend was observed among subjects with monomicrobial infections (Table 57). The presumed microbiological eradication was higher in the rifamycin treatment group than ciprofloxacin treatment group of infections caused by ETEC heat-labile toxin (85.7% v. 50%) and ETEC heat-labile/stable toxin (50.0% v. 0%).

There were no significant difference in clinical response and microbiological eradication between treatment groups among subjects with invasive bacterial pathogens other than diarrheagenic *E. coli* that showed.

Table 56: Microbiological eradication, clinical cure and Median TLUS by patient and pathogen type (RIT-1/AID, MITT population)

	Microbiological Eradication		Clinical Cure		TLUS in hours	
Pathogen	**Rifamycin MMX (n = 420)**	**Ciprofloxacin (n=415)**	**Rifamycin MMX (n = 420)**	**Ciprofloxacin (n=415)**	**Rifamycin MMX (n = 420) Median {95% CI]**	**Ciprofloxacin (n=415) Median {95% CI]**
E. coli	**94/206 (43.3%)**	**94/217 (45.6%)**	**183/206 (84.3%)**	**179/217 (86.9%)**	**45.2 [40.1, 48.0]**	**39.9 [30.9, 45.4]**
ETEC heat stable toxin	65/142 (45.8%)	69/138 (50.0%)	116/142 (81.7%)	123/138 (89.1%)	47.2 [41.0, 54.8]	39.8 [30.2, 47.0]
ETEC heat labile toxin	9/16 (56.3%)	3/11 (27.3%)	14/16 (87.5%)	9/11 (81.8%)	37.4 [18.1, 49.2]	43.6 [19.9, 71.6]
ETEC heat stable/labile	11/17 (64.7%)	6/9 (66.7%)	17/17 (100%)	8/9 (88.9%)	46.3 [18.3, 54.8]	21.7 [18.3, 101]
EAEC	67/112 (59.8%)	66/98 (67.3%)	100/112 (89.3%)	87/98 (88.8%)	41 [30.0, 46.5]	33.1 [27.3, 45.4]
Other Bacterial pathogens	**27/47 (57.5%)**	**32/55 (58.2%)**	**33/47 (70.2%)**	**47/55 (85.5%)**	**55.3 [46.6, 71.2]**	**40.0 [22.0, 51.9]**
Aeromonas spp.	5/8 (62.5%)	8/9 (88.9%)	6/8 (75.0%)	9/9 (100.0%)	46.6 [21.3, 55.3]	20.8 [6.7, 41.8]
Campylobacter jejuni	17/20 (85.0%)	19/26 (73.1%)	14/20 (70.0%)	23/26 (88.5%)	50.3 [35.4, 56.0]	22 [18.8, 51.9]
Salmonella spp.	7/7 (100.0%)	10/12 (83.3%)	6/7 (85.7%)	10/12 (83.3%	50.2 [12, 71.8]	32.5 [18.8, 61]
Shigella spp.	6/9 (66.7%)	7/8 (87.5%)	5/9 (55.6%)	5/8 (62.5%)	71.6 [11, NC]	63.1 [41.5, NC]
Plesiomonas spp.	3/4 (75.0%)	3/3 (100.0%)	3/4 (75.0%)	3/3 (100.0%)	63.8 [35.0, NC]	40.0 [34.0 48.5]
Protozoa or Viruses	**31/68 (45.6%)**	**20/51 (39.2%)**	**60/68 (88.2%)**	**45/51 (88.2%)**	**31.3 [21.7, 46.6]**	**27.5 [20.5, 42]**
Giardia lamblia	27/48 (56.3%)	20/35 (57.1%)	43/48 (89.6%)	31/35 (88.6%)	45.7 [41.5, 48]	24.9 [19.5, 33.3]
Entamoeba histolytica	1/1 (100.0%)	3/3 (100.0%)	1/1 (100.0%)	3/3 (100.0%)	45.8	20.3 [19.3, 29.6]
Cryptosporidium parvum	13/14 (92.9%)	5/6 (83.3%)	12/14 (83.3%)	5/6 (85.7%)	27.9 [11.4, 51.8]	32.4 [4.65, NC]
Norovirus	8/11 (72.7%)	6/12 (50.0%)	8/11 (72.7%)	11/12 (91.7%)	48.2 [27.3, NC]	43.4 [11.9, 57]

TLUS Source: RIT-1/AID1 Table 14.2.1.2, 14.2.1.10, Microbiological cure Source: 14.2.2.28, 14.2.2.3

Source: Created by reviewer using datasets ADSL.xpt, ADMS.xpt, ADTTE.xpt, ADEFF2.xpt

Table 57: Microbiological eradication, clinical cure and Median TLUS by patient and pathogen type with a single infection (RIT-1/AID, MITT Population)

MONOMICROBIAL INFECTIONS	(n = 130)	(n = 149)	(n = 130)	(n = 149)	(n = 130)	(n = 149)
	Microbiological Eradication		Clinical Cure		TLUS in hours	
Pathogen	**Rifamycin MMX (n = 157)**	**Ciprofloxacin (n=165)**	**Rifamycin MMX (n = 157)**	**Ciprofloxacin (n=165)**	**Rifamycin MMX (n = 157) Median {95% CI]**	**Ciprofloxacin (n=165) Median {95% CI]**
E. coli	**61/117 (52.1%)**	**69/130 (53.1%)**	**95/117 (81.2%)**	**111/130 (85.4%)**	**46.5 [40.3, 49.2]**	**43.7 [33,47.7]**
ETEC heat stable toxin	28/62 (45.2%)	43/82 (52.4%)	46/62 (74.2%)	71/82 (86.6%)	52.5 [42, 70.8]	44.0 [31.5, 48]
ETEC heat labile toxin	6/7 (85.7%)	1/2 (50.0%)	6/7 (85.7%)	1/2 (50.0%)	33 [17.9, 45.2]	NC
ETEC heat stable/labile	1/2 (50.0%)	0/1 (0.0%)	2/2 (100%)	0/1 (0.0%)	59.7 [54.8, 64.5]	NC
EAEC	27/46 (58.7%)	31/48 (64.6%)	41/46 (89.1%)	42/48 (87.5%)	39.4 [26, 47.8]	34.5 [25.8, 49.5]
Other Bacterial pathogens	**17/22 (77.3%)**	**17/24 (70.8%)**	**15/22 (68.2%)**	**22/24 (91.7%)**	**56.2 [32, NC]**	**35.7 [18.2, 46]**
Aeromonas spp.	3/3 (100.0%)	3/3 (100.0%)	2/3 (66.7%)	3/3 (100.0%)	56 [55.3, NC]	6.7 [4.2, 44.1]
Campylobacter jejuni	7/10 (70.0%)	7/12 (58.3%)	8/10 (80.0%)	12/12 (100.0%)	50.9 [16, 56.3]	20.4 [17.2, 58.3]
Salmonella spp.	3/3 (100.0%)	4/5 (80.0%)	3/3 (100.0%)	3/5 (60.0%)	71.2 [12, 71.8]	46 [18.5, NC]
Shigella spp.	4/6 (66.7%)	2/3 (66.7%)	2/6 (33.3%)	3/3 (100.0%)	NC	47 [41.5, 54.3]
Plesiomonas spp.	--	1/1 (100.0%)	--	1/1 (100.0%)	--	NC
Parasites and Viruses	**17/28 (60.7%)**	**9/25 (36.0%)**	**22/28 (78.6%)**	**23/25 (92.0%)**	**40.8 [21, 48.2]**	**35 [20.5, 44.8]**
Giardia lamblia	9/16 (56.3%)	7/12 (58.3%)	12/16 (75.0%)	11/12 (91.7%)	28.7 [15.3, 61]	28.2 [16.6, 44.4]
Cryptosporidium parvum	3/3 (100.0%)	1/2 (50.0%)	2/3 (66.7%)	2/2 (100.0%)	47.9 [19.5, NC]	53.0 [21.3, 84.8]
Norovirus	8/11 (72.7%)	6/12 (50.0%)	8/11 (72.7%)	11/12 (91.7%)	48.2 [27.3, NC]	43.4 [11.9, 57]

TLUS Source: RIT-1/AID1 Table 14.2.1.2, 14.2.1.10, Microbiological cure Source: 14.2.2.28, 14.2.2.3

Source: Created by reviewer using datasets ADSL.xpt, ADMS.xpt, ADTTE.xpt, ADEFF2.xpt

Table 58 shows the *in vitro* antimicrobial activity of rifamycin against all bacterial pathogens isolated from study subjects before and after treatment. In the pre-treatment isolates against diarrheagenic *E. coli*, the rifamycin MICs ranged from 8 – >1024 µg/mL with $MIC_{50/90}$ values of 16 and 64 µg/mL, respectively. Increases in MIC for rifamycin s were observed in post-treatment isolates with MICs increasing at least 8-fold to 64-fold. Against the other invasive pathogens, the rifamycin MIC values ranged from 2 – 8 µg/mL for *Aeromonas* spp., 4 – 1024 µg/mL for *Campylobacter* spp, 2 – 4 µg/mL for *Plesiomonas* spp., 32 – 128 µg/mL for *Salmonella* spp, and 8 – 16 µg/mL for *Shigella* spp. There was no direct correlation between MIC and clinical response or bacterial eradication. Pathogens with high MICs (> 8 µg/mL) to rifamycin had clinical cures.

Table 58: Activity (MIC) of rifamycin in pre-treatment and post-treatment bacterial in the rifamycin treatment group

Pathogen group	Pre-Treatment				Post-Treatment			
	N	Range	MIC_{50}	MIC_{90}	N	Range	MIC_{50}	MIC_{90}
Diarrheagenic *E. coli* group	**287**	**8 - >1024**	**16**	**64**	**94**	**8 - >=1024**	**16**	**>=1024**
Enterotoxigenic *E. coli* heat stable toxin	142	8 – 1024	16	64	60*	16 - >1024	16	>=1024
Enterotoxigenic *E. coli* heat labile toxin	16	8 - 1024	16	64	6*	8 - >1024	16	>=1024
Enterotoxigenic *E. coli* heat stable/labile toxin	17	8 – 64	16	64	6	16 - >1024	16	512
Enteraggregative *E. coli*	112	8 - 1024	16	64	22*	8 - >1024	16	>=1024
Invasive pathogens	**48**	**2 - >1024**	**32**	**>=1024**	**7**	**8 - >1024**	**256**	**>1024**
Aeromonas spp.	8	2 – 8	4	8	2*	8	--	--
Campylobacter jejuni	20	4 - 1024	128	1024	3	>=1024	--	--
Plesiomonas spp.	4	2 – 4	2	4	1	256	--	--
Salmonella spp.	7	32 – 128	32	128	--	--		
Shigella spp.	9	8 – 16	16	16	1*	64	--	--

*Not all isolates that were failures were cultured and identified

Reviewer comments: *The overall median TLUS was longer in the rifamycin treatment group (44.3 hours) compared to ciprofloxacin (40.3 hours). The clinical cure at 120 hours after treatment was achieved in 85.0% in the rifamycin treatment group compared to 84.8% in the ciprofloxacin treatment group. However, microbiological eradication (defined as the absence of the baseline pathogen after treatment) was 31.2% in the rifamycin group and 30.4% of in the ciprofloxacin group. The most frequently isolated pathogen from subjects across both treatment groups was diarrheagenic E. coli (76.7%). The pre-treatment rifamycin MICs observed in the clinical study were similar to those observed in surveillance studies. There was no direct correlation between MIC and clinical response or bacterial eradication. Subjects with pathogens with high MICs (> 8 µg/mL) to rifamycin often had clinical cures.*

8.8 Summary and Conclusions

Rifamycin belongs to the ansamycin class of antibacterial drugs and acts by inhibiting the beta-subunit of the bacterial DNA-dependent RNA polymerase, blocking one of the steps in DNA transcription. This results in inhibition of bacterial synthesis and consequently growth of bacteria.

Similar to other ansamycins, resistance have been associated with mutations in the *rpoB* gene which results in changes in the binding site and decreased affinity to DNA dependent RNA polymerase. The spontaneous mutation frequency rate for rifamycin ranged from (b) (4) to 10^{-10} at 4x – 16x MIC. Isolates showing an increase in MICs were observed both *in vitro* and while on treatment following exposure to rifamycin.

Two clinical studies (C2009-201 and RIT-1/AID) evaluated the clinical efficacy of 400 mg rifamycin administered twice daily for 3 days in subjects with traveler's diarrhea. Most isolates were *E. coli*. There was a clinical benefit of rifamycin over placebo in subjects in study C2009-201; rifamycin appeared numerically similar to ciprofloxacin- in study RIT-1/AID in treatment of traveler's diarrhea due to pathogenic *E. coli*. Overall the rifamycin MIC values from the clinical trials (MIC_{90}, 64 µg/mL) against pathogenic *E. coli* including enterotoxigenic and enteroaggregative producing isolates were comparable to isolates from surveillance studies (MIC_{90} , 64 µg/mL). There was no direct correlation with MIC and clinical response or bacterial eradication, since subjects with TD due pathogens with high MICs (> 8 µg/mL) to rifamycin had clinical cures. There were few isolates due to other invasive bacterial pathogens in both studies. These included *Aeromonas* spp. (n =8), *Salmonella* spp. (n =7), *Shigella* spp. (n=9) and *Plesiomonas* spp. (n =4). Most of these isolates were found in mixed infections, often with a diarrheagenic *E. coli*; thus, there were not enough isolates to make a conclusive determination concerning clinical efficacy of rifamycin against bacterial pathogens other than *E. coli*.

The "first list" of microorganisms will include only *Escherichia coli* (enterotoxigenic and enteroaggregative) isolates. No interpretive criteria are recommended.

9 Review of Safety

In this NDA, the Applicant submitted safety data for 761 subjects studied in the development program (phase 1 to 3) who received at least one dose of rifamycin (any dose) and had at least 1 post baseline safety assessment. However, the clinical safety data discussed in this review are primarily derived from two phase 3 trials evaluating rifamycin for the treatment of TD, Studies C2009-0201 and RIT-1/AID. These studies enrolled a total of 619 subjects (199 and 420 in studies C2009-0201 and RIT-1/AID, respectively) who received rifamycin at the dosing regimen proposed for marketing: 400 mg twice a day for 3 days. Phase 3 data were collected from subjects enrolled in 4 countries: India, Mexico, Guatemala, and Ecuador.

Overall, the safety data and analyses provided by the Applicant in this submission were of adequate quality.

Key safety findings include the following:

- **Deaths:** There were no deaths that occurred in clinical studies during the product development.

- **Serious Adverse Events (SAEs), Discontinuations and Dose Modifications:**

    - Only 3 SAEs were reported in the product development of rifamycin; two in C2009-0201, and one in a phase 2 study (see Table 59 below). Only one of these events was deemed to be possibly related to rifamycin, abdominal pain and vomiting 3 days after receiving the study medication leading to hospitalization in a 20 year old female; symptoms resolved after 3 days.

    - Only 6 subjects (1%) receiving rifamycin in phase 3 trials discontinued study medication due to treatment emergent adverse events.

    - A total of 4 rifamycin recipients (1%) in phase 3 studies had dose modifications following treatment emergent adverse events.

- **Treatment Emergent Adverse Events (TEAEs):** The only adverse events that occurred at rate of 2% or greater and were more frequent in the rifamycin group were constipation (3.5% of rifamycin subjects compared with 1.5% of placebo recipients in C2009-0201) and headache (3.3% of rifamycin subjects compared with 1.9% of those receiving ciprofloxacin in RIT-1/AID).

- No clinically significant treatment differences in blood chemistry or hematologic parameters were observed.

- **QT interval:** ECG data were not collected in phase 3 trials. In phase 1-2 studies, no clinically significant changes in the QT interval were observed.

These findings are discussed in more detail below.

9.4.2 Safety Review Approach

The safety review concentrated on Studies C2009-0201 and RIT-1/AID. In C2009-0201, a total of 199 subjects received rifamycin 200 mg orally twice a day for three days. Safety data from Study RIT-1/AID comprised an additional 420 rifamycin-treated subjects. In Safety Section tables, the results for the two studies are presented side by side, where relevant. Phase 2 dose-ranging study CB-01-01-11/03, which included subjects who received up to 1200 mg per day for 7 days for a maximum total dose of 8400 mg was also reviewed for safety.

9.4.3 Review of the Safety Database

Overall Exposure

Table 59: Rifamycin-Exposed Subjects During Product Development – ITT/FAS (Safety Population

Clinical Trial Groups In CSR[1] and (ISS)[2] safety datasets	Rifamycin[2] n=776 (761)	Active Control n=473 (468)	Placebo n=65
Controlled trials for travelers' diarrhea (Phase 3)			
• **C2009-0201** (Rifamycin 400 mg BID for 3 days or 2400 mg total dose)	199	--	65
• **RIT-1/AID** (Rifamycin 400 mg BID for 3 days vs. Ciprofloxacin 500 mg BID for 3 days)	420	415 (414)	--
Controlled trials for infectious diarrhea (Phase 2)			
• **CB-01-11/02** (Rifamycin 400 mg BID for 3 days vs. Rifaximin 200 mg QID)	57 (45)	58 (54)	--
• **CB-01-11/03** (Rifamycin 200 mg BID or 400 mg BID or 400 mg TID for up to 7 days; maximum total dose of 8400 mg)	40 (37)	--	--
Healthy volunteer studies (Phase 1)			
• **CB-01-11** (Rifamycin 2400 mg total dose)	12	--	--
• **CB-01-11/10** (Rifamycin 400 mg single dose oral vs 250 mg IV; 400 mg oral – fed vs 400 mg oral – fasting)	24	--	--
• **CB-01-12/11** (Rifamycin 200 mg QID for 3 days or 400 mg BID for 2 days)	24	--	--

Source: Adapted from M5 5.3.5.2 - Integrated Summary of Safety (ISS), Table 2.1

[1] CSR dataset: Subjects who received at least one dose of rifamycin, whether or not any post-baseline safety information was available

[2] Integrated Summary of Safety (ISS or Safety Population) dataset: Subjects who received at least one dose of rifamycin and who had at least one baseline safety assessment. ISS numbers presented only if different from CSR.

