CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211672Orig1s000
211673Orig1s000

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)	211672, 211673
Priority or Standard	Priority
Submit Date(s)	19 December 2018
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PDUFA Goal Date	19 August 2019
Division/Office	DAIP/OAP
Review Completion Date	12 August 2019
Established/Proper Name	Lefamulin
(Proposed) Trade Name	XENLETA
Pharmacologic Class	Pleuromutilin
Code name	BC-3781
Applicant	Nabriva Therapeutics
Dosage form	150 mg for injection and 600 mg oral tablet
Applicant proposed Dosing Regimen	150 mg IV every 12 hours; 600 mg PO every 12 hours
Applicant Proposed Indication(s)/Population(s)	Community-Acquired Bacterial Pneumonia (CABP) in adults
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	53084003 |Bacterial pneumonia (disorder)|
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	Community-Acquired Bacterial Pneumonia (CABP) in adults
Recommended SNOMED CT Indication Disease Term for each Indication (if applicable)	53084003 |Bacterial pneumonia (disorder)|
Recommended Dosing Regimen	150 mg IV every 12 hours; 600 mg PO every 12 hours

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Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CABP	community-acquired bacterial pneumonia
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
ECG	electrocardiogram
ECR	early clinical response
eCTD	electronic common technical document
EOT	end of treatment
ETASU	elements to assure safe use
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
GRMP	good review management practice
IACR	investigator assessment of clinical response
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
LEF	lefamulin
LFU	late follow up
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat

MOX	moxifloxacin
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PCS	Potentially Clinically Significant
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
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
SGE	special government employee
SOC	standard of care
TOC	test of cure
TEAE	treatment emergent adverse event

1 Executive Summary

1.1. Product Introduction

Lefamulin (XENLETA) is a pleuromutilin antibacterial drug available as oral and IV formulations. The proposed dose is 150 mg intravenously (IV) every 12 hours or 600 mg oral every 12 hours for a total duration of 5 to 7 days.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness of lefamulin for the treatment of CABP due to the designated susceptible bacteria in adults from two adequate and well-controlled Phase 3 trials (Studies 3101 and 3102). In Study 3101, subjects were randomized to receive either IV lefamulin or IV moxifloxacin with the option to switch to oral lefamulin or oral moxifloxacin, respectively, after 3 days. In Study 3102, subjects were randomized to receive either oral lefamulin or oral moxifloxacin. Lefamulin was noninferior to moxifloxacin in both trials for the primary endpoint of early clinical response rates (ECR). Consistent results were observed for secondary efficacy endpoints of investigator assessed clinical responses at the test of cure visit, 5-10 days after completing therapy and up to 30 days after starting therapy. ECR rates were similar in the treatment groups in various demographic and baseline health status subgroups in both trials.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

In NDAs 211672 and 211673, the Applicant is seeking approval of lefamulin injection and tablets respectively, for the treatment of CABP in adults due to designated susceptible bacteria. Lefamulin is a pleuromutilin antibacterial drug with oral and IV formulations. CABP is a serious infection associated with significant morbidity and mortality, especially those who are older and have comorbidities. Although there are many antibacterial drugs approved to treat CABP, antimicrobial resistance, safety profile, and lack of oral formulations for some drugs may limit their use in certain patients. Therefore, it is important to have different therapeutic options available for the treatment of CABP to meet patient needs.

In two Phase 3 trials, lefamulin was noninferior to moxifloxacin for the treatment of CABP. In Study 3101, subjects with Pneumonia Outcome Research Team (PORT) scores of ≥ III were randomized to receive either IV lefamulin or IV moxifloxacin with the option to switch to the respective oral formulations after 3 days. In Study 3102, subjects with PORT scores of II, III, or IV and able to take oral medication were randomized to receive either oral lefamulin or oral moxifloxacin. The primary efficacy endpoint in both trials was early clinical response (ECR) which included improvement in at least two patient-reported symptoms without any worsening 3 days after starting therapy. In Study 3101, the ECR rate was 87.3% for lefamulin and 90.2% for moxifloxacin with a difference of -2.9% (95% CI, -8.5, 2.8). In Study 3102, the ECR rate was 90.8% for lefamulin and 90.8% for moxifloxacin with a difference of 0.0% (95% CI, -4.4, 4.5). The ECR rates for lefamulin were noninferior to moxifloxacin in both studies and the difference between the treatment groups met the predefined noninferiority margin. Lefamulin had similar ECR rates compared to moxifloxacin in various demographic and baseline health status subgroups in both trials. In addition, investigator assessed clinical response at the test of cure visit, 5-10 days after completing therapy and up to 30 days after starting therapy did not show meaningful differences between the treatment groups.

The safety database is comprised of 641 patients who received IV or oral lefamulin for CABP at the proposed dose and duration. Additional safety information was provided by 71 subjects enrolled in a Phase 2 trial (Study 2001) for Acute Bacterial Skin and Skin Structure Infections (ABSSSI). In the Phase 3 CABP trials, rates of deaths, serious adverse events, and treatment-emergent adverse events were similar between subjects treated with lefamulin and moxifloxacin. There were more lung infections reported as serious adverse events in the Phase 3 trials among lefamulin-treated subjects compared to moxifloxacin-treated subjects (12 versus 6). Review of the cases suggested that these likely represented lack of efficacy of the study drug to treat the pneumonia, many of which may have been caused by pathogens not covered by lefamulin, including Enterobacteriaceae. Of note, these serious adverse events of treatment failure were captured as failures in the efficacy

analyses. Enterobacteriaceae are not common causes of CABP; subsection 12.4 of the label will reflect that lefamulin does not have antibacterial activity against Enterobacteriaceae.

QT prolongation was a safety issue identified early in drug development. The Phase 3 trials confirmed lefamulin prolongs the QT interval to a similar extent as moxifloxacin and this is included in the Warnings and Precautions section of the label. Animal studies showed fetal malformations, postimplantation fetal loss, stillbirths, as well as additional rat pup deaths during early lactation, in rats and rabbits treated during the period of organogenesis or in rats treated from the beginning of organogenesis through the time of weaning. Labeling will include a statement in the Warnings and Precautions section advising females of reproductive potential to use effective contraception during treatment and for 2 days after the final dose and a recommendation that the pregnancy status be verified in females of reproductive potential prior to initiating therapy. Animal studies indicate that lefamulin was concentrated in the milk of lactating rats. Subsection 8.2 of the label advises lactating women to pump and discard milk during treatment with lefamulin. Administration site reactions with the IV formulation and nausea and vomiting with the oral formulation were more commonly seen with lefamulin; they were mostly mild to moderate in severity and did not result in treatment discontinuation in most patients.

A postmarketing requirement (PMR) for a pregnancy surveillance program will collect information on pregnancy complications and birth outcomes in women exposed to lefamulin during pregnancy. The Applicant will conduct two studies as PMRs to assess the genotoxicity of lefamulin and its main metabolite, BL-8041, using in vitro assays as mutagenicity testing was not valid for these compounds.

In summary, there are adequate data to support the efficacy of lefamulin for the treatment of CABP with an acceptable safety profile. Safety issues will be addressed in product labeling and the required postmarketing studies will evaluate the risk of genotoxicity and provide outcome data on use in pregnancy.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• CABP is an acute lung infection in patients without recent healthcare exposure. It is characterized by symptoms of chest pain, cough, sputum production, difficulty breathing, chills, rigors, and fever. • Common pathogens that cause CABP include *S. pneumoniae*, *H. influenzae*, *S. aureus*, *M. catarrhalis*, *C. pneumoniae*, *M. pneumoniae*, and *L. pneumophila*.	CABP is a serious infection that causes significant morbidity and mortality in patients, especially those who are older and have medical comorbidities.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• The incidence of CABP is 24.8 per 10,000 adults but is higher with older age. CABP can be severe and require hospitalization especially for older patients and those with medical comorbidities. Among hospitalized patients, the mortality can be as high as 23%.	
Current Treatment Options	• There are many FDA-approved antibacterial drugs for the treatment of CABP including macrolides, fluoroquinolones, cephalosporins, and beta-lactam drugs. Some of the available drugs have IV and oral formulations, but others have only IV formulations. • Some of the available drugs have known adverse reactions including QT prolongation, tendonitis, and neuropathy. • The choice of an antibacterial drug depends on the severity of the patient's illness, underlying comorbidities, the likely pathogen, and the adverse event profile of the drug.	There are many antibacterial drugs approved to treat CABP, but antimicrobial resistance, adverse reactions, and lack of oral formulations may limit their use in certain patients.
Benefit	• The efficacy of lefamulin in the treatment of CABP was demonstrated in two adequate and well-controlled noninferiority trials in which lefamulin was compared to moxifloxacin. • Most subjects in Study 3101 were PORT risk class III (72.2%) and received IV therapy with an option to switch to oral therapy. • In Study 3102 about half of the subjects were PORT risk class II (50.8%) with the rest being PORT risk class III or IV. All subjects received oral therapy. • Lefamulin was noninferior to moxifloxacin at the early clinical response evaluation (ECR, Day 4) in both trials. — In Study 3101, the ECR rate was 87.3% for lefamulin and 90.2% for	The effectiveness of lefamulin for the treatment of CABP was demonstrated in two adequate and well-controlled trials. Lefamulin was noninferior to moxifloxacin in both trials with respect to the primary endpoint of early clinical response. Consistent results were seen at later time points as well.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	moxifloxacin with a difference of -2.9% (95% CI, -8.5% to 2.8%). — In Study 3102, the ECR rate was 90.8% for lefamulin and 90.8% for moxifloxacin with a difference of 0% (95% CI, -4.4% to 4.5%). • The ECR rates between lefamulin and moxifloxacin did not differ substantially in various demographic or baseline health status subgroups in either trial. • Consistent results were seen for the secondary endpoints of investigator assessed clinical response at the test of cure (5-10 days after completing treatment) and at the late follow up visit (30 days post therapy).	
Risk and Risk Management	• The safety database included 1242 subjects who received varying doses of lefamulin. • The primary safety population included 641 lefamulin-treated subjects with CABP from two Phase 3 trials who received the proposed dosing regimen. • Rates of deaths, SAEs, and TEAEs were similar between subjects treated with lefamulin and moxifloxacin. • There was a 1% difference in the number of subjects with lung infections categorized as SAEs; 12 (1.9%) lefamulin subjects compared to 6 (0.9%) moxifloxacin subjects. Many of these lefamulin treated subjects grew an Enterobacteriaceae from sputum cultures for which lefamulin does not have antibacterial activity. • Prolongation of the QT interval occurred to a similar extent in both arms; 17.9% of lefamulin subjects and 22.3% of moxifloxacin subjects had an increase in the QTcF interval of more than 30 msec.	The two Phase 3 trials provided an adequate safety database. The identified safety issues (e.g. QT prolongation, embryo-fetal toxicity) did not preclude approval. Overall, there was an acceptable risk profile for an effective antibacterial drug for CABP. QT prolongation, gastrointestinal side effects with oral lefamulin, and administration site reactions with IV lefamulin were noted in the Phase 3 trials. These adverse reactions are included in the label. The risk of QT prolongation is included in the Warnings and Precautions section of the label. The labeling includes a Warning and Precaution regarding embryo-fetal toxicity and

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	(Moxifloxacin has also been shown to prolong the QT interval.) • Diarrhea, nausea, and vomiting were more common with the oral formulation of lefamulin compared to oral moxifloxacin; diarrhea occurred in 12.2% of lefamulin subjects compared to only 1.1% of moxifloxacin subjects. These adverse events were mild to moderate in severity. • Administration site reactions with the IV formulation of lefamulin occurred in 7.3% of lefamulin subjects compared to 2.6% of moxifloxacin subjects. The reactions were mostly mild with only 3 lefamulin subjects (1.1%) having severe reactions and 2 (0.7%) who discontinued study drug due to the reaction. • Animal studies of lefamulin indicate an increased incidence of postimplantation fetal loss, stillbirths, and pup death during lactation in rats and rabbits. In addition, rare malformations in rats at systemic exposures less than the systemic exposure in CABP patients raise a concern for embryo-fetal toxicity. • Mutagenicity testing of lefamulin and and its main metabolite, BL-8041, were not adequately assessed with valid assays.	recommend against prescribing lefamulin to pregnant women. Additionally, the label will recommend that women pump and discard human milk for the duration of treatment with lefamulin and for 2 days after the final dose. The Applicant will initiate a pregnancy surveillance program as a PMR to collect information on pregnancy complications and birth outcomes in women exposed to lefamulin during pregnancy. Labeling notes that the mutagenicity of lefamulin and its main metabolite, BL-8041, were not adequately assessed. The Applicant will conduct two studies as PMRs to assess the mutagenicity of lefamulin and its main metabolite, BL-8041, using in vitro assays.

1.4. Patient Experience Data

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

x	The patient experience data that were submitted as part of the application include:			Section of review where discussed, if applicable
	x	Clinical outcome assessment (COA) data, such as		
		x	Patient reported outcome (PRO)	Sections 8.1.1, 8.1.3
		□	Observer reported outcome (ObsRO)	
		x	Clinician reported outcome (ClinRO)	Sections 8.1.1, 8.1.3
		□	Performance outcome (PerfO)	
	□	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		
	□	Natural history studies		
	□	Patient preference studies (e.g., submitted studies or scientific publications)		
	□	Other: (Please specify):		
□	Patient experience data that were not submitted in the application, but were considered in this review:			
	□	Input informed from participation in meetings with patient stakeholders		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		
	□	Other: (Please specify):		
□	Patient experience data was not submitted as part of this application.			

2 Therapeutic Context

2.1. Analysis of Condition

Community-acquired bacterial pneumonia (CABP) is defined as an acute bacterial infection of the lung parenchyma that patients develop while in the community and is a separate entity from hospital-acquired or ventilator-associated bacterial pneumonia. Patients present with some combination of chest pain, cough, sputum production, difficulty breathing, chills, rigors, and fever. The diagnosis of CABP is made clinically and includes new infiltrates on chest imaging. The usual bacterial pathogens that cause CABP include *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis, Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*.

The annual incidence of CABP requiring hospitalization in the United States was recently found to be 24.8 per 10,000 adults with a higher incidence in older patients (Jain et al.). Compared to the incidence in adults 18 to 49 years old, the incidence among adults 50 to 64 years old, 65 to 79 years old, and 80 years or older were approximately 4, 9, and 25 times as high.

CABP has a significant impact on American society. While most patients with CABP are treated as outpatients, the mortality of those needing hospitalization was reported as high as 23% (File and Marrie). In 2005, there were more than 60,000 deaths due to pneumonia in the United States (File and Marrie). In 2011, the aggregate cost of pneumonia hospitalizations in the United States was estimated to be $10.6 billion (Pfunter et al.).

When evaluating patients with CABP, physicians need to decide if patients require hospitalization or can be treated with oral medication as an outpatient. In addition to clinical judgement, there are two main scoring systems for risk stratification, the PSI/PORT and CURB-65. The PSI uses 20 variables and assigns patients to 1 of 5 categories, while the CURB-65 uses 5 variables and assigns patients to 1 of 3 categories. The PSI/PORT system was used to stratify patients in the trials from this application and uses information from the patient's demographics, comorbidities, physical exam findings, and lab and radiographic data. The scoring system and associated mortality data are listed in the table below (Fine et al.).

Table 1. PSI/PORT Score for CABP Risk Stratification

PORT Score	PORT Risk Class	Predicted Mortality (%)
No points from comorbidities, physical exam findings, or lab findings	I	0.1
≤70	II	0.6
71–90	III	0.9
91–130	IV	9.3
>130	V	27.0

PSI = pneumonia severity index; PORT = Pneumonia Outcomes Research Team; CABP = community-acquired bacterial pneumonia

Overall, CABP is a serious condition associated with mortality especially in the elderly and those with comorbidities.

2.2. Analysis of Current Treatment Options

There are several antibacterial drugs that are FDA-approved for the treatment of CABP (or indications such as "community acquired pneumonia" or "lower respiratory tract infections") and are recommended by the Infectious Diseases Society of America as standard of care for the indication (Table 2). They include macrolides (azithromycin and clarithromycin), respiratory fluoroquinolones (moxifloxacin and levofloxacin), cephalosporins (cefotaxime and ceftriaxone), doxycycline, linezolid (if MRSA is a concern), and aztreonam (for patients with penicillin allergy). If *Pseudomonas* is a consideration, empiric treatment for CABP could include piperacillin/tazobactam, cefepime, or imipenem. Other beta-lactam/beta-lactamase inhibitor combination drugs, cephalosporins, and carbapenems which are not labeled for CABP are often used to treat patients when resistant organisms are suspected to be the cause or when patients do not respond to first-line therapy. Oral antibacterial therapy is used when patients do not need hospitalization and are able to take oral medication. Hospitalized patients are started on IV antibacterial therapy and switched to oral medication when they are clinically improved. Overall, there are many options for clinicians to use to treat CABP. However, there are limitations of the current drugs, including lack of oral options for some drugs, antibacterial resistance, and drug safety issues. Additional options for the treatment of CABP that have both IV and oral formulations and a broad-spectrum of antibacterial activity would be beneficial to patients.

Table 2. Summary of Available Antibacterial Drugs for Treatment of CABP

Product(s) Name	Relevant Indication	Dosing/ Administration	Important Safety and Tolerability Issues
Fluoroquinolones (moxifloxacin, levofloxacin)	CAP	Oral and IV	Tendinitis and tendon rupture, peripheral neuropathy, central nervous system effects
Macrolides (azithromycin, clarithromycin)	CAP	Oral and IV	Prolongation of QT interval
Cephalosporins (cefotaxime, ceftriaxone, cefepime)	LRTI	Oral and IV	N/A
Piperacillin/tazobactam	CAP	IV	Hematological effects (bleeding, leukopenia, and neutropenia), nephrotoxicity
Carbapenems (imipenem)	LRTI	IV	Seizure potential
Aztreonam	LRTI	IV	N/A
Linezolid	CAP	Oral and IV	Myelosuppression, peripheral and optic neuropathy, serotonin syndrome
Doxycycline	RTI	Oral and IV	Fetal effects on tooth development, photosensitivity

CAP = community-acquired pneumonia; CABP = community-acquired bacterial pneumonia; LRTI = lower respiratory tract infections; RTI = respiratory tract infections; IV = intravenous

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Lefamulin is a new molecular entity and is not currently marketed in the United States or the rest of the world.

3.2. Summary of Presubmission/Submission Regulatory Activity

The Applicant opened two INDs to support the development of lefamulin. The first IND (#106594) for the IV formulation was submitted in October 2009. The second IND (#125546) for the oral formulation was submitted in January 2015. The Sponsor's initial development plan included (b) (4) to pursue an indication for the treatment of CABP. In March 2013, they proposed a Phase 3 trial of IV lefamulin with optional switch to oral lefamulin for the treatment of CABP. In January 2014, the Sponsor provided additional details regarding the Phase 3 CABP trial. One major feedback item from FDA to the Sponsor was that the proportion of subjects receiving prior short-acting antibacterial drug therapy should be limited to 25%. In May 2015, the Sponsor submitted a Special Protocol Amendment (SPA) for the IV to oral lefamulin trial in CABP (NAB-BC-3781-3101). FDA notified the Sponsor that the proposed study would not address the objectives needed for regulatory submission. Specifically, FDA felt the trial should not allow

coadministration of penicillins and fosfomycin and exclude patients with *S. aureus* bacteremia. At that time, FDA also informed the Sponsor that if they were only seeking the CABP indication they would need two adequate and well-controlled trials in CABP. In September 2015, FDA notified the Sponsor that their revised protocol for Trial 3101 was acceptable. In December 2015, the Sponsor submitted a second Phase 3 CABP protocol (NAB-BC-3781-3102) which would study only the oral formulation of lefamulin. At that time, FDA informed the Sponsor that a 12.5% noninferiority margin would be acceptable for the primary endpoint if patients with a PORT Risk Class of II were limited to no more than 25% of the study population. In February 2016, the Sponsor submitted an amendment for the oral only CABP trial (3102) which FDA found acceptable. The major changes in that submission were to change the randomization scheme from 2:1 to 1:1 and to revise the NI margin from 12.5% to 10% which allowed for enrollment of a higher percentage of PORT Risk Class II subjects given that Trial 3102 only studied the oral formulation and would likely enroll more outpatients. The change in the NI margin from 12.5% to 10% increased the estimated ITT population size from 573 (2:1 randomization) to 738 (1:1 randomization). The Sponsor also applied for and was granted Fast Track and Qualified Infectious Disease Product designations for CABP (b) (4) on 11 Sept 2014 (for IV use) and 21 Jan 2016 (for the oral tablet).

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

4.1. Office of Scientific Investigations (OSI)

The Office of Scientific Investigations conducted clinical site inspections at 3 sites which were chosen based on high enrollment, high rates of deaths and AEs, and high efficacy rates. Two of the sites enrolled subjects in both Phase 3 studies (Dr. Joven Roque Gonong in the Philippines and Dr. Tatjana Pejcic in Serbia). The other site (Dr. Vojislav Radosavljevic in Serbia) only enrolled subjects in Study 3102.

Per the OSI report, the study data derived from these clinical sites are considered reliable in support of the NDA. Of note, one subject at Dr. Pejcic's site was misclassified as PORT Risk Class II when in fact he was PORT Risk Class I because an incorrect birth date was used. Therefore, this subject was ineligible to participate in the study.

M.O. Comment*: The exclusion of a single subject is unlikely to make a significant difference in the efficacy analyses.*

4.2. Product Quality

NDA 211672, as amended, has provided adequate CMC information to assure the identity, strength, purity, and quality of the proposed drug product. 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 and an overall "Approve" recommendation was entered into Panorama by the Office of Process and Facilities (OPF) on May 8, 2019. Therefore, this NDA is recommended for Approval by the Office of Pharmaceutical Quality (OPQ).

NDA 211673, as amended, has provided adequate CMC information to assure the identity, strength, purity, and quality of the proposed drug product. 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 and an overall "Approve" recommendation was entered into Panorama by the Office of Process and Facilities (OPF) on May 6, 2019. Therefore, this NDA is recommended for Approval by OPQ.

From a Pharmacology/Toxicology perspective, mutagenicity testing of some of the potentially genotoxic impurities (PGIs) was not valid. In the absence of valid in vitro data, those PGIs should be considered to be mutagens and treated accordingly. This information is included in section 13.1 of the label.

4.3. Clinical Microbiology

The clinical microbiology review evaluated the mechanism of action, development of resistance, and the activity of lefamulin in vitro, in vivo and in clinical studies. From a clinical microbiology perspective, the information provided by the Applicant supports the efficacy of lefamulin for the treatment of susceptible bacteria for CABP, and approval of this product is recommended, based on the evidence provided by the Applicant and summarized below. Please refer to Section 17 for the full clinical microbiology review. A summary of the clinical microbiology review is below:

Mechanism of Action

The mechanism of action studies support that lefamulin is a member of the the pleuromutilin class of antibacterials. Lefamulin inhibits prokaryotic ribosomal protein synthesis by binding to the peptidyl transferase center (PTC) at the 50S subunit of the bacterial ribosome, while mammalian protein synthesis appears to be unaffected. In the eukaryotic transcription/translation assay, the IC50 values for *S. aureus* were 0.29µM but 952µM for the eukaryotic system tested (rabbit reticulocyte lysate).

Activity In Vitro

The assessment of lefamulin activity came from individual study collections, clinical trials and the SENTRY global surveillance programs (2015-2017). Information was provided on the in vitro activity (MIC_{90} and MIC range) of lefamulin against organisms associated with CABP.

Information on pathogens was pooled from surveillance and the combined Phase 3 studies. Among the first list organisms, the MIC_{90}s were as follows: 0.25 mcg/mL for 7753 *S. pneumoniae* isolates, 0.12 mcg/mL for 6492 methicillin-susceptible *S. aureus* (MSSA), 1 mcg/mL for 44 *L. pneumophila*, 0.002 mcg/mL for 61 *M. pneumoniae*, and 0.04 mcg/mL for 50 *C. pneumoniae*. Lefamulin was found to be bactericidal in vitro against *S. pneumoniae*, *H. influenzae* and *M. pneumoniae,* and bacteriostatic against *S. aureus* and *S. pyogenes*. It also had intracellular antibacterial activity, which is important for intracellular CABP pathogens such as *C. pneumoniae* and some *H. influenzae*.

Resistance

The resistance frequency to lefamulin due to spontaneous mutations in vitro at 2-8 times the MIC was $2x10^{-9}$ to $<2x10^{-11}$ for *S. aureus*, $<1x10^{-9}$ to $<3x10^{-10}$ for *S. pneumoniae,* and $<4x10^{-9}$ to $<2x10^{-10}$ for *S. pyogenes*.

Resistance mechanisms that affected lefamulin activity included specific protection or modification of the ribosomal target by ABC-F proteins such as *vga* (A, B, E), *lsa*(E), *sal*(A), and Cfr methyl transferase, or by mutations of ribosomal proteins L3 and L4. Most of these were identified in *Staphylococcus* or *Streptococcus* spp. during lefamulin surveillance studies 2010 and 2015-2016. Additionally, Cfr methyl transferase has the potential to mediate cross-resistance between lefamulin and phenicols, lincosamides, oxazolidinones, and streptogramin A antibacterials. This phenotype is called PhLOPS-resistance. Evidence of these mechanisms was provided in in vitro assays, from published literature, as well as from recent lefamulin surveillance studies.

Activity In Vivo

The activity of lefamulin was assessed in the murine systemic infection model of *S. aureus* where the in vivo protective efficacy was evaluated against the MSSA strain *S. aureus* B9 (MIC0.06 mcg/mL) and an ED50 (effective dose for protection of 50% of infected mice) was 1.77 mg/kg/day subcutaneously and 9.97 mg/kg/day orally.

- In a murine *S. aureus* bacteremia model, lefamulin showed activity in vivo against *S. aureus* that was comparable to daptomycin and vancomycin (approximately 4 log10 CFU/mL reduction). Lefamulin had more activity (4.5 log 10 CFU/mL reduction) in vivo in this model compared to linezolid and tigecycline (2 and 3 log reduction in CFU/mL, respectively).
- In a murine pulmonary infection model of *S. pneumoniae*, lefamulin was given subcutaneously in comparison to moxifloxacin and linezolid. The ED50±SE for lefamulin in mg/kg/day was 14.34±2.33 QD, and 44.06±16.75 TID. This was in comparison to moxifloxacin 31.14±7.98 QD and linezolid 63.05±30.85 QD. (QD is once daily dosing and TID is three times daily dosing).

- In a murine thigh infection model of MSSA (*S. aureus* B399) in neutropenic mice, the change in log10 CFU/thigh for lefamulin was -2.66 subcutaneously and -3.76 orally.
- Other animal models were designed to test efficacy of lefamulin against MRSA, including the pulmonary infection model of MRSA pneumonia and an immunocompetent and neutropenic murine thigh infection models with *S. aureus* B29 (MRSA).

Clinical Studies

Lefamulin efficacy in adult patients with CABP was established in two pivotal Phase 3 studies, Studies 3101 and 3102. Pathogen identification included molecular and standard culture methods. Molecular methods were used by the Applicant because of poor diagnostic yield with traditional sputum cultures for some bacteria and to maximize the identification of baseline CABP pathogens. The clinical trial data were evaluated by the Agency's clinical microbiology group, and decision-making focused primarily on culture where culture was available for a particular pathogen. If no (or limited) culture data were available due to the fastidious nature of the organism, then emphasis was placed on FDA-cleared tests first, followed by serology. Reliance on non-cleared PCR-based tests was not necessary.

Susceptibility Interpretive Criteria

The following is a summary of the Agency's breakpoint rationale followed by labeling recommendations:

Agency's Breakpoint Rationale:

- Breakpoints were not provided for *H. parainfluenzae*, *M. catarrhalis*, beta-hemolytic *Streptococcus* spp. or Viridans Group *Streptococcus* spp. due to insufficient clinical information. These organisms are included in the second list (i.e.; *H. parainfluenzae*, *M. catarrhalis* and *S. pyogenes*, *S. agalactiae, S. anginosus, S. salivarius, and S. mitis*).
- Breakpoints are provided for MSSA. MRSA is included in the second list. For MSSA, a susceptible only breakpoint was provided as shown in the table below. The susceptible breakpoint of ≤ (b) (4) (proposed by the Applicant) is not supported by the probability of PK-PD target attainment (b) (4) or by clinical data. The PTA was ~90% at MIC of 0.25 mcg/mL, supporting a susceptible breakpoint of ≤0.25 mcg/mL. Note that the susceptible breakpoint of (b) (4) mcg/mL is greater than MIC_{90} of 0.12 mcg/mL. At MIC ≤0.25 mcg/mL, the clinical success rate was 100% (16/16) in clinical trials (early clinical response in Studies 3101 and 3102); at MIC of 0.25 mcg/mL, the clinical success rate was 100% (4/4). No clinical data are available at MIC above 0.25 mcg/mL, so an intermediate breakpoint cannot be established.
- For *S. pneumoniae*, a susceptible only breakpoint was provided as shown in the table below. Similar to *S. aureus*, the PTA does not support the Applicant's proposed

breakpoint of (b) (4) The PTA was ~90% at MIC of 0.5 mcg/mL. Additionally, a susceptible breakpoint of 0.5 mcg/mL is above the MIC_{90} of 0.25mcg/mL for *S. pneumoniae*. At MICs ≤0.5 mcg/mL for *S. pneumoniae,* the clinical success rates were 51/60 (85%) overall and 18/22 (82%) for *S. pneumoniae* excluding those identified from a nasopharyngeal culture; clinical response rate at MIC 0.5 mcg/mL was 78% (7/9). No clinical data were available at MIC above 0.5 mcg/mL.

- For *H. influenzae*, a susceptible only breakpoint was provided as shown in the table below. At MIC of 2mcg/mL, the susceptible breakpoint is at the MIC_{90} for *H. influenzae* of 2 mcg/mL. The susceptible breakpoint of ≤2mcg/mL is supported by the clinical data with 18/19 (95%) clinical successes at or below an MIC of 2 mcg/mL. With only 1 isolate with MIC above 2 mcg/mL, there were not enough clinical data to propose a higher susceptible breakpoint.

Table 3. Agency's MIC Breakpoints for Lefamulin

	Minimum Inhibitory Concentrations (mcg/mL)		
Pathogen	**S**	**I**	**R**
S. aureus (MSSA)	≤0.25	---	---
S. pneumoniae	≤0.5	---	---
H. influenzae	≤2	---	---

S = Susceptible; I = Intermediate; R = Resistant; MIC = minimum inhibitory concentration; MSSA = methicillin-susceptible *Staphylococcus aureus*
Note: The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC results other than "Susceptible" should be submitted to a reference laboratory for further testing.

MIC-Disk Correlation

The Agency's disk diffusion breakpoints were determined based on the correlation of the disk diffusion diameter to the Agency's MIC susceptible breakpoint for the proposed organisms. The rationale is below using re-analysis of the data submitted in the NDA and generally accepted methodology as described in the CLSI guidelines.

Table 4. CLSI Guideline Acceptable Discrepancy Rate (Without Intermediate Range)

	Discrepancy Rates		
MIC Range	**Very Major**	**Major**	**Minor**
≥R+1	<2%	NA	-----
R+S	<10%	<10%	-----
≤S-1	NA	<2%	-----

Note: If there are no intermediate ranges for both disk diffusion and dilution testing minor discrepancies are not a consideration. R is the resistant breakpoint MIC; S is the susceptible breakpoint MIC.
CLSI = Clinical and Laboratory Standards Institute

(b) (4)

Reviewer's Comment: *For an MIC of ≤0.25 mcg/mL for* S. aureus *(MSSA): The susceptible breakpoint for disk that correlates with the lowest error rate is ≥22 mm for a larger collection of* S. aureus *and 23 mm for MSSA. This gives no very major or major error rates. A susceptible breakpoint was set at ≥23 mm for MSSA.*

(b) (4)

Reviewer's Comment: *For an MIC of ≤0.5 mcg/mL for* S. pneumoniae*: The susceptible breakpoint for disk that correlates with the lowest error rate is ≥17 mm. This gives no very major or major error rates.*

Reviewer's Comment: *For an MIC of ≤2 mcg/mL for* H. influenzae*: The susceptible breakpoint for disk that correlates with the lowest error rate is ≥17 mm. This gives no very major or major error rates. The susceptible breakpoint was established at ≥17 mm, because the isolate with the MIC correlating with ≥17 mm (2mcg/mL) was considered susceptible.*

The disk susceptibility interpretive criteria are below:

Table 5. Agency's Disk Interpretive Criteria for Lefamulin

	Disk Diffusion (Zone Diameter in mm)		
Pathogen	**S**	**I**	**R**
Staphylococcus aureus (methicillin-susceptible isolates)	≥23	-	-
Streptococcus pneumoniae	≥17	-	-
Haemophilus influenzae	≥17	-	-

S = Susceptible; I = Intermediate; R = Resistant

Note: The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC results other than "Susceptible" should be submitted to a reference laboratory for further testing.

4.3.1. Final Clinical Microbiology Recommendations

From a clinical microbiology perspective, the information provided by the Applicant supports the efficacy of lefamulin for the treatment of susceptible bacteria listed in the product labeling for the indication of CABP. The following is a summary of the Agency's proposed clinical microbiology labeling changes and rationale:

- Subsection 12.4 has been updated in accordance with the FDA guidances for industry *Microbiology Data for Systemic Antibacterial Drugs-Development, Analysis, and Presentation* (February 2018) and Systemic *Antibacterial and Antifungal Drugs: Susceptibility Test Interpretive Criteria Labeling for NDAs and ANDAs* (December 2017).

- Quality Control ranges used for susceptibility testing have been accepted by the Clinical and Laboratory Standards Institute (CLSI) and are recommended here as published in the current CLSI document M100.
- The mechanism of action subsection was revised for clarity, brevity and accuracy in comparison to current literature and submitted study reports.
- The resistance section was modified to describe the frequency of resistance for specific pathogens and the lefamulin concentration.
- The list of resistance mechanisms was updated to include *Isa*(E) which was identified among isolates with elevated lefamulin MICs (>32 mcg/mL) in *S. aureus* and beta-hemolytic *Streptococcus* spp. including *S. agalactiae*. A mechanism of resistance to lefamulin found in *Staphylococcus* spp., *sal*(A) was also added.
- A cross-resistance statement was added, "Cfr methyl transferase has the potential to mediate cross-resistance between lefamulin and phenicols, lincosamides, oxazolidinones, and streptogramin A antibacterials", based on the reference: Veve, et al.; Lefamulin: Review of a Promising Novel Pleuromutilin Antibiotic. *Review of Therapeutics*. 18 July 2018.
- The multidrug resistant claim for [redacted] (b) (4) was removed from the first list of bacteria.
- The statement, "XENLETA has demonstrated synergy in vitro with doxycycline against *S. aureus* [redacted] (b) (4) was revised, as Study Report: 10-19-2016-Nabrivia 2v3 FINAL Report stated that [redacted] (b) (4)
- [redacted] (b) (4) was removed from the first list of bacteria because there were less than 10 isolates (n=8) from the Phase 3 clinical trials. It was moved to the second list.
- [redacted] (b) (4) was moved from the first list of bacteria to the second list because of lack of clinical data from culture and FDA cleared tests (4 isolates were obtained, 3 with a favorable clinical response at the ECR visit).
- Headings in the second list, "[redacted] (b) (4)." and "[redacted] (b) (4)." were removed and specific species tested individually, because not all species were relevant to the indications. The following were listed instead (*S. pyogenes, S. agalactiae*, *S. anginosus*, *S. salivarius, S. mitis*). "[redacted] (b) (4)" was removed from the label because it was not relevant to CABP.
- The breakpoints are shown in the table below. The Applicant's proposal for breakpoints was revised based on the Agency's analysis of PK/PD taking fasting and fed states into consideration, use of standard culture-based tests, and lefamulin activity in vitro and in CABP clinical trials.

Table 6. Agency's Interpretive Criteria for Lefamulin

	Minimum Inhibitory Concentrations (mcg/mL)			Disk Diffusion (Zone Diameter in mm)		
Pathogen	**S**	**I**	**R**	**S**	**I**	**R**
Staphylococcus aureus (methicillin-susceptible isolates)	≤0.25	-	-	≥23	-	-
Streptococcus pneumoniae	≤0.5	-	-	≥17	-	-
Haemophilus influenzae	≤2	-	-	≥17	-	-

S = Susceptible; I = Intermediate; R = Resistant
Note: The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC results other than "Susceptible" should be submitted to a reference laboratory for further testing.

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Lefamulin is a pleuromutilin antibacterial drug that has been developed for the treatment of community-acquired bacterial pneumonia (CABP). The clinical dose described in proposed labeling is 150 mg q12h IV (300 mg/day, $AUC_{0\text{-}24h}$ =28.6 mcg*hr/mL), or 600 mg q12h (1200 mg/day, $AUC_{0\text{-}24h}$ =32.7 mcg*hr/mL).

A battery of safety pharmacology studies was conducted for lefamulin. In vitro, hERG assays and a Purkinje fiber assay demonstrated that lefamulin has the potential for QT/QTc prolongation and proarrhythmic potential. In telemetered monkeys, prolongation of QT/QTc was observed by as much as 42 msec, but no effect on respiratory function was noted. Potential for lefamulin to prolong QT/QTc interval was confirmed in clinical trials. Irwin tests in rats following a single dose or following repeated dosing in a general toxicology study revealed no effect on the central nervous system.

General toxicology studies were conducted in rats and cynomolgus monkeys for 4 weeks and 13 weeks by the IV route and for 4 weeks by the oral route. Injection site reactions and inflammatory changes were noted in IV studies in both species, as was evidence of regenerative anemia, and intestinal and fecal changes.

Additional findings in rats after 4 weeks of IV treatment included increased fibrinogen and increased coagulation times in high dose animals that were reversible. The NOAEL in this study was determined to be the high dose, 37.5 mg/kg BID (75 mg/kg/day, AUC≈10,000–12,000 ng*hr/mL). After 13 weeks of IV treatment in rats, decreased body weight gain, decreased food consumption, and mortality were noted in mid-and high dose animals leaving the low dose of 18.75 mg/kg/day BID (37.5 mg/kg/day) to be the NOAEL (AUC_{12h} =4536 and 4754 ng*hr/mL in

males and females, respectively at Week 13). Additional findings in monkeys following 4 weeks of IV treatment included histological findings of pancreatic microvesicular vacuolization of acinar cells noted at 120 mg/kg/day, that was not evident after the recovery period. The clinical significance of this finding is unclear, but established the NOAEL to be the next lower dose, 70 mg/kg/day (35 mg/kg BID; $AUC_{0\text{-}inf}$ approximately 17,000 ng*hr/mL on Day 1). This pancreatic lesion was also observed at all completed doses in the 13 week IV study in monkeys, and again was not noted in the recovery animals. Alveolar macrophage infiltrates and/or thrombosis were noted in the lungs of monkeys. A NOAEL was not identified in the 13-week IV monkey study. The lowest dose, 60 mg/kg/day, was the LOAEL (mean $AUC_{0\text{-}inf}$ ranged from 13,000–13,900 ng*hr/mL on Day 1 and 14,700 and 23,900 on Days 28 and 91).

In the four-week oral toxicology studies, moribundity and deaths were seen in high dose rats, while severe clinical signs in high dose monkeys necessitated a dosing holiday and dose reduction. Gastrointestinal signs were seen in both species, including hypersalivation and fecal changes in both species, distended abdomen (correlating with intestinal/cecal dilation) in rats, and emesis in monkeys. Additionally, findings in rats included degenerative changes in the stomach at the mid- and high doses (partially reversible), and organ weight and/or histological evidence of lymphoid (all doses) and hemopoietic (high dose) depletion that appeared to be reversible. The NOAEL was the mid-dose, 150 mg/kg/day BID ($AUC_{0\text{-}12h}$ ranged from 7810 ng*hr/mL to 13043 ng*hr/mL). Additional findings in the monkey included QT/QTc prolongation in high dose males that was statistically significant but reversible, increased myocardial vacuolation with fibrosis was observed in three animals at the end of treatment and in one recovery animal. The mid-dose (35 mg/kg BID, or 70 mg/kg/day) was considered to be the NOAEL. At that dose, on Day 28, $AUC_{0\text{-}inf}$ was 8090 ng*hr/mL in mid-dose males (n=1) and 4660 ng*hr/mL in mid-dose females (n=4).

A battery of genetic toxicology tests was conducted, consisting of a bacterial reverse mutation (Ames) assay, a mouse lymphoma assay (MLA), and an in vivo rat micronucleus assay. Lefamulin demonstrated antibacterial activity in the Ames assay, and the MLA was not evaluated at doses reaching 10% to 20% relative total growth (RTG) as recommended in guidances for the appropriate conduct of this assay, rendering both assays invalid to determine the mutagenic potential of the drug and the main human metabolite (2*R*-hydroxy lefamulin). The in vivo rat micronucleus assay was negative for clastogenicity.

No adverse effects on fertility were noted with IV lefamulin at doses up to 75 mg/kg/day ($AUC_{0\text{-}24h}$ approximately 20.6 mcg*hr/mL) in males, and up to 50 mg/kg/day ($AUC_{0\text{-}24h}$ approximately 13.4 mcg*hr/mL) in females. At the highest dose tested, 75 mg/kg/day, abnormal estrous cycling was seen in 40% of the female rats, and 10% had a high degree of postimplantation loss.

In an embryo-fetal development (EFD) study with IV lefamulin in rats, there were four late resorptions in the high dose group, compared to one each in the control and mid-dose groups. Malformations at the mid-dose included one fetus with cleft palate and short lower jaw, along with gross disruption of the vertebral column (scoliosis). At the high dose, one fetus had a

similar spectrum of defects: cleft palate, short lower jaw, malformed ribs (oriented cranially), and malformed thoracic vertebrae; a second fetus in another high dose litter had an enlarged ventricular heart chamber with a thin ventricular wall. These findings were rare or nonexistent in the historical database and concurrent controls. Decreased or no ossification in a number of skeletal elements in all treatment groups exhibited dose-related increases in incidence relative to controls and may indicate treatment-related developmental delay at all doses. The level of concern may be higher, since developmental adverse effects were seen despite the fact that a maternally toxic dose was not reached in this study, and all doses resulted in exposures that were lower than clinical exposure. Assuming that the delays in skeletal ossification at the lowest dose would not be adverse, the fetal NOAEL in this study would be the low dose, 50 mg/kg/day, divided BID (mean C_{max} =5612–7058 ng/mL, steady state $AUC_{0\text{-}24h}$ approximately 10.8 mcg*hr/mL).

In the EFD study with IV lefamulin in rabbits, low numbers of live fetuses were found in all treated groups. Comparisons were made between control and high dose groups only due to low numbers of live fetuses, revealing significantly lower pup and litter weights, higher percentage of small fetuses, and an increased incidence of decreased or no ossification in high dose litters relative to control. Due to low numbers of live fetuses and lack of complete evaluation of low and mid-dose groups, a NOAEL was not found. The low dose, 20 mg/kg/day, resulted in an AUC in a dose range-finding study of approximately 1920 ng*hr/mL, or approximately 0.1 times exposure in CABP patients treated IV).

In a pre- and postnatal development (PPND) study with IV lefamulin in rats the pup live birth index was markedly reduced in the high dose group (87.4% compared with 98.7% in the control). There was no reported effect of maternal treatment on pup observations, including preweaning physical or functional development of the F1 pups, neurobehavioral tests (learning and memory in the water maze, motor activity in an open field) and sensory function (auditory startle response).

There were apparent findings that differed from concurrent controls that were at the upper end of the historical control range that may still represent effects in this study, including lower mean number of implantation sites in mid- and high dose F0 females, lower mean number of pups delivered in the mid- and high dose groups, higher numbers of dead pups during lactation in treated groups, lower F1 body weights persisting through mating, apparent delays in sexual maturation, and higher pre- and or post- implantation loss in mid- and/or high dose F1 females in reproductive performance testing of the offspring. The No Observed Adverse Effect Level (NOAEL) for embryo-fetal and pre- and postnatal development in the rat and subsequent reproductive performance of the offspring was considered to be the mid-dose, 2x37.5 mg/kg/day, based on the observed decrease in live births in the high dose group. Based on pharmacokinetic data from the rat EFD study, mean $AUC_{0\text{-}12h}$ ranged from 8592 ng*hr/mL to 13042 ng*hr/mL at that dose.

Evaluation of local tolerance of IV administered lefamulin in rats revealed dose-dependent necrosis around the tail vein (injection site) when administered as 30 minute infusions, but was well tolerated when administered as 24-hour infusions.

In accordance with the FDA guidance for industry *Safety Testing of Drug Metabolites* (November 2016), the main human metabolite, 2*R*-hydroxy lefamulin, was evaluated as described for human metabolites that are disproportionally higher in humans than in animals or are present as greater than 10 percent of total drug-related exposure at steady state in clinical subjects (See Section 6 Clinical Pharmacology; the metabolite was present at steady state at greater than 10% of the parent drug after oral administration to clinical trial subjects). 2*R*-hydroxy lefamulin exhibited hERG inhibition in vitro, but the IC_{50} for hERG inhibition was an order of magnitude higher than for the parent drug in the same experiment. It was toxic to test bacteria in a bacterial reverse mutation test and was tested in the in vitro MLA assay for mutagenicity in mammalian cells. However, the highest doses evaluated in the MLA did not reach 10% to 20% RTG, so did not provide valid evidence that the metabolite was not mutagenic. In an EFD study with the metabolite in rats, malformations of the heart (enlarged ventricular chamber, thin ventricular wall) or great vessels in 2 mid-dose and 1 high dose litters were consistent with those reported in the rat EFD study of lefamulin that were rare in the historical database and nonexistent in concurrent controls. In that study, again, a maternally toxic dose was not reached. The fetal NOAEL in was the low dose, 10 mg/kg/day, divided BID (mean C_{max} =3416–4500 ng/mL, mean $AUC_{0\text{-}12h}$ =1705–2135 ng.h/mL).

The Applicant has proposed limits of (b) (4)% for the impurity (b) (4) and (b) (4)% for the impurity (b) (4) in the drug substance, indicating that these impurities were qualified in a 14-day general toxicology studies in cynomolgus monkeys (Study no. (b) (4).298.3) and in rats (Study no. 73925-02). Data from the monkey study support the safety of those levels of the impurities following IV or oral dosing. Using the LOAEL dose in rats for comparison to clinical dosing, the proposed acceptance criteria would be supported for IV dosing, but not at the higher oral dose. However, since this rat study used IV administration, and the toxicity at the lowest dose was related to irritation/inflammation at the injection site, it is reasonable that the proposed limits should be acceptable for the oral formulation.

A number of additional impurities were identified by the Applicant as potentially genotoxic impurities (PGIs). In mutagenicity testing (Ames assay), two of these were found to be negative in valid assays, while one, (b) (4) was positive. The Applicant proposed controlling this genotoxic impurity and a genotoxic process impurity, (b) (4), to approximately (b) (4) mcg each for total daily intake. Six other PGIs, (b) (4) were toxic to the test bacteria, rendering the assays invalid. The amounts present (or that can be identified based on the limits of sensitivity of the assays) would exceed the total daily intake for all genotoxic impurities as described in the ICH M7 guidance. The Applicant chose not to test these compounds for mutagenicity in mammalian cell assays as recommended. In the absence of valid data or the ability to control these impurities to the prescribed levels, the Applicant and the Division agreed that their presence and potential for

mutagenicity will be described in labeling. Although the clinical significance of the total (known and potential) mutagenic impurities exceeding ICH M7 limits is unclear, the short duration of clinical treatment (5 to 7 days) may minimize risk. Ultimately, if each PGI were to be tested in a mammalian cell assay and found to be positive, it is likely that the positive results would be similarly addressed in labeling.

From a pharmacology/toxicology perspective, the application is approvable. The Applicant has agreed to a postmarketing requirement to repeat the MLAs for lefamulin and 2*R*-hydroxy lefamulin to provide data for mutagenicity.

5.2. Referenced INDs, NDAs, BLAs, DMFs

IND 106594 for lefamulin administered by the IV route.
IND 125546 for lefamulin administered by the oral route.

5.3. Pharmacology

Cardiovascular System

(b) (4) *Study No.: 99910 (Nabriva Project No.: 03781A-SP03-001 GxP): BC-3781.Ac:*[1] *Effect on HERG Tail Currents recorded from Stably Transfected CHO cells*

(From Dr. M. Rivera's review of the original submission of IND 106594)

BC-3781.Ac (lefamulin) was tested at concentrations of 3, 10, 30, and 100mcM. A concentration dependent inhibition was observed at all doses (12, 26, 49, and 83%, respectively). The IC_{50} was 27mcM (14 mcg/mL). The positive control (100nM E-4031) showed 99% inhibition.

(b) (4) *Project no. 489527: The Ability Of Bc-3781.Ac to Block the HERG Current In Stably Transfected HEK-293 Cells*

(From Dr. M. Rivera's review of the original submission of IND 106594)

BC-3781.Ac was tested at concentrations of 3, 10, 30, and 100mcM. A concentration dependent inhibition was observed at doses greater than or equal to 10mcM (15.2, 37.5, and 71.2%, respectively). The IC_{50} was 47mcM (24 mcg/mL in terms of free base). The positive control (100nM E-4031) showed 86% to 95% inhibition.

[1] Nomenclature: (Laboratory Code) BC-3781.Acetate, BC-3781.Ac, BC-3781, lefamulin, lefamulin acetate

Study No. 12.0275 (Applicant Study Code No. 03781a-Sp01-003-Gxp): Evaluation of Arrhythmogenic Risk for Bc-3781.Ac in an In Vitro model (Purkinje fiber) in the Rabbit

Purkinje fiber preparations were made from six male New Zealand White (NZW) rabbits (body weight: 2.079–2.997 kg). Evaluation of the test article (BC-3781.Ac batch no. Q000000484) and the positive control (cisapride, 100nM) was conducted in a single fiber. BC-3781.AC dosing solutions were 0.5, 3, and 10 µg/mL (free base), administered as a superfusion of 3mL/min of ascending concentrations at intervals of approximately 36 minutes each.

Parameters evaluated were resting membrane potential, maximal upstroke velocity, action potential amplitude, action potential duration at 30, 60, and 90% depolarization, action potential triangulation and absence or presence of early after depolarizations (EADs). During the first 30 minutes, the fiber was driven at 60 pulses/min (1 Hz). Afterwards, the stimulation rate was reduced to 20 pulses/min (0.33 Hz) for 3 minutes and then to 12 pulses/min (0.20 Hz) for 3 further minutes, to elicit early after depolarizations (EADs). Recordings were taken before and every 5 minutes after the beginning of each 30-minute superfusion period at 60 pulses/min. The number of Purkinje fibers showing EADs was determined during each period where stimulation frequency was reduced to 20 and 12 pulses/min.

The report states that dosing formulations were found to be within 81.9% to 102.7% of the nominal concentrations, which was within the limit of 80% to 120% specified in the study plan, but probably should have been more tightly controlled.

No substantial or biologically relevant effects of BC-3781.Ac were reported on resting membrane potential (RMP), maximal upstroke velocity (V_{max}), action potential amplitude (APA), and action potential duration at 30% repolarization (APD_{30}) over the 30-minute superfusion period at any of the three doses. At 0.5, 3 and 10 µg/ml, BC-3781.Ac did not provoke any EADs during low stimulation rates of 20 or 12 pulses/min.

BC-3781.Ac had no significant effects on action potential duration at 60% repolarization (APD_{60}) over the 30-minute superfusion period at 0.5 µg/mL, but, at 3 and 10 µg/ml, BC-3781.Ac progressively lengthened APD_{60} over the 30-minute superfusion period (+13% at T30min, $p<0.001$ and +7% at T30min, $p<0.05$, respectively).

At 0.5, 3 and 10 µg/ml, BC-3781.Ac progressively lengthened action potential duration at 90% repolarization (APD_{90}) over the 30-minute superfusion period (at 0.5 µg/ml: +6% at T30min, $p<0.001$ and at 3 and 10 µg/ml: +13% at T30min, $p<0.001$ for each). These were interpreted as suggestive of a blockade of the delayed rectifier potassium channels by BC-3781.Ac from 0.5 µg/ml.

At 0.5 and 3 µg/ml, BC-3781.Ac had no significant effects on action potential triangulation (APT). In contrast, at 10 µg/ml, BC-3781.Ac increased APT over the 30-minute superfusion period (+41% at T30min, $p<0.05$).

The positive control, cisapride (100nM) had no substantial effects on RMP, Vmax, APA and APD_{30} over the 30-minute superfusion period, but lengthened APD_{60} (+28% at T30min) and APD_{90} (+39% at T30min) and increased APT (+134% at T20min) over the 30-minute superfusion period. The latter effects were reported to be consistent with historical control data. In this fiber, cisapride did not provoke any EADs during low stimulation rates of 20 or 12 pulses/min, although it was said to have produced EADs in historical control experiments.

The report concluded that BC-3781.Ac was found to block the delayed rectifier potassium channel from the lowest concentration tested (0.5 µg/ml) with increased action potential triangulation at 10 µg/ml. The positive control, cisapride, exhibited lengthened APD_{60} and APD_{90} and increased action potential triangulation. At the tested concentrations (0.5, 3 and 10 µg/ml), BC-3781.Ac did not induce the occurrence of EADs at low pacing rates (20 and 12 pulses/min), although no EADs were seen under the same conditions with the positive control. The report states that, based on these results, BC-3781.Ac showed a potential for QT/QTc interval prolongation at all tested concentrations and proarrhythmic potential at 10 µg/ml.

Cardiovascular and Respiratory Systems

Study Number: (b) (4).289.02 (Applicant Reference Number: 03781A-SP01-002-GxP): A Cardiovascular and Respiratory Safety Pharmacology Study of BC-3781.Ac Intravenously Administered to Telemetry-Instrumented Conscious Male Cynomolgus Monkeys

(From Dr. M. Rivera's review of the original submission of IND 106594)

Four male monkeys (4.5 yrs to 6.5 yrs old; 4.6 kg to 5.9 kg) were given BC-3781.Ac (lot # 76943-04) at doses of 0, 7.5, 15, and 40 mg/kg in a 4x4 latin square design with a 7-day washout period between doses. The dose level was expressed in terms of the free base. The vehicle (0.9% sodium chloride) and test article were given as a 30-min IV infusion via a catheter placed in the femoral vein at a dose volume of 15 mL/kg. Parameters evaluated by telemetry included arterial blood pressure (systolic, diastolic, and mean), HR, respiratory rate, and EKG (lead II) parameters (QRS duration, and RR, PR, and QT intervals). QT was corrected by both Bazett's (QTcB) and Fridericia's (QTcF) formulas. Clinical signs, body weights, food consumption, and arterial blood gases (pCO2, pO2, oxyhemoglobin, and oxygen hemoglobin saturation) including pH were also assessed. The animals were given a second cycle of doses administered in the same manner as in the safety study for TK analysis.

At doses greater than or equal to 15 mg/kg, there was a statistically significant increase in QTc above baseline levels. The increase was observed from 0.42 hrs to 1.5 hrs at 15 mg/kg (mean max prolongation of 21 msec by both formulas) and 0.25 hrs to 3 hrs at 40 mg/kg (mean max prolongation of 42 msec by QTcB and 37 msec by QTcF). This effect was reversible; baseline values were restored within 2 hrs to 3 hrs postdose at 15 mg/kg and 4 hrs to 5 hrs postdose at 40 mg/kg. The Applicant selected 15 mg/kg as the NOAEL based on the consideration that a QTc

prolongation in excess of 25 ms to 30 ms (about 10%) is considered potentially adverse as the risk for precipitating TdP increases above those levels. However, E14 ICH Guidance for Industry sets a conservative threshold of concern in the clinic for any drug that causes a mean increase in QTc of 5 ms and a high level of concern for an increase of 20 ms. Therefore, the reviewer believes 7.5 mg/kg should be selected as the NOAEL.

TK analysis showed plasma levels at the end of infusion of 0.723 +/-0.130, 1.66 +/-0.350, and 4.64+/-1.09 mcg/mL and $AUC_{0\text{-}inf}$ of 3.04 +/- 0.123, 6.23 +/- 0.594, and 16.5 +/-2.13 mcg*hr/mL at 7.5, 15, and 40 mg/kg, respectively.

The Applicant acknowledged the potential risk to human of this finding and noted that QT prolongation was observed in the first in human study at doses greater than or equal to100 mg, i.e., mean increases of 2.4 msec at 100 mg, 7.0 msec at 200 mg, 15.9 msec at 300 mg, and 19.3 msec at 400 mg.

No test article-related effect was observed in respiratory rate, arterial blood gas parameters (pCO2, pO2, oxyhemoglobin, oxygen hemoglobin saturation, and pH).

Central Nervous System

(b) (4) *Project No.: 073823 (Nabriva Project No.: 03781A-SP02-001-GxP): Influence of a Single Oral Application on the Central Nervous System in the Rat of BC-3781. Ac*

(From Dr. M. Rivera's review of the original submission of IND 106594)

Wistar rats (8 weeks to 12 weeks of age, 5/sex/dose) were given a single oral gavage dose of BC-3781.Ac (lot # 73925-02) at doses of 0, 25, 75, and 150 mg/kg (in terms of free base). BC-3781.Ac was dissolved in water and administered at a volume of 10 mL/kg. Clinical observations according to the Irwin test were performed immediately before and 1, 2, 4, 6, and 24 hours postdose. At the same time points, the spontaneous activity was assessed in the open field. No test article-related adverse effects were apparent. The highest dose, 150 mg, is equivalent to a human dose of 24 mg/kg, or approximately 1.5 g for a 60 kg human.

Irwin screen conducted as part of Study no. AA97305 (4-week general toxicology study of IV lefamulin in rats)

An Irwin test was conducted on the first 3 animals/sex/group (approx. 9 weeks of age) on Day 0 and Day 1. Observations time points were 5, 15, and 25 minutes (presumably postdose). Observations included home cage observations, observations in a room dedicated to the Irwin test, and open field testing. No adverse treatment-related findings or changes in CNS parameters on Irwin screen were reported. Monitoring of rectal temperatures did reveal a slight decrease in mid- and high -dose animals, but the changes were minimal and not considered to be toxicologically relevant.

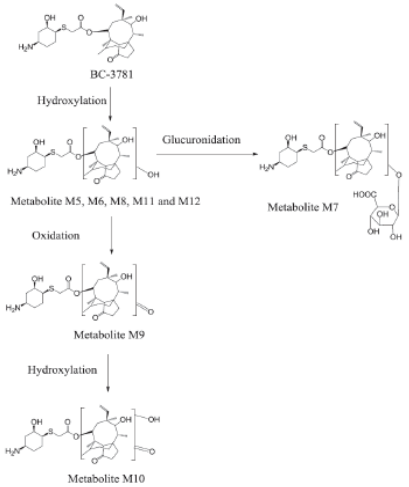
5.4. ADME/PK

Table 7. Summary of Studies and Major Findings

Type of Study	Major Findings
Absorption	
Study # NABRIVA 2008-25 PKB	Six female Sprague-Dawley (SD) rats were given a single BC-3781.Ac dose of 10 mg/kg (free base) IV into the tail vein (5 mL/kg in saline). BC-3781 showed a bi- or triphasic disposition; initial $t_{1/2}$ of 1 hr and terminal $t_{1/2}$ of 2.14 hr. The C_{max} and $AUC_{0-\infty}$ were 9.58 mcg/mL and 2.1 mcg•hr/mL, respectively. The V_{ss} (9.82 L/kg) suggest wide distribution into tissues. The renal clearance (ClR) was lower than the nonrenal CL (CLNR), i.e., 0.28 L/hr/kg versus 4.47 L/hr/kg. Higher amounts of BC-3781 were found in the feces (28.5% dose) compared to urine (5.95% dose). The feces were the major route of elimination for BC-3781.
Study # NABRIVA 2009-11 PKPD	Female Sprague-Dawley rats were dosed BC-3781.Ac (free base) either orally (gavage) at 5, 10, 20, 30, and 60 mg/kg (10 mL/kg in sterile water) or IV into the tail vein at 20 mg/kg (5 mL/kg in saline). After oral administration, the increase in BC-3781 plasma exposure was greater than dose-proportional based on $AUC_{0-\infty}$ and nearly dose-proportional based on C_{max}. The mean terminal elimination $t_{1/2}$ ranged from 1.51 hrs to 2.08 hrs. The C_{max} and $AUC_{0-\infty}$ ranged from 0.132 mcg/mL to 1.65 mcg/mL and 0.416 mcg*hr/mL to 8.70 mcg•hr/mL at 5 mg/kg to 60 mg/kg, respectively. The mean bioavailability increased with dose and ranged between 39.4% to 68.8%. The mean ClR was lower than the mean CLNR, i.e., 0.244 L/hr/kg to 0.341 L/hr/kg versus 7.03 L/hr/kg to 13.01 L/hr/kg. Higher amounts of BC-3781 were found in the feces (~30% to 50% dose) compared to urine (1.81% to 4.31% dose). After 20 mg/kg IV, the C0 and $AUC_{0-\infty}$ were 20.78 mcg/mL and 4.26 mcg•hr/mL, respectively, the terminal elimination $t_{1/2}$ was 2.48 hrs, the ClR and CLNR were 0.188 L/hr/kg and 4.502 L/hr/kg, respectively, and 4% of the dose was found in the urine versus 19% of the dose in the feces. The feces were the major route of elimination for BC-3781 for both routes of administration.
Study #NBR/02	A 10 mg/kg dose of radiolabeled lefamulin was administered IV to 5/sex SD rats. No statistical difference was reported in PK parameters between genders, and radioactivity was below the limit of detection after 12h.