9.4.4 Adequacy of the Safety Database

The overall number of patients exposed to rifamycin in the clinical development program appeared adequate to perform a safety evaluation. The numbers of patients in the greater than 65 years or in other demographic categories were insufficient to conduct subgroup analyses.

9.4.5 Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

The Phase 3 protocols utilized different definitions of TEAEs and recorded enteric symptoms differently. In Study C2009-0201, travelers' diarrhea pre-specified events were recorded as TEAEs. In Study RIT-1/AID, these events were recorded on a separate subject diary, and sites were instructed to only record these types of events as AEs if they significantly worsened or were still present at the end of the dosing period. The Applicant asserts that these different approaches were responsible for the noticeably different incidences for these AEs in the two Phase 3 studies.

Categorization of Adverse Events

All AEs and SAEs were coded using MedDRA Version 12.0. The number and percentage of patients reporting at least one AE/SAE for each System Organ Class and Preferred Term were tabulated. Per the Applicant, for multiple occurrences of the same AE with different severities, the AE with the highest severity was tabulated. For multiple occurrences of the same AE with different relationships to the study drug (related and not related), the AE was tabulated as related. Narratives were prepared for each SAE. Because all subjects enrolled in the phase 3 studies had travelers' diarrhea with accompanying gastrointestinal symptoms, treatment emergent AEs and traveler's diarrhea-related AEs assembled from patient diaries were analyzed and presented by the Applicant separately as TEAEs and diarrhea and other enteric symptoms (see Tables 60-63 below).

Clinical Reviewer Comments: *Analyses on TEAEs and enteric symptoms were repeated using J Review and the Applicant's results were confirmed. Note: In addition to the TEAE analysis excluding SOC for gastrointestinal tract, SOC for Infections and Infestations (which included preferred term such as pyrexia) were also excluded from the applicant's analysis of TEAE but included in the analysis of diarrhea and enteric symptoms. This is a reasonable approach given that all subjects had gastrointestinal symptoms due to the underlying condition.*

Laboratory Findings

The phase 2 and 3 clinical studies included routine laboratory testing of blood chemistries, liver profile, and hematology panels.

Vital Signs

Vital signs, including temperature, systolic and diastolic blood pressure and pulse were monitored for clinically significant changes. No meaningful differences were observed in changes from baseline values between treatment groups.

Electrocardiograms (ECGs)

ECGs were not performed Phase 3 trials but were conducted in the phase 2 protocol CB-01-11/02 as discussed below.

Immunogenicity

Not applicable.

9.4.6 Safety Results

Table 60: Summary of Treatment Emergent Events (TEAEs) for Phase 3 Trials (ISS Safety Population)

TEAEs N subjects (%)	C2009-0201		RIT-1/AID	
	Rifamycin 400 mg BID N=199	Placebo N=65	Rifamycin 400 mg BID N=420	Cipro 500 mg BID N=414
TEAEs	131 (65.8)	46 (70.8)	60 (14.3)	61 (14.7)
• General	43 (21.6)	16 (24.6)	41(9.8)	35 (8.5)
• Diarrhea and other enteric symptoms	119 (59.8)	46 (70.8)	26 (6.2)	31 (7.5)
Serious TEAEs	2 (1.0)	1 (1.5)	0	0
TEAEs leading to discontinuation of dosing	5 (2.5)	9 (13.8)	1 (0.2)	1 (0.2)
TEAEs leading to dose modification	4 (2.0)	2 (3.1)	0	0
TEAEs leading to study withdrawal	0	0	1 (0.2)	0
TEAEs leading to death	0	0	0	0

Adapted from M5 5.3.5.3 ISS Table 8.1

Table 61: Common Treatment Emergent Adverse Events (TEAEs) by System Organ Class (SOC) and Preferred Term for Phase 3 Trials; SOC Incidence ≥ 1% (ISS Safety Population)

TEAEs SOC n (%) • Preferred Term n (%)	C2009-0201		RIT-1/AID	
	Rifamycin 400 mg BID N=199	Placebo N=65	Rifamycin 400 mg BID N=420	Cipro 500 mg BID N=414
All General TEAEs	**43 (21.6)**	**16 (24.6)**	**41 (9.8)**	**35 (8.5)**
Nervous System Disorders	**18 (9.0)**	**7 (10.8)**	**15 (3.6)**	**9 (1.9)**
• Dizziness	1 (0.5)	1 (1.5)	1 (0.2)	1 (0.2)
• Headache	16 (8.0)	6 (9.2)	14 (3.3)	8 (1.9)
• Somnolence	1 (0.5)	0	0	0

TEAEs SOC n (%) • Preferred Term n (%)	C2009-0201 Rifamycin 400 mg BID N=199	 Placebo N=65	RIT-1/AID Rifamycin 400 mg BID N=420	 Cipro 500 mg BID N=414
Gastrointestinal disorders	**9 (4.5)**	**3 (4.6)**	**9 (2.1)**	**5 (1.2)**
• Abdominal Distension	1 (0.5)	0	0	0
• Abdominal tenderness	0	0	0	2 (0.5)
• Aphthous ulcer	0	0	1 (0.2)	9
• Constipation	7 (3.5)	1 (1.5)	2 (0.5)	1 (0.5)
• Diarrhea Hemorrhagic	0	1 (1.5)	2 (0.5)	2 (0.5)
• Dry Mouth	0	0	1 (0.2)	0
• Dyspepsia	1 (0.5)	0	1 (0.2)	0
• Eructation	0	0	1 (0.2)	0
• Feces soft	0	0	1 (0.2)	0
• Gastritis	0	0	0	1 (0.2)
• Gastrointestinal disorder	0	0	0	1 (0.2)
• Hyperchlorhydria	0	0	1 (0.2)	0
Musculoskeletal and connective tissue disorders	**3 (1.5)**	**1 (1.5)**	**7 (1.7)**	**7 (1.7)**
• Arthralgia	1 (0.5)	0	2 (0.5)	1 (0.2)
• Back pain	0	0	2 (0.5)	1 (0.2)
• Flank pain	0	0	0 (0.0)	1 (0.2)
• Muscle spasms	2 (1.0)	1 (1.5)	1 (0.2)	3 (0.7)
• Muscular weakness	0	0	1 (0.2)	0
• Myalgia	0	0	0	1 (0.2)
• Pain in extremity	0	0	0	0
General disorders and administration site conditions	**2 (1.0)**	**1 (1.5)**	**7 (1.7)**	**6 (1.4)**
• Asthenia	0	1 (1.5)	1 (0.2)	1 (0.2)
• Chest pain	0	0	0	1 (0.2)
• Fatigue	2 (1.0)	0	1 (0.2)	0
• Malaise	0	0	1 (0.2)	0
Infections and infestations	**7 (3.5)**	**6 (9.2)**	**2 (0.5)**	**6 (1.4)**
• Amoebic dysentery	0	2 (3.1)	0	1 (0.2)
• Bronchitis	2 (1.0)	0	0	0
• *Clostridium difficile* colitis	0	1 (1.5)	0	0
• *Cyclosporidium* infection	0	0	0	1 (0.2)
• Gastrointestinal infection	0	2 (3.1)	0	0
• Nasopharyngitis	1 (0.5)	0	0	0
• Parasitic gastroenteritis	2 (1.0)	1 (1.5)	0 (0.0)	2 (0.5)
• Paronychia	0	0	0	1 (0.2)
• Pharyngitis	1 (0.5)	0	0	0
• Tonsillitis	0	0	0	1 (0.2)
• Urinary tract infection	2 (1.0)	0	2 (0.5)	1 (0.2)

Adapted from M5 5.3.5.3 ISS Table 9.1
AE terms are coded with MedDRA Version 19.0

Clinical Reviewer Comments: *In phase 3 trials, the treatment emergent adverse event profile of rifamycin was similar to placebo and the active comparator (ciprofloxacin). The only adverse events occurring at rate >1% that was more common in participants receiving rifamycin compared with placebo was constipation in Study C2009-0201(3.5% rifamycin vs. 1.5% placebo), and headache in Study RIT-1/AID (3.3% rifamycin, 1.9% ciprofloxacin).*

C. difficile testing was at the discretion of the clinical investigator and was not routinely performed in the clinical studies of AEMCOLO. Only one participant (placebo in *C2009-0201*) in the phase 3 trials tested positive for *C. difficile.*

Table 62: General Treatment-Emergent Adverse Events (TEAES)[1] by Preferred Term for Phase 3 Trials, Incidence ≥ 0.3% (ISS Safety Population)

TEAEs	C2009-0201		RIT-1/AID	
Preferred Term n (%)	Rifamycin 400 mg BID N=199	Placebo N=65	Rifamycin 400 mg BID N=420	Cipro 500 mg BID N=414
All General TEAEs	43 (21.6)	16 (24.6)	41 (9.8)	35 (8.5)
Headache	16 (8.0)	6 (9.2)	14 (3.3)	8 (1.9)
Constipation	7 (3.5)	1(1.5)	2 (0.5)	2 (0.5)
Pain	1 (0.5)	0	4 (1.0)	4 (1.0)
Urinary tract infection	2 (1.0)	0	2 (0.5)	2 (0.5)
Arthralgia	1 (0.5)	0	2 (0.5)	1 (0.2)
Dysmenorrhea	3 (1.5)	0	0	0
Dyspepsia	1	0	2 (0.5)	0
Fatigue	2 (1.0)	0	1 (0.2)	0
Muscle spasms	2 (1.0)	1 (1.5)	1 (0.2)	3 (0.7)
Vertigo	0	0	3 (0.7)	1 (0.2)
Back pain	0	0	2 (0.5)	1 (0.2)
Bronchitis	2 (1.0)	0	0	0
Conjunctivitis	2 (1.0)	0	0	0
Dizziness	1 (0.5)	1 (1.5)	1 (0.2)	0
Dysuria	2 (1.0)	0	0	2 (0.5)
Parasitic gastroenteritis	2 (1.0)	1 (1.5)	0	2 (0.5)

Adapted from M5 5.3.5.3 ISS Table 9.1
AE terms are coded with MedDRA Version 19.0
[1]General TEAEs excluded enteric infections signs and syndromes; these are summarized separately, in table below.

Table 63: Diarrhea and Other Enteric Symptoms for Phase 3 Trials (ISS Safety Population)

Preferred Term n (%)	C2009-0201		RIT-1/AID	
	Rifamycin 400 mg BID N=199	Placebo N=65	Rifamycin 400 mg BID N=420	Cipro 500 mg BID N=414
Diarrhea and other enteric symptoms	119 (59.8)	46 (70.8)	26 (6.2)	31 (7.5)
Diarrhea	10 (5.0)	6 (9.2)	12 (2.9)	9 (2.2)
Diarrhea infections	10 (5.0)	5 (7.7)	0	0
Other Enteric symptoms	114 (57.3)	45 (69.2)	19 (4.5)	27 (6.5)
Rectal tenesmus	54 (27.1)	25 (38.5)	0	0
Abdominal pain	41 (20.6)	16 (24.6)	4 (1.0)	7 (1.7)
Flatulence	38 (19.1)	19 (29.2)	7 (1.7)	9 (2.2)

Preferred Term n (%)	C2009-0201		RIT-1/AID	
	Rifamycin 400 mg BID N=199	Placebo N=65	Rifamycin 400 mg BID N=420	Cipro 500 mg BID N=414
Nausea	31 (15.6)	16 (24.6)	6 (1.4)	7 (1.7)
Defecation urgency	27 (13.6)	16 (24.6)	2 (0.5)	3 (0.7)
Vomiting	8 (4.0)	2 (3.1)	1 (0.2)	4 (1.0)
Pyrexia	5 (2.5)	4 (6.2)	3 (0.7)	4 (1.0)
Abdominal pain, upper	1 (0.5)	1 (1.5)	2 (0.5)	2 (0.5)

Adapted from M5, 5.3.5.3 ISS Table 11.1
AE terms are coded with MedDRA Version 19.0

Clinical Reviewer Comments: *The Applicant's analysis separated patient diary data by whether AE was treatment emergent or related to travelers' diarrhea. Under protocol C2009-0201, "if the progression of the underlying condition might be reasonably anticipated given the nature and severity of the underlying condition, then the progression of the underlying condition per se will not constitute an AE." The above table demonstrates similar rates of diarrhea and other gastrointestinal symptoms in participants receiving rifamycin compared with either placebo or ciprofloxacin, with slightly more symptoms noted in the comparator arms. The percentage of subjects reporting symptoms was consistently higher in C2009-0201 than RIT-1/AID which may be explained, at least in part, to the RIT-1/AID protocol which directed clinical investigators to record these events only if they significantly worsened or were still present at the end of the dosing period.*

Deaths

No deaths were observed in the clinical development program.

Serious Adverse Events

Table 64: SAE Summary in Clinical Development of Rifamycin

Study	Subject ID	Treatment	Description
C2009-0201	(b) (6)	**Rifamycin**	**28 yo female w/ neuroblastoma 1 month after completing study**
C2009-0201		**Rifamycin**	**20 yo female hospitalized with abdominal pain and vomiting 3 days after last dose of study treatment; Symptoms resolved after 3 days**
C2009-0201		Placebo	21 yo male hospitalized after first dose for *C.difficile* colitis
CB-01-11/02		**Rifamycin**	**18 yo male involved in MVA on first day of study treatment (fracture left femur, chest contusion); hospitalized in intensive care; DC after 2 weeks**
CB-01-11/02		Rifaximin	25 yo male hospitalized after first dose with bacterial gastroenteritis

Study	Subject ID	Treatment	Description
CB-01-11/02	(b) (6)	Rifaximin	22 yo male with history of seizures starting 3 weeks before study enrollment, experienced seizure within 1 day of first dose.

Dropouts and/or Discontinuations Due to Adverse Effects

Adverse events did not appear to be a significant contributor to subject discontinuation.

In C2009-0201, of the 264 subjects enrolled in the study 231 (87.5%) completed the study, with 53 (82.5%) of subjects receiving placebo, and 178 (89.5%) of subjects receiving rifamycin. Discontinuation occurred in 25 (9.5%) due to rescue medication use, 4 (1.5%) for non-compliance or use of prohibited concomitant medication, 3 (1.1%) for withdrawal of consent, and 1(0.4) subject due to discretion of the investigator. Five subjects receiving rifamycin had adverse events leading to discontinuation of dosing. These adverse events are summarized below. No subjects were categorized as withdrawing from the study due to adverse events.

In RIT-1/AID, 814 (97.5%) of 835 subjects enrolled completed the study; 409 (97.4%) receiving rifamycin and 405 (97.6%) receiving ciprofloxacin. The reasons for study discontinuation in subjects receiving rifamycin included: lack of efficacy in 4 (1.0%), lack of patient cooperation in 6 (1.4%) and intolerable adverse events in 1 (0.2%) subject.

Table 65: Adverse Events Leading to Study Discontinuation or Discontinuation of Dosing (Phase 3 Studies)

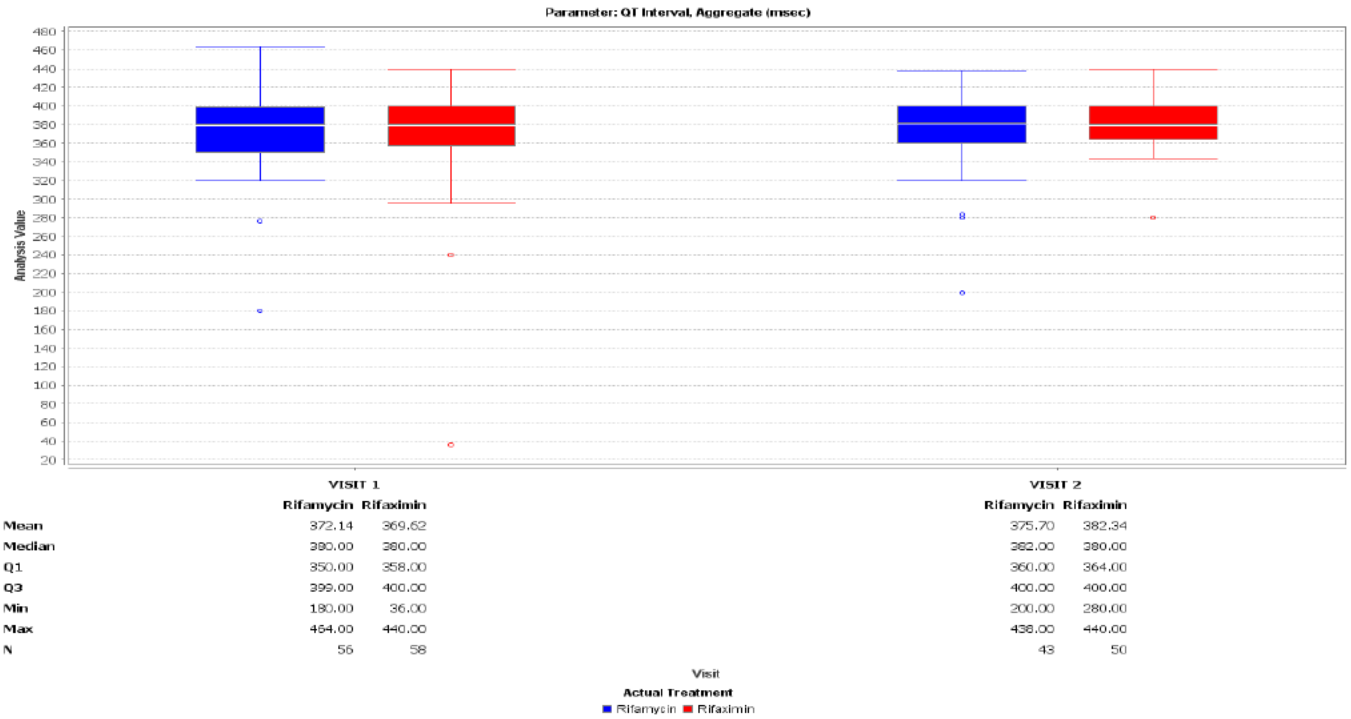
Study Number	Subject Number	AE Leading to Study Discontinuation	AE Leading to Discontinuation of Study Dosing
C2009-0201	(b) (6)	N/A	22 year old Asian male experienced abdominal pain and fever one day after first dose. Study drug was discontinued and subject was started on ciprofloxacin
C2009-0201		N/A	21 year old White male with abdominal pain, fecal urgency, nausea, rectal tenesmus, diarrhea and vomiting one day after first dose. Study drug was discontinued and subject was started on ceftriaxone, ciprofloxacin, and metronidazole.
C2009-0201		N/A	22 year old White male with fever, defecation urgency, fatigue, worsening diarrhea, and headache on day after first dose. Study drug was discontinued and subject was treated with azithromycin and paracetamol.
C2009-0201		N/A	25 year old White male with abdominal pain, defecation urgency, flatulence, rectal tenesmus, vomiting, and worsening of diarrhea on day after first dose. Study drug

Study Number	Subject Number	AE Leading to Study Discontinuation	AE Leading to Discontinuation of Study Dosing
			was discontinued 3 days after first dose and subject was started on azithromycin.
C2009-0201	(b) (6)	N/A	20 year old African American male with loss of appetite one day after first dose and worsening diarrhea 2 days after the first dose. Study medication was discontinued and participant was treated with ciprofloxacin.
RIT-1/AID		68 year old White male experienced worsening of diarrhea 2 days after first dose. Subject was withdrawn from study and treated with ofloxacin.	
RIT-1/AID		N/A	50 year old White male experienced worsening of diarrhea, moderate dehydration, and mild anxiety 2 days after first dose. Study medication was discontinued and subject was treated with cefixime/clavulanic acid.