Type of Study	Major Findings
Study #A136/09, corresponding to CIT Study # 36074 PAP	Four male cynomolgus monkeys/dose were given a single BC-3781 dose of 15 or 40 mg/kg IV infusion (in saline) over 30 min into the saphenous vein. BC-3781 showed a multiphasic decline; terminal elimination $t_{1/2}$ of 7.37 hr (15 mg/kg) and 6.47 hrs (40 mg/kg). The increase in exposure showed dose proportionality based on both C_{max} and AUC. The C_{max} was 3.79 and 8.86 mcg/mL at 15 and 40 mg/kg, respectively. The corresponding $AUC_{0-\infty}$ values were 5.01 and 13.8 mcg•hr/mL, respectively. The total clearance was 3.11 and 2.97 L/hr/kg, and the total volume of distribution was 33 and 27.6 L/kg at 15 and 40 mg/kg, respectively. The V_{ss} suggests wide distribution into tissues.
Studies #8NABRP3 and #8NABRP5R2-3781	In vitro evaluation demonstrated that lefamulin is a P-gp substrate and a weak inhibitor of P-gp-mediated efflux transport.
Distribution	
Study #NBR/02	Sprague-Dawley rats (5/sex) were given a single ^{14}C-BC-3781.Ac dose of 10 mg/kg (free base) IV into the tail vein (5 mL/kg in saline). Mean blood plasma ratios were 1.45 (males) and 1.35 (females) indicating some degree of binding/association with RBC. Whole body autoradiography showed rapid distribution (within 5 min) to most tissues evaluated. In males, highest concentrations of radioactivity (22.7 mcg to 94.0 mcg equiv/g within 5 min postdose) were observed in the GI tract followed by the kidney (cortex and medulla), thyroid gland, myocardium, adrenal gland, urinary bladder, pituitary gland, and preputial gland. In females, highest concentrations of radioactivity were observed in the GI tract followed by the urinary bladder, kidney (cortex and medulla), myocardium, thyroid gland, adrenal gland, lungs, pituitary gland, liver, and lacrimal glands. In both males and females, low levels of radioactivity were observed in the brain (≤0.093 mcg equiv/g). By 72 hrs, radioactivity levels were below the lower limit of quantitation in most tissues; low levels (0.066 mcg to 2.44 mcg equiv/g) of radioactivity were still detected in the GI tract, kidney cortex and medulla, liver, lung (males only), spleen (males only), testis, and the clitoral/preputial, Harderian, pituitary, and thyroid glands.
Study #NABRIVA 2010-27 PKPD	After a single dose to noninfected mice, plasma and bronchoalveolar lavage samples were collected and analyzed for lefamulin. After 35 mg/kg IV, lefamulin exhibited a bi- or tri-phasic disposition in both plasma and epithelial lining fluid (ELF). The total AUC_{ELF}/AUC_{plasma} ratio was 4.7, 2.4, 2.0 after IV, subcutaneous (35 mg/kg), and oral (100 mg/kg) administration of lefamulin, respectively.

Type of Study	Major Findings
Study # EVT-00756-3781	Binding to plasma proteins was determined by equilibrium dialysis at concentrations of BC-3881.Ac of 1, 3, and 10 mcg/mL. Binding was dose-dependent in humans with values of 73% to 88%. Binding in rat, mouse, and monkey plasma proteins showed saturation and ranged between 76% to 81% in rats, 79% to 81% in mouse, and 61% to 64% in monkeys. Therefore, monkeys had a higher level of unbound BC-3781 compared to the other species.
Study #00000APP99001	In vitro, at concentrations of 1.6mcM to 200mcM, lefamulin exhibited low binding affinity for human serum albumin and human alpha-acid glycoprotein.
(b) (4) Study no. NBR/04: [^{14}C]-BC-3781: Placental transfer and milk secretion studies in rats	Placental transfer: Following a single intravenous administration of [^{14}C]-BC-3781 to pregnant female rats on Day 17 of gestation, one rat per time point was killed and subjected to quantitative whole-body autoradiography. The lower limit of quantification (LLOQ) was 0.155 mcg equivalent of [^{14}C]-BC-3781/g of tissue. The upper limit of accurate quantification was 267 mcg equivalent of [^{14}C]-BC-3781/g of tissue. At 10 minutes postdose, absorption of radioactivity was widespread, with greatest concentrations of maternal radioactivity associated with the myocardium (134 mcg equivalents/g), thyroid gland (134 mcg equivalents/g), adrenal gland (121 mcg equivalents/g), pancreas (117 mcg equivalents/g), salivary gland (105 mcg equivalents/g) and liver (96.6 mcg equivalents/g). Radioactivity was visible in fetal tissue, with greatest concentrations measured in the placenta and fetal liver (34.3 and 8.26 mcg equivalents/g respectively). Radioactivity in fetal tissues generally declined rapidly after this first sampling time, with radioactivity associated with the fetus itself below the limit of quantification by 12 hours postdose, and it was considered unlikely for the drug to be retained or accumulate in fetal tissues. Radioactivity in the placenta was initially high (34.3 mcg equivalents/g), but declined rapidly and was BLQ by 24 hours after dosing. Concentrations of radioactivity in the amniotic sac remained measurable at the final sampling time (72 hours), peaking at 6 hours postdose. The amniotic fluid did not contain radioactivity at any time after dose administration. Maternal radioactivity was generally greatest in glandular tissues and tissues associated with elimination of the test material. At 72 hours after dose administration, greatest concentrations of radioactivity were measured in contents of the GI tract (7.99 mcg to 11.9 mcg equivalents/g), the pituitary gland (9.93 mcg equivalents/g) and uterus (9.30 mcg equivalents/g), with radioactivity in remaining tissues associated only with the Harderian gland, amniotic sac, spleen, ex-orbital lachrymal gland, liver and kidney cortex. High concentrations of radioactivity in contents of the gastrointestinal tract were considered to be associated with biliary excretion. Milk secretion: Groups of female rats at approximately 14 days postparturition were administered [^{14}C]-BC-3781 as a single intravenous dose of 30 mg free base/kg. Milk and plasma were

Type of Study	Major Findings
	collected from three rats at each of 0.25, 1, 3, 6 and 24 hours following dose administration. Mean concentrations of radioactivity in plasma were maximal at 0.25 hour post dose (3.29±0.19 mcg equiv./g). Twenty four hours post dose, it was markedly reduced with a value of 0.00663±0.01147 mcg equiv./g. Mean concentrations of radioactivity in milk were maximal at 0.25 hour post dosing (10.7±1.8 mcg equiv./g). Twenty four hours post dose, it was markedly reduced with a value of 0.0700±0.0143 mcg equiv./g . Milk/plasma ratios increased from 3.27 to 8.33 between 0.25 hours to 6 hours post dosing. The data indicate that it is likely that pups would be exposed to the test article in milk.
Metabolism	
	In vitro assessment in primary hepatocytes (**Study #NABRIVA 2008-22 ANC, Study #NABRIVA 2008-23 ANC, Study #NABRIVA 2009-15 ALL**) demonstrated similar metabolism between human, mouse, rat, rabbit, and cynomolgus monkey, consisting primarily of CYP450 phase I reactions and suggested that metabolism can be saturated at higher lefamulin concentrations. Lefamulin was a substrate only of CYP3A4 and CYP3A5 (**Study #15570v3**). Potential for inhibition of ofCYP2C8, CYP3A4, and CYP3A5 was demonstrated in several studies. Results of **Study #XT153113** indicated that induction of CYP1A2, CYP2B6, or CYP3A4 would be unlikely in a clinical setting. In vivo, metabolism following IV and oral administration was evaluated in rats (**Studies #1281-043 and #BC3-TX-01**) and cynomolgus monkeys (**Studies #1281-044 and #BC3-TX-02**). In general, unchanged lefamulin was the predominant circulating compound in plasma, less than 40% was excreted unchanged in urine or bile, and metabolism was primarily by hydroxylation pathways, with at least one mono-hydroxy metabolite undergoing glucuronide conjugation. From the Applicant's written summary: Figure 12: Proposed biotransformation pathway of lefamulin (BC-3781) in rat 

Type of Study	Major Findings

Figure 13: Proposed biotransformation pathway of lefamulin (BC-3781) in cynomolgus monkey

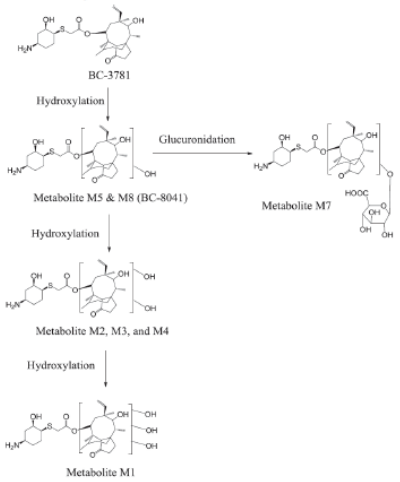


Table 11: Metabolite codes used in different studies

Type of metabolite / name	Code used internally at Nabriva	Species (Study number)		
		Rat (BC3-TX-01)	Monkey (BC3-TX-02)	Human (NAB-BC-3781-1013)
Tri-hydroxy lefamulin	M(556a)	a)	*M1*	a)
Di-hydroxy lefamulin	M(540a)	a)	*M2*	M7
Di-hydroxy lefamulin	M(540a)	a)	*M3*	a)
Di-hydroxy lefamulin	M(540a)	a)	*M4*	a)
Mono-hydroxy lefamulin	M(524b)	*M5*	*M5*	M10
Mono-hydroxy lefamulin	Not separated from M(524b)	*M6*	a)	a)
Lefamulin glucuronide	M(700a)	*M7*	*M7*	a)
2*R*-hydroxy lefamulin	M(524c) = BC-8041	*M8*	*M8*	M13
Lefamulin ketone	M(522a)	*M9*	a)	a)
Mono-hydroxy lefamulin ketone	M(538a)	*M10*	a)	a)
Mono-hydroxy lefamulin	M(524d)	*M11*	a)	a)
Mono-hydroxy lefamulin	M(524e)	*M12*	a)	a)
Lefamulin		*BC-3781*	*BC-3781*	M19 (P)

a) Not detected or below the limit of quantification

The main human metabolite, 2*R*-hydroxy lefamulin, corresponds to M8 in the rat and monkey and M13 in the human.

Excretion	
	Based on Study #NBR/02, #NBR/03, and #1281-044 of IV and orally administered radio-labelled lefamulin in rats (2 studies) and cynomolgus monkeys, respectively, the fecal route was the primary route of elimination, with excretion of lesser amounts in the urine.
TK data from general toxicology studies	
Study no. AA97305 – 4-week IV study in rats	The NOAEL was determined to be the high dose, 37.5 mg/kg BID (75 mg/kg/day, $AUC_{0\text{-}12h}$**≈10,000–12,000 ng*hr/mL)**. $T_{1/2}$: 2.43–2.73 hours on Day 0 Increases in systemic exposure appeared to be linear and dose-proportional, with no evidence of accumulation.

Type of Study	Major Findings
Study no. AB21053 – 13-week IV study in rats	The NOAEL was the low dose of 18.75 mg/kg/day BID (37.5 mg/kg/day, AUC_{12h} =4536 and 4754 ng*hr/mL in males and females, respectively at Week 13). $T_{1/2}$: 1.90–4.31 hours There was no accumulation after 13 weeks of treatment. The increase in AUC_{last} with increasing doses was generally linear but slightly greater than dose-proportional.
Study no. AB16227 – 4-week oral study in rats	The NOAEL was the mid-dose, 150 mg/kg/day BID (AUC_{0-12h} 7810–13043 ng*hr/mL). Variability in plasma concentrations was high. No accumulation of the test item or metabolite was observed after 4 weeks of treatment. Increases in systemic exposure and C_{max} were generally dose-related.
Study no. (b) (4).289.15 – 4-week IV study in cynomolgus monkeys	The NOAEL was the MD, 70 mg/kg/day (35 mg/kg BID; AUC_{0-inf} approximately 17,000 ng*hr/mL on Day 1, dose solution concentration 1.17 mg/mL). Systemic exposure was greater than dose-proportional with the suggestion of accumulation with repeated dosing over time. Half-life also increased with repeated dosing.
Study no. (b) (4) 289.19 – 13-week IV study in cynomolgus monkeys	The LD, 60 mg/kg/day, was the LOAEL (Mean AUC_{0-inf} was 13,000–13,900 ng*hr/mL on Day 1 and 14,700 and 23,900 on Days 28 and 91). $T_{1/2}$: 3.85–5.59 h. Increases in C_{max} and AUC were generally dose-proportional on Day 1 and more variable at later collection times. Accumulation ratios were less than 2-fold.
Study no. 8275686 – 4-week oral study in cynomolgus monkey	The MD (35 mg/kg BID, or 70 mg/kg/day) was the NOAEL. At that dose, AUC_{0-inf} on Day 1 was 2230 ng*hr/mL in males (n=1) and 1120 ng*hr/mL in females (n=2). On Day 28, AUC_{0-inf} was 8090 ng*hr/mL in MD males (n=1) and 4660 ng*hr/mL in MD females (n=4). $T_{1/2}$: 3.6–7.2 hours Mean C_{max} and exposure increased in an approximately dose-proportional manner. There was evidence of accumulation of BC-3781 following repeated administration. Values for the main metabolite suggested saturation of metabolism.

Type of Study	Major Findings
TK data from reproductive toxicology studies	
Rat fertility and early embryonic development studies	In a fertility (Segment I) study in male rats (**Study no. AA97303**), the NOAEL was the high dose, 75 mg/kg/day IV (free base), divided into 2 doses given 12 hours apart (HED =12.5 mg/kg/day, or 750 mg/day for a 60 kg human). At that dose, $AUC_{0\text{-}12h}$, based on the 4-week IV general toxicology study, was 10289 ng*hr/mL on Day 26 (**$AUC_{0\text{-}24h}$ approximately 20.6 mcg*hr/mL**). In a fertility (Segment I) study in female rats (**Study no. AA97304**), the NOAEL was the mid-dose, 50 mg/kg/day IV divided into 2 doses given 12 hours apart (HED =8.3 mg/kg/day, or 500 mg/day for a 60 kg human). At that dose, $AUC_{0\text{-}12h}$, based on the 4-week IV general toxicology study, was 6722 ng*hr/mL on Day 26 (**$AUC_{0\text{-}24h}$ approximately 13.4 mcg*hr/mL**).
Study no. AA97308 Rat embryo-fetal development study	In the rat embryo-fetal development study, a maternally toxic dose was not reached. Systemic exposure at all doses was lower than that of clinical patients. Assuming that the delays in skeletal ossification would not be adverse, the fetal NOAEL in this study would be the low dose, 50 mg/kg/day, divided BID (mean C_{max} =5612–7058 ng/mL, mean $AUC_{0\text{-}12h}$ =5378–8056 ng*h/mL; steady state **$AUC_{0\text{-}24h}$ approximately 10.8 mcg*hr/mL**).
Study no. 82750 Rabbit embryo-fetal development study	In the embryofetal development study in rabbits, due to low numbers of live fetuses and lack of complete evaluation of low and mid-dose groups, a NOAEL was not found
Study no. AB21312 Rat pre- and postnatal development study	In the rat pre- and postnatal development study, the NOAEL was considered to be the mid-dose, 2x37.5 mg/kg/day. Based on pharmacokinetic data from the rat EFD study, **mean $AUC_{0\text{-}12h}$ ranged from 8592–13042 ng*hr/mL** at that dose.

IV = intravenous; C_{max} = maximum concentration; $AUC_{0\text{-}\infty}$ = area under the concentration-time curve from time zero to infinity; V_{ss} = apparent volume of distribution at steady state; PK = pharmacokinetic; RBC = red blood cell; GI = gastrointestinal; NOAEL = no-observed-adverse-effect level; BID = twice a day; MD = mid dose; LD = low dose

5.5. Toxicology

5.5.1. General Toxicology

GLP-compliant toxicology studies with lefamulin included 4-week oral and IV studies in the rat and cynomolgus monkey and 3-month IV studies in the rat and cynomolgus monkey.

By the Intravenous Route

Study no. AA97305: BC-3781 – 4-week toxicity study in the Sprague-Dawley rat by intravenous injection (bolus) in surgically implanted animals followed by a 4-week treatment-free period

- Transient hypersalivation was seen immediately after injection for animals at 50 and 75 mg/kg/day, and there were isolated findings of soft and/or discolored feces. Body weight gain was lower in treated animals during the recovery period.
- Evidence of slight anemia at all doses was reported with evidence of regeneration at 50 and 75 mg/kg/day; this was thought to be due to the hemolytic properties of the test article.
- Macroscopic necropsy findings were limited to firm areas at the injection sites that correlated with histological findings of phlebitis, periphlebitis, peripheral inflammation and thrombosis.
- The NOAEL was determined to be the high dose, 37.5 mg/kg BID (75 mg/kg/day, AUC ≈ 10,000–12,000 ng*hr/mL).

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

Table 8. Study No. AA97305: Methods

Study Method	Details
Dose and frequency of dosing	0, 12.5, 25, and 37.5 mg/kg twice daily, for total daily doses of 0, 25, 50, and 75 mg/kg/day (as the free base)
Route of administration	IV bolus
Formulation/vehicle	0.9% NaCl
Species/strain	Sprague-Dawley rats (Crl:OFA(SD))
Number/sex/group	10, plus 5/sex in each group for recovery
Age	9 weeks
Satellite groups/unique design	3/sex in the control group and 6/sex in each treatment group for toxicokinetics. Animals were implanted with a polyurethane catheter in the caudal vena cava via the left femoral vein. Patency was maintained by continuous infusion with physiological saline.
Deviation from study protocol affecting interpretation of results	No

IV = intravenous

Table 9. Study No. AA97305: Observations and Results: Changes From Control

Parameters	Major Findings
Mortality	No test article-related deaths were reported.
Clinical signs	Transient hypersalivation was seen immediately after injection for animals in the mid- and high dose groups. Isolated findings of soft and/or discolored feces were considered to be incidental.
Body weights	There was no treatment related effect on body weight gain during the treatment period reported. However, there were statistically significant decreases in mean body weight gain in the treated groups between Days 27 and 35 in males and females, and between Days 42 and 55 in females during the recovery period, relative to controls.
Ophthalmoscopy	No treatment-related findings were reported.
Hematology	Dose-related slight decreases in mean red blood cell parameters (RBC, Hb, and PCV) were seen in all treated groups relative to controls at the end of the treatment period. There were also statistically significant increases in MCV, MCH, and MCHC at all doses, as well as increased mean reticulocyte counts at the mid and high doses, which were suggestive of a regenerative effect. At the end of the recovery period, values had partially returned to control values.
Clinical chemistry	No treatment-related findings were reported.
Urinalysis	No treatment-related findings were reported.
Gross pathology	No treatment-related findings were reported, other than phlebitis, peri-phlebitis, peripheral inflammation and thrombosis considered to be associated with the administration procedure.
Organ weights	At the end of treatment, mean absolute and relative testes and epididymis weights were decreased in all male dose groups, but only the relative mean weights were statistically significant relative to controls. There were no correlating microscopic findings reported, and the effect could have been due to slightly higher terminal body weights. No organ weight differences were reported at the end of recovery.
Histopathology Adequate battery: A full set of tissues was collected, but examination was limited to control and high dose animals.	At the terminal sacrifice, there were no treatment-related findings reported. Microscopic findings were reported to be typical of those seen in infusion studies in the rat, including thickening of the intima, phlebitis, periphlebitis, and thrombosis at the injection site, and multifocal perivascular inflammation/alveolitis/alveolar hemorrhage and multiple granulomas in the lungs.
[Other evaluations]	Irwin tests (described under CNS safety pharmacology) revealed no adverse test article-related findings. Rectal temperatures were slightly lower in mid- and high -dose animals.

LD = low dose; MD = mid dose; HD = high dose; RBC = red blood cell; Hb = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; CNS = central nervous system

Toxicokinetics

Toxicokinetic parameters are shown in the Applicant's table below:

Table 10. Study No. AA97305: Toxicokinetic Parameters

Occasion	Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12h} (ng.h/mL)
day 0	2 x 12.5	3547	0.05	3268
	2 x 25	5736	0.05	7895
	2 x 37.5	9166	0.05	11950
day 14	2 x 12.5	3517	0.05	3518
	2 x 25	6865	0.05	6336
	2 x 37.5	9677	0.05	10433
day 26	2 x 12.5	3743	0.05	3211
	2 x 25	6529	0.05	6722
	2 x 37.5	9024	0.05	10289

C_{max} = maximum concentration; T_{max} = time to reach maximum concentration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration

Increases in systemic exposure appeared to be linear and dose-proportional. There were no gender differences reported. T_{max} was the first time point, 3 minutes postdose. The test article underwent rapid elimination, with half-life ranging from 2.43 hours to 2.73 hours on Day 0. Clearance was reported to be 3.03 L/hr/kg to 3.72 L/hr/kg, and volume of distribution was reported to be 10.8 L/kg to 14.1 L/kg. The Applicant stated that the large volume of distribution was suggestive of extensive extravascular distribution. Accumulation was not apparent in this species in this study.

No evaluation of the main human metabolite, 2*R*-hydroxy lefamulin, was reported.

Study no. AB21053 (Applicant reference no. LMU_SS_02_001): BC-3781.Ac – 13-week toxicity study by intravenous (bolus) route in the rat followed by a 4-week treatment-free period

- Body weight gain and food consumption were decreased in MD and HD males.
- Increased production of feces in treated groups was attributed to alteration in intestinal flora.
- Decreased red blood cell parameters were seen in males at all doses and in HD females at the end of treatment. This finding was partially resolved after the recovery period.
- Intestinal dilatation (primarily cecum) was noted at all doses, and was dose-related in severity in females.
- Vascular inflammatory and thrombotic changes appeared to be exacerbated by the test article in a dose-related manner.

- Based on mortality due to the test article-related effects at the mid- and high doses, the low dose of 18.75 mg/kg/day BID (37.5 mg/kg/day) was considered to be the NOAEL (AUC_{12h} =4536 and 4754 ng*hr/mL in males and females, respectively at Week 13). The formulation used for the low dose had a nominal test item concentration of 1.875 mg/mL (in terms of free base).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (OECD)

Table 11. Study No. AB21053: Methods

Study Method	Details
Dose and frequency of dosing	0, 37.5 (2x18.75), 75 (2x37.5), and 125 (2x62.5) mg/kg/day
Route of administration	IV bolus twice daily, q12h
Formulation/vehicle	10mM citrate-buffer normal saline, pH 5.0
Species/strain	Sprague-Dawley rats (Crl:OFA(SD))
Number/sex/group	10
Age	10 weeks at the start of treatment
Satellite groups/ unique design	2/sex (control group) or 6/sex (treated groups) were included for toxicokinetics. 5/sex/group were included for recovery. A polyurethane catheter was surgically implanted into the posterior vena cava via the left femoral vein. The catheter was attached to an infusion pump via a tether system and a swivel joint (up to 8 animals of the same group and sex per infusion pump). Animals were maintained on continuous infusion (0.4 mL/hour/animal) with physiological saline (Lavoisier) between implantation and the start of treatment and between the two daily treatments.
Deviation from study protocol affecting interpretation of results:	No

Table 12. Study No. AB21053: Observations and Results: Changes From Control

Parameters	Major Findings
Mortality	During the treatment period, 1 male treated at 75 mg/kg/day, and 4 males and 1 female at 125 mg/kg/day were sacrificed for ethical reasons. In these animals, swelling at the injection and implantation site progressed to marked changes at and around the site of injection resulting in the poor clinical condition of the animals. One female treated at 37.5 mg/kg/day was sacrificed due to critical respiratory changes attributed to a technical accident (presence of air in the infusion system).

Parameters	Major Findings
Clinical signs	Increased production of feces was noted in treated animals and was attributed to perturbation of intestinal flora.
Body weights	During the treatment period, mid- and high-dose males exhibited lower body weight gains than controls, correlating with lower food consumption. At the end of the treatment period, statistically significantly lower mean body weight was noted in these animals (-7% and -13% respectively, p≤0.01, per the pathology report), relative to controls. After the 4-week recovery period, lower body weight persisted in high dose males (-11% relative to controls, p≤0.05). In females, body weight and food consumption were comparable to controls.
Ophthalmoscopy	No treatment-related findings were reported.
Hematology	At the end of treatment, decreased red blood cell (RBC) parameters (RBC count, hemoglobin concentration, and packed cell volume) were seen in all treated males and in high -dose females, relative to controls. Increased mean relative neutrophil count was noted in all treated animals, and a slight decrease in mean platelet count in all treated males. Partial recovery was noted at the end of the treatment free-period.
Clinical chemistry	At the end of the treatment period, dose-related decreases in mean protein, albumin and globulin concentrations were noted in all treated males, relative to controls. These changes appeared to resolve in low and mid-dose males during the recovery period, but persisted in high dose males. The report attributed these changes to “the digestive and/or the inflammatory changes.”
Urinalysis	At the end of the treatment period, decreased mean urinary volume and pH and increased specific gravity were noted in all treated males (dose-related) and in mid- and high-dose females (not dose-related), relative to controls. At the end of the recovery period, these findings persisted in high dose males only.
Gross pathology	For the rats sacrificed in moribund condition, abdominal distension, distension of intestinal segments (primarily the cecum), firm/edematous areas at the injection site accompanied by adherences around tissue/organs (abdominal/thoracic skin, hind limb skeletal muscles, prostate, seminal vesicles), and dilatation of the urinary bladder and renal pelvis were reported. No gross findings were reported for the LD female that was

Parameters	Major Findings
	euthanized due to an apparent technical error. At the end of the treatment period, intestinal distension was observed in some treated rats at all doses, but abdominal distension was not reported. Firm areas at the injection site were reported for 2 MD and 2 HD animals. Renal pelvic dilatation was reported for one LD male, one MD female, and one HD female. At the end of the recovery period, no test article-related gross findings were reported.
Organ weights	Terminal body weights were decreased in MD and HD males. The following organ weight changes at the end of treatment were attributed to stress: Adrenal gland weights were higher than control in HD males and MD and HD females, correlating with cortical hypertrophy in HD animals. Spleen weights lower than control in all treated groups in both males and females, correlating with decreased peri-arteriolar lymphoid sheath in HD females. Thymus weights were decreased relative to control in MD and HD males and in HD females, correlating at the HD with cortical atrophy. Following the recovery period, no test article-related organ weight changes were reported. Terminal body weights were decreased in HD males, but were partially resolved.
Histopathology Adequate battery: Yes. While the full tissue list was collected for all animals, the pathology report indicates that only heart, kidney, liver, injection sites and lungs were examined for "intermediate" (presumably low and mid-dose) groups. Although not stated in the report, gross lesions, notably in the cecum, were also examined. It is also notable that not all lesions noted at the HD were also examined in the LD and MD groups, including thymus, spleen, and adrenals.	Premature decedents: • The firm/edematous appearance at injection sites correlated microscopically with moderate to severe perivascular inflammation and moderate to severe thrombosis at or beyond the tip of the catheter. • In two high-dose males, inflammatory/ thrombotic changes around tissues/organs were stated in the pathology report to have resulted in microscopic findings in the kidneys (slight dilatation of the renal pelvis and/or renal tubules) and urinary bladder (slight serosa inflammation and dilatation). • Distended intestinal segments, mainly in the caecum (minimal to marked luminal dilatation) were reported. • Three high-dose male moribund rats had additional findings of minimal/slight adrenocortical hypertrophy and slight/moderate thymic cortical atrophy, considered to be related to stress.

Parameters	Major Findings
	• For the low-dose female that was sacrificed due to an apparent technical error, minimal dilatation of the cecum was observed histologically. End of treatment sacrifice: • Minimal to moderate dilatation in the cecum was reported in all treated groups that was dose-related in severity in females. This was not associated with any degenerative changes in the wall of the cecum. • At the injection sites, vascular inflammation and thrombosis were noted in treated animals at all doses, as well as in control animals. Perivascular inflammation was limited to a few treated animals only. Catheter-related changes at the LD and MD were reported to be generally less prevalent and less severe than those observed at the HD. • The increased severity of findings with dose at the injection sites was considered as an exacerbation by the test article of background infusion-related lesions. • Changes considered to be secondary to inflammatory changes included lung granulomas (aggregates of macrophages and a few multinucleated cells associated with foreign bodies) in all groups, including controls, and unilateral renal pelvic dilatation in one HD female. • Changes reflective of stress included thymic atrophy, increased apoptosis and decreased size of the marginal zone in the spleen, and adrenocortical hypertrophy at the HD. Recovery sacrifice: • Evaluation of the cecum was not performed, but macroscopic dilation was not observed. • Changes at the injection sites exhibited partial resolution. • The report states that adrenal or thymic changes in HD rats were not observed, however, summary tables in the pathology report do not indicate that these tissues were examined.

LD = low dose; MD = mid dose; HD = high dose.

Toxicokinetics

Plasma concentrations were generally quantifiable in most plasma samples from treated animals up to 12 hours (i.e., just before the second daily dosing). The half-life values ranged

from 1.90 hours to 4.31 hours. No sex-related difference and no accumulation after 13 weeks of treatment were observed for C_{max} and AUC_{last}. The increase in AUC_{last} with increasing doses was generally linear but slightly greater than dose-proportional.

Toxicokinetic parameters are shown in Table 13.

Table 13. Study No. AB21053: Toxicokinetic Parameters

Occasion	Dose (mg/kg/adm)	Sex	C_{max} (ng/mL)	AUC_{last} (ng.h/mL)
Day 1	18,75	Male	3316	4139
		Female	3412	3766
		mean	*3364*	*3953*
	37,5	Male	6517	8993
		Female	6502	10908
		mean	*6510*	*9951*
	62,5	Male	11332	18892
		Female	10437	22689
		mean	*10885*	*20790*
Week 4	18,75	Male	3358	4375
		Female	3409	3545
		mean	*3384*	*3960*
	37,5	Male	6104	8582
		Female	6799	9613
		mean	*6452*	*9098*
	62,5	Male	12065	17530
		Female	11119	16180
		mean	*11592*	*16855*
Week 13	18,75	Male	3560	4536
		Female	3693	4754
		mean	*3626*	*4645*
	37,5	Male	6045	8656
		Female	6769	10076
		mean	*6407*	*9366*
	62,5	Male	13247	21020
		Female	11408	15990
		mean	*12328*	*18505*

AUC_{last} = $AUC_{0\text{-}12h}$, except for females at 18.75 mg/kg/day on Day 1 and in Week 4 ($AUC_{0\text{-}6h}$)

C_{max} = maximum drug concentration; AUC_{last} = area under the concentration-time curve from time zero to time of last measurable concentration

No evaluation of the main human metabolite, 2*R*-hydroxy lefamulin, was reported.

Study no. (b) (4).289.15 (Applicant reference no. 03781A-ST08-001-GxP): A 4-week intravenous toxicity study of BC-3781.Ac in cynomolgus monkeys followed by a 4-week recovery period

- Sporadic hypoactivity or lethargy was reported in treated animals.
- Decreased red blood cell mass with evidence of a regenerative response was reported in 120 mg/kg/day animals and was attributed to hemolysis at the injection site by the higher concentrations of test article. Red blood cell parameters had recovered by the end of the recovery period.

- Histologically, pancreatic microvesicular vacuolization of acinar cells was noted at the 120 mg/kg/day, but was not evident after the recovery period. Vascular inflammatory changes and thrombus formation were noted at the injection site.
- Systemic exposure was greater than dose-proportional with the suggestion of accumulation with repeated dosing over time. The half-life also increased with repeated dosing.
- The NOAEL was reported by the Applicant to be the high dose, 120 mg/kg/day, in light of the magnitude and reversibility of the findings. However, it is unclear whether or not the pancreatic lesions may represent a clinical risk, in which case the NOAEL may be better estimated as 70 mg/kg/day (35 mg/kg BID; $AUC_{0\text{-}inf}$ approximately 17,000 ng*hr/mL on Day 1, dose solution concentration 1.17 mg/mL).

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

Table 14. Study No. (b) (4) .289.15: Methods

Study Method	Details
Dose and frequency of dosing	0, 20, 35, 60 mg/kg BID for total daily doses of 0, 40, 70, and 120 mg/kg/day (in terms of the free base)
Route of administration	IV infusion over 1 hour
Formulation/vehicle	0.9% sodium chloride for injection, USP
Species/strain	Cynomolgus monkey
Number/sex/group	4
Age	3–7 years
Satellite groups/unique design	2/sex for recovery in each dose group All animals were implanted with a femoral venous catheter for test article administration. Patency was maintained by continuous saline infusion at 0.05 mL/minute. Animals were fasted prior to procedures involving sedation or anesthesia and prior to collection of samples for clinical pathology.
Deviation from study protocol affecting interpretation of results	No

USP = U.S. Pharmacopeia

Table 15. Study No. (b) (4).289.15: Observations and Results: Changes From Control

Parameters	Major Findings
Mortality	None
Clinical signs	Hypoactive or lethargic behavior was noted sporadically and mostly in the first two weeks of treatment in males and females in the low- and high-dose groups. Eyelid closure was noted in males in all treated groups and in high-dose females. Findings were sporadic, and the former finding was without a clear dose-response relationship (no occurrences noted in mid-dose group, although incidence was dose related in groups exhibiting this sign), but did occur only in treated animals and only during the dosing period, arguing for a relationship to treatment.
Body weights	Weight gain over the study was slower in the mid- and high-dose groups, but body weights were comparable to controls by the end of treatment.
Ophthalmoscopy	No test article-related changes were reported. One animal (#SSAN32; mid-dose male) had retinal lesions in the left eye on Day 57 that were considered to be possibly due to an embolic event, but these were not considered to be treatment-related.
ECG	Not performed
Hematology	Decreased red blood cell parameters (RBC, hemoglobin, and hematocrit) reached statistical significance in the high -dose group on Days 15 and 29. Increased reticulocytes and red cell distribution width indicated a regenerative response. This finding was attributed to potential hemolytic properties of high -dose test article concentrations (2 mg/mL) at the infusion site. Alterations to white blood cell (WBC) counts (increased WBC, neutrophils and/or monocytes) occurred in individual animals in the low- and high-dose groups on Day 29. These changes, along with decreased lymphocytes, serum chemistry changes, and increased fibrinogen were considered to be indicative of an “acute phase response.” Coagulation assessment revealed increased fibrinogen in individual males in all treated groups and females in the high -dose group on Days 15 and/or 29. In some of these animals, the report states that associated changes in hematology and serum chemistry were suggestive of an “acute phase response.” All parameters were reported to have returned to baseline by Day 57. No treatment-related changes in PT or APTT were reported.
Clinical chemistry	Individual animals in the low- and high -dose groups on Days 15 and/or 29 had decreased albumin and A/G ratio, and increased alkaline phosphatase and globulin. Higher C-reactive protein and haptoglobin were found on Day 29. All of these findings were considered to be indicative of an “acute phase response.” All of these parameters returned to baseline by Day 57. Mild increases in AST and ALT were seen on Days 15 and 29 in mid- and high -dose males that were statistically significant at the high dose. Creatine kinase was also increased in those animals. The report states that, since similar findings were seen in control and treated females, and since these findings did not worsen with subsequent dosing, this was likely due to stress.

Parameters	Major Findings
Urinalysis	Urine was collected in cage pans once pretest and on Days 15, 29, and 57. Urine testing positive for blood was more common in treated males after the start of treatment. However, since there were isolated instances in control and mid-dose animals noted pretest, the relationship to treatment is unclear. The Applicant considered this to be an incidental finding, but could be related to intravascular hemolysis that was thought to affect red blood cell parameters on hematology evaluation.
Gross pathology	No test article related findings were reported from either the terminal or recovery sacrifice. Vascular inflammatory changes, edema and discoloration at the injection site, and thrombus formation were attributed to IV catheter placement and the IV dosing procedure.
Organ weights	Decreased absolute and relative heart weights were seen in mid- and high -dose males at the terminal necropsy that were statistically significantly different from control. There were no histopathological correlates to heart weight changes.
Histopathology Adequate battery: Yes	Minimal or greater microvesicular vacuolization of acinar cells in the pancreas was considered to be test article-related. It was seen in all four high-dose males and one of four high-dose females at terminal necropsy. The finding was more severe in males. There were no apparent clinical pathology correlates or effect on food consumption or weight gain. The finding was no longer apparent at the end of recovery. Findings secondary to continuous indwelling catheters were seen, including vascular/ perivascular inflammation and thrombosis/embolism at injection sites, eosinophilic perivascular infiltration, arterial hyperplasia, and thrombosis/embolism in the lung.

LD = low dose; MD = mid dose; HD = high dose; IV = intravenous; PT = prothrombin time; APTT = activated partial thromboplastin time; A/G ratio = albumin to globulin ratio; AST = aspartate aminotransferase; ALT = alanine aminotransferase

Toxicokinetics

Systemic exposure was demonstrated on Days 1 and 28 at all three doses. The time course was biphasic, with a rapid distribution phase followed by slower elimination phase. Group mean toxicokinetic parameters are shown in the Applicant's table below:

Table 16. Study No. (b) (4).289.15: Group Mean Toxicokinetic Parameters

Group mean TK results

Group/ Dose Level (mg/kg/ administration)	Dose Day	Sex	N	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-12hr} (hr*ng/mL)	AUC_{inf} (hr*ng/mL)	HL (hr)
2/20	1	F	6	0.765	3710	7830	8520	3.80
		M	6	0.848	3670	8720	9830*	4.63
3/35		F	6	0.936	6540	15200	17000	4.15
		M	6	0.940	7090	17300	20000	4.64
4/60		F	6	0.933	12800	25300	28500	4.45
		M	6	0.935	15100	31500	36300*	5.05
2/20	28	F	6	0.871	4540	11600	13500	4.65
		M	6	0.838	5020	13600	15900**	5.28
3/35		F	6	0.826	8980	21900	25800	4.77
		M	6	0.753	8270	24700	27500**	5.40
4/60		F	6	0.870	15400	34300	36500*	4.38
		M	6	0.744	16200	45300	52600*	5.18

* AUC_{inf} values derived from n=5, ** AUC_{inf} value derived from n=4

C_{max} = maximum drug concentration; AUC_{0-12hr} = area under the concentration-time curve from time 0 to 12 hours after drug administration; AUC_{inf} = area under the concentration-time curve from time zero to infinity

C_{max} and AUC increased with increasing dose and were slightly higher following repeated doses, suggesting accumulation. C_{max} was slightly greater than dose-proportional after a single dose and was variable after repeated dosing. C_{max} was comparable between males and females. AUC was generally dose-proportional in females on Days 1 and 28, but was greater than dose-proportional in males. AUC tended to be greater for males than for females. Half-life was longer for males and was longer with repeated dosing. T_{max} was generally seen at the first or second time point after the start of infusion.

For BC-8041, the major metabolite, T_{max} was 1 hour after the start of infusion in both males and females. C_{max} and AUC were not dose-proportional; both parameters increased in a greater than dose-proportional manner on Day 1. No gender differences were noted, and terminal half-lives were highly variable. After repeated dosing, on Day 28, T_{max} was unchanged from Day 1. At steady state, C_{max} and AUC still increased in a greater than dose-proportional manner. The values for these parameters were approximately 2-fold higher, again suggesting accumulation. Metabolite trough values were reported to be consistent between the two sampling times. Group mean toxicokinetic parameters are shown in the Applicant's table below:

Table 17. Study No. (b) (4).289.15: Group Mean Toxicokinetic Parameters (Day 1 vs. Day 28)

Dose BC-3781		Study Day: Day 1 C_{max} [ng/ml]	Day 1 AUC_{0-12h} [ng·h/ml]	Day 28 C_{max} [ng/ml]	Day 28 AUC_{0-12h} [ng·h/ml]
20 mg/kg q12h (*n* = 12)	Mean	40.2	178	56.1	334
	SD	27.3	43.6	21.8	67
	CV [%]	68.0	24.5	38.9	20.1
35 mg/kg q12h (*n* = 12)	Mean	80.5	363	167	755
	SD	24.4	109	91	314
	CV [%]	30.4	30.1	54.6	41.5
60 mg/kg q12h (*n* = 12)	Mean	443	1350	816	3130
	SD	230	521	480	1500
	CV [%]	51.9	38.6	58.8	47.9

No sex-related differences in the exposure or C_{max} of BC-8041 could be identified, irrespective of the BC-3781 dose level.

C_{max} = maximum drug concentration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration; SD = standard deviation; CV = coefficient of variation

Study no. (b) (4).289.19 (Applicant Reference No. LMU_SS_02_003): A 13-Week Intravenous Toxicity Study of BC-3781.Ac in Cynomolgus Monkeys Followed by a 4-Week Recovery Period

- Animals at all doses exhibited emesis, lethargy, prostration in a dose-related incidence. Clinical pathology findings consistent with inflammatory changes included increased neutrophils at MD and HD, increased monocytes at HD, mild to moderate regenerative anemia at MD, and increased C-reactive protein (CRP) at LD and MD (dose-related incidence). Findings were severe enough in HD animals to terminate that group early.
- At all doses, inflammatory changes, thickening, abscesses, granulation tissue, and fibrosis were seen at the proximal and distal ends of the IV catheter, with thrombosis and inflammation at distant sites (dose-related incidence and severity). Renal vein and artery changes (inflammation and fibrosis) were also attributed to proximity to the catheter. Abscesses, inflammatory cell infiltrates and granulation tissue were considered to be direct effects of the test article, while other injection site findings were considered to be exacerbation of catheter-related injury by the test article. Incidence and severity appeared to be dose-related.
- Additional test article-related findings included: vacuolation of acinar cells in the pancreas at the LD and MD in males and in the MD in females (resolved in recovery

animals), minimal alveolar macrophage infiltrates in the lung in LD and MD animals and thrombosis in the lung at the LD and MD in males, and an abdominal cavity abscess in one MD male, confirmed on histology, near the injection site.

- Findings resolved at least partially by the end of the recovery period. The lowest dose, 60 mg/kg/day, may be considered a LOAEL (Mean $AUC_{0\text{-}inf}$ ranged from 13,000–13,900 ng*hr/mL on Day 1 and 14,700–23,900 on Days 28 and 91).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 18. Study No. (b) (4).289.19: Methods

Study Method	Details
Dose and frequency of dosing	0 (vehicle), 60, 120, 200 mg/kg/day, divided into BID doses The high-dose group received 120 mg/kg/day for Days 1–2, 160 mg/kg/day for Days 3–4, then 200 mg/kg/day from Day 5 through Day 61 or 64, when that group was terminated due to poor condition.
Route of administration	IV infusion over 1 hour twice daily via an indwelling femoral catheter
Formulation/vehicle	10mM citrate-buffered saline (pH 5)
Species/strain	Cynomolgus monkeys (Cambodian)
Number/sex/group	4, with an additional 2/sex/group for recovery
Age	2–5 years
Satellite groups/unique design	Dosing for the high dose group was step-wise (see above). Individual animals with declining clinical condition and clinical pathology changes indicative of inflammation were placed on a dosing holiday ranging from 1–10 days in duration. The high dose group was terminated early (Day 64 for males or Day 61 for females).
Deviation from study protocol affecting interpretation of results	Yes. The high dose group could not be fully evaluated relative to groups that completed the study. Dosing holidays in high dose animals were reported to have impacted TK and toxicity profiles. Dosing holidays affected TK sample collection in one mid-dose animal.

Table 19. Study No. (b) (4).289.19: Observations and Results: Change From Control

Parameters	Major Findings
Mortality	One control animal was euthanized on Day 49 due to a catheter failure. Findings in that animal were limited to increased creatine kinase on Day 22 and minimal endothelial hypertrophy in the renal vein and artery (considered to be associated with the indwelling catheter) at necropsy. Six HD animals were euthanized between Days 43 and 63 due to declining condition. Findings included emesis, lethargy and prostration, clinical pathology findings attributed to inflammation, and thickening, abscessation, inflammation, granulation tissue and fibrosis at the injection sites. Findings of thrombosis and inflammation were also seen in multiple distant tissues. The remaining six HD animals were euthanized and necropsied on Day 64 (males) or 61 (females). Findings in these animals were similar to but not as severe as in previously euthanized HD animals, and there was concern that the number of surviving animals would be insufficient for statistical analysis.
Clinical signs	Clinical signs at all doses included emesis, eyes shut, lethargy, hunched posture, and prostration. Incidence (in terms of recorded observations) appeared to be dose-related, as was the number animals affected in each group (3 at the LD, 10 at the MD, and all 12 at the HD).
Body weights	No test article-related body weight changes were reported.
Ophthalmoscopy	No test article-related findings were reported.
ECG	Not performed
Hematology	In the six HD animals euthanized in extremis, increased neutrophil counts correlated with abscesses at the injection site at necropsy. Monocytes were increased in two of the males. Mild to moderate anemia in these animals (decreased RBC count, hemoglobin, and/or hematocrit) appeared to be regenerative (increased reticulocytes, and red cell distribution width). In the remaining four HD animals at the early termination of that group, minimally to mildly higher neutrophil counts correlated in three animals with abscessation at the renal artery/vein and/or the injection site. Near the end of treatment, increased neutrophil counts were seen at the MD correlating with injection site abscesses. Minimal to mild decreases in red blood cell parameters were seen in MD animals along with evidence of regeneration (increased MCV, RDW, and reticulocyte counts). Near the end of recovery, increased neutrophils were seen in 3 control animals and 1 MD male; of these 2 control males had pulmonary abscesses. These findings were considered to be secondary to the indwelling catheters. The absence of dose-related findings was considered to be evidence of reversibility. No test article-related changes in coagulation parameters were reported at the LD or MD.

Parameters	Major Findings
Clinical chemistry	Minimal to moderate increases in CRP in individuals at LD, MD (dose-related incidence) correlated with perivascular abscesses at the proximal and distal injection sites. Variability in TP, albumin, globulin, and A:G ratio was evident in all groups. Lower albumin in one MD female on Days 80 and 85 may have been reflective of poor body condition. Mild to moderately increased ALT in that female and another MD female did not correlate to any reported microscopic findings. By Day 113 (recovery), CRP, albumin and ALT were similar between control and treated animals, with the exception of minimally higher CRP in 2 LD females.
Urinalysis	No test article-related findings were reported during the dosing or recovery periods in LD and MD groups or in the HD group through the last urine collection on Day 22.
Gross pathology	Terminal necropsy on Day 92 (control, LD, and MD only): • Dose-related incidence of thickened proximal and distal (catheter tip) injection sites were seen in LD and MD males and MD females. The primary histologic correlate was abscess. • Abdominal abscess in 1 MD male • Increased size of iliac lymph nodes in 1 LD male • Decreased size of thymus in 1 LD and 1 MD female Recovery necropsy on Day 120 (control, LD, and MD only): • Thickened proximal and distal injection sites in 1 control and one LD female • Cyst in the liver of 1 MD male

Parameters	Major Findings
Organ weights	No test article-related findings were reported.
Histopathology Adequate battery: Yes	Terminal necropsy on Day 92 (control, LD, and MD only): Test article-related findings at the injection sites included:

Finding	Injection Site	Incidence					
		Male*			Female**		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Infiltration, Mixed Inflammatory Cells (vein, endothelium)	Proximal	0/2	2/4	3/4	1/4	1/4	3/4
	Distal	0/3	2/4	2/4	0/3	0/4	4/4
Granulation Tissue (perivascular, vein)	Proximal	0/2	2/4	3/4	0/4	1/4	3/4
	Distal	0/3	1/4	1/4	0/3	0/4	3/4
Abscess (perivascular)	Proximal	0/2	1/4	1/4	0/4	0/4	3/4
	Distal	0/3	1/4	1/4	0/3	0/4	2/4
Fibrosis (intravenous, perivascular, tunica intima)	Proximal	1/2	0/4	0/4	0/4	3/4	0/4
	Distal	1/3	1/4	1/4	0/3	0/4	0/4

Finding	Injection Site	Incidence					
		Male*			Female**		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Thrombosis (chronic, focal)	Proximal	0/2	0/4	0/4	1/4	1/4	0/4
	Distal	0/3	0/4	1/4	0/3	1/4	0/4
Brown Pigment, Intracellular	Proximal	1/2	0/4	0/4	0/4	0/4	1/4
	Distal	1/3	1/4	0/4	0/3	0/4	0/4
Hemorrhage	Proximal	0/2	0/4	2/4	0/4	0/4	0/4
	Distal	0/3	0/4	1/4	0/3	0/4	0/4
Edema (vein, perivascular)	Proximal	0/2	1/4	0/4	0/4	0/4	0/4
	Distal	0/3	0/4	0/4	1/3	0/4	2/4
Hypertrophy (endothelium)	Proximal	0/2	0/4	0/4	0/4	0/4	0/4
	Distal	0/3	0/4	0/4	1/3	0/4	0/4
Necrosis (vein)	Proximal	0/2	0/4	1/4	0/4	0/4	0/4
	Distal	0/3	0/4	0/4	0/3	0/4	0/4

*Male: proximal injection site was missing in 1 of 3 control vehicle animals
**Female: distal injection site was missing in 1 of 4 control vehicle animals

Abscesses, mixed inflammatory cell infiltrates and granulation tissue considered most likely related to the test article. Other injection site findings were considered to either represent direct effects of the test article or an exacerbation of catheter-related injection site injury.

Other findings included:

- Minimal mixed inflammatory cell infiltrates and minimal fibrosis in the renal artery/vein (distal to injection site) of one MD male, and mild fibrosis in the renal artery/vein of one LD female
- Minimal vacuolation of acinar cells in the pancreas at the LD and MD in males and in the MD in females (resolved in recovery animals)
- Minimal alveolar macrophage infiltrates in the lung in LD and MD animals and thrombosis in the lung at the LD and MD in males
- An abdominal cavity abscess in one MD male, confirmed on histology, near the injection site.
- Mixed inflammatory cell infiltrate in liver, gall bladder, kidney spleen and stomach in control and treated animals were considered to be incidental.

Parameters	Major Findings
	Recovery necropsy on Day 120 (control, LD, and MD only): Test article-related findings at the injection sites included:

Finding	Injection Site	Incidence					
		Male			Female		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Infiltration, Mixed Inflammatory Cells (vein, endothelium)	Proximal	1/2	1/2	2/2	1/2	1/2	1/2
	Distal	1/2	0/2	0/2	2/2	1/2	1/2
Granulation Tissue (perivascular, vein)	Proximal	0/2	0/2	1/2	1/2	1/2	0/2
	Distal	0/2	0/2	0/2	2/2	1/2	0/2
Fibrosis (vein/perivascular, tunica intima)	Proximal	1/2	2/2	2/2	0/2	1/2	1/2
	Distal	1/2	1/2	0/2	0/2	1/2	2/2
Thrombosis (chronic)	Proximal	0/2	0/2	0/2	1/2	1/2	0/2
	Distal	0/2	0/2	0/2	1/2	1/2	1/2
Brown Pigment, Intracellular	Proximal	0/2	0/2	1/2	0/2	1/2	0/2
	Distal	1/2	0/2	0/2	0/2	0/2	0/2
Hemorrhage	Proximal	0/2	0/2	0/2	0/2	0/2	0/2
	Distal	0/2	0/2	0/2	0/2	1/2	1/2
Hypertrophy (endothelium)	Proximal	0/2	0/2	0/2	0/2	0/2	0/2
	Distal	0/2	1/2	0/2	0/2	0/2	0/2

Mixed inflammatory cell infiltrates and granulation tissue were partially resolved, but still present.
Alveolar macrophage infiltrates were reported in 1 female in each of vehicle, LD and MD groups.

LD = low dose; MD = mid dose; HD = high dose; RBC = red blood cell; MCV = mean corpuscular volume; RDW = red cell distribution width; TP = total protein; A:G ratio = albumin to globulin ratio; CRP = c-reactive protein; ALT = alanine aminotransferase

Toxicokinetics

In treated animals, after the first IV infusion, BC-3781 exhibited biphasic disposition with a rapid initial distribution phase followed by a slower elimination phase. T_{max} for BC-3781 was at either the first (0.5 hours) or second (1 hour) time point following start of infusion, while T_{max} for the metabolite BC-8041 between 1 hour to 1.25 hours after start of infusion.

C_{max} and AUC values for BC-3781 increased with increasing dose, and were generally dose-proportional on Day 1 and more variable at later collection times. Accumulation ratios were less than 2-fold. C_{max} and AUC values for the metabolite BC-8041 were more variable, but were greater than dose-proportional, with greater accumulation observed. No significant gender differences in C_{max} or AUC were reported for either BC-3781 or BC-8041.

Half-life values over the sampling time points ranged from 3.85h to 5.59h for BC 3781. Half-life values for the metabolite tended to be longer and more variable, with half-life decreasing as doses increased.

Toxicokinetic parameters are shown in the table from the study report below:

Table 20. Study No. (b) (4).289.19: Toxicokinetic Parameters

Dose Day	Group / Dose Level (mg/kg)*	Sex	BC-3781					BC-8041				
			T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}12hr}$ (hr*ng/mL)	AUCinf (hr*ng/mL)	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}12hr}$ (hr*ng/mL)	AUC_{inf} (hr*ng/mL)	$t_{1/2}$ (hr)
1	2 / 30	M	0.7	5040	12100	13900	4.49	1.0	52.9	279	639	13.2
		F	0.8	5340	11600	13000	4.28	1.0	64.8	333	681	11.4
	3 / 60	M	0.8	9310	22400	24800	3.85	1.0	173	670	1240	8.10
		F	0.7	10000	23600	27100	4.57	1.0	338	1130	837	6.65
	4 / 60	M	0.8	10700	24200	26800	3.93	1.0	210	734	1020	6.86
		F	1.0	10400	24300	27100	3.99	1.0	212	845	1150	6.63
28	2 / 30	M	0.8	6910	19000	23900	5.59	1.0	113	597	1480	11.9
		F	0.8	6250	14800	17900	5.16	1.0	74.8	451	565	10.7
	3 / 60	M	0.9	11500	32100	37900	4.81	1.0	282	1250	2280	7.78
		F	0.7	12200	33500	39700	4.90	0.9	411	1810	1580	6.61
	4 / 100	M	0.7	19600	58200	70700	5.39	1.1	1070	4680	5650	5.45
		F	0.8	20400	53100	63200	5.05	1.1	831	3590	4490	5.28
64	4 / 100	M	0.8	19700	NC	NC	NC	1.2	1450	NC	NC	NC
61		F	0.8	22400	NC	NC	NC	1.1	950	NC	NC	NC
91	2 / 30	M	0.8	7030	16500	19000	4.66	1.0	170	655	1250	8.22
		F	0.8	5310	12900	14700	4.41	1.0	105	483	579	7.78
	3 / 60	M	0.8	12200	31800	37900	4.93	1.0	448	1660	2310	6.72
		F	0.7	13000	30800	35400	4.66	1.0	655	2130	2840	5.41

*Dose level of BC-3781.Ac per administration
M – Male; F – Female
NC – Not calculated

T_{max} = time to reach maximum concentration; C_{max} = maximum drug concentration; $AUC_{0\text{-}12hr}$ = area under the time-concentration curve from time 0 to 12 hours after drug administration; AUC_{inf} = area under the time-concentration curve from time zero to infinity; $t_{1/2}$ = half-life

By the oral route

Study no. AB16227 (Applicant reference no. 03781A-ST04-002-GxP): BC-3781.Ac: 4-week oral (gavage) toxicity study in the rat followed by a 4-week treatment-free period

- Moribundity and deaths were seen at the high -dose; clinical signs included hypersalivation, fecal changes, and distended abdomen in mid- and high -dose groups and decreased activity, piloerection, and partially closed eyes at the high dose.
- Findings in animals surviving until the end of the study included intestinal and/or cecal dilatation at all doses (partially reversible during the recovery period), degenerative changes in the stomach at the mid- and high -doses (partially reversible), and organ weight and/or histological evidence of lymphoid (all doses) and hemopoietic (high -dose) depletion that appeared to be reversible.
- The NOAEL was the mid-dose, 150 mg/kg/day BID ($AUC_{0\text{-}12h}$ ranged from 7810–13043 ng*hr/mL).

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

Table 21. Study No. AB16227: Methods

Study Method	Details
Dose and frequency of dosing	0 (vehicle), 25, 300, 600/450 mg/kg/day, divided into BID doses In the high -dose group and satellites, the dose level was decreased on Day 7 to 450 mg/kg/day due to severe clinical signs, and a drug holiday on Days 12 and 13 was taken due to marked effects.
Route of administration	Oral gavage
Formulation/vehicle	Water for injection
Species/strain	Sprague-Dawley rats (Crl:OFA (SD))
Number/sex/group	10 For the recovery period, an additional 5/sex in the control and high dose group and an additional 3/sex in the low- and mid-dose groups were included.
Age	Approximately 8 weeks
Satellite groups/unique design	3/sex in the control group and 9/sex in test article-treated groups were included for toxicokinetics.
Deviation from study protocol affecting interpretation of results	No

BID = twice a day

Table 22. Study No. AB16227: Observations and Results: Change From Control

Parameters	Major Findings
Mortality	Two males and two females at the HD were sacrificed in extremis between Days 4 and 8; clinical signs in these animals included decreased activity, abnormal feces, soft distended abdomen, abnormal breathing, piloerection, red stained fur around the muzzle and/or partially closed eyes. After dose reduction, one HD male was found dead on Day 12, and one HD female was sacrificed in extremis on Day 13; clinical signs were consistent with earlier decedents plus findings of cold to the touch, thin appearance and/or soiled urogenital region. Deaths were attributed to intestinal dilation in 2 animals, marked or severe tracheal epithelial necrosis in 2 animals (considered to be aspiration following reflux of high gastric volume, which may indicate that the dose volume was excessive), and slight or moderate ulcerative inflammation of the nonglandular stomach in 2 animals. One MD male was euthanized on Day 17 due to what initially appeared to be a gavage error (swelling of the ventral neck and thorax), and not test article-related. On necropsy, death was attributed to marked necrotic inflammation of the skin.
Clinical signs	LD: No clinical signs were noted. MD: Soft feces from the first week of treatment, soft distended abdomen from approximately Day 16 through the end of treatment, and/or hypersalivation (considered to be indicative of bad taste of the test article) from the first week of treatment through the end of the treatment period. All resolved in the recovery period.

Parameters	Major Findings
	HD: Soft/liquid/pale feces and/ or soft distended abdomen were observed from Day 3 through the end of the treatment period; all surviving animals affected by the end of the first week. Decreased activity, piloerection, and/or partly closed eyes were seen at higher incidence in males than females. Hypersalivation throughout the treatment period was considered to reflect the bad taste of the test article. No clinical signs were reported in surviving animals during the recovery period.
Body weights	LD: No test article-related effect was reported. MD: Body weights of males were not affected. In females, effects were similar to those seen in HD females HD: In males, mean body weight gain between Days 0 and 11 was lower than control by 69.8%. Gain was similar to controls thereafter (after dose reduction). Mean body weight at the end of treatment was 8.4% lower than control. During the recovery period, weight gain was variable but lower than control for the first week, but improved, resulting in similar body weight to controls at the end of the study. In females, mean body weight gain in the first week of treatment was higher than controls (+46.6%) and persisted during treatment. At the end of treatment, mean body weight was greater than control (+7%). During the recovery period, body weight loss or decreased weight gain resulted in mean body weight that was similar to control by the end of the study.
Ophthalmoscopy	No test article-related findings were reported.
Hematology	There were no changes that were considered to be toxicologically relevant.
Clinical chemistry	Total protein was decreased at all doses, reflecting lower albumin and globulin; this persisted at the end of the recovery period. Cholesterol was lower in MD and HD females (no values were reported for treated males), but was reversible. Some of these findings could indicate decreased synthesis in the liver. Urea was decreased, but was not dose-related in females, and was only statistically significant in LD and MD females; this finding was reversible. All of these mean values were reported to be within the range of historical controls. Decreased bilirubin in LD females and MD and HD males was observed at the end of treatment, but was reversible. Serum ALT was increased in MD and HD males and females and was reversible. No pathological correlates to these findings were reported.

Parameters	Major Findings
Urinalysis	In males at all doses, urine volume was decreased in a dose-related manner, correlating with higher specific gravity. Urinary pH was lower than controls at the MD and HD. At the end of the recovery period, lower urine volume persisted in MD males, and urinary pH was higher than controls at the MD and HD. The report states that all mean values were within or close to the background control range.
Gross pathology	At the end of treatment, the cecum was distended by fluid/dark material or by gas at all doses; incidence was dose-related. The duodenum was distended by fluid/dark material or by gas in 2 MD and 2 HD males. The duodenal wall was thickened in the 2 HD males. The ileum and the colon were distended by fluid/ material at the MD and HD (mostly males). Pale liver was observed in 1 HD male and 1 HD female, with no histological correlates. No treatment-related findings were reported at the recovery necropsy. The length of the cecum was greater than controls at all doses in a dose-related manner, persisting at all doses after the recovery period, but decreased in magnitude, indicating partial reversibility.
Organ weights	Mean absolute cecum weight was greater than controls at all doses, and was dose-related in magnitude, correlating with dilation on histology, and exhibiting partial reversibility after the recovery period. Mean and absolute and relative spleen weights were decreased at the MD and HD, correlating with decreased white pulp in HD females and exhibiting partial reversibility after the recovery period. Mean absolute and relative thymus weights were decreased in HD males and mean relative thymus weights were decreased in MD females. There were no histological correlates, but the finding coincided with decreased lymphoid tissue in the spleen. This finding reversed by the end of the recovery period. Mean absolute and relative adrenal weights were greater than controls in HD animals and mean absolute weight was increased in MD females. The report did not consider this finding to be treatment-related, but likely reflected a degree of stress in treated animals.
Histopathology Adequate battery: Yes At the recovery necropsy, only the mandibular and mesenteric lymph nodes, spleen, sternal bone marrow, stomach, duodenum, jejunum, ileum, cecum, colon, and gross lesions were examined in the LD and MD groups.	In the gastrointestinal tract: Stomach/duodenum: • Minimal, focal or multifocal, glandular degeneration in the stomach in 5 HD animals and 1 MD female was reported. • Slight erosion in the stomach of 1 HD animal, and focal glandular atrophy in stomach with a slight duodenal erosion in a second HD animal were reported. One MD male had minimal erosion in the stomach.