Electrocardiograms

In CB-01-11-02, a graphical analysis of the QT change from baseline is found below.

Figure 11: CB-01-11-02 -- ECG Change from Baseline - QT



Source: J Review analysis Study CB-01-11-02 (Safety Population)

Clinical Reviewer Comments: *In phase 2 study CB-01-11-/02 which compared rifamycin to rifaximin, no absolute QT prolongation observed. Ten of 43 (23.3%) of subjects receiving rifamycin had changes from baseline QT > 30 ms compared with 6 of 50 (12.0%) rifaximin recipients, but no difference was observed between groups in change from baseline QT > 60.*

9.4.7 Analysis of Submission-Specific Safety Issues

General safety issues with rifamycin class of antimicrobials include hepatoxicity, flu-like syndrome at higher doses, induction of cytochromes P450 (particularly CYP3A4) and attendant drug-drug interactions, and inhibition of hepatic transporters. (Aristoff, Garcia et al. 2010) Specific toxicities observed with rifamycin following its initial introduction in Europe included allergic reactions and fever (in less than 1%) and liver effects including jaundice at higher concentrations. (Bergamini and Fowst 1965). Possible mechanisms for the liver toxicity include glutathione depletion and generation of reactive oxygen in liver microsomes and inhibition of OATP2. (Vavricka, Van Montfoort et al. 2002)

In the literature, a modest and temporary elevation of bilirubin following intravenous rifamycin administration was first described in an early healthy volunteer study following a 2 hour infusion of 1 gram of rifamycin (Acocella, Nicolis et al. 1965). The increase in total bilirubin was observed one hour after starting the infusion, reaching its maximum within two hours following completion, with peak bilirubin of 0.8 to 3.3 mg/d; eight hours following the end of the infusion the serum bilirubin was at pre-infusion levels. In intravenous preparations of rifamycin approved in Europe such as ROFOCINE elevations of bilirubin and transaminases have been reported and are included in the summary of product characteristics (Sanofi Belgium).

The Applicant's analysis of subjects with elevated LFTs at any post baseline visit is presented in Table 66 below.

Table 66: Subjects in Phase 3 Trials with elevated LFTs at any post-baseline visit (ISS Population)

	C2000-0201		RIT-1/AID	
Laboratory Test Criterion, n1/n2 (%)	**Rifamycin 400 mg BID N=199**	**Placebo N=65**	**Rifamycin 400 mg BID N=420**	**Cipro 500 mg BID N=414**
Total Subjects with any Elevated Liver Tests	4/188 (2.1)	0/59 (0.0)	20/381 (5.2)	21/380 (5.5)
AST Elevation				
>3 x ULN	1/188 (0.5)	0/59 (0.0)	6/380 (1.6)	12/380 (3.2)
>5 x ULN	0/188 (0.0)	0/59 (0.0)	4/380 (1.1)	4/380 (1.1)
>10 x ULN	0/188 (0.0)	0/59 (0.0)	2/380 (0.5)	1/380 (0.3)
ALT Elevation				
>5 x ULN	0/188 (0.0)	0/59 (0.0)	5/381 (1.3)	4/380 (1.1)
>10 x ULN	0/188 (0.0)	0/59 (0.0)	3/381 (0.8)	0/380 (0.0)

Laboratory Test Criterion, n1/n2 (%)	C2000-0201		RIT-1/AID	
	Rifamycin 400 mg BID N=199	Placebo N=65	Rifamycin 400 mg BID N=420	Cipro 500 mg BID N=414
AST or ALT Elevation				
>3 x ULN	1/188 (0.5)	0/59 (0.0)	11/380 (2.9)	16/380 (4.2)
>5 x ULN	0/188 (0.0)	0/59 (0.0)	5/380 (1.3)	7/380 (1.8)
>10 x ULN	0/188 (0.0)	0/59 (0.0)	3/380 (0.8)	1/380 (0.3)
TBL Elevation				
>1.5 x ULN	3/188 (1.6)	0/59 (0.0)	7/381 (1.8)	2/380 (0.5)
>2 x ULN	1/188 (0.5)	0/59 (0.0)	4/381 (1.0)	0/380 (0.0)
ALP Elevation				
>2 x ULN	0/188 (0)	0/59 (0)	5/381 (1.3)	4/380 (1.1)
ALT or AST and TBL Elevation				
(ALT > 3x ULN or AST > 3x ULN) and (TBL > 2x ULN)	0/188 (0.0)	0/59 (0.0)	2/381 (0.5)	0/380 (0.0)
(ALT > 3x ULN or AST > 3x ULN) and (TBL > 2x ULN and no ALP >=2x ULN)	0/188 (0.0)	0/59 (0.0)	2/381 (0.5)	0/380 (0.0)

Adapted from M5, 5.3.5.3 ISS Table 30.1

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TBL: Total Bilirubin; ALP: Alkaline Phosphatase; n1 = Number of subjects with an elevated value (worst case scenario); n2 = number of subjects with at least one post-baseline value

Clinical Reviewer Comment: *According to the Applicant's analysis of phase 3 studies, two rifamycin-treated subjects (0.4%) and no subjects in the comparator groups had post-baseline elevations of ALT or AST >3x ULN and total bilirubin >2x ULN without an elevation of ALP ≥2x ULN at the Day 4 (post-treatment) visit. The Applicant states that "these abnormal values were attributed by the investigator to laboratory technical failure and were not accompanied by any adverse clinical sequalae or AEs." (from Module 2, ISE, Clinical Overview, 5.4 Clinical Laboratory Parameters.) The Applicant's response to an information request regarding these two subjects is discussed below*

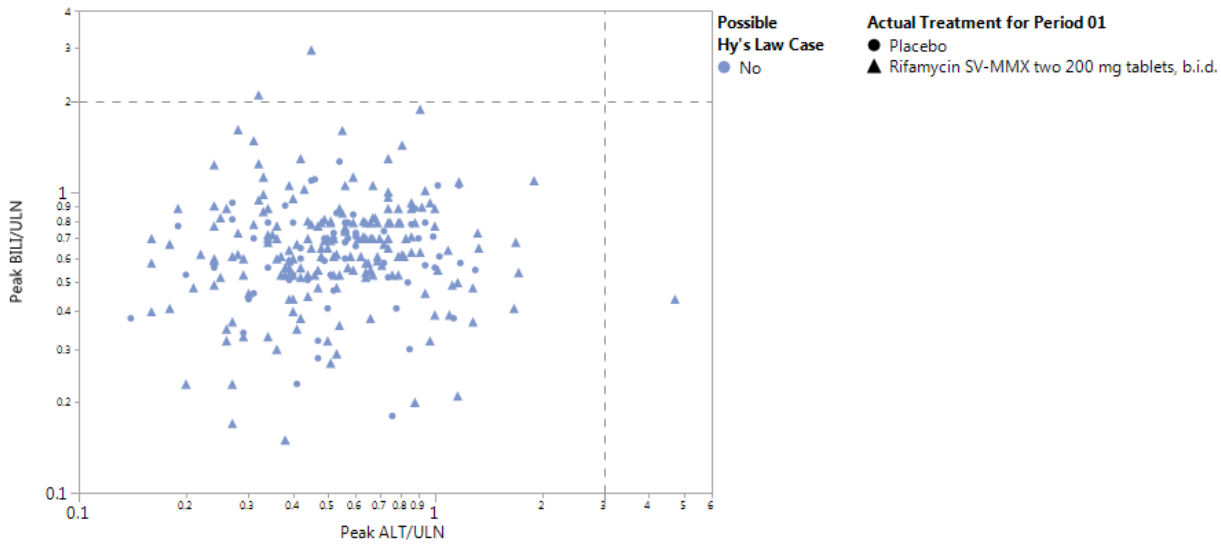
The following figures present the clinical reviewer's analysis of LFT elevation and possible Hy's Law cases using J Review and JMP Clinical.

Clinical Reviewer Comments:

These analyses indicate that some subjects receiving rifamycin experienced a change in baseline bilirubin levels and transaminases compared with those receiving ciprofloxacin. To determine whether any subjects met Hy's Law criteria, data from C2009-0201 and RIT-1/AID were evaluated in more detail.

Hy's Law screening was conducted for the two phase 3 trials; see JMP Clinical analysis below.

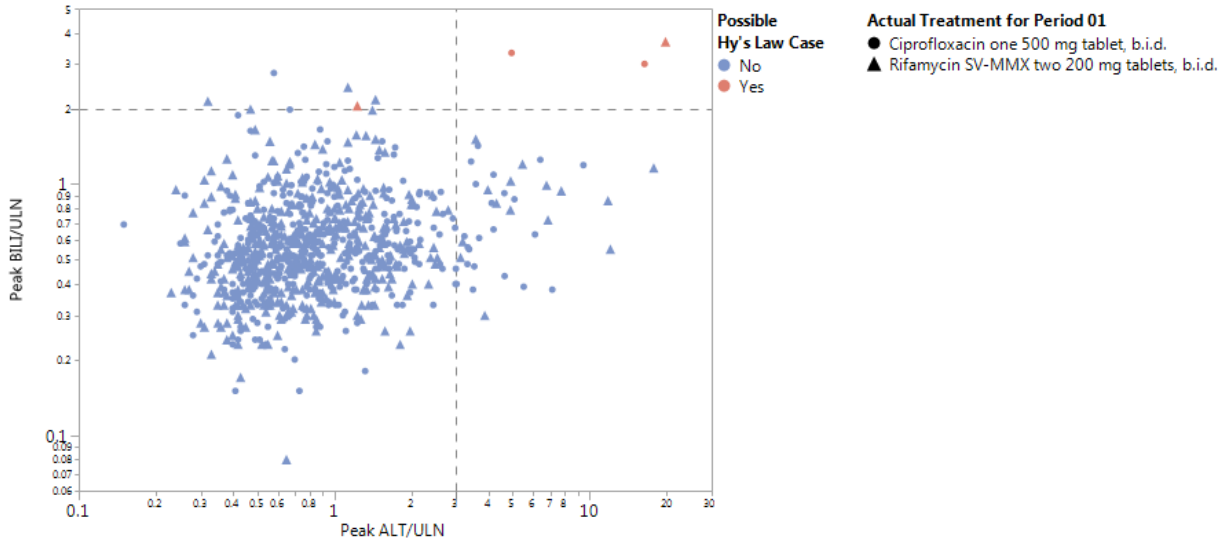
Figure 12: C2009-0201 - Hy's Law Screening (ITT)



Source: JMP Clinical

No Hy's Law Cases were found based on ALT or AST >=3*ULN and BILI >= 2*ULN within 0 Days of ALT/AST peak.

Figure 13: RIT-1/AID Hy's Law Screening (ITT)



Source: JMP Clinical

Possible Hy's Law Cases are flagged if ALT or AST >= 3*ULN and BILI >= 2*ULN within 0 Days of ALT/AST peak.

Possible Hy's Law Cases for <u>Rifamycin:</u>

- Subject ID (b) (6)
- Subject ID (b) (6)

Possible Hy's Law Cases for <u>Ciprofloxacin:</u>

- Subject ID (b) (6)

- Subject ID (b) (6)

Clinical Reviewer Comments: *Screening for Hy's Law in C2009-0201 did not reveal any study subjects meeting the criteria. Screening for Hy's Law in RIT-1/AID identified two subjects in the rifamycin group, and 2 subjects in the ciprofloxacin group. The subjects receiving rifamycin were from two sites,* (b) (6) *and* (b) (6)*.*

In the IR responses dated 10 September and 18 September 2018, the Applicant confirmed that the two subjects they identified with elevated LFTs who received rifamycin were the same subjects identified in the clinical reviewer's Hy's Law screening. Both were enrolled in Study RIT-1/AID. A summary of the clinical information on each subject is found below:

- Subject ID (b) (6) (also identified by randomization number (b) (6)) was a 36-year old Asian male who developed travelers' diarrhea two weeks after arriving in India from Japan. He was randomized and started on rifamycin one day after onset of symptoms. No adverse events were reported, and his symptoms of travelers' diarrhea had resolved by the follow up visit on Day 4. A follow up phone call one month later noted that his general well-being had returned to normal with no adverse events. Selected baseline and follow up laboratory values are found in the table below. No follow up laboratory tests were performed.

Table 67: RIT-1/AID Subject (b) (6) Lab Results

Laboratory Test [normal range]	Visit 1 (Day 1)	Visit 3 (Day 4)	Follow up Testing
ALT [4-36 U/L]	37.05	487.39	none
AST [8-33 U/L]	45.07	712.2	"
Alk Phosphatase [30-120 U/ml]	135.33	112.84	"
Total Bilirubin [0.1-1.2 mg/dL]	0.95	4.43	"
GGT [< 55 U/L]	307.86	46.32	"
Serum Potassium [3.5-5.1 mEq/L]	4.95	5.0	"
Hematocrit [36-46%]	40.50	30.40	"
BUN [8-23 mg/dL]	13.69	8.99	"
Creatinine [0.6-1.2 mg/dL]	1.07	0.92	"

- Subject (b) (6) (also identified by randomization number (b) (6)) was a 42 year old white female who traveled from Russia to India and developed diarrhea two days after arrival. Five days after arrival she was enrolled, randomized and received rifamycin. No adverse events were noted and the symptoms of travelers' diarrhea resolved by Day 4. A follow up phone call one month later also reported no adverse events and noted that her wellbeing had returned to normal. Selected baseline and follow up laboratory values are found in the table below. No follow up laboratory tests were performed.

Table 68: RIT-1/AID Subject (b) (6) Lab Results

Laboratory Test [normal range]	Visit 1 (Day 1)	Visit 3 (Day 4)	Follow up Testing
ALT [4-36 U/L]	30.77	44.29	none

Laboratory Test [normal range]	Visit 1 (Day 1)	Visit 3 (Day 4)	Follow up Testing
AST [8-33 U/L]	31.99	152.11	"
Alk Phosphatase [30-120 U/ml]	143	193.72	"
Total Bilirubin [0.1-1.2 mg/dL]	0.62	2.46	"
GGT [< 38 U/L]	57.2	222.69	"
Serum Potassium [3.5-5.1 mEq/L]	7.22	7.73	"
Hematocrit [36-46%]	29.7	47.2	"
BUN [8-23 mg/dL]	19.4	33.19	"
Creatinine [0.6-1.2 mg/dL]	2.06	2.25	"

Clinical Reviewer Comments: *The Applicant reported that for both these subjects, no adverse events were reported at baseline, during, or after the trial and no significant medical history, concomitant medications, or clinical evidence of liver impairment or drug or alcohol abuse were noted. In both cases the clinical investigators ascribed the LFT elevations to laboratory "technical failure." For Subject* (b) (6) *the clinical investigator's explanation for the technical failure was hemolysis of the laboratory sample, noting the high serum potassium. Hemolysis of blood specimens can impact a wide range of laboratory values, including LFTs. (Agarwal, Vargas et al. 2015) For Subject* (b) (6)*, no explanation for the technical failure was provided, laboratory tests were not repeated, and the Applicant provided no evidence that alternative causes such as viral hepatis were investigated. However, rifamycin is unlikely to have had a causal effect given its negligible systemic absorption.*

9.2.7 Specific Safety Studies/Clinical Trials

No specific safety studies were conducted by the Applicant.

8.2.8 Additional Safety Explorations

Human Reproduction and Pregnancy

During the clinical development of rifamycin for travelers' diarrhea, women who were pregnant, breastfeeding, or not using adequate birth control were excluded from enrollment. There were no reports of pregnancy occurring during clinical trials of this product.

A literature review did not find evaluation of rifamycin in pregnant or lactating women.

Clinical Reviewer Comments: One non-clinical study evaluated the kinetics of rifamycin in serum and milk following a single intravenous infusion in lactating ewes and found a t1/2 of 1.1 hours with approximately 20% of the drug available in milk. Rifamycin was not found in breast milk until 2 hours after intramuscular injection. (Ziv and Sulman 1974) Because systemic absorption of rifamycin in humans is negligible following oral administration of the recommended dose, maternal use of AEMCOLO is unlikely to affect maternal milk production or the breastfed infant.

Human Carcinogenicity or Tumor Development

Pediatrics and Assessment of Effects on Growth

No studies in pediatrics were conducted. See Section 11.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

No cases of overdose were observed during clinical development of rifamycin.

To determine if there are were any cases of overdose from other rifamycin preparations (IV, ocular, topical) approved outside the US, reports submitted to FDA's FAERS system were reviewed. See discussion in next section for details. Review of the 55 FAERS reports identified no overdose cases.

Based on rifamycin's low systemic absorption and good tolerability profile, supportive treatment should be adequate if overdose occurs.

Regarding the potential for accidental ingestion of rifamycin by children, the Applicant plans to use a novel "lock and key" packaging to satisfy requirements for dispensing of AEMCOLO in child-resistant containers. No pediatric overdose cases were found in a literature review. The "Summaries of Product Characteristics" [Sanofi-Aventis 2011] of Rifocin® lists acute toxicity (death) in mice and rats with oral administration, with the dose that causes death in 50% (LD50) of 2120 mg/kg and 2680 mg/kg for mice and rats respectively and requested, translating to a Human Equivalent Dose (HED) of 169.6 mg/kg HED. In response to an IR requesting justification for their risk analysis in pediatric patients, the Applicant submitted a test report on the child-resistant folding box demonstrating that only one of 51 children was able to open the package compared with 50 of 50 adults. In addition, the Applicant noted that AEMCOLO is poorly absorbed from the gastrointestinal tract and ingestion of more than a few tablets of AEMCOLO by children is unlikely due to the large tablet size (19 mm), bitter taste, and the delayed-release coating and matrix which makes chewing difficult. The Applicant has agreed to conduct a Human Factors Study under a Postmarketing Commitment to further evaluate whether the intended population for AEMCOLO can effectively use the packaging to self-administer recommended doses and maintain the child-resistant features.

9.4.8 Safety in the Postmarket Setting (Outside the U.S.)

Because rifamycin is approved in IV, IM and ophthalmic formulations in other countries, FDA's Adverse Event Reporting System (FAERS) database was searched via the Mercado OND Drug Safety Analytics platform. When "rifamycin" was entered into the product active ingredient search, 55 reports were retrieved. Product names included rifamycin, rifamycin S, and rifamycin sodium. Note: For the purposes of this review, a case-level analysis was not performed on all reports. Report counts may include duplicate reports for the same patient from multiple reporters (e.g., manufacturer, family member, physician, pharmacist, nurse), miscoded reports, or unrelated reports.

FDA receipt dates ranged from 1997 to 2018. Forty-one reports were from France, with remaining reports from US (5), Italy (3) and one each from Austria, Great Britain, Japan, Thailand, Turkey, and Taiwan. Thirty-one (56.4%) reports involved female patients, 21 (38.2%) involved males, and 3 (5.5%) did not have gender identified. Age range for cases were as follows: 9 (16.4%) were less than 1 year, 3 (5.5%) were 7 to <17 years, 21 (38.2%) were 17 to < 65 years, 17 (30.9%) were >= 65 years with 5 (9.1%) cases not reported. Most reports (54or 98.0%) were deemed serious, 1 (2.0%) was deemed non-serious.

Additional details on the reports are found in Tables 69 - 71 and discussion below.

Table 69: FAERS Reports by Reported Outcome (%)

Reported Outcomes	Total Cases (%)
Death	10 (18.2)
Hospitalized	38 (69.1)
Life Threatening	2 (3.6)
Disabled	0
Congenital Anomaly	1 (1.8)
Required Intervention	1 (1.8)
Other Outcome	12 (21.8)
Total (Distinct Cases)	**55**

Source: FAERS OND Mercado Drug Safety Analytics Dashboard

Table 70: FAERS Reports – Reported Indication for Rifamycin

Reported Reason For Use	Total Cases	% of Cases
Not Reported	16	29.1%
Infection prophylaxis, prophylaxis, local bacterial prophylaxis, antibiotic prophylaxis	14	25.5%
Product used for unknown indication	4	7.3%
Conjunctivitis	4	7.3%
Dermatitis	3	5.5%
Bronchitis	2	3.6%
Skin disorder	2	3.6%
Toothache	2	3.6%
Tuberculosis, lymph node tuberculosis	2	3.6%
Antiretroviral therapy	1	1.8%
Cataract operation	1	1.8%
Herpes ophthalmic	1	1.8%
Infection	1	1.8%
Mycobacterial infection	1	1.8%
Otitis media acute	1	1.8%
Postoperative care	1	1.8%
SARCOIDOSIS	1	1.8%
Total	**55**	**100.0%**

Source: Adapted from FAERS OND Mercado Drug Safety Analytics Dashboard

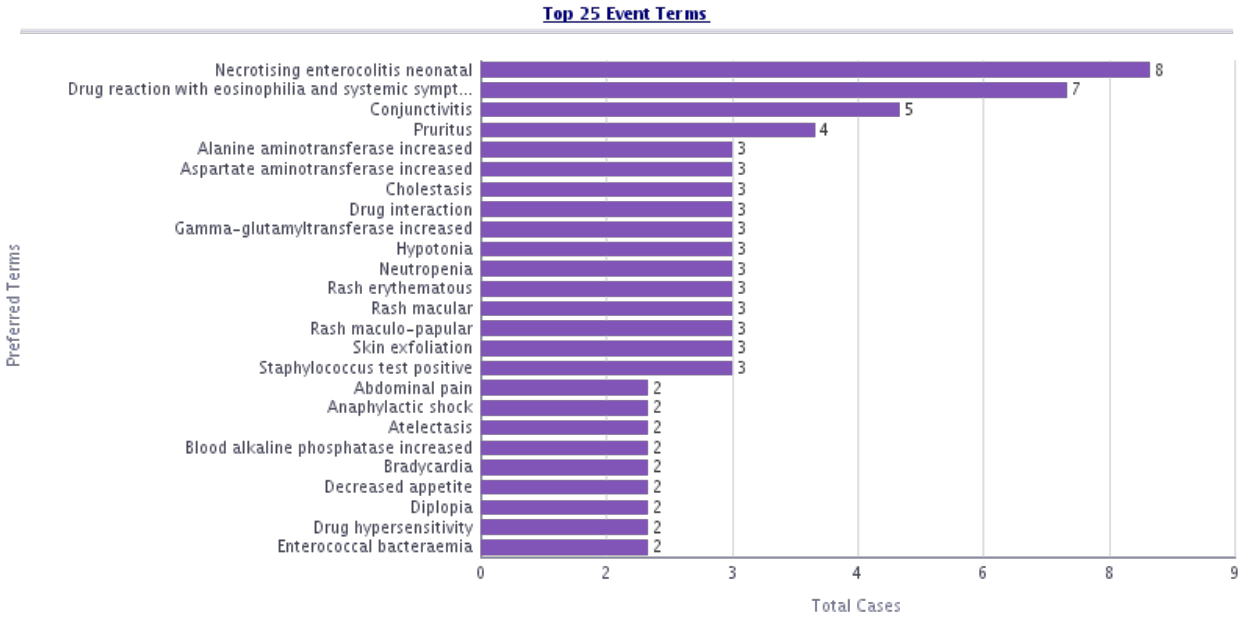
*Total number of cases exceeds reports due to more than one reason provided for use.

Table 71: FAERS Reports by System Organ Class

System Organ Class	Total Reports	% of Reports
Skin and subcutaneous tissue disorders	26	47.3%
Immune system disorders	20	36.4%
Infections and infestations	17	30.9%
General disorders and administration site conditions	16	29.1%
Gastrointestinal disorders	13	23.6%
Investigations	13	23.6%
Vascular disorders	12	21.8%
Eye disorders	11	20.0%
Injury, poisoning and procedural complications	11	20.0%
Blood and lymphatic system disorders	10	18.2%
Pregnancy, puerperium and perinatal conditions	10	18.2%
Musculoskeletal and connective tissue disorders	9	16.4%
Hepatobiliary disorders	8	14.5%
Respiratory, thoracic and mediastinal disorders	8	14.5%
Metabolism and nutrition disorders	6	10.9%
Nervous system disorders	6	10.9%
Cardiac disorders	5	9.1%
Renal and urinary disorders	4	7.3%
Endocrine disorders	3	5.5%
Ear and labyrinth disorders	2	3.6%
Psychiatric disorders	2	3.6%
Reproductive system and breast disorders	2	3.6%
Surgical and medical procedures	2	3.6%
Congenital, familial and genetic disorders	1	1.8%
Total	**55**	**100.0%**

Source: FAERS OND Mercado Drug Safety Analytics Dashboard
*Each case report may contain more than one AE.

Figure 14: FAERS Reports by Preferred Term (Top 25 Event Terms)



Source: FAERS OND Drug Safety Analytics Dashboard

Each of the 55 FAERS reports for rifamycin included concomitant medications, usually multiple. Only one report listed rifamycin IV as the primary suspect product, where a 54 year old female patient with tuberculosis and *Candida* sepsis, also on voriconazole, fluconazole, cefmetazone, and streptomycin experienced pyrexia and chills.

The reports included pediatric patients with necrotizing enterocolitis. The most common adverse events in the FAERS reports were skin reactions and rashes, including DRESS syndrome (five cases), macular-papular eruptions, exfoliation, and pruritis. When reports involving elevated LFTs were combined, these constituted the second most common adverse event group.

Four FAERS cases were associated with rifamycin given orally. All four were submitted as expedited reports from outside the US and were categorized as "serious"; two of the cases appeared to be duplicates and one case appeared to be misclassified in FAERS as being associated with rifamycin as the case report refers to rifampicin. A brief summary of two cases involving orally administered rifamycin follows:

1. Case #3773918 described a 65-year old female hospitalized for septic knee arthritis, thrombocytopenia, and intestinal obstruction. Sodium valproate was started and she experienced acute renal failure and confusion. In addition to rifamycin, additional co-suspect medications included heparin, oxacillin, pefloxacin.
2. Cases #14882530 and #14972887 described a 26-year old female who experienced anaphylactic shock and urticaria on the same day she received spiramycin, riflumic acid, rifamycin, and metronidazole for a toothache. All drugs were discontinued, and patient

recovered.

There were no reports of overdoses or accidental ingestions with rifamycin in FAERS. Nine reports involved children less than 5 years of age (range newborn to 11 weeks). Further review revealed that the case involving the newborn did not involve rifamycin (the exposure was to rifabutin). The remaining 8 reports involved pre-term infants who received rifamycin in an ophthalmic preparation. These infants had multiple medical problems and also received several other concomitant medications; rifamycin was unlikely to be the cause of the AEs reported.

***Clinical Reviewer Comments**:*

Reports to FAERS involved postmarketing use of rifamycin formulations approved outside the U.S. and did not involve AEMCOLO. Most reported IV, IM, ophthalmic, and topical routes of administration; the most common AE system organ class involved skin and subcutaneous disorders such as DRESS. Of note, most FAERS reports involved patients who had serious medical underlying conditions and multiple concomitant medications, so it was difficult to ascertain the role of rifamycin in the etiology of the reported adverse events.

While hypersensitivity reactions have not been reported during the clinical development of AEMCOLO, such adverse events have been reported with other rifamycin products; therefore, it is prudent to include known hypersensitivity to rifamycin as a contraindication in the AEMCOLO label.

Expectations on Safety in the Postmarket Setting

If approved, the Applicant intends to market AEMCOLO using a novel lock and key cardboard carton containing a blister pack of 12 rifamycin tablets. Previous U.S. experience with this packaging has been in products marketed solely for administration by healthcare providers in healthcare settings. As this product is intended for use in adults in an outpatient settings, improper use or damage to the packaging in the outpatient setting could effectively eliminate the child-resistant feature, potentially exposing small children to harm if the contents were inadvertently ingested. The Applicant has agreed to a human factor validation study as a reportable Postmarketing Commitment. (See Section 9.2.8, Additional Safety Explorations -- Overdose) and Section 14, Postmarketing Requirements and Commitments)

9.5 Integrated Assessment of Safety

The safety database included approximately 619 subjects with travelers' diarrhea receiving rifamycin at a dose of 800 mg per day for 3 days.

AEMCOLO is formulated with an enteric coating and controlled-release excipients intended to delay delivery until the colon. When administered orally, rifamycin is poorly absorbed with approximately 83% excreted unchanged in the stool.

In phase 3 studies, rifamycin was well tolerated. In C2009-0201, constipation was the only adverse event that occurred more often in the rifamycin-treated patients at rate of ≥ 2% (3.5% vs. 1.5% of rifamycin and placebo recipients, respectively). In RIT-1/AID, headache was most common, occurring in 3.3% of rifamycin subjects compared with 1.9% of those receiving ciprofloxacin.

There were no deaths in the rifamycin development program. Only three subjects (0.5%) receiving rifamycin in the pooled phase 2-3 trials experienced serious adverse events, with only one (abdominal pain) possibly related to be related to the study medication. Only 6 (1.0 %) subjects in phase 3 studies of travelers' diarrhea discontinued treatment due to adverse events.

Two cases that met laboratory criteria for Hy's Law were identified in phase 3 trials. These cases were not associated with adverse events and did not lead to subject discontinuation. The elevated liver enzymes in one case was thought to be related to a hemolyzed blood sample and in the second case no other obvious reason for the elevated liver enzymes was noted. As no work up for other etiologies was performed, it is difficult to ascertain the reason for the elevated liver enzymes. Given the very limited systemic absorption of rifamycin, it is unlikely to be drug-related.

Based on limited data from phase 2 studies, no clinically significant changes in QT were observed in subjects receiving rifamycin.

Overall, rifamycin administered at a dose of 400 mg twice a day for 3 days was shown to have an acceptable safety profile.

10 Advisory Committee Meeting and Other External Consultations

This application was not discussed at an advisory committee meeting as there were no issues that needed input from the committee.

11 Pediatrics

Under the agreed initial Pediatric Study Plan (iPSP) (FDA Correspondence 10/12/2017), the Applicant submitted a request for partial waiver of the requirements of Pediatric Research Equity Act (PREA) to provide data from pediatric studies of AEMCOLO for the treatment of travelers' diarrhea for the pediatric age group from 0 to 5 years.

The Applicant requested a deferral from the requirements of PREA to provide data from pediatric studies of AEMCOLO for the treatment of traveler's diarrhea for the 6-17 year age group.

For the 6-11 year age group, deferral was requeste (b) (4) Protocol (b) (4) (b) (4) (b) (4) *study to evaluate the safety and efficacy of* (b) (4) *rifamycin* (b) (4) *in the treatment of traveler's diarrhea in children age 6 to 11 years,* is planned (b) (4) The Applicant notes that completion may be hampered due to the limited pediatric patient population in travel clinics, where subjects are recruited.

In the 12-17 year population, the Applicant requested a deferral pending rifamycin approval in adults. The proposed study (b) (4) (b) (4) *study to evaluate the safety and efficacy of* (b) (4) *Rifamycin* (b) (4) *, in the treatment of traveler's diarrhea in children 12 to 17 years,* (b) (4)

At the Pediatric Review Committee (PeRC) meeting on September 5, 2018, the PeRC concurred with the agreed iPSP for a deferral in patients 6 to 17 years. The PeRC also agreed with DAIP's recommendation to change the primary endpoint of the pediatrics studies to reflect (b) (4) The PeRC concurred with the agreed iPSP plan for a partial waiver in children ≤ 5 years, but recommended the Division send an Information Request (IR) to the Applicant requesting documentation (b) (4) .

Clinical Reviewer Comments: The Applicant's IR Response dated September 28, 2018 summarized efforts (b) (4)

12 Labeling Recommendations

12.4 Prescribing Information

High level changes to the proposed labeling are summarized in the Table below

Table 72: Summary of Significant Labeling Changes

Section	Proposed Labeling	Approved Labeling
1. Indications and Usage	AEMOCOLO (b) (4) is indicated for the treatment of travelers' diarrhea in adults. Limitations of Use: (b) (4)	AEMCOLO (b) (4) is indicated for the treatment of travelers' diarrhea caused by noninvasive strains of *Escherichia coli* in adults. Limitations of Use: AEMCOLO is not indicated in patients with diarrhea complicated by fever or blood in the stool or due to pathogens other than *Escherichia coli.* Do NOT take AEMCOLO concomitantly with alcohol.
2. Dosage and Administration	The recommended dose is (b) (4) mg (two tablets) orally twice daily for three days.	The recommended dose is 388 mg (two tablets) twice daily for three days.
5. Warning and Precautions	5.1 (b) (4)	Risk of Persistent or Worsening Diarrhea (b) (4) Complicated by Fever and/or Bloody Stool
6. Adverse Reactions	"Adverse reactions reported in (b) (4) of patients…"	"Adverse reactions in < 2% of patients…" *Note:* (b) (4) *were deleted.*
7. Drug Interactions	(b) (4)	No clinical Drug-Drug Interactions (DDIs) have been studied. Based on the minimally observed rifamycin systemic concentrations after the recommended dose of AEMCOLO, clinically relevant DDIS are not expected.
8. Use in Specific Populations	8.1 Pregnancy 8.2 Lactation	• *New Animal Data section added* • Systemic absorption of AEMCOLO in humans is negligible following oral administration of the recommended dose of AEMCOLO *[see Clinical Pharmacology (12.3)]*. (b) (4)
12. Clinical Pharmacology		*Edits made to section to make it more informative for healthcare practitioners.*
12.4 Microbiology	Resistance	• *Edits made to reflect observed increases in the minimum inhibitory concentrations in vitro and following receipt of rifamycin as well as the development of cross-resistance between rifamycin and other ansamycins.* • *Text concerning* (b) (4) *deleted because* (b) (4) *has*

Section	Proposed Labeling	Approved Labeling
		not been demonstrated.
13. Nonclinical Toxicology	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility (b) (4)	• (b) (4) (b) (4) • (b) (4)
14. Clinical Studies	• Applicant included text and table on (b) (4). • Paragraph on (b) (4)	• *Removed language on* (b) (4) • (b) (4)
16 How Supplied/Storage and Handling		AEMCOLO delayed-release tablets contain 194 mg of rifamycin (equivalent to 200 mg of rifamycin sodium), and are yellow brown, ellipsoidal and film coated
17. Patient Counseling Information	• (b) (4) • Administration Instructions	• *Additional text added for clarification.* • *Added sentence:* Instruct the patient that they must not take AEMCOLO concomitantly with alcohol.