Parameters	Major Findings
	• Findings were partially reversible during the recovery period. Ileum/jejunum: • Minimal dilatation in the jejunum was reported in 3 of 7 HD animals and 2 of 9 MD animals, and was considered to be reversible in the recovery period. • Minimal to slight dilatation of the ileum was reported in MD and HD males, and minimal dilatation of the ileum was reported in females at all doses (including one control, but incidence was higher in treated females). There did not appear to be any treatment-related dilatation in ileum at the end of recovery. Cecum/colon: • Minimal to moderate dilatation in the cecum was reported at all doses, was dose-related in severity, and was reversible in the recovery period. • Minimal to moderate dilatation was present in the colon at all doses, but less frequently than in the cecum, and was also reversible. In lymphoid tissue: • Decreased lymphoid follicle development (minimal to marked) was reported at all doses in the mandibular and mesenteric lymph nodes at all doses, and was dose-related in incidence and severity. Minimal decreased paracortex accompanied this finding at all doses in the mesenteric lymph node. Minimal or slight congestion, hemorrhage, erythrophagocytosis and/or increased incidence of macrophages were noted in the mesenteric node at the MD and HD. These findings appeared to be reversible. • In the spleen, minimal decreased white pulp development was reported in 3 of 8 HD females. After the recovery period, this finding was reported in 2 LD and 2 HD recovery animals; it is unclear whether or not the recovery finding was related to treatment. • In sternal bone marrow, minimal to moderate decreased cellularity was noted in 7 of 15 HD animals, but was reversible in the recovery period.

LD = low dose; MD = mid dose; HD = high dose; ALT = alanine aminotransferase

Toxicokinetics

Variability in plasma concentration between animals was described as “very high.” Toxicokinetic parameters for test article and metabolite were not calculated at 12.5 mg/kg BID, due to insufficient quantifiable concentrations (except for test article BC-3781 on Day 0 for females). No clear sex-related differences were noted. No accumulation of the test item or metabolite was observed after 4 weeks of treatment.

Increases in systemic exposure and C_{max} were generally dose-proportional between the MD and HD for the test article in males and females and for the metabolite in males. In females, the increase in systemic exposure of the metabolite was generally less than dose-proportional between the MD and HD. The systemic exposure to the test item BC-3781 was markedly higher than that to the metabolite BC-8041.

Pharmacokinetic parameters for the test article and metabolite are shown in the following two tables from the study report:

Table 23. Study No. AB16227: Test Article Toxicokinetic Parameters

Test item (BC-3781) mean toxicokinetic parameters:

Occasion	Dose (mg/kg/adm)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12h} (ng.h/mL)
day 0	12.5	Male	NA	NA	NA
		Female	94.3	6	457 [a]
	150	Male	1077	3	7810
		Female	2110	3	13043
	300	Male	1775	3	12607
		Female	2469	3	16814
day 14	150	Male	1203	0.25	8890
		Female	1701	0.25	9992
	225	Male [b]	2833	0	9766
		Female	1868	3	11424
day 27	150	Male	1380	0.25	9707
		Female	1588	1	10971
	225	Male	1669	3	12671
		Female	1687	0.5	13627

a: AUC_{0-6h} instead of AUC_{0-12h}, b: atypical profile since the C_{max} was noted before dosing.
The value was confirmed by reanalysis of the same aliquot of plasma samples.

C_{max} = maximum drug concentration; T_{max} = time to reach maximum concentration after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration

Table 24. Study No. AB16227: Metabolite Toxicokinetic Parameters

Metabolite (BC-8041) mean toxicokinetic parameters:

Occasion	Dose (mg/kg/adm)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12h} (ng.h/mL)
day 0	150	Male	50.7	0.5	239
		Female	77.0	0.25	211
	300	Male	69.2	0.25	375
		Female	55.9	0.25	234
day 14	150	Male	33.8	0.25	184
		Female	48.5	0.25	187
	225	Male [a]	669	0	269
		Female	40.3	0.5	183
day 27	150	Male	39.8	0.25	200
		Female	54.8	1	259
	225	Male	74.1	0.25	291
		Female	38.8	0.5	223

a: atypical profile since the C_{max} was noted before dosing. The value was confirmed by reanalysis of the same aliquot of plasma samples.

C_{max} = maximum plasma concentration of drug; T_{max} = time to reach maximum plasma concentration after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration

Study no. 8275686 (Applicant reference no. 03781A-ST08-002-GxP): BC-3781.Ac 4-week oral (gavage) administration toxicity study in the cynomolgus monkey with a 4-week recovery phase

- Clinical signs at all doses included diarrhea and emesis, with salivation also seen in high dose animals. Severe clinical signs in high dose animals (dosed at 100 mg/kg BID), in addition to diarrhea and emesis, included hypoactivity, movement abnormalities, and/or poor physical condition, recumbency, and severe body weight losses in animals. Three of these animals underwent a dosing holiday, and the dose was reduced for the group to 70 mg/kg BID on Day 9, after which the condition of high dose animals improved.
- In high dose males, QT/QTc prolongation was statistically significant but reversible.
- Increased myocardial vacuolation with fibrosis was observed in two high-dose animals and one low-dose female at the end of treatment. At the end of the recovery period, one mid-dose male had similar findings with greater severity and increased heart weight.
- The mid-dose (35 mg/kg BID, or 70 mg/kg/day) was considered to be the NOAEL. At that dose, AUC_{0-inf} on Day 1 was 2230 ng*hr/mL in males (n=1) and 1120 ng*hr/mL in females (n=2). On Day 28, AUC_{0-inf} was 8090 ng*hr/mL in mid-dose males (n=1) and 4660 ng*hr/mL in mid-dose females (n=4).

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

Table 25. Study No. 8275686: Methods

Study Method	Details
Dose and frequency of dosing:	0 (vehicle), 12.5, 35, or 100/70 mg/kg BID, for daily doses of 0, 25, 70, or 200/140 mg/kg/day
Route of administration:	Oral (gavage) to nonfasted animals
Formulation/vehicle:	Water
Species/strain:	Cynomolgus monkeys (*Macaca fascicularis*), Mauritian (purpose-bred)
Number/sex/group:	5 (3/sex/group for main study and 2/sex/group for recovery)
Age:	5–6 years
Satellite groups/unique design:	Severe clinical signs and poor condition in HD animals led to dosing holidays in 2 males and 1 female. The HD dose level was reduced from 100 mg/kg BID to 70 mg/kg BID after 8 days.
Deviation from study protocol affecting interpretation of results:	No

BID = twice a day; HD = high dose

Table 26. Study No. 8275686: Observations and Results: Changes From Control

Parameters	Major Findings
Mortality	None
Clinical signs	HD: Diarrhea, emesis, recumbency, hypoactivity, movement abnormalities/ uncoordinated movement, and poor physical condition were observed from Study Days 1 to 8. After dose reduction on Study Day 9, emesis, salivation, and diarrhea were noted at decreased incidence, and diarrhea resolved by Study Day 17. LD and MD: Emesis and diarrhea were reported during the first half of the treatment period.
Body weights	HD: During Study Days 1 to 8, all males lost body weight (200 g –600 g), and 4/5 females lost 100 g to 300 g body weight. After dose reduction, 2 animals continued to lose weight for another week, while the rest stabilized or gained weight. Marked body weight increase was noted during recovery. LD and MD: No effect of treatment was reported.
Ophthalmoscopy	No treatment-related findings were reported.
ECG	Dose-related QT/QTc interval prolongation was noted in males at all doses, but was >15% to 20% and statistically significant only at the high dose. This finding was no longer evident at the end of the recovery period. Transient decreases in systolic blood pressure were reported in HD males on Study Days 1 and 24 at 2 hours postdose.

Parameters	Major Findings
Hematology	No treatment-related findings were reported.
Clinical chemistry	No treatment-related findings were reported.
Urinalysis	No treatment-related findings were reported.
Gross pathology	No treatment-related findings were reported.
Organ weights	No treatment-related findings were reported for the end of treatment necropsy. At the end of recovery, 1 (of 2) MD male (no. 27179) had increased heart weight that was approximately twice that of the highest value recorded in the concurrent control group or in the historical control range.
Histopathology Adequate battery: Yes	At the end of treatment, vacuolation in the myocardium of the left ventricle and/or septum exceeded background severity in 2 HD animals and one LD female, accompanied by minimal fibrosis, karyomegaly, and/or interstitial cell hyperplasia. At the end of recovery, 1 (of 2) MD male (no. 27179) had moderate myocardial vacuolation associated with moderate fibrosis, moderate karyomegaly, slight interstitial cell hyperplasia, and minimal inflammatory cell foci. These findings correlated with increased heart weight in this animal.

LD = low dose; MD = mid dose; HD = high dose.

Toxicokinetics

Following a single administration of BC-3781.Ac, all treated animals were exposed to both BC-3781 and its metabolite, BC-8041, within 0.75 hours of dose administration, indicating rapid drug absorption and rapid biotransformation at all dose levels. The mean C_{max} for BC-3781 was 4.4 hours after dose administration, and the mean C_{max} for the metabolite, BC-8041, was 3.4 hours after dose administration. Mean maximum plasma concentrations and exposure for the parent drug increased in an approximately dose-proportional manner, while mean maximum concentrations and exposure for the metabolite increased in a dose-proportional manner between the low and mid doses. The changes for BC-8041 at the high dose were less than dose-proportional, possibly indicating that the biotransformation pathways were becoming saturated.

Half-lives of both BC-3781 and BC-8041 ranged from 3.6 hours to 7.2 hours with no notable trend relating to dose level, sex or analyte. There was evidence of accumulation of both BC-3781 and BC-8041 following repeated administration indicating saturation of routes of elimination and/or biotransformation. The metabolite to parent ratios decreased with increasing dose level, again indicating saturation metabolism.

Toxicokinetic parameters are summarized for BC-3781 and BC-8041 in the following tables from the study report:

Table 27. Study No. 8275686: Mean Toxicokinetic Parameters of BC-3781 on Day 1

Mean toxicokinetic parameters of BC-3781 on Day 1

	12.5 mg/kg BC-3781.Ac		35 mg/kg BC-3781.Ac		100 mg/kg BC-3781.Ac	
	Males (n=5)[1]	Females (n=5)[1]	Males (n=5)[1]	Females (n=5)[1]	Males (n=5)[1]	Females (n=5)[1]
Cmax (ng/mL)	57.9 ± 21.1	116 ± 40.2	263 ± 78.2	360 ± 132	445 ± 190	695 ± 371
tmax (h)	4.2 ± 2.6	2.1 ± 1.2	3.5 ± 1.1	2.7 ± 1.8	4.4 ± 2.2	2.3 ± 1.6
t½ (h)	5.22 (n=1)	3.57 ± 0.508 (n=4)	4.55 (n=1)	4.24 (n=2)	4.54 (n=1)	4.66 ± 0.566 (n=3)
AUC(0-t) (ng.h/mL)	402 ± 152	465 ± 157	2090 ± 717	2080 ± 1160	2800 ± 1180	3060 ± 1020
AUC(0-12) (ng.h/mL)	402 ± 152	465 ± 157	2090 ± 717	2080 ± 1160	2800 ± 1180	3060 ± 1020
AUC(0-∞) (ng.h/mL)	575 (n=1)	485 ± 190 (n=4)	2230 (n=1)	1120 (n=2)	2680 (n=1)	3490 ± 1570 (n=3)
Cmax/D	4.63 ± 1.69	9.29 ± 3.21	7.52 ± 2.23	10.3 ± 3.76	4.45 ± 1.90	6.95 ± 3.71
AUC(0-t)/D	32.2 ± 12.2	37.2 ± 12.6	59.7 ± 20.5	59.4 ± 33.2	28.0 ± 11.8	30.6 ± 10.2

[1] Unless otherwise stated

C_{max} = maximum plasma concentration of drug; t_{max} = time to reach maximum plasma concentration after administration; $t_{1/2}$ = half-life; AUC_{0-t} = area under the concentration-time curve from time 0 to time t after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity

Table 28. Study No. 8275686: Mean Toxicokinetic Parameters of BC-3781 on Day 28

Mean toxicokinetic parameters of BC-3781 on Day 28

	12.5 mg/kg BC-3781.Ac		35 mg/kg BC-3781.Ac		100/70 mg/kg BC-3781.Ac	
	Males (n=5)	Females (n=5)	Males (n=5)1	Females (n=5) 1	Males (n=5) 1	Females (n=5) 1
C_{max} (ng/mL)	211 ± 78.6	206 ± 80.5	523 ± 206	757 ± 179	991 ± 222	1250 ± 254
t_{max} (h)	1.8 ± 0.3	1.7 ± 0.3	3.6 ± 0.9	2.1 ± 1.1	2.1 ± 1.2	3.1 ± 1.2
$t_{½}$ (h)	5.42 ± 0.818	5.31 ± 0.807	5.27 (n=1)	4.20 ± 0.351 (n=4)	6.53 ± 1.79 (n=4)	5.33 (n=2)
$AUC_{(0-t)}$ (ng.h/mL)	1160 ± 312	816 ± 294	4070 ± 1250	4180 ± 1160	7610 ± 1590	9130 ± 2180
$AUC_{(0-12)}$ (ng.h/mL)	1160 ± 312	816 ± 294	4070 ± 1250	4180 ± 1160	7610 ± 1590	9130 ± 2180
$AUC_{(0-\infty)}$ (ng.h/mL)	1520 ± 474	1010 ± 355	8090 (n=1)	4660 ± 1440 (n=4)	11400 ± 3390 (n=4)	9930 (n=2)
C_{max}/D	16.9 ± 6.29	16.5 ± 6.44	15.0 ± 5.89	21.6 ± 5.11	14.2 ± 3.18	17.9 ± 3.62
$AUC_{(0-t)}/D$	93.0 ± 25.0	65.3 ± 23.5	116 ± 35.6	119 ± 33.1	109 ± 22.7	130 ± 31.1
RAC_{max}	4.18 ±2.57	1.80 ± 0.589	2.02 ± 0.534	2.21 ± 0.523	2.83 ± 2.17	2.18 ± 1.00
RA_{AUC}	3.15 ± 1.17	1.83 ± 0.654	2.03 ± 0.533	3.11 ± 3.26	3.24 ± 1.94	3.49 ± 2.17

[1] Unless otherwise stated

C_{max} = maximum plasma concentration of drug; t_{max} = time to reach maximum plasma concentration after administration; $t_{1/2}$ = half-life; AUC_{0-t} = area under the concentration-time curve from time 0 to time t after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity; RAC_{max} = accumulation ratio based on C_{max}; RA_{AUC} = accumulation ratio based on AUC

Table 29. Study No. 8275686: Mean Toxicokinetic Parameters of BC-8041 on Day 1

Mean toxicokinetic parameters of BC-8041 on Day 1

	12.5 mg/kg BC-3781.Ac		35 mg/kg BC-3781.Ac		100 mg/kg BC-3781.Ac	
	Males (n=5) 1	Females (n=5)1	Males (n=5) 1	Females (n=5)1	Males (n=5) 1	Females (n=5)
C_{max} (ng/mL)	22.6 ± 14.9	35.3 ± 8.78	70.0 ± 28.8	81.7 ± 32.7	58.0 ± 17.1	92.5 ± 49.0
t_{max} (h)	1.1 ± 0.4	2.1 ± 1.2	1.6 ± 1.4	3.4 ± 1.5	2.7 ± 1.2	1.5 ± 0.7
$t_{½}$ (h)	5.78 ± 0.692 (n=4)	3.63 ± 0.528 (n=4)	5.30 ± 0.363 (n=4)	5.23 (n=1)	5.67 (n=2)	5.07 ± 1.27
$AUC_{(0-t)}$ (ng.h/mL)	111 ± 72.4	146 ± 33.8	487 ± 248	455 ± 234	361 ± 108	369 ± 207
$AUC_{(0-12)}$ (ng.h/mL)	111 ± 72.4	146 ± 33.8	487 ± 248	455 ± 234	361 ± 108	369 ± 207
$AUC_{(0-\infty)}$ (ng.h/mL)	105 ± 42.4 (n=4)	157 ± 42.4 (n=4)	584 ± 360 (n=4)	217 (n=1)	400 (n=2)	449 ± 246
C_{max}/D	1.80 ± 1.19	2.83 ± 0.703	2.00 ± 0.824	2.33 ± 0.936	0.580 ± 0.171	0.925 ± 0.490
$AUC_{(0-t)}/D$	8.85 ± 5.79	11.7 ± 2.70	13.9 ± 7.08	13.0 ± 6.68	3.61 ± 1.08	3.69 ± 2.07

[1] Unless otherwise stated

C_{max} = maximum plasma concentration of drug; t_{max} = time to reach maximum plasma concentration after administration; $t_{1/2}$ = half-life; AUC_{0-t} = area under the concentration-time curve from time 0 to time t after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity

Table 30. Study No. 8275686: Mean Toxicokinetic Parameters of BC-8041 on Day 28

Mean toxicokinetic parameters of BC-8041 on Day 28

	12.5 mg/kg BC-3781.Ac		35 mg/kg BC-3781.Ac		100/70 mg/kg BC-3781.Ac	
	Males (n=5)1	Females (n=5)	Males (n=5)1	Females (n=5)1	Males (n=5)1	Females (n=5) 1
C_{max} (ng/mL)	72.7 ± 42.7	56.2 ± 16.3	110 ± 47.5	169 ± 44.3	216 ± 60.1	343 ± 113
t_{max} (h)	2.2 ± 1.0	1.5 ± 0.4	2.8 ± 1.1	2.2 ± 1.0	3.1 ± 1.2	3.5 ± 1.1
$t_{½}$ (h)	7.01 ± 1.43 (n=4)	5.06 ± 1.04	5.09 ± 0.0839 (n=3)	3.97 ± 0.542 (n=4)	6.84 (n=2)	7.19 (n=1)
$AUC_{(0-t)}$ (ng.h/mL)	428 ± 272	237 ± 74.6	827 ± 326	1020 ± 323	1570 ± 380	2290 ± 759
$AUC_{(0-12)}$ (ng.h/mL)	428 ± 272	237 ± 74.6	827 ± 326	1020 ± 323	1570 ± 380	2290 ± 759
$AUC_{(0-\infty)}$ (ng.h/mL)	648 ± 437 (n=4)	289 ± 103	1270 ± 434 (n=3)	1140 ± 405 (n=4)	2260 (n=2)	2830 (n=1)
C_{max}/D	5.82 ± 3.41	4.49 ± 1.31	3.14 ± 1.36	4.83 ± 1.27	3.09 ± 0.858	4.91 ± 1.61
$AUC_{(0-t)}/D$	34.3 ± 21.7	18.9 ± 5.97	23.6 ± 9.33	29.2 ± 9.23	22.5 ± 5.42	32.7 ± 10.8
RAC_{max}	4.60 ± 3.40	1.60 ± 0.297	1.69 ± 0.598	2.20 ± 0.576	3.90 ± 1.49	5.20 ± 3.96
RA_{AUC}	4.51 ± 2.76	1.63 ± 0.423	1.96 ± 0.864	2.88 ± 2.24	4.52 ± 1.29	8.13 ± 6.16

[1] Unless otherwise stated

C_{max} = maximum plasma concentration of drug; t_{max} = time to reach maximum plasma concentration after administration; $t_{1/2}$ = half-life; AUC_{0-t} = area under the concentration-time curve from time 0 to time t after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity; RAC_{max} = accumulation ratio based on C_{max}; RA_{AUC} = accumulation ratio based on AUC

General Toxicology; Additional Studies

From Dr. Maria Rivera's review of the original submission of IND 106594:

Repeated-dose toxicity studies of up to 14 days duration were conducted in monkeys and rats by both the oral and IV routes of administration. BC-3781.Ac was better tolerated in monkeys.

In monkeys, a slight but reversible decrease in RBC parameters was the only finding when BC-3781 was given as total daily doses up to 80 mg/kg/day, administered as two 40 mg/kg/day 30-min IV infusion 8 hrs apart. After oral administration of 25 or 50 mg/kg, findings were limited to soft feces and emesis. The plasma exposure at 80 mg/kg/day (IV) and 50 mg/kg/day (PO) were ~30 mcg*hr/mL ($AUC_{0-24hrs}$) and 5 mcg*hr/mL (AUC_{0-inf}), respectively.

On the other hand, after IV administration to rats at total daily doses up to 100/75 (males) and 75 mg/kg/day (females), also as a 30-min IV infusion 8-hrs apart, mortalities were observed at greater than or equal to 50 mg/kg/day. The animals receiving ≥50 mg/kg/day that died (unscheduled) presented with signs of right foreleg drawn up, local swelling in the neck or thorax region, and hunched posture. These mortalities were associated with injection site reactions (phlebitis/periphlebitis, thrombosis, peripheral inflammation, necrosis, and edema) and inflammation of surrounding tissues (trachea, thymus, mandibular glands, sublingual glands, and thyroid gland). Neither a NOEL nor a NOAEL could be established due to the findings observed microscopically at the injection site and surrounding tissues at all dose levels. After oral (gavage) administration to rats at doses up to 150 mg/kg/day, mortalities and intestinal bloating (meteorism) were noted at the high dose. Other findings included thymic atrophy and splenic lymphoid depletion at the high dose. The NOEL was 50 mg/kg/day. The plasma exposures were ~11 mcg*hr/mL in males and ~5 mcg*hr/mL in females (AUC_{0-8hrs}) at 50 mg/kg/day (IV) and ~7 mcg*hr/mL (AUC_{0-inf}) at 150 mg/kg/day (PO).

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study no. AA72083: BC-3781.Ac – Bacterial reverse mutation test (plate incorporation and preincubation methods

Key Study Findings:

- The study was uninterpretable due to the high degree of toxicity to the test bacteria.

GLP compliance: Yes

Test system: *Salmonella typhimurium* strains TA98, TA100, TA 1535, TA 1537, and TA 102

Study is valid: No, the test article was toxic to the bacterial strains, allowing assessment only at very low doses (0.5 mcg/plate to 16 mcg/plate). No analysis of dosing solutions was performed.

In Vitro Assays in Mammalian Cells

Study no. AA70859: BC-3781.Ac – In vitro *mammalian cell gene mutation test on L5178Y mouse lymphoma cells $TK^{+/-}$ (microwell method)*

Key Study Findings:

- BC-3781.Ac did not increase the mutant frequency under the conditions of the study. However, the study was not valid, based on established guidance for the conduct and interpretation of the mouse lymphoma assay.

GLP compliance: Yes

Test system: L5178Y $TK^{+/-}$ mouse lymphoma cells

Study is valid: No. No analysis of dosing solutions was performed. High cytotoxicity only allowed evaluation of the lowest doses. The RTG (relative total growth) at the highest evaluated dose should be between 10% to 20%; in this study, it was 22% for the 4-hour incubation in the absence of S9, 34% for the 4-hour incubation in the presence of S9, and 46% for the 24-hour incubation in the absence of S9.

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study no. 35972 MAR (Applicant Project no. 03781A-SG07-001-GxP): Bone marrow micronucleus test by intraperitoneal route in rats

Key Study Findings:

- Under the conditions of the study, BC-3781.Ac was not genotoxic.

GLP compliance: Yes (OECD) except for dose solution analysis

Test system: Sprague-Dawley rats

Study is valid: Yes. Positive (cyclophosphamide) and negative (vehicle, aqueous 0.9% NaCl solution) controls yielded expected results. In test article-treated animals, the ratio of polychromatic erythrocytes to normochromatic erythrocytes was decreased relative to controls; this was considered to be evidence that bone marrow cells were exposed to the test article.

Other Genetic Toxicity Studies

See "Other Toxicology Studies" for genetic toxicology testing of metabolites and impurities.

5.5.3. Carcinogenicity

Not performed.

5.5.4. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study no. AA97303 (Applicant reference no. 03781A-SR01-001GxP): BC-3781 – Fertility toxicity study by intravenous injection (bolus) in surgically implanted Sprague-Dawley male rats (Segment I)

Key Study Findings:

- No adverse effects on male fertility were seen.
- The NOAEL for male fertility was the high dose, 75 mg/kg/day IV (free base), divided into 2 doses given 12 hours apart (HED =12.5 mg/kg/day, or 750 mg/day for a 60 kg human).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (OECD)

Table 31. Study No. AA97303: Methods

Study Method	Details
Dose and frequency of dosing	0, 12.5, 25, and 37.5 mg/kg BID, for total daily doses of 0, 25, 50, and 75 mg/kg/day (in terms of free base)
Route of administration	IV bolus
Formulation/vehicle	Sterile physiological saline (0.9% NaCl)
Species/strain	Sprague-Dawley rats, Crl:OFA(SD)
Number/sex/group	20 males/dose group
Satellite groups	None
Study design	Each animal was surgically implanted with a catheter into the caudal vena cava for test article administration. Continuous saline infusion at 0.4 mL/hour/animal maintained patency. Males were treated during a 2-week premating period, an up-to-2-week mating period and through the day before necropsy (following caesarean section of females at gestation day 13; at least 5 weeks of treatment). Doses were selected based on previous 2- and 4-week studies in rats (b) (4) study no. C06271 and (b) (4) study no. AA97305). Males were mated to untreated females. Those females were Caesarean-sectioned on Day 13 for evaluation of the reproductive tract and conceptuses.
Deviation from study protocol affecting interpretation of results	No

IV = intravenous; BID = twice a day

Table 32. Study No. AA97303: Observations and Results

Parameters	Major Findings
Mortality	No treatment-related deaths were reported.
Clinical signs	Transient hypersalivation was noted immediately after injection for most males at the high-dose and a few at the mid-dose sporadically on Days 3 to 37. Soft or bright feces were noted for 7 mid-dose and 8 high dose males during the premating period.
Body weights	A decrease in mean body weight gain was noted in all treated male groups in a dose-related manner that was statistically significant at the mid- and high-doses between Days 3 and 7 only. Terminal body weights were comparable among treated groups, but treated groups were still lower than controls throughout the study.
Necropsy findings *[Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc]*	Sperm analysis revealed no differences from control in mean sperm count, mean percentage of motile sperm, or motility parameters in any group. Precoital interval was less than 4 days in all groups and was considered to be normal. No adverse effect on fertility was reported. One mated female did not become pregnant in each of the low- and high-dose groups, but this was considered to be incidental. Another low-dose male failed to mate. Copulation and fertility indices ranged from 95% to 100%.

Parameters	Major Findings
	Pre-implantation data (number of corpora lutea, number of implantations, and % preimplantation loss) were reported to be comparable in all groups with historical controls. However, the low- dose group had statistically lower total implantations and statistically lower preimplantation loss, presumably due to the lower number of pregnant females. Post implantation data indicated no influence of male treatment on embryo survival in any group. Mean live litter size was comparable between groups.

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

Study no. AA97304 (Applicant reference no. 03781A-SR01-002-GxP: BC-3781 – Fertility toxicity study by intravenous injection (bolus) in surgically implanted Sprague-Dawley female rats (Segment I)

Key Study Findings:

- Eight (of 20) females in the high-dose group had abnormal estrous cycling, and two high-dose females had a large percent postimplantation loss. However, group mean values for reproductive indices, estrous cycles, microscopic examination, and reproductive organ weights did not provide any evidence of adverse effects on female gonadal function, mating behavior, or fertility.
- No effect of treatment on embryo survival was reported. Mean live litter size was comparable in all groups.
- The NOAEL for female fertility was determined to be the highest dose tested, 75 mg/kg/day IV (divided BID), however, based on potential effects on estrous cycling and the higher incidence of resorptions in that group, the mid-dose may be a better estimate of the NOAEL, 50 mg/kg/day IV divided into 2 doses given 12 hours apart (HED =8.3 mg/kg/day, or 500 mg/day for a 60 kg human).

Conducting laboratory and location [redacted] (b) (4)

GLP compliance: Yes (OECD)

Table 33. Study No. AA97304: Methods

Study Method	Details
Dose and frequency of dosing	0, 12.5, 25, and 37.5 mg/kg BID, for total daily doses of 0, 25, 50, and 75 mg/kg/day (in terms of free base
Route of administration	IV bolus
Formulation/vehicle	Sterile physiological saline (0.9% NaCl)
Species/strain	Sprague-Dawley rats, Crl:OFA(SD)
Number/sex/group	20 females per dose group
Satellite groups	None
Study design	Each animal was surgically implanted with a catheter into the posterior vena cava via the femoral vein for test article administration. Continuous saline infusion at 0.4 mL/hour/animal-maintained patency. Females were treated for a 2-week premating period, during mating (up to 2 weeks), and through the seventh day of gestation. Doses were selected based on a previous 4-week study in rats (b) (4) study no. AA97305). Untreated males were mated to treated females (paired 1:1). Those females were Caesarean-sectioned on Day 13 for evaluation of the reproductive tract and conceptuses.
Deviation from study protocol affecting interpretation of results	No

BID = twice a day; IV = intravenous

Table 34. Study No. AA97304: Observations and Results

Parameters	Major Findings
Mortality	No treatment-related deaths were reported. One low-dose female was found dead during the mating period, and was not pregnant; that death was considered to be incidental.
Clinical signs	Transient hypersalivation was seen immediately after injection for 7 high-dose and 2 mid-dose females on Study Days 7 and 14; the severity was stated to be minimal.
Body weights	There were fluctuations in mean body weight and weight gain. It is unclear whether or not the differences were treatment-related. Overall, there did not appear to be adverse effects on body weight change.
Necropsy findings *[Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc]*	Eight of the 20 high-dose females were acyclic for all or part of the treatment period, while only one of the control females was acyclic. All but one of these animals had positive evidence of mating. Of the animals that cycled normally, mean cycle length and % days in estrus were comparable to controls. All females mated with the exception of one in each of the mid- and high-dose groups; these were thought to be

Parameters	Major Findings
	incidental due to pseudopregnancy induced by vaginal smearing. Most females showed evidence of insemination within the first 4 days of pairing. Mean precoital interval for treated groups was comparable to or shorter than control. The fertility index was comparable between groups. There were 2, 3, 1, and 3 mated females that did not become pregnant in the control, low, mid-, and high-dose groups, respectively. There were 18, 16, 18, and 16 pregnant females at terminal C-section. All had viable embryos except for one high dose and one control dam. There was no effect of treatment on the mean numbers of corpora lutea, implantations or % preimplantation loss. Total postimplantation loss was 24 in the high-dose group, compared to 13 in the control group, and was 10.2% of implantations, compared to 5.4% in the control group. The difference was attributed to one female that had 10 resorptions from 21 implantation sites and a second high-dose female with 4 resorptions and no viable embryos that affected the group mean. One control animal had a single resorption and no viable embryos. These findings were considered to be sporadic and incidental by the Applicant, but relationship to treatment cannot be ruled out. Mean live litter size was unaffected; it was comparable to or slightly greater than control in all treated groups.

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

Embryo-Fetal Development

Study no. AA97308: BC-3781 – Embryo-fetal development toxicity study in the pregnant Sprague-Dawley rat by intravenous injection (bolus) in surgically implanted animals

Key Study Findings:

- There were four late resorptions in the high-dose group, compared to one each in the control and mid-dose groups. Malformations at the mid-dose included one fetus that had a cleft palate and short lower jaw, along with gross disruption of the vertebral column (scoliosis). At the high-dose, one fetus had a similar spectrum of defects: cleft palate, short lower jaw, malformed ribs (oriented cranially), and malformed thoracic vertebrae. A second fetus in another high-dose litter had an enlarged ventricular heart chamber with a thin ventricular wall. These findings were rare or nonexistent in the historical database and concurrent controls.

- Decreased or no ossification in a number of skeletal elements in all treatment groups were increased in incidence relative to controls in a dose-related manner and may indicate treatment-related developmental delay at all doses.
- A maternally toxic dose was not reached, increasing the level of concern of the findings observed in this study.
- Assuming that the delays in skeletal ossification at the lowest dose would not be adverse, the fetal NOAEL in this study would be the low dose, 50 mg/kg/day, divided BID (mean C_{max} =5612–7058 ng/mL, mean $AUC_{0\text{-}12h}$ =5378–8056 ng*h/mL).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (OECD)

Table 35. Study No. AA97308: Methods

Study Method	Details
Dose and frequency of dosing	0 (vehicle), 25, 37.5, and 50 mg/kg BID (0, 50, 75, and 100 mg/kg/day) in terms of the free base
Route of administration	IV via implanted catheter into the vena cava
Formulation/vehicle	Sterile physiological saline (0.9% NaCl), USP
Species/strain	Sprague-Dawley rats (Crl:OFA (SD))
Number/sex/group	25 mated females per group
Satellite groups	An additional 6 mated female rats per group were sampled for toxicokinetics on GD 6 and 17.
Study design	Prior to study initiation, all animals were implanted with a polyurethane catheter into the posterior vena cava via the left femoral vein. Animals were maintained on continuous infusion with physiological saline (0.4 mL/hour/animal). Animals were treated from gestation days (GD) 6–17. Caesarean section and sacrifice were on GD 20. After gross examinations, half of the fetuses were processed for skeletal examination. The remaining fetuses were preserved for fixed visceral examination.
Deviation from study protocol affecting interpretation of results	No

BID = twice a day; IV = intravenous; USP = U.S. Pharmacopeia

Table 36. Study No. AA97308: Observations and Results

Parameters	Major Findings
Mortality	No treatment-related deaths were reported.
Clinical signs	Transient hypersalivation immediately after dose injection was noted for 16 high-dose females and six mid-dose females. Soft and/or clear feces were noted on a few occasions for 11 mid-dose females and 10 high dose females.
Body weights	No effect on mean body weight gain was reported.
Necropsy findings	• There were 25/25, 24/24, 25/25, and 24/25 pregnant females in Groups 1

Parameters	Major Findings
Cesarean section data	through 4, respectively, at termination. All pregnant animals at termination had viable fetuses and no dead fetuses. • The report states that preimplantation data were comparable between treated groups and controls. • Mean live litter size was comparable to control, and the report states that there were no obvious treatment-related effects on postimplantation survival, although there were four late resorptions in the high-dose group, compared to one each in the control and mid-dose groups. • Mean fetal weights in treated groups were slightly lower than controls, but without statistical significance. No effect on fetal sex ratio was reported.
Necropsy findings Offspring	<u>Malformations</u> • Control: 1 fetus in 1litter had anal atresia, acaudia, and gross disruption of the vertebral column (short trunk) • LD: None reported • MD: 2 fetuses in 2 litters had malformations: 1) one fetus had a cleft palate and short lower jaw, along with gross disruption of the vertebral column (scoliosis), and 2) one fetus in a second litter had a cyst in the neck region with a compressed thyroid. • HD: 2 fetuses in 2 litters had malformations: 1) one fetus had cleft palate, short lower jaw, malformed ribs (oriented cranially), and malformed thoracic vertebrae (*Reviewer's comment: These seem to represent an increased severity of the malformations seen at the mid-dose.*), and 2) one fetus in another litter had an enlarged ventricular heart chamber with a thin ventricular wall. • The malformations in the mid- and high-dose fetuses seem to be a cluster of skeletal findings that increased in severity with dose. The cardiac malformation at the high dose may also be of concern. Historical data indicate that between 2005 to 2007, cleft palate and dilated heart ventricle were each observed in one fetus out of 2012 fetuses in 15 studies, and neither were observed in any fetuses between 2008 to 2010 (out of 975 fetuses in 8 studies). Those malformations would seem to be rare enough in the historical databases to assume that these two observations may be treatment-related. • Soft tissue variations included renal pelvis dilation in one low-dose fetus, convoluted ureters or dilated ureters in all groups, with highest litter incidence in the control group. The report states that these "did not suggest any influence of treatment," and comparison with the historical database confirms this. • Additional findings in all treated groups included reduced skeletal ossification (consistent with findings in the rabbit EFD study below). While the incidence in some parts of the skeleton was not vastly different from historical or concurrent controls, incidences in the cranium and facial bones were more than twice that of controls and often showed a dose-response relationship. Unossified sternebrae and vertebrae were also more than twice that of controls in some treated groups. These may represent a treatment-

Parameters	Major Findings
	related delay in skeletal development.

LD = low dose; MD = mid dose; HD = high dose; EFD = embryo-fetal development

Toxicokinetics:

No quantifiable test article was detected in plasma from control animals. Systemic exposure was demonstrated in all treated satellite animals. Toxicokinetic parameters are shown in the Applicant's table below:

Table 37. Study No. AA97308: Toxicokinetic Parameters

Gestational Day	Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}12h}$ (ng.h/mL)
GD 6	50	7058	0.05	8056
	75	9446	0.05	13042
	100	13351	0.05	19351
GD 17	50	5612	0.05	5378
	75	7687	0.05	8592
	100	10556	0.05	12178

C_{max} = maximum plasma concentration of drug; T_{max} = time to reach maximum plasma concentration after administration; $AUC_{0\text{-}12h}$ = area under the concentration-time curve from time 0 to 12 hours after drug administration

Following IV bolus administration, half-life values ranged from 2.83 hours to 3.27 hours, indicating rapid elimination. Clearance ranged from 2.47 and 2.93 L/h/kg. Volume of distribution ranged from 10.5 L/kg to 12.7 L/kg. No accumulation was evident with repeated dosing; exposure appeared to decrease on GD17 relative to that on GD 6. On both GD 6 and GD 17, exposure was approximately linear and dose-proportional between 50 and 100 mg/kg/day.

Study no. 82750: BC-3781.Ac – Embryo-foetal development study in rabbits by intravenous administration

Key Study Findings:

- Low numbers of live fetuses were found in all treated groups.
- Comparisons were made between control and high-dose groups only due to low numbers of live fetuses in treated groups. Pup and litter weights were significantly lower at the high dose relative to control. Eighty-eight percent of high-dose litters had small fetuses compared to 33% of control litters. An increased incidence of decreased or no ossification was seen in high-dose litters, and was attributed to maternal toxicity.
- Due to low numbers of live fetuses and lack of complete evaluation of low- and mid-dose groups, a NOAEL was not found. The low dose, 20 mg/kg/day would be equivalent to approximately 6.7 mg/kg/day, or 400 mg/day for a 60 kg patient. In a dose range-finding study, in which the low and mid doses were not considered to be maternally toxic, the AUC at the low dose was approximately 2000 ng*hr/mL.

Conducting laboratory and location [redacted] (b) (4)

GLP compliance: Yes

Table 38. Study No. 82750: Methods

Study Method	Details
Dose and frequency of dosing:	0 (vehicle), 20, 40, or 60 mg/kg/day BC-3781 (in terms of free base), divided into two daily doses, on gestation days (GD) 6 to 18
Route of administration:	Intravenous infusion
Formulation/vehicle:	0.9% physiological saline; filtered using 0.2 µm filter
Species/strain:	New Zealand White rabbits
Number/sex/group:	31, 18, 18, and 38 mated females in the 0, 20, 40, and 60 mg/kg/day groups, respectively
Satellite groups:	None
Study design:	The rabbits were surgically implanted with a polyurethane catheter into the vena cava via the femoral vein and connected to a vascular access port located in the subcutis of the dorsum of each animal, at least one week prior to treatment. Beginning the day before treatment began, the animals were placed on a continuous infusion with physiological saline at 1 mL/hr using an infusion pump. Test article was administered by infusion twice daily on Gestation Day (GD) 6 to 18. Dams were sacrificed and Caesarean-sectioned on GD 29. Statistical comparisons were made between the high dose and control groups only.
Deviation from study protocol affecting interpretation of results:	No

Table 39. Study No. 82750: Observations and Results

Parameters	Major Findings
Mortality	Mortality was high in all groups, including control, some of which appeared to be procedure-related. However, total deaths and abortions/premature births were higher in the high-dose group.
Clinical signs	Decreased water consumption, decreased feces, abnormally colored urine, red staining in the cage tray, and decreased motor activity were seen in treated groups, beginning approximately one week after the start of treatment. Evidence of abortions began just before the end of treatment or several days after the end of treatment. At postdose observations, decreased (61%) or increased (2.8%) motor activity was noted in the high-dose group. Mid- and high-dose animals had semi-closed eyes on several occasions. Pallor was noted in 3 mid-dose females.

Parameters	Major Findings
Body weights	Weight reduction was noted in all treated groups. The decrease in body weight (9% to 10%) in high-dose animals was statistically significant relative to controls on GD 18 and from GD 24 until sacrifice. Reduced body weight gain was evident from GD 9 onwards and was statistically significant on GD 12 and GD 29. Statistically significantly lower terminal body weight and gravid uterine weight were recorded at the terminal sacrifice in the high dose group relative to controls.
Necropsy findings *[Mating/fertility index, corpora lutea, preimplantation loss, etc]*	Percentages of dams with live fetuses were as follows: • Control – 48%, • Low dose – 16% • Mid-dose – 11% • High dose – 21% Due to the low number of dams with live fetuses in the low- and mid-dose groups, group data were evaluated in the control and high-dose groups only. Statistically significant reductions were noted in pup weight (23%) and litter weight in the high-dose group relative to controls. The total numbers of fetuses were 115, 29, 8, and 54 in the control, low-, mid- and high-dose groups. External examination revealed small fetuses in the control, low-, and high-dose groups. Eighty-eight percent of high-dose litters had small fetuses, compared to 33% of controls. Pup weights and litter weights were statistically significantly lower in the high-dose group relative to controls. Skeletal examination revealed increased incidence of incomplete or no ossification in high-dose fetuses. Most affected were forelimbs, hindlimbs, forepaws, hind paws, and pelvic girdle. Fetuses with very low weight also had reduced ossification of ribs, thoracic centra, hyoid body, hyoid horns, astragalus, calcaneum, and generally incomplete ossification of all skull bones. One fetus in each of the control and high-dose groups had pelvic girdle with the articulation point absent. Two high-dose fetuses showed changes in lumbar vertebrae – hemivertebra, arch abnormal shape, and hypoplastic with centrum absent. Most changes were considered to be due to very low fetal weight and/or maternal toxicity.

LD = low dose; MD = mid dose; HD = high dose; GD = gestation day

Prenatal and Postnatal Development

Study no. AB21312 (Applicant no. LMU_SS_03_007): BC-3781.Ac – Pre- and postnatal development study by the intravenous route (bid injection) in the rat (Segment III)

Key Study Findings:

- There were 25, 24, 24 and 25 pregnant females in the control, 2x25, 2x37.5 and 2x50 mg/kg/day groups, respectively, that completed delivery. The pup live birth index was

markedly reduced in the high-dose group (87.4% compared with 98.7% in the control, with 33 stillborn/dead pups on PND 0 compared with 4 in the control group), associated with partial or total litter death of 4/25 litters.

- There was no reported effect of maternal treatment on pup observations, including preweaning physical or functional development of the F1 pups, neurobehavioral tests (learning and memory in the water maze, motor activity in an open field) and sensory function (auditory startle response), sexual maturation (although developmental anatomical landmarks were marginally delayed) and subsequent reproductive performance (mating, fertility and pre- and postimplantation data, although pre- and postimplantation losses in mid and/or high dose groups were slightly greater than controls) of the F1 animals in any group.
- It was concluded that the No Observed Adverse Effect Level (NOAEL) for embryo-fetal and pre- and postnatal development in the rat and subsequent reproductive performance of the offspring was considered to be the mid-dose, 2x37.5 mg/kg/day, based on the observed decrease in live births in the high-dose group. Based on pharmacokinetic data from the rat EFD study, mean $AUC_{0\text{-}12h}$ ranged from 8592 ng*hr/mL to 13042 ng*hr/mL at that dose.
- There were, however, additional findings in treated groups that differed from concurrent controls but were within the range of historical controls that may be considered equivocal, including lower mean number of implantation sites in mid- and high-dose F0 females, lower mean number of pups delivered in the mid- and high-dose groups, higher numbers of dead pups during lactation in treated groups, lower F1 body weights persisting through mating, apparent delays in sexual maturation, and higher pre- and or post- implantation loss in mid- and/or high-dose F1 females.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (OECD)

Table 40. Study No. AB21312: Methods

Study Method	Details
Dose and frequency of dosing:	0 (vehicle), 25, 37.5 and 50 mg/kg/ BC-3781.Ac (in terms of free base) twice daily for daily doses of 50, 75, and 100 mg/kg/day
Route of administration:	Intravenous, via indwelling catheter
Formulation/vehicle:	10mM citrate-buffered normal saline, pH 5.0
Species/strain:	Sprague-Dawley rats [Crl:OFA(SD)]
Number/sex/group:	F0 - 25 mated females per dose group F1 – 20/sex/group
Satellite groups:	None
Study design:	A polyurethane catheter was implanted into the caudal vena cava via the left femoral vein of each animal. The catheter was attached to the delivery system via a tether

Study Method	Details
	and a swivel joint and was connected to an infusion pump that served up to 8 animals. Animals were maintained on continuous infusion (0.4 mL/hour/animal) with physiological saline. Groups of 25 mated female Sprague-Dawley rats (F0 females) were given twice daily intravenous administrations of 0 (vehicle), 2x25, 2x37.5 and 2x50 mg/kg/day BC-3781.Ac (in terms of free base) from gestation day (GD) 6 to PND 20). The F0 females were allowed to give birth and the preweaning viability, growth and development of the offspring were evaluated. Litter sizes were culled to a maximum of 4 male and 4 female pups on PND 4. F0 females and offspring that were not selected for postweaning tests and reproduction were necropsied at the time of weaning of F1 pups. The F1 generation (20/sex/group) was selected from the offspring and was maintained, untreated, for postweaning development, behavioral tests and mating. The study was terminated with the necropsy of the F1 males after the caesarean examination of the F1 females on GD 13. All F1 animals underwent a macroscopic examination. The pregnancy status, number of corpora lutea and numbers and types of uterine implantations were determined for the F1 females. In order to assess maternal and pup plasma exposure to the test article, selected F0 dams and their offspring were sampled on lactation days (LD) 4 and 20. F1 pups were observed for the onset and duration of pinna unfolding, incisor eruption, and eye opening. Surface righting reflex was assessed on PND 8. Gripping reflex was assessed on PND 17. Pupillary reflex and auditory startle reflex were assessed on PND 21. Evaluation of sexual maturation was performed on F1 animals selected at weaning. Females were examined from PND 28 to detect the day of vaginal opening; the body weight was recorded on the day of occurrence. Males were examined from PND 38 to detect the day of balano-preputial skinfold separation; the body weight was recorded on the day of occurrence. At least one male and one female pup per litter were selected for postweaning behavioral tests (water maze at 8 and 9 weeks of age, open field at 10 weeks of age,

Study Method	Details
	auditory startle response (habituation) at 10 weeks of age) and mating, for a total of 20 males and 20 females per group. At approximately 11 weeks of age, these rats were paired on the basis of one male and one female from the same group for up to 21 days. Daily vaginal smears were made to confirm the day of mating (GD 0). Mated females were separated from the males once mating had been confirmed.
Deviation from study protocol affecting interpretation of results:	No

PND = postnatal day

Table 41. Study No. AB21312: Observations and Results

Generation	Major Findings
F0 dams	One HD female was euthanized in extremis on LD 8. Severe local reactions at the catheter implantation site were considered to be secondary to extravasation of the test article. Increased fecal output during the gestation day (GD) 18 or 20 through lactation was reported. Higher body weight gain between GD 6 and GD 9 was associated with and lower food consumption in all treated groups during that time frame. Lower mean body weight gain in MD and HD females from GD15-GD20 was considered to be related to lower mean live litter size at birth. Distended digestive tract at necropsy was noted in all test article-treated groups relative to control; these findings were attributed to test article effects on intestinal flora. Total litter loss was reported for one control and three high-dose litters. Duration of gestation was approximately 22 days in all groups. There were 25, 24, 24, and 25 (24 surviving to termination) pregnant females in the control, low-, mid-, and high- dose groups, respectively. The mean number of implantation sites was lower in the mid- and high-dose groups, relative to concurrent and historical controls, but the report states that the mean percentage of prenatal loss in treated groups was comparable to controls. The mean number of pups delivered in the mid- and high-dose groups was lower than control but was stated to be within the historical control range. In contrast, there were 2, 0, 1, and 6 females in the control, low-, mid-, and high-dose groups, respectively, with stillborn/dead pups (4, 0, 1, and 33 pups, respectively). Three high dose females (nos. 81, 87 and 100) had total litter loss, and a fourth (no. 93) had only 4 live pups from a total of 10 delivered. The pup live birth index was consequently lower in the high-dose group

Generation	Major Findings
	(87.4%) compared to controls (98.7%). Twenty-four females in each of the control, low-, and mid-dose groups and 21 in the high- dose group successfully reared their offspring to weaning. The mean percentage of males per litter was approximately 50% in all groups.
F1 generation	Following birth, 2, 1, and 3 live-born pups in the low-, mid-, and high-dose groups, respectively, died between LD 0 and LD 1. During lactation, the number of dead pups from LD 1 to LD 20 was higher in treated groups (total of 6, 10 and 5 at 50, 75 and 100 mg/kg/day, respectively; all died by PND 7) than in the concurrent control (2 dead pups by PND 7). Both the viability and lactation indices were said to be comparable with the concurrent and historical controls; this finding was not considered to be related to treatment but may better be considered equivocal when considered in concert with findings from other developmental toxicology study findings related to mortality of offspring. Mean pup weights after PND 1 through PND 21 were lower in treated groups than in concurrent controls. In the postweaning period, mean body weights of high-dose males and females were lower than control at selection (approximately 3 weeks of age) and through the first two weeks of the premating period. Body weights in high dose F1 females caught up with controls during gestation. No effect of treatment was noted on pinna unfolding, incisor eruption, eye opening, surface righting reflex, gripping, pupillary reflex or auditory reflex. There were no notable necropsy findings in culled pups. The mean time of balano-preputial separation was later in mid- and high-dose groups (46.7 and 46.3 days) relative to control (44.7 days). The mean time of vaginal opening was at 36.5 days in high-dose animals and at 35 days in the control group. This may be related to body weights lagging behind those of concurrent controls. These values were near the upper end of the range of historical controls. Intergroup differences in water maze, open field activity, and auditory startle habituation were not considered to be relevant; most were stated to be consistent with historical control data. There was no apparent effect of maternal treatment on the fertility of F1 offspring. On Caesarean section, there were 19, 16, 19, and 20 pregnant females in the control, low-, mid-, and high-dose groups, respectively, all of which had viable embryos. Mean preimplantation loss was greater in the high-dose group (7.2%, driven by one female no. 245 with 58.8% preimplantation loss) relative to concurrent control (3.8%), but was reported to be within the range of historical controls.

Generation	Major Findings
	Post-implantation loss was higher in the mid- (9.0%) and high-dose (6.3%) groups relative to control (3.4%) but was reported to be within the range of historical controls.
F2 generation	No evaluation of the F2 generation was performed.

LD = low dose; MD = mid dose; HD = high dose; PND = postnatal day

Toxicokinetics

In order to assess maternal and pup plasma exposure to the test article, selected F0 dams (3/group) and their offspring (pooled samples from 2 to 3 culled pups from 3 litters/group on PND 4; 1/sex from each of the 3 litters were sampled on PND 20) were sampled at 30 minutes and 90 minutes postdose on lactation days (LD) 4 and 20. No test item was quantified in maternal and pup plasma from the control group. Test article exposure was demonstrated in all treated dams and in only one litter (out of 3 tested) in each of the mid- and high-dose groups on PND 4 only. In F0 dams, no obvious accumulation was observed between LD 4 and LD 20. The increase in mean concentration was approximately dose-proportional on LD 4 and less than dose-proportional on LD 20.

Plasma concentrations are shown in the Applicant's tables below:

Table 42. Study No. AB21312: Plasma Concentrations for Dams

Mean BC-3781.Ac plasma concentrations
Dams

Occasion (day)	Dose (mg/kg/day)	Theoretical Time (minute)	Mean concentration (ng/mL)	SD	CV (%)	N
L4	0	3	BLQ	NA	NC	3
		120	BLQ	NA	NC	3
	50 (2x25)	3	5328	496	9.31	3
		120	706	61.0	8.63	3
	75 (2x37.5)	3	9082	563	6.20	3
		120	1990	988	49.7	3
	100 (2x50)	3	8919	520	5.83	3
		120	1456	116	7.98	3
L20	0	3	BLQ	NA	NC	3
		120	BLQ	NA	NC	3
	50 (2x25)	3	7665	2678	34.9	3
		120	1292	1052	81.4	3
	75 (2x37.5)	3	8931	818	9.16	3
		120	1575	217	13.8	3
	100 (2x50)	3	10084	722	7.16	3
		120	1365	322	23.6	3

SD = standard deviation; CV = coefficient of variation

Table 43. Study No. AB21312: Plasma Concentrations for Pups

Mean BC-3781.Ac plasma concentrations
Pups

Occasion (day)	Dose (mg/kg/day)	Sex	Theoretical Time (minute)	Mean concentration (ng/mL)	SD	CV (%)	N
PND4	0	NA	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
	50 (2x25)		30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
	75 (2x37.5)		30	33.1	57.3	173	3
			90	28.1	48.6	173	3
	100 (2x50)		30	21.8	37.7	173	3
			90	BLQ	NA	NC	3
PND20	0	Male	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
		Female	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
	50 (2x25)	Male	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
		Female	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
	75 (2x37.5)	Male	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
		Female	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
	100 (2x50)	Male	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3
		Female	30	BLQ	NA	NC	3
			90	BLQ	NA	NC	3

SD = standard deviation; CV = coefficient of variation

5.5.5. Other Toxicology Studies

Local Tolerance

From Dr. Maria Rivera's review of the original submission of IND 106594:

Local tolerance studies were conducted in rats. When BC-3781.Ac was administered by 30-min IV tail vein infusion 2x/day (8-hr apart) to Sprague-Dawley rats for a total dose of 20 mg/kg/day to 150 mg/kg/day for 7 days, dose-dependent tail necrosis was observed at greater than or equal to 40 mg/kg/day leading to early sacrifice of the animals. In a second study, BC-3781.Ac was administered by IV infusion at 75 or 150 mg/kg/day for a period of 7 days either by a 30-min infusion 2x/day or by a 24-hr infusion. BC-3781.Ac was well tolerated at 75 mg/kg/day when infused over a period of 24 hrs. All other conditions resulted in adverse clinical signs and/or mortalities.

Metabolites

BC-3781.Ac; BC-8041.HCl: Effect on hERG Tail Currents Recorded from Stably Transfected CHO Cells (Study number A0520)

Whole cell patch clamp technique was used to evaluate test article effects in CHO cells stably expressing hERG potassium channels (n=3). The study was GLP-compliant.

BC-3781.Ac was tested at concentrations of 10, 30, 100, and 300µM. The metabolite, BC-8041.HCl, was tested at the same concentrations. Statistically significant ($p<0.05$) and concentration-dependent inhibition was observed at the top three doses of BC-3781.Ac (21, 58, and 89% at 30, 100, and 300µM, respectively). Concentration-dependent inhibition was observed for BC-8041.HCl that was statistically significant at the top two doses (15 and 33% at 100 and 300µM, respectively).

The IC_{50} for BC-3781.Ac was estimated to be 78.18µM, and the IC_{50} for BC-8041.HCl was estimated to be 702.184µM. The positive control (100nM E-4031) resulted in 94% inhibition of hERG tail current.

Study no. AB08824: BC-8041.HCl – Bacterial reverse mutation test (plate incorporation and preincubation methods)

Key Study Findings:

- The study was uninterpretable due to high degree of toxicity to the test bacteria.

GLP compliance: Yes, except for test article characterization

Test system: *Salmonella typhimurium* strains TA98, TA100, TA 1535, TA 1537, and TA 102

Study is valid: No. BC-8041.HC1 was tested in triplicate up to the maximum recommended dose level of 5000 mcg/plate. Signs of cytotoxicity were noted both in the absence and in the presence of metabolic activation from doses ≥1600 mcg/plate when using the plate incorporation method and from doses ≥784 mcg/plate when using the preincubation method. Precipitate was noted in all strains at doses ≥1400 mcg/plate both with and without metabolic activation when using the preincubation method. No statistically and/or biologically significant increase in the number of revertants was noted in any strain either in the absence or in the presence of metabolic activation using either method. However, the apparent antibacterial activity of this metabolite makes it difficult to reach a conclusion that it is not mutagenic at sufficiently high exposures.

Study no. AB14823: BC-8041.HCl – In vitro *mammalian cell gene mutation test on L5178Y mouse lymphoma cells $TK^{+/-}$ (microwell method)*

Key Study Findings:

- BC-8041.HCl was negative for induction of mutation under the conditions of the study. However, the study was not valid, based on established guidance for the conduct and interpretation of the mouse lymphoma assay.

GLP compliance: Yes

Test system: L5178Y $TK^{+/-}$ mouse lymphoma cells

Study is valid: No. The report states that the highest test article doses resulted in a Relative Total Growth (RTG) below the 15±5% acceptable level of cytotoxicity, but the highest doses evaluated had RTGs of 59% to 65%. No statistically and biologically significant increases in the mutant frequency were noted for the long treatment period (~24 hours) in the absence of metabolic activation and for the short treatment period (~4 hours), either with or without metabolic activation at any dose levels ranging from 0.022 µg/mL to 625 µg/mL. However, the doses evaluated did not reach the target RTG.

Study no. AB03683 (Applicant reference no. 03781A-SR03-GxP): BC-3781 – BC-8041.HCl - Embryo toxicity study by intravenous injection in the Sprague-Dawley rat (Segment II)

Key Study Findings:

- Malformations of the heart (enlarged ventricular chamber, thin ventricular wall) or great vessels were reported in two MD and one HD litters. Heart malformations were consistent with those reported in the rat EFD study of lefamulin that were rare in the historical database and nonexistent in concurrent controls.
- A maternally toxic dose was not reached.
- The fetal NOAEL in this study would be the low dose, 10 mg/kg/day, divided BID (mean C_{max} =3416–4500 ng/mL, mean $AUC_{0\text{-}12h}$ =1705–2135 ng.h/mL).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (OECD), with the exception of bioanalysis

Table 44. Study No. AB03683: Methods

Study Method	Details
Dose and frequency of dosing:	0 (vehicle), 2 x 5, 2 x 10 and 2 x 20 (i.e., 10, 20, and 40) mg/kg/day BC-8041.HCl (in terms of free base) by twice daily intravenous bolus injection into a tail vein
Route of administration:	IV bolus injection into a tail vein, using a microflex infusion set and a Harvard PHD 2000 infusion pump (Ealing)
Formulation/vehicle:	Sterile physiological saline (0.9% NaCl), USP
Species/strain:	Sprague-Dawley rats (Crl:OFA (SD))
Number/sex/group:	25 mated females per group
Satellite groups:	An additional 6 mated female rats per group were sampled for toxicokinetics on GD 6 and 17.
Study design:	Animals were treated on gestation day (GD) 6 through GD 17. Caesarean section and sacrifice were on GD 20. After gross examinations, half of the fetuses were processed for skeletal examination. The remaining fetuses were preserved for fixed visceral examination.
Deviation from study protocol affecting interpretation of results:	No

IV = intravenous

Table 45. Study No. AB03683: Observations and Results

Parameters	Major Findings
Mortality	No treatment-related deaths were reported.
Clinical signs	The only treatment-related clinical sign reported was noisy breathing for less than a minute immediately after treatment, usually only on a single day for 15 of 25 of HD females (and just before treatment in 2 HD females) between GD 13 and GD 17.
Body weights	A transient reduction in mean body weight gain and food consumption was reported in the MD and HD groups between GD 6 (for food consumption) or GD 9 (for body weight gain) and GD 12 relative to concurrent control. Thereafter, food consumption and terminal body weights were similar in treated groups to control. The report describes this finding as “nonadverse,” but also cites it as evidence that dosing reached a maternally toxic dose. A maternally toxic dose was not reached.
Necropsy findings Cesarean section data	There were 24/25, 25/25, 25/25, and 25/25 pregnant females in Groups 1 through 4, respectively, at termination. All pregnant animals at termination had viable fetuses, with the exception of one female in the LD group. There was a slightly higher percentage of postimplantation loss in the LD group compared with the concurrent and historical control data, due to a single female (#31) that had 3 implantation sites and no viable fetuses. Mean live litter size at the MD and HD was comparable to control, and the report states that there were no obvious treatment-related effects on postimplantation survival.

Parameters	Major Findings
	No treatment-related effect on mean fetal weight or sex ratio was reported.
Necropsy findings Offspring	Malformations Control and LD: None reported MD: 3 fetuses in 3 litters had malformations: 1) two fetuses from separate litters had either an enlarged left or right ventricular chamber; one also had a thin ventricular wall (*Reviewer's comment: These findings are consistent with findings at the high dose in the rat EFD study of the parent drug, and as discussed in the review of that study, appear to be relatively rare.*), and 2) one fetus in a third litter had marked shortening of the intestines. HD: 1 fetus in 1 litter had malformed major blood vessels. The report argues that the ventricular enlargement was not treatment-related, stating that enlarged ventricular chamber is part of the background of changes noted for the strain of rat used in the study (1 out of 141 fetuses (0.7%) were affected in 2005). *Reviewer's comment: This appears to be a selective sample from the historical control database appended to this report that also indicates that this was the only fetus affected from 2005 through 2010 out of a total of 2987 fetuses in 23 studies.* The report also states that enlarged ventricular chamber (unilateral or bilateral) has also been observed among the treated groups in two contemporary studies performed at the Testing Facility in 2011 in the same strain of rat. In those two studies, the data indicate that there was no incidence of this alteration in 48 control litters in 2011, and that it occurred only in a total of three litters in MD (2 of 49) and HD (1 of 49) treated groups (test article not specified) in that year. These data do not provide evidence that this would be a spontaneous background finding in untreated litters. The appended historical control database indicates that malformed great vessels were recorded in 1 fetus of 2012 (from 15 studies) between 2005 and 2007 and in 1 of 975 fetuses (from 8 studies) between 2008 and 2010. In that same database, dilated ventricle was recorded in 1 fetus of 2012 between 2005 and 2007 and in 0 of 975 fetuses between 2008 and 2010. The rarity or absence of these findings in untreated animals increases the likelihood that this is a treatment-related effect. Later communications regarding this finding included a statement from and expert pathologist that dilated ventricle could be related to valve or great vessel malformations that might go undetected using the method of fetal sectioning and evaluation that was employed. The report states, "The incidences of other less severe soft tissue anomalies and variations, which principally included slight renal pelvic dilatation and convoluted or slightly/moderate dilated ureters, did not suggest any influence of treatment." Similar renal lesions

Parameters	Major Findings
	were noted in the rat EFD study of lefamulin, and were not considered to be treatment-related. The lack of relationship of these findings to treatment appears to be supported by the appended historical control database. The report states that fetal and litter incidences of the degree of ossification did not show any statistically or biologically significant differences between the groups.

LD = low dose; MD = mid dose; HD = high dose; EFD = embryo-fetal development

Toxicokinetics

Maximum plasma concentrations of BC-8041 were observed at 1.5 minutes after administration. On GD 6, BC-8041 plasma concentration time curves showed a biphasic decline with a rapid first distributional phase (0h and 0.75h) followed by an extended elimination phase with half-life ranging between 3.06 hours and 3.39 hours. No significant accumulation of BC-8041 was observed between GD 6 and GD 17. The increase in systemic exposure was reported to be linear and dose-proportional between 5 and 20 mg/kg/administration. Clearance and volume of distribution were constant regardless of dose, ranging between 2.57 mL/h/kg and 2.89 mL/h/kg and between 11.5 mL/kg and 13.9 mL/kg, respectively.

No quantifiable test article was detected in plasma from control animals. Systemic exposure was demonstrated in all treated satellite animals. Toxicokinetic parameters are shown in the Applicant's table below:

Table 46. Study No. AB03683: Toxicokinetic Parameters

Occasion	Dose (mg/kg/adm)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-12h} (ng.h/mL)
G6	5	3413	0.025	1705
	10	6866	0.025	3436
	20	15031	0.025	7609
G17	5	4500	0.025	2135
	10	7824	0.025	3788
	20	16768	0.025	8486

C_{max} = maximum plasma concentration of drug; T_{max} = time to reach maximum plasma concentration after administration; AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration

Impurities

The Applicant has proposed limits of (b) (4)% for the impurity (b) (4) and (b) (4)% for the impurity (b) (4) in the drug substance, indicating that these impurities were qualified in 14-day general toxicology studies in cynomolgus monkeys (Study no. (b) (4).298.3) and in rats (Study no. 73925-02). For Study no. (b) (4).298.3 in cynomolgus monkeys, the NOAEL dose was 80 mg/kg/day

(HED =26 mg/kg/day). (b) (4) was present as (b) (4)% of the test article in that study, so the NOAEL dose of (b) (4) was (b) (4) mg/kg/day (HED = (b) (4) mg/kg/day). (b) (4) was present as (b) (4)% of the test article in the cynomolgus monkey study, so the NOAEL dose of (b) (4) was (b) (4) mg/kg/day (HED = (b) (4) mg/kg/day).

The proposed clinical IV dose of 150 mg q12h =300 mg/day, or 5 mg/kg/day for a 60 kg patient. (b) (4) at the proposed limit of (b) (4)% would be administered at a dose of (b) (4) mg/kg/day, and (b) (4) at the proposed limit of (b) (4)% would be administered at a dose of (b) (4) mg/kg/day. Therefore, the proposed limits are supported by the data from the 14-day general toxicology study in cynomolgus monkeys.

The proposed clinical oral dose of 600 mg q12h =1200 mg/day, or 20 mg/kg/day for a 60 kg patient. (b) (4) at the proposed limit of (b) (4)% would be administered at a dose of (b) (4) mg/kg/day, and (b) (4) at the proposed limit of (b) (4)% would be administered at a dose of (b) (4) mg/kg/day. The data from the 14-day general toxicology study in cynomolgus monkeys also support the proposed limits for these two impurities in the oral formulation.

In the rat study (Study no. 73925-02), there was no NOAEL, but the LOAEL was 25 mg/kg/day (HED =4.0 mg/kg/day). (b) (4) was present as (b) (4)% of the test article, so the "NOAEL" dose of that impurity was (b) (4) mg/kg/day (HED = (b) (4) mg/kg/day), and (b) (4) was present as (b) (4)% of the test article, so the "NOAEL" dose of that impurity was (b) (4) mg/kg/day (HED = (b) (4) mg/kg/day). Using the LOAEL dose in rats for comparison to clinical dosing, the proposed acceptance criteria would be supported for the IV formulation, but not at the higher oral dose. However, since this rat study used IV administration, and the toxicity at the lowest dose was related to irritation/inflammation at the injection site, it is reasonable that the proposed limits should be acceptable for the oral formulation. It is also noteworthy that the proposed clinical IV dose of the drug substance (5 mg/kg/day) exceeds the human equivalent of the lowest dose in the rat study (4 mg/kg/day).

Potentially Genotoxic Impurities:

In the Quality section, the application states that a Genotoxic Impurity Risk Assessment was performed to identify potentially genotoxic impurities, but the report of that assessment was not provided for review. The Applicant communicated in their response to an Agency information request that no such report was generated and that the risk assessment consisted of their noting specific chemical structures that could be associated with genetic toxicity. The application states that the impurities in the table below were selected for genetic toxicology testing. The Applicant has referenced the ICH M7 guidance as indicating that for marketed products with a treatment duration of >1 month but <12 months, the acceptable intake of an individual impurity is 20 mcg/day. They calculate proposed limits of (b) (4) ppm by the IV route and (b) (4) ppm by the PO route, based on a daily IV dose of 340 mg (300 mg free base) and a daily PO dose of 1360 mg (1200 mg free base). ICH M7 also indicates that the Acceptable Total Daily Intake for multiple impurities over that duration of time is 60 mcg/day.

Reviewer's Comment: *The proposed labeling indicates that treatment duration is 5 to 7 days; it is unclear why the Applicant chose to apply daily limits based on a longer duration of dosing.*

Genetic toxicity testing was performed for the following impurities. For each, the initial assay in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 employed doses of 0.5, 1.6, 5, 16, 50, 160, 500, 1600 and 5000 mcg/plate, plus vehicle and positive controls.

Table 47. Potentially Genotoxic Impurities for Lefamulin

Impurity	Assay	Result	Is the Study Valid?
(b) (4)	Bacterial reverse mutation assay (b) (4) study no. 8313936)	Negative	Not valid; toxicity noted at 50 mcg/plate and above in TA100 -S9; at 500 and/or 1600 mcg/plate and above in TA100, TA1537 and TA102 +S-9; and at 5000 mcg/plate in TA1535 and TA102 -S-9.
(b) (4)	Bacterial reverse mutation assay (b) (4) study no. 8313937)	Positive for mutagenicity in *S. typhimurium* strains TA100 -S9, and TA1535 +/- S9	Yes
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8313938)	Negative	No. The test article demonstrated excessive toxicity to the test bacteria. Toxicity was observed at 6.4 mcg/plate and above in strains TA100 and TA1537 +/- S9 or at 16 and/or 40 mcg/plate and above in strains TA98, TA1535 and TA102 +/- S9.
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8313939)	Negative	No. The report cites evidence of toxicity or complete killing of the test bacteria at 50 mcg/plate and above in all strains +/- S9, and for strain TA100 -S9 at 16 mcg/plate.
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8313940)	Negative	No. Toxicity was observed at 50 and/or 500 mcg/plate and above in all strains +/- S9.
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8313941)	Negative	Possibly valid. Toxicity was observed at 1600 and/or 5000 mcg/plate in all strains +/- S9.

Impurity	Assay	Result	Is the Study Valid?
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8388424)	Negative	No. Toxicity was observed at 160 or 500 mcg/plate and above in all strains +/- S9.
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8388426)	Negative	No. Toxicity was observed at 160 or 500 mcg/plate and above in all strains +/- S9.
(b) (4)	Bacterial reverse mutation assay (b) (4) study no 8388427)	Negative	Yes. Toxicity was observed only at 5000 mcg/plate in strain TA102 +/- S9, and in strains TA98, TA100 and TA1537 -S9.

Of these, (b) (4) was positive and should be controlled in accordance with ICH M7. According to the CMC drug substance review, this (b) (4) was found to be (b) (4) ppm in registration batches. The Applicant proposes a limit of less than or equal to (b) (4) ppm (b) (4) mcg/day for the oral dose) for this impurity. The Applicant also proposes a limit of less than or equal to (b) (4) ppm ((b) (4) mcg/day for the oral dose) for (b) (4) that is genotoxic. According to ICH M7, for a drug used for treatment for less than or equal to 1 month, the limit for total daily intake for an individual genotoxic impurity would be 120 mcg/day, and the limit for total daily intake for total genotoxic impurities would also be 120 mcg/day; the proposed limits for these two impurities are in accordance with M7.

A (Q)SAR analysis was performed by the CDER Computational Toxicology group. That analysis indicated that (b) (4) should be negative in mutagenicity assays. Using this as the first screen for impurities, an in vitro mutagenicity assay would not be needed for this compound, and it may be removed from the list of PGIs. The remaining five PGIs of concern were shown likely to be positive in multiple genotoxicity assays.

In the Applicant's bacterial reverse mutation testing, (b) (4) exhibited excessive toxicity to the bacterial strains used in the assay and should be tested for mutagenicity in an assay in mammalian cells or controlled as a genotoxic impurity per ICH M7. Based on information provided by the drug substance reviewer:

- (b) (4) was present as < (b) (4) % in all registration and Phase 3 clinical batches. For the daily oral dose of 1360 mg (1200 mg free base), (b) (4) % would result in a daily exposure to (b) (4) mg ((b) (4) mcg) which **exceeds the 120 mcg/day limit** described in ICH M7 for a 5 to 7 day treatment for a genotoxic impurity.
- (b) (4) was present as < (b) (4) ppm ((b) (4) %) in all registration and Phase 3 clinical batches. For the daily oral dose of 1360 mg (1200 mg free base), (b) (4) %would result in a daily exposure (b) (4) mg ((b) (4) mcg) which is **below the 120 mcg/day limit** described in ICH M7 for a 5 to 7 day treatment for a genotoxic impurity.

- [redacted] was present as < [redacted] ppm ([redacted] %) in clinical batches and < [redacted] ppm ([redacted] %) in registration batches. For the daily oral dose of 1360 mg (1200 mg free base), the lower value for registration batches would result in a daily exposure [redacted] mg ([redacted] mcg) which is **below the 120 mcg/day limit** described in ICH M7 for a 5 to 7 day treatment for a genotoxic impurity.
- [redacted] was present as < [redacted] ppm ([redacted] %) in all registration and Phase 3 clinical batches. For the daily oral dose of 1360 mg (1200 mg free base), that would result in a daily exposure [redacted] mg ([redacted] mcg) which is **below the 120 mcg/day limit** described in ICH M7 for a 5 to 7 day treatment for a genotoxic impurity.
- [redacted] was present as < [redacted] ppm ([redacted] %) in all registration and Phase 3 clinical batches. For the daily oral dose of 1360 mg (1200 mg free base), that would result in a daily exposure [redacted] mg ([redacted] mcg) which is **below the 120 mcg/day limit** described in ICH M7 for a 5 to 7 day treatment for a genotoxic impurity.

(Even in cases where the impurity was considered to be below the lower limit of quantitation, in absence of negative mutagenicity results or a more sensitive assay, it will have to be assumed that the genotoxic impurity is present at or just below the LLOQ for the purposes of determining the possible total exposure genotoxic impurities.)

The total exposure for the latter four would be [redacted] mcg/day, which is still below the 120 mcg daily limit for total genotoxic impurities. The application acknowledges confirmed genotoxic impurities, [redacted] and [redacted] and proposes to limit each of these to [redacted] ppm ([redacted] %, or [redacted] mg, or [redacted] mcg) or less. Addition of this maximum for each of these compounds to the total results in [redacted] mcg/day. In order to remain below the 120 mcg/day limit, [redacted] and probably others would need to be more tightly controlled, unless they can be demonstrated to not be genotoxic in a valid assay.

In the absence of mutation assays in mammalian cells, the following PGIs should be treated as genotoxic: [redacted]. If these and the two positive genotoxic impurities cannot be controlled in accordance with ICH M7, all seven impurities should be noted in the label under section 13.1 as known or potential genotoxicants, the total of which exceed the acceptable total daily intake, with the acknowledgement that the short (5 to 7 day) duration of treatment minimizes the risk.

6 Clinical Pharmacology

6.1. Executive Summary

The clinical pharmacology information in this NDA supports approval of XENLETA [established name lefamulin (LEF)] injection and tablets for the treatment of adult patients with CABP caused by susceptible microorganisms. Pivotal evidence of efficacy and safety are provided by two Phase 3 trials for CABP (Studies NAB-BC-3781-3101 and NAB-BC-3781-3102) (see Sections 8.1 and 8.2). The following four important issues were identified during the review:

(1) Plasma protein binding (PPB). We have determined that the plasma protein binding of LEF is 94% to 97%. The Applicant had proposed 73% to 88% based on the results of one study where PPB was determined using 85% (v/v) plasma (see Plasma Protein Binding in Section 6.3.2 for details). This difference significantly influences the probability of PK-PD target attainment analyses which are entirely based on unbound drug concentrations.

(2) Dosage adjustment for patients with hepatic impairment. Protein binding of LEF is reduced and, accordingly, unbound (biologically active) LEF concentrations increased in patients with hepatic impairment. The LEF half-life was increased in patients with hepatic impairment. Therefore, we recommend the following dosages in patients with hepatic impairment:

Table 48. Recommended Dosages of Lefamulin for Patients With Hepatic Impairment

	XENLETA	
Degree of Hepatic Impairment	**Injection**	**Tablets**
Mild (child-pugh A)	150 mg infused over 1 hr q12 hrs	600 mg q12 hr
Moderate (child-pugh B)	150 mg infused over 1 hr q12 hrs	Not recommended
Severe (child-pugh C)	150 mg infused over 1 hr q24 hrs	Not recommended

(b) (4) . See *Patients With Hepatic Impairment* section for further discussion of this observation (i.e., unchanged total drug concentrations despite a decrease in PPB).

(3) How to take XENLETA tablets with regard to food intake. We recommend that XENLETA tablets be taken at least 1 hour before a meal or 2 hours after a meal, to be consistent with Phase 3 trial dosing instructions. (b) (4) See *Food-Drug Interaction* section for further details.

(4) Concomitant use of XENLETA tablets and strong CYP3A inhibitors or P-gp inhibitors. We recommend avoiding coadministering XENLETA Tablets with strong CYP3A inhibitors or P-gp inhibitors because coadministration increased LEF exposure (AUC) 2.65-fold. (b) (4)

[redacted] (b) (4) (See *Drug-Drug Interaction* for details).

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Table 49. Summary of the Clinical Pharmacokinetics of Lefamulin (LEF)

Pharmaceutical Properties	
Bridge between to-be marketed and clinical trial formulations	The to-be-marketed LEF tablet formulation is the same as the Phase 3 IR tablet formulation used in the Phase 3 trials; only differing in appearance (color and imprint). The two in vitro dissolution profiles were similar (f_2>50). See *Comparison Between Phase 3 Tablet and to-be-Marketed Tablet*.
Drug product formulation	XENLETA for injection. 150 mg LEF solution infused over 60 min XENLETA tablets. 600 mg immediate release tablet taken 1-hr before or 2-hr after a meal.
ADME Properties	
Absorption	Double peak phenomena were observed following oral administration, but not IV administration. T_{max1} was 20 min to 1 hr and T_{max2} was 1 to 4 hrs postdose. LEF exposure (C_{max} and $AUC_{0\text{-}inf}$) following PO administration of LEF tablets with a high fat meal was, on average, approximately 20% lower compared with PO administration under fasting conditions.
Distribution	LEF plasma protein binding (PPB) is concentration-dependent at the clinically achievable concentrations (ranged from 94.5% to 97.2%). The mean (min to max) volume of distribution is 552 L (376 L to 929 L) Epithelial lining fluid (ELF) concentrations, determined from bronchoalveolar lavage, approximated total plasma concentrations with parallel kinetics over time following a single IV dose of 150 mg in healthy adult subjects. The ratio of AUC_{ELF}: free-drug AUC_{plasma} was approximately 20.
Elimination	The mean (min to max) LEF half-life is 8 h (3.5 h to 20.1 h) The mean (min to max) LEF clearance is 90.3 L/h (18.8 L/h to 227 L/h)

Pharmaceutical Properties	
	Metabolism CYP3A4 is the primary LEF metabolizing enzyme; however, in vitro data suggest flavin-containing monooxygenases (FMOs) may also contribute. BC-8041 is the major systemic metabolite, not active at the clinically relevant concentration range, in plasma with the $AUC_{metabolite}/AUC_{parent}$ ratio of 0.14 to 0.22 following oral administration. The $AUC_{metabolite}/AUC_{parent}$ ratio following IV administration was <0.1.
	Excretion Unchanged LEF in feces and urine were 4.2% to 9.1% and 9.6% to 14.1% of the dose, respectively, following IV administration of the radiolabeled drug.

T_{max} = time to reach maximum plasma concentration after administration; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The Applicant's proposed dosage regimens for the treatment of adult patients with CABP are acceptable based on the Phase 3 trials demonstrating noninferiority to moxifloxacin and acceptable safety profile (see Section 8). The intravenous (IV) and oral (PO) dosages and mean treatment durations from the Phase 3 trials guided the proposed dosage regimens as follows:

- 150 mg every 12 hours (q12hr) by IV infusion over 1 hr for 5 days to 7 days, or
- 150 mg q12hr by IV infusion over 1 hr then switch to 600 mg PO q12hr (at discretion of physician) for 5 to 7 days (total), or
- 600 mg PO q12hr for 5 days

(b) (4) we recommend that XENLETA tablets be taken — as studied in the Phase 3 trials – 1 hour before or 2 hours after a meal (See Food-Drug Interaction in Section 6.3.2)

Dosage Adjustment in Patients with Hepatic Impairment

We recommend XENLETA tablets not be used in patients with moderate (Child-Pugh Class B) and severe (Child-Pugh Class C) hepatic impairment. However, no dosage adjustment of XENLETA tablets is necessary in patients with mild (Child-Pugh Class A) hepatic impairment.

For XENLETA injection, a dose reduction (150 mg every 24 hours) is recommended for patients with severe (Child-Pugh Class C) hepatic impairment. No dosage adjustment of XENLETA injection is necessary for patients with mild (Child-Pugh Class B) and moderate (Child-Pugh Class

A) hepatic impairment. (b) (4) See *Patients With Hepatic Impairment* in Section 6.3.2 for details.

Outstanding Issues

There are no outstanding issues.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

Table 50. Summary of Pharmacologic Activity and Clinical Pharmacology

Characteristic	Drug Information
Pharmacologic Activity	
Mechanism of action	LEF inhibits bacterial protein synthesis via interruption of the peptidyl transferase center of the bacterial ribosome.
Antibacterial activity	The PK-PD index of the antibacterial activity of LEF was the ratio of free-drug AUC_{0-24} to MIC (*f*AUC/MIC). BC-8041 (metabolite): The main metabolite, BC-8041, is not expected to be active at the clinically relevant concentration range.
Active moieties	LEF is the active moiety.
QT prolongation	LEF: The mean QTcF increase was 14 and 10 ms at a C_{max} of 3.5 (IV-steady state) and 2.24 (PO-steady state) mcg/mL in Phase 3 trials. Clinical experience up to a mean LEF C_{max} of 4.4 mcg/mL has been studied in healthy adults (400 mg IV dose infused over 30 min). BC-8041: hERG assay results suggest BC-8041 does not prolong the QT interval at clinically relevant concentrations. In addition, the mean change in QT prolongation was less in patients received LEF tablets compared to that in patients received LEF injection in Phase 3 trials, despite greater BC-8041 exposure following PO compared to IV administration, supporting the hERG assay results.
General Information	
Bioanalysis	Validated HPLC/MS/MS methods were used to determine the concentrations of LEF, BC-8041, and coadministered drugs in various biological matrices as applicable to individual studies.
Healthy versus patients	LEF: The mean AUC_{0-24} and C_{max} in CABP patients was approx. 1.73- and 1.3-fold greater compared to adults without pneumonia following the therapeutic IV and PO dosing regimens on Day 1.
Drug exposure at steady state (SS) following the therapeutic dosing	150 mg LEF injection infused over 1 hr Q12 hr– SS in CABP patients (n=252)
	Parameter / LEF [Geometric mean (%CV)]

<table>
<tr><td>regimen</td><td>
<table>
<tr><td>AUC (mcg*hr/mL)</td><td>26.3 (40)</td></tr>
<tr><td>C_{max} (mcg/mL)</td><td>3.6 (13.7)</td></tr>
</table>
<table>
<tr><td colspan="2">600 mg LEF tablets Q12 hr – SS in CABP patients (n=230)</td></tr>
<tr><td>Parameter</td><td>LEF [Geometric mean (%CV)]</td></tr>
<tr><td>AUC (mcg*hr/mL)</td><td>29.4 (45)</td></tr>
<tr><td>C_{max} (mcg/mL)</td><td>2.09 (38)</td></tr>
</table>
LEF tablets was administered 1 hr before or 2 hr after a meal.</td></tr>
<tr><td>Range of effective dose or exposure</td><td>One dosage was evaluated in efficacy studies. No relationship was observed between LEF exposures (i.e., AUC, C_{max}, or fAUC/MIC and Phase 3 efficacy endpoints following doses of 150 mg IV and 600 mg PO q12hr.</td></tr>
<tr><td>Maximally tolerated dose or exposure</td><td>Subjects tolerated single doses of LEF up to 400 mg IV and 750 mg PO and multiple doses up to 200 mg IV and 600 mg PO every 12 hours for 6 or 10 days, respectively. Higher doses have not been evaluated.

Average drug exposures following single and multiple administration of the highest dose in healthy subjects were:
Single Dose
IV 400 mg - 4.4 mcg/mL (C_{max});16.5 mcg*hr/mL (AUC_{0-inf})
PO 600 mg - 1.35 mcg/mL (C_{max}); 8.2 mcg*hr/mL (AUC_{0-inf})
Multiple Dose (Q12 hr)
IV 200 mg – 3 mcg/mL (C_{max}); 9.07 (AUC_{0-12})
PO 600 mg - 2.07 mcg/mL (C_{max}); 11.3 mcg*hr/mL (AUC_{0-12}).</td></tr>
<tr><td>Dose proportionality</td><td>IV (Dose Range: 25 mg–400 mg): LEF AUC increased dose proportionally. However, changes in the LEF C_{max} were subproportional to dose.
PO (Dose Range:500 mg–750 mg) LEF AUC was supraproportional to dose.
(See section 16.3.2.1)</td></tr>
<tr><td>Accumulation</td><td>Accumulation ratio (assessed by AUC) was less than 2 irrespective of formulation in CABP patients.</td></tr>
<tr><td>Absorption</td><td></td></tr>
<tr><td>Bioavailability</td><td>Absolute bioavailability of LEF tablets: 25%</td></tr>
<tr><td>Food effect</td><td>The ratio of PK parameters (fed/fasted) following administration of LEF tablets: Geometric mean (90% CI)
<table>
<tr><th>AUC_{0-inf}</th><th>C_{max}</th><th>T_{max}</th></tr>
<tr><td>0.82 (0.75,0.88)</td><td>0.77 (0.68, 0.88)</td><td>T_{max} prolonged from 1.76 hr (fasted) to 5.0 hr (fed)</td></tr>
</table>
Fed state =30 minutes from completion of high-fat, high-calorie breakfast</td></tr>
<tr><td>Distribution</td><td></td></tr>
<tr><td>Volume of distribution</td><td>The mean (min to max) estimate is 552 L (376L to 929 L)</td></tr>
<tr><td>Plasma protein binding</td><td>Human plasma protein binding of LEF is 97.2% to 94.5%.</td></tr>
</table>

ELF and intracellular accumulation	Total-drug epithelial lining fluid (ELF) concentrations in healthy subjects were approximately 20 times free-drug plasma concentrations. The impact of infection on drug exposures in the lung has not been studied. Intracellular LEF concentrations were 30 to 40 times extracellular LEF concentrations in a murine macrophage cell line after 1 hr and 50 times after 5 hr.
As substrate of transporters	LEF is a substrate of P-gp transporter.
Elimination	
Mass balance results	Following IV administration, 77.3% and 15.5% of total radioactivity was recovered in feces and urine, respectively. Unchanged LEF in feces and urine was 4.2% to 9.1% and 9.6% to 14.1% of the dose administered, respectively. Following PO administration, 88.5% of total radioactivity was excreted in feces. Unchanged LEF in feces was 7.8% to 24.8% of the dose administered. Unchanged LEF in urine was not determined. Predominant radioactivity recovered in feces is BC-8041. The plasma $AUC_{BC-8041}/AUC_{LEF}$ ratio was 0.14 to 0.22 and <0.1 following PO and IV administration, respectively.
Clearance	The mean (min to max) estimate is 90.3 L/hr (18.8 L/hr to 227 L/hr)
Terminal elimination half-life	The mean (min to max) estimate is 8.0 hr (3.5 to 20.1)
Primary metabolic pathway(s)	LEF: CYP3A
Drug Interaction Liability (Drug as Perpetrator)	
Inhibition/induction of metabolism	LEF inhibits CYP3A
Inhibition/induction of transporter systems	LEF is not expected to inhibit major transporters at the clinical dose.

PO = oral; IV = Intravenous; LEF = lefamulin; C_{max} = maximum plasma concentration of drug; MIC = minimum inhibitory concentration; PK = pharmacokinetic; PD = pharmacodynamic; AUC_{0-24} = area under the concentration-time curve from time 0 to 24 hours after drug administration; hERG = human ether-a-go-go-related gene; *f*AUC/MIC = ratio of free drug area under the concentration-time curve to MIC over a 24-hour period; IV = intravenous; AUC_{0-inf} = area under the concentration-time curve from time 0 to infinity; CABP = community-acquired bacterial pneumonia; T_{max} = time to reach maximum plasma concentration after administration

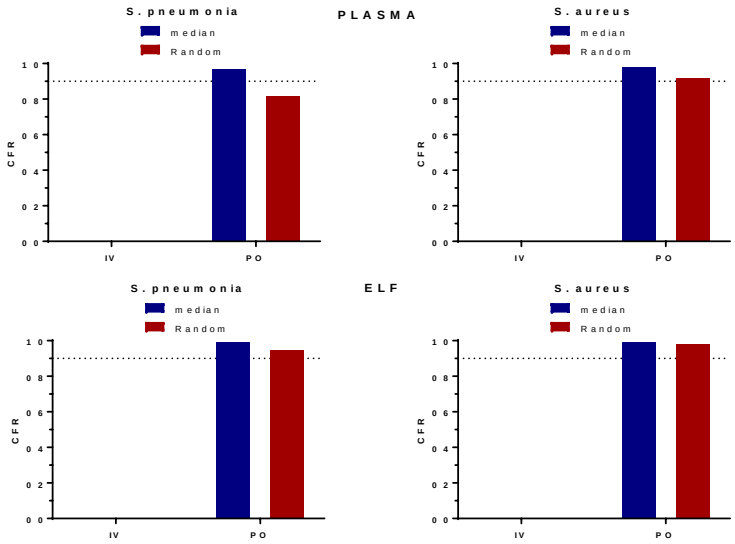
6.3.2. Clinical Pharmacology Questions

6.3.2.1. Does the clinical pharmacology program provide supportive evidence of effectiveness?

Yes. While no clinical exposure-response relationships were observed in the Phase 3 trials, the review team's probability of PK-PD target attainment (PTA) analyses support the clinical efficacy observed. Day 1 drug exposures (free-drug plasma and total-drug epithelial lining fluid (ELF) achieved in CABP patients following the proposed IV and PO doses were adequate based on PTA analyses incorporating CABP PK variability, the distribution of MICs observed in Phase 3 trials, and the PK-PD target(s) obtained from murine models of acute *S. pneumoniae* and *S. aureus* pneumonia (i.e., the free drug AUC/MIC ratio for 1-log CFU reduction from baseline).

The approximately 90% cumulative probability to reach the PK-PD target (irrespective of exposure-site) suggests a high likelihood for treatment success, supporting the effectiveness observed for lefamulin in CABP patients infected with *S. pneumoniae* and *S. aureus*. (Figure 4). See Section 16.3.2.5.1 for further details and discussion.

Figure 4. Predicted Cumulative Probability to Reach the 1-Log_{10} Bacterial Kill PK-PD Target on Day 1 for *S. pneumoniae* and *S. aureus* in a Virtual Phase 3 CABP Patient Population



CFR = cumulative fractional response; PK = pharmacokinetic; PD = pharmacodynamic; CABP = community-acquired bacterial pneumonia; ELF = epithelial lining fluid
2 This is the expected population probability of target attainment for a specific drug dose and a specific population of bacteria

The Monte Carlo simulations incorporated PK variability and bacterial MIC distributions observed from patients in Phase 3 studies, as well as either a single point estimate of the PK-PD target (i.e., median) or a random allocation of the PK-PD target drawn from a truncated log10-normal distribution (±2 standard deviations). Dosing regimens were 150 mg LEF IV (1-hr infusion) or 600 mg PO (fasting) LEF every 12 hr. Free-drug plasma or total-drug ELF AUC24 were simulated with plasma unbound fraction of 0.0379. The PK-PD target (fAUC24/MIC) associated with a 1-log CFU reduction from baseline determined from murine models of acute pneumonia were used.

From a clinical pharmacology perspective, plasma and ELF concentrations/exposures are important considerations for proper clinical interpretation. In adults without pneumonia, rapid equilibration between ELF and plasma, with nearly identical total (bound+unbound) LEF concentration-time profiles in ELF and plasma, were observed (NAB-BC-3781-1005). Based on a

[2] Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. J Antimicrob Chemother. 2005;55(5):601-7.

PPB estimate of approximately 96% and an assumption of negligible protein binding in ELF, unbound concentrations (biologically active) were approximately 20-fold greater in ELF compared to plasma (by $AUC_{0\text{-}8,\ total\ ELF}$ / $AUC_{0\text{-}8,\ free\ plasma}$). Thus target-site (i.e., ELF) exposure appears favorable for the pneumonia indication.

From a regulatory standpoint, there are two limitations to make clinical decisions based upon ELF assessment alone. First, BAL sampling and ELF drug concentration are likely more qualitative than quantitative because there are considerable technical challenges associated with the methods to estimate ELF drug concentrations and drug binding to protein has never been definitively determined.[3][4] Second, bacterial pneumonia is not always confined superficially to the luminal airway surface. Invasion of the pulmonary parenchyma and hematogenous dissemination need to be considered for appropriate care.

Accordingly, the review team has determined that use of LEF PK in plasma is the most appropriate exposure metric when assessing the probability of target attainment and likelihood of a therapeutic response. A higher $AUC_{ELF}/AUC_{free,plasma}$ ratio in humans compared to that in mice suggests that use of unbound LEF PK in plasma, as the exposure metric for the PTA analyses, would be a cautious approach to superficial lung infections as it would underestimate target attainment at that biophase (ELF). However, as discussed above, ELF is not the only site of infection that requires adequate LEF exposure (lung parenchyma and plasma). From a population perspective, the use of unbound LEF exposure in plasma minimizes the likelihood of therapeutic failure.

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

Yes. Efficacy (noninferiority to moxifloxacin) and safety were demonstrated for both IV and PO dosage regimens in adults with CABP. See Sections 8.1 and 8.2 for further details on efficacy and safety.

Supportive Efficacy Information

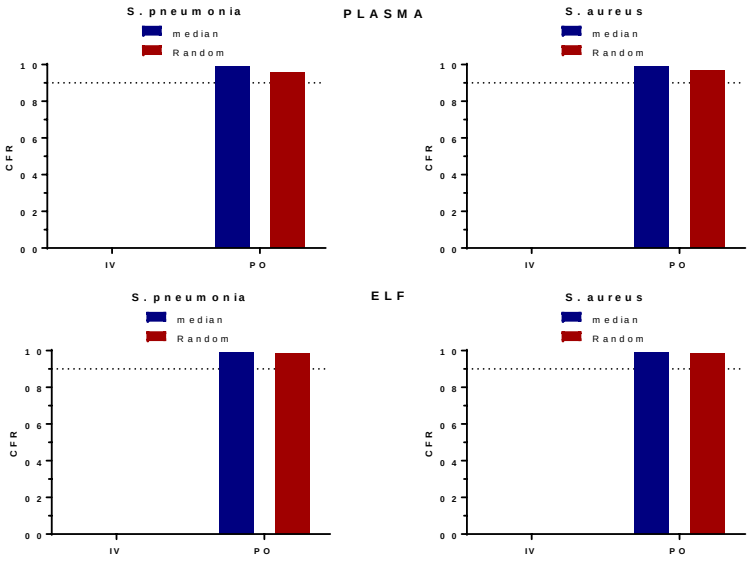
No relationship between LEF plasma exposure ($AUC_{0\text{-}24}$ and $AUC_{0\text{-}24}$:MIC) and Phase 3 clinical efficacy against *S. pneumoniae* (most common pathogen) infection was identified probably because of broadly similar LEF exposures, limited MIC range, and high success rates (See Section 16.3.2.5.1).

[3] Rodvold KA, Yoo L, George JM. Penetration of anti-infective agents into pulmonary epithelial lining fluid: focus on antifungal, antitubercular and miscellaneous anti-infective agents. Clin Pharmacokinet. 2011;50(11):689-704.

[4] Kiem S, Schentag JJ. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. Antimicrob Agents Chemother. 2008;52(1):24-36

PTA analyses for efficacy against *S. pneumoniae* or *S. aureus* incorporating the Phase 3 PK data and their expected global MIC distributions (based on MIC surveillance data) was conducted. Results suggest a high likelihood (probability >90%) of target attainment against the bacterial populations likely encountered by the general CABP patient population (Figure 5).

Figure 5. Predicted Cumulative Probability to Reach the 1-Log_{10} Bacterial Kill PK-PD Target on Day 1 for *S. pneumoniae* and *S. aureus* in a Virtual General CABP Patient Population by Monte Carlo Simulations



CFR = cummulative fractional response; PK = pharmacokinetic; PD = pharmacodynamic; CABP = community-acquired bacterial pneumonia; ELF = epithelial lining fluid

The modeling approach incorporated PK variability and bacterial MIC distributions observed from global SENTRY surveillance data, as well as either a single point estimate of the PK-PD target (i.e., median) or a random allocation of the PK-PD target drawn from a truncated log10-normal distribution (±2 standard deviations). Dosing regimen used was 600 mg PO LEF every 12 hr. Free-drug plasma or total-drug ELF exposure (AUC24) was determined (PPB =0.0379). The PK-PD target associated with a 1-log CFU reduction in murine models of acute pneumonia was used.

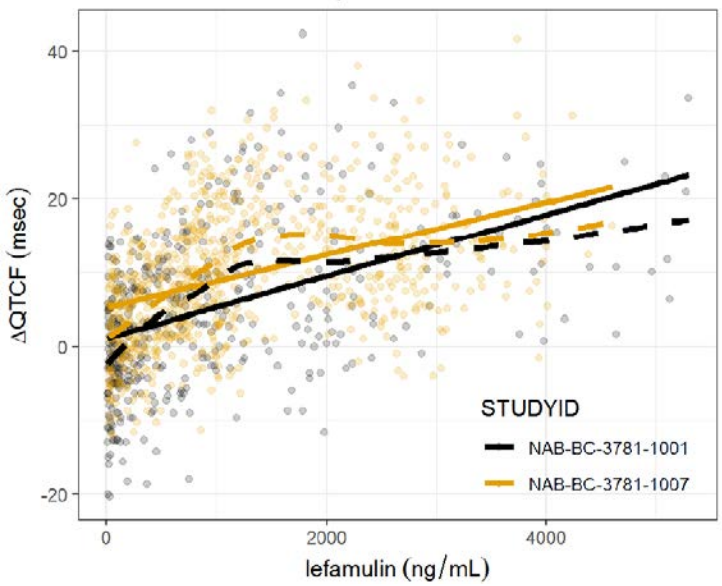
Supportive Safety Information

QT-prolongation is potentiated by LEF. The mean placebo-corrected changes in QTcF from baseline (ΔQTcF) were 13.6 ms and 9.3 ms following administration of 150 mg LEF IV infused over 1 hr q12 hr and 600 mg LEF tablets q12 hr, respectively, in the two Phase 3 trials (Studies NAB-BC-3781-3101 and 3102). LEF and moxifloxacin appear equipotent with minimal clinical risk, in terms of QT-prolongation, at clinically recommended doses. The relationship between drug concentration and ΔQTcF was evaluated by the QT-interdisciplinary review team using the data from Phase 1 Studies 1001 and 1007 (single IV doses up to 400 mg; C_{max} approx. 4.4

mcg/mL). From this analysis, a saturable nonlinear relationship between LEF concentration and ΔQTcF was observed, suggesting a ceiling effect with QT prolongation (Figure 6).

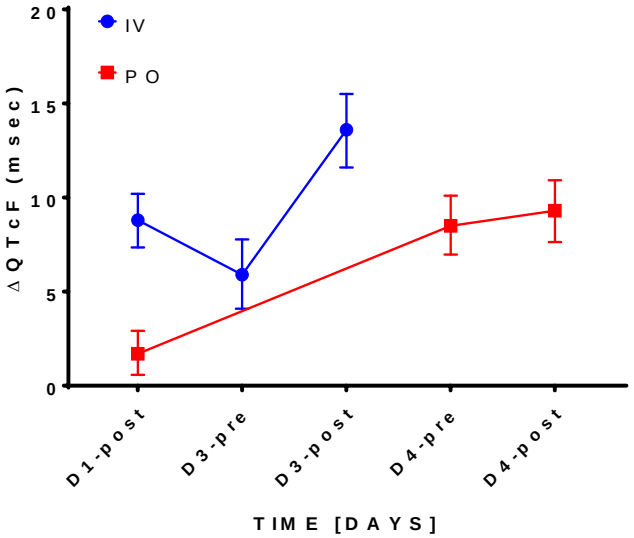
Interestingly, based on the Applicant's time-point analysis, there was a significant increase in QTcF from baseline that occurred between Day 1 and Day 3 (Figure 7). Given that LEF accumulation is minimal (approximately 20%), other PK drivers of the QT-prolongation effect such as cumulative and/or total LEF exposure (AUC) cannot be ruled out. The review team agrees with the Applicant that a warning in the proposed label (Section 5.1) along with a recommendation not to exceed the rate of infusion of the IV formulation is adequate to minimize the QT prolongation effect in the general CABP patient population.

Figure 6. Assessment of Linearity of Lefamulin Concentration-QTc Response



Data are represented as individual data (dots) and either linear or nonlinear (Emax) model-fitted lines. Note the use of ng/mL used here.
Source: QT-IRT report, Figure 6; pg 17.

Figure 7. Mean Change in QTcF From Baseline Over Time



Phase 3 IV (Trial 3101) and PO (Trial 3102) data are displayed relative to pre- or postdose administration. Day is abbreviated D. Mean and 90% confidence intervals based on the Applicant's linear mixed-effects model.
Source: Cardiac Safety Report, Table 6-2a,b, pgs 30 and 31.

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

Patients With Hepatic Impairment

We recommend XENLETA tablets not be used for patients with moderate (Child-Pugh Class B) and severe (Child-Pugh Class C) hepatic impairment. However, no dosage adjustment of XENLETA tablets is necessary for patients with mild (Child-Pugh Class A) hepatic impairment.

For XENLETA injection, a dose reduction (i.e., 150 mg every 24 hours) is recommended for patients with severe (Child-Pugh Class C) hepatic impairment. No dosage adjustment of XENLETA injection is necessary for patients with mild (Child-Pugh Class A) and moderate (Child-Pugh Class B) hepatic impairment. (b) (4)

Results from a dedicated hepatic impairment study (Study NAB-BC-3781-1010) showed similar total (bound plus unbound) LEF AUCs in adults with moderate and severe hepatic impairment compared to adults with normal hepatic function (Table 51). (b) (4) LEF PPB was reduced (Table 52) and, therefore, the unbound (biologically active) LEF AUC was increased (Table 51) in patients with moderate (~2 fold) and severe (~3 fold) hepatic impairment. Such

observations (i.e., no change in total drug concentration despite an increase in unbound drug fraction) can occur when an increase in the unbound drug fraction is offset by a decrease in intrinsic hepatic clearance. In addition, because LEF is a drug with a low extraction ratio and LEF PPB is saturable, unbound concentrations are supposed to be inversely related to intrinsic hepatic clearance and, thus, an increase in the unbound fraction and unbound concentrations of LEF may occur in patients with hepatic impairment. The LEF half-lives in subjects with moderate and severe hepatic impairment were greater compared with that in subjects with normal hepatic function, while unbound C_{max} in subjects with moderate or severe hepatic impairment are relatively comparable to that in subjects with normal hepatic function (Table 51).

Table 51. Lefamulin Exposure Across Hepatic Stages

	Normal	Moderate	Severe		
Single IV dose (mg)	150	150	150		
Total (Bound + Unbound) LEF Exposure					
$AUC_{0\text{-}inf}$ (ng*h/mL)	7,615	8,233	8,938		
C_{max} (ng/mL)	2,463	1,746	1,468		
CL (L/h)	20.5	19.6	17.4		
$t_{1/2}$ (h)	11.5	13.6	17.5	**Fold Change**	
Unbound LEF Exposure				**Mod/Norm**	**Sev/Norm**
$AUC_{0\text{-}inf}$ (ng*h/mL)	294	693	903	2.3	3
C_{max} (ng/mL)	128	180	194	1.4	1.5

The arithmetic means for subjects without pneumonia with normal hepatic function (NORMAL) or hepatic impairment (MODERATE, SEVERE) following administration of LEF injection. Unbound LEF concentrations for the NORMAL, MODERATE, and SEVERE groups were approximated by multiplying the total LEF concentrations by the plasma protein binding estimate from the time interval which the concentration fell within (0-2, 3-6, >8 hr; Table 6). Average exposures were compared to subjects with normal hepatic function (fold-change). Source: Adopted with modification from NAB-BC-3781-1010-pharmacokinetic report.
LEF = lefamulin; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; CL = total body clearance of drug from plasma; $t_{1/2}$ = half-life; C_{max} = maximum plasma concentration of drug; IV = intravenous

Table 52. LEF Plasma Protein Binding as a Function of Time After the Beginning of Infusion

	Normal (CV%)	Moderate (CV%)	Severe (CV%)
Time (h)	**N=11**	**N=8**	**N=8**
1	94.8 (1.4)	89.2 (3.6)	86.5 (3.8)
3	97.0 (0.6)	91.8 (3.1)	89.6 (2.5)
8	97.1 (0.6)	92.8 (3.1)	90.8 (3.1)

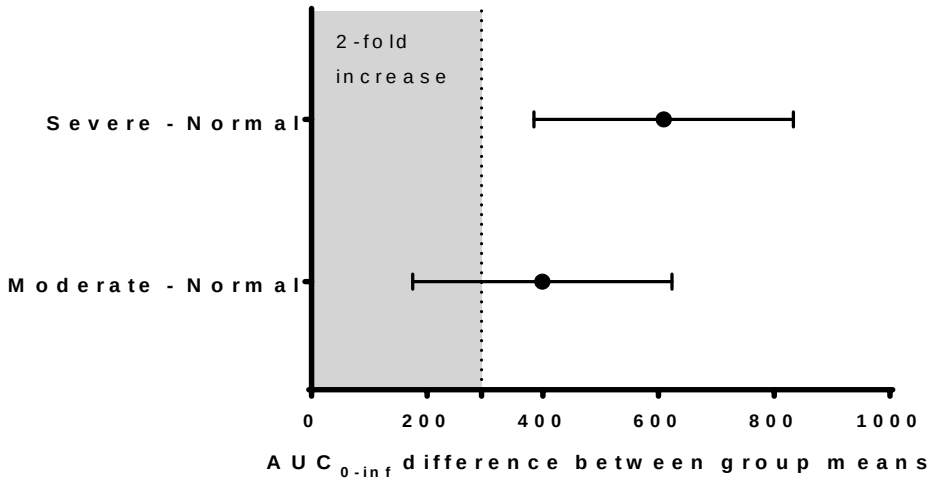
The arithmetic mean and coefficient of variation expressed as a percent (%CV) for subjects with normal hepatic function (Norm) and hepatic impairment (Mod = Child-Pugh B, Sev = Child-Pugh C).
Source: Study Report NAB-BC-3781-1010-pharmacokinetic, Table 9, pg 36.

For patients with moderate hepatic impairment, dosage adjustment of XENLETA injection does not appear to be needed because potential increases in the unbound LEF $AUC_{0\text{-}inf}$ may not be clinically significant. Although the mean unbound $AUC_{0\text{-}inf}$ in subjects with moderate hepatic impairment was approximately 2-fold greater compared with subjects with normal hepatic function, the lower bound of the 90% CI of the mean change in unbound $AUC_{0\text{-}inf}$ was less than 2-fold (Figure 8). Considering the variability of LEF exposure in CABP patients observed in Phase

3 trials and associated adverse event profiles, the extent of unbound LEF exposure in patients with moderate hepatic impairment does not appear to warrant dosage adjustment of XENLETA injection. Any risk to safety is further managed by patient hospitalization and direct clinical observation and care. Therefore, we recommend no dose adjustment of XENLETA injection for patients with moderate hepatic impairment, but those patients be treated with caution and appropriately monitored for adverse events associated with XENLETA throughout the treatment period.

However, in patients with severe hepatic impairment, the increase in mean unbound $AUC_{0\text{-}inf}$ is greater than 3-fold and the lower bound of the 90% CI of the mean change in $AUC_{0\text{-}inf}$ was greater than 2-fold (Figure 8). Because there is no clinical evidence to determine whether 3-fold higher unbound LEF concentrations is safe, dosage adjustment of XENLETA injection is needed for patients with severe hepatic impairment to manage this concern. Note that CABP patients with moderate or severe hepatic impairment were not enrolled in Phase 3 trials. Considering the prolonged LEF half-life and relatively smaller change in the unbound LEF C_{max} compared to LEF $AUC_{0\text{-}inf}$, we recommend the dosing interval for XENLETA injection be extended to every 24 hr from every 12 hr for patients with severe hepatic impairment. Patients should be treated with caution and close monitoring for adverse reactions, as well as treatment response.

Figure 8. Comparative Differences in Unbound LEF Exposure ($AUC_{0\text{-}inf}$) by Hepatic Impairment



Shown are the 95% confidence intervals and point estimates of unbound LEF $AUC_{0\text{-}inf}$ differences between adults without pneumonia with moderate hepatic impairment or normal hepatic function and adults without pneumonia with severe hepatic impairment or normal hepatic function. The gray box denotes the decision boundary defined as a 2-fold increase in $AUC_{0\text{-}inf}$ from the average unbound exposure observed in adults without pneumonia with normal hepatic function and is based on the review team's assessment of safety data.
$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration

There are not enough data to propose dosage adjustment recommendations for XENLETA tablets in moderate and severe hepatic impairment. Although a decrease in PPB and, accordingly, an increase in unbound LEF exposure related to the degree of hepatic impairment is presumed to be similar to observations following IV administration (Study NAB-BC-3781-

1010), no dedicated PK study with PO administration evaluated potential increases in LEF bioavailability. Literature suggests that hepatic impairment may reduce intestinal intrinsic drug clearance and increase intestinal permeability[5,6,7,8]. Presumably then, the effect of hepatic impairment on LEF PK following PO administration may be greater than that following IV administration considering that LEF saturates its own enzyme metabolism (CYP3A4) and P-gp efflux; both integral to LEF significant intestinal first-pass metabolism (~25% bioavailability). Therefore, the effect of hepatic impairment on the PK of lefamulin following oral administration may not be extrapolated from the effect of hepatic impairment on the PK of lefamulin following IV administration. Furthermore, because the patients receiving XENLETA tablets are more likely to be in the ambulatory setting, direct observation and care cannot be performed to help manage risks. Thus, we recommend XENLETA tablets not be used for patients with moderate or severe hepatic impairment. However, based on our judgement regarding the impact of LEF PK following IV administration to patients with moderate hepatic impairment and pathophysiologic considerations of mild hepatic impairment, we recommend that XENLETA tablets be used without dosage adjustment in patients with mild hepatic impairment.

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

Yes, there are clinically relevant food-drug or drug-drug interactions.

Food-Drug Interaction

The administration of LEF tablets with a high fat meal decreased PO LEF exposure by approximately 20% as determined by $AUC_{0\text{-}inf}$ or 30% as determined by $AUC_{0\text{-}12}$ (the dosing interval) compared to fasting conditions. The food effect on the oral bioavailability of LEF over the dosing interval is clinically relevant especially because the PTA at an MIC at or near the susceptibility breakpoint is affected substantially by small changes in drug exposure. According to the FDA's breakpoint selection for *S. pneumoniae* (0.5 mcg/mL) and *S. aureus* (0.25 mcg/mL), the review team found that the PTA is substantially affected by food intake at these MICs (Table 142; PTA <70%)**.** Thus, we recommend that XENLETA tablets be administered under the same conditions as the Phase 3 trials— 1 hour before or 2 hours after a meal (Study NAB-BC-3781-3102). Note that the review team used the above two pathogens for benchmarking food-effects because there are no nonclinical PK-PD data for other pathogens (see Section 16.3.2.5.1).

[5] Büdingen FV, Gonzalez D, Tucker AN, Derendorf H. Relevance of Liver Failure for Anti-Infective Agents: From Pharmacokinetic Alterations to Dosage Adjustments. Ther Adv Infect Dis. 2014;2(1):17-42
[6] Mcconn DJ, Lin YS, Mathisen TL, et al. Reduced duodenal cytochrome P450 3A protein expression and catalytic activity in patients with cirrhosis. Clin Pharmacol Ther. 2009;85(4):387-93.
[7] Chalasani N, Gorski JC, Patel NH, Hall SD, Galinsky RE. Hepatic and intestinal cytochrome P450 3A activity in cirrhosis: effects of transjugular intrahepatic portosystemic shunts. Hepatology. 2001;34(6):1103-8.
[8] Andersen V, Pedersen N, Larsen NE, Sonne J, Larsen S. Intestinal first pass metabolism of midazolam in liver cirrhosis --effect of grapefruit juice. Br J Clin Pharmacol. 2002;54(2):120-4.

Phase 1 PK studies were conducted to determine oral bioavailability of LEF tablets in the fed and fasted state. In Study 1107, LEF oral bioavailability was 25.8% and 21.1% in the fasted state (>8 hours fasting prior to PO LEF administration) and in the fed state (LEF administration with a high-fat meal), respectively. The average relative difference in the bioavailability between PO LEF given in the fasted and fed condition was 22.9% [90% CI: 32.3; 12.2], 18.43% [90% CI: 24.7; 11.7], and 27.57% [CI: 20.19; 34.26] for C_{max}, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}12}$, respectively suggesting that food reduces the oral bioavailability rate and extent of LEF. Study 1106 showed that the bioavailability when LEF was given 1 hr before a meal is comparable to LEF under fasting conditions (see Section 16.3.2.5.1). It is important to note that the food-effect on the bioavailability of LEF tablets was known prior to the Phase 3 study and, thus, the Applicant chose administration of LEF tablets 1 hr before or 2 hr after a meal in the Phase 3 study. Taken together, the review team recommends XENLATA tablets be administered 1 hr before or 2 hr after a meal as conducted in the Phase 3 trial.

Drug-Drug Interaction

There are PK and PD drug-drug interactions (DDIs) that pose a clinically significant risk (efficacy loss or adverse events).

PK DDIs

The review team agrees with the Applicant's proposal that concomitant use of IV LEF with strong and moderate CYP3A inducers be avoided based on a risk of loss of efficacy. In addition, the review team agrees with the Applicant's proposal that concomitant use of XENLETA tablets with strong CYP3A inhibitors — with the addition of P-gp inhibitors — be avoided because an observation of 2.6-fold increase in LEF exposure. For concomitant use of XENLETA tablets with moderate CYP3A inhibitors, the review team recommends caution and monitoring because clinical data are limited and the PBPK model was not validated to estimate the potential DDIs quantitatively. Lastly, the review team agrees with the Applicant's proposal (sections 4 and 5 of the label) that CYP3A substrates that prolong the QT interval be contraindicated. Otherwise, monitoring for adverse effects is adequate.

PD DDIs

The review team recommends that concomitant use of IV or PO LEF be avoided with Class Ia and III antiarrhythmics, antipsychotics, erythromycin, moxifloxacin, and tricyclic antidepressants that affect cardiac conduction because the potential PD interaction to prolong the QT_c interval of the electrocardiogram is unknown.

Summary of In Vitro DDI Studies

The clinical potential of LEF as a substrate, inhibitor, or inducer of membrane transporters and metabolism was assessed through in vitro studies consistent with the 2017 FDA Draft In Vitro DDI Guidance. The results suggest that LEF is: (i) a substrate of P-gp and OCT1, (ii) an inhibitor

of BCRP (gut), P-gp (gut), and MATE1, (iii) a substrate of CYP3A4, and to a lesser extent flavin containing monooxygenases (FMOs), and (iv) an inhibitor of CYP3A4. The Applicant subsequently conducted clinical DDI studies with IV and PO LEF to address DDI potential of IV and PO LEF either as a victim or perpetrator drug (see below for the results of clinical DDI studies). Note that no clinical evaluation of BCRP, MATE1, and pH-dependent DDIs was performed (see below evaluation of potential DDIs without clinical study).

Summary of Clinical PK DDI Studies With PO LEF

LEF as Victim (The Effects of Other Drugs on LEF)

- CYP3A4 and P-gp inhibitor: PO ketoconazole (strong inhibitor) increased the arithmetic mean C_{max} and $AUC_{0\text{-}inf}$ of LEF by 58% and 165%, respectively, when co-administered. There are limited data to support a >2-fold increase in LEF exposure would be safe. Therefore, the review team recommends that concomitant use of XENLETA tablets with strong CYP3A4 or P-gp inhibitors be avoided. Note that XENLETA tablets would be used mostly in out-patient settings where close monitoring for adverse events is difficult. There are no data to estimate the effect of moderate CYP3A4 and P-gp inhibitors on the PK of PO LEF. However, it is reasonable to presume that concomitant use with a moderate CYP3A4 and P-gp inhibitor may increase the LEF AUC by approximately <2-fold. Given that the duration of treatment is limited to approximately 5 to 7 days, we recommend caution and monitoring for adverse reactions for concomitant use of XENLETA tablets with moderate CYP3A4 and P-gp inhibitors, as the Applicant proposed.
- CYP3A4 and P-gp inducer: PO rifampin (strong inducer) reduced the arithmetic mean C_{max} and $AUC_{0\text{-}inf}$ of LEF by 57% and 72%, respectively, when coadministered. Because of potential efficacy loss due to low exposure of LEF, coadministration of LEF with moderate and strong CYP3A4 or P-gp inducers should be avoided.

LEF as Perpetrator (The Effects of LEF on Other Drugs)

- CYP3A4 substrate: LEF increased the arithmetic mean C_{max} and $AUC_{0\text{-}inf}$ of PO midazolam (substrate) by approximately 100% and 200%, respectively, when administered at 0, 2 or 4 hr after administration of PO LEF. The review team finds the risk to safety unacceptable with concomitant administration of PO LEF with CYP3A4 substrates (e.g., pimozide) that prolong the QTc interval. Therefore, the review team agrees with the Applicant that concomitant administration with CYP3A4 substrates that prolong the QTc interval be contraindicated. For other strong CYP3A4 substrates (e.g., alprazolam, diltiazem, verapamil), it may be needed to monitor patients closely for concentration-dependent adverse effects associated with these CYP3A substrates. The review team agrees with the Applicant's recommendation that adverse events associated with the CYP3A4 substrate be carefully monitored when administered concomitantly with XENLETA tablets. For weak CYP3A4 substrates, a potential DDI with PO LEF is judged not to be clinically significant based on an expected modest increase in exposure and relatively short duration of coadministration with XENLETA tablets (i.e., 5 to 7 days).

- P-gp substrate: Coadministation with PO LEF did not affect the exposure of PO digoxin, indicating a minimal effect of LEF on the PK of P-gp substrates although in vitro studies showed that LEF is an inhibitor of P-gp.

Summary of Clinical PK DDI Studies With IV LEF

LEF as Victim (The Effects of Other Drugs on LEF)

- CYP3A4 and P-gp inhibitor: PO ketoconazole (inhibitor) increased the arithmetic mean C_{max} and $AUC_{0\text{-}inf}$ of IV LEF 6% and 31%, respectively, when coadministered. The extent of the increase in LEF exposure is not judged to be clinically significant given the tolerability of higher LEF exposures in the clinical development program.
- CYP3A4 inducers: PO rifampin (inducer) reduced the arithmetic mean C_{max} and $AUC_{0\text{-}inf}$ of LEF by 8% and 28%, respectively, when coadministered. Strong and moderate CYP3A4 inducers should be avoided as the reduction in daily LEF exposure (AUC) will approximate the clinically relevant reduction noted for the food-effect. Because of potential efficacy loss, concomitant LEF administration with CYP3A4 inducers should be avoided.

LEF as Perpetrator (The Effects of LEF on Other Drugs)

- CYP3A4 inhibitor: The effect of LEF on the disposition of PO midazolam (CYP3A4 substrate) was minimal (i.e., <20% increase in midazolam exposure)

Evaluation of Potential DDIs Without Clinical Study

Co-administration of LEF With a MATE1 Substrate

As discussed above, in vitro findings suggest LEF is an inhibitor of the MATE1 transporter. However, the interaction between LEF and MATE1 substrates may not be clinically meaningful because most MATE1 substrates have a wide therapeutic window. The safety concern associated with an increase in exposure of MATE1 substrates, like metformin, is limited due to the short duration of LEF treatment (5 to 7 days). Meanwhile, it is recommended that coadministration of LEF with dofetilide (a MATE1 substrate with narrow therapeutic index) be contraindicated mainly because of the PD interaction (QT prolongation).

Co-administration of LEF with a BCRP Substrate

In vitro findings suggest LEF is an inhibitor of the BCRP transporter. However, the strength of the interaction between LEF and a BCRP substrate was less than that between LEF and a P-gp substrate, indicating LEF is more potent at P-gp inhibition (IC_{50}=3 mcg/mL) than BCRP inhibition (IC_{50}=21 mcg/mL). A clinical DDI study with coadministration of LEF and digoxin, a Pgp substrate, did not reveal a clinically relevant interaction. Therefore, it is not expected that LEF will inhibit BCRP to a clinically significant extent.

pH dependent Drug-Drug Interaction

Based on the formulation composition and the Applicant's Biopharmaceutics Classification System (Class 3) of the LEF tablets, gastric pH is not expected to affect LEF dissolution or absorption. In vitro dissolution data and a Phase 3 subgroup analysis also suggest that LEF absorption is not affected by gastrointestinal pH. In an in vitro dissolution study with Phase 1 600 mg IR tablets, with comparable in vitro dissolution and clinical PK profiles with the Phase 3 600 mg IR tablets, the dissolution rate is comparable at pH 1.0 and pH 6.8 (3.2.P.5 Control of Drug Product, pg 13, Fig 6). Additionally, a subgroup analysis of PK data from Phase 3 study (Study NAB-BC-3781-3102) showed that mean LEF AUC (30.3 versus 30.6 mg*h/L) and C_{max} (2.3 versus 2.2 mg/L) on Day 1 were comparable between patients who received LEF with proton pump inhibitors (PPIs) (n=34) versus patients who received LEF without PPIs (n=297), suggesting no clinically meaningful drug-drug interaction between gastric acid inhibitors such as PPIs and XENLETA tablets.

Note, the review team could not validate the Applicant's physiologically based PK (PBPK) model for use in evaluating clinical potential risks regarding pH, transporter and metabolic DDIs. See Section 16.3.2.6 for PBPK details and Section 16.3.2.2 and 16.3.1.2 for further DDI details.