12.5 Patient Labeling

No patient labeling was proposed or deemed necessary for this NDA.

13 Risk Evaluation and Mitigation Strategies (REMS)

Based on the favorable safety profile of this product, risk evaluation and mitigation measures beyond labeling are not warranted at this time.

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14 Postmarketing Requirements and Commitments

As of the date of this application's submission, AEMCOLO has not been marketed anywhere in the world.

Under the agreed initial Pediatric Study Plan (iPSP), the Applicant has submitted a partial waiver of the requirements of the (PREA) to provide data from pediatric studies of Rifamycin SV-MMX for the treatment of travelers' diarrhea for the pediatric age group from 0 to 5 years.

During NDA review, the Applicant agreed to adhere to the Pediatric PMR milestones specified in the agreed iPSP.

Two studies were agreed upon under PREA (See Section 11, Pediatrics):

1. Protocol (b) (4) (b) (4) *study to evaluate the safety and efficacy of* (b) (4) *rifamycin* (b) (4) *in the treatment of traveler's diarrhea in children age 6 to 11 years,* is planned following development of the pediatric formulation.

   Agreed PMR Milestone Dates (assuming formulation is completed in a timely manner):
   Draft Protocol Submission: 07/2019
   Final Protocol Submission: 12/2019
   Study Completion: 06/2022
   Final Report Submission: 12/2022

2. Protocol (b) (4) (b) (4) *study to evaluate the safety and efficacy of* (b) (4) *Rifamycin* (b) (4) *, in the treatment of traveler's diarrhea in children 12 to 17 years,* (b) (4)

   Agreed PMR Milestone Dates:
   Draft Protocol Submission: Submitted
   Final Protocol Submission: 03/2019
   Study Completion: 06/2021
   Final Report Submission: 12/2021

During the NDA review the Applicant agreed to conduct a Section (o) human factor study PMC entitled, "Human Factors Validation Study for Rifamycin Packaging" to evaluate the novel lock and key carton containing the blister packs of AEMCOLO. Previous experience with this packaging has been in marketed products for use solely in healthcare settings. As this product is proposed for use in home or travel settings, human factor validation is being sought due to

concerns that damage to or improper use of the packaging could effectively eliminate the child-resistant feature and potentially lead to harm if a child inadvertently ingested the product. (See Additional Safety Explorations -- Overdose.)

Agreed PMR Milestone Dates:

Draft Protocol Submission:	Submitted
Final Protocol Submission:	12/2018
Study Completion:	06/2019
Final Report Submission:	09/2019

15 Appendices

15.1 References

Acocella, G., F. B. Nicolis and L. T. Tenconi (1965). "The effect of an intravenous infusion of rifamycin SV on the excretion of bilirubin, bromsulphalein, and indocyanine green in man." Gastroenterology **49**(5): 521-525.

Agarwal, S., G. Vargas, C. Nordstrom, E. Tam, G. J. Buffone and S. Devaraj (2015). "Effect of interference from hemolysis, icterus and lipemia on routine pediatric clinical chemistry assays." Clin Chim Acta **438**: 241-245.

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15.2 Financial Disclosure

For clinical investigators participating in Studies CB-01-11/02, CB-01-11/03, C2009-0201, and RIT-1/AID, the Applicant certified that there were no financial arrangements that could affect the outcome of the study as defined in 21 CFR 54.2(a) and that the clinical investigators had no reportable financial disclosures as defined in 21 CFR 54.2(b). The Applicant also certified that no investigator was the recipient of significant payments as defined in 21 CFR 54.2(f).

Covered Clinical Study (Name and/or Number): Study C2009-0201 and Study RIT-1/AID

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 50		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): None		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): None		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 50		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

15.3 OCP Appendices (Technical documents supporting OCP recommendations)

15.3.1 Individual Summary Report of Clinical Studies

STUDY #	CB-01-11/10	**STUDY PERIOD:** May 2009 through June 2009
TITLE	A Phase 1, open labelled, randomized, cross-over single dose study to assess the absolute bioavailability and food effect on the oral absorption of Rifamycin SV-MMX® 200 mg tablets in healthy male and female volunteers.	

TRIAL SUMMARY (As Reported by the Applicant)
RATIONALE
The Applicant conducted two exploratory Phase 2 efficacy trials using Rifamycin SV-MMX® 200 mg tablets. Although CB-01-11/03 showed no significant dose-dependent effect on time to last unformed stool (TLUS), the Applicant concluded that the total daily dose of 800 mg (2 tablets twice daily) was most effective. CB-01-11/02 showed non-inferiority to active comparator (rifaximin) at the total daily dose of 800 mg (1 tablet four times daily). CB-01-11/10 was designed to assess absolute bioavailability and investigate food-effects on oral absorption of 400 mg (two tablets given once) Rifamycin SV-MMX®.
DESIGN AND PK ASSESSMENTS
Formulation(s): Rifamycin SV MMX® (as sodium salt) 200 mg, batch # 6107, expiration JAN 2010; Rifocine® (Rifamycin SV) 500 mg/10mL, batch # A8001, expiration JUL 2011
Trial Design, PK Sampling and Pharmacokinetic Assessments: In period 1 (P1), all subjects received a single *i.v.* injection of 250 mg rifamycin SV. After a washout of 7 days, subjects received a single dose of 400 mg rifamycin SV MMX (2x200 mg tablets) either under fasting conditions (no food or drink allowed for at least 10 hours prior to dosing and then up to 5 hours after dosing) or fed conditions (high fat high calorie breakfast [1000 Kcal; 50 % Kcal derived from fat) in a crossover manner in two consecutive periods (Period 2 [P2] and Period 3[P3]). Each period was separated by a 7-day washout. In period 1, plasma and urine sampling was conducted up to 12 hr post-dose. In period 2 and 3, plasma and urine sampling was conducted up to 24 hr post-dose and in period 2, fecal samples were collected from 12 fasting subjects prior to the first dose and then every 24-hours until the end of day 4 (84 hr post-dose) Blood, urine, and feces PK parameters were determined based on data from all evaluable subjects at the scheduled sampling times using a noncompartmental analysis approach.
RESULTS
Study Subjects

Subject Disposition									Protocol Deviations	Treatment
Enrolled	Completed	Discontinued	PK Population			Safety Population			# Occurrences	Compliance
			P1	P2	P3	P1	P2	P3		
24	22	2[a]	24	22	22	24	24	24	12[b]	100%

[a]During P2 for personal reasons not related to safety
[b]deviation from scheduled sampling times (all ≤ 4 min)

Demographics

	Male/Female	Age (yr)	Weight[a] (kg)	BMI (kg/m²)
P1	12/12	32.8 (19 to 49)	68.3 (45.6 to 90.6)	24 (18.5 to 28.8)
P2 and P3	12/12	33.7 (19 to 49)	67.4 (45.6 to 90.6)	23.9 (18.5 to 28.8)

[a]Weight did not change over study course.

Pharmacokinetics: Data summarized as mean ± SD unless otherwise stated. Study link: CB-01-11/10

Rifamycin SV PK Parameters from Plasma after IV Administration:

Table 73: Plasma PK Parameter Estimates After Single IV Dose

N=24	$C_0 = C_{max}$ ng/mL	T_{max} min	AUC_{0-t} ng/mL * h	$AUC_{0-\infty}$ ng/mL * h	$T_{1/2}$ h	MRT h	Cl_t L/h	V_d L
Mean	36,025	5	11,840.5	11,865.21	3.04	0.49	23.29	101.79
SD[a]	± 8,569.32	± 0	± 4,002.83	± 4,007.18	± 0.73	± 0.06	± 7.25	± 40.34

[a]Standard deviation
Source: CB-01-11/10 Study Report, pg 45

Rifamycin SV PK Parameters from Plasma after Oral Administration:

Plasma PK parameter estimates after single oral dose administration under fasting and fed conditions could not be calculated due to lack of quantifiable plasma concentrations. Plasma concentration data was only available from 5 individuals (only 2 of these 5 individuals had serially measurable plasma concentrations). In total, 8 plasma samples with measurable plasma concentration were available.

Rifamycin SV PK Parameters from Urine after IV Administration

Table 74: Urinary PK Parameter Estimates After Single IV Dose

Time (hr)	Xu (µg)	Xu (% of dose)	dXu/dt (µg/h)	∑Xu (µg)	∑Xu (% of dose)	Cl_r (L/h)
0-4	6,398.1 ± 2,132.5	2.56 ± 0.85	1,599.5 ± 533.1	6,539.1 ± 2,153.7	2.62 ± 0.86	0.6 ± 0.1
4-8	111.9 ± 58.0	0.04 ± 0.02	28.0 ± 14.5			
8-12	29.1 ± 26.9	0.01 ± 0.01	7.3 ± 6.7			

Source: CB-01-11/10 Study Report, pg 47

Rifamycin SV PK Parameters from Urine after Oral Administration

Table 75: Urine PK Parameter Estimates After Single Oral Dose Under Fasting and Fed Conditions

Condition	Time (hr)	Xu (ng)	Xu (% of dose)	dXu/dt (ng/h)	∑Xu (ng)	∑Xu (% of dose)
Fast	**0-6**	<0.1	<0.0001	<0.1	3,966.1 ± 6,166.3	0.001 ± 0.0015
	6-12	1,612.8 ± 3,338.4	<0.0001	268.8 ± 556.4		
	12-24	2,353 ± 3,231.4	0.0003 ± 0.0007	196.1 ± 268.5		
Fed	**0-6**	<0.1	<0.0001	<0.1	1,500.5 ± 3,049.1	0.0004 ± 0.0008
	6-12	103.2 ± 335.7	<0.0001	17.2 ± 55.9		
	12-24	1,397.3 ± 2,990.5	0.0003 ± 0.0007	116.4 ± 249.2		

Source: CB-01-11/10 Study Report, pg 48

Rifamycin SV Fecal Excretion after Oral Administration

Table 76: Fecal PK Parameter Estimates After Single Oral Dose Under Fasting Conditions

Time (hr)	Sample Size	Xu (µg)	Xu (% of dose)	dXu/dt (µg/h)	∑Xu (mg)	∑Xu (% of dose)
Pre-Dose	--	NC	NC	NC	N=22	N=22
0-24	N=19	74,559.8 ± 115,987	18.64 ± 29	3,106.7 ± 4,832.8	331.6 ± 69	82.9 ± 17.3
24-48	N=20	200,044.5 ± 134,718.6	50.01 ± 33.68	8,335.2 ± 5,613.3		
48-72	N=22	84,148.3 ± 98,101.0	21.04 ± 24.53	3,506.2 ± 4,087.5		
72-84	N=22	1,250 ± 2,856.5	0.31 ± 0.71	104.2 ± 238		

Source: CB-01-11/10 Study Report, pg 50

Assessment of Absolute Bioavailability

Table 77: Absolute Bioavailability (Fabs) Using Total Urinary Excretion Amounts (Xu) After a Single I.V. Injection and Oral Dose Under Fasting Conditions

$F_{abs} = \frac{\sum Xu\ (oral)/Dose\ (oral)}{\sum Xu\ (i.v.)/Dose\ (i.v.)}$	0.041 ± 0.062 %

Source: CB-01-11/10 Study Report, pg 49

Note: Assessment of absolute bioavailability and the effect of food on the pharmacokinetics of rifamycin SV was not conducted using plasma PK parameters due to lack of quantifiable plasma concentrations in majority of subjects after oral administration.

Safety:

No Grade 3 or 4 AEs, serious adverse events (SAEs), AEs leading to study discontinuation, or deaths were reported during the study.

REVIEWER ASSESSMENT

Study Design Acceptable ☒ Yes ☐ No

Study Conduct

- Analytical method performance is acceptable (*FDA BMV Guidance 2018*) ☒ Yes ☐ No
- Protocol deviations affect the integrity of the study ☐ Yes ☒ No ☐ N/A

Study Results

The study results are acceptable as reported by the sponsor ☑ Yes ☐ No

Summary

1) Absolute bioavailability (F) was reported by the Sponsor to be 0.041 ± 0.062 %. Because the 400 mg dose is expressed as the sodium salt form, the amount of rifamycin SV base is slightly less than 400 mg (i.e., 388 mg). Adjusting for this difference, absolute bioavailability is 0.043%.
2) The excreted urine fraction as a % of the total dose following a single 400 mg dose under fasting conditions is 0.001; calculated with dose expressed as rifamycin SV base. Furthermore, the excreted fecal fraction as a % of the total dose following a single 400 mg dose under fasting conditions is approximately 86% when calculated with dose expressed as rifamycin SV base.
3) Effect of Food: Although there wasn't sufficient plasma PK data to assess the effect of food on the pharmacokinetics of rifamycin SV, greater number of subjects with quantifiable concentrations after fasted state and higher amount of drug excreted in the urine in the fasted state suggests increased absorption under fasting conditions.

4) Drug release targeting the colon. Urinary drug excretion of rifamycin SV began after 6 hr with oral administration, whereas near complete urine excretion of rifamycin SV occurred between 0-4 hr (97% of excreted amount) with *i.v.* administration.

Relevant Links and information on clinical and bioanalytical sites:

Table 78: Summary of Bioanalytical Methods Used in CB-01-11/10[a] to Quantify Rifamycin SV Concentrations

Biological Matrix	Plasma	Urine	Feces
Validation Report[b]	(b) (4) 9589	(b) (4) 9591	(b) (4) 9593
Inter-Study Analytical Report[b]	9596	9597	9598
Detection Method	HPLC-MS/MS[b]	HPLC-MS/MS[b]	HPLC-MS/MS[b]
Lower Limit of Quantification (ng/mL)	2	2	10[c]
Validation Range (ng/mL)[c]	2 to 2,000	2 to 2,000	10 to 10,000

[a]Clinical Site: (b) (4)

[b]Bioanalytical Site: (b) (4)

[c]Over the curve sample dilutions were validated and support the concentrations of rifamycin SV greater than 2000 ng/mL after intravenous administration.

STUDY #	CB-01-11/11	STUDY PERIOD: April 2009 through May 2009
TITLE	A phase I, open labelled, parallel group, multiple dose study to assess the pharmacokinetics of Rifamycin SV-MMX® 200 mg tablets administered b.i.d. or q.i.d. to healthy male and female volunteers.	

TRIAL SUMMARY (As Reported by the Applicant)

RATIONALE

The Applicant conducted two exploratory Phase 2 efficacy trials using Rifamycin SV-MMX® 200 mg tablets. Although CB-01-11/03 showed no significant dose-dependent effect on time to last unformed stool (TLUS), the Applicant concluded that the 800 mg (2 tablets twice daily) dose was most effective. CB-01-11/02 showed non-inferiority to active comparator (rifaximin) at a total daily dose of 800 mg (1 tablet four times daily). CB-01-11/11 was designed to investigate the PK of rifamycin SV following repeat administration for the two previously experienced dose regimens.

DESIGN AND PK ASSESSMENTS

Formulation(s):

Rifamycin SV MMX® 200 mg (as sodium salt) tablets, batch # 6107, expiration JAN 2010

Trial Design, PK Sampling and Pharmacokinetic Assessments:

Cohort 1 (C1): 400 mg of oral rifamycin SV (2 tablets of Rifamycin SV-MMX®) twice daily (b.i.d., τ =12 hr) for a total of 6 administrations. The first dose was administered at 18:00± 1h of day 1.
Cohort 2 (C2): 200 mg of oral rifamycin SV (1 tablet of Rifamycin SV-MMX®) four times daily (q.i.d., τ = 6 hr) for a total of 12 administrations. The first dose was administered at 12:00± 1h of day 1.

Each cohort received rifamycin SV for 3-days followed by intensive plasma and urine PK sampling from 0 to 12-hours post-dose after the last dose on day 4. Doses were given irrespective of food; however, grapefruit juice was not allowed starting 24 hr prior to study initiation.

Blood and urine PK parameters were determined from all evaluable subjects at the scheduled sampling times using a noncompartmental analysis approach.

RESULTS

Study Subjects

Subject Disposition							Protocol Deviations	Treatment
Enrolled	Completed	Discontinued	PK Population		Safety Population		# Occurrences	Compliance
			C1	C2	C1	C2		
24	24	0[a]	12	12	12	12	11[a]	100%

[a]deviation from scheduled sampling times (one delay of 10 min; all others ≤ 5 min)

Demographics

	Male/Female	Age (yr)	Weight[a] (kg)	BMI (kg/m^2)
C1	6 /6	32.2 (20 to 51)	66.6 (47.1 to 84.3)	23.2 (18.4 to 29.0)
C2	6 /6	33.2 (19 to 55)	71.7 (56.1 to 90.1)	24.7 (20.6 to 28.8)

[a]Weight did not change over study course.
[#]Data are summarized as mean (min to max)

Pharmacokinetics: Data summarized as mean ± SD unless otherwise stated. Study link: CB-01-11/10

Rifamycin SV PK Parameters from Plasma after Oral Administration:

Table 79: Plasma rifamycin SV Concentration Estimates After Repeat Oral Dosing of 200 mg q.i.d. and 400 mg b.i.d. rifamycin SV for 3 consecutive days (concentrations measured after the last dose on day 4).

Time	Mean rifamycin SV plasma concentrations (ng/mL) ±SD	
	200 mg Rifamycin SV-MMX® tablets q.i.d.	400 mg Rifamycin SV-MMX® tablets b.i.d.
	N=12	N=12
Pre-dose	BLQL	1.41±2.49**
1 h	BLQL	0.40±0.93°°
2 h	BLQL	BLQL
3 h	BLQL	BLQL
4 h	BLQL	0.25±0.87°
5 h	BLQL	0.80±1.20**
6 h	0.75±1.39*	1.57±2.71**
8 h	BLQL	0.77±1.94°°
10 h	BLQL	0.44±1.52°
12 h	BLQL	0.87±1.58*

Source: Table 14.2.1.1 and Table 14.2.1.2
°: one quantifiable value and 11 BLQL values;
°°: 2 quantifiable values and 10 BLQL values;
*: 3 quantifiable values and 9 BLQL values;
**: 4 quantifiable values and 8 BLQL values;

Source: CB-01-11/11 Study Report, pg 37

Note: The third column indicates "400 mg Rifamycin SV-MMX tablets b.i.d". The correct description should be "2 X 200 mg Rifamycin SV-MMX tablets b.i.d.) considering that only the 200 mg Rifamycin SV tablet was used in the trial.