6.3.2.5. Question on clinically relevant specifications

Plasma Protein Binding

We do not agree with the Applicant's LEF plasma protein binding (PPB) estimate. We find PPB of LEF to be 94% to 97% (Studies NAB-BC-3781-1010, NAB-BC-3781-1011, and XS-1103) in contrast to the Applicant's 73% to 88% (Study EVT-00756-3781). The Applicant conducted all PK-PD analyses with the LEF PPB estimate of 73% to 88% without any explanation for the discrepancies in PPB values from other studies. We found that the discrepancy could be explained by diluted plasma proteins. In Study EVT-00756-3781, LEF PPB was evaluated in 85% (v/v) plasma. In contrast, LEF PPB was evaluated with 100% (v/v) plasma (i.e., without dilution) in Studies NAB-BC-3781-1010 and NAB-BC-3781-1011. Additionally, Study XS-1103 demonstrated an increase in LEF PPB in adult or adolescent plasma compared to infant or toddler plasma —where the protein concentrations may be lower than in adult and adolescent plasma. The review team concludes that the PPB of LEF appears to be underestimated in Study EVT-00756-3781 because of dilution of plasma. Accordingly, we reconducted all PK-PD analyses with LEF PPB of 94% to 97%.

All discussions and conclusions in this review are based on the results of the PTA analyses conducted with LEF PPB of 94% to 97%. Note all studies used the same method (equilibrium dialysis). See Section 16.3.1.1 for further details on protein binding. Of note, LEF PPB in mouse was approximately 21% and 25% at 3 mcg/mL estimated with 85% (v/v) plasma or 100% serum (i.e., without dilution), respectively.

Susceptibility Breakpoint Determination: PK/PD Cutoffs

The PK-PD cutoffs for *S. pneumoniae* or *S. aureus* based on the PTA analyses ranged between 0.5 mcg/mL (median PD target approach) to 0.25 mcg/mL (PD target variability approach) and 0.25 mcg/mL (median PD target approach) to 0.125 mcg/mL (PD target variability approach), respectively (see Table 142, free-LEF plasma exposure). Together with MIC distribution and clinical response as a function of MIC, the susceptibility breakpoints for these pathogens were established (see Section 4.3 for further details). Note that these PK-PD cutoffs were established based on the LEF PPB of 94% to 97% and the recommended IV and PO LEF dosages. For the PO dosage, the PK data following administration of LEF tablets without food (i.e., 1 hour before or 2 hours after a meal) were used for the PTA analyses. It also should be noted that the PTA estimates at the MICs of 0.25 and 0.5 mcg/mL for *S. aureus* and *S. pneumoniae* were 69% and 47%, respectively, when LEF tablets are administered with food (median PD target approach).

We note that the cumulative fractional response (CFR) (overall expectation) in the general patient population is reasonably high (probability >0.9) when considering the expected MIC distribution in this patient population for either bacterial species. However, from a labeling standpoint, the decision to recommend (b) (4) was based on the susceptibility interpretive criteria or breakpoint. This breakpoint separates strains with high versus low likelihood of treatment success based on LEF concentrations (MICs) which are helpful when guiding therapy for the individual. In addition, it is not sensitive to changes in resistance patterns over time (MIC creep); a limitation of the CFR approach. See Section 16.3.2.5 for further details regarding probability of target attainment (PTA) methods.

Comparison Between Phase 3 Tablet and To-Be-Marketed Tablet

The Clinical Pharmacology reviewer agrees with the Biopharmaceutics reviewer that the Phase 3 immediate release (IR) tablets and final commercial image tablets (to-be-marketed) are adequately bridged. Tablet composition, manufacturing process, and manufacturers remain the same; only a change in appearance (color and imprint) was made (Table 53). In vitro dissolution testing demonstrates that the two dissolution profiles were similar (f_2>50). Please see the Biopharmaceutics review (part of the CMC quality assessment) for further details.

Table 53. Composition Comparison Between Phase 3 LEF Tablets and To-Be-Marketed LEF Tablets

	Phase 3 Tablet (mg/tablet)	To-Be-Marketed Tablet (mg/tablet)
Manufacturer	(b) (4)	(b) (4)
(b) (4)		
Lefamulin acetate	671	671
Lefamulin free base	600	600
Mannitol	(b) (4)	(b) (4)
Povidone K30		

	Phase 3 Tablet (mg/tablet)	To-Be-Marketed Tablet (mg/tablet)
(b) (4)		
Microcrystalline cellulose Croscarmellose sodium Talc Colloidal silicon dioxide Magnesium stearate	(b) (4)	(b) (4)
Coating		
Opadry II	(b) (4) yellow	(b) (4) blue
Printing		
Opacode monogramming ink black	-----	(b) (4)
Total	1030	1030
Batch size	(b) (4) kg	(b) (4) kg
Tablet dimensions	19.0 x 10.5 mm	19.6 x 9.5 mm
Granulation process	(b) (4)	

LEF = lefamulin
Source: Summary of Biopharmaceutic Studies and Associated Analytical Methods Report, Table 5, pg 12

7 Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 54. Listing of Clinical Trials

Trial Identity	NCT No.	Trial Design	Regimen/Schedule/Route/ Treatment Duration	Study Endpoints	Follow Up	No. of Subjects Enrolled	Study Population	No. of Centers and Countries
Controlled Studies to Support Efficacy and Safety								
Study 3101	NCT 02559310	Phase 3, randomized, double-blind, double-dummy, active-control, noninferiority	Investigational drug: Lefamulin 150 mg IV q12h for at least 3 days; optional switch to 600 mg PO q12h to complete 5–10 days total Comparator: Moxifloxacin 400 mg IV q24h for at least 3 days; optional switch to 400 mg PO q24h to complete 7–10 days total	Percentage of subjects with Early Clinical Response at 96 +/- 24 hours after the first dose of study drug in the ITT population	27–34 days	551 (276 in LEF arm; 275 in MOX arm)	Adult patients with PORT III-V CABP	66 study sites in 18 countries
Study 3102	NCT 02813694	Phase 3, randomized, double-blind, double-dummy, active-control, noninferiority	Investigational drug: Lefamulin 600 mg PO q12h for 5 days Comparator: moxifloxacin 400 mg PO q24h for 7 days	Percentage of subjects with Early Clinical Response at 96 +/- 24 hours after the first dose of study drug in the ITT population	27–34 days	738 (370 in LEF arm; 368 in MOX arm)	Adult patients with PORT II-IV CABP	99 study sites in 19 countries

Trial Identity	NCT No.	Trial Design	Regimen/Schedule/Route/ Treatment Duration	Study Endpoints	Follow Up	No. of Subjects Enrolled	Study Population	No. of Centers and Countries
Studies to Support Safety								
Study 2001	NCT 01119105	Phase 2, randomized, double-blind, active-control	Investigational drug: Lefamulin 100 mg or 150 mg IV q12h for 5–14 days Comparator: vancomycin 1 g q12h for 5–14 days	Clinical success rate at TOC visit (7–14 days after final dose of study drug) in the CE and MITT populations	30 days post final treatment	210 (72 in LEF 150 mg arm; 70 in LEF 100 mg arm; 68 in vancomycin arm)	Adults patients with ABSSSI	20 study sites in the United States

LEF = lefamulin; PORT = Pneumonia Outcomes Research Team; CABP = community-acquired bacterial pneumonia; PO = by mouth; ABSSSI = Acute Bacterial Skin and Skin Structure Infections; MOX = moxifloxacin; ITT = intent-to-treat; TOC = test-of-cure; MITT = modified intent-to-treat

7.2. Review Strategy

The review of clinical efficacy and safety of lefamulin for the indication of CABP was conducted using Studies 3101 and 3102 (Table 54). Supplementary safety data were obtained from Study 2001 in ABSSSI. In addition to confirming the efficacy and safety analyses conducted by the Applicant, the clinical and statistical reviewers also conducted additional exploratory safety analyses, particularly regarding cases of pneumonia and other lung infections in lefamulin subjects.

8 Statistical and Clinical Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Trial 3101 – Study Design

Trial Design

This was a Phase 3 multicenter, multinational, double-blind, double-dummy, randomized noninferiority trial to evaluate the efficacy and safety of lefamulin versus moxifloxacin for the treatment of adults with CABP. 551 subjects with CABP in 66 centers were randomized to the lefamulin versus moxifloxacin arms in a 1:1 ratio within randomization strata defined by geographic region (U.S. versus non-U.S.), prior use or not of a single dose of a short-acting antibacterial drug, and Pneumonia Outcomes Research Team (PORT) risk class (III versus IV/V). Enrollment of subjects using prior short-acting antibacterial drugs was capped at 25% and enrollment of subjects with a PORT risk class of III was capped at 75%.

Subjects with CABP that was not caused by MRSA received 7 days of study medication, the first 3 days administered via IV and the remaining 4 days by IV or oral administration. Subjects in the lefamulin arm receiving IV medication got 150 mg every 12 hours and those receiving oral medication got 600 mg every 12 hours (plus moxifloxacin placebo every 24 hours). Subjects in the moxifloxacin arm receiving IV medication got 400 mg every 24 hours (plus IV lefamulin placebo 12 hours after each administration of IV moxifloxacin) and those receiving oral medication got 400 mg every 24 hours (plus lefamulin placebo every 12 hours).

Subjects with CABP that was caused by MRSA were to receive 10 days of study medication, the first 3 days administered via IV and the remaining 7 days by IV or oral administration. Subjects were to be dosed similarly as described above, except that moxifloxacin subjects also received 600 mg linezolid every 12 hours over the 10 days, administered either IV or orally. Lefamulin subjects were to receive a placebo linezolid. However, no subjects with CABP due to MRSA were enrolled.

Study visits were scheduled at baseline, at 96 +/-24 hours after the first dose of study drug (early clinical assessment, or ECA), within 2 days after the last dose of study drug (end of treatment, or EOT), at 5 days to 10 days after the last dose of study drug (test of cure, or TOC), and between study days 27 to 34 inclusive (late follow up, or LFU).

When ECA symptom data were obtained for about 330 subjects, an interim analysis to perform a blinded sample size re-estimation was to be conducted. This could not lead to decreasing the initial sample size of 550 but could lead to an increase up to as many as 626 subjects.

Key inclusion criteria include:

- Age ≥18 years
- Acute illness with at least three symptoms of CABP
  - Dyspnea
  - Cough
  - Purulent sputum production
  - Chest pain
- At least two vital sign abnormalities
  - Body temperature >38°C or <35°C
  - Systolic blood pressure <90 mmHg
  - Heart rate >100 beats/min
  - Respiratory rate >20 breaths/min
- At least one other clinical or laboratory finding of CABP
  - Oxygen saturation <90% on room air or PaO2<60 mmHg
  - Auscultatory or percussion findings consistent with pneumonia
  - WBC count >10,000 cells/mm^3 or <4500 cells/mm^3, or >15% bands
- Evidence of pneumonia on chest x-ray or CT scan
- PORT Risk Class ≥ III and require IV antibacterial therapy as initial treatment for the current episode of CABP.

***M.O. Comment**: The inclusion criteria follow the draft CABP guidance and are similar to other trials in the treatment of CABP.*

Key exclusion criteria include:

- Receipt of more than a single dose of a short-acting antibacterial drug within 72 hours before randomization
- Have risk for major cardiac events (QT prolongation, unstable cardiac disease, recent receipt of Class IA or Class III anti-arrhythmic medications)
- Concomitant treatment with a strong p-glycoprotein inhibitor or strong CYP3A inducer or inhibitor
- Creatinine clearance ≤30 mL/min

M.O. Comment*: The inclusion/exclusion criteria were acceptable.*

Study Endpoints

The Applicant defined a primary endpoint and several secondary endpoints. The definitions are consistent with the CABP guidance.

Intention-to-Treat Analysis Populations

Intention-to-treat (ITT) analysis set includes all randomized subjects (whether or not any study drug was administered).

Modified Intention-to-treat (mITT) analysis set includes all randomized subjects who received any study drug.

Microbiological ITT (microITT) analysis set includes all subjects in the ITT set who have at least one CABP-causing pathogen at baseline.

Efficacy Endpoints

Primary endpoint

Early clinical response (*ECR*): This is a binary variable indicating whether a subject is a responder at 96+/-24 hours after the first dose of study drug. As a primary endpoint, this is assessed in the ITT analysis set.

Responder must satisfy all four bullet points, otherwise is a nonresponder.

- Alive by time for assessment of 4 symptoms (dyspnea, cough, production of purulent sputum, chest pain).

- Improvement in at least 2 of 4 symptoms (decrease of at least one level of severity).
- No worsening in any of the 4 symptoms (increase of at least one level of severity).
- Did not receive a concomitant antibacterial drug for treatment of CABP by time of assessment.

Subjects with missing data such that response/lack-of-response cannot be determined are considered to have an indeterminate response. Subjects who did not have at least 2 of the 4 symptoms at baseline are also considered to have an indeterminate response (this did not occur in the study).

Secondary endpoints assessed on intention-to-treat populations

- *Investigator's Assessment of Clinical Response* (*IACR*) at TOC in the mITT analysis set. *IACR success*: subject's clinical signs and symptoms have resolved or improved so that no additional antibacterial therapy is administered for the current CABP episode. *IACR failure*: death from any cause OR administration of nonstudy antibacterial therapy due to lack of improvement in (i) CABP signs/symptoms, (ii) measures of inflammation, or (iii) bacteremia, OR administration of nonstudy antibacterial therapy due to occurrence of an adverse event requiring discontinuation of study drug. *IACR indeterminate*: insufficient information available to determine success or failure, specifically lost to follow-up.
- *ECR* in the microITT analysis set.
- *ECR plus improvement in vital signs* in the ITT analysis set. More specifically:
- All vital signs that were abnormal at baseline return to normal.
- All vital signs that were normal at baseline do not worsen.
- *IACR* at TOC in the microITT analysis set.
- *By-pathogen microbiological response* at TOC in the microITT analysis set. *Success*: eradication OR presumed eradication. *Failure*: persistence OR presumed persistence. *Indeterminate*: IACR at TOC indeterminate and culture not repeated at TOC and no cultures demonstrated persistence between EOT and TOC. The values *eradication* and *persistence* are based on analyses of cultures obtained between EOT and TOC indicating that the baseline pathogen is absent or persistent, respectively. The values *presumed eradication* and *presumed persistence* are assigned in the absence of repeat cultures, and are based on whether the IACR at TOC is success or failure.
- *All-cause mortality (ACM)* through day 28 in the ITT analysis set.

Statistical reviewer comment*: Other than a small number of indeterminate responses, all values for by-pathogen microbiological response were either "presumed eradication" or "presumed persistence." Hence, these values were determined from the IACR at TOC rather than from any repeat cultures. In the following, therefore, we refer to "by-pathogen IACR response at TOC" rather than "by-pathogen microbiological response at TOC."*

Statistical Analysis Plan

Interim Analysis

The blinded sample size re-estimation analysis noted above is performed by the independent interim analysis committee (IAC) when ECR data have been obtained for 330 ITT analysis set subjects. The overall ECR response rate is computed (pooled across arms), and Table 2 in the IAC charter is referenced to determine the appropriate sample size. This table indicates, given the observed overall ECR response rate, what total sample size would be needed to provide 90% power for a continuity-corrected z-test of noninferiority for the primary efficacy endpoint (see below), under the assumption that both arms have the same ECR response rate. Per the table, if the overall ECR response rate is 74% or greater, then the proposed sample size of 550 suffices; if this rate is 73%, then an increase to a sample size of 562 is needed; and so on. If the overall ECR response rate is at least 67%, then a sample size of 626 or smaller suffices. If the overall ECR response rate is lower than 67%, then the protocol might be amended to ensure an appropriately large sample size.

Analysis of Primary Efficacy Endpoint

The Applicant proposed a one-sided continuity-corrected z-test to test the noninferiority of lefamulin to moxifloxacin. The null and alternative hypotheses are, respectively, H0: *p1 – p2 <= -.125* versus H1: *p1 – p2 > -.125*, where *p1* is the true success rate for the lefamulin arm, *p2* is the true success rate for the moxifloxacin arm, and the noninferiority margin is 12.5%. That is, H0 states that the lefamulin success rate is at least 12.5% smaller than the moxifloxacin success rate, and H1 states that any lefamulin-versus-moxifloxacin success rate deficit is less extreme than 12.5%. We conclude that lefamulin is noninferior to moxifloxacin if H0 is rejected.[9] This z-test rejects H0 when z >1.96. Equivalently, one can perform the noninferiority test by computing the corresponding 2-sided continuity-corrected 95% confidence interval and rejecting H0 if the interval's lower bound is larger than -.125.

The Applicant also specified several sensitivity analyses. These include:

- Repeating the just-described noninferiority test, but handling missing observations differently than in the primary analysis (see below), by treating them as ECR responders.
- A covariate-adjusted noninferiority analysis via Miettinen and Nurminen 95% confidence intervals, stratifying by the randomization stratum a subject was randomized to and using Cochran-Mantel-Haenszel stratum weights.

[9] Technical note: For formulas for the continuity-corrected z-test and confidence interval, see Fleiss, Levine, and Paik (2013, chapter 3).

Analysis of Secondary Efficacy Endpoints

- *Investigator's Assessment of Clinical Response* (*IACR*) at TOC in the mITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in success rates will be computed using a continuity-corrected z-test.
- *ECR* in the microITT analysis set: two-sided unadjusted 95% confidence intervals for the difference in responder rates will be computed using a continuity-corrected z-test.
- *ECR plus improvement in vital signs* in the ITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in responder rates will be computed using a continuity-corrected z-test.
- *IACR* at TOC in the microITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in success rates will be computed using a continuity-corrected z-test.
- *By-pathogen microbiological response* at TOC in the microITT analysis set: arm-specific response proportions will be computed.
- *All-cause mortality (ACM)* through day 28 in the ITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in survival rates will be computed using a continuity-corrected z-test.

Handling Missing Data

For the ECR endpoint, if any of the four components is missing (unless subject dies or is deemed a failure prior to this time point), OR if the subject does not have at least two of the four cardinal symptoms of CABP at baseline, then ECR is defined as indeterminate. In data analyses of the primary endpoint and of secondary endpoints involving ECR, indeterminate values are treated as failures.

For the IACR endpoint, a missing IACR at TOC is considered indeterminate, unless IACR at EOT is failure, in which case IACR at TOC is also considered failure. In data analyses of IACR at TOC, indeterminate values are treated as failure.

For by-pathogen IACR response, an indeterminate value is treated as a failure. ACM missing values will not be imputed and only observed values used in data analyses.

Statistics reviewer comment*: Regarding the ACM endpoint, it is valuable to consider treating missing values as deaths. Analyses using this approach to missing data are presented below.*

Handling Familywise Type I Error

None of the secondary efficacy endpoints are analyzed via hypothesis tests, and hence no adjustment for multiple testing is made.

Protocol Amendments

The original protocol was finalized in July 2015, and the first subject was enrolled in February 2016. There were two important protocol amendments, both implemented in March 2016:

- The noninferiority margin for the primary endpoint ECR was increased from 10% to 12.5%, allowing a consequent decrease in planned sample size from 738 to 550. The 12.5% noninferiority margin accords with the suggested margin in the CABP guidance.
- In the original protocol, subjects with CABP caused by MRSA, *S. pneumoniae* with bacteremia or *Legionella pneumophila* also were to receive 10 days of active treatment. All other subjects were to receive either 5 days of active treatment (lefamulin arm) or 7 days of active treatment (moxifloxacin arm). The protocol amendment simplified the treatment scenarios to decrease the burden on study sites and reduce the risk of medication errors. In the protocol amendment, all subjects with CABP not caused by MRSA were to receive 7 days of active treatment.

8.1.2. Trial 3101 - Study Results

Compliance With Good Clinical Practices

The Applicant states in the clinical study report that, "This clinical study was conducted in compliance with the protocol, ethical principles that have their origin in the Declaration of Helsinki..., the guidelines of International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) (CPMP/ICH/135/95), ... and Code of Federal Regulation Title 21, Parts 50, 56 and 312..."

Financial Disclosure

The Applicant certified that none of the investigators for Trial 3101 had any disclosable financial interests or arrangements with the Sponsor.

Patient Disposition

The following table presents the composition of the three intention-to-treat analysis sets by arm.

Table 55. Trial 3101: Composition of Intention-to-Treat Analysis Sets

Analysis Set	Lefamulin	Moxifloxacin
ITT	276	275
mITT	273	273
microITT	159	159

Notes: ITT analysis set includes all randomized subjects. mITT analysis set includes all randomized subjects who received any study drug. microITT analysis set includes members of the ITT analysis set who were infected with at least one CABP-causing pathogen at baseline. No subjects had pathogens resistant to moxifloxacin.
ITT = intent-to-treat; mITT = modified intent-to-treat

The next table presents the per-arm proportions of subjects who withdrew from the study or discontinued treatment.

Table 56. Trial 3101: Study Withdrawals and Treatment Discontinuations in the ITT Analysis Set

	Lefamulin	Moxifloxacin
Premature withdrawal from study	27/276 (9.8%)	19/275 (6.9%)
Did not complete ECA visit	9/276 (3.3%)	14/275 (5.1%)
Did not complete TOC visit	16/276 (5.8%)	11/275 (4.0%)
Reason for premature withdrawal		
Lost to follow-up	5/276 (1.8%)	3/275 (1.1%)
Withdrawal by subject	13/276 (4.7%)	9/275 (3.3%)
Physician decision	2/276 (0.7%)	1/275 (0.4%)
Sponsor decision	0/276 (0.0%)	1/275 (0.4%)
Death	4/276 (1.4%)	3/275 (1.1%)
Other	3/276 (1.1%)	2/275 (0.7%)
Premature discontinuation from study drug	29/276 (10.5%)	27/275 (9.8%)
Reason for premature discontinuation		
Adverse event	8/276 (2.9%)	11/275 (4.0%)
Lack of efficacy	5/276 (1.8%)	4/275 (1.5%)
Lost to follow-up	1/276 (0.4%)	0/275 (0.0%)
Physician decision	1/276 (0.4%)	1/275 (0.4%)
Sponsor decision	2/276 (0.7%)	1/275 (0.4%)
Withdrawal by subject	8/276 (2.9%)	7/275 (2.5%)
Randomized but did not receive study drug	3/276 (1.1%)	2/275 (0.7%)
Other	1/276 (0.4%)	1/275 (0.4%)

ECA = early clinical assessment; TOC = test-of-cure; ITT = intent-to-treat

There were 2.9% more study withdrawals in the lefamulin arm than in the moxifloxacin arm (9.8% versus 6.9%), but the breakdowns by reason for withdrawal were quite similar. There were 0.7% more study drug discontinuations in the lefamulin than the moxifloxacin arm (10.5% versus 9.8%), and again the breakdowns by reason were very similar.

Protocol Violations/Deviations

The following table documents the significant protocol deviations by arm. Per the CSR, a *significant protocol deviation* has the potential to affect efficacy assessments, placement into

analysis populations, ability to monitor safety, or the study's scientific value. *CE-analysis-set excluding protocol deviations* are considered more serious and are detailed in the table.

Table 57. Trial 3101: Significant Protocol Deviations in ITT Analysis Set

	Lefamulin	Moxifloxacin
Subjects with a significant protocol deviation[a]	146/276 (52.9%)	149/275 (54.2%)
Subjects with a significant deviation that excludes them from the CE analysis sets[b]	42/276 (15.2%)	40/275 (14.5%)
Type of CE-analysis-sets-excluding protocol deviation[b]		
Accidental unblinding	0/276 (0.0%)	2/275 (0.7%)
Exclusion criteria	4/276 (1.4%)	5/275 (1.8%)
Inclusion criteria	4/276 (1.4%)	4/275 (1.5%)
Study procedures/assessments	34/276 (12.3%)	30/275 (10.9%)

Notes: A *significant* deviation has the potential to affect efficacy assessments, placement into analysis populations, ability to monitor safety, or the study's scientific value. The CE (clinically evaluable) analysis sets (CE-EOT, CE-TOC, and CE-LFU analysis sets) include subjects in the ITT analysis set who (i) meet key inclusion criteria, (ii) received at least the prespecified minimal intended dose of study drug, (iii) do not have an indeterminate response on the IACR at EOT/TOC/LFU, (iv) did not receive concomitant antibacterial therapy that is potentially effective against CABP pathogens through EOT/TOC/LFU, and (v) had no other confounding factors that interfere with endpoint assessment.
[a] There were a total of 528 significant protocol deviations (254 lefamulin, 274 moxifloxacin); the table gives the number of subjects with at least 1 such deviation.
[b] There were a total of 86 CE-analysis-set-excluding deviations (44 lefamulin, 42 moxifloxacin); the table gives the number of subjects with at least 1 such deviation. These deviations are considered more consequential than other protocol deviations.
CE = clinically evaluable; ITT = intent-to-treat

The most common types of significant protocol deviations involved study procedures and assessments (88 subjects in lefamulin arm, 84 in moxifloxacin arm; most common were LFU visit out of window and OP swab not done), assignment to incorrect randomization strata (27 in lefamulin, 27 in moxifloxacin), exclusion criteria (30 in lefamulin arm, 21 in moxifloxacin arm), and study treatment administration (17 in lefamulin arm, 33 in moxifloxacin arm). There were 22 subjects who used prohibited medications (14 lefamulin, 8 moxifloxacin), but none used prohibited antibacterials and none of the prohibited uses were CE-analysis-set excluding. The most common type of CE-analysis-set excluding deviation involved study procedures or assessments, and most of these involved subjects whose LFU visit occurred out-of-window (28 in the lefamulin arm, 19 in the moxifloxacin arm). Note that out-of-window LFU visits do not compromise the validity of the primary or secondary endpoints.

Demographic Characteristics

The following table examines baseline balance between the lefamulin and moxifloxacin arms on demographic characteristics.

Table 58. Trial 3101: Demographic Characteristics of the ITT Analysis Set

Demographic Parameters	Lefamulin (N=276) n (%)	Moxifloxacin (N=275) n (%)	Standardized Difference[1]
Sex			
Male	170 (61.6)	160 (58.2)	0.07
Female	106 (38.4)	115 (41.8)	-0.07
Age			
Mean years (SD)	61.0 (16.3)	59.6 (14.9)	0.09
Median (years)	64	61	NA
Min, max (years)	19,91	20,90	NA
Age group			
<65 years	144 (52.2)	167 (60.7)	-0.17
≥65 years	132 (47.8)	108 (39.3)	0.17
Race			
White	239 (86.6)	239 (86.9)	-0.01
Black or African American	11 (4.0)	12 (4.4)	-0.02
Asian	24 (8.7)	20 (7.3)	0.05
American Indian or Alaska Native	0 (0.0)	1 (0.4)	NA
Native Hawaiian or other Pacific Islander	0 (0.0)	0 (0.0)	NA
Other	2 (0.7)	3 (1.1)	-0.04
Ethnicity			
Hispanic or Latino	8 (2.9)	10 (3.6)	-0.04
Not Hispanic or Latino	268 (97.1)	265 (96.4)	0.04
Region			
North America[2]	2 (0.7)	1 (0.4)	0.05
Latin America	4 (1.4)	10 (3.6)	-0.14
Eastern Europe	218 (79.0)	217 (78.9)	0.00
Western Europe	17 (6.2)	14 (5.1)	0.05
Rest of the world	35 (12.7)	33 (12.0)	0.02

[1] The standardized difference is the difference between the means in the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled standard deviation term. It gives the effect size difference between the two arms.
[2] All 3 North American participants were from the United States.
NA = not applicable; ITT = intent-to-treat; SD = standard deviation

The largest standardized baseline difference between the two arms was on age group, as the lefamulin arm had a larger proportion of subjects who were age 65 or older (47.8% versus 39.3%).

Other Baseline Characteristics (e.g., Disease Characteristics, Important Concomitant Drugs)

The following table examines baseline balance between the lefamulin and moxifloxacin arms on health status characteristics.

Table 59. Trial 3101: Baseline Health Status of the ITT Analysis Set

Health Status Parameters	Lefamulin (N=276) n (%)	Moxifloxacin (N=275) n (%)	Standardized Difference[1]
PORT class[2]			
II	0 (0.0)	1 (0.4)	-0.09
III	196 (71.0)	201 (73.1)	-0.05
IV	76 (27.5)	70 (25.5)	0.05
V	4 (1.4)	3 (1.1)	0.03
Prior antibacterial drug use			
Yes	71 (25.7)	71 (25.8)	0.00
No	205 (74.3)	204 (74.2)	0.00
Baseline pathogen detected[3]			
Yes	159 (57.6)	159 (57.8)	0.00
No	117 (42.4)	116 (42.2)	0.00
Respiratory disease			
Yes	60 (21.7)	49 (17.8)	0.10
No	216 (78.3)	226 (82.2)	-0.10
Renal impairment[4]			
Normal functioning	121 (44.2)	134 (48.9)	-0.10
Mild impairment	89 (32.5)	75 (27.4)	0.11
Moderate impairment	61 (22.3)	62 (22.6)	-0.01
Severe impairment	3 (1.1)	3 (1.1)	0.00
Heart disease			
Yes	64 (23.2)	63 (22.9)	0.01
No	212 (76.8)	212 (77.1)	-0.01

[1] The standardized difference is the difference between the means in the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled standard deviation term. It gives the effect size difference between the two arms.
[2] This trial intended to only include subjects from PORT classes III, IV, and V.
[3] No subjects were infected with MRSA.
[4] Three subjects had missing data. They are not included in computations of percentages or the standardized difference.
ITT = intent-to-treat; PORT = Pneumonia Outcomes Research Team

The largest standardized baseline differences between the two arms were with regard to the presence of respiratory disease and the presence of renal impairment. A larger proportion of subjects in the lefamulin arm suffered from respiratory disease (21.7% versus 17.8%), and similarly a larger proportion of lefamulin subjects had mild renal impairment (32.5% versus 27.4%).

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The following table documents the extent of study drug noncompliance in the mITT analysis set. The Applicant defined noncompliance as either using less than 90% of the intended total dose or using greater than 100% of the intended total dose.

Table 60. Trial 3101: Study Drug Treatment Non-Compliance in the mITT Analysis Set

Study Drug	Lefamulin (N=273)	Moxifloxacin (N=273)
Intravenous	5/273 (1.8%)	7/273 (2.6%)
Oral	9/104 (8.7%)	9/121 (7.4%)
Intravenous or oral	13/273 (4.8%)	14/273 (5.1%)

mITT = modified intent-to-treat

All participants in the mITT analysis set started with IV study drug. In the lefamulin arm, 104 of 273 participants (38.1%) switched to oral medication at some point during treatment, and in the moxifloxacin arm, 121 of 273 participants (44.3%) switched at some point. In both arms, most of the noncompliance occurred prior to the protocol amendment that simplified the treatment regimens (described above). In the lefamulin arm, 10 of the 13 participants with intravenous or oral noncompliance were enrolled prior to the protocol amendment, though only 70 of 273 (25.6%) participants were enrolled preamendment. In the moxifloxacin arm, 8 of the 14 participants with intravenous or oral noncompliance were enrolled prior to the protocol amendment, though only 70 of 273 (25.6%) participants were enrolled preamendment.

The following table provides a high-level overview of the use of concomitant medications after study entry.

Table 61. Trial 3101: Post Study Entry Concomitant Medication Use in the ITT Analysis Set

Drug Category	Lefamulin	Moxifloxacin
Antibacterials for systemic use	47/276 (17.0%)	43/275 (15.6%)
Other anti-infectives for systemic use	16/276 (5.8%)	11/275 (4.0%)
Alimentary tract and metabolism	57/276 (20.7%)	77/275 (28.0%)
Antineoplastic and immunomodulating agents	1/276 (0.4%)	3/275 (1.1%)
Blood and blood forming agents	33/276 (12.0%)	39/275 (14.2%)
Cardiovascular system	39/276 (14.1%)	43/275 (15.6%)
Dermatologicals	4/276 (1.4%)	2/275 (0.7%)
Genito urinary system and sex hormones	0/276 (0.0%)	1/275 (0.4%)
Musculoskeletal system	18/276 (6.5%)	14/275 (5.1%)
Nervous system	30/276 (10.9%)	31/275 (11.3%)
Respiratory system	65/276 (23.6%)	34/275 (12.4%)
Sensory organs	0/276 (0.0%)	1/275 (0.4%)
Systemic hormonal preparations (excluding sex hormones and insulins)	19/276 (6.9%)	18/275 (6.5%)
Other	14/276 (5.1%)	3/275 (1.1%)

There were 1133 uses of post study entry concomitant medication (584 lefamulin, 549 moxifloxacin). There were 301 subjects who used post study entry concomitant medications (155/276 lefamulin (56.2%), 146/275 moxifloxacin (53.1%)).
ITT = intent-to-treat

The largest differences in between-arm concomitant medication usage rates are in medications targeting alimentary tract and metabolism problems (20.7% lefamulin versus 28.0% moxifloxacin) and those targeting respiratory problems (23.6% lefamulin versus 12.4% moxifloxacin). The alimentary tract medication difference is mostly due to use of antidiarrheals and intestinal anti-inflammatory/anti-infective agents (six subjects in lefamulin arm versus 25 in

moxifloxacin arm). Regarding the respiratory system medication difference, recall, per Table 59 above, that the lefamulin arm had a somewhat higher baseline rate of respiratory disease than the moxifloxacin arm. The difference in use of respiratory system medications was largely due to drugs for obstructive airway diseases (37 subjects in the lefamulin arm, 18 in moxifloxacin arm) and cough and cold preparations (28 in lefamulin arm, 23 in moxifloxacin arm).

M.O. Comment*: In Trial 3101, there were more moxifloxacin subjects with diarrhea as an adverse event compared to lefamulin subjects which likely explains the imbalance in antidiarrheal medication use. Regarding the respiratory system medication use imbalance, inhalers and other drugs for COPD accounted for most of the difference. As there were more subjects with underlying respiratory disease in the lefamulin arm at baseline, this imbalance is not surprising.*

The next table provides additional detail on the use of concomitant systemic antibacterial medication. Recall that the usage rates were 17.0% in the lefamulin arm versus 15.6% in the moxifloxacin arm.

Table 62. Trial 3101: Post-Study Entry Concomitant Systemic Antibacterial Medication Use in the ITT Analysis Set

	Lefamulin	Moxifloxacin
Reason for use		
Concomitant infection, unrelated to CABP	7/276 (2.5%)	7/275 (2.5%)
Insufficient therapeutic effect of study drug	32/276 (11.6%)	27/275 (9.8%)
Treatment limiting AE resulting in discontinuation of study drug	4/276 (1.4%)	7/275 (2.5%)
Other	7/276 (2.5%)	2/275 (0.7%)
Antibacterial category		
Aminoglycoside antibacterials	9/276 (3.3%)	4/275 (1.5%)
Beta-lactam antibacterials, penicillins	11/276 (4.0%)	4/275 (1.5%)
Other beta-lactam antibacterials	20/276 (7.2%)	24/275 (8.7%)
Macrolides, lincosamides, and streptogramins	11/276 (4.0%)	6/275 (2.2%)
Quinolone antibacterials	26/276 (9.4%)	14/275 (5.1%)
Sulfonamides and trimethoprim	0/276 (0.0%)	2/275 (0.7%)
Tetracyclines	3/276 (1.1%)	1/275 (0.4%)
Combinations of antibacterials	2/276 (0.7%)	1/275 (0.4%)
Other antibacterials	4/276 (1.4%)	8/275 (2.9%)

Notes: There were 179 prescriptions for post study entry concomitant systemic antibacterial medication (106 lefamulin, 73 moxifloxacin). There were 90 subjects who used post study entry concomitant systemic antibacterial medications (47/276 lefamulin (17.0%), 43/275 moxifloxacin (15.6%)).
CABP = community-acquired bacterial pneumonia; AE = adverse event; ITT = intent-to-treat

Rescue antibacterial medication (due to insufficient therapeutic effect of study drug or due to treatment-limiting adverse events resulting in discontinuation of study drug) was administered to 36 subjects in the lefamulin arm (13.0%) and 34 subjects in the moxifloxacin arm (12.4%).

M.O. Comment*: Non-study antibacterial drug use was balanced between the study arms and was most commonly administered for lack of efficacy.*

Results of the Interim Analysis

The interim analysis committee concluded that no modification of the initial sample size was needed.

Efficacy Results – Primary Endpoint

The table below presents results of the analysis of the primary efficacy endpoint, ECR, on the ITT analysis set.

Table 63. Trial 3101: Results of Analyses of Early Clinical Response (ECR) in ITT Analysis Set

Version of ECR	Estimated Lefamulin Response Rate (# Successes/Arm Size)	Estimated Moxifloxacin Response Rate (# Successes/Arm Size)	Estimated Difference in Response Rates	95% Confidence Interval
Applicant	87.3% (241/276)	90.2% (248/275)	-2.9%	(-8.5, 2.8)
Worst case	87.3% (241/276)	92.4% (254/275)	-5.0%	(-10.4, 0.3)

The ECR data contained 6 indeterminate responses in the lefamulin arm (2.2%) and 6 indeterminate responses in the moxifloxacin arm (2.2%). In *Applicant* version of ECR, all indeterminate ECR values are changed to treatment nonresponse. In *Worst Case* version of ECR, indeterminate ECR values in the moxifloxacin arm are changed to treatment response and indeterminate ECR values in the lefamulin arm are changed to treatment nonresponse. 95% confidence interval computed based on continuity-corrected z-test.
ITT = intent-to-treat

Using the Applicant's version of the ECR, which treats indeterminate responses as treatment nonresponses, we conclude that lefamulin is noninferior to moxifloxacin, p-value for noninferiority test =0.0003. When we instead use the "worst-case" version of ECR, which fills in indeterminate responses in the manner most prejudicial to lefamulin vis a vis moxifloxacin, we still conclude that lefamulin is noninferior to moxifloxacin, p-value for noninferiority test =0.003. We additionally computed stratified Miettinen and Nurminen 95% confidence intervals, using the four strata defined by prior use of or having not received a single dose of short-acting antibacterial drug by PORT risk class (III versus IV/V). Geographic region (U.S. versus non-U.S.) was not used to define strata, as only three subjects were from the United States. When using the Applicant's version of ECR, the 95% confidence interval was (-8.1, 2.6), and when using the "worst-case" version, the confidence interval was (-10.0, 0.1). These confidence intervals are slightly narrower than their unstratified continuity-corrected analogues and again lead to statistically-significant support for the noninferiority of lefamulin vis a vis moxifloxacin.

Data Quality and Integrity

The data quality was acceptable and allowed the statistical reviewer to replicate the Applicant's data analyses.

Efficacy Results – Secondary and other relevant endpoints

The following table presents the extent of indeterminate values in the secondary efficacy endpoints.

Table 64. Trial 3101: Indeterminate Data Values in Secondary Efficacy Endpoints

Endpoint	Analysis Set	Indeterminate Values in Lefamulin Arm	Indeterminate Values in Moxifloxacin Arm
IACR at TOC	mITT	7/273 (2.6%)	3/273 (1.1%)
ECR	microITT	2/159 (1.3%)	2/159 (1.3%)
ECR + vital signs	ITT	14/276 (5.1%)	21/275 (7.6%)
IACR at TOC	microITT	1/159 (0.6%)	4/159 (2.5%)
Survival at 28 days[a]	ITT	10/276 (3.6%)	5/275 (1.8%)

[a] We report survival at Day 28 rather than mortality at Day 28.
IACR = investigator's assessment of clinical response; ECR = early clinical response; mITT = modified intent-to-treat; ITT = intent-to-treat; TOC = test of cure

The largest indeterminacy rates are for the *ECR + vital signs* endpoint. This is due to the fact that a subject's value can be indeterminate due to the lack of an ECA assessment or to the lack of assessment of vital signs. The most important secondary endpoint is *IACR at TOC* in the mITT analysis set. It has small indeterminacy rates in both arms. More generally, indeterminacy rates are small for all endpoints except *ECR + vital signs*.

The next table presents the results of the analyses of the five secondary efficacy endpoints. For the first four endpoints in the table, the results pertain to the Applicant's version, which treats indeterminate values as treatment failures. The Applicant did not specify any noninferiority margins for these four endpoints' analyses for the FDA, and the CABP guidance does not specify margins for them, so no tests of noninferiority are reported in the table. For the fifth endpoint, survival at 28 days, however, the CABP guidance specifies an M_1 margin, and test results relying on this margin are given.

Table 65. Trial 3101: Results of Analyses of Secondary Efficacy Endpoints

Endpoint	Analysis Set	Estimated Lefamulin Success Rate (# Successes/Arm Size)	Estimated Moxifloxacin Success Rate (# Successes/Arm Size)	Estimated Difference In Success Rates	95% Confidence Interval
IACR at TOC[a]	mITT	81.7% (223/273)	84.2% (230/273)	-2.6%	(-9.2, 4.1)
ECR	microITT	87.4% (139/159)	93.1% (148/159)	-5.7%	(-12.8, 1.5)
ECR + vital signs	ITT	72.8% (201/276)	76.0% (209/275)	-3.2%	(-10.8, 4.5)
IACR at TOC	microITT	79.9% (127/159)	85.5% (136/159)	-5.7%	(-14.6, 3.3)
Survival at 28 days[bc]	ITT	94.6% (261/276)	96.7% (266/275)	-2.2%	(-5.9, 1.6)

[a] We also analyzed IACR at TOC over the full ITT analysis set. The estimated lefamulin success rate is 80.8% (223/276) and the estimated moxifloxacin success rate is 83.6% (230/275), giving an estimated difference in success rates of -2.8%, with 95% confidence interval (-9.6, 3.9).
[b] We report survival at day 28 rather than mortality at day 28. The results in the table are based on treating missing values as deaths. The Applicant's analysis, however, excluded subjects with missing status. It estimated a difference in success rates of -0.4% (98.1% lefamulin vs. 98.5% moxifloxacin), with a 95% confidence interval of (-2.9, 2.2).
[c] The CABP guidance specifies an M_1 margin of 15% for the survival endpoint. This can be used to perform a noninferiority test of whether lefamulin has therapeutic effect. Since -.15 is below the lower bounds of the confidence intervals reported in the table and in table note a, we conclude that lefamulin is effective, $p < .05$, whether missing values are treated as deaths or ignored.
IACR = investigator's assessment of clinical response; ECR = early clinical response; mITT = modified intent-to-treat; ITT = intent-to-treat; TOC = test of cure

The estimated lefamulin-versus-moxifloxacin differences in success rates are uniformly small, with the most extreme estimated differences being -5.7% for ECR and for IACR at TOC in the microITT analysis set.

For the EMA, IACR at TOC in the mITT analysis set was the primary efficacy endpoint, and the Applicant stipulated that it be used to test the noninferiority of lefamulin to moxifloxacin, employing a margin of 10% and computing a stratified Miettinen and Nurminen 95% confidence interval. Using the four strata defined by prior use or not of single dose of short-acting antibacterial drug by PORT risk class (III versus IV/V), as discussed above, the 95% confidence interval computed using the Applicant's version of the endpoint is (-8.8, 3.9). Using the "worst-case" version instead, the corresponding 95% confidence interval is (-9.9, 2.7). Hence, for both versions of the endpoint, the hypothesis of noninferiority is supported, as -10% is below the lower bound of both confidence intervals.

The following table presents by-pathogen IACR at TOC results for individuals infected at baseline.

Table 66. Trial 3101: By-Pathogen IACR at TOC in the MicroITT Analysis Set

Baseline Pathogen	Lefamulin N=159	Moxifloxacin N=159
Gram-positive bacteria (aerobes)		
Staphylococcus aureus	8/10 (80.0%)	4/4 (100%)
Streptococcus pneumoniae	79/93 (84.9%)	85/97 (87.6%)
Streptococcus pyogenes	0/0	1/1 (100%)
Gram-negative bacteria (aerobes)		
Acinetobacter baumannii	1/1 (100%)	0/0
Acinetobacter calcoaceticus- A. baumannii complex	0/0	2/2 (100%)
Acinetobacter junii	1/1 (100%)	0/0
Acinetobacter lwoffii	2/2 (100%)	0/0
Acinetobacter species	0/0	1/1 (100%)
Burkholderia cepacia	0/0	1/1 (100%)
Citrobacter koseri	1/1 (100%)	0/0
Enterobacter aerogenes	1/1 (100%)	1/1 (100%)
Enterobacter cloacae	2/3 (66.7%)	0/0
Escherichia coli	0/0	1/2 (50.0%)
Haemophilus influenzae	43/51 (84.3%)	48/57 (84.2%)
Haemophilus parainfluenzae	3/3 (100%)	2/2 (100%)
Klebsiella pneumoniae	3/3 (100%)	2/2 (100%)
Moraxella catarrhalis	20/25 (80.0%)	11/11 (100%)
Pseudomonas aeruginosa	1/1 (100%)	0/0
Serratia marcescens	1/1 (100%)	0/0
Atypical pathogens		
Chlamydophila pneumoniae	8/11 (72.7%)	13/19 (68.4%)
Legionella pneumophila	14/18 (77.8%)	11/14 (78.6%)
Mycoplasma pneumoniae	16/19 (84.2%)	19/20 (95.0%)

Indeterminate responses are treated as clinical nonresponse.
TOC = test of cure; IACR = investigator's assessment of clinical response

At baseline, the most common Gram-positive bacterium was *Streptococcus pneumoniae*, and the two arms had similar clinical response rates (lefamulin 84.9% versus moxifloxacin 87.6%). The most common baseline Gram-negative bacterium was *Haemophilus influenzae*, and again the arms had similar clinical response rates (lefamulin 84.3% versus moxifloxacin 84.2%). At baseline, each of the atypical pathogens infected at least 30 subjects, and the clinical response rate for *Mycoplasma pneumoniae* was somewhat higher in the moxifloxacin arm (95.0% versus 84.2%).

***M.O. Comment**: The by-pathogen clinical response rates in the microITT population do not reveal any meaningful differences between the treatment arms for any particular pathogen noting that some pathogens were isolated from relatively small numbers of subjects. It is notable that some lefamulin subjects in whom Enterobacteriaceae and Pseudomonas aeruginosa were identified in sputum at baseline were clinical successes despite lefamulin having no microbiological activity against these organisms. It is possible that these organisms were not true pathogens in these subjects.*

Dose/Dose Response

Not applicable.

Durability of Response

Regarding the durability of the treatment effects, we examined IACR at the LFU visit for the mITT analysis set (recall that the key secondary endpoint IACR at TOC was analyzed on the mITT analysis set). There were 13 indeterminate responses in the lefamulin arm (4.8%) and 8 indeterminate responses in the moxifloxacin arm (2.9%). Treating indeterminate responses and relapses as treatment failures, the estimated success rate in the lefamulin arm was 78.4% (214/273) and the estimated success rate in the moxifloxacin arm was 82.1% (224/273). This gives an estimated lefamulin-versus-moxifloxacin difference in success rates of -3.7%, with a 95% confidence interval of (-10.7, 3.4).

In addition, we examined the different patterns of treatment success or failure at the ECA, TOC, and LFU visits, looking at the ECR at the first visit and the IACR at the latter two visits, using the ITT analysis set. The results are given in the table below.

Table 67. Trial 3101: Patterns of Treatment Success at ECA, TOC, and LFU Visits in the ITT Analysis Set

Pattern			Lefamulin N=276	Moxifloxacin N=275
ECA visit	**TOC visit**	**LFU visit**		
Failure	Failure	Failure	27 (9.8%)	22 (8.0%)
Success	Failure	Failure	26 (9.4%)	23 (8.4%)
Success	Success	Failure	9 (3.3%)	6 (2.2%)
Failure	Success	Success	8 (2.9%)	5 (1.8%)
Success	Success	Success	206 (74.6%)	219 (79.6%)

Indeterminate values are treated as failures.
ECA = early clinical assessment; TOC = test of cure; ITT = intent-to-treat; LFU = late follow-up

The pattern breakdown was similar for the two arms. In the lefamulin arm, 74.6% of subjects were treatment successes at all three visits, 9.8% were treatment failures at all three visits, and the remaining 15.6% showed a mixed pattern. The corresponding percentages for the moxifloxacin arm were 79.6%, 8.0%, and 12.4%, respectively.

Persistence of Effect

Not applicable.

Efficacy Results – Secondary or exploratory COA (PRO) endpoints

Not applicable.

Additional Analyses Conducted on the Individual Trial

The two Trial 3101 tables below present estimated differences in lefamulin versus moxifloxacin ECR response rates within subgroups defined in terms of demographic characteristics and baseline health status variables, respectively.

Table 68. Trial 3101: Early Clinical Response (ECR) Rates in Demographic Subgroups of the ITT Analysis Set

Subgroup	Lefamulin (N=276) n (%)	Moxifloxacin (N=275) n (%)	Difference (95% Confidence Interval)
Sex			
Male	144/170 (84.7%)	143/160 (89.4%)	-4.7% (-12.5,3.2)
Female	97/106 (91.5%)	105/115 (91.3%)	0.2% (-8.1,8.5)
Age Group			
<65 years	122/144 (84.7%)	156/167 (93.4%)	-8.7% (-16.3,1.1)
≥65 years	119/132 (90.2%)	92/108 (85.2%)	5.0% (-4.3,14.2)
Race			
White	208/239 (87.0%)	219/239 (91.6%)	-4.6% (-10.5,1.3)
Black or African American	9/11 (81.8%)	12/12 (100%)	-18.2% (-49.7,13.3)
Asian	22/24 (91.7%)	14/20 (70.0%)	21.7% (-5.8,49.2)
American Indian or Alaska Native	0/0	1/1 (100%)	NA
Other	2/2 (100%)	2/3 (66.7%)	33.3% (NA)
Ethnicity			
Hispanic or Latino	8/8 (100%)	8/10 (80.0%)	20.0% (NA)
Not Hispanic or Latino	233/268 (86.9%)	240/265 (90.6%)	-3.6% (-9.4,2.1)
Region			
North America[1]	1/2 (50.0%)	1/1 (100%)	-50.0% (NA)
Latin America	4/4 (100%)	8/10 (80.0%)	20.0% (NA)
Eastern Europe	191/218 (87.6%)	200/217 (92.2%)	-4.6% (-10.7,1.6)
Western Europe	13/17 (76.5%)	12/14 (85.7%)	-9.2% (-43.0,24.5)
Rest of the World	32/35 (91.4%)	27/33 (81.1%)	9.6% (-9.4,28.7)

[1] All 3 North American participants were from the United States.
NA = not applicable due to small sample size; ITT = intent-to-treat

Table 69. Trial 3101: Early Clinical Response (ECR) Rates in Baseline Health Status Subgroups of the ITT Analysis Set

Subgroup	Lefamulin (N=276) n (%)	Moxifloxacin (N=275) n (%)	Difference (95% Confidence Interval)
PORT class[1]			
III	175/196 (89.3%)	187/201 (93.0%)	-3.7% (-9.8,2.3)
IV	63/76 (82.9%)	57/70 (81.4%)	1.5% (-12.3,15.3)
V	3/4 (75.0%)	3/3 (100%)	-25.0% (NA)
Prior antibacterial drug use			
Yes	62/71 (87.3%)	61/71 (85.9%)	1.4% (-11.2,14.0)
No	179/205 (87.3%)	187/204 (91.7%)	-4.3% (-10.8,2.1)

Subgroup	Lefamulin (N=276) n (%)	Moxifloxacin (N=275) n (%)	Difference (95% Confidence Interval)
Baseline pathogen detected[2]			
Yes	139/159 (87.4%)	148/159 (93.1%)	-5.7% (-12.8,1.5)
No	102/117 (87.2%)	100/116 (86.2%)	1.0% (-8.6,10.6)
Respiratory disease			
Yes	54/60 (90.0%)	46/49 (93.9%)	-3.9% (-15.9,8.1)
No	187/216 (86.6%)	202/226 (89.4%)	-2.8% (-9.3,3.7)
Renal impairment[3]			
Normal functioning	109/121 (90.1%)	126/134 (94.0%)	-3.9% (-11.4,3.5)
Mild impairment	73/89 (82.0%)	66/75 (88.0%)	-6.0% (-18.1,6.1)
Moderate impairment	56/61 (91.8%)	53/62 (85.5%)	6.3% (-6.5,19.1)
Severe impairment	2/3 (66.7%)	3/3 (100%)	-33.3% (NA)
Heart disease			
Yes	56/64 (87.5%)	55/63 (87.3%)	0.2% (-12.9,13.3)
No	185/212 (87.3%)	193/212 (91.0%)	-3.8% (-10.2,2.6)
Bacteremia			
Yes	4/7 (57.1%)	2/3 (66.7%)	-9.5% (NA)
No	237/269 (88.1%)	246/272 (90.4%)	-2.3% (-7.9,3.2)

[1] One subject had a PORT class of II and is not included in computations of percentages.
[2] No subjects were infected with MRSA.
[3] Three subjects had missing data. They are not included in computations of percentages.
NA = not applicable due to small sample size; PORT = Pneumonia Outcomes Research Team

***M.O. Comment**: The ECR rates for LEF subjects were similar to MOX subjects among those with PORT IV CABP, moderate renal impairment, and history of heart and lung disease. This is reassuring as patients in these subgroups typically have worse outcomes.*

Because of their modest statistical power and lack of adjustment for multiple testing, subgroup analyses are difficult to interpret. In the two tables above, the estimated differences in ECR response rates in all subgroups with at least 50 subjects roughly support the comparability of the lefamulin rates to the moxifloxacin rates, but it is not possible to rigorously assess differences in rate differences between subgroups.

Integrated Review of Effectiveness for Trial 3101

Trial 3101 was conducted in a manner consistent with the CABP guidance and provides very strong evidence that lefamulin is noninferior to moxifloxacin for the treatment of CABP. This is based on the following:

- Analyses of the primary endpoint, ECR on the ITT analysis set, strongly support noninferiority. Whether using the Applicant's version of ECR or the "worst case" version, the null hypothesis of inferiority (i.e., the hypothesis that the ECR response rate for the lefamulin arm is at least 12.5% worse than the ECR response rate for the moxifloxacin arm) is rejected at p=.0003 and p=.003, respectively. Using the Applicant's version of the

ECR, the estimated response rate for the lefamulin arm is 2.9% less than the estimated moxifloxacin response rate (87.3% versus 90.2%).

- Analyses of the key secondary endpoint, IACR at TOC on the mITT analysis set, also strongly support the finding of noninferiority.
- Formal testing of the noninferiority of lefamulin relative to moxifloxacin was not conducted with any of the other secondary endpoints (e.g., survival at 28 days, ECR plus improvement in vital signs). Nonetheless, analyses of these endpoints support the noninferiority of lefamulin: while the estimated success rates for lefamulin were always smaller than the corresponding estimated success rates for moxifloxacin, they were always within 5.7% of the estimated moxifloxacin rates.
- Regarding IACR at TOC within groups of subjects having specific pathogens detected at baseline:
  - The estimated lefamulin and moxifloxacin clinical response rates for the most common Gram-positive bacterium, *S. pneumoniae*, were 84.9% and 87.6%, respectively.
  - The estimated lefamulin and moxifloxacin clinical response rates for the most common Gram-negative bacterium, *H. influenzae*, were 84.3% and 84.2%, respectively.
- Regarding IACR at the LFU visit for the mITT analysis set, the estimated success rate in the lefamulin arm was 78.4% and the estimated success rate in the moxifloxacin arm was 82.1%, giving an estimated difference in success rates of -3.7%.

In sum, analyses of the efficacy endpoints strongly support the noninferiority of lefamulin relative to moxifloxacin.

8.1.3. Trial 3102 – Study Design

Trial Design

This was a Phase 3 multicenter, multinational, double-blind, double-dummy, randomized noninferiority trial to evaluate the efficacy and safety of lefamulin versus moxifloxacin for the treatment of adults with CABP. 738 subjects with CABP in 99 centers were randomized to the lefamulin versus moxifloxacin arms in a 1:1 ratio within randomization strata defined by geographic region (US versus non-US), prior use or not of a single dose of a short-acting antibacterial drug, and PORT risk class (II versus III/IV). No more than 25% of subjects were to have received a single dose of a short-acting antibacterial drug, and at least 50% of subjects were to have a PORT risk class of III or IV.

Blinded study drug administration lasted 7 days. Subjects in the lefamulin arm received oral lefamulin 600 mg twice daily, for 5 days, and 7 days of daily oral moxifloxacin placebo. Subjects

in the moxifloxacin arm received 7 days of daily oral moxifloxacin 400 mg and oral lefamulin placebo twice daily, for 5 days.

Study visits were scheduled at baseline, at 96 +/- 24 hours after the first dose of study drug (early clinical assessment, or ECA), within 2 days after the last dose of study drug (end of treatment, or EOT), at 5 to 10 days after the last dose of study drug (test of cure, or TOC), and study day 30 (+/- 3 days) (late follow up, or LFU).

Key inclusion criteria include:

- Age ≥18 years
- Acute illness with at least 3 symptoms of CABP
  - Dyspnea
  - Cough
  - Purulent sputum production
  - Chest pain
- At least two vital sign abnormalities
  - Body temperature >38°C or <35°C
  - Systolic blood pressure <90 mmHg
  - Heart rate >100 beats/min
  - Respiratory rate >20 breaths/min
- At least one other clinical or laboratory finding of CABP
  - Oxygen saturation <90% on room air or PaO2<60 mmHg
  - Auscultatory or percussion findings consistent with pneumonia
  - WBC count >10,000 cells/mm3 or <4500 cells/mm3, or >15% bands
- Evidence of pneumonia on chest x-ray or CT scan
- Pneumonia Outcomes Research Team (PORT) Risk Class of II, III, or IV and be a candidate for oral antibacterial therapy as treatment for the current episode of CABP.

Key exclusion criteria include:

- Receipt of more than a single dose of a short-acting antibacterial drug within 72 hours before randomization
- Have risk for major cardiac events (QT prolongation, unstable cardiac disease, recent receipt of Class IA or Class III anti-arrhythmic medications)
- Concomitant treatment with a strong p-glycoprotein inhibitor or strong CYP3A inducer or inhibitor
- Creatinine clearance ≤30 mL/min

***M.O. Comment**: The inclusion/exclusion criteria were acceptable.*

Study Endpoints

The Applicant defined a primary efficacy endpoint and several secondary endpoints. The definitions of these endpoints, and the study populations they are defined in reference to, are identical to those from Trial 3101, and are consistent with the CABP guidance. Please refer back to the discussion of the Trial 3101 evaluation of efficacy for these definitions.

Intention-to-treat Analysis Populations

- *Intention-to-treat (ITT) analysis set*.
- *Modified Intention-to-treat (mITT) analysis set*.
- *Microbiological ITT (microITT) analysis set.*

Efficacy Endpoints

Primary endpoint
The primary efficacy endpoint was *Early clinical response* (*ECR*) as assessed in the ITT analysis set.

Subjects with missing data such that response/lack-of-response cannot be determined are considered to have an indeterminate response.

Secondary endpoints (assessed on intention-to-treat populations)

- Investigator's Assessment of Clinical Response (*IACR*) at TOC in the mITT analysis set.
- ECR in the microITT analysis set.
- IACR at TOC in the microITT analysis set.
- By-pathogen microbiological response at TOC in the microITT analysis set.
- All-cause mortality (ACM) through day 28 in the ITT analysis set.

Statistical reviewer comment*: Only four by-pathogen microbiological response values were based on repeat cultures, and these values matched the corresponding four IACR at TOC values. The remaining by-pathogen microbiological response values were based on IACR at TOC. In the following, therefore, we refer to "by-pathogen IACR response at TOC" rather than "by-pathogen microbiological response at TOC."*

Statistical Analysis Plan

Interim Analysis

Trial 3102 did not include an interim analysis.

Analysis of Primary Efficacy Endpoint

The Applicant proposed and used an upper-tailed continuity-corrected z-test, since the hypotheses are H0: *p1 – p2 <= -.10* versus H1: *p1 – p2 > -.10*, where *p1* is the true success rate

for the lefamulin arm, *p2* is the true success rate for the moxifloxacin arm, and the noninferiority margin is 10%.

Analysis of Secondary *Efficacy Endpoints*

- *Investigator's Assessment of Clinical Response* (*IACR*) at TOC in the mITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in success rates will be computed using a continuity-corrected z-test.
- ECR in the microITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in responder rates will be computed using a continuity-corrected z-test.
- IACR at TOC in the microITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in success rates will be computed using a continuity-corrected z-test.
- By-pathogen microbiological response at TOC in the microITT analysis set: descriptive statistics.
- All-cause mortality (ACM) through Day 28 in the ITT analysis set: 2-sided unadjusted 95% confidence intervals for the difference in survival rates will be computed using a continuity-corrected z-test.

Handling Missing Data

The handling of missing data was identical to that utilized in Trial 3101 and described above.

Handling Familywise Type I Error

As in Trial 3101, none of the secondary efficacy endpoints are analyzed via hypothesis tests, and hence no adjustment for multiple testing is made.

Protocol Amendments

The original protocol was finalized in December 2015, and the first subject was enrolled in August 2016. There were several important protocol amendments, all implemented in February 2016 in response to requests from the FDA that were conveyed at a January 2016 Type C meeting:

- Having confirmed or suspected CABP caused by MRSA became an exclusion criterion.
- A minimum of 50% (instead of 25%) of all subjects were required to have a PORT risk class of III or IV.
- The noninferiority margin for the primary endpoint ECR was decreased from 12.5% to 10%.
- The lefamulin-versus-moxifloxacin randomization ratio was changed from 2:1 to 1:1, and the sample size was increased from 573 to 738.

8.1.4. Trial 3102 - Study Results

Compliance With Good Clinical Practices

The Applicant states in the clinical study report that, "This clinical study was conducted in compliance with the protocol, ethical principles that have their origin in the Declaration of Helsinki..., the guidelines of International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) (CPMP/ICH/135/95), ... and Code of Federal Regulation Title 21, Parts 50, 56 and 312..."

Financial Disclosure

The Applicant certified that none of the investigators for Trial 3102 had any disclosable financial interests or arrangements with the Sponsor.

Patient Disposition

The following table presents the composition of the three intention-to-treat analysis sets by arm.

Table 70. Trial 3102: Composition of Intention-to-Treat Analysis Sets

Analysis Set	Lefamulin	Moxifloxacin
ITT	370	368
mITT	368	368
microITT	205	186

ITT analysis set includes all randomized subjects. mITT analysis set includes all randomized subjects who received any study drug. microITT analysis set includes members of the ITT analysis set who were infected with at least one CABP-causing pathogen at baseline. Resistance to the control is not a concern because there were no subjects with pathogens resistant to moxifloxacin in the moxifloxacin treatment arm.
ITT = intent-to-treat; mITT = modified intent-to-treat

The next table presents the per-arm proportions of subjects who withdrew from the study or discontinued treatment.

Table 71. Trial 3102: Study Withdrawals and Treatment Discontinuations in the ITT Analysis Set

	Lefamulin	Moxifloxacin
Premature withdrawal from study	17/370 (4.6%)	14/368 (3.8%)
Did not complete ECA visit	14/370 (3.8%)	6/368 (1.6%)
Did not complete TOC visit	15/370 (4.1%)	14/368 (3.8%)
Reason for premature withdrawal		
Lost to follow-up	1/370 (0.3%)	1/368 (0.3%)
Withdrawal by subject	10/370 (2.7%)	9/368 (2.4%)
Physician decision	0/370 (0.0%)	1/368 (0.3%)
Randomized but did not receive study drug	2/370 (0.5%)	0/368 (0.0%)
Death	3/370 (0.8%)	3/368 (0.8%)
Other	1/370 (0.3%)	0/368 (0.0%)
Premature discontinuation from study drug	25/370 (6.8%)	28/368 (7.6%)
Reason for premature discontinuation		
Adverse event	11/370 (3.0%)	8/368 (2.2%)
Lack of efficacy	8/370 (2.2%)	9/368 (2.4%)
Lost to follow-up	0/370 (0.0%)	1/368 (0.3%)
Physician decision	0/370 (0.0%)	2/368 (0.5%)
Sponsor decision	0/370 (0.0%)	4/368 (1.1%)
Withdrawal by subject	4/370 (1.1%)	3/368 (0.8%)
Randomized but did not receive study drug	2/370 (0.5%)	0/368 (0.0%)
Other	0/370 (0.0%)	1/368 (0.3%)

ECA = early clinical assessment; TOC = test of cure; ITT = intent-to-treat

There were 0.8% more study withdrawals in the lefamulin arm than in the moxifloxacin arm (4.6% versus 3.8%), and the breakdowns by reason for withdrawal were quite similar. There were 0.8% fewer study drug discontinuations in the lefamulin than the moxifloxacin arm (6.8% versus 7.6%), and again the breakdowns by reason were similar. Note, though, that in the moxifloxacin arm six subjects had study medication discontinued due to physician or sponsor decision, whereas this did not happen in the lefamulin arm.

Protocol Violations/Deviations

The following table documents the significant protocol deviations by arm. Per the CSR, a *significant protocol deviation* has the potential to affect efficacy assessments, placement into analysis populations, ability to monitor safety, or the study's scientific value. *CE-analysis-set excluding protocol deviations* are considered more serious and are detailed in the table.

Table 72. Trial 3102: Significant Protocol Deviations in ITT Analysis Set

	Lefamulin	Moxifloxacin
Subjects with a significant protocol deviation[a]	184/370 (49.7%)	162/368 (44.0%)
Subjects with a significant deviation that excludes them from the CE analysis sets[b]	59/370 (15.9%)	57/368 (15.5%)
Type of CE-analysis-sets-excluding protocol deviation[b]		
Exclusion criteria	1/370 (0.3%)	2/368 (0.5%)
Inclusion criteria	4/370 (1.1%)	9/368 (2.4%)
Study procedures/assessments	57/370 (15.4%)	49/368 (13.3%)

The CE (clinically evaluable) analysis sets (CE-EOT, CE-TOC, and CE-LFU analysis sets) include subjects in the ITT analysis set who (i) meet key inclusion criteria, (ii) received at least the prespecified minimal intended dose of study drug, (iii) do not have an indeterminate response on the IACR at EOT/TOC/LFU, (iv) did not receive concomitant antibacterial therapy that is potentially effective against CABP pathogens through EOT/TOC/LFU, and (v) had no other confounding factors that interfere with endpoint assessment.

[a] There were a total of 575 significant protocol deviations (317 lefamulin, 258 moxifloxacin); the table gives the number of subjects with at least 1 such deviation.

[b] There were a total of 137 CE-analysis-set-excluding deviations (69 lefamulin, 68 moxifloxacin); the table gives the number of subjects with at least 1 such deviation.

ITT = intent-to-treat; CE = clinically evaluable; LFU = late follow-up; CABP = community-acquired bacterial pneumonia

The most common types of significant protocol deviations involved study procedures and assessments (131 subjects in lefamulin arm, 120 in moxifloxacin arm; most common were LFU visit out of window and ECG performed after randomization but prior to first dose), assignment to incorrect randomization strata (38 in lefamulin, 26 in moxifloxacin), exclusion criteria (25 in lefamulin arm, 17 in moxifloxacin arm), and CABP signs and symptoms not assessed in person within the ECR window (22 in lefamulin arm, 16 in moxifloxacin arm). There were 16 subjects who used prohibited medications (6 lefamulin, 10 moxifloxacin), but none used prohibited antibacterials and none of the prohibited uses were CE-analysis-set excluding. The most common type of CE-analysis-set excluding deviation involved study procedures or assessments, and most of these involved subjects whose LFU visit occurred out-of-window (44 in the lefamulin arm, 34 in the moxifloxacin arm). Note that out-of-window LFU visits do not compromise the validity of the primary or secondary endpoints.

Table of Demographic Characteristics

The following table examines baseline balance between the lefamulin and moxifloxacin arms on demographic characteristics.

Table 73. Trial 3102: Demographic Characteristics of the ITT Analysis Set

Demographic Parameters	Lefamulin (N=370) n (%)	Moxifloxacin (N=368) n (%)	Standardized Difference[1]
Sex			
Male	207 (55.9)	180 (48.9)	0.14
Female	163 (44.1)	188 (51.1)	-0.14
Age			
Mean years (SD)	57.4 (16.4)	57.7 (16.2)	-0.02
Median (years)	59	59.5	NA
Min, max (years)	19, 97	19, 93	NA

Demographic Parameters	Lefamulin (N=370) n (%)	Moxifloxacin (N=368) n (%)	Standardized Difference[1]
Age group			
<65 years	234 (63.2)	227 (61.7)	0.03
≥65 years	136 (36.8)	141 (38.3)	-0.03
Race			
White	274 (74.1)	270 (73.4)	0.02
Black or African American	19 (5.1)	22 (6.0)	-0.04
Asian	48 (13.0)	52 (14.1)	-0.03
American Indian or Alaska Native	24 (6.5)	16 (4.3)	0.09
Other	5 (1.4)	8 (2.2)	-0.06
Ethnicity			
Hispanic or Latino	45 (12.2)	38 (10.3)	0.06
Not Hispanic or Latino	325 (87.8)	330 (89.7)	-0.06
Region			
North America[2]	11 (3.0)	12 (3.3)	-0.02
Latin America	38 (10.3)	34 (9.2)	0.03
Eastern Europe	236 (63.8)	218 (59.2)	0.09
Western Europe	17 (4.6)	19 (5.2)	-0.03
Rest of the world	68 (18.4)	85 (23.1)	-0.12

[1] The standardized difference is the difference between the means in the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled standard deviation term. It gives the effect size difference between the two arms.
[2] All 23 North American subjects were from the United States.
NA = not applicable; ITT = intent-to-treat; SD = standard deviation

The demographic variables exhibiting the largest standardized differences between arms are gender (44.1% female in the lefamulin arm versus 51.1% female in the moxifloxacin arm) and whether enrolled outside of the Americas and Europe (18.4% in the lefamulin arm, 23.1% in the moxifloxacin arm).

Other Baseline Characteristics (e.g., Disease Characteristics, Important Concomitant Drugs)

The following table examines baseline balance between the lefamulin and moxifloxacin arms on health status characteristics.

Table 74. Trial 3102: Baseline Health Status of the ITT Analysis Set

Demographic Parameters	Lefamulin (N=370) n (%)	Moxifloxacin (N=368) n (%)	Standardized Difference[1]
PORT class[2]			
I	1 (0.3)	2 (0.5)	-0.04
II	183 (49.5)	189 (51.4)	-0.04
III	145 (39.2)	133 (36.1)	0.06
IV	40 (10.8)	42 (11.4)	-0.02
V	1 (0.3)	2 (0.5)	-0.04
Prior antibacterial drug use			
Yes	80 (21.6)	79 (21.5)	0.00
No	290 (78.4)	289 (78.5)	0.00

Demographic Parameters	Lefamulin (N=370) n (%)	Moxifloxacin (N=368) n (%)	Standardized Difference[1]
Baseline pathogen detected			
Yes	205 (55.4)	186 (50.5)	0.10
No	165 (44.6)	182 (49.5)	-0.10
Lung disease			
Yes	71 (19.2)	67 (18.2)	0.03
No	299 (80.8)	301 (81.8)	-0.03
Renal impairment			
Normal functioning	190 (51.4)	178 (48.4)	0.06
Mild impairment	112 (30.3)	117 (31.8)	-0.03
Moderate impairment	64 (17.3)	70 (19.0)	-0.04
Severe impairment	4 (1.1)	3 (0.8)	0.03
Heart disease			
Yes	43 (11.6)	51 (13.9)	-0.07
No	327 (88.4)	317 (86.1)	0.07

[1] The standardized difference is the difference between the means in the two arms (for a binary variable, the difference in proportions) divided by the square root of a pooled standard deviation term. It gives the effect size difference between the two arms.
[2] The trial intended to only include subjects from PORT classes II, III, and IV.
PORT = Pneumonia Outcomes Research Team; ITT = intent-to-treat

The baseline health status variable exhibiting the largest standardized difference between arms is whether a pathogen was detected at baseline (55.4% detected in the lefamulin arm versus 50.5% detected in the moxifloxacin arm).

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

We use the Applicant's definition of compliance from Trial 3101: a subject was compliant in taking his/her medication if at least 90% and no more than 100% of the intended dosage was used. Three subjects had missing data for medication compliance (1 in the lefamulin arm, 2 in the moxifloxacin arm). Ignoring these three subjects, the mITT analysis set noncompliance rate was 2.5% (9/367) in the lefamulin arm and 1.6% (6/366) in the moxifloxacin arm. If we count the subjects with missing data as noncompliant, then the noncompliance rates are 2.7% and 2.2%, respectively.

The following table provides a high-level overview of the use of concomitant medications after study entry.

Table 75. Trial 3102: Post Study Entry Concomitant Medication Use in the ITT Analysis Set

Drug Category	Lefamulin	Moxifloxacin
Antibacterials for systemic use	49/370 (13.2%)	33/368 (9.0%)
Other anti-infectives for systemic use	10/370 (2.7%)	7/368 (1.9%)
Alimentary tract and metabolism	57/370 (15.4%)	53/368 (14.4%)
Antineoplastic and immunomodulating agents	1/370 (0.3%)	1/368 (0.3%)
Antiparasitic product, insecticides, and repellents	0/370 (0.0%)	1/368 (0.3%)
Blood and blood forming agents	25/370 (6.8%)	31/368 (8.4%)
Cardiovascular system	28/370 (7.6%)	31/368 (8.4%)

Drug Category	Lefamulin	Moxifloxacin
Dermatologicals	2/370 (0.5%)	3/368 (0.8%)
Genito urinary system and sex hormones	2/370 (0.5%)	1/368 (0.3%)
Musculoskeletal system	17/370 (4.6%)	16/368 (4.3%)
Nervous system	24/370 (6.5%)	33/368 (9.0%)
Respiratory system	46/370 (12.4%)	55/368 (14.9%)
Sensory organs	3/370 (0.8%)	0/368 (0.0%)
Systemic hormonal preparations (excluding sex hormones and insulins)	11/370 (3.0%)	16/368 (4.3%)
Other	8/370 (2.2%)	6/368 (1.6%)

There were 1008 uses of post study entry concomitant medication (521 lefamulin, 487 moxifloxacin). There were 259 subjects who used post study entry concomitant medications (132/370 lefamulin (35.7%), 127/368 moxifloxacin (34.5%)).
ITT = intent-to-treat

The largest difference in between-arm concomitant medication usage rates was in antibacterials for systemic use (13.2% in lefamulin arm versus 9.0% in moxifloxacin arm). The next table provides additional detail on the use of concomitant systemic antibacterial medication.

Table 76. Trial 3102: Post Study Entry Concomitant Systemic Antibacterial Medication Use in the ITT Analysis Set

	Lefamulin	Moxifloxacin
Reason for use		
Concomitant infection, unrelated to CABP	10/370 (2.7%)	6/368 (1.6%)
Insufficient therapeutic effect of study drug	32/370 (8.6%)	22/368 (6.0%)
Treatment limiting AE resulting in discontinuation of study drug	7/370 (1.9%)	4/368 (1.1%)
Other	3/370 (0.8%)	2/368 (0.5%)
Antibacterial category		
Aminoglycoside antibacterials	5/370 (1.4%)	3/368 (0.8%)
Beta-lactam antibacterials, penicillins	10/370 (2.7%)	5/368 (1.4%)
Other beta-lactam antibacterials	27/370 (7.3%)	16/368 (4.3%)
Macrolides, lincosamides, and streptogramins	8/370 (2.2%)	7/368 (1.9%)
Quinolone antibacterials	18/370 (4.9%)	14/368 (3.8%)
Sulfonamides and trimethoprim	2/370 (0.5%)	1/368 (0.3%)
Tetracyclines	1/370 (0.3%)	2/368 (0.5%)
Combinations of antibacterials	1/370 (0.3%)	1/368 (0.3%)
Other antibacterials	10/370 (2.7%)	4/368 (1.1%)

There were 167 prescriptions for post study entry concomitant systemic antibacterial medication (109 lefamulin, 58 moxifloxacin). There were 82 subjects who used post study entry concomitant systemic antibacterial medications (49/370 lefamulin (13.2%), 33/368 moxifloxacin (9.0%)).
ITT = intent-to-treat; CABP = community-acquired bacterial pneumonia; AE = adverse event

Rescue antibacterial medication (due to insufficient therapeutic effect of study drug or to treatment-limiting adverse event resulting in discontinuation of study drug) was administered to 39 subjects in the lefamulin arm (10.5%) and 26 subjects in the moxifloxacin arm (7.1%).

M.O. Comment*: This imbalance in nonstudy antibacterial drug use appears to be driven by use of penicillins and other beta-lactam antibacterial drugs. This nonstudy antibacterial drug use was mostly accounted for by subjects who required alternative treatment/rescue therapy for the primary pneumonia because of treatment failure of the study drug or a treatment-limiting AE*

from the study drug. Of the 39 LEF and 26 MOX subjects who received nonstudy antibacterial therapy for these reasons all were counted as failures at the LFU timepoint.

Efficacy Results – Primary Endpoint

The table below presents results of the analysis of the primary efficacy endpoint, ECR, on the ITT analysis set.

Table 77. Trial 3102: Results of Analyses of Early Clinical Response (ECR) on ITT Analysis Set

Version of ECR	Estimated Lefamulin Success Rate (# Successes/Arm Size)	Estimated Moxifloxacin Success Rate (# Successes/Arm Size)	Estimated Difference in Success Rates	95% Confidence Interval
Applicant	90.8% (336/370)	90.8% (334/368)	0.0%	(-4.4, 4.5)
Worst Case	90.8% (336/370)	91.6% (337/368)	-0.8%	(-5.1, 3.6)

The ECR data contained 5 indeterminate responses in the lefamulin arm (1.4%) and 3 indeterminate responses in the moxifloxacin arm (0.8%). In *Applicant* version of ECR, all indeterminate ECR values are changed to treatment failure. In *Worst Case* version of ECR, indeterminate ECR values in the moxifloxacin arm are changed to treatment success and indeterminate ECR values in the lefamulin arm are changed to treatment failure. 95% confidence interval computed based on continuity-corrected z-test.
ITT = intent-to-treat

Using the Applicant's version of the ECR, which treats indeterminate responses as treatment nonresponses, we conclude that lefamulin is noninferior to moxifloxacin, p-value for noninferiority test <0.0001. When we instead use the "worst-case" version of ECR, which fills in indeterminate responses in the manner most prejudicial to lefamulin vis a vis moxifloxacin, we still conclude that lefamulin is noninferior to moxifloxacin, p-value for noninferiority test <0.0001.

We additionally computed stratified Miettinen and Nurminen 95% confidence intervals, using the six strata defined by prior use or not of single dose of short-acting antibacterial drug by PORT risk class (II versus III versus IV). Geographic region (U.S. versus non-U.S.) was not used to define strata, as only 23 subjects were from the United States. When using the Applicant's version of ECR, the 95% confidence interval was (-4.3, 4.2), and when using the "worst-case" version, the confidence interval was (-5.0, 3.3). These confidence intervals are slightly narrower than their unstratified continuity-corrected analogues and again lead to statistically-significant support for the noninferiority of lefamulin vis a vis moxifloxacin.

Data Quality and Integrity

Data quality was acceptable and allowed the statistical reviewer to replicate the Applicant's data analyses.

Efficacy Results – Secondary and other relevant endpoints

The following table presents the extent of indeterminate values in the secondary efficacy endpoints.

Table 78. Trial 3102: Indeterminate Data Values in Secondary Efficacy Endpoints

Endpoint	Analysis Set	Indeterminate Values in Lefamulin Arm	Indeterminate Values in Moxifloxacin Arm
IACR at TOC	mITT	2/368 (0.5%)	8/368 (2.2%)
ECR	microITT	2/205 (1.0%)	1/186 (0.5%)
IACR at TOC	microITT	1/205 (0.5%)	2/186 (1.1%)
Survival at 28 days[ab]	ITT	3/370 (0.8%)	1/368 (0.3%)

[a] We report survival at day 28 rather than mortality at day 28.
IACR = investigator's assessment of clinical response; ECR = early clinical response; TOC = test of cure; mITT = modified intent-to-treat; ITT = intent-to-treat

The per-arm indeterminacy rates are quite small for all secondary endpoints: all less than 2.5%, with the largest being IACR at TOC in the mITT analysis set for the moxifloxacin arm.

The next table presents the results of the analyses of the four secondary efficacy endpoints. For the first three endpoints in the table, the results pertain to the Applicant's version, which treats indeterminate values as treatment failures. The Applicant did not specify any noninferiority margins for these three endpoints' analyses for the FDA, and the CABP guidance does not specify margins for them, so no tests of noninferiority are reported in the table. For the fourth endpoint, survival at 28 days, however, the CABP guidance specifies an M_1 margin, and test results relying on this margin are given.

Table 79. Trial 3102: Results of Analyses of Secondary Efficacy Endpoints

Endpoint	Analysis Set	Estimated Lefamulin Success Rate (# Successes/Arm Size)	Estimated Moxifloxacin Success Rate (# Successes/Arm Size)	Estimated Difference in Success Rates	95% Confidence Interval
IACR at TOC[a]	mITT	87.5% (322/368)	89.1% (328/368)	-1.6%	(-6.5, 3.3)
ECR	microITT	90.7% (186/205)	93.0% (173/186)	-2.3%	(-8.2, 3.6)
IACR at TOC	microITT	85.9% (176/205)	87.6% (163/186)	-1.8%	(-9.0, 5.5)
Survival at 28 days[ab]	ITT	98.4% (364/370)	98.9% (364/368)	-0.5%	(-2.5, 1.4)

[a] We also analyzed IACR at TOC over the full ITT analysis set. The estimated lefamulin success rate is 87.0% (322/370) and the estimated moxifloxacin success rate is 89.1% (328/368), giving an estimated difference in success rates of -2.1%, with 95% confidence interval (-7.0, 2.8).
[b] We report survival at Day 28 rather than mortality at Day 28. The results in the table are based on treating missing values as deaths. The Applicant's analysis, however, excluded subjects with missing status. It estimated a difference in success rates of 0.0% (99.2% lefamulin vs. 99.2% moxifloxacin), with a 95% confidence interval of (-1.6, 1.6).
[c] The CABP guidance specifies an M1 margin of 15% for the survival endpoint. This can be used to perform a noninferiority test of whether lefamulin has therapeutic effect. Since -.15 is below the lower bounds of the confidence intervals reported in the table and in table note a, we conclude that lefamulin is effective, $p < .05$.
IACR = investigator's assessment of clinical response; ECR = early clinical response; ITT = intent-to-treat; mITT = modified intent-to-treat; TOC = test of cure

The estimated lefamulin-versus-moxifloxacin differences in success rates are uniformly small, with the most extreme estimated differences being -2.3% for ECR in the microITT analysis set.

For the EMA, IACR at TOC in the mITT analysis set was the primary efficacy endpoint, and the Applicant stipulated that it be used to test the noninferiority of lefamulin to moxifloxacin, employing a margin of 10% and computing a stratified Miettinen and Nurminen 95% confidence

interval. Using the six strata defined by prior use or not of single dose of short-acting antibacterial drug by PORT risk class (II versus III versus IV), as discussed above, the 95% confidence interval computed using the Applicant's version of the endpoint is (-6.6,2.7). Using the "worst-case" version instead, the corresponding 95% confidence interval is (-8.6,0.4). Hence, for both versions of the endpoint, the null hypothesis of inferiority is rejected, as -10% is below the lower bound of both confidence intervals.

The following table presents by-pathogen IACR at TOC results for individuals infected at baseline.

Table 80. Trial 3102: By-pathogen IACR by TOC Results in the MicroITT Analysis Set

Baseline Pathogen	Lefamulin N=205	Moxifloxacin N=186
Gram-positive bacteria (aerobes)		
Beta hemolytic streptococcus	2/2 (100%)	1/1 (100%)
Staphylococcus aureus	12/13 (92.3%)	5/6 (83.3%)
Streptococcus agalactiae	2/2 (100%)	0/0
Streptococcus pneumoniae[a]	105/123 (85.4%)	108/126 (85.7%)
Streptococcus pyogenes	0/0	1/1 (100%)
Gram-negative bacteria (aerobes)		
Achromobacter xylosoxidans	0/0	1/1 (100%)
Acinetobacter calcoaceticus	0/0	1/1 (100%)
Acinetobacter ursingii	1/1 (100%)	0/0
Aeromonas caviae complex	1/1 (100%)	0/0
Citrobacter freundii complex	0/0	0/1 (0%)
Enterobacter cloacae	0/0	1/1 (100%)
Escherichia coli	1/1 (100%)	0/0
Haemophilus influenzae	52/56 (92.9%)	40/48 (83.3%)
Haemophilus parainfluenzae	6/6 (100%)	2/2 (100%)
Klebsiella oxytoca	1/1 (100%)	0/0
Klebsiella pneumoniae	4/5 (80%)	2/2 (100%)
Klebsiella variicola	0/1 (0%)	0/0
Moraxella catarrhalis	17/21 (81.0%)	11/11 (100%)
Pasteurella pneumotropica	0/0	0/1 (0%)
Proteus mirabilis	1/1 (100%)	0/0
Pseudomonas aeruginosa	2/4 (50%)	2/3 (66.7%)
Pseudomonas luteola	0/0	1/1 (100%)
Stenotrophomonas maltophilia	0/1 (0%)	1/1 (100%)
Atypical pathogens		
Chlamydophila pneumoniae	12/16 (75%)	10/12 (83.3%)
Legionella pneumophila	13/16 (81.3%)	15/17 (88.2%)
Mycoplasma pneumoniae	19/20 (95%)	14/14 (100%)

Indeterminate responses are treated as clinical nonresponse.
[a] There was 1 indeterminate response in the lefamulin arm and 2 indeterminate responses in the moxifloxacin arm.
TOC = test of cure; IACR = investigator's assessment of clinical response

At baseline, the most common Gram-positive bacterium was *Streptococcus pneumoniae*, and the two arms had similar clinical response rates (lefamulin 85.4% versus moxifloxacin 85.7%). The most common baseline Gram-negative bacterium was *Haemophilus influenzae*, and the

lefamulin arm had a somewhat better clinical response rate (92.9% versus 83.3%). The clinical response rates for the arms were similar for each of the three atypical pathogens.

M.O. Comment*: Similar to Trial 3101, the by-pathogen clinical response rates in the microITT population for Trial 3102 do not reveal any meaningful differences between the treatment arms for any particular pathogen noting that some pathogens were isolated from relatively small numbers of subjects.*

Dose/Dose Response

Not applicable.

Durability of Response

Regarding the durability of the treatment effects, we examined IACR at the LFU visit for the mITT analysis set (recall that the key secondary endpoint IACR at TOC was analyzed on the mITT analysis set). There were 5 indeterminate responses in the lefamulin arm (1.4%) and 7 indeterminate responses in the moxifloxacin arm (1.9%). Treating indeterminate responses and relapses as treatment failures, the estimated success rate in the lefamulin arm was 86.7% (319/368) and the estimated success rate in the moxifloxacin arm was 89.1% (328/368). This gives an estimated lefamulin-versus-moxifloxacin difference in success rates of -2.4%, with a 95% confidence interval of (-7.4, 2.5).

In addition, we examined the different patterns of treatment success or failure at the ECA, TOC, and LFU visits, looking at the ECR at the first visit and the IACR at the latter two visits, using the ITT analysis set. The results are given in the table below.

Table 81. Trial 3102: Patterns of Treatment Success at ECA, TOC, and LFU Visits in the ITT Analysis Set

Pattern			Lefamulin	Moxifloxacin
ECA Visit	TOC Visit	LFU Visit	(N=370)	(N=368)
Failure	Failure	Failure	25 (6.8%)	18 (4.9%)
Success	Failure	Failure	23 (6.2%)	21 (5.7%)
Success	Success	Failure	3 (0.8%)	1 (0.3%)
Success	Failure	Success	0 (0%)	1 (0.3%)
Failure	Success	Success	9 (2.4%)	16 (4.3%)
Success	Success	Success	310 (83.8%)	311 (84.5%)

Indeterminate values are treated as failures.
ECA = early clinical assessment; TOC = test of cure; LFU = late follow-up; ITT = intent-to-treat

The pattern breakdown was similar for the two arms. In the lefamulin arm, 83.8% of subjects were treatment successes at all three visits, 6.8% were treatment failures at all three visits, and the remaining 9.5% showed a mixed pattern. The corresponding percentages for the moxifloxacin arm were 84.5%, 4.9%, and 10.6%, respectively.

Persistence of Effect

Not applicable.

Efficacy Results – Secondary or Exploratory COA (PRO) Endpoints

Not applicable.

Additional Analyses Conducted on the Individual Trial

The two Trial 3102 tables below present estimated differences in lefamulin versus moxifloxacin ECR response rates within subgroups defined in terms of demographic characteristics and baseline health status variables, respectively.

Table 82. Trial 3102: Early Clinical Response (ECR) Rates in Demographic Subgroups of the ITT Analysis Set

Subgroup	Lefamulin (N=370) n (%)	Moxifloxacin (N=368) n (%)	Difference (95% Confidence Interval)
Sex			
Male	186/207 (89.9%)	158/180 (87.8%)	2.1% (-4.8,8.9)
Female	150/163 (92.0%)	176/188 (93.6%)	-1.6% (-7.6,4.4)
Age group			
<65 years	211/234 (90.2%)	210/227 (92.5%)	-2.3% (-7.9,3.2)
≥65 years	125/136 (91.9%)	124/141 (87.9%)	4.0% (-3.8,11.8)
Race			
White	252/274 (92.0%)	247/270 (91.5%)	-0.5% (-4.5,5.5)
Black or African American	15/19 (78.9%)	20/22 (90.9%)	-12.0% (-38.8,14.9)
Asian	41/48 (85.4%)	45/52 (86.5%)	-1.1% (-16.8,14.5)
American Indian or Alaska Native	24/24 (100%)	16/16 (100%)	0.0% (-5.2,5.2)
Other	4/5 (80.0%)	6/8 (75.0%)	5.0% (-57.4,67.4)
Ethnicity			
Hispanic or Latino	43/45 (95.6%)	35/38 (92.1%)	3.5% (-9.5,16.4)
Not Hispanic or Latino	293/325 (90.2%)	299/330 (90.6%)	-0.5% (-5.3,4.4)
Region			
North America[1]	7/11 (63.6%)	9/12 (75.0%)	-11.4% (-57.6,34.9)
Latin America	37/38 (97.4%)	32/34 (94.1%)	3.3% (-8.9,15.4)
Eastern Europe	217/236 (91.9%)	205/218 (94.0%)	-2.1% (-7.2,3.0)
Western Europe	14/17 (82.4%)	14/19 (73.7%)	8.7% (-23.7,41.1)
Rest of the world	61/68 (89.7%)	74/85 (87.1%)	2.6% (-8.8,14.1)

[1] All 23 North American participants were from the United States.
NA = not applicable; ITT = intent-to-treat

Table 83. Trial 3102: Early Clinical Response (ECR) Rates in Baseline Health Status Subgroups of the ITT Analysis Set

Subgroup	Lefamulin (N=276) n (%)	Moxifloxacin (N=275) n (%)	Difference (95% Confidence Interval)
PORT class[1]			
II	168/183 (91.8%)	176/189 (93.1%)	-1.3% (-7.2,4.6)
III	132/145 (91.0%)	120/133 (90.2%)	0.8% (-6.8,8.4)
IV	34/40 (85.0%)	36/42 (85.7%)	-0.7% (-18.5,17.0)
Prior antibacterial drug use			
Yes	75/80 (93.8%)	70/79 (88.6%)	5.1% (-4.9,15.2)
No	261/290 (90.0%)	264/289 (91.3%)	-1.3% (-6.4,3.7)
Baseline pathogen detected			
Yes	186/205 (90.7%)	173/186 (93.0%)	-2.3% (-8.2,3.6)
No	150/165 (90.9%)	161/182 (88.5%)	2.4% (-4.5,9.4)
Respiratory disease			
Yes	63/71 (88.7%)	60/67 (89.6%)	-0.8% (-12.7,11.0)
No	273/299 (91.3%)	274/301 (91.0%)	0.3% (-4.6,5.1)
Renal impairment			
Normal functioning	177/190 (93.2%)	167/178 (93.8%)	-0.7% (-6.2,4.9)
Mild impairment	102/112 (91.1%)	102/117 (87.2%)	3.9% (-5.0,12.8)
Moderate impairment	54/64 (84.4%)	63/70 (90.0%)	-5.6% (-18.5,7.2)
Severe impairment	3/4 (75.0%)	2/3 (66.7%)	8.3% (NA)
Heart disease			
Yes	40/43 (93.0%)	42/51 (82.4%)	10.7% (-4.4,25.8)
No	296/327 (90.5%)	292/317 (92.1%)	-1.6% (-6.3,3.1)
Bacteremia			
Yes	4/6 (66.7%)	8/9 (88.9%)	-22.2% (-79.1,34.6)
No	332/364 (91.2%)	326/359 (90.8%)	0.4% (-4.0,4.8)

[1] 3 subjects were PORT class I (1 lefamulin, 2 moxifloxacin) and 3 subjects were PORT class V (1 lefamulin, 2 moxifloxacin). These 6 subjects were excluded from subgroup analyses, as they were not intended to be included in the trial.
NA = not applicable due to small sample size; PORT = Pneumonia Outcomes Research Team

***M.O. Comment**: The ECR rates for LEF subjects were similar to MOX subjects among those with PORT III and IV CABP, renal impairment, and history of heart and lung disease. This is reassuring as patients in these subgroups typically have worse outcomes.*

Because of their modest statistical power and lack of adjustment for multiple testing, subgroup analyses are difficult to interpret. In the two tables above, the estimated differences in ECR response rates in all subgroups with at least 50 subjects, roughly support the comparability of the lefamulin rates to the moxifloxacin rates, but it is not possible to rigorously assess differences in rate differences between subgroups.

Integrated Review of Effectiveness for Trial 3102

Trial 3102 was conducted in a manner consistent with the CABP guidance and provides very strong evidence that lefamulin is noninferior to moxifloxacin for the treatment of CABP. This is based on the following:

- Analyses of the primary endpoint, ECR on the ITT analysis set, strongly support noninferiority. Whether using the Applicant's version of ECR or the "worst case" version, the null hypothesis of inferiority (i.e., the hypothesis that the ECR response rate for the lefamulin arm is at least 10% worse than the ECR response rate for the moxifloxacin arm) is rejected at $p<0.0001$. Using the Applicant's version of the ECR, the estimated response rate for the lefamulin arm (90.8%) is equal to the estimated moxifloxacin response rate.
- Analyses of the key secondary endpoint, IACR at TOC on the mITT analysis set, also strongly support the findings of noninferiority.
- Formal testing of the noninferiority of lefamulin relative to moxifloxacin was not conducted with any of the other secondary endpoints (e.g., survival at 28 days). Nonetheless, analyses of these endpoints support the noninferiority of lefamulin: while the estimated success rates for lefamulin were never larger than the corresponding estimated success rates for moxifloxacin, they were always within 2.3% of the estimated moxifloxacin rates.
- Regarding IACR at TOC within groups of subjects having specific pathogens detected at baseline:
  - The estimated lefamulin and moxifloxacin clinical response rates for the most common Gram-positive bacterium, *S. pneumoniae*, were 85.4% and 85.7%, respectively.
  - The estimated lefamulin and moxifloxacin clinical response rates for the most common Gram-negative bacterium, *H. influenzae*, were 92.9% and 83.3%, respectively.
- Regarding IACR at the LFU visit for the mITT analysis set, the estimated success rate in the lefamulin arm was 86.7% and the estimated success rate in the moxifloxacin arm was 89.1%, giving an estimated difference in success rates of -2.4%.

In sum, analyses of the efficacy endpoints strongly support the noninferiority of lefamulin relative to moxifloxacin.

8.1.5. Assessment of Efficacy Across Trials

Pooled efficacy analyses of Trials 3101 and 3102 were conducted by baseline pathogen. The following table summarizes IACR rates at TOC by the most common baseline pathogens across

both trials in the microITT Analysis Set, which comprised all randomized patients with at least 1 baseline pathogen.

Table 84. Investigator-Assessed Clinical Response Rates at TOC by Baseline Pathogen in Trial 3101 and Trial 3102 (MicroITT Analysis Set)

Pathogen	Lefamulin n/N (%)	Moxifloxacin n/N (%)*
Streptococcus pneumoniae	184/216 (85.2)	193/223 (86.5)
Methicillin-susceptible *Staphylococcus aureus* (MSSA)	14/16 (87.5)	5/5 (100.0)
Haemophilus influenzae	95/107 (88.8)	88/105 (83.8)
Mycoplasma pneumoniae	35/39 (89.7)	33/34 (97.1)
Legionella pneumophila	27/34 (79.4)	26/31 (83.9)
Chlamydophila pneumoniae	20/27 (74.1)	23/31 (74.2)

*Trial 1 compared lefamulin to moxifloxacin + linezolid.
TOC = test of cure

Primary Endpoints

Not applicable.

Secondary and Other Endpoints

Not applicable.

Subpopulations

Not applicable.

Additional Efficacy Considerations

Not applicable.

8.1.6. Integrated Assessment of Effectiveness

Phase 3 Trials 3101 and 3102 demonstrate the noninferiority of lefamulin relative to moxifloxacin for the treatment of CABP:

- They used the same primary efficacy endpoint, ECR on the ITT analysis set, and their ECR analyses used acceptable noninferiority margins. Whether using the Applicant's version of ECR or a "worst-case" version (these versions differed in how missing data were handled), testing yielded statistically significant support for the noninferiority of lefamulin.
- The trials also used the same key secondary endpoint, IACR at TOC on the mITT analysis set. Whether using the Applicant's version of IACR or a "worst-case" version (these versions differed in how missing data were handled), yielded consistent results.

- For other secondary endpoints and for by-pathogen clinical response endpoints, formal testing of the noninferiority of lefamulin was not conducted. However, in both trials the response rates for lefamulin were close to the response rates for moxifloxacin.
- In examining ECR values within subgroups defined in terms of demographic or baseline health status characteristics, no subgroups of a nontrivial size in either trial gave strong evidence that lefamulin was not noninferior to moxifloxacin.

8.2. Review of Safety

8.2.1. Safety Review Approach

The safety of IV and oral lefamulin for the treatment of CABP was evaluated primarily using the safety data from 641 subjects with CABP enrolled in two randomized, controlled trials (Table 85). Additional safety data were obtained from 71 subjects enrolled in a Phase 2 trial (Study 2001) for ABSSSI. See Table 54 for more information on the individual studies. Two safety review issues identified during early drug development that needed particular attention were: administration site reactions and QT prolongation.

8.2.2. Review of the Safety Database

Overall Exposure

In total, the lefamulin safety database includes 1988 subjects (1242 received lefamulin) who received at least one dose of study drug (Table 85). In Phase 1 studies, there were 460 subjects who received single or multiple doses of lefamulin; 280 were exposed to IV doses and 200 to oral doses. Single doses ranged from 25 mg to 400 mg IV and 100 mg to 750 mg orally. Multiple dose IV regimens included up to 150 mg q12h for 10 days or 200 mg q12h for 6 days. Multiple dose oral regimens included up to 600 mg q12h for 10 days. Of the 460 Phase one subjects, 391 received IV or oral doses at or above the proposed dose for CABP. In the Phase 2 trial (Study 2001), subjects with ABSSSI were treated with lefamulin IV 100 mg or 150 mg q12h for between 5 and 14 days. In the Phase 3 IV to oral CABP trial (Study 3101), subjects were initially treated with IV lefamulin 150 mg q12h or IV moxifloxacin 400 mg q24h. After 3 days of IV therapy, subjects could be switched to oral lefamulin 600 mg q12h or oral moxifloxacin 400 mg q24h. Subjects in both arms received a median total duration of study drug treatment of 7 days. In the second Phase 3 CABP trial (Study 3102), subjects were treated with oral lefamulin 600 mg q12h or oral moxifloxacin 400 mg q24h. Median duration of study drug treatment was 5 days for lefamulin and 7 days for moxifloxacin which reflects the intended duration in the protocol.

The Applicant pooled subjects into 3 groups for the safety analysis. Pool 1 consisted of 428 healthy volunteers from the 24 Phase 1 studies but did not include 32 subjects with hepatic or renal impairment, from Studies 1010 and 1011 respectively, who were analyzed separately.

Pool 3 consisted of subjects from the Phase 3 CABP Studies 3101 and 3102 who received lefamulin (IV and oral) compared to the active control, moxifloxacin (IV and oral). Pool 2-3 consisted of Pool 3 plus subjects from the Phase 2 ABSSSI study who received the 150 mg IV q12h dose of lefamulin (n=71) compared to IV vancomycin (n=66). For all studies, the safety population was defined in the protocols as subjects who received at least one dose of study drug.

M.O. Comment*: The Applicant's pooling strategy was acceptable. For most of the safety analyses, Pool 3 is used as it matches the proposed indication, dose, and duration. Pool 2-3 provided additional safety data in patients infected with CABP or ABSSSI.*

Table 85. Safety Database for the Lefamulin Development Program

	N=1988*		
Clinical Trial Groups	**Lefamulin (N=1242)**	**Active Control (N=707)**	**Placebo (N=39)**
Controlled trials conducted for this indication (CABP; Pool 3)	Lefamulin (n=641)	Moxifloxacin (n=641)	-
Study 3101	273	273	-
Study 3102	368	368	-
Controlled trials conducted for other indications (ABSSSI)	Lefamulin (n=141)	Vancomycin (n=66)	-
Study 2001	141**	66	-
Phase 1 trials	Lefamulin (n=460)	-	Placebo (n=39)
Healthy adults in 24 Phase 1 studies (Pool 1)	428	-	39
Subjects with hepatic and renal impairment in 2 Phase 1 studies	32	-	-

* Sum of all available numbers from the columns below
** Only 71 subjects received the 150 mg IV q12h dose and are included in Pool 2-3
ABSSSI = Acute Bacterial Skin and Skin Structure Infection; CABP = community-acquired bacterial pneumonia

Across the 3 Phase 2/3 studies, there were 10 subjects who were randomized but not treated and were not included in the safety analysis.

The demographic characteristics of Pool 3 (primary safety population) is summarized in the table below. These characteristics were well-balanced between the treatment groups. The patient population was mostly White (79.3%), non-Hispanic (92.1%), and male (55.6%). 40.2% of the population were over the age of 65 years and 17% of subjects were over the age of 75 years. Unless otherwise specified, the following safety analyses will be based on Pool 3 (the pooled Phase 3 CABP safety population) and will be referred to as the "Phase 3 Safety Population" throughout the remainder of this review.

Table 86. Demographic and Other Baseline Patient Characteristics of Pool 3 (Phase 3 Safety Population) by Actual Arm

	Lefamulin N=641	Moxifloxacin N=641	Combined N=1282
Age (years), mean	58.9	58.5	58.7
Age (years), median	61	60	61
Categorical age (years), n (%)			
18–64	374 (58.3)	393 (61.3)	767 (59.8)
65–74	152 (23.7)	145 (22.6)	297 (23.2)
>74	115 (17.9)	103 (16.1)	218 (17.0)
Sex, n (%)			
Female	267 (41.7)	302 (47.1)	569 (44.4)
Male	374 (58.3)	339 (52.9)	713 (55.6)
Race, n (%)			
White	508 (79.3)	508 (79.3)	1016 (79.3)
Black	30 (4.7)	34 (5.3)	64 (5.0)
Asian	72 (11.2)	71 (11.1)	143 (11.2)
Amer. Indian or Alaska Native	24 (3.7)	17 (2.7)	41 (3.2)
Other	7 (1.1)	11 (1.7)	18 (1.4)
Ethnicity, n (%)			
Hispanic or Latino	53 (8.3)	48 (7.5)	101 (7.9)
Not Hispanic or Latino	588 (91.7)	593 (92.5)	1181 (92.1)
Geographic region, n (%)			
North America[1]	13 (2.0)	13 (2.0)	26 (2.0)
Latin America	42 (6.6)	44 (6.9)	86 (6.7)
Eastern Europe	451 (70.4)	434 (67.7)	885 (69.0)
Western Europe	32 (5.0)	33 (5.1)	65 (5.1)
Rest of the world	103 (16.1)	117 (18.3)	220 (17.2)
PORT risk class, n (%)			
Class I	1 (0.2)	2 (0.3)	3 (0.2)
Class II	183 (28.5)	190 (29.6)	373 (29.1)
Class III	337 (52.6)	333 (52.0)	670 (52.3)
Class IV	115 (17.9)	111 (17.3)	226 (17.6)
Class V	5 (0.8)	5 (0.8)	10 (0.8)
Kidney disease[2], n (%)			
Normal	310 (48.4)	311 (48.5)	621 (48.4)
Mild renal impairment	198 (30.9)	192 (30.0)	390 (30.4)
Moderate renal impairment	125 (19.5)	132 (20.6)	257 (20.0)
Severe renal impairment	7 (1.1)	6 (0.9)	13 (1.0)
History of lung disease[3], n (%)			
Yes	134 (20.9)	126 (19.7)	260 (20.3)
No	507 (79.1)	515 (80.3)	1022 (79.7)
History of heart disease[4], n (%)			
Yes	110 (17.2)	120 (18.7)	230 (17.9)
No	531 (82.8)	521 (81.3)	1052 (82.1)
History of diabetes mellitus, n (%)			
Yes	80 (12.5)	87 (13.6)	167 (13.0)
No	561 (87.5)	554 (86.4)	1115 (87.0)

[1]All North American subjects were from the United States
[2]One subject in the LEF arm was missing renal impairment status
[3]Based on having a medical history term in the SOC of Respiratory disorders
[4]Based on having a medical history term in the SOC of Cardiac disorders

PORT = Pneumonia Outcomes Research Team

Adequacy of the safety database

The safety database includes 641 subjects with CABP and another 71 subjects with ABSSSI who all received the intended dose (150 mg IV or 600 mg PO). Only 2% of subjects in the Phase 3 safety population were from the United States.

M.O. Comment*: The size of the safety database is adequate per the draft CABP guidance which states a minimum of 700 patients be included. The number of subjects with a history of kidney, lung, and heart disease is adequate. The diversity in race and geography is not ideal, but in general, the patient population enrolled is similar to the U.S. population.*

8.2.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

There were no major issues regarding data integrity for these applications. The submitted materials were generally organized well. Please refer to Section 4.1 for details on the OSI clinical site inspections.

Categorization of Adverse Events

There were no identified issues with the coding or categorizing of AEs. The Applicant used MedDRA version 20.0 to code AEs for both Phase 3 trials. AEs and TEAEs were defined appropriately in the protocols. AEs were reported from subject consent to the TOC visit (5 to 10 days after the last dose of study drug) and SAEs from consent to the LFU visit (Day 30 +/- 3 days).

Routine Clinical Tests

Overall, the routine clinical testing done in the two Phase 3 studies was adequate. Subjects had vital signs recorded daily (heart rate, blood pressure, temperature, respiratory rate, and oxygen saturation). Regular laboratory testing including chemistry, hematology, and urinalysis. Of note, chemistry laboratory testing did not include serum bicarbonate levels as this was not specified in either study protocol. ECGs were performed at baseline and again at Day 3 or 4.

8.2.4. Safety Results

Deaths

There were 19 deaths in the lefamulin Phase 3 clinical development program: 11 deaths in Study 3101 and eight deaths in Study 3102. In Study 3101, six subjects died in the lefamulin (LEF) arm and five died in the moxifloxacin (MOX) arm. Of note, two of the deaths (1 from each arm) occurred after Day 28. In Study 3102, five subjects died in the lefamulin arm and three died in the moxifloxacin arm. Of note, two of the deaths (both in the lefamulin arm) occurred after Day 28. Therefore, in the two Phase 3 trials, a total of 15 deaths occurred by Day 28: eight deaths in the LEF arm (1.2%) compared to seven deaths in the MOX arm (1.1%). The Applicant prespecified 28-day all-cause mortality in the ITT analysis set as a secondary endpoint. Table 87 provides additional details about the deaths from the two Phase 3 trials. There were no deaths in the Phase 2 ABSSSI study (2001) or in the Phase 1 clinical program.

***M.O. Comment**: Overall, deaths were balanced between the treatment groups.*

Table 87. Summary of Deaths in the Phase 3 Safety Population

Age/Sex/Race	Subject ID	Cause of Death	Last Day of Study Drug	Day of Death
Study 3101 (IV/Oral)				
Lefamulin				
72/M/Asian	(b) (6)	Unknown (presumed ventricular arrhythmia; patient died at home after severe dyspnea; no autopsy)	2*	20
87/F/Asian		Sepsis from HABP (BAL culture positive for *Citrobacter koseri*)	8	32
65/M/White		Congestive heart failure	3	4
78/F/White		Unknown (presumed myocardial infarction; patient died at home after chest pain; no autopsy)	8	23
59/F/White		Respiratory failure from pneumonia	2	3
84/M/White		Respiratory failure from COPD	6	6
Moxifloxacin				
66/M/Asian	(b) (6)	Stroke	3	4
26/M/White		Testicular cancer with lung metastasis	8	48
78/F/White		Hemorrhagic shock from hematemesis	1	1
77/M/White		Cardiac arrest	9	18
61/M/Black		Unknown (died at home in bed; no autopsy)	8	18
Study 3102 (Oral)				
Lefamulin				
25/M/Asian	(b) (6)	Acute respiratory distress syndrome	1	2
70/F/White		Acute myeloid leukemia	5	271
80/M/White		Endocarditis (blood culture positive for *Enterococcus faecalis)*	5	57
70/M/White		Myocardial infarction	2	3
80/F/White		Pulmonary edema	1	1

Age/Sex/Race	Subject ID	Cause of Death	Last Day of Study Drug	Day of Death
Moxifloxacin				
75/F/White	(b) (6)	Respiratory failure	4	4
68/M/White		Unknown (died at home after collapsing; no autopsy)	7	12
53/M/Black		Stroke	7	18

* Study drug was stopped as subject had abnormal baseline ECG findings, elevated cardiac enzymes, and complicated presentation with pneumothorax.
IV = intravenous; COPD = chronic obstructive pulmonary disease; HABP = hospital-acquired bacterial pneumonia; BAL = bronchoalveolar lavage

Death Narratives

Study 3101

Lefamulin arm

- Subject (b) (6) was a 72-year-old male from the Philippines with a history of previously treated pulmonary tuberculosis, heavy tobacco use, coronary artery disease, and COPD who died on study day 20. He presented with CABP complicated by pneumothorax that required aspiration. The baseline pathogens were *H. influenzae* and *M.* catarrhalis. On presentation he also had "borderline elevated" cardiac enzymes (values not provided). On study day 2, he was noted to have QT prolongation (up to 503 ms). Based on the patient's medical history, new ECG findings, and complicated presentation it was decided the patient was inappropriately enrolled in the study and lefamulin was discontinued on the same day (study day 2), but he was continued in the study for safety monitoring. He was started on piperacillin/tazobactam and levofloxacin for the CABP on study day 3. Of note, the QT interval was reduced to 367 ms. He was diagnosed with hospital-acquired pneumonia on study day 7; a follow-up X-ray showed new infiltrates in the left lower lobe and lingula. He left the hospital on study day 17 against medical advice but was continued on oral antibacterial therapy for pneumonia with amoxicillin/clavulanate. On study day 20, he had severe dyspnea, loss of consciousness, and died. A fatal arrhythmia was suspected. No autopsy was performed.

M.O. Comment*: The patient could have died from an arrhythmia, pulmonary embolism, or another cause. However, this death is unlikely to be related to study drug as it was stopped 18 days before death. Notably, lefamulin was stopped early (after only 2 days) because the subject had a history of heart disease with elevated cardiac enzymes, pneumothorax at presentation, and QT prolongation. Based on the elevated cardiac enzymes and chest pain at presentation, he could have met the exclusion criterion of "active myocardial ischemia." However, the chest pain was pleuritic in nature making cardiac ischemia less likely.*

- Subject (b) (6) was an 87-year-old female from the Philippines with a history of COPD and hypertension who died on study day 32 from sepsis. The patient received 8 days of IV study drug for *H. influenzae* CABP, but immediately afterward required additional treatment with piperacillin/tazobactam and azithromycin for hospital-acquired bacterial pneumonia (HABP) and insufficient response to study drug. The HABP was diagnosed

based on new radiographic findings in a different location compared to baseline and worsening symptoms. A BAL culture from day 12 grew *Citrobacter koseri*, which was resistant to lefamulin (as are all Enterobacteriaceae). The piperacillin/tazobactam and azithromycin were administered from day 8 to day 17. Starting from study day 18, the patient received several additional antibacterial drugs to treat the HABP including meropenem, levofloxacin, gentamicin, ceftazidime, and cefepime. On study day 31, while the patient was still in the hospital, she was diagnosed with sepsis after having sudden obtundation and hypotension. The next day, she was taken home against medical advice and died.

M.O. Comment*: The patient's death could have been from sepsis but with the information provided, a stroke could also explain the events. Regardless, this death is unlikely to be related to study drug toxicity as it occurred 23 days after lefamulin was discontinued. This case is an example of the development of pneumonia reported as a TEAE in which a culture on day 12 showed a secondary pneumonia from an Enterobacteriaceae that was likely acquired in the hospital.*

- Subject (b) (6) was a 65-year-old male from Bosnia and Herzegovina with a history of arteriosclerosis and aortic bypass who died on study day 4 from congestive heart failure. He was admitted to the hospital and treated for CABP with study drug. The baseline pathogen was *S. pneumoniae*. Methylprednisolone was given concomitantly for "respiratory failure." On study day 2, he developed atrial fibrillation which was treated with dalteparin, digoxin, and propafenone. On study day 3, he developed congestive heart failure and study drug was stopped. No symptoms of CHF were provided. Ceftriaxone and azithromycin were started for CABP and CHF was treated with furosemide, amiodarone, and oxygen. He died on study day 4. No autopsy was performed. The death certificate listed pneumonia as the immediate cause of death with decompensated cardiomyopathy and exacerbated COPD as conditions that led to the immediate cause of death.

M.O. Comment*: Decompensated cardiomyopathy and exacerbated COPD were listed on the death certificate, but neither condition was listed in the patient's medical history. It appears the patient likely had these underlying conditions which were exacerbated by pneumonia and led to his death. Atrial fibrillation may have worsened these conditions. If the study drug led to the arrhythmia, it may have contributed to this death.*

- Subject (b) (6) was a 78-year-old female from the country of Georgia with a history of hypertension, diabetes mellitus with retinopathy, and mild aortic and mitral valve stenosis who died on study day 23 from a presumed myocardial infarction. She was admitted to the hospital with CABP and treated with study drug for 8 days. The baseline pathogen was *S. aureus*. She responded well and was discharged home. QT intervals were normal during treatment. On study day 23, she had chest pain while at home and died. There was no autopsy.

***M.O. Comment**: The cause of death could have been myocardial infarction as proposed by the study site, but pulmonary embolism could also have explained the events. Regardless, this death is unlikely to be related to lefamulin given the death occurred 15 days after the end of study therapy.*

- Subject (b) (6) was a 59-year-old female from Serbia with no reported medical history who died on study day 3 from respiratory failure. She was admitted to the hospital for CABP and treated with study drug. The baseline pathogen was *S. pneumoniae* which grew from blood culture and was identified by NP swab PCR, sputum PCR, and urinary antigen. On study day 3, the patient became somnolent with hypoxemia and signs of cardiorespiratory failure. Despite treatment with mannitol, dalteparin, intravenous fluids (0.9% saline and 5% dextrose), and oxygen she died. Her blood pressure was normal during her clinical course (100/50 on day 1, 120/80 on day 2, and 120/70 on day 3) and no vasopressors were administered. An autopsy showed evidence of bacterial pneumonia and medium grade myocardial hypertrophy of the left ventricle. The death certificate listed pneumonia and respiratory failure as the causes of death.

***M.O. Comment**: This death was from severe pneumonia leading to respiratory failure and unlikely a result of toxicity from study drug. However, the M.O. cannot rule out lack of efficacy of the study drug.*

- Subject (b) (6) was an 84-year-old male from Serbia with history of COPD who died from respiratory failure on study day 6. Prior to admission he was on chronic treatment for COPD with inhaled fenoterol/ipratropium and budesonide/formoterol and oral theophylline. He was admitted to the hospital and treated with study drug for CABP. The baseline pathogen was not specified. On study day 4, he developed a COPD exacerbation which progressed despite treatment with methylprednisolone and oxygen. He had hypercarbic and hypoxemic respiratory failure and died on study day 6.

***M.O. Comment**: This subject likely had severe COPD as he was taking multiple inhalers and oral theophylline prior to his admission for CABP. As a result, this death is unlikely to be related to study drug unless evidence is found to implicate lefamulin with worse respiratory outcomes. However, the M.O. cannot rule out lack of efficacy of the study drug.*

Moxifloxacin arm

- Subject (b) (6) was a 66-year-old male from the Philippines with a history of diabetes mellitus and congestive heart failure who died on study day 4 from a stroke. He was admitted to the hospital and treated with study drug for CABP. The baseline pathogen was not specified. In addition, he had a CHF exacerbation on admission (prior to study drug) and was treated with furosemide. Also prior to first dose of study drug, the patient

was noted to have bigeminy and trigeminy on cardiac monitoring in the ICU. He was treated with amiodarone for the arrhythmia on study day 2, which was a prohibited medication. The study drug was stopped on study day 3 because of the arrhythmia and treatment with ceftriaxone was started for CABP. On study day 4, he developed cardiogenic shock and stroke and died the same day. The death certificate listed uncal herniation as the immediate cause of death with cerebrovascular disease as the antecedent cause of death.

M.O. Comment*: The subject experienced arrhythmia and CHF exacerbation prior to study drug administration and then experienced the TEAEs of shock and stroke. The M.O. cannot rule out the possibility that the study drug may have worsened the arrhythmia and contributed to the cardiac disease, but the stroke is unlikely to be related to study drug.*

- Subject (b) (6) was a 26-year-old male from Bulgaria with no known prior medical history who died on study day 48 with likely metastatic testicular cancer. He was admitted to the hospital and treated with study drug for CABP caused by *S. pneumoniae* for 8 days and responded well to treatment. However, during the hospitalization he was found to have a pulmonary mass which on biopsy was found to be "bronchial carcinoma." On study day 21 he was noted to have testicular seminoma from which he died on study day 48. No autopsy was performed, and the death certificate was not available.

M.O. Comment*: This patient likely had metastatic testicular cancer prior to study drug administration and therefore this death is not related to study drug.*

- Subject (b) (6) was a 78-year-old female from Bulgaria with a history of chronic heart failure, hypertension, and Graves disease who died on study day 1 from hemorrhagic shock. She was admitted to the hospital and treated with study drug for CABP. The baseline pathogen was not specified. However, on the evening of the first study day she vomited a large amount of blood and lost consciousness. Despite treatment with epinephrine, atropine, etamsylate, and fluids she died the same day. There was no autopsy and no death certificate was available. One risk factor for a gastrointestinal hemorrhage in this case was use of diclofenac prior to admission.

M.O. Comment*: This death is unlikely to be related to study drug as it occurred so quickly after starting antibacterial therapy.*

- Subject (b) (6) was a 77-year-old male from Russia with a history of COPD, hypertension, ischemic heart disease with MI, and CHF who died on study day 18 from cardiac arrest. He was admitted to the hospital and received 9 days of study drug for CABP caused by *H. influenzae*. No ECGs showed QT prolongation. However, after treatment he was noted to have leukocytosis, cough, and shortness of breath and was treated with cefoperazone/sulbactam for refractory pneumonia. The investigator

considered treatment with study drug as a "failure." On study day 18, while still in the hospital, the patient had a cardiac arrest with an idioventricular rhythm. Despite resuscitative efforts, the patient died. No autopsy was performed, and the death certificate was not available.

***M.O. Comment**: This death is unlikely to be related to study drug as postdose ECGs were normal and the event occurred 9 days after the last dose of study drug. However, the M.O. cannot rule out lack of efficacy leading to treatment failure.*

- Subject (b) (6) was a 61-year-old male from South Africa with a history of asthma who died from unknown causes on study day 18. He was treated as an outpatient for CABP caused by *M. catarrhalis* with IV study drug for 8 days. Post-dose ECGs showed inverted T waves, ventricular premature complexes, and sinus tachycardia. The baseline QTcF value was 383 ms and all postdose triplicate mean QTcF values were <403 ms. Assessments on study days 9 and 17 were recorded as clinical success. However, the patient died at home in bed on study day 18 without any reported symptoms. There was no autopsy and the death certificate listed "natural causes" as the cause of death.

***M.O. Comment**: Even though the cause of death in this case is not known, it is unlikely to be related to study drug given the 9-day gap between last dose of study drug and death.*

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Lefamulin arm

- Subject (b) (6) was a 25-year-old male from the Philippines with no reported past medical history who died on study day 2 from acute respiratory distress syndrome (ARDS). He presented with high fever (40.5°C), dyspnea, productive cough, chest pain. Oxygen saturation was 90%, HR was 131 beats/min, and BP was 90/60 mmHg. Notable laboratory findings included a WBC count of 36.3 x 10^9 /L. He was started on study drug one day after admission to the hospital (day 1). Baseline pathogens included *H. influenzae, M. catarrhalis, and S. pneumoniae* (from sputum PCR and NP swab). Blood cultures were negative. On day 2, he developed ARDS requiring intubation and mechanical ventilation. No vital signs were reported from day 2. Despite these interventions, the patient had cardiac arrest and died.

***M.O. Comment**: It is unclear why this acutely ill patient was admitted to the hospital but not given IV antibacterial therapy immediately rather than oral therapy one day after admission. Though the M.O. cannot completely rule out lack of efficacy of study therapy, this death appears to be a result of severe CABP and delayed initiation of antibacterial treatment.*

- Subject (b) (6) was a 70-year-old female from Hungary with history of COPD and hypertension who died on study day 271 from acute myeloid leukemia (AML). She was

initially treated with 5 days of oral lefamulin for CABP and responded well. Baseline pathogens included *H. influenzae*, *H. parainfluenzae*, and *M. pneumoniae*. Months later (264 days after her last dose of lefamulin) she was admitted with respiratory failure, was diagnosed with AML, and died.

M.O. Comment: *Though it is unlikely that the study drug caused the AML, the M.O. cannot completely rule this out because there are no long-term carcinogenicity studies in animals with lefamulin.*

- Subject (b) (6) was an 80-year-old male from Hungary with history of myocardial ischemia, aortic stenosis, rheumatoid arthritis, COPD, and HTN who died on study day 57 from endocarditis. He was initially treated with 5 days of lefamulin for CABP and responded well. Of note, a BAL culture grew *S. aureus*. On study day 23, the patient presented with dyspnea, but the etiology was unclear. He received antibacterial drugs (amoxicillin/clavulanate, moxifloxacin), methylprednisolone, and diuretics presumably to treat pneumonia, COPD exacerbation, and heart failure, respectively. However, a cardiac echocardiogram on study day 33 showed an aortic valve vegetation and a blood culture from study day 46 grew *Enterococcus faecalis*. Taken together, these two findings were used to make the diagnosis of endocarditis. He was treated with ampicillin and gentamicin from study day 48 to his death on study day 57. No details regarding his death such as a death certificate or autopsy information were provided.