In comparison to the Applicant's calculated concentrations using zero as the value for samples reported below quantification, estimates of rifamycin SV concentrations in plasma should be calculated as the mean of only those that have quantifiable concentrations. For example, 6 hr post-dose rifamycin SV concentrations are 2.98 ng/mL and 5.13 ng/mL for the 200 mg q.i.d. and 400 mg b.i.d. dosing regimen respectively. The maximum rifamycin SV concentration observed was 8.72 ng/mL 6 hr post-dose in the 400 mg b.i.d. dose group.

Note: Plasma PK parameters were not calculated due to scarcity of quantifiable concentrations.

Rifamycin SV PK Parameters from Urine after Oral Administration

Table 80: Urinary rifamycin SV PK Parameter Estimates After Repeat Oral Dosing of 200 mg q.i.d. and 400 mg b.i.d. rifamycin SV for 3 consecutive days (urinary rifamycin SV concentrations measured after the last dose on day 4).

Treatment		Xu (ng)	Xu (% of dose)	dXu/dt (ng/h)	ΣXu (ng)	ΣXu (% of dose)
200 mg Rifamycin SV-MMX® tablets q.i.d.	0-3 h	1873.9±1405.8	0.0009±0.0007	624.6±468.6	7172.7±4180.0	0.0036±0.0021
	3-6 h	1611.1±931.2	0.0008±0.0005	537.0±310.4		
	6-12 h	3687.7±2377.6	0.0018±0.0012	614.6±396.3		
400 mg Rifamycin SV-MMX® tablets b.i.d.	0-3 h	3216.9±2437.2	0.0008±0.0006	1072.3±812.4	10914.5±5309.3	0.0027±0.0013
	3-6 h	2713.3±1249.5	0.0007±0.0003	904.4±416.5		
	6-12 h	4984.3±3029.6	0.0012±0.0008	830.7±504.9		

Source: Table 14.2.1.7, 14.2.1.8, 14.2.1.9, 14.2.1.10, 14.2.1.11, 14.2.1.12, 14.2.2.3 and 14.2.2.4

Source: CB-01-11/10 Study Report, pg 38

Note: The second row indicates "400 mg Rifamycin SV-MMX tablets b.i.d". The correct description should be "2 X 200 mg Rifamycin SV-MMX tablets b.i.d.) considering that only the 200 mg Rifamycin SV tablet was used in the trial.

Safety:
No Grade 3 or 4 AEs, serious adverse events (SAEs), AEs leading to study discontinuation, or deaths were reported during the study.

REVIEWER ASSESSMENT	
Study Design Acceptable ☒ Yes ☐ No	
Study Conduct	
▪ Analytical method performance is acceptable (*FDA BMV Guidance 2018*)	☒ Yes ☐ No
▪ Protocol deviations affect the integrity of the study	☐ Yes ☒ No ☐ N/A
Study Results	
The study results are acceptable as reported by the Sponsor ☑ Yes ☐ No	
Summary	
1) After oral administration of rifamycin SV 400 mg b.i.d for three days (clinically recommended regimen), the majority of the plasma concentrations were below the limit of quantification suggesting very limited systemic absorption. 2) Although the excreted urine fraction as a % of the total dose (400 mg expressed as rifamycin SV sodium) suggest accumulation following multiple dosing (b.i.d.) (0.001 vs 0.0027 for single dose), this difference is not expected to be clinically relevant given the overall limited absorption. If expressed as rifamycin SV base, the dose is approximately 388 mg (instead of 400 mg which reflects the dose of rifamycin SV sodium). The excreted urine fraction as a % of the total dose following multiple dosing (b.i.d.) is 0.0028 when calculated with dose expressed as rifamycin SV base.	
Relevant Links and information on clinical and bioanalytical sites: The bioanalytical methods used to quantify rifamycin in plasma and urine are the same as used for trial CB-01-11-10.	

15.3.2 Individual Summary Report of Pre-Clinical Studies

Enzymes Involved in Biotransformation of Rifamycin SV in Man In Vitro	
Study #	ADME-TOX Study of Rifamycin SV Sodium EP (16108)
Objectives	Determine enzymes involved in the metabolism of rifamycin SV
METHODS	
System	Human recombinant enzyme
Test (substrate) concentration	Rifamycin SV sodium EP 1.0E-6 M
Appropriate system?	☑ Yes ☐ No
RESULTS	
Controls (Concentrations and Parent Remaining)	

Enzyme	Concentration (1.0E-6 M)	Positive Control (% Remaining)	Rifamycin SV (% Remaining)
CYP1A2	Ethoxy Resorufin	0	105
	Propranolol	52	
CYP2B6	Benzphetamine	6	99
	Propranolol	93	
CYP2C8	Paclitaxel	26	94
	Propranolol	88	
CYP2C9	Diclofenac	0	102
	Propranolol	90	
CYP2C19	Omeprazole	21	107
	Propranolol	79	
CYP2D6	Propranolol	22	107
	Terfenadine	84	
CYP2E1	Chlorzoxazone	28	96
	Propranolol	91	
CYP3A4	Propranolol	86	88
	Terfenadine	14	
CYP3A5	Propranolol	86	100
	Terfenadine	6	

Source: Table 3.2.1., page 10 and Table 3.2.3., page 11 of report 16108.

REVIEWER ASSESSMENT

Experimental Approach (Draft *FDA in vitro metabolism and transporter DDI Guidance 2017*)

Controls/concentrations appropriate? ☑ Yes ☐ No

Test concentrations appropriate? ☑ Yes ☐ No

Integrated Summary

1) Rifamycin SV does not appear to be metabolized by CYP enzymes *in vitro* which is also supported by *in vivo* observations where 86% of the drug is eliminated unchanged in the feces. Furthermore, limited absorption suggests lack of pre-systemic and systemic metabolism of rifamycin SV by CYP enzymes.
2) Lack of CYP involvement in the metabolism of rifamycin SV suggest no dose adjustments are necessary when rifamycin SV is concomitantly administered with CYP inducers or inhibitors.

Evaluation of Induction Potential of Cytochrome P450 Isoforms by rifamycin SV 1	
Title (Study #)	ADME-TOX Study of Rifamycin SV Sodium EP (16108)
Objectives	Determine if rifamycin SV is an inducer of CYP enzymes
METHODS	
System	Human hepatocytes (n=3) incubated for 48 hr with rifamycin SV. Extent of enzyme activity was measured by probe substrates after a 30-min exposure to cell system.
Probe Substrates	

Enzyme	Probe Substrate (M)	Positive Control (M)
CYP1A	Ethoxyresorufin (2.0E-6)	Omeprazole (5.0E-5)
CYP2B6	Bupropion (2.0E-4)	Phenobarbital (1.0E-3)
CYP3A	Midazolam (1.0E-5)	Rifampin (1.0E-5)

RESULTS

	Lot[a]	Positive Control (Fold Induction)	Enzyme Activity (% Positive Control)[a]		
			Rifamycin SV (M)		
			1.0E-6	1.0E-5	1.0E-4
CYP1A2	1	5	-2	-2	-3
	2	25	1	1	1
	3	8	0	0	-1
CYP2B6	1	7	26	38	6
	2	13	34	36	49
	3	5	24	32	23
CYP3A4/5	1	37	50	63	7
	2	27	104	100	92
	3	33	77	79	26

[a]Each lot represents a different donor and performed in triplicate

Source: Table 3.2.5; Page 11 of report.

REVIEWER ASSESSMENT

Experimental Approach (Draft *FDA in vitro metabolism and transporter DDI Guidance 2017*)

Appropriate system? ☑ Yes ☐ No

Appropriate controls/concentrations ☑ Yes ☐ No

Appropriate test concentrations? ☑ Yes ☐ No

Integrated Summary

1) *In vitro* data suggest potential of rifamycin SV to induce CYP3A4 and CYP2B6, however, follow up quantitative predictions (as outlined in the 2017 draft FDA in vitro metabolism and transporter DDI Guidance) rule out the potential for rifamycin to induce CYP3A4 and CYP2B6 *in vivo*.
2) CYP3A4 and CYP2C9 are induced *via* activation of pregnane X receptor. In this study, rifamycin SV was shown to induce CYP3A4 and CYP2B6, hence it may also induce CYP2C9 *in vitro*. Considering that systemic CYP3A4 induction is not expected to be clinically relevant (based on quantitative predictions outlined in the 2017 draft FDA *in vitro* metabolism and transporter DDI Guidance), CYP2C9 induction (even if it observed *in vitro*) is also not expected to be clinically relevant.

Evaluation of Induction Potential of Cytochrome P450 Isoforms by rifamycin SV 2	
Titles (Study #s)	Rifamycin SV: Potential induction of activity levels of the main CYP450 enzymes in cryopreserved human hepatocytes (CB-01-11/07). Calculation of In Vivo Prediction of the Induction Potential towards CYP2B6 and CYP3A4 (R3 and AUCR Parameters) (CB-01-11/37).
Objectives	Determine if rifamycin SV is an inducer of main CYP enzymes
METHODS	
System	Human hepatocytes (n=1) incubated for 48 hr with potential perpetrator (rifamycin SV). Extent of enzyme activity was measured by probe substrates after a 1hr (CYP1A2) or 2 hr (CYP2B6, CYP3A4) exposure to cell system.
Controls	

Enzyme	Probe Substrate (M)	Positive Control (M)
CYP1A2	Ethoxyresorufin (1.0E-4)	Omeprazole (5.0E-5)
CYP2B6	S-Mephenytoin (2.0E-4)	Phenobarbital (1.0E-3)
CYP3A4	Testosterone (2.5E-4)	Rifampin (2.5E-5)

RESULTS

	Lot[a]	Positive Control (Fold Induction)	Enzyme Activity (% Positive Control)[a]			Induction Concentration (EC_{50})
			Rifamycin SV (M)			Rifamycin SV (M)
			1.0E-6	1.0E-5	5.0E-5	
CYP1A2	1	5.7	2.3	-0.2	-2.7	---
CYP2B6	1	3.3	10.6	46.2	38.1	2.14E-6
CYP3A4/5	1	20.5	35.2	57.0	101.7	1.11E-5

[a]One donor performed in triplicate
Source: Table 1, 2, and 3; Page 11 and 12 of report CB-01-11/07.

	Model Predictions for Clinical Potential of Drug Induction Interactions	
	R3	AUCR
CYP2B6	0.994	NA
CYP3A4	0.986	0.854

[†]induction cutoff of >0.8 for R3 and AUCR suggest drug has no effect on enzyme levels
Source: Table 3 and Appendix 2; Page 6 and 10 of report CB-01-11/37.

REVIEWER ASSESSMENT

Experimental Approach (*FDA in vitro metabolism and transporter DDI Guidance 2017*)

Appropriate system? ☑ Yes ☐ No
Appropriate controls/concentrations ☑ Yes ☐ No
Appropriate test concentrations? ☑ Yes ☐ No

Notes:

1) Clinically relevant concentrations were incorporated as recommended per guidance using static mechanistic modeling.
2) Guidance cites a limitation of using only enzyme activity in the context of simultaneous induction and inhibition, however, the use of only enzyme activity is not expected to alter the conclusions considering that rifamycin SV is not an inhibitor of CYP enzymes (per the results of CPS-11).
3) Guidance recommends use of hepatocyte lots from three donors

4) A subsequent study (16108) was more extensive (e.g., multiple donor hepatocytes, use of midazolam as a more sensitive CYP3A4 marker, and use of a more specific *in vitro* CYP2B6 marker).
5) The reviewer used a fraction unbound of 0.2 to estimate CYP2B6 and CYP3A4 R3 estimates [redacted] (b) (4). R3 was found to be 0.976 and 0.948 respectively. Estimates are still above the cutoff value recommended by 2017 draft FDA DDI guidance.

Integrated Summary

1) *In vitro* data demonstrate rifamycin SV is a direct inducer of 3A4/5 and 2B6. Rifamycin SV was most potent at inducing CYP3A4/5 with an EC_{50} of 11.1 µM. CYP1A2 and CYP2B6 displayed a low capacity for induction with a maximum of ca. 2.3- and 46% respectively. Despite limitations, this *in vitro* study demonstrated a concentration-response relationship from which the Sponsor conducted a model-based *in vivo* DDI prediction assessment for CYP3A4/5.
2) Systemic/hepatic/Gut Drug-Drug Interaction Potential via CYP Induction. The Sponsor conducted a static mechanistic model (AUCR) to account for both liver and intestine effects on CYP3A4/5. Using midazolam as a substrate, it was predicted that alterations of systemic midazolam exposure would not be affected by Rifamycin SV MMX®. As a worst-case scenario, the review team also considered a high intestinal extracted drug (e.g., tacrolimus). While diminution of the AUCR was noted (AUCR midazolam 0.91 vs AUCR tacrolimus 0.86) it did not reach the critical cutoff predictive of *in vivo* clinical importance. Lastly, considering the experimental limitations of this study, the review team used the data from study 16108 to conduct additional analysis. Using an EC_{50} of 1 µM results in an AUCR value of 0.48 which may suggest potentially clinically relevant CYP3A induction by rifamycin SV, nevertheless because of the limited treatment duration (3 days), and since most of the drug is expected to be available in the colon (where CYP3A is thought minimal[1]) the review team does not expect that rifamycin will be a clinically significant inducer. In addition, despite a marginal induction ofCYP2B6 observed *in vitro* (i.e., 46%), the review team does not expect the *in vivo* potential for rifamycin SV MMX® to induce CYP2B6 to be clinically significant given the regional distribution pattern of CYP2B6 (chiefly in the liver[2,3]) and minimally observed systemic drug exposures (R3 model results).

References:

1. Xu J, Lin Y, Boulas P, Peterson ML. Low colonic absorption drugs: risks and opportunities in the development of oral extended release products. Expert Opin Drug Deliv. 2018;15(2):197-211.
2. Thelen K, Dressman JB. Cytochrome P450-mediated metabolism in the human gut wall. J Pharm Pharmacol. 2009;61(5):541-58.
3. Xie F, Ding X, Zhang QY. An update on the role of intestinal cytochrome P450 enzymes in drug disposition. Acta Pharm Sin B. 2016;6(5):374-383.

Evaluation of Inhibition Potential of Cytochrome P450 Isoforms by rifamycin SV	
Title (Study #)	Assessment of the potential to inhibit human hepatic cytochrome P450 (CYP) enzymes in vitro (CPS/11)(Study Report Amendment)
Objectives	Determine if rifamycin SV is an inhibitor of CYP enzymes
METHODS	
System	Pooled human liver microsomes (n=150 donors) were incubated with 0, 1.0E-7, 1.0E-5, 3.4E-5, 1.0E-4, or 3.4E-4 M rifamycin SV with and without enzyme cofactor for 0 or 30 min prior to selective CYP probe substrate addition. Enzyme activity was measured 10 min post substrate addition. Positive control incubations contained selective CYP probe inhibitors in place of test perpetrator (rifamycin SV). Experiments performed in triplicate.

Enzyme	Probe substrate (M)	Probe inhibitor (> 2 Log_{10} concentration range)
CYP1A2	Phenacetin (2.0E-5)	Direct: α-Naphthoflavone
		Time-dependent: Furafylline
CYP2B6	Bupropion (8.0E-5)	Direct: N/A
		Time-dependent: Phencyclidine
CYP2C8	Amodiaquine (2.0E-6)	Direct: Quercetin
		Time-dependent: Pheneizine
CYP2C9	Tolbutamide (2.0E-4)	Direct: Sulphaphenazole
		Time-dependent: Tienilic acid
CYP2C19	S-Mephenytoin (2.0E-5)	Direct: Modafinil
		Time-dependent: Ticlopidine
CYP2D6	Bufuralol (1.0E-5)	Direct: Quinidine
		Time-dependent: Paroxetine
CYP3A4/5	Midazolam (5.0E-6)	Direct: Ketoconazole
	Testosterone (7.5E-5)	Time-dependent: Azamulin

Source: Table 1; Page 10 of report CPS/11.

RESULTS

Table 81: Summary of the Effect of CYP-Selective Control Inhibitors

CYP enzyme	Inhibitor	0-minute pre-incubation		30-minute pre-incubation with cofactor		30-minute pre-incubation without cofactor		TDI potential* [fold-shift in IC_{50}]
		% Inhibition of metabolite formation at highest inhibitor concentration	IC_{50} (µM) ± SE	% Inhibition of metabolite formation at highest inhibitor concentration	IC_{50} (µM) ± SE	% Inhibition of metabolite formation at highest inhibitor concentration	IC_{50} (µM) ± SE)	
CYP1A2	Furafylline	51.1	2.86 ± 0.38	94.0	0.206 ± 0.009	51.1	2.84 ± 0.14	Detected [13.9]
CYP2B6	Phencyclidine	54.7	81.1 ± 13.1	86.8	6.79 ± 0.81	60.0	32.8 ± 19.0	Detected [11.9]
CYP2C8	Phenelzine	53.2	907 ± 0	81.3#	81.2 ± 13.6	76.8	239 ± 38	Detected [11.2]
CYP2C9	Tienilic acid	54.7	2.36 ± 0.06	77.2	0.635 ± 0.068	54.3	2.76 ± 0.89	Detected [3.75]
CYP2C19	Ticlopidine	78.5	0.883 ± 0.038	>93.0	0.432 ± 0.022	80.2	0.749 ± 0.359	Detected [2.04]
CYP2D6	Paroxetine	65.1	1.30 ± 0.08	91.1	0.108 ± 0.012	63.2	1.43 ± 0.15	Detected [12.0]
CYP3A4/5 (T)	Azamulin	78.9	0.316 ± 0.115	97.3	0.0421 ± 0.0034	86.8	0.174 ± 0.024	Detected [7.51]
CYP3A4/5 (M)		85.3	0.155 ± 0.003	92.7	0.0094 ± 0.0025	87.9	0.113 ± 0.004	Detected [16.5]

(T)= testosterone

(M) = midazolam

*TDI potential is indicated if the IC_{50} is halved (fold-shift >2) following 30 min pre-incubation with cofactor

> limit of quantification used to determine degree of inhibition

#No two values were within 20% deviation of mean; all values excluded. Results from next highest concentration reported.

Source: Table 15; Page 39 of report CPS/11.

Table 82: Summary of Inhibitory Effects of Rifamycin SV towards CYP Enzyme Activities

CYP enzyme	Assay	Direct inhibition (0 minute pre-inc.)		30 minute pre-inc. with cofactor^		30 minute pre-inc. without cofactor#		TDI potential*
		Inhibition at 340 µM (%)	IC_{50} (µM) ± SE	Inhibition at 340 µM (%)	IC_{50} (µM) ± SE	Inhibition at 340 µM (%)	IC_{50} (µM) ± SE	
CYP1A2	Phenacetin O-deethylation to acetaminophen	35.2	>340¥	47.7	>340¥	55.6	286 ± 29	Not detected [-]
CYP2B6	Bupropion hydroxylation to hydroxybupropion	18.8	>340¥	14.4	>340¥	57.7	171 ± 191	Not detected [-]
CYP2C8	Amodiaquine N-deethylation to N-desethylamodiaquine	96.6	15.6 ± 3.0	95.5	28.3 ± 3.3	98.4	35.5 ± 2.0	Not detected [0.551]
CYP2C9	Tolbutamide hydroxylation to 4-hydroxytolbutamide	92.5	28.0 ± 0.7	92.0	37.2 ± 2.4	92.9	39.2 ± 1.8	Not detected [0.753]
CYP2C19	S-Mephenytoin hydroxylation to 4'-hydroxymephenytoin	77.8	99.9 ± 6.8	74.2	115 ± 6	80.7	90.1 ± 7.6	Not detected [0.869]
CYP2D6	Bufuralol hydroxylation to 1'-hydroxybufuralol	72.9	123 ± 9	71.3	149 ± 7	77.2	97.3 ± 6.0	Not detected [0.826]
CYP3A4/5	Testosterone hydroxylation to 6β-hydroxytestosterone	93.0	16.7 ± 7.3	92.4	48.1 ± 4.3	85.6	128 ± 95	Not detected [0.347]
	Midazolam hydroxylation to 1'-hydroxymidazolam	98.1	8.47 ± 0.58	98.7	17.7 ± 0.5	99.0	17.0 ± 0.6	Not detected [0.479]

inc = incubation

SE = standard error

^Assessed with cofactor to determine total time-dependent inhibition (TDI)

#Assessed without cofactor to determine contribution of mechanism-based inhibition (MBI-irreversible)

*TDI potential is indicated if the IC_{50} is halved (fold-shift >2) following a 30 min pre-incubation

[] indicates fold shift in IC_{50} following incubation for 30 min with cofactor

¥Insufficient inhibition observed at 340 µM rifamycin SV to accurately determine IC_{50}

-not calculated; no IC_{50} determined

Source: Table 2; Page 11 of report CPS/11.

Table 83: Model Prediction for Clinical Potential of Drug Inhibition Interactions

	R1	R1, gut	AUCR
CYP2C8	1.00	NA	NA

CYP2C9	1.00	NA	NA
CYP2C19	1.00	NA	NA
CYP2D6	1.00	NA	NA
CYP3A4/5 (T)	1.00	276	NA
CYP3A4/5 (M)	1.00	542	1.01

(T) = testosterone, (M) = midazolam
NA= not applicable; R1,gut is not required.
Source: Table 16; Page 5 of report CPS/11 Final Report Amendment.

$R_1 = 1+(I_{max,u} / K_i)$; where $I_{max,u}$ is the maximal unbound plasma concentration; based on the highest observed C_{max} of 8.72 ng/mL following repeat dosing and a fraction unbound of 5%. Ki is the inhibition constant; estimated from IC50/2 (competitive inhibition)

$R_{1,gut} = 1+(I_{gut} / K_i)$, where I_{gut} is the intestinal luminal concentration of rifamycin SV calculated as the maximum dose/250 mL; Based on a rifamycin SV dose of 400 mg.

$AUCR = (1/(A_g \times B_g \times C_g) \times (1-F_g) + F_g) \times (1/(A_h \times B_h \times C_h) \times f_m + (1-f_m))$; where A = effect of reversible inhibition ($A_g = (1/(1 +[I]_g / K_i))$; $A_h = (1/(1 +[I]_h / K_i))$, B = effect of time-dependent inhibition (not observed set to 1), and C = effect of induction (not recommended together set to 1). $[I]g = F_a \times k_a \times dose / Q_{en}$ and represents the enterocyte concentration, $[I]h = f_{u,p} \times (C_{max} + F_a \times k_a \times dose / Qen / R_b)$ and represent the hepatic inlet unbound concentration, $F_g = 0.51$; fraction midazolam available after intestinal metabolism, $f_m = 0.9$; fraction of systemic clearance of the substrate that is subject to inhibition/induction, $F_a = 0.041\%$; fraction rifamycin SV absorbed after oral administration, $k_a = 0.1$ min^{-1}; assumed first order absorption rate constant in vivo, $Q_{en} = 18$ L/hr/70 kg; assumed blood flow through enterocytes, $f_{u,p} = 5\%$; fraction of rifamycin SV unbound in plasma, $C_{max} = 8.72$ ng/mL; maximal total rifamycin SV concentration in plasma, $Q_h = 97$ L/hr/70 kg; assumed hepatic blood flow, $R_b = 1$; assumed blood to plasma concentration ratio, dose = 400 mg; represent current proposed regimen (400 mg b.i.d., K_i = inhibition constant estimated from $IC_{50}/2$, g- denotes the gut, h- denotes the liver.

REVIEWER ASSESSMENT

Experimental Approach (*FDA in vitro metabolism and transporter DDI Guidance 2017*)

Appropriate system? ☑ Yes ☐ No

Appropriate controls/concentrations ☑ Yes ☐ No

Appropriate test concentrations? ☑ Yes ☐ No

Note(s):

1) Five concentrations that spanned 4 orders of magnitude
2) Enterocyte concentration estimated to be 78 nM ($[I]_g = F_a \times k_a \times Dose/Q_{en}$)

Integrated Summary

1) All R_1 values were less than 1.02, the cut off outlined in the FDA DDI guidance to determine the need for an *in vivo* study. In addition, given that the lowest IC_{50} values noted in the study (i.e.,CYP3A4) are significantly higher than the concentrations observed *in vivo*, the potential for rifamycin SV to inhibit CYP enzymes systemically appears to be low.
2) $R_{1,gut}$ values for CYP3A4 were greater than 11; the threshold outlined in the FDA DDI guidance to further explore interaction potential by static mechanistic modeling or *in vivo* study. The predicted AUCR for midazolam (most sensitive) using static mechanistic modeling was <1.25; the cut off for clinical investigations. As a worst-case scenario, we considered a higher intestinal extracted drug (e.g., tacrolimus). Negligible changes in AUCR were noted (AUCR midazolam 1.01 vs AUCR tacrolimus 1.02). In conjunction with a proposed limited treatment duration and colon release, the risk of rifamycin to significantly affect CYP3A4 interaction appears minimal.
3) No Time dependent Inhibition (TDI) or mechanism based inhibition (MBI) mechanisms were noted for any CYPs.

Enzymes Involved in Biotransformation of Rifamycin SV in Man In Vitro	
Title (Study #)	Assessment of the potential to inhibit human hepatic cytochrome P450 (CYP) enzymes in vitro (CPS/11)(Study Report Amendment)
Objectives	Determine stability of rifamycin SV
METHODS	
System	Pooled human liver microsomes (n=150 donors) at 0.025 and 0.3 mg microsomal protein/mL were incubated with rifamycin SV (0.1- or 340 µM) in the presence and absence of enzyme cofactor. Samples were taken at 0, 10, and 40 minutes and analyzed by LC-MS/MS to quantify rifamycin SV concentrations.
RESULTS	

0.025	Yes	0	325/ 0.268	100/ 100
		10	323/ 0.254	99.4/ 94.8
		40	**412/ 0.199**	**127/ 74.3**
	No	0	360/ 0.380	100/ 100
		10	353/ 0.177	98.1/ **46.6**
		40	**474/ 0.151**	**132/ 39.7**
0.3	Yes	0	325/ 0.275	100/ 100
		10	381/ 0.201	117/ **73.1**
		40	295/ 0.214	90.8/ **77.8**
	No	0	551/ 0.249	100/ 100
		10	580/ 0.239	105/ 96.0
		40	**316/ 0.163**	**57.4/ 65.5**

[a]Target rifamycin SV: 340 / 0.1 µM
[b]relative to 0 min
[c]Outside 20% target window

REVIEWER ASSESSMENT
Experimental Approach (*FDA in vitro metabolism and transporter DDI Guidance 2017*) Controls/concentrations appropriate? ☑ Yes ☐ No Test concentrations appropriate? ☑ Yes ☐ No
Integrated Summary 1) Drug stability (within 20% of nominal target concentration) was demonstrated for: i) 340 µM rifamycin SV and 0.025 mg/mL microsomal protein (± cofactor), and ii) 0.3 mg/mL microsomal protein (with cofactor) and 340 µM rifamycin SV. 2) Drug stability was not achieved for i) 340 µM rifamycin SV and 0.3 mg/mL microsomal protein (without cofactor), and ii) 0.1 µM rifamycin SV and 0.3 mg/mL microsomal protein (with and without cofactor). 3) Time dependent drug loss was observed for 0.1 µM rifamycin SV in the absence of cofactor for both 0.025 and 0.3 mg/mL microsomal protein. 4) Results seem to suggest technical error (i.e., pipetting) rather than compound instability as: i) the 340 µM rifamycin SV target concentration in 0.3 mg/mL microsomal protein without cofactor was measured at 551 µM (at 0 min). Subsequent time points demonstrated concentrations above (580 µM at 10 min) or slightly below (316 µM at 40 min) the nominal target. Furthermore, in the nominal 340 µM group, the lowest concentration measured was 295 µM at 40 min (cofactor added). Interestingly, all the 0.1 µM rifamycin SV target concentrations studied were observed to be >0.15 µM irrespective of time or cofactor. Thus, the reported % differences are deceptive as concentrations are near the nominal concentration

or greater in all instances. Collectively, data suggests technical error and preclude definitive conclusions.

5) Despite these inconclusive findings, the review team does not believe it impacts *in vitro* CYP inhibition study assessments conducted using microsomes as: i) the true concentration would be expected to be broadly the nominal concentrations and thus changes in experimentally determined IC_{50}'s would be similar or slightly higher, and ii) as calculations evaluating drug interaction potential already incorporate high µM IC_{50}'s compared with low nM enterocyte rifamycin SV concentrations, changes in the IC_{50} value would not significantly impact predictions.

Title: Rifamycin SV: Drug Transporter Interactions	Study Report CPS/10
Cell System and Objective	Monolayers of P-gp or BCRP overexpressing Caco-2 and MDCKII cells assessed rifamycin SV substrate potential.
Investigational drug (concentration)	Rifamycin SV (0.344 – 34.4 µM) for 120 minutes
Probe Substrate (concentration)	P-gp: Digoxin (10 µM) BCRP: Prazosin (1 µM)
Reference Inhibitor (concentration):	P-gp: Verapamil (50 µM) BCRP: Ko134 (1 µM)
Bidirectional Markers (confirm monolayer polarity)	Atenolol and Propranolol (10 µM)

Results

Table 84: Permeability of Rifamycin SV Across the Caco-2 Cell Monolayer[a]

Rifamycin SV (uM)	A to B		B to A		Efflux Ratio (B-A/A-B)
	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD	
0.344	NA	NC	15.0	0.6	NE
3.44	0.722	0.087	4.27	0.6	5.98
10.3	0.513	0.131	3.17	0.17	6.42
34.4	1.12	NC	4.53	0.067	3.97

[a]Data represent the120 minute incubation period
P_{app} data reported as mean of at least 2-3 values
NA – no two values within <30% deviation of mean; all data points excluded
NC- standard deviation (SD) not calculable
NE- no efflux data could be calculated
Source: CPS-10, Table 3, pg 45

Table 85: Efflux of Rifamycin SV Across the Cell Membrane of P-gp Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)		Net Flux Ratio (ER_t/ER_w)
	MDCKII-WT	MDCKII-P-gp	
0.344	1.84[b]	13.0[b]	7.07
3.44	2.08[b]	17.8	8.56
10.3	2.42[b]	16.8	6.94
34.4	2.62[b]	5.70	2.18
0 +Digoxin	122	300	2.46
0.344 + VPM	0.897	4.28[b]	4.77
3.44 + VPM	1.99	10.3	5.18
10.3 + VPM	2.37	5.37	2.27
34.4 + VPM	1.94	3.15	1.62
0+Digoxin + VPM	80.5[b]	97.9[b]	1.22

Source: CPS-10, Table 14, pg 56

Table 86: Inhibition of Rifamycin SV Efflux by Verapamil Using the P-gp Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)		% Inhibition by VPM
	Without VPM	With VPM	
0.344	13.0[b]	4.28[b]	67.1
3.44	17.8	10.3	42.1
10.3	16.8	5.37	68.0
34.4	5.70	3.14	44.7
0 +Digoxin	300	97.9[b]	67.4

Source: CPS-10, Table 15, pg 57

Table 87: Efflux of Rifamycin SV Across the Cell Membrane of BCRP Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)		Net Flux Ratio (ER_t/ER_w)
	MDCKII-WT	MDCKII-BCRP	
0.344	0.682[b]	0.347	0.509
3.44	1.21[b]	0.416[b]	0.344
10.3	1.13[b]	0.535[b]	0.473
34.4	1.26[b]	0.369[b]	0.293
0 +Prazosin	0.736	23.4	31.8
0.344 + K	1.35[b]	0.265[b]	0.196
3.44 + K	3.09	0.260[b]	0.0841
10.3 + K	2.85	0.407[b]	0.143
34.4 + K	2.38	0.431[b]	0.181
0+Prazosin + K	0.704	0.588	0.835

Source: CPS-10, Table 18, pg 60

Table 88: Inhibition of Rifamycin SV Efflux by Ko143 using the BCRP Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)		% Inhibition by Ko143
	Without Ko143	With Ko143	
0.344	0.347	0.265[b]	23.6
3.44	0.416[b]	0.260[b]	37.5
10.3	0.535[b]	0.407[b]	23.9
34.4	0.369[b]	0.431[b]	0
0 +Prazosin	23.4	0.588	97.5

Source: CPS-10, Table 19, pg 61

[a]Data represent the120 minute incubation period
[b]Post experimental Lucifer yellow test failed to meet acceptance criterion in at least one replicate; value is included for analysis here
ER_t = efflux ratio transfected cells
ER_w = efflux ratio wild-type cells

REVIEWER ASSESSMENT

Experimental Approach (*FDA in vitro metabolism and transporter DDI Guidance 2017*)

Controls/concentrations appropriate? ☑ Yes ☐ No
Test concentrations appropriate? ☑ Yes ☐ No
Assay System Functional? ☑ Yes ☐ No

Integrated Summary

1) Intestinal absorption: Low apical to basolateral transport and extensive basolateral to apical transport in Caco-2 cells suggest that efflux transporters serve as a rate limiting barrier to

absorption. The efflux ratio greater than 2 for rifamycin SV prompted the Sponsor to further assess P-gp and BCRP mediated transport with specific probes and inhibitors.

2) P-gp substrate: Rifamycin SV appears to be a substrate of P-gp based on a >2-fold increase in the ER and NFR in transporter over-expressing cells compared to wildtype cells. Decreases in ER and NFR in the presence of verapamil (P-gp inhibitor) further support the conclusion.
3) BCRP Substrate: Rifamycin SV does not appear to be a substrate for BCRP based on the <2-fold difference in ER between transporter over-expressing and wildtype cells. No significant decreases in ER and NFR in the presence of Ko143 (BCRP inhibitor) further support the conclusion.
4) P-gp inhibitor effects on rifamycin SV exposure with Rifamycin SV MMX®: Minimal systemic exposure (bioavailability ca. 0.04%) in conjunction with poor drug solubility, proposed limited treatment duration, and targeted colon release the review team does not expect an interaction and increase in systemic rifamycin SV exposure when concomitantly administered with P-gp inhibitors to be clinically significant.
5) P-gp inducer effects on rifamycin SV exposure in the gastrointestinal tract with rifamycin SV MMX®: As mentioned above, rifamycin SV MMX® exerts its actions locally in the lumen therefore risk of treatment failure would not be expected as more drug would be effluxed back to the lumen.