M.O. Comment*: This death is unlikely to be related to the study drug as the patient had underlying cardiac valve disease (aortic stenosis) which predisposed him to Enterococcus faecalis endocarditis.*

- Subject (b) (6) was a 70-year-old male from Hungary with history of tobacco use and coronary artery bypass and stent placement who died on study day 3 from myocardial infarction. The baseline CABP pathogen was unknown. The patient died suddenly, and resuscitation efforts were not successful. There was no report an of ECG performed at the time. The autopsy showed recurrent myocardial infarction that may have been exacerbated by acute pneumonia.

M.O. Comment*: This death is unlikely related to the study drug as the patient had underlying cardiovascular disease.*

- Subject (b) (6) was an 80-year-old female from Serbia with history of diabetes mellitus and HTN who died on study day 1 with pulmonary edema. The baseline CABP pathogen was unknown. The patient was admitted to the hospital and given oral study drug the same day. The baseline (predose) ECG showed left ventricular hypertrophy, ST depression, and T-wave inversion. A 1-hour postdose ECG showed left bundle branch block, QTc prolongation, and sinus tachycardia. Later that day, she developed acute

hypoxic respiratory failure and died. The autopsy showed severe pulmonary edema and severe myocardial hypertrophy.

M.O. Comment*: This death is unlikely to be related to study drug as the patient had underlying cardiac hypertrophy which led to acute pulmonary edema.*

Moxifloxacin arm

- Subject (b) (6) was a 75-year-old female from Hungary with a history of COPD, myocardial ischemia, and hypertension who died on study day 4 from respiratory failure. The patient was admitted to the hospital and given oral study drug the following day for *S. pneumoniae* CABP. On day 4, the patient development atrial fibrillation with a heart rate of 141. Arterial blood gas showed pH 7.23, pCO2 60 mmHg, and pO2 41 mmHg. She was treated with furosemide and methylprednisolone but died later the same day. The autopsy showed acute respiratory failure with pneumonia and underlying COPD as the cause of death.

M.O. Comment*: This death is unlikely to be related to study drug as the patient had underlying lung disease which in combination with CABP may have led to the respiratory failure. Though less likely, the M.O. cannot completely rule out that the study drug may have contributed to the atrial fibrillation or that lack of efficacy of the study drug led to treatment failure.*

- Subject (b) (6) was a 68-year-old male from South Africa with history of diabetes mellitus, hypertension, and prostate cancer who died on study day 12 from unknown causes. The baseline CABP pathogen was unknown. He received a single oral dose of amoxicillin/clavulanate for CABP one day prior to the start of study drug. He completed 7 days of study drug for CABP and responded well. On study day 12, the patient was at home and reportedly without complaints. He later collapsed and did not recover. An autopsy was not performed.

M.O. Comment*: A cardiac arrhythmia could have caused this death. If so, the M.O. cannot rule out that the study therapy may have contributed to the development of the arrhythmia as moxifloxacin is known to cause QT prolongation.*

- Subject (b) (6) was a 53-year-old male from South Africa with a history of stroke and hemiplegia who died on study day 18 from a stroke. The patient completed 7 days of study drug for CABP and responded well. The baseline CABP pathogen was unknown. On study day 17, the patient was admitted to the hospital for worsening hemiplegia, aspiration pneumonia, and peptic ulcer. He died the next day. An autopsy was not performed.

M.O. Comment*: This death is unlikely to be related to study drug as the patient had underlying cerebrovascular disease which led to his death.*

Serious Adverse Events

In the two Phase 3 CABP studies, there were 36 subjects in the lefamulin group (5.6%) and 31 subjects in the moxifloxacin group (4.8%) who experienced at least one treatment-emergent SAE. The table below provides an overview of SAEs in the Phase 3 safety population.

Table 88. Treatment-Emergent Serious Adverse Events in the Phase 3 Safety Population by System Organ Class and Preferred Term

System Organ Class/Preferred Term	Lefamulin N=641 n (%)	Moxifloxacin N=641 n (%)
Infections and infestations*	17 (2.7)	9 (1.4)
Pneumonia[1]	9	2
Urinary tract infection	2	1
Empyema	1	0
Endocarditis	1	0
Infectious pleural effusion	1	1
Lung abscess	1	3
Pulmonary tuberculosis	1	1
Sepsis	1	0
Viral pharyngitis	1	0
Tuberculous pleurisy	0	1
Respiratory, thoracic and mediastinal disorders	8 (1.2)	4 (0.6)
Acute respiratory distress syndrome	2	0
Acute respiratory failure	1	1
Bronchial disorder	1	0
Chronic obstructive pulmonary disease	1	0
Pleurisy	1	0
Pulmonary embolism	1	1
Pulmonary edema	1	0
Pulmonary necrosis	0	1
Respiratory failure	0	1
Cardiac disorders*	6 (0.9)	5 (0.8)
Myocardial infarction[2]	3	3
Atrial fibrillation	2	0
Ventricular arrhythmia	1	0
Cardiac failure congestive	1	0
Cardiac arrest	0	1
Cardiogenic shock	0	1
Myocardial ischemia	0	1

System Organ Class/Preferred Term	Lefamulin N=641 n (%)	Moxifloxacin N=641 n (%)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)*	4 (0.6)	3 (0.5)
Acute myeloid leukemia	1	0
Lung neoplasm	1	0
Renal cancer	1	0
Squamous cell carcinoma of lung	1	1
Bronchial carcinoma	0	1
Small cell lung cancer	0	1
Testicular seminoma (pure)	0	1
Investigations	3 (0.5)	1 (0.2)
Alanine aminotransferase increased	1	0
Liver function test increased	1	0
Nuclear magnetic resonance imaging brain abnormal	1	0
Hepatic enzyme increased	0	1
General disorders and administration site conditions	1 (0.2)	2 (0.3)
Injection site reaction	1	0
Death	0	2
Nervous system disorders	0	4 (0.6)
Cerebrovascular accident	0	2
Embolic stroke	0	1
Cerebral infarction	0	1
Gastrointestinal disorders	0	2 (0.3)
Hematemesis	0	1
Inguinal hernia strangulated	0	1
Skin and subcutaneous tissue disorders	0	2 (0.3)
Angioedema	0	2
Metabolism and nutrition disorders	0	1 (0.2)
Hypokalemia	0	1
Vascular disorders	0	1 (0.2)
Shock hemorrhagic	0	1
Hepatobiliary disorders	0	1 (0.2)
Cholecystitis acute	0	1
Blood and lymphatic system disorders	0	1 (0.2)
Anemia	0	1

*Note: Subjects with more than one TEAE in the same SOC were counted only once in that SOC.
[1]Includes the preferred terms: "pneumonia" and "pneumonia bacterial."
[2]Includes the preferred terms: "myocardial infarction" and "acute myocardial infarction."

M.O. Comment: *Review of the case narratives showed that the only cases with a clear relationship between lefamulin exposure and an SAE were one case of an injection site reaction and two cases of liver enzyme elevation. Regarding the injection site reaction, the subject developed pain and redness at two different study drug administration sites. The investigator reported the subject had difficulty using the affected arm, but the symptoms resolved by Day 11 (7 days after last dose of study drug). Of note, administration site reactions are discussed*

further in Section 8.2.5.1. The two cases of liver enzyme elevations (maximum ALT 600 U/L in one case and 172 U/L in the other) were asymptomatic and resolved after study drug was discontinued. The incidence of liver enzyme SAEs was similar between the two groups.

In the SOC of Infections and Infestations, there were 17 subjects in the LEF arm with SAEs compared to 9 in the MOX arm. Of the 17 LEF subjects, 12 had lung infections (PTs of pneumonia, infectious pleural effusion, lung abscess, pneumonia bacterial, and empyema). Of the 9 MOX subjects, 6 had lung infections. Case narratives for these 18 subjects with lung infections as SAEs are below.

LEF Subjects

- (b) (6) was an 81-year-old male from the Philippines with a history of cerebrovascular disease, hypertension, and remote pulmonary TB who received 7 days of LEF (3 days IV; 4 days oral) for PORT risk class III CABP. The baseline pathogen was *S. pneumoniae*. However, a sputum culture grew *K. pneumoniae*, but the Gram stain morphology was not consistent and so it did not qualify as a baseline pathogen. Screening chest X-ray showed infiltrates in the left lower lobe. The subject initially responded well to treatment and was discharged home on day 8. He was a responder for ECR. On day 17 (10 days after completing study drug), he was diagnosed with a **"relapse" of CABP** with fever, leukocytosis, and infiltrates in the left lower lobe. No additional microbiology results are available. He was treated with piperacillin-tazobactam from day 18 to day 22. He was discharged on day 22 and the pneumonia was noted as resolved. The IACR at EOT and TOC were both noted as success but was deemed a relapse at LFU.

***M.O. Comment**: The* K. pneumoniae *may not have been a pathogen associated with the initial episode of CABP as the subject improved on LEF treatment despite it having no activity against Enterobacteriaceae. The "relapse" of pneumonia did not have new radiographic findings but was associated with signs and symptoms that would be consistent with pneumonia. Overall the AE of pneumonia appears to be a second, separate diagnosis as he was improved after receiving study drug.*

- (b) (6) was a 72-year-old male from the Philippines with a history of COPD, CAD, and pulmonary TB who received 2 days of IV LEF for PORT risk class IV CABP. The baseline pathogens were *H. influenzae* and *M. catarrhalis*. However, a sputum culture also grew *K. oxytoca*, but the sputum was not considered adequate (the Gram stain PMN count was too low and the squamous cell count was too high). On initial presentation, the subject was noted to have a right lower lobe infiltrate and a left pneumothorax. The pneumothorax was drained, and he was enrolled in the study. However, on day 2 it was decided he was not an appropriate subject for the study given his complicated presentation and the finding of elevated cardiac enzymes (without cardiac-type chest pain) and ECG findings (right bundle branch block, ST depression, and inverted T waves).

Treatment with study drug was stopped and levofloxacin and piperacillin/tazobactam were started instead. He was a nonresponder for ECR. On day 7 (while still on antibacterial therapy), he was diagnosed with **hospital acquired pneumonia** based on new infiltrates in the right and left lower lobes on X-ray. He died on day 20 after suddenly losing consciousness at home. See "Deaths" section for details on this aspect of the case. He was noted to be a failure by IACR at all time points.

M.O. Comment*: This case is complicated, but the diagnosis of HABP appears valid. It should be noted that the HABP was diagnosed 5 days after stopping lefamulin and that the subject only received 2 days of study drug.*

- (b) (6) was an 87-year-old female from the Philippines with a history of COPD and hypertension who received 8 days of IV LEF for PORT risk class III CABP. The baseline pathogen was *H. influenzae*. Screening chest X-ray showed infiltrates in the right lower lobe. She was a responder for ECR. However, on the last day of study drug (day 8), she was diagnosed with **hospital acquired pneumonia** with increased symptoms and infiltrates in the right middle and lower lobes, left upper and lower lobes, and lingula on X-ray. She was started on piperacillin/tazobactam and azithromycin for the HABP. A BAL culture from day 12 grew *Citrobacter koseri*, which was resistant to lefamulin (as are all Enterobacteriaceae). She died on day 32 related to sepsis. See "Deaths" section for details on this aspect of the case. She was noted to be a failure by IACR at all time points.

M.O. Comment*: The diagnosis of hospital acquired bacterial pneumonia appears valid with new infiltrates on X-ray.*

- (b) (6) was a 59-year-old female from Serbia with no documented medical history who received 2 days of IV LEF for PORT risk class III CABP. The baseline pathogen was *S. pneumoniae*. Screening chest X-ray showed infiltrates in the right middle/lower lobe, left lower lobe, and lingula. On day 3, the subject experienced **"respiratory stasis due to bacterial pneumonia."** She was somnolent, with tachypnea and hypoxemia, but with normal temperature and blood pressure. Despite treatment with oxygen she died on day 3. See "Deaths section" for details on this aspect of the case. She was noted to be a failure by IACR at all time points and a nonresponder for ECR.

M.O. Comment*: The verbatim term of "respiratory stasis due to bacterial pneumonia" was coded as "pneumonia" but could have been coded differently. For example, "respiratory failure" or "acute respiratory failure" appear to more accurately reflect the events of this case. Pneumonia was likely a key contributor to the outcome, but the AE was not a pneumonia.*

- (b) (6) was an 84-year-old male from Ukraine with a history of atrial fibrillation, cerebral arteriosclerosis, chronic cardiac failure, coronary artery disease, encephalopathy, and hypertension who received 5 days of IV LEF for PORT risk class IV

CABP. The baseline pathogens were *E. cloacae* and *S. pneumoniae*. Screening chest X-ray showed infiltrates in the right upper lobe. On day 6, the subject continued to have a fever (38.1 C) and respiratory symptoms and so study drug was stopped for lack of efficacy. Sputum culture on day 6 grew *Haemophilus haemolyticus* and pleural fluid culture on day 13 grew *K. pneumoniae*, *E cloacae*, and *E. faecalis*. Alternative therapy with vancomycin, ceftriaxone, and azithromycin was started on day 6. He was a nonresponder for ECR. On day 14 (9 days after last dose of study drug), he was diagnosed with an **infectious pleural effusion** on CT scan which required thoracentesis and transfer to another hospital. He was noted to be a failure by IACR at all time points.

M.O. Comment*: This empyema is unlikely to be related to study drug as it occurred several days after stopping LEF. However, one of the baseline pathogens (E. cloacae) and the pleural fluid pathogens are not covered by LEF and could have led to the treatment failure which necessitated alternative therapy.*

- (b) (6) was a 64-year-old female from the United States with a history of asthma, cardiovascular disorder, iron deficiency anemia, and sinusitis who received 1 day of oral LEF for PORT risk class III CABP. The baseline pathogen was *S. pneumoniae*. Screening chest X-ray showed a hazy opacity at the right lung base. A CT scan on the same day revealed a **lung abscess** in the right lower lobe that was documented as a SAE. In addition, the subject had elevated troponin levels and was diagnosed with acute myocardial infarction also as an SAE. Because of both SAEs, the study drug was stopped on day 1 and alternative antibacterial drugs (meropenem, linezolid, clindamycin) were started on day 2. She was noted to be a failure by IACR at all time points and a nonresponder for ECR.

M.O. Comment*: The lung abscess appears to have been present at baseline, so in actuality was not truly a TEAE and is not related to study drug. However, the AE was reported as such as it was discovered postrandomization.*

- (b) (6) was a 68-year-old female from the Philippines with a history of C-section and partial thyroidectomy who received 5 days of oral LEF for PORT risk class II CABP. The baseline pathogen was *S. pneumoniae*. However, sputum cultures from day 1 grew *K. pneumoniae*, *Klebsiella variicola*, and *E. cloacae* and from day 2 grew *E. coli* and *K. pneumoniae* but neither sputum was not considered adequate. Screening chest X-ray showed infiltrates in the right upper lobe. On day 8, she was discharged from the hospital and assessed as a success by the investigator (EOT). She was also a responder for ECR. On day 12, 7 days after the last dose of LEF, the subject was admitted to the hospital with fever, cough, and pleuritic chest pain. At this time the chest X-ray showed infiltrates in the right middle and lower lobes and she was diagnosed with pneumonia. After treatment with several antibacterial drugs, the pneumonia was considered resolved on day 22. She was noted to be a failure by IACR at TOC and LFU.

M.O. Comment*: This pneumonia appears to be a separate diagnosis from the initial pneumonia as the subject was improved after completing LEF and then later developed symptoms and new X-ray findings. Regarding the baseline pathogens, it appears the Enterobacteriaceae that grew from sputum culture on days 1 and 2 were likely not pathogens as the subject improved initially without adequate coverage of these organisms.*

- (b) (6) was a 45-year-old male from Peru with a history of obesity who received 5 days of oral LEF for PORT risk class II CABP. The baseline pathogen was *S. pneumoniae*. Screening chest X-ray showed infiltrates in the right lower lobe with right diffuse opacities. He was a responder for ECR. However, on day 5 (last day of LEF), the subject had fever (38.2 C), moderate dyspnea, and production of purulent sputum. Also, on day 5, a sputum culture was positive for *Klebsiella pneumoniae*. On day 8 (3 days after stopping LEF), the subject was diagnosed with bacterial pneumonia and treated with nonstudy antibacterial drugs. X-ray at this time showed pleural effusion. The AE of pneumonia was considered resolved by day 29. He was noted to be a failure by IACR at all time points.

M.O. Comment*: Although the pneumonia AE was diagnosed 3 days after stopping LEF, the subject had continued symptoms of pneumonia at the end of treatment. In addition, a nonbaseline sputum culture grew Klebsiella pneumoniae which was not covered by LEF. As a result, I would classify this case as treatment failure of LEF which is captured in the IACR.*

- (b) (6) was a 63-year-old male from Hungary with a history of COPD, hypertension, pneumonia 4 months prior to admission, and salivary gland adenoma who received 5 days of oral LEF for PORT risk class III CABP. The baseline pathogens were *S. pneumoniae* and *C. pneumoniae*. In addition, a sputum culture from day 1 grew *H. parainfluenzae* but the sputum was not considered adequate. Screening chest X-ray showed infiltrates in the right lower lobe. At the EOT visit on day 8 he only had mild cough as a reported symptom and was assessed as a success by IACR. He was also a responder for ECR. However, he was diagnosed with an AE of **pneumonia** on day 9 but did not receive treatment (no symptoms reported). On day 12 he was admitted to the hospital with moderate dyspnea and cough, WBC 15.6 (up from 12.4 on day 8), and unchanged chest radiograph. He was started on nonstudy antibacterial drugs on day 13. On day 14, WBC improved to 8.4. The AE of pneumonia was considered resolved by day 20. He was noted to be a failure by IACR at TOC and LFU.

M.O. Comment*: In this case, it is difficult to determine if the AE of pneumonia was a separate diagnosis or failure of study drug treatment. It appears LEF did improve the subject's symptoms, but WBC was still elevated suggesting continued inflammation likely from the original pneumonia. Therefore, I would deem this case as a treatment failure.*

- (b) (6) was a 67-year-old male from Russia with a history of stable angina, heart failure, COPD, hypertension, glucose intolerance, and pulmonary fibrosis who received 5 days of oral LEF for PORT risk class II CABP. The baseline pathogen was unknown. Screening chest X-ray showed infiltrates in the left lower lobe. On day 3, the subject experienced a nonserious AE of COPD exacerbation that required supplemental oxygen at 3 L/min. On the last day of treatment (day 5), the subject had moderate dyspnea, cough, production of purulent sputum, and chest pain. He was a nonresponder for ECR. On day 8, the symptoms continued, and chest pain was rated severe. On the same day, he was started on nonstudy antibacterial drugs, but was only noted to have an AE of **pneumonia** on day 15. The AE of pneumonia was considered resolved on day 26. He also had an AE of respiratory tract infection from days 29 to 35 which was treated with xylometazoline (a decongestant). He was noted to be a failure by IACR at all time points.

M.O. Comment*: The subject experienced symptoms of pneumonia at the end of treatment which worsened over time requiring nonstudy antibacterial drugs. As a result, this case appears to be treatment failure of LEF.*

- (b) (6) was a 57-year-old male from Russia with a history of cataract operation, hypertension, nephrolithiasis, and type 2 diabetes mellitus who received 3 days of oral LEF for PORT risk class II CABP. The baseline pathogen was *M. catarrhalis*. Screening chest X-ray showed infiltrates in the right lower lobe and right pleural effusion. However, on day 2, the X-ray showed infiltrates in the right lower, middle, and upper lobes. On day 3, he was noted to have mild dyspnea and chest pain, and moderate cough. Study drug was withdrawn on day 3 for lack of efficacy. He was a nonresponder for ECR. A respiratory culture on day 4 grew *E. coli*. Non-study antibacterial drugs were started on day 4. On day 8, the subject was noted to have an **empyema** which was drained on day 10. Pleural fluid culture results were not available. He was noted to be a failure by IACR at all time points.

M.O. Comment*: The subject had treatment failure of LEF for the original pneumonia based on needing alternative therapy on day 4 and the finding of* E. coli *which is not covered by LEF. Later, he also experienced an empyema which could be considered consequences of the treatment failure.*

- (b) (6) was a 45-year-old male from Ukraine with a history of varicose veins who received 5 days of oral LEF for PORT risk class II CABP. The baseline pathogen was *M. catarrhalis*. However, the screening sputum sample grew *K. pneumoniae* and *Pseudomonas putida,* but the Gram stain morphology was not consistent. Screening chest X-ray showed infiltrates in the right lower lobe. Chest X-ray on day 6 was unchanged. The IACR at EOT (day 8) was a success. He was also a responder for ECR. However, he was noted to have an AE of **pneumonia** on day 12 which was considered worsening of the original diagnosis and nonstudy antibacterial drugs were started. He was noted to have moderate dyspnea and mild cough and production of purulent

sputum. An X-ray showed left upper lobe infiltrates. Symptoms resolved by day 30. He was noted to be a failure by IACR at TOC and LFU.

M.O. Comment*: The fact that the original sputum specimen grew organisms which were not covered by LEF suggests this is a case of treatment failure. However, the apparent improvement with study drug and new infiltrates on X-ray suggest a new diagnosis of pneumonia. Overall, I would consider this a treatment failure.*

MOX Subjects

- (b) (6) was a 65-year-old female from the Philippines with a history of pulmonary TB who received 2 days of IV MOX for PORT risk class IV CABP. The baseline pathogen was unknown. However, the screening sputum culture grew *Moraxella* species, but the sputum specimen was not considered adequate. Screening chest X-ray showed bilateral diffuse opacities and infiltrates in the right lower lobe and left lower lobe. On day 2, she experienced myocardial ischemia requiring aspirin and clopidogrel treatment. On day 3, chest X-ray showed new infiltrates in the right middle lobe. Also, on day 3, she had decreased sensorium and dyspnea and was diagnosed with acute respiratory failure and community acquired pneumonia. Study drug was stopped, and alternative antibacterial treatment was started. She was noted to be a failure by IACR at all time points and a nonresponder for ECR.

M.O. Comment*: This appears to be a case of treatment failure that required alternative treatment early in the course of the pneumonia. I interpreted that the AE of pneumonia was that the original pneumonia was not improving.*

- (b) (6) was an 84-year-old male from Peru with no documented medical history who received 3 days of IV MOX for PORT risk class IV CABP. The baseline pathogen was unknown. However, sputum culture grew *K. pneumoniae,* but the Gram stain morphology was not consistent. Screening chest X-ray showed infiltrates in the right lower lobe and a right pleural effusion. After three days of treatment the subject did not improve, and alternative antibacterial treatment was started. An AE of empyema was noted on day 4 based on the results of a pleural culture which grew *Streptococcus anginosus*. He was noted to be a failure by IACR at all time points and a nonresponder for ECR.

M.O. Comment*: This appears to be a case of treatment failure that required alternative treatment early in the course of the pneumonia.*

- (b) (6) was a 42-year-old female from Ukraine with a history of obesity who received 7 days of MOX (3 days IV; 4 days oral) for PORT risk class III CABP. The baseline pathogen was unknown. Screening chest X-ray showed infiltrates in the right upper lobe. She was a responder for ECR. On day 7, a follow up X-ray showed right middle lobe infiltrates and

acute abscess of the right lung. Alternative antibacterial treatment was started on day 8. The lung abscess was considered resolved on day 28. She was noted to be a failure by IACR at all time points.

***M.O. Comment**: The fact that a lung abscess developed while on study drug and that alternative antibacterial drugs needed to be started right after the course of study treatment was completed makes it likely that this was a case of treatment failure.*

- (b) (6) was a 49-year-old female from the United States with a history of anxiety, asthma, low back pain, bronchitis, GERD, and hypertension who received 4 days of oral MOX for PORT risk class II CABP. The baseline pathogen was *S. pneumoniae*. Screening chest X-ray showed infiltrates in the right lower lobe with right diffuse opacities. At baseline she reported moderate dyspnea, cough, and production of sputum. On day 4 she was noted to have severe shortness of breath with fever and tachypnea. This event was categorized as an AE of pneumonia and study drug was stopped. She was a nonresponder for ECR and was admitted to the hospital on day 6 with severe dyspnea, cough, and production of purulent sputum. Non-study antibacterial drugs were started for pneumonia. An X-ray did not show new findings. The pneumonia was considered resolved on day 14. She was noted to be a failure by IACR at all time points.

***M.O. Comment**: This case appears to be a treatment failure as the subject developed worsening symptoms while on study drug.*

- (b) (6) was a 63-year-old female from Hungary with a history of hypercholesterolemia, hypertension, tobacco use, and type 2 diabetes mellitus who received 7 days of oral MOX for PORT risk class III CABP. The baseline pathogen was unknown. Screening chest X-ray showed infiltrates in the left lower lobe. She was a responder for ECR. However, on day 6, a CT scan showed left lower lobe infiltrate with a cavity and associated diagnosis of lung abscess. The subject underwent bronchoscopy which showed a large amount of pus in the left lower lobe. On day 8 (one day after the last dose of study drug), the subject was started on additional nonstudy IV MOX which continued through day 12 as the investigator felt there was insufficient therapeutic effect of the study drug. The subject later underwent left lower lobectomy and received additional nonstudy oral MOX as prophylaxis. She was noted to be a failure by IACR at all time points.

***M.O. Comment**: The development of a lung abscess while on study drug and the need for additional antibacterial drugs make this case likely a treatment failure of study drug.*

- (b) (6) was a 54-year-old male from Ukraine with a history of aortic valve disease, chronic cardiac failure, hypertensive heart disease, coronary artery disease, cerebrovascular accident, and hemiparesis who received 7 days of oral MOX for PORT risk class IV CABP. The baseline pathogen was *L. pneumophila*. However, the sputum

culture grew *E. coli*, but the Gram stain morphology was not consistent. Screening chest X-ray showed infiltrates in right lower lobe with right pleural effusion. He was a responder for ECR. On day 8 (one day after last dose of study drug), the subject had mild cough without other associated symptoms. X-ray showed the same right lower lobe infiltrates seen at baseline. However, the investigator felt the CABP was unresolved and started nonstudy antibacterial drugs due to insufficient therapeutic effect of study medication. A lung abscess was diagnosed on day 15 after evaluation by a surgeon. Non-study antibacterial drugs were continued. The lung abscess was considered resolved on day 30. He was noted to be a failure by IACR at all time points.

M.O. Comment*: The need for additional antibacterial drugs immediately after stopping study drug makes this case likely a treatment failure of study drug.*

Regarding these 18 cases of SAEs related to lung infections, most were treatment failures of the study drug with a few cases of a separate infection. In addition, 8 of 12 LEF-treated subjects had a positive culture for Enterobacteriaceae which are not covered by LEF. As a result, most of the treatment failures in LEF subjects are likely a result of inadequate antibacterial coverage. Of note, 17 of 18 subjects were noted as failures at the TOC visit by IACR; all were either a relapse or failure at LFU. To examine whether the finding of positive cultures for Enterobacteriaceae was coincidental among treatment failures, I searched the microbiology dataset to find all subjects with a positive culture for Enterobacteriaceae. Using this list, I found success by IACR at LFU for subjects with a positive Enterobacteriaceae culture was 78% compared to 85% for the remainder of the study population. This difference was present overall and in LEF-treated and MOX-treated subjects. In addition, 10% of subjects with LFU successes had positive Enterobacteriaceae cultures compared to 16% of nonsuccesses. By treatment arm, 13.6% of LEF subjects had a positive Enterobacteriaceae culture versus 9.2% of MOX subjects. These data show that having a positive culture for Enterobacteriaceae was associated with a higher chance of nonsuccess by IACR at LFU and that the finding of positive Enterobacteriaceae cultures in LEF-treated subjects with lung infections is likely not a coincidence. In addition, Enterobacteriaceae may have been selected for in LEF subjects given the drug's lack of coverage of these organisms. It is possible some subjects developed secondary pneumonia that was not covered by lefamulin but may have been covered by moxifloxacin. Overall, this appears to be an issue of some subjects receiving inadequate treatment rather than a direct safety issue and was captured by the IACR at LFU. Of note, the proposed product label states that lefamulin is not active against Enterobacteriaceae. Azithromycin, which is approved for the treatment of CABP, also does not have activity against Enterobacteriaceae and may serve as a useful comparator for the clinical utility of lefamulin.

An alternative explanation for increased reporting of lung infections in the LEF arm is that lefamulin is associated with an inflammatory process in the lung that could be misinterpreted as an infectious process, but there is no evidence to support this theory. Treatment failure, likely related to inadequate antibacterial coverage, is the most likely explanation.

In the SOC of Respiratory disorders, there were eight subjects in the LEF arm and 4 in the MOX arm with SAEs. Of the 8 LEF subjects, 6 experienced SAEs which could have been related to their pneumonia or worsened by it (PTs of pleurisy, COPD, ARDS, acute respiratory failure, and pulmonary edema). Of the 4 MOX subjects with respiratory SAEs, 2 had conditions which could have been related to their pneumonia (PTs of acute respiratory failure and respiratory failure).

M.O. Comment*: In the Respiratory disorders SOC, it appears most of the SAEs were related to treatment failure and there is an imbalance with more subjects in the LEF arm having SAEs in the SOC. Review of the microbiology results from the LEF subjects only showed 2 of 8 grew organisms in their sputum which were not covered by LEF (*P. aeruginosa *and* E. cloacae*). As a result, there is no microbiological evidence to explain these treatment failures. Of note, almost all of the subjects with respiratory SAEs (10 of 12) were counted as failures at the TOC and LFU visits by IACR. Again, this does not appear to be a direct safety issue.*

In the Investigations SOC, there were three subjects in the LEF arm compared to one subject in the MOX arm who experienced an SAE. Three of these subjects had elevations in their liver enzymes (2 in the LEF arm and 1 in the MOX arm). SAEs in the other SOCs were balanced between the treatment arms or had more subjects in the comparator arm (MOX).

Dropouts and/or Discontinuations Due to Adverse Effects

In the Phase 3 safety population, 42 subjects discontinued study drug due to at least one TEAE. These subjects were balanced between the treatment arms with 21 in the LEF arm (3.3%) and 21 in the MOX arm (3.3%). The table below provides an overview of dropouts and discontinuations due to a TEAE in the Phase 3 safety population.

Table 89. Dropouts and Discontinuations Due to Treatment-Emergent Adverse Events in the Phase 3 Safety Population by System Organ Class and Preferred Term

System Organ Class/Preferred Term	Lefamulin N=641 n (%)	Moxifloxacin N=641 n (%)
Investigations*	4 (0.6)	4 (0.6)
Electrocardiogram QT prolonged	2	3
Alanine aminotransferase increased	1	0
Aspartate aminotransferase increased	1	0
Blood alkaline phosphatase increased	1	0
Creatinine renal clearance decreased	1	0
Gamma-glutamyltransferase increased	1	0
Hepatic enzyme increased	0	1
Cardiac disorders	4 (0.6)	2 (0.3)
Myocardial infarction[1]	2	0
Bradycardia	1	0
Cardiac failure congestive	1	0
Atrial fibrillation	0	1
Palpitations	0	1

System Organ Class/Preferred Term	Lefamulin N=641 n (%)	Moxifloxacin N=641 n (%)
Infections and infestations	4 (0.6)	6 (0.9)
Infectious pleural effusion	1	2
Lung abscess	1	0
Pneumonia	1	2
Pulmonary tuberculosis	1	0
Cystitis	0	1
Urinary tract infection	0	1
Respiratory, thoracic and mediastinal disorders	4 (0.6)	4 (0.6)
Acute respiratory distress syndrome	1	0
Acute respiratory failure	1	1
Chronic obstructive pulmonary disease	1	0
Pulmonary edema	1	0
Dyspnea	0	1
Pulmonary embolism	0	1
Respiratory failure	0	1
Gastrointestinal disorders	3 (0.5)	1 (0.2)
Vomiting	2	1
Abdominal pain upper	1	0
General disorders and administration site conditions	2 (0.3)	1 (0.2)
Infusion site phlebitis	1	0
Injection site reaction**	1	0
Infusion site erythema	0	1
Hepatobiliary disorders	1 (0.2)	0
Hepatitis toxic	1	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	1 (0.2)
Small cell lung cancer	0	1
Nervous system disorders	0	2 (0.3)
Dizziness	0	2
Psychiatric disorders	0	1 (0.2)
Confusional state	0	1
Skin and subcutaneous tissue disorders	0	3 (0.5)
Urticaria	0	2
Angioedema	0	1
Vascular disorders	0	2 (0.3)
Shock hemorrhagic	0	1
Hypertension	0	1

* Note: Subjects with more than one TEAE in the same SOC were counted only once in that SOC.

** One subject in the LEF arm (b) (6) was not counted as a discontinuation due to a TEAE by the Sponsor but discontinued oral study drug because of injection site reactions that occurred while receiving IV lefamulin.

[1]Includes the preferred terms: "myocardial infarction" and "acute myocardial infarction."

***M.O. Comment**: All case narratives for subjects who discontinued study drug due to a TEAE were reviewed.*

- *The subject who discontinued study drug due to "creatinine renal clearance decreased" in fact had an increased creatinine clearance at study drug discontinuation compared to baseline so it is unclear why study drug was stopped.*
- *One LEF subject ([redacted] (b) (6)) had elevations in four different liver enzymes leading to study drug discontinuation that was likely related to LEF as there were no concomitant medications or medical conditions to explain the enzyme elevation. The peak ALT was 653 U/L (13x ULN), the peak AST was 227 U/L (4.5x ULN), and the peak alkaline phosphatase was 187 U/L (1.5x ULN). The serum bilirubin was normal. This subject was asymptomatic, and the enzymes returned to normal by the end of the study. In addition, the LEF subject with "hepatitis toxic" ([redacted] (b) (6)) had elevations in AST and ALT between 5x and 10x the ULN that returned to baseline levels by the end of the study. This case was likely related to study drug, but the subject received a single dose of amoxicillin/clavulanate which could have contributed to the liver enzyme elevations.*
- *The cases of "electrocardiogram QT prolonged" were similar in that subjects were asymptomatic and QTcF returned to baseline after study drug discontinuation; QT prolongation was likely related to study drug.*
- *Review of the cases in the cardiac disorders SOC showed that the case of bradycardia in a LEF subject and palpitations and dizziness in a MOX subject could have been related to study drug.*
- *Most of the TEAEs in the infections and infestations SOC and respiratory disorders SOC leading to drug discontinuation appeared to be related to treatment failure or progression/complications of the underlying pneumonia.*
- *The three cases of vomiting were likely to be related to study drug as they occurred immediately after starting therapy.*
- *The two cases of urticaria and one case of angioedema in the MOX arm were likely to be allergic reactions related to moxifloxacin based on the timing of the events and resolution after drug discontinuation.*
- *Although administration site reaction was a common TEAE associated with LEF, only 2 LEF subjects and 1 MOX subject discontinued study drug because of an administration site reaction.*
- *Overall, the study drug discontinuations were balanced between the treatment arms.*

Significant Adverse Events

This section will discuss treatment-emergent adverse events that were not considered serious but rated severe by the investigator. There were 14 subjects in the Phase 3 safety population with severe but not serious TEAEs; 8 in the LEF arm and 6 in the MOX arm. Notably 3 LEF subjects and 1 MOX subject had severe, but not serious administration site reactions. All four subjects' reactions were resolving or had resolved at the end of the study. Two LEF subjects had

severe, but not serious TEAEs of nausea after receiving oral lefamulin that resolved by the end of the study.

***M.O. Comment**: Severe, but not serious TEAEs were not common in the Phase 3 safety population and were balanced between the treatment groups overall. The finding of more administration site reactions and nausea in the LEF arm is consistent with the data for all TEAEs that will be discussed in the following section.*

Treatment Emergent Adverse Events and Adverse Reactions

An overview of TEAEs in the Phase 3 safety population are summarized in the tables below.

Table 90. Treatment-Emergent Adverse Events in the Phase 3 Safety Population by Study, Treatment Group, and System Organ Class

	Study 3101		Study 3102		Pooled	
	LEF N=273 n (%)	MOX N=273 n (%)	LEF N=368 n (%)	MOX N=368 n (%)	LEF N=641 n (%)	MOX N=641 n (%)
Subjects with any TEAE	104 (38.1)	103 (37.7)	120 (32.6)	92 (25.0)	224 (34.9)	195 (30.4)
Blood and lymphatic system disorders	6 (2.2)	3 (1.1)	3 (0.8)	6 (1.6)	9 (1.4)	9 (1.4)
Cardiac disorders	8 (2.9)	11 (4.0)	8 (2.2)	9 (2.4)	16 (2.5)	20 (3.1)
Ear and labyrinth disorders	1 (0.4)	1 (0.4)	1 (0.3)	0	2 (0.3)	1 (0.2)
Eye disorders	0	1 (0.4)	0	2 (0.5)	0	3 (0.5)
Gastrointestinal disorders	18 (6.6)	37 (13.6)	66 (17.9)	28 (7.6)	84 (13.1)	65 (10.1)
General disorders and administration site conditions	24 (8.8)	15 (5.5)	4 (1.1)	2 (0.5)	28 (4.4)	17 (2.7)
Hepatobiliary disorders	2 (0.7)	4 (1.5)	4 (1.1)	2 (0.5)	6 (0.9)	6 (0.9)
Infections and infestations	20 (7.3)	22 (8.1)	27 (7.3)	18 (4.9)	47 (7.3)	40 (6.2)
Investigations	17 (6.2)	14 (5.1)	14 (3.8)	12 (3.3)	31 (4.8)	26 (4.1)
Metabolism and nutrition disorders	10 (3.7)	10 (3.7)	6 (1.6)	8 (2.2)	16 (2.5)	18 (2.8)
Musculoskeletal and connective tissue disorders	4 (1.5)	7 (2.6)	4 (1.1)	4 (1.1)	8 (1.2)	11 (1.7)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	3 (1.1)	2 (0.7)	2 (0.5)	2 (0.5)	5 (0.8)	4 (0.6)
Nervous system disorders	8 (2.9)	9 (3.3)	8 (2.2)	13 (3.5)	16 (2.5)	22 (3.4)
Psychiatric disorders	10 (3.7)	7 (2.6)	2 (0.5)	5 (1.4)	12 (1.9)	12 (1.9)
Renal and urinary disorders	3 (1.1)	6 (2.2)	1 (0.3)	5 (1.4)	4 (0.6)	11 (1.7)
Reproductive system and breast disorders	0 (0.0)	1 (0.4)	2 (0.5)	3 (0.8)	2 (0.3)	4 (0.6)
Respiratory, thoracic and mediastinal disorders	16 (5.9)	13 (4.8)	13 (3.5)	15 (4.1)	29 (4.5)	28 (4.4)
Skin and subcutaneous tissue disorders	1 (0.4)	3 (1.1)	2 (0.5)	7 (1.9)	3 (0.5)	10 (1.6)
Vascular disorders	3 (1.1)	10 (3.7)	8 (2.2)	7 (1.9)	11 (1.7)	17 (2.7)

TEAE = treatment-emergent adverse event; LEF = lefamulin; MOX = moxifloxacin

***M.O. Comment**: TEAEs were more common in the LEF arm compared to the MOX arm in the overall and in the SOCs of gastrointestinal disorders, general disorders and administration site conditions, infections and infestations, and investigations. Administration site conditions will be discussed in Section 8.2.5.1.*

Table 91. Treatment-Emergent Adverse Events Occurring in ≥1% of Subjects by Preferred Term in the Phase 3 safety population

Preferred Term	LEF N=641 n (%)	MOX N=641 n (%)
Diarrhea	47 (7.3)	25 (3.9)
Nausea	27 (4.2)	13 (2.0)
Vomiting	15 (2.3)	4 (0.6)
Headache	9 (1.4)	11 (1.7)
Pneumonia[1]	10 (1.6)	2 (0.3)
Alanine aminotransferase increased	8 (1.2)	10 (1.6)
Chronic obstructive pulmonary disease	8 (1.2)	3 (0.5)
Hypokalemia	8 (1.2)	7 (1.1)
Infusion site pain	8 (1.2)	0
Insomnia	8 (1.2)	9 (1.4)
Hypertension	7 (1.1)	11 (1.7)
Abdominal pain[2]	7 (1.1)	5 (0.8)

[1]Includes preferred terms of "pneumonia" and "pneumonia bacterial"
[2]Includes preferred terms of "abdominal pain" and" "abdominal pain upper"
LEF = lefamulin; MOX = moxifloxacin

TEAEs occurring in less than 1% of LEF-treated subjects are listed in Table 148 in the Appendices. TEAEs occurring in greater than 2% of LEF-treated subjects in each Phase 3 trial are listed in Table 149 and Table 150 in the Appendices.

***M.O. Comment**: The GI TEAEs and TEAEs recorded as pneumonia are discussed in the next two subheadings. Administration site reactions are summarized in Section 8.2.5.1. Regarding the imbalance of chronic obstructive pulmonary disease cases, the Applicant provided narrative summaries in response to our information request. Review of these cases revealed that several subjects developed symptoms of COPD several days (3 days to 24 days) after completing study drug, making it less likely the COPD was related to study drug. In addition, it appears that some subjects had underlying COPD prior to the study but were only diagnosed while receiving medical care for their CABP. Focusing on cases in which the TEAE of COPD was reported while subjects received study drug, there were three subjects in the LEF arm and three subjects in the MOX arm. As there is not an imbalance in COPD TEAEs reported while subjects received study drug, it appears unlikely the TEAE of COPD is related to LEF.*

TEAEs in the Gastrointestinal Disorders SOC

Notably, in the Gastrointestinal disorders SOC, the rates of TEAEs varied between the studies and treatment arms. These data are summarized in the table below.

Table 92. Treatment-Emergent Adverse Events in the Gastrointestinal Disorders SOC Occurring in ≥3 Subjects Overall by Preferred Term in the Phase 3 Safety Population

	Study 3101		Study 3102		Pooled	
	LEF N=273 n (%)	MOX N=273 n (%)	LEF N=368 n (%)	MOX N=368 n (%)	LEF N=641 n (%)	MOX N=641 n (%)
Subjects with any TEAE in gastrointestinal disorders SOC	18 (6.6)	37 (13.6)	66 (17.9)	28 (7.6)	84 (13.1)	65 (10.1)
Diarrhea	2 (0.7)	21 (7.7)	45 (12.2)	4 (1.1)	47 (7.3)	25 (3.9)
Nausea	8 (2.9)	6 (2.2)	19 (5.2)	7 (1.9)	27 (4.2)	13 (2.0)
Vomiting	3 (1.1)	1 (0.4)	12 (3.3)	3 (0.8)	15 (2.3)	4 (0.6)
Constipation	2 (0.7)	3 (1.1)	0 (0.0)	3 (0.8)	2 (0.3)	6 (0.9)
Dyspepsia	0 (0.0)	3 (1.1)	3 (0.8)	1 (0.3)	3 (0.5)	4 (0.6)
Abdominal pain*	3 (1.1)	3 (1.1)	4 (1.1)	2 (0.5)	7 (1.1)	5 (0.8)
Gastritis	0 (0.0)	0 (0.0)	4 (1.1)	2 (0.5)	4 (0.6)	2 (0.3)
Abdominal distension	0 (0.0)	3 (1.1)	0 (0.0)	1 (0.3)	0 (0.0)	4 (0.6)
Chronic gastritis	0 (0.0)	1 (0.4)	0 (0.0)	2 (0.5)	0 (0.0)	3 (0.5)

*Includes preferred terms of abdominal pain and abdominal pain upper
LEF = lefamulin; MOX = moxifloxacin; TEAE = treatment-emergent adverse event; SOC = system organ class

***M.O. Comment**: In Study 3101, in which all subjects started with IV study drug, diarrhea was more common in the MOX arm compared to the LEF arm. However, in Study 3102, in which all subjects received oral study drug, diarrhea was more common in the LEF arm compared to the MOX arm. In addition, nausea and vomiting were more common in Study 3102 in subjects exposed to LEF. In Study 3102, the GI TEAEs in LEF-treated subjects were mostly mild and none were severe.*

TEAEs in the Infections and Infestations SOC

Table 93. Treatment-Emergent Adverse Events in the Infections and Infestations SOC Occurring in ≥3 Subjects Overall by Preferred Term in the Phase 3 Safety Population

	Study 3101		Study 3102		Pooled	
	LEF N=273 n (%)	MOX N=273 n (%)	LEF N=368 n (%)	MOX N=368 n (%)	LEF N=641 n (%)	MOX N=641 n (%)
Subjects with any TEAE in the infections and infestations SOC	20 (7.3)	22 (8.1)	27 (7.3)	18 (4.9)	47 (7.3)	40 (6.2)
Urinary tract infection	2 (0.7)	4 (1.5)	3 (0.8)	6 (1.6)	5 (0.8)	10 (1.6)
Pneumonia*	5 (1.8)	1 (0.4)	5 (1.4)	1 (0.3)	10 (1.6)	2 (0.3)

	Study 3101		Study 3102		Pooled	
	LEF N=273 n (%)	MOX N=273 n (%)	LEF N=368 n (%)	MOX N=368 n (%)	LEF N=641 n (%)	MOX N=641 n (%)
Respiratory tract infection viral	0	0	5 (1.4)	1 (0.3)	5 (0.8)	1 (0.2)
Lung abscess	0	2 (0.7)	1 (0.3)	2 (0.5)	1 (0.2)	4 (0.6)
Oral candidiasis	2 (0.7)	3 (1.1)	0	0	2 (0.3)	3 (0.5)
Pharyngitis	1 (0.4)	3 (1.1)	1 (0.3)	0	2 (0.3)	3 (0.5)
Infectious pleural effusion	2 (0.7)	2 (0.7)	0	0	2 (0.3)	2 (0.3)
Pulmonary tuberculosis	1 (0.4)	1 (0.4)	0	1 (0.3)	1 (0.2)	2 (0.3)
Sepsis	1 (0.4)	0	2 (0.5)	0	3 (0.5)	0

* Includes PTs of Pneumonia and Pneumonia bacterial
LEF = lefamulin; MOX = moxifloxacin; SOC = system organ class; TEAE = treatment-emergent adverse event

***M.O. Comment**: Similar to SAEs, pneumonia as a TEAE is more common in the LEF arm compared to the MOX arm. Respiratory tract viral infections were also more common in the LEF arm. Taken together, these data suggest failure of treatment in these subjects, but inflammation in the lung from a drug effect cannot be excluded.*

Additional details on the 12 subjects with pneumonia as a TEAE are provided in the tables below.

Table 94. Clinical Data on 12 Subjects With TEAEs of Pneumonia in the Phase 3 Safety Population

Subject ID	Study/ Treatment Arm	Age (Years)/Sex/ PORT Risk Class	Medical History	Days of Study Drug Exposure	Start of Alternative Antibacterial Drugs	Reported Onset of Pneumonia TEAE (Study Day)	Death	Early Clinical Response Status (~Day 4)	IACR at TOC (5–10 Days Post Last Dose)	IACR at LFU (~Day 30)
(b) (6)	3101/LEF	46/M/IV	Depression, Diabetes mellitus type 2, fatty liver, GERD, obesity, OSA, pulm. HTN, heart failure	8	22	18	No	Responder	Success	Sustained success
(b) (6)	3101/LEF	81/M/III	Cerebrovascular disease, HTN, pulm. TB	7	18	17	No	Responder	Success	Relapse
(b) (6)	3101/LEF	72/M/IV	COPD, CAD, pulm. TB	2	3	7	Yes (on Day 20)	Non-responder	Failure	Failure
(b) (6)	3101/LEF	87/F/III	COPD, HTN	8	8	8	Yes (on Day 32)	Responder	Failure	Failure
(b) (6)	3101/LEF	59/F/III	None	2	N/A	3	Yes (on Day 3)	Non-responder	Failure	Failure
(b) (6)	3102/ LEF	68/F/II	C-section, partial thyroidectomy	5	12	12	No	Responder	Failure	Failure
(b) (6)	3102/ LEF	45/M/II	Obesity	5	8	8	No	Responder	Failure	Failure
(b) (6)	3102/ LEF	63/M/III	COPD, HTN, pneumonia, salivary gland adenoma	5	13	9	No	Responder	Failure	Failure
(b) (6)	3102/ LEF	67/M/II	Stable angina, heart failure, COPD, HTN, glucose intolerance, pulm. fibrosis	5	8	15	No	Non-responder	Failure	Failure
(b) (6)	3102/ LEF	45/M/II	Varicose veins	5	12	12	No	Responder	Failure	Failure

Subject ID	Study/ Treatment Arm	Age (Years)/Sex/ PORT Risk Class	Medical History	Days of Study Drug Exposure	Start of Alternative Antibacterial Drugs	Reported Onset of Pneumonia TEAE (Study Day)	Death	Early Clinical Response Status (~Day 4)	IACR at TOC (5–10 Days Post Last Dose)	IACR at LFU (~Day 30)
(b) (6)	3101/MOX	65/F/IV	Tubal ligation, pulm. TB	2	3	3	No	Non-responder	Failure	Failure
(b) (6)	3102/MOX	49/F/II	Anxiety, asthma, low back pain, bronchitis, GERD, HTN	4	6	4	No	Non-responder	Failure	Failure

IACR = Investigator assessment of clinical response; HTN = hypertension; TB = tuberculosis; GERD = gastroesophageal reflux disease; COPD = chronic obstructive pulmonary disease; OSA = obstructive sleep anpea; CAD = coronary artery disease; LEF = lefamulin; PORT = Pneumonia Outcomes Research Team; TEAE = treatment-emergent adverse event

M.O. Comment*: 11 of 12 subjects with pneumonia as a TEAE were counted as either failures or relapses for the IACR at the LFU visit. As a result, the longer-term efficacy endpoints captured these cases as treatment failures. Of note, the lone success at LFU (* (b) (6) *) developed pneumonia about 10 days after completing study drug. The investigator believed this later pneumonia was not related to the original pneumonia and therefore did not consider nonstudy antibacterial drug therapy as disqualifying for IACR success.*

Table 95. Microbiological Data on 12 Subjects With TEAEs of Pneumonia in the Phase 3 Safety Population

Subject ID	Study/ Treatment Arm	Baseline Pathogens* (Source)	LEF MIC (mcg/mL)/ Interpretation	MOX MIC (mcg/mL)/ Interpretation	Additional Culture Results (Source)
(b) (6)	3101/LEF	None	N/A	N/A	N/A
(b) (6)	3101/LEF	*S. pneumoniae* (NP swab culture and PCR, sputum PCR, urine antigen)	0.5/S	0.12/S	*K. pneumoniae* (day 1 sputum culture)
(b) (6)	3101/LEF	*H. influenzae* and *M. catarrhalis* (sputum PCR)	N/A	N/A	*Klebsiella oxytoca* (day 1 sputum culture)
(b) (6)	3101/LEF	*H. influenzae* (sputum PCR)	N/A	N/A	*Citrobacter koseri* (day 12 BAL culture)
(b) (6)	3101/LEF	*S. pneumoniae* (blood culture, NP swab PCR, sputum PCR, urine antigen)	0.5/S	0.12/S	N/A
(b) (6)	3102/ LEF	*S. pneumoniae* (sputum PCR)	N/A	N/A	*K. pneumoniae*, *Klebsiella variicola*, *E. cloacae* (day 1 sputum culture); *E. coli*, *K. pneumoniae* (day 2 sputum culture)
(b) (6)	3102/ LEF	*S. pneumoniae* (sputum PCR, urine antigen)	N/A	N/A	*K. pneumoniae* (day 5 sputum culture)
(b) (6)	3102/ LEF	*C. pneumoniae* (serology), *S. pneumoniae* (sputum PCR, urine antigen)	N/A	N/A	*H. parainfluenzae* (day 1 sputum culture)
(b) (6)	3102/ LEF	None	N/A	N/A	N/A
(b) (6)	3102/ LEF	*M. catarrhalis* (sputum PCR)	N/A	N/A	*K. pneumoniae* and *Pseudomonas putida* (day 1 sputum culture)
(b) (6)	3101/MOX	None	N/A	N/A	*Moraxella* species (day 1 sputum culture)
(b) (6)	3102/MOX	*S. pneumoniae* (sputum PCR)	N/A	N/A	N/A

*An organism was considered a baseline pathogen if the specimen was obtained within 24 hours of the first dose of study drug. In addition, depending on the organism, it had to originate from an adequate specimen (>25 PMNs/LPF, <10 SECs/LPF) and have a consistent Gram stain (e.g., Gram-negative rods for Enterobacteriaceae). Cultured pathogens which did not meet these criteria are listed in the final column.
LEF = lefamulin; NP = nasopharyngeal; MIC = minimum inhibitory concentration; MOX = moxifloxacin; TEAE = treatment-emergent adverse event; PCR = polymerase chain reaction

M.O. Comment*: The baseline pathogen criteria may have been overly strict as the growth of* K. pneumoniae *was not categorized as a baseline pathogen in several subjects despite the known association of this organism with pneumonia. Of note,* K. pneumoniae *has been associated with COPD, which was a common medical comorbidity in this population. Also, 6 of 10 LEF-treated subjects had cultures growing Enterobacteriaceae which are not covered by LEF. As a result,*

some of the TEAEs of pneumonia may have been a result of inadequate antibacterial coverage of LEF. Overall, this does not appear to be an issue of LEF causing pneumonias, but rather in some cases subjects having pneumonia caused by an organism not covered by LEF.

TEAEs in the Investigations SOC

Table 96. Treatment-Emergent Adverse Events in the Investigations SOC Occurring in ≥3 Subjects Overall by Preferred Term in the Phase 3 Safety Population

	Study 3101		Study 3102		Pooled	
	LEF N=273 n (%)	MOX N=273 n (%)	LEF N=368 n (%)	MOX N=368 n (%)	LEF N=641 n (%)	MOX N=641 n (%)
Subjects with any TEAE in the investigations SOC	20 (7.3)	22 (8.1)	27 (7.3)	18 (4.9)	47 (7.3)	40 (6.2)
Alanine aminotransferase increased	5 (1.8)	6 (2.2)	3 (0.8)	4 (1.1)	8 (1.2)	10 (1.6)
Aspartate aminotransferase increased	4 (1.5)	2 (0.7)	2 (0.5)	4 (1.1)	6 (0.9)	6 (0.9)
Electrocardiogram QT prolonged	3 (1.1)	5 (1.8)	1 (0.3)	0	4 (0.6)	5 (0.8)
Gamma-glutamyltransferase increased	4 (1.5)	1 (0.4)	2 (0.5)	1 (0.3)	6 (0.9)	2 (0.3)
Blood pressure increased	1 (0.4)	0	2 (0.5)	2 (0.5)	3 (0.5)	2 (0.3)
Blood alkaline phosphatase increased	2 (0.7)	0	2 (0.5)	0	4 (0.6)	0
Blood creatine phosphokinase increased	1 (0.4)	0	2 (0.5)	1 (0.3)	3 (0.5)	1 (0.2)
White blood cell count increased	1 (0.4)	3 (1.1)	0	0	1 (0.2)	3 (0.5)
Hepatic enzyme increased	0	0	0	3 (0.8)	0	3 (0.5)
Lymphocyte count decreased	1 (0.4)	2 (0.7)	0	0	1 (0.2)	2 (0.3)
Transaminases increased	1 (0.4)	0	1 (0.3)	1 (0.3)	2 (0.3)	1 (0.2)

TEAE = treatment-emergent adverse event; SOC = system organ class; MOX = moxifloxacin; LEF = lefamulin

***M.O. Comment**: The PTs of gamma-glutamyltransferase increased and alkaline phosphatase increased were more common in the LEF arm, but still relatively uncommon. Otherwise, elevations in other liver enzymes and QT prolongation noted as TEAEs were balanced between the treatment arms.*

Laboratory Findings

Review of the electrolyte, renal, liver, and hematology laboratory data in the Phase 3 safety population revealed no clinically meaningful differences in mean values between the treatment arms at the different timepoints of the studies. Of note, serum bicarbonate values were not reported from either Phase 3 study and so were not available for review. With regards to hepatoxicity, one subject in the MOX arm and none in the LEF arm met laboratory criteria for Hy's Law. Examination of the data using the "potentially clinically significant" (PCS) criteria defined in the SAP revealed a higher proportion of LEF subjects compared to MOX subjects with

any PCS laboratory value (15.2% versus 10.2%). The subjects with PCS values for selected laboratory parameters of interest are summarized in the table below.

Table 97. Subjects With Potentially Clinically Significant (PCS) Laboratory Parameters of Interest by Treatment Arm in the Phase 3 Safety Population

Laboratory Parameter (PCS Criteria)	LEF n=641 n/N (%)	MOX n=641 n/N (%)
Low hemoglobin (<0.8 x LLN and decrease >20% from baseline)	7/548 (1.3)	4/559 (0.7)
High platelets (>1.5 x ULN and increase of >100% from baseline)	20/529 (3.8)	12/540 (2.2)
High leukocytes (>1.6 x ULN and increase of >100% from baseline)	9/548 (1.6)	6/559 (1.1)
High neutrophils (>1.6 x ULN and increase of >100% from baseline)	20/547 (3.7)	10/558 (1.8)
Low neutrophils (<0.65 x LLN and decrease >75% from baseline)	9/547 (1.6)	4/558 (0.7)
High creatinine (>2.0 x ULN and increase >100% from baseline)	5/606 (0.8)	0/615
High potassium (>1.2 x ULN and increase >20% from baseline)	7/605 (1.2)	3/604 (0.5)
Low potassium (<0.8 x LLN and decrease >20% from baseline)	4/605 (0.7)	5/604 (0.8)
High calcium (>1.3 x ULN and increase >30% from baseline)	0/607	1/615 (0.2)
Low calcium (<0.7 x LLN and decrease >30% from baseline)	4/607 (0.7)	1/615 (0.2)
High AST (>3.0 x ULN and increase >200% from baseline)	11/553 (2.0)	7/572 (1.2)
High ALT (>3.0 x ULN and increase >200% from baseline)	20/573 (3.5)	18/583 (3.1)
High GGT (>3.0 x ULN and increase >200% from baseline)	18/606 (3.0)	8/613 (1.3)
High ALP (>2.0 x ULN and increase >100% from baseline)	7/607 (1.2)	3/613 (0.5)
High bilirubin (≥2.0 x ULN and increase >150% from baseline)	1/574 (0.2)	1/585 (0.2)

n = number subjects with PCS value; N = number of subjects with both a baseline and subsequent value for the laboratory parameter; ULN = upper limit of normal; LLN = lower limit of normal; LEF = lefamulin; MOX = moxifloxacin

M.O. Comment:

- *There was an imbalance with more subjects in the LEF arm with low hemoglobin and neutrophils, but the difference was small. In addition, the level of decline in these two laboratory values in the LEF arm was not significant.*
- *Further analysis of the high platelet, WBC, and neutrophil counts showed that most of these high values occurred later in the treatment course or posttreatment. Of note, there were only two subjects with both elevated WBC and platelet counts. The elevated platelet or WBC counts could suggest that inflammation from the CABP may not have been sufficiently treated in these subjects. However, the sustained success rates at LFU for these subjects were similar between the treatment arms [27/39 (69%) for LEF and 16/22 (73%)] for MOX).*
- *The 5 LEF subjects with increased creatinine were initially concerning for acute kidney injury related to LEF but review of the cases revealed 4 of five subjects had elevations starting after stopping study drug which suggests alternative causes. In addition, the remaining subject was receiving diclofenac (an NSAID) which could have also contributed to the elevated creatinine.*
- *High potassium was noted in more LEF subjects with several subjects having levels >7.3 mEq/L. Review of these cases revealed that several of the LEF subjects with elevated potassium levels had the high levels after LEF treatment was completed. In addition, 3 of*

the LEF subjects blood specimens likely were not processed correctly as other tests run on the same blood draw returned as "beyond stability" which may explain the high values. Eliminating these likely spurious results and examining only cases in which the high potassium level occurred while on study drug, there was no imbalance as two subjects in each arm had high potassium levels.

- *Hypocalcemia was noted more frequently in LEF subjects, but the calcium levels were not very low (between 5.3 mg/dL to 5.5 mg/dL at EOT).*
- *AST and ALT increases were relatively common and balanced between the treatment arms.*
- *More LEF subjects had elevations in GGT and alkaline phosphatase suggesting biliary injury, but notably bilirubin increases were not observed.*
- *There were no potential Hy's Law cases in the LEF arm.*

Vital Signs

In the Phase 3 safety population, there were modest decreases in mean pulse rate, temperature, and respiratory rate over the course of the study consistent with resolving infections, but no meaningful differences between the treatment arms were noted. Similarly, mean systolic and diastolic blood pressure and oxygen saturation increased over the course of the study without differences in the treatment arms. The proportion of subjects with "potentially clinically significant" changes in postbaseline vital signs (defined in the SAP) were similar between the treatment arms.

M.O. Comment*: Review of the vital signs data did not reveal any notable differences between the treatment arms.*

Electrocardiograms (ECGs)

In Study 3101 (IV administration with optional oral switch), ECGs were obtained on Day 1 and Day 3 both before and within 15 minutes after the infusion of study drug. In Study 3102 (oral), ECGs were obtained on Day 1 and Day 4 both before and 1 to 3 hours after study drug administration. In addition, ECGs were obtained as clinically indicated. At each timepoint, ECGs were obtained in triplicate within a 5-minute interval. A total of 15,630 ECGs were performed during the two Phase 3 studies. ECGs were reviewed by the investigator at the time they were obtained and were also sent to a core laboratory for summary analysis. The major finding from review of the ECG data was QT prolongation, which is discussed below. The only other notable ECG finding was decreased mean heart rate at Day 3/4 compared to baseline of between 6 to 8 beats/min in each arm.