Title: Rifamycin SV: Drug Transporter Interactions	Study Report CPS/10
Cell System and Objective	Monolayers of P-gp or BCRP overexpressing Caco-2 and MDCKII cells assessed rifamycin SV inhibitor potential.
Investigational Drug (concentrations)	Rifamycin SV (0.344, 3.44, 10.3, 34.4 µM)
Probe Substrate (concentration)	P-gp: Digoxin (10 µM) BCRP: Prazosin (1 µM)
Control Inhibitor (concentration)	P-gp: Verapamil (50 µM) BCRP: Ko143 (1 µM)
Bidirectional Markers	Atenolol and Propranolol (10 µM)

Results

Table 89: Inhibitory Effects of Rifamycin SV Toward P-gp using Caco-2 Cell Monolayer[a]

Rifamycin SV (uM)	A to B		B to A		Efflux Ratio (B-A/A-B)
	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD	
0	1.11	0.17	5.24	0.37	4.75
0.344	1.08	0.07	5.07[b]	0.12	4.72[b]
3.44	1.03	0.12	4.51	0.15	4.42
10.3	1.09	0.18	4.07	0.33	3.83
34.4	1.28	0.16	4.69	0.27	3.69
VPM	2.41	0.27	1.64	0.10	0.687

[a]Data represent the 120-minute incubation period
[b]Post experimental Lucifer yellow test failed to meet the acceptance criterion in at least one replicate; failed still reported
P_{app} data reported as mean of at least 2 values
NC- standard deviation (SD) not calculable
Efflux ratio reported as mean of at least two values
VPM – verapamil (reference inhibitor)
Digoxin – Probe substrate
Source: CPS-10, Table 4, pg 46

Table 90: Inhibition of P-gp by Rifamycin SV using Caco-2 Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)	% Inhibition
0	4.75	0
0.344	4.72[b]	0.632[b]
3.44	4.42	6.95
10.3	3.83	19.4
34.4	3.69	22.3
VPM	0.687	85.5

[a]Data represent the 120-minute incubation period
[b]Post experimental Lucifer yellow test failed to meet the acceptance criterion in at least one replicate; failed still reported
% inhibition relative to 0 µM
Digoxin – Probe Substrate
Source: CPS-10, Table 5, pg 47

Table 91: Inhibitory Effects of Rifamycin SV Toward P-gp using P-gp Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	MDCKII-WT				MDCKII-P-gp			
	A to B		B to A		A to B		B to A	
	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD
0	1.30	0.09	4.57	0.11	0.497	0.051	8.37	0.88
0.344	1.22	0.12	4.17	0.49	0.385	0.029	8.43	0.42
3.44	1.24	0.21	2.02	NC	0.375	0.042	6.57	0.72
10.3	1.20	0.31	1.88	0.35	0.452	0.041	6.05	0.27
34.4	1.60	0.14	1.86	0.32	0.389	0.05	6.31	1.25
VPM	1.83	0.25	1.02	0.09	1.01	0.08	4.31	0.49

[a]Data represent the120 minute incubation period
[b]Post experimental Lucifer yellow test failed to meet the acceptance criterion in at least one replicate; failed still reported
P_{app} data reported as mean of at least 2 values
WT- Wild-Type
NC- standard deviation (SD) not calculable
VPM – verapamil (reference inhibitor)
Digoxin – Probe substrate
Source: CPS-10, Table 6, pg 48

Table 92: Net Flux of Digoxin Across the P-gp Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)		Net Flux Ratio (ER_t/ER_w)
	MDCKII-WT	MDCKII-P-gP	
0	3.53	17.0	4.82
0.344	3.46	21.9	6.33
3.44	1.77	17.7	10.0
10.3	1.62	13.5	8.33
34.4	1.16	16.2	14.0
VPM	0.562	4.3	7.65

Efflux ratio reported as mean of at least two values
WT – Wild-Type
VPM – verapamil (reference inhibitor)
Digoxin – Probe substrate
Source: CPS-10, Table 7, pg 49

Table 93: Inhibition of P-gp by Rifamycin SV using MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)	% Inhibition
0	17.0	0
0.344	21.9	0
3.44	17.7	0
10.3	13.5	20.6
34.4	16.2	4.71
VPM	4.30	74.7

[a]Data represent the120 minute incubation period
% inhibition relative to 0 µM
VPM – verapamil (reference inhibitor)
Digoxin – Probe Substrate
Source: CPS-10, Table 8, pg 50

Table 94: Inhibitory Effects of Rifamycin SV Toward BCRP using BCRP Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	MDCKII-WT				MDCKII-BCRP			
	A to B		B to A		A to B		B to A	
	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD	P_{app} ($x10^{-6}$ cm/s)	±SD
0	27.9	1.2	21.5	0.9	1.60	0.03	41.0	1.9
0.344	28.2	0.4	24.2	1.0	1.49	0.17	50.7	2.3
3.44	24.5	2.8	20.3	1.6	1.99	0.08	46.4	1.9
10.3	26.2	3.5	18.0	NC	1.92	0.05	49.4	4.5
34.4	27.3	1.0	23.3	1.6	2.98	0.22	47.4	2.7
Ko143	27.9	3.7	23.1	0.6	22.9	2.4	17.8	0.5

[a]Data represent the120 minute incubation period
[b]Post experimental Lucifer yellow test failed to meet the acceptance criterion in at least one replicate; failed still reported
P_{app} data reported as mean of at least 2 values
WT- Wild-Type
NC- standard deviation (SD) not calculable
Ko143 - reference inhibitor
Digoxin – Probe substrate
Source: CPS-10, Table 9, pg 51

Table 95: Net Flux of Prazosin Across the BCRP Transfected MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)		Net Flux Ratio (ER_t/ER_w)
	MDCKII-WT	MDCKII-BCRP	
0	0.769	25.6	33.3
0.344	0.860	34.3	39.9
3.44	0.831	23.4	28.2
10.3	0.743	25.7	34.6
34.4	0.855	15.9	18.6
Ko143	0.836	0.783	0.937

[a]Data represent the120 minute incubation period
Efflux ratio reported as mean of at least two values
WT – Wild-Type
Ko143 – reference inhibitor
Prazosin – Probe substrate
Source: CPS-10, Table 10, pg 52

Table 96: Inhibition of BCRP by Rifamycin SV using MDCKII Cell Monolayer[a]

Rifamycin SV (uM)	Efflux Ratio (B-A/A-B)	% Inhibition
0	25.6	0
0.344	34.3	0
3.44	23.4	8.59
10.3	25.7	0
34.4	15.9	37.9
Ko143	0.783	96.9

[a]Data represent the120 minute incubation period
% inhibition relative to 0 µM
Ko143 – reference inhibitor
Prazosin – Probe Substrate
Source: CPS-10, Table 11, pg 53

REVIEWER ASSESSMENT
Experimental Approach (*FDA in vitro metabolism and transporter DDI Guidance 2017*) Controls/concentrations appropriate? ☑ Yes ☐ No Test concentrations appropriate? ☑ Yes ☐ No Assay System Functional? ☑ Yes ☐ No Note(s): 1. A plasma C_{max} =**0.012 µM** was observed following repeat Rifamycin SV MMX® administration (400 mg b.i.d. for 3 days) 2. Luminal gut concentration estimated at 2293 µM rifamycin SV base (I_{gut} = Dose [molar units]/250 mL) 3. IC_{50} is the inhibitor concentration showing 50% inhibition 4. Caco-2 determined rifamycin SV IC_{50} for P-gp is 6.54 **µM.** 5. **MDCKII rifamycin SV IC_{50}** for BCRP could not be determined (>34.4 **µM**)
Integrated Summary 1) Inhibition of P-gp transporters due to systemic levels of rifamycin SV: Based on the highest observed mean plasma C_{max} after repeat administration of 400 mg Rifamycin SV MMX®, inhibition of P-gp or BCRP transporters systemically are not likely. 2) Effect of rifamycin SV on P-gp transporters in the gastrointestinal tract: The draft 2017 FDA DDI guidance recommends the following cut off criteria to assess the potential for a drug to inhibit P-gp: I_{gut}/IC_{50}> 10. Based on the reviewer's estimate, an I_{gut}/IC_{50} of ca. 351 suggests that rifamycin SV may be an inhibitor of P-gp transporters in the gut. 3) Effect of rifamycin SV on BCRP transporters in the gastrointestinal tract: The draft 2017 FDA DDI guidance recommends the same cutoff criteria as above to assess the potential for a drug to inhibit BCRP. At the maximum concentration tested (34.4 µM), ca. 38% inhibition was observed. It is unclear from the data whether greater concentrations would result in more inhibition. Therefore, since theoretical concentrations in the lumen of the gastrointestinal tract could reach 2293 µM the review team chose to use 34.4 µM as the IC_{50} (worst-case) to calculate the I_{gut}/IC_{50}. A ratio of 67.7 suggest that rifamycin SV may be an inhibitor of BCRP in the gut.

Title: Rifamycin SV: Drug Transporter Interactions	Study Report CPS/10
Cell System and Objective	Monolayers of SLC transporter (OAT1, OAT3, OCT2, MATE1 or MATE2-K) overexpressing HEK 293 cells assessed rifamycin SV inhibitor potential.
Investigational Drug (concentrations)	Rifamycin SV (5.25, 52.5, 158, and 525 nM OAT1 /OAT3 and 0.525, 5.25, 15.8, 52.5 nM OCT2/ MATE1/ MATE2-K)
Probe Substrate (concentration)	OAT1: p-Aminohippuric acid (30 µM) OAT3: Estrone 3-sulphate (2 µM) OCT2/MATE1/MATE2-K: Metformin (100 µM)
Control Inhibitor (concentration)	OAT1/OAT3: Probenecid (50 µM) OCT2: Decynium-22 (10 µM) MATE1/MATE2-K: Pryimethamine (10 µM)
Bidirectional Markers	N/A

Results

Transporter	SLC Uptake Ratio[a]	Maximum Inhibition (% Control)				IC_{50} Values (nM)	
	Substrate	Investigational Drug		Control Inhibitor		Investigational Drug	
		Wildtype	Overexpressing	Wildtype	Overexpressing	Wildtype	Overexpressing
OAT1	204	0.1	10.1	-26.0	86.2	>525[c]	>525[c]
OAT3	20.3	4.1	18.7	56.5	83.6	>525[c]	>525[c]
OCT2	29.4	-4.9	5.4	20.8	82.5	>52.5[c]	>52.5[c]
MATE1	7.44	0.2	7.5	-43.0	96.3	>52.5[c]	>52.5[c]
MATE2-K	**0.606**	0.2	11.7	-43.0	74.0	>52.5[c]	>52.5[c]

[a]ratio calculated as mean uptake rate of known probe substrate in overexpressing / wildtype cells
[b]Represents the highest maximum inhibition in the concentration range tested
[c]Insufficient inhibition at highest tested concentration to determine IC_{50}
Source: CPS-10, Adopted from Tables 20-25

REVIEWER ASSESSMENT

Experimental Approach (*FDA in vitro metabolism and transporter DDI Guidance 2017*)

Controls/concentrations appropriate? ☑ Yes ☐ No
Test concentrations appropriate? ☑ Yes ☐ No
Assay System Functional? ☑ Yes ☐ No
Note(s):

1. A plasma C_{max} =**0.012 µM** was observed following repeat rifamycin SV MMX® administration (400 mg b.i.d. for 3 days)
2. $C_{max,\ unbound}$ = 12 nM x 0.2 (fraction unbound) = 2.4 nM
3. IC_{50} is the inhibitor concentration showing 50% inhibition

Integrated Summary

1) The draft FDA 2017 guidance indicates that a $C_{max,unbound}/IC_{50}$ >0.1 indicates that the drug may be an inhibitor of renal uptake transporters (OAT1, OAT3, and OCT3). Based on the highest observed plasma C_{max} of rifamycin SV after administration of 400 mg rifamycin SV MMX® (~0.012 µM or 12 nM) and a fraction unbound of 0.20, the unbound C_{max}/IC_{50} is significantly lower than 0.1, hence rifamycin SV is not expected to inhibit OAT1, OAT3, and OCT2 transporters *in vivo*.
2) The draft FDA 2017 guidance indicates that a $C_{max,unbound}/IC_{50}$ >0.2 indicates that the drug may be an inhibitor of renal uptake transporters (MATE-1 and MATE-2K). Based on the highest observed plasma C_{max} of rifamycin SV after administration of 400 mg rifamycin SV MMX® (~0.012 µM or 12

nM) and a fraction unbound of 0.20, the unbound C_{max}/IC_{50} is significantly lower than 0.1, hence rifamycin SV is not expected to inhibit MATE-1 or MATE-2K transporters *in vivo*.

3) No *in vitro* experiments were conducted to determine if rifamycin SV is a substrate of OAT1, OAT3, OCT2, MATE1, or MATE2-K. The review team finds this acceptable as the primary elimination rifamycin SV is not active renal secretion and thus follows recommendations laid out in draft FDA 2017 guidance

Title: Subgroup Analysis Comparing Rifamycin SV-MMX® and Ciprofloxacin Clinical Cure Rates with Respect to Medications for Acid Related Disorders	Study Report: RIT-1/AID pg 106
Investigational Drug	200 mg Rifamycin SV MMX® tablet
Alcohol	0.1M HCL solution at 40% ethanol

Results

Table 97: Clinical Cure Rate by Subgroup Analysis (Acid Suppressants and Propulsive/Motility Agents)

	Rifamycin SV-MMX® (1) (N = 420)	Ciprofloxacin (2) (N = 415)	Difference (1) – (2) (%)
With Drugs for Acid Related Disorders			
n	53	43	
Yes	44 (83.0%)	38 (88.4%)	-5.4
No	9 (17.0%)	5 (11.6%)	
95% CI (Response)	[72.9, 93.1]	[78.8, 98.0]	[-19.3, 8.6]
p-value (chi square test)			0.4599
Without Drugs for Acid Related Disorders			
n	367	372	
Yes	313 (85.3%)	314 (84.4%)	0.9
No	54 (14.7%)	58 (15.6%)	
95% CI (Response)	[81.7, 88.9]	[80.7, 88.1]	[-4.3, 6.0]
p-value (chi square test)			0.7394
With Motility Drugs			
n	8	10	
Yes	8 (100.0%)	6 (60.0%)	40.0
No	0 (0.0%)	4 (40.0%)	
95% CI (Response)	[100.0, 100.0]	[29.6, 90.4]	[9.6, 70.4]
p-value (chi square test)			0.0425
Without Motility Drugs			
n	412	405	
Yes	349 (84.7%)	346 (85.4%)	-0.7
No	63 (15.3%)	59 (14.6%)	
95% CI (Response)	[81.2, 88.2]	[82.0, 88.9]	[-5.6, 4.2]
p-value (chi square test)			0.7718

Source: RIT-1/AID, Table 27, pg 106

REVIEWER ASSESSMENT

Integrated Summary

Acid Suppressant Drug Interaction. Concomitant proton pump inhibitor use was ca. 95% for each treatment arm in the "Drugs for Acid Related Disorders" subgroup. No statistical differences were noted in Clinical Cure Rate. Overall, rifamycin SV is expected to primarily undergo colonic drug release based on the following considerations:

a) Presence of a film coat and controlled release excipients (biopharm summary, pg.8) to ensure that rifamycin SV is released directly into the colon.
b) Dissolution data where the batch of rifamycin SV RV used for Phase 3 trials and the registration batch showed minimal dissolution at pH1 and pH 7.2 (after 1 hour) (page 11 of the biopharma summary)
c) Available PK data from the BA and FE assessment where most the concentrations above BLQ are observed after 6 hours (which seems to correspond to the dissolution data).
d) Theoretical basis which suggest that the small intestine is the major site of drug absorption[1]

Reference:
1.http://www.derangedphysiology.com/main/cicm-primary-exam/required-reading/pharmacokinetics/Chapter%201.3.4/drug-absorption-small-intestine

Reviewer Analysis

Title: *In Vivo* Inhibition Potential of the OATP1B1 Transporter by Rifamycin SV MMX®: An *In Vitro* and *In Silico* approach.

Rationale/Background: Rifamycin SV is found on the FDA Interactions and Labeling website as an *in vitro* inhibitor for the OATP1B1 and OATP1B3 transporters. Furthermore, an article by Acocella *et al* demonstrated elevated bilirubin following rifamycin SV intravenous administration(Acocella, Nicolis et al. 1965).

Elevations in total bilirubin was noted in ca. 1-2% of subjects (.M5, 5.3.5.3, ISS table 30.1). Because bilirubin is a substrate of OATP1B1 and OATP1B3 transporters, the potential for rifamycin SV (after administration of rifamycin SV MMX tablets) to inhibit OATP1B1 and OATP1B3 transporters was assessed as outlined in the draft FDA 2012 DDI guidance.

Objective: Assess potential clinical significance for a concentration-related interaction between Rifamycin SV MMX® and OATP1B1.

Methods: A basic static physiologically based PK model for OATP1B1/3 inhibition was utilized using Sponsor and published literature data as recommended by FDA guidance[3]. Definitions of terms can also be found in FDA guidance[3].

$$R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50})$$

IC_{50} = 2 µm (Vavricka et al(Vavricka, Van Montfoort et al. 2002))

$[I]_{in,\,max}$ = 0.026 µM

$$[I]_{in,max} = (I_{max} + (F_a \times F_g \times K_a \times Dose))/(Q_h/R_b)$$

MW = 697.77 g/mol (Sponsor data)

Dose = 400 mg (Sponsor data; proposed dose)

I_{max} = 0.012 µM (Sponsor data)

K_a = 0.1 min-1 (FDA Guidance)

$F_{u,p}$ = 0.2 (Sponsor data)

Q_h = 1.62 L/min (FDA Guidance)

R_b = 1 (FDA Guidance)

F_g = 1 (Estimated from Sponsor data)

F_a = 0.004 (Sponsor data)

	Model Predictions for Clinical Potential of Drug Inhibition Interactions
	AUCR
OATP1B1	1.00

Conclusion:

1. The estimated maximum hepatic inlet concentration achieved for rifamycin SV MMX® is ca. 0.149 uM and the reported IC_{50} ca. 2 µM; ca. 1 order of magnitude greater (base Log_{10}). hence, rifamycin SV is unlikely to inhibit OATP transporters *in vivo*, when given as rifamycin SV MMX® tablet formulation. The model does predict that rifamycin SV may inhibit OATP transporters after intravenous administration and may help to explain, in part, the observation of increases in bilirubin after intravenous administration of rifamycin SV (as described in Acocella *et al* article).
2. No *in vitro* studies were conducted by the Sponsor to evaluate rifamycin SV as an OATP1B1 or OATP1B3 substrate. The review team find this acceptable as systemic absorption is (<0.1%) and follows recommendations set forth in FDA guidance[3].

References:

3. Food and Drug Administration. 2017. *In Vitro Metabolism - and Transporter - Mediated Drug- Drug Interaction Studies Guidance for Industry*. [ONLINE] Available at: https://www.fda.gov/downloads/Drugs/Guidances/UCM581965.pdf. [Accessed 14 June 2018]

16 Division Director (Clinical)

Concur with review.

17 Office Director (OAP)

Concur with review.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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