M.O. Comment*: The decrease in mean heart rate is consistent with improvement in the CABP.*

QT

QT prolongation was identified as a potential safety issue early in the lefamulin development program. The FDA Interdisciplinary Review Team for QT studies (QT-IRT) was consulted and determined that a thorough QT study was not necessary. From two of the Phase 1 studies, 1001 and 1007, the team concluded that lefamulin prolongs the QT interval in a nonlinear and concentration-dependent manner. From the two Phase 3 studies (3101 and 3102), the team found that the IV dose of 150 mg twice daily was associated with a mean change from baseline of the QTcF interval of 13.6 ms. The change from baseline was 9.3 ms for oral administration. The difference in QTcF interval prolongation between the IV and oral formations likely results from differences in the peak lefamulin concentration (2240 ng/mL for oral versus 3030 ng/mL for IV). The QT-IRT team also recommended the following language be included in section 12.2 (Pharmacodynamics) of the product label.

"The QTcF interval prolongation risk of Xenleta was evaluated using 2 randomized, double-blind, double-dummy, active controlled (moxifloxacin 400 mg once daily), parallel group, phase-3 studies in adult patients with community-acquired bacterial pneumonia. A concentration dependent QTc prolongation effect of Xenleta was observed. The mean placebo-corrected change from baseline QTcF (90% two-sided upper confidence interval) values around T_{max} were 13.6 ms (15.5 ms) for 150 mg injection administered twice daily as infusion and 9.3 ms (10.9 ms) at 600-mg tablet administered twice daily."

See Section 8.2.5.2 for further analysis of the QT prolongation data.

Immunogenicity

Not applicable for this NDA.

8.2.5. Analysis of Submission-Specific Safety Issues

8.2.5.1. Administration site reactions

Nonclinical and early clinical studies of IV lefamulin identified administration site irritation and inflammation to be a safety issue. In Study 3101 (IV with optional oral switch), 21 subjects in the LEF arm (7.7%) and 10 subjects in the MOX arm (3.7%) experienced a TEAE in the high-level group term of administration site reactions. This includes the high-level terms (HLTs) of infusion site reactions, injection site reactions, administration site reactions NEC, and implant and catheter site reactions. A closer analysis shows that TEAEs in the HLT of administration site reactions NEC describe issues with venipuncture sites for blood draws and not reactions to the study drug. Eliminating that HLT results in 20 subjects in the LEF arm (7.3%) and seven subjects in the MOX arm (2.6%) with administration site reactions. The preferred terms describe pain, phlebitis, inflammation, erythema, reaction, bruising, and coldness at the infusion site, injection site, or catheter site. These reactions were mostly mild, but three subjects in the LEF arm and

one subject in the MOX arm had severe reactions. Of the subjects with severe reactions, 2 in the LEF arm and 1 in the MOX arm discontinued the study drug due to the AE.

***M.O. Comment**: Of note, the Applicant did not consider catheter site inflammation in their analysis used to generate the adverse reactions tables in the prescribing information, so there is a slight discrepancy in the results. Overall, administration site reactions in the Phase 3 safety population were more frequent in subjects exposed to IV lefamulin compared to moxifloxacin but were generally mild and did not result in study drug discontinuation.*

In the Phase 2 ABSSSI Study 2001, administration site reactions occurred in 12.7% of subjects in the lefamulin 150 mg arm compared to 3.0% of subjects in the vancomycin arm. Most of the reactions were mild, but one subject (1.4%) in the lefamulin 150 mg arm had a severe reaction resulting in study drug discontinuation.

***M.O. Comment**: The Phase 2 study corroborates the finding of increased administration site reactions among subjects who received lefamulin IV 150 mg. In addition, the reactions were mostly mild and did not result in study drug discontinuation.*

8.2.5.2. QT prolongation

Nonclinical toxicity studies showed lefamulin reduced the amplitudes of the hERG-mediated potassium channel currents in a concentration-dependent manner which suggested it would cause QT prolongation in humans. Early Phase 1 studies confirmed dose-related QT prolongation. In the Phase 3 safety population, ECGs were obtained in triplicate before and after the first dose of study drug and again at Day 3 or Day 4. Analysis of all postbaseline QTcF values showed the proportions of subjects exposed to LEF versus MOX had similar degrees of QT prolongation. These data are summarized in the table below.

Table 98. Measures of Post-Baseline QTcF Prolongation in the Phase 3 Safety Population

Measure of QTcF Prolongation at Any Post-Baseline Timepoint (msec)	LEF[1] N=636 n (%)	MOX[1] N=636 n (%)
Mean max change in QTcF from baseline (msec)	16.8	19.3
Value >480	20 (3.1)	21 (3.3)
Value >500	2 (0.3)	6 (0.9)
Increase of >30 from baseline	114 (17.9)	142 (22.3)
Increase of >60 from baseline	11 (1.7)	16 (2.5)
Increase of >30 from baseline & value >480	9 (1.4)	11 (1.7)
Increase of >30 from baseline & value >500	2 (0.3)	3 (0.5)
Increase of >60 from baseline & value >480	1 (0.2)	4 (0.6)
Increase of >60 from baseline & value >500	0	1 (0.2)

[1]Demoninator is all subjects in each arm with both a baseline and at least one postbaseline QTcF value

LEF = lefamulin; MOX = moxifloxacin

***M.O. Comment**: QT prolongation was seen in subjects in the LEF arm, but extreme prolongation was rare and by each measure, no worse than the comparator. However, moxifloxacin is a known QT prolonger. The product label for LEF will need to have similar language about QT prolongation to what is in the moxifloxacin label.*

A similar analysis of the QT prolongation data from the Phase 2 ABSSSI Study 2001, in which vancomycin was the comparator, is shown in the table below.

Table 99. Measures of Post-Baseline QTcF Prolongation in Phase 2 Study in ABSSSI (2001)

Measure of QTcF Prolongation at Any Post-Baseline Timepoint (msec)	LEF 100 mg N=70 n (%)	LEF 150 mg N=71 n (%)	Vancomycin 1g N=66 n (%)
Mean max change in QTcF from baseline (msec)	20.4	22.0	16.0
Value >450	5 (7.1)	2 (2.8)	2 (3.0)
Value >500*	0	0	0
Increase of >30 from baseline	15 (21.4)	16 (22.5)	8 (12.1)
Increase of >45 from baseline*	0	3 (4.2)	0
Increase of >30 from baseline & value >450	1 (1.4)	2 (2.8)	2 (3.0)
Increase of >45 from baseline & value >450	0	0	0

*No subjects had postbaseline QTcF values >480 or an increase from baseline of >60
ABSSSI = Acute Bacterial Skin and Skin Structure Infection; LEF = lefamulin

***M.O. Comment**: QT prolongation of between 30 and 45 msec is noted in the two LEF arms. A few subjects in the vancomycin arm also had QT prolongation which is unusual as vancomycin is not usually associated with that finding. In addition, the mean maximum change in QTcF was fairly high in the vancomycin subjects. As a result, the extent of QT prolongation in the LEF arms is likely exaggerated in this analysis. Overall, these data corroborate the finding of QT prolongation in LEF-exposed subjects.*

8.2.6. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

There are no COA data that are applicable to the safety analysis.

8.2.7. Safety Analyses by Demographic Subgroups

The numbers of deaths, SAEs, and dropouts due to study drug in the Phase 3 safety population were too low to allow a meaningful analysis of these data by subgroups. Therefore, the focus of

this section is on the proportion of subjects with at least one TEAE in different demographic and other baseline characteristic-based subgroups (Table 100).

Table 100. Proportion of Subjects with at least one TEAE by Demographic Subgroups in the Phase 3 Safety Population

Subgroup	LEF n/N (%)	MOX (%) n/N (%)
All subjects	224/641 (34.9)	195/641 (30.4)
Sex		
Female	97/267 (36.3)	90/302 (29.8)
Male	127/374 (34.0)	105/339 (31.0)
Categorical age (years)		
18–64	143/374 (38.2)	115/393 (29.3)
65–74	34/152 (22.4)	46/145 (31.7)
>74	47/115 (40.9)	34/103 (33.0)
Race		
White	167/508 (32.9)	140/508 (27.6)
Black	8/30 (26.7)	11/34 (32.4)
Asian	38/72 (52.8)	34/71 (47.9)
Amer. Indian or Alaska Native	8/24 (33.3)	5/17 (29.4)
Other	3/7 (42.9)	5/11 (45.5)
Ethnicity		
Hispanic or Latino	22/53 (41.5)	14/48 (29.2)
Not Hispanic or Latino	202/588 (34.4)	181/593 (30.5)
Geographic region		
North America[1]	8/13 (61.5)	8/13 (61.5)
Latin America	19/42 (45.2)	12/44 (27.3)
Eastern Europe	132/451 (29.3)	113/434 (26.0)
Western Europe	19/32 (59.4)	11/33 (33.3)
Rest of the world	46/103 (44.7)	51/117 (43.6)
PORT risk class		
Class I	0/1 (0.0)	1/2 (50.0)
Class II	72/183 (39.3)	45/190 (23.7)
Class III	97/337 (28.8)	98/333 (29.4)
Class IV	52/115 (45.2)	46/111 (41.4)
Class V	3/5 (60.0)	5/5 (100.0)
Kidney disease[2]		
Normal	103/310 (33.2)	81/311 (26.0)
Mild renal impairment	67/198 (33.8)	63/192 (32.8)
Moderate renal impairment	50/125 (40.0)	48/132 (36.4)
Severe renal impairment	4/7 (57.1)	3/6 (50.0)
History of lung disease[3]		
Yes	48/134 (35.8)	50/126 (39.7)
No	176/507 (34.7)	145/515 (28.2)
History of heart disease[4]		
Yes	41/110 (37.3)	43/120 (35.8)
No	183/531 (34.5)	152/521 (29.2)
History of diabetes mellitus		
Yes	29/80 (36.3)	29/87 (33.3)
No	195/561 (34.8)	166/554 (30.0)

[1]All North American subjects were from the United States

[2]One subject in the LEF arm was missing renal impairment status
[3]Based on having a medical history term in the SOC of Respiratory disorders
[4]Based on having a medical history term in the SOC of Cardiac disorders
n = number of subjects with at least one TEAE; N = all subjects in the subgroup; TEAE = treatment-emergent adverse event; LEF = lefamulin; MOX = moxifloxacin; PORT = Pneumonia Outcomes Research Team;

M.O. Comment:

- *When reviewing these data, it should be noted that there was an imbalance overall between the treatment arms for subjects with at least one TEAE (35% versus 30%).*
- *Considering this overall imbalance between the treatment arms and that there were small numbers for many subgroups, there was not a significant additional imbalance based on sex, age, race, ethnicity, or geographic region.*
- *The higher proportion of Asians with at least one TEAE in both arms might be a result of AE reporting tendencies at certain sites. Most Asian subjects were at clinical sites in the Philippines.*
- *There was an imbalance with more subjects in the LEF arm with PORT Risk Class II with at least one TEAE. This imbalance in AEs is mostly driven by the PTs of diarrhea and nausea, which were more common in Study 3102 in which subjects with PORT Risk Class II were enrolled.*
- *There was no imbalance based on history of diabetes mellitus, kidney, lung, or heart disease.*

8.2.8. Specific Safety Studies/Clinical Trials

There were no specific safety studies for this NDA.

8.2.9. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

In the Phase 3 safety population, five subjects in the LEF arm (0.8%) and four subjects in the MOX arm (0.6%) had TEAEs in the neoplasms SOC. These included lung cancer and liver hemangioma in both arms, AML and renal cancer in the LEF arm, and testicular seminoma, splenic neoplasm, and lymphoproliferative disorder in the MOX arm. None of these cases appear to be related to study drug.

M.O. Comment*: There is little concern for human carcinogenicity for lefamulin given the planned short treatment duration.*

Human Reproduction and Pregnancy

The Phase 2 and 3 clinical trials excluded pregnant women and women of childbearing potential who were not on contraceptives. In addition, no subjects became pregnant during any of the clinical trials. As a result, there are no data on the effect of lefamulin on human reproduction or pregnancy.

Pediatrics and Assessment of Effects on Growth

Lefamulin was not studied in children so there are no data on pediatric safety or the effects of lefamulin on growth.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Lefamulin does not have any known potential for drug abuse or dependence. With respect to overdose, single doses of lefamulin 400 mg IV and 750 mg oral did not result in any SAEs in healthy volunteers. Supportive treatment only is recommended for cases of overdose.

8.2.10. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

Lefamulin is not approved in the United States or in other countries so there is no postmarket experience.

Expectations on Safety in the Postmarket Setting

Per the proposed product label, lefamulin is only indicated for the treatment of CABP. However, it is possible physicians would prescribe it off-label for longer durations of treatment. For example, patients with chronic infections such as osteomyelitis may be treated with lefamulin for weeks to months. This longer duration of treatment was not studied in the drug development program.

8.2.11. Integrated Assessment of Safety

The safety of lefamulin (LEF) in the treatment of CABP was evaluated mainly through data from two Phase 3 trials which compared LEF to moxifloxacin (MOX). The pooled data from these trials included 273 subjects who received IV to oral LEF and 368 subjects who received oral LEF only. Supportive data were also obtained from a Phase 2 trial for ABSSSI. The Phase 3 pooled population was balanced between the treatment arms with respect to age, sex, race, and medical comorbidities. Most subjects were White and from Eastern Europe, but CABP in this population is likely similar to that in the United States. In addition, subjects with underlying

cardiac and respiratory disorders, as well as, diabetes mellitus were well represented in the primary safety population. There were no major imbalances between the LEF and MOX subjects in deaths, SAEs, dropouts due to study drug, or TEAEs overall. However, there were several issues identified during the review, which will be summarized in this section.

An important issue identified in the review, was an imbalance of SAEs with more cases of pneumonia and other lung infections in LEF subjects compared to MOX subjects (12 versus 6). Similarly, there was an imbalance of respiratory SAEs with more LEF subjects having events related to treatment failure such as respiratory failure (6 versus 2). Further analysis of these cases revealed most to be failure of the study drug to adequately treat the primary pneumonia. In addition, many LEF-treated subjects that experienced treatment failure grew an Enterobacteriaceae from their sputum culture which is not included in the antibacterial spectrum of activity of LEF. As a result, these LEF subjects may not have been adequately treated for their primary pneumonia and thus experienced treatment failure. It is notable that nearly all of these cases were categorized as failures by IACR at LFU and thus these failures were included in the efficacy analyses.

Prolongation of the QT interval was another issue that was identified early in the development of LEF. In the Phase 3 trials, the extent of QT prolongation was similar to moxifloxacin, a drug that has been shown to prolong the QT interval. For example, 17.9% of LEF subjects and 22.3% of MOX subjects had an increase in the QTcF interval of more than 30 msec. In addition, a similar number of subjects in each arm discontinued study drug because of QT prolongation (2 versus 3) and there was no imbalance in SAEs or TEAEs in the cardiac disorders SOC suggesting any effects of QT prolongation were also balanced between the treatment arms. However, MOX is a known prolonger of the QT interval so LEF should contain appropriate safety labeling communicating the risk of QT prolongation. The label includes a warning regarding risk of QT prolongation associated with lefamulin use.

Another issue that was known early in the development of LEF was administration site reactions with the IV formulation. More LEF subjects experienced an administration site reaction in Study 3101 compared to MOX subjects (7.3% versus 2.6%). These reactions included inflammation, pain, and phlebitis at the administration site. However, the reactions were mostly mild and rarely resulted in study drug discontinuation. The risk of administration site reactions will be communicated in product labeling.

Gastrointestinal adverse events were common with the oral formulation of LEF. In Study 3102 (oral LEF versus oral MOX), 17.9% of LEF subjects compared to 7.6% of MOX subjects experienced a TEAE in the gastrointestinal disorders SOC. Diarrhea was the most frequently reported AE with 12.2% of LEF subjects compared to only 1.1% of MOX subjects. Nausea (5.2% versus 1.9%) and vomiting (3.3% versus 0.8%) were also common GI TEAEs in subjects treated with oral LEF compared to oral MOX. However, these events were not serious and only rarely resulted in study drug discontinuation. In addition, there were no severe GI TEAEs among LEF-

treated subjects in Study 3102. The risk of gastrointestinal adverse events will be communicated in product labeling.

In the Phase 3 pooled data, laboratory data and TEAEs did not show a clear imbalance between LEF- and MOX-treated subjects who had elevations in AST, ALT, or bilirubin. More LEF subjects compared to MOX subjects had elevations in GGT (3.0% versus 1.3%) and alkaline phosphatase levels (1.2% versus 0.5%). However, without concomitant elevations in bilirubin, elevations in GGT and alkaline phosphatase do not have a clear clinical consequence. In addition, there were no cases of Hy's law in LEF-treated subjects making drug-induced liver injury related to LEF less likely. The risk of liver enzyme elevations will be communicated in product labeling.

An imbalance of subjects who experienced "chronic obstructive pulmonary disorder" (COPD) as an AE was seen: 8 LEF subjects versus 3 MOX subjects. However, review of these cases showed that several of the LEF subjects developed symptoms of COPD several days after completing study drug. In addition, examining only cases in which COPD was reported while subjects received study drug, the imbalance was not present. Taken together, it is unlikely the COPD AEs were related to LEF.

In summary, the safety issues of lefamulin in the treatment of CABP include QT prolongation that is similar to moxifloxacin, mild to moderate gastrointestinal adverse events with the oral formulation, and administration site reactions with the IV formulation. In addition, the safety data revealed that some LEF-treated subjects likely did not have adequate antibacterial coverage of their pneumonia resulting in treatment failure given that LEF does not cover Enterobacteriaceae. However, these treatment failures were captured in the efficacy analyses which demonstrated noninferiority between lefamulin and moxifloxacin at early and later timepoints.

8.3. Statistical Issues

The Applicant's proposed statistical methods were sensible but not always optimal. For the primary efficacy endpoint, ECR, the Applicant used continuity-corrected z-tests and associated confidence intervals to perform noninferiority tests. However, the use of standard (uncorrected) z-tests would have been better, as these tests are more powerful and still maintain the nominal alpha level, given the two Phase 3 trials' sample sizes. In addition, since both trials used randomization strata, basing noninferiority testing on the so-called standardization estimator (which combines stratum-specific estimates of the between-arm differences in success rates) would also have yielded more powerful tests. The Applicant proposed reasonable sensitivity analyses for this endpoint, but didn't include the most rigorous one, namely, the "worst-case" sensitivity analysis that treats missing endpoint values in the moxifloxacin arm as treatment successes but missing endpoint values in the lefamulin arm as treatment failures. Nonetheless, the combination of the Applicant's continuity-corrected tests

and the reviewer-implemented worst-case analysis yielded strong support for the noninferiority of lefamulin to moxifloxacin for the treatment of CABP.

8.4. Conclusions and Recommendations

The efficacy and safety of lefamulin for the treatment of adults with CABP were demonstrated in two adequate and well-controlled Phase 3 trials in which lefamulin was compared to moxifloxacin. Regarding efficacy, lefamulin was found to be noninferior to moxifloxacin on the primary endpoint (ECR) with consistent results for the key secondary endpoint (IACR at TOC). In addition, subgroup analyses including by-pathogen analyses did not show a meaningful difference in the clinical response rates of lefamulin and moxifloxacin. Taken together, these findings demonstrate that lefamulin is noninferior to moxifloxacin for the treatment of CABP.

Regarding safety, there were no major safety issues identified in the Phase 3 trials that cannot be mitigated with product labeling. While there were more lung infections reported as serious adverse events among lefamulin subjects compared to moxifloxacin subjects (12 versus 6), review of the cases suggests these reported infections likely represented failure of the study drug to treat the primary pneumonia, many of which may have been caused by pathogens not covered by lefamulin, including Enterobacteriaceae. Of note, these treatment failures were captured as failures in the efficacy analyses which demonstrated noninferiority between lefamulin and moxifloxacin at early and later timepoints. QT interval prolongation and elevation of liver enzymes were noted with lefamulin, but to a similar extent as with moxifloxacin. Administration site reactions with the IV formulation and nausea and vomiting with the oral formulation were seen with lefamulin, but these adverse reactions were mostly mild to moderate in severity and rarely resulted in treatment discontinuation.

In summary, the Applicant has provided substantial evidence for the effectiveness of lefamulin for the treatment of CABP and sufficient safety information. The safety issues identified in the clinical trials can be mitigated with appropriate product labeling.

9 Advisory Committee Meeting and Other External Consultations

No advisory committee meeting was held, and no external consultations were obtained as there were no issues that needed input from external experts

10 Pediatrics

There are currently no clinical data available with lefamulin in the treatment of pediatric CABP. An initial Pediatric Study Plan (iPSP) for lefamulin for the treatment of CABP in patients 2

months to less than 18 years old was submitted to INDs 106594 (IV formulation) and 125546 (tablet) on 02 June 2017. The Division confirmed initial agreement of the iPSP on 11 December 2017.

The Applicant requested deferral of the pediatric clinical study in CABP patients 2 months to <18 years of age (b) (4) The Applicant requested a waiver from studying pediatric patients less than 2 months of age, (b) (4)

A review by the PeRC committee was conducted on 10 July 2019. PeRC agreed with granting the deferral and waiver as presented in the Agreed iPSP.

Please also see Section 13 of this review regarding the postmarketing requirement to study lefamulin in pediatric patients with CABP.

11 Labeling Recommendations

11.1. Prescription Drug Labeling

Table 101. Significant High-Level Labeling Changes (Not Direct Quotations)

Section	Proposed Labeling	Tentative Labeling
1 INDICATIONS AND USAGE	(b) (4)	
M.O. Comment: (b) (4) *In general, the threshold for inclusion in the first list and indication is 10 subjects.* (b) (4) *was also deleted from the indication statement because of a lack of sufficient data from clinical cultures or FDA cleared tests. Both of these organisms were moved to the second list. Reference to* (b) (4) *was deleted. Susceptibiity to the particular drug is of clinical utility rather than resistance to other classes of drugs.*		
2 DOSAGE AND ADMINISTRATION	• (b) (4) • (b) (4)	• Recommend adjusting the dose of IV lefamulin in patients with hepatic impairment. • Dosing with the oral formulation is not recommended in patients with moderate or severe hepatic impairment. • Administration instructions for lefamulin tablets modified to include taking at least 1 hour before or 2 hours after a meal.
M.O. Comment*: There is concern for increased unbound drug exposure in patients with hepatic impairment. Also, the oral formulation of lefamulin was not studied in patients with hepatic impairment in whom there may be erratic bioavailability. With regard to food effect, administration instructions for the lefamulin tablets were modified to resemble the instructions used in the Phase 3 protocols.*		
4 CONTRAINDICATIONS	• No contraindication for concomitant use of lefamulin with CYP3A4 substrates that prolong the QTc interval	• Added contraindication for concomitant use of lefamulin with CYP3A4 substrates that prolong the QTc interval
M.O. Comment*: A contraindication was added as concomitant administration of oral lefamulin with CYP3A4 substrates that prolong the QTc interval could lead to development of torsades de pointes.*		
5 WARNINGS AND PRECAUTIONS	• No information on embryo-fetal toxicity	• Added information regarding animal data on embryo-fetal toxicity and recommendation against use in pregnancy

		• Warning statement includes verifying pregnancy status in females of reproductive potential and advising females of reproductive potential to use effective contraception during treatment with lefamulin and for 2 days (5 to 6 times the half-life) after the final dose.
***M.O. Comment**: A warning for embryo-fetal toxicity was added because nonclinical studies demonstrated an increased incidence of postimplantation fetal loss and stillbirths in rats or rabbits treated during the period of organogenesis or in rats treated from the beginning of organogenesis through the time of weaning. Additional rat pup deaths were observed during early lactation that were likely related to maternal treatment with lefamulin. Malformations were noted in rats at systemic exposures lower than the systemic exposure expected in CABP patients.*		
6 ADVERSE REACTIONS	• Summary of clinical trial experience and adverse events.	• Minor modifications to some adverse event totals • Split adverse reactions from each trial into separate tables for ease of reading • Combined related adverse event terms, such as, abdominal pain and gastritis.
7 DRUG INTERACTIONS		• Reorganized subsections into the following categories: effect of other drugs on lefamulin, effect of lefamulin on other drugs, and drugs that prolong the QT interval • Removed subsections which only (b) (4)
8 USE IN SPECIFIC POPULATIONS	• Brief statement on the lack of data for the use of lefamulin in pregnancy and during breastfeeding • (b) (4)	• Lefamulin may cause fetal harm when given to pregnant women • Verify pregnancy status in females of reproductive potential prior to considering lefamulin as a therapeutic option • Added information on pregnancy pharmacovigilance program • Breastfeeding is not recommended during lefamulin treatment • For patients with severe hepatic impairment, the lefamulin injection dose should be reduced by extending the dosing interval to q24hrs • Insufficient information to recommend lefamulin tablets in patients with moderate or severe hepatic impairment. No dosage adjustment for patients with mild hepatic impairment.

M.O. Comment: *Nonclinical studies showed lefamulin was concentrated in the milk of lactating rats suggesting lefamulin would be present in human breast milk. As a result, lactating women are recommended to pump and discard breast milk during treatment with lefamulin and for two days afterward.*		
12 CLINICAL PHARMACOLOGY	• Minimized potential effect of food on the bioavailability of oral lefamulin. • Protein binding noted to be (b) (4) %. • Noted no clinically meaningful changes in PK parameters of lefamulin in subjects with hepatic impairment compared to healthy subjects.	• Noted approximately 20% reduction in bioavailability of oral lefamulin in the presence of a high fat, high calorie meal. • Estimated protein binding revised to 95 to 97%. • Revised discussion of exposure in subjects with hepatic impairment. — 3-fold increase in exposure in patients with severe hepatic impairment compared to those with normal hepatic function. — Recommendation to reduce the dose of IV lefamulin in patients with severe hepatic impairment. — Note that there is no information to evaluate the effect of moderate or severe hepatic impairment on the disposition of lefamulin following administration of tablets. — Lefamulin tablets are not recommended in patients with moderate or severe hepatic impairment • Removed information on (b) (4) • (b) (4) were moved to the second list
M.O. Comment*: The clinical pharmacology review team differed from the Applicant in the interpretation of these data which led to revised dosing recommendations for patients with hepatic impairment and for administration with food.*		
13 NONCLINICAL TOXICOLOGY	• (b) (4) • NOAEL for female fertility was (b) (4) • General toxicology data repetitive of findings in human subjects	• Added information about possible genotoxic impurities. • Specified that there are no valid in vitro assays for mutagenicity of lefamulin and its metabolite as the MLAs did not meet the standards for a valid assay. • NOAEL for female fertility corrected and effects seen at the higher dose

		described. • General toxicology data revised to limit to primarily clinically relevant findings not already described in human subjects.
***M.O. Comment**: Regarding the possible genotoxic impurities, there are at least 6 impurities which may exceed the total daily intake recommendations. However, the amounts of several of these impurities are below the lower limit of detection of the assay used to measure the level. This suggests that at least some of these impurities may be at lower levels. In addition, the short duration of treatment may reduce the risk from these possible genotoxic impurities.*		
14 CLINICAL STUDIES	• Summary of efficacy data from two Phase 3 trials	• Changed by-pathogen clinical response data from (b) (4) to investigator-assessed response at the test-of-cure timepoint • Removed (b) (4)
17 PATIENT COUNSELING INFORMATION	• (b) (4)	• Changed food recommendation to include taking at least 1 hour before or 2 hours after a meal • Added information for patients regarding embryo-fetal toxicity and lactation
***M.O. Comment**: These changes reflect nonclinical and clinical pharmacology data discussed in sections 8 and 12 of the product label.*		

12 Risk Evaluation and Mitigation Strategies (REMS)

No risk evaluation and mitigation strategies are needed at this time. The risks of lefamulin may be adequately managed in the postmarketing setting through labeling.

13 Postmarketing Requirements and Commitment

PREA PMRs

(1) Conduct a single-dose study to evaluate pharmacokinetics and safety of intravenous XENLETA (lefamulin) in children from birth to less than 18 years with suspected or confirmed bacterial infections receiving standard of care.

- Final protocol submission: 04/2018 (submitted)
- Study completion: 06/2024
- Final report submission: 12/2024

***M.O. Comment**: This study was initiated in May 2018 and is ongoing.*

(2) Conduct a single-dose study to evaluate pharmacokinetics and safety of oral XENLETA (lefamulin) in children from birth to less than 18 years of age with suspected or confirmed bacterial infections receiving standard of care.

- Final protocol submission: 05/2021
- Study completion: 12/2024
- Final report submission: 06/2025

(3) Conduct a randomized active-controlled, study to assess the safety and pharmacokinetics of XENLETA (lefamulin) in children from 2 months to less than 18 years of age with community-acquired bacterial pneumonia (CABP).

- Draft protocol submission: 09/2020
- Final protocol submission: 12/2020
- Study completion: 12/2024
- Final report submission: 06/2025

505(o) Safety PMR

(4) Conduct a United States surveillance study for 5 years from the date of marketing to determine if resistance to XENLETA (lefamulin) has developed in those organisms specific to the indication in the label.

- Final protocol submission: 09/2019
- Interim study report: 06/2020
- Interim study report: 06/2021
- Interim study report: 06/2022
- Interim study report: 06/2023
- Interim study report: 06/2024
- Study completion: 09/2024
- Final report submission: 12/2024

(5) Conduct a pregnancy surveillance program to collect and analyze information for a minimum of 10 years on pregnancy complications and birth outcomes in women exposed to XENLETA (lefamulin) during pregnancy.

- Final protocol submission: 08/2019 (submitted)
- Interim study report: 08/2020
- Interim study report: 08/2021
- Interim study report: 08/2022
- Interim study report: 08/2023
- Interim study report: 08/2024
- Interim study report: 08/2025
- Interim study report: 08/2026
- Interim study report: 08/2027
- Interim study report: 08/2028
- Study completion: 08/2029
- Final report submission: 08/2030

M.O. Comment*: DPMH recommended a study in lactating women who are receiving therapeutic doses of lefamulin to determine the concentration of lefamulin in human breast milk. After further discussion, including conversations with DPMH it was agreed to not require the Applicant to conduct a lactation study due to the following reasons: (1) the planned duration of therapy with lefamulin is short (5 to 7 days); (2) to the label will recommend that women not breastfeed while on lefamulin; and (3) lefamulin has a limited spectrum of antibacterial activity and other treatment options are available that would not pose a potential risk to a breastfed baby. It is not anticipated that lefamulin would be a first-choice antibacterial drug for lactating women with CABP.*

Nonclinical PMRs

(6) Conduct an *in vitro* Mouse Lymphoma Assay (MLA) that evaluates higher doses of lefamulin reaching 10-20% Relative Total Growth (RTG) and in accordance with the Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals #476.

- Draft protocol submission: 01/2020
- Final protocol submission: 03/2020
- Study completion: 06/2020
- Final study report submission: 08/2020

(7) Conduct an *in vitro* Mouse Lymphoma Assay (MLA) that evaluates higher doses of the lefamulin metabolite BC-8041 reaching 10-20% Relative Total Growth (RTG) and in accordance with the Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals #476.

- Draft protocol submission: 01/2020
- Final protocol submission: 03/2020
- Study completion: 06/2020
- Final study report submission: 08/2020

14 Division Director (DAIP) Comments

I concur with the review team's assessment and recommendations.

15 Office Director Comments

I concur with the review team's assessment and recommendations.

16 Appendices

16.1. References

File TM and Marrie TJ. Burden of community-acquired pneumonia in North American adults. Postgrad Med. 2010 Mar;122(2):130-41.

Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, Coley CM, Marrie TJ, Kapoor WN. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997 Jan 23;336(4):243-50.

Jain S, Self WH, Wunderink RG et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. N Engl J Med. 2015 Jul 30;373(5):415-27.

Pfuntner A, Wier LM, Steiner C; Agency for Healthcare Research and Quality. Costs for hospital stays in the United States. http://www.hcup-us.ahrq.gov/reports/statbriefs/sb168-Hospital-Costs-United-States-2011.jsp. Accessed Dec 12, 2018.

16.2. Financial Disclosure

There were two covered clinical studies in this NDA which were the two Phase 3 studies (3101 and 3102).

Covered Clinical Study (Name and/or Number): NAB-BC-3781-3101

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 104		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
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 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) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

Covered Clinical Study (Name and/or Number): NAB-BC-3781-3102

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 161		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
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 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) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

16.3. OCP Appendices (Technical Documents Supporting OCP Recommendations)

16.3.1. Nonclinical Studies

16.3.1.1. Protein binding

Lefamulin (LEF) plasma protein binding (PPB) has been studied in mouse plasma (Study 03781A-PP04-001: in vivo assay) and human plasma (Studies EVT-00756-3781 and XS-1103: in vitro assays and Studies 1010 and 1011: in vivo assays). PPB was determined by equilibrium dialysis methods.

Murine In Vivo PPB (Study 03781A-PP04-001)

Mean unbound fraction of LEF, expressed as a percentage, in infected mice increased from 20.8% to 24.6% when the LEF concentrations increased from 0.12 mcg/mL to 3.25 mcg/mL (pooled serum (i.e., 99% serum); equilibrium dialysis).

Human In Vitro PPB (Studies EVT-00756-3781 and XS-1103)

Both studies demonstrated that LEF PPB in human plasma is concentration-dependent as observed in mouse plasma. However, the mean unbound fractions of LEF were substantially different between the two studies. In Study EVT007-3781, the mean unbound fraction, expressed as a percentage, was 12.1, 17.1, and 2d7.3% at the LEF concentrations of 1, 3, and 10 mcg/mL, respectively. In Study XS-1103, the corresponding mean unbound fractions were 3.1, 6.4, and 14.5%. The Applicant did not provide a reason for this discrepancy. The most likely explanation is the difference in plasma concentrations used (% v/v). In Study EVT-00756-3781,

LEF PPB was evaluated in 85% plasma. In contrast, in Study XS-1103, LEF PPB was evaluated in 99% plasma. Study XS-1103 also demonstrated an increase in LEF PPB (i.e., a decrease in unbound fraction) in pooled adult or adolescent plasma compared to pooled infant or toddler plasma (where the protein concentrations may be lower than in adults and adolescents), supporting that different plasma concentrations used in Studies EVT-00756-3781 and XS-1103 may result in the different PPB estimates (Table 102). Other differences such as the anticoagulant (EVT-00756-3781: Lithium Heparin; XS-1103: K2EDTA) were noted as possible influencing factors, but lack of data do not allow evaluation.

Table 102. Human In Vitro LEF Plasma Protein Binding Comparison Between Studies

			LEF (mcg/ml)		
Study	**Group**	**Age**	**1**	**3**	**10**
XS-1103			**% Bound**		
	Infant	0 to <2 mo	84	76	68
		2 to ≤6 mo	87	81	74
		6 to <12 mo	92	88	79
	Toddler	1 to 2 yrs	94	91	82
	Adolescent	2 to 17 yrs	96	94	85
	Adult	38 to 53 yrs	97	94	86
EVT-00756-3781	Not specified	---	88	83	73

LEF = lefamulin

Human In Vivo PPB

In Phase 1 clinical adult studies (Studies 1010 (hepatic impairment) and 1011 (renal impairment)), LEF PPB was also concentration-dependent with a higher mean unbound fraction immediately after the end of a 1-hr IV infusion compared to that at 3, and 8 hr after the start of infusion (equilibrium dialysis and LC-MS/MS). The mean unbound LEF fractions, expressed as a percentage and obtained after pooling these two studies (in patients with normal hepatic and renal function), was 5.5, 3.1, and 2.8% at 1, 3, and 8 hr after the start of infusion (single dose of 150 mg LEF IV), respectively. Maximum LEF concentrations achieved in these studies were between 1 and 3 mcg/mL. PPB results are greater than those observed in Study EVT-00756-3781, but in line with findings in Study XS-1103.

Binding Affinity

The binding affinity of LEF to human serum albumin (HSA) and alpha-1 acid glycoprotein (AGP) was analyzed over a concentration range of 1.6µM to 200µM (ca. 0.8 mcg/mL to 101.5 mcg/mL) (surface plasmon resonance (SPR) biosensor). The lefamulin AGP K_d was 118µM. No K_d could be calculated for HSA. The K_d for the prototypical AGP drug dipyridamole was 57µM for benchmark comparison, indicating that lefamulin exhibits weaker binding affinity than dipyridamole to AGP. No information regarding variables such as free-fatty acids, lipoproteins, or ionized calcium were included.

Collectively, LEF PPB, expressed as a percentage, in humans without pneumonia is concentration-dependent, ranging between 94.5% to 97.2% at LEF concentrations achieved in the clinic. The observed mean unbound LEF fractions, expressed as a percentage, from pooled clinical data (excluding hepatic impairment) across time, is 3.8%. PK and PK-PD analyses were updated and reassessed with this information.

16.3.1.2. Evaluation of enzyme or transporter-mediated drug-drug interactions

Table 103. In Vitro Assessment of Lefamulin as a Substrate, Inhibitor, or Inducer of Metabolism

	In Vitro Findings				In Vivo		
Enzyme	% Drug Remaining After Incubation[a,b]	IC_{50} [µM][d,e]	IC_{50} Shift	Induction FC[g,h]	Potential Substrate/ Inhibitor/ Inducer	Rationale/ Interpretation Reviewer Analysis	Applicant Action
CYP1A2	105.5	>200[f]	---	0.52–1.33	---	NC	---
CYP2B6	115.6	>200[f]	---	0.52–1.5	---	NC	---
CYP2C8	102.3	41[c]	1.26	---	---	R1=1.0<1.02	PBPK
CYP2C9	116.5	>200[f]	---	---	---	NC	---
CYP2C19	107	>200[f]	---	---	---	NC	---
CYP2D6	113.6	>200[f]	---	---	---	NC	---
CYP3A4/5	0.4/ 47.1	15 (T) 0.86[c] (M)	2.2 (T) 0.86 (M)	0.68–1.51	Substrate Inhibitor	AUCR (M) =2.73>1.25	Clinical (M)(K)

[a] human recombinant CYP450 Isoenzymes
[b] Lefamulin metabolism was saturable (i.e., concentration dependent) at higher concentrations (24.6µM)
[c] Ki [µM] experimentally determined. CYP2C8 and CYP3A4 exhibited mixed and direct inhibition, respectively.
[d] human liver microsomes (pooled)
[e] nominal drug concentrations
[f] >70% parent drug remaining at 200µM
[g] human hepatocytes (mRNA expression); all enzyme responses <20% of positive control
[h] cellular viability issues limited higher concentrations (>15µM)
Model Assumptions: Dose (lefamulin base) =600 mg or 1.18 mmol (PO); 150 mg or 0.30 mmol infused over 1 hr (IV); [I]g = Dose/250 mL =2.4 mg/mL or 4726.9µM; C_{max},Day1=2.24 mcg/mL or 4.41µM (PO); 3.50 mcg/mL or 6.89µM (IV) Patients with CABP; fu, p =0.04 based on plasma protein binding from clinical studies; Ka =0.033 min-1 (fastest absorption rate from PPK model); Fa =0.258 (absolute bioavailability); fm =0.9 and fg =0.51 for midazolam.
Refer to FDA Draft In Vitro Guidance for all equations and other default parameter specifics.
T = testosterone; M = midazolam; K = ketoconazole; Ki = inhibition constant; NC = not calculated; FC = fold change; IC_{50} = half-maximal inhibitory concentration; AUCR = ratio of area under concentration-time curve

Table 104. In Vitro Assessment of Lefamulin as a Substrate or Inhibitor of Human Uptake and Efflux Transporters

	In Vitro Findings				
Transporter	Max Flux Rate Ratio	IC_{50} [µM]	In Vivo Potential Substrate/Inhibitor	Rationale/Interpretation Reviewer Analysis	Applicant Action
BCRP	1.45	42.2	Inhibitor	$R_{1, gut}$ =113≥11	PBPK
P-gp	68	6.2	Substrate and inhibitor	ER >2 $R_{1, gut}$ =763≥11	Clinical (D) (K)
BSEP	1.1	24.5		$I_{max, u}/IC_{50}$ =0.01[b] ≤0.02	NT[c]
OATP1B1	0.86	122		R =1.0≤1.1	PBPK
OATP1B3	0.63	122		R =1.0≤1.1	PBPK
OCT1	4.2	20.3	Substrate and inhibitor	ER >2[b] $I_{max, u}/IC_{50}$=0.01[b] ≤0.02	PBPK[c]
OAT1	NT[a]	>122	---	---	---
OAT3	NT[a]	>122	---	---	---
OCT2	NT[a]	>122	---	---	---
MATE1	1.88	0.297	Inhibitor	$I_{max, u}/IC_{50}$=0.93≥0.02	PBPK
MATE2	1.53	76.4	---	---	---

[a]renal clearance <25% of total lefamulin clearance
[b]EMA cut-off; not specified in FDA guidance
[c]Not specified in in vitro DDI draft guidance
Model Assumptions: Dose (lefamulin base) =600 mg or 1.18 mmol (PO); 150 mg or 0.30 mmol infused over 1 hr (IV); $[I]_g$ = Dose/250 mL =2.4 mg/mL or 4726.9µM; $C_{max,Day1}$=2.24 mcg/mL or 4.41µM (PO); 3.50 mcg/mL or 6.89µM (IV) Patients with CABP; $f_{u, p}$ =0.04 based on in vitro plasma protein binding from clinical studies; K_a =0.033 min^{-1} (fastest absorption rate from PPK model); F_a =0.258 (absolute bioavailability); Refer to FDA Draft In Vitro Guidance for all equations and other default parameter specifics.
BCRP = breast cancer resistance protein; MATE = multiantimicrobial extrusion protein; OATP = organic-anion-transporting polypeptide; P-gp = P-glycoprotein; NT = not tested; ER = efflux rate ratio; D = digoxin; K = ketoconazole; PBPK = physiologically-based pharmacokinetic; IC_{50} = half-maximal inhibitory concentration; I_{max} = maximum inhibition

Metabolic Profiling and Phenotyping of Lefamulin

In vitro metabolic profiles of lefamulin in primary hepatocytes revealed monohydroxylated metabolites (2.4% to 23.3% area), dihydroxylated metabolites (0.29% to 5% area), and trihydroxylated metabolites (0.12% to 0.82% area) as the predominate metabolites. Phase II conjugates (methylation) of parent or metabolite phase I species were observed but to a lesser extent (0.1% to 1.3% area). No glucuronidation was observed in human cells.

In vitro reaction phenotyping studies suggest the prevailing metabolizing enzyme responsible for lefamulin (0.5µM [ca. 284 ng/mL]) breakdown is CYP3A4/5 based on pooled human liver microsome (HLM) and human recombinant CYP450 isoenzyme studies. The extent of metabolism was near complete for CYP3A4 (0.4% remaining) and partial (47.1% remaining) for CYP3A5 at 60 min (Study 15570v3; Table 2-1, pg. 18). Recovery was ≥100% for CYP1A2, 2C8, 2C9, 2C19, 2D6, and 2B6. Additionally, HLM studies suggest the Phase I flavin-containing monooxygenases (FMOs) are also involved in lefamulin metabolism (NADPH-dependent stability; incomplete inhibition by ketoconazole). Importantly, lefamulin metabolism or stability was concentration-dependent in a pooled primary human hepatocyte model (lefamulin recovery: 50% at 0.1 mcg/mL and ≥90% at 12.5- and 25 mcg/mL).

P-gP Efflux Saturation Potential

Potential intraenterocyte efflux (B-A direction) saturation of LEF [8 concentrations (0µM to 500µM; limit of tolerability)] was evaluated in a Caco-2 cell system with and without a chemical inhibitor. A plot of the net transport rates suggests a nonlinear dose-response (saturation) at higher LEF concentrations with near complete saturation of its own efflux around 220µM (5% of an estimated initial intestinal luminal concentration [600 mg/250 mL; 4727µM].

On the other hand, LEF transport from the gut lumen across the apical enterocyte membrane was not saturable at concentrations studied.

16.3.1.3. Drug activity

Minimum Inhibitory Concentrations (MIC) of Lefamulin and Its Major Metabolite (BC-8041)

LEF and BC-8041 MICs for *S. aureus*, *S. epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *S. pneumoniae*, *M. catarrhalis*, *K. pneumoniae*, *A. baumannii*, and *H. influenzae* were conducted under standard broth dilution methods. LEF MICs ranged between ≤0.03 mcg/mL to 4 mcg/mL. BC-8041 MICs ranged between 8 mcg/mL to ≥256 mcg/mL. In vitro data demonstrate that BC-8041 antibacterial activity is less potent than lefamulin. Clinical exposure data (average C_{max} 3.5 mcg/mL after a single 150 mg lefamulin IV dose in CABP) suggest minimal BC-8041 antibacterial activity in vivo.

Lung Surfactant Effects on Lefamulin Antibacterial Activity

LEF and daptomycin MICs against 1 to 3 isolates of *S. pneumoniae*, *S. aureus*, *H. influenzae*, and β-lactamase producing *E. coli* were determined with and without increasing concentrations of bovine lung surfactant (0.06% to 4% v/v Survanta™) using a checkerboard broth microdilution method. The fold change in lefamulin MICs (with surfactant compared to without) were always ≤2. For benchmark comparison, the prototypical surfactant labile antibiotic, daptomycin, exhibited fold changes in MICs ≥160.

Intracellular Lefamulin Penetration, Accumulation, Killing

Intracellular concentrations (C_i) and extracellular concentrations (C_e) of LEF were determined in murine macrophage cells (J774). LEF's penetration ratio was approx. 30- to 40-fold (C_i / C_e) and 50-fold after 1 hr and 5 hr incubation, respectively. Antibacterial activity against *Chlamydophila pneumoniae* in HEp-2 cells suggests drug activity is maintained within the cell.

Lefamulin Exposure-Bacterial Kill Response Relationship

The PK-PD indices best correlated with bacterial reduction in a *S. pneumoniae* or *S. aureus* neutropenic murine thigh infection model after a single lefamulin dose were free-drug AUC_{0-24}/MIC ($fAUC_{0-24}$/MIC) and % time to dosing interval for free-drug concentrations to exceed the MIC ($fT > MIC$). Coefficients of determination (R^2) in the *S. pneumoniae* model were 0.80 and 0.68 for $fAUC_{0-24}$/MIC and $fT > MIC$, respectively, while R^2 values in the *S. aureus* model were approximately 0.78 for both indices. A modest postantibiotic effect (PAE ca. 1 hr to 3 hr) observed in these model systems support a $fAUC_{0-24}$/MIC as the best PK-PDindex correlated with antibacterial activity of LEF.

LEF pharmacodynamic (PD) studies using *S. pneumoniae* and *S. aureus* lung infected mice were used to derive the nonclinical PK-PD targets for lefamulin.

Table 105. Observed Free-Drug[a] AUC[b]/MIC Targets in Neutropenic Lung-Infected Mice.

	1-log_{10} CFU Reduction[c]		2-log_{10} CFU Reduction[c]	
	Plasma	**ELF[b]**	**Plasma**	**ELF**
S. pneumoniae (n=5; MIC range: 0.12–0.5 mcg/mL)				
Mean	2.43	24.9	3.91	39.9
Median (min to max)	1.37 (0.67, 6.05)	14.0 (6.84, 61.8)	2.15 (1.06, 10.7)	22.0 (10.8, 109)
>75% percentile	4.39	44.85	7.33	74.75
S. aureus (n=5; MIC range: 0.06–0.5 mcg/mL)				
Mean	2.97	30.4	6.96	71.2
Median (min to max)	2.13 (0.76, 5.94)	21.7 (7.72, 60.7)	6.24 (1.42, 15.3)	63.9 (14.5, 157)
>75% percentile	5.14	52.6	11.85	121.35

[a] value of 20% unbound lefamulin was used based on in vitro and in vivo protein binding assays.
[b] Lung penetration ratio (ELF AUC_{0-24} / free plasma AUC_{0-24}) of 10.2 was determined from a noninfection murine model at two dose levels.
[c] baseline corrected
¥ Mean dose-normalized AUC_{0-24} for plasma of 0.11 and 0.136 hrs·mcg·mL-1/mg·kg-1 were used to translate the dose into lefamulin exposure for *S. pneumoniae* and *S. aureus* respectively and determined from noninfected mice.
¥¥ Max daily subcutaneous doses of 320- and 160-mg/kg were administered in *S. pneumoniae* and *S. aureus* studies respectively. Broadly, dose proportionality (plasma AUC_{0-24}) was shown in murine thigh infection models across lefamulin doses of 10 mg/kg to 160 mg/kg.
Lefamulin's MIC at which ≥90% of strains for the patient population are inhibited (MIC_{90}) against *S. pneumoniae* and *S. aureus* are 0.5 and 0.25 mcg/mL respectively (Phase 3 MIC surveillance data).
¥¥¥¥ No statistical differences were found between 1-log compared to 2-log targets for either bacterial species. Furthermore, no statistical differences between bacterial species for 1-log or 2-log PD targets were found (Mann-Whitney U test).
MIC = minimum inhibitory concentration; ELF = epithelial lining fluid; AUC = area under the concentration-time curve

16.3.2. Clinical Studies

16.3.2.1. ADME

Mass Balance

Study 1013 was a single [^{14}C] lefamulin dose, open label, 1-period, IV and PO cohort study. Each administration route consisted of 5 healthy males 31 to 60 years of age. Oral drugs were

administered with 240 mL of water after an overnight fast of ≥10 hr. IV solution was administered as a 60 min infusion.

- PO: 3x200 mg (early Phase 1 capsules) [ca. 600 mg (~112 µCi); range: 607.7–607.8 mg]
- IV: 150 mg/ 15 mL conc. in 250 mL CBNS [ca. 150 mg (~117 µCi); range: 125–134.6 mg]

Blood, urine, and fecal samples were collected for at least 168 hours postdose to measure total radioactivity (whole blood, plasma, urine, and feces), lefamulin and metabolite BC-8041 concentrations (plasma only) and metabolic profiles (plasma, urine, and feces).

- Mean radioactive recoveries in total excreta (urine+feces), urine, and feces
  - IV: ca. 92.9% (min to max: 89.8% to 96.5%), 15.5%, and 77.3% respectively.
  - PO: ca. 93.9% (min to max: 89% to 97.2%), 5.3%, and 88.5% respectively.
- Circulating plasma lefamulin radioactivity
  - Lefamulin: 76% (IV) and 58% (PO)
  - BC-8041 (major metabolite): 0.8% to 6.7% (IV) and 8.3% to 22.0% (PO)
- Parent/Metabolite profiling and identification in feces
  - Lefamulin PO only
  - Metabolites from mono- and di-hydroxylation, phase II pentose conjugation of mono-hydroxylated metabolites and direct conjugation of lefamulin with pentose.
- Absolute bioavailability was determined to be ca. 27%
- Median terminal half-life
  - Lefamulin: 18 (IV) and 16 (PO) hr
  - BC-8041: 11 (IV) and 17 (PO) hr

Single Ascending Dose

Intravenous

Study 1001: A randomized, placebo-controlled, cross-over, two-cohort, 6-period study to assess safety, tolerability and plasma and urine PK of single ascending doses of lefamulin administered IV (25 mg to 400 mg).

- Cohort 1: Placebo (0.9% saline), 25-, 50-, 100-mg lefamulin dosed one week apart in ascending order; However, placebo treatment was randomly assigned.
- Cohort 2: Placebo (0.9% saline), 200-, 300-, and 400-mg lefamulin dosed one week apart in ascending order; However, placebo treatment was randomly assigned.

The two cohorts consisted of 9 or 8 healthy males respectively 26 to 45 years of age. Plasma lefamulin PK samples were collected up to 48 hrs and Urine lefamulin PK obtained from 24 hrs urine collection after the start of drug infusion.

Table 106. LEF PK Parameters Following Single IV Dose

Dose Level (mg)	$AUC_{0-\infty}$ (ng·hr·mL^{-1})	C_{max} (ng·mL^{-1})	$T_{1/2}$ (hr)	A_e (mcg)
25[a]	1480 (447)	1255 (304)	8.56 (0.81)	1932 (160)
50[a]	3211 (928)	2081 (427)	8.56 (0.87)	4237 (531)
100	4897 (1004)	1953 (306)	9.14 (0.46)	7980 (882)
200	8511 (2333)	2734 (617)	10.92 (1.16)	24482 (2023)
300	12953 (3117)	3776 (652)	11.72 (0.98)	38052 (4127)
400	16880 (3966)	4484 (685)	11.26 (0.79)	54365 (4945)

[a] 30 minute infusion; all others 60 min infusion
[¥] Data presented as arithmetic mean (SD)
AUC = area under the concentration-time curve; SD = standard deviation; IV = intravenous; $T_{1/2}$ = half-life; LEF = lefamulin; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; A_e = cumulative amount of unchanged drug excreted into the urine

Table 107. Summary of Dose Proportionality; Statistical Analyses (One-Way ANOVA)

	$AUC_{0-\infty}$ (ng·hr·mL^{-1})	C_{max} (ng·mL^{-1})	$T_{1/2}$ (hr)
Slope (95% CI)	0.93 (0.87, 0.98)	0.44 (0.38, 0.50)	0.06 (0.04, 0.09)

$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; ANOVA = analysis of variance; geometric mean (95% confidence interval).

Plasma concentration-time profiles follow a biexponential decline after the end of infusion.

A 15- to 30-min lag time was noted between maximum plasma concentrations and maximum QTcF prolongation. The mean change from baseline values in QTcF at T_{max} was 4.9 7.9 21.7 23.8 msec for 100, 200, 300, and 400 mg, respectively.

Study 1005: An open-label, nonrandomized, single-center, single dose, tissue distribution study in 12 healthy males 20 to 48 years of age.

Following single IV 1-hr infusion of 150 mg LEF, plasma and interstitial microdialysate (adipose and muscle) samples were taken predose and up to 24 hr after the start of infusion. Bronchoalveolar lavage fluid samples were also taken up to 8 hr after the start of infusion (1 time point per subject was pooled to calculate an AUC_{ELF}).

Table 108. LEF PK Parameters in Various Body Compartments Following Single IV 1-hr Infusion of 150 mg LEF

Site	AUC_{0-12} (ng·hr·mL^{-1})	C_{max} (ng·mL^{-1})	$T_{1/2}$ (hr)	$f^{d}AUC_{0-8}$ Ratios (Site: Plasma)
Plasma	6022 (1365)	205.1 (90.3)	9.56 (1.92)	
Muscle[a]	678.8 (232.5)	761.9 (393.3)	9.8 (2.03)	0.84
Adipose[b]	675.3 (206.9)	1203 (407)	9.88 (1.95)	0.84
ELF	3871[c] (NC)	932		5.8[c] or 19.3[e]

Data presented as arithmetic mean (SD)
[a] Skeletal tissue
[b] Subcutaneous tissue
[c] AUC_{0-8}
[d] Free drug fraction =0.13; interstitial fluids and ELF were assumed to have a free drug fraction of 1.
efree drug fraction =0.038 based time averaged unbound LEF fraction from NAB-BC-3781-1010 and 1011.
AUC_{0-12h} = area under the concentration-time curve from time 0 to 12 hours after drug administration; SD = standard deviation, ELF = epithelial lining fluid; NC = not calculated; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; LEF = lefamulin; IV = intravenous; PK = pharmacokinetic

Lefamulin concentrations in ELF, as well as muscle and adipose tissue interstitial fluid reached equilibrium fast (within 1 hr after the end of infusion). With regards to microdialysis, five subjects had predose baseline concentrations. Two subjects had concentrations >5% of the $C_{max.}$ With regards to urea quantification in BAL, no data were provided to assess the robustness of analytical method. No BAL cellularity data were provided to assess issues such as bleeding or intracellular lysis.

Oral

Study 1101: A double-blind, randomized, placebo-controlled, 5-period, cross-over study evaluating the safety, tolerability, and PK of lefamulin oral doses (100 mg to 400 mg).

Eight healthy males 24 to 44 years of age received ascending single oral doses at least 5-days apart with ≥8 hr fasting in the first 4 periods and 40 min after consumption of a high fat meal in period 5. Plasma lefamulin PK samples were obtained up to 36 hrs postdose. Urine lefamulin PK samples were obtained from 24 hr void collection postdose. Oral lefamulin capsules (early Phase I) were given with 250 mL water.

Table 109. LEF PK Parameters Following Single Oral Dose

Dose Level (mg)	$AUC_{0-\infty}$ (ng·hr·mL^{-1})	C_{max} (ng·mL^{-1})	$T_{1/2}$ (hr)	A_e (mcg)
100	696.8 (392.9)	205.1 (90.3)	9.56 (1.92)	774.1 (454.3)
200	3210 (1315)	761.9 (393.3)	9.8 (2.03)	3318.3 (981.1)
400 (fasting)	6647 (1593)	1203 (407)	9.88 (1.95)	7340 (1074)
400 (fed)	5150 (1074)	759.6 (233.4)	9.56 (0.99)	7607 (2267)
400 Fed/Fasting ratio	0.78 (0.64, 0.95)	0.64 (0.49, 0.82)		

$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; LEF = lefamulin; PK = pharmacokinetic; A_e = cumulative amount of unchanged drug excreted into the urine; SD = standard deviation, CI = confidence interval

¥Data presented as arithmetic mean (SD) except Fed/Fasting ratio which is presented as geometric mean (90% CI)

Plasma concentration curves demonstrated an early peak with T_{max} at approximately 0.5 hr postdose for all dose levels. Lefamulin postpeak concentrations exhibited a slight shoulder or second peak and declined biexponentially. The binomial peaks were not dose-dependent and, therefore, not supportive of gastric muscle relaxant effects. No humps around other meal times were observed, minimizing potential enterohepatic recirculation concerns. Under the fed condition, a single peak was observed at around 4 hr on average. Additionally, no shoulder or second peak was observed under the fed condition.

Arithmetic mean dose-normalized AUC_{0-inf} was approximately dose-proportional at 200 and 400 mg when LEF was administered in the fasted condition. Arithmetic mean C_{max} was less than dose-proportional across dosing groups.

Study 1104: A randomized, double-blind, placebo-controlled, single ascending, 3-treatment, 2-part crossover safety, tolerability, PK and comparative bioavailability study of lefamulin 500 and 750-mg doses (Phase 1 IR tablet).

The Study enrolled 13 males, 29 to 55 years of age. 12 subjects completed all treatments (1-dropped for personal reasons). Plasma lefamulin and BC-8041 PK samples were obtained up to 36 hrs postdose.

Part 1: Fasting (≥8 hr overnight)

- Treatment 1: Lefamulin 500 mg (2x 250 mg IR tablets)
- Treatment 2: 750 mg (3x 250 mg IR tablets)
- Treatment 3: Placebo

Part 2: Fed (1 hr after a high-fat, high calorie meal)

- Treatment 1: 500 mg (2x 250 mg IR tablets)

Table 110. PK Parameters of LEF Following a Single Oral Administration

Dose Level (mg)	$AUC_{0-\infty}$ (ng·hr·mL^{-1})	C_{max} (ng·mL^{-1})	$T_{1/2}$ (hr)
500	5235 (2088)	1142 (544)	8.12 (0.92)
750	8561 (2738)	1396 (381)	7.93 (0.85)
500 (fed)	3732 (1003)	682 (216)	7.87 (1.16)
500 fed/fasting	0.78 (0.69, 0.88)	0.63 (0.52, 0.76)	0.97 (0.93, 1.01)

LEF = lefamulin; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; PK = pharmacokinetic

Table 111. PK Parameters of BC-8041 Following a Single Oral Administration

Dose Level (mg)	$AUC_{0-\infty}$ (ng·hr·mL^{-1})	C_{max} (ng·mL^{-1})	$T_{1/2}$ (hr)
500	978 (412)	197 (81)	7.06 (0.79)
750	1499 (531)	211 (69)	7.18 (0.66)
500 (fed)	724 (277)	119 (57)	7.08 (1.25)
500 Fed/Fasting	0.79 (0.68, 0.92)	0.60 (0.48, 0.74)	1.0 (0.96, 1.04)

LEF = lefamulin; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; PK = pharmacokinetic
¥Data presented as arithmetic mean (SD) except Fed/Fasting ratio which is presented as geometric mean (90% CI)

Multiple Ascending Dose

Intravenous

Study 1007: A randomized, double-blind, placebo-controlled, single center, two-part crossover, safety, tolerability, and PK study with two different formulations of lefamulin under single and repeat ascending IV doses (150 mg to 400 mg).

A total of six male subjects were enrolled in Part A and a total of 24 male subjects were enrolled in Part B.

- Part A:
  - — Cohort 1: single 400 mg lefamulin IV dose infused over 1 hr in citrate buffered saline (CBNS), then normal saline (NS), or NS alone (placebo).
- Part B:
  - — Cohort 1: Repeat 150 mg lefamulin infused over 1 hr in CBNS q12hr for 5 days
  - — Cohort 2: Repeat 200 mg lefamulin infused over 1 hr in CBNS q12hr for 5 days

There was at least a 5-day washout period from the start of study drug infusion between each Part/Period.

Following a single IV dose of 400 mg lefamulin (CBNS), the arithmetic mean (SD) $AUC_{0\text{-}inf}$, $AUC_{0\text{-}12}$, C_{max}, and $T_{1/2}$ were 15,252 (1623) $ng{\cdot}hr{\cdot}mL^{-1}$, 11046 (963) $ng{\cdot}hr{\cdot}mL^{-1}$, 3,952 (390) $ng{\cdot}mL^{-1}$, and 11.8 (1.49) hr, respectively. Nearly identical values were observed with the normal saline formulation.

Following repeat 150 mg lefamulin (CBNS), the arithmetic mean (SD) $AUC_{0\text{-}12}$, C_{max}, and $T_{1/2}$ were 7342 (1087) $ng{\cdot}hr{\cdot}mL^{-1}$, 2681 (324) $ng{\cdot}mL^{-1}$, and 13.8 (1.13) hr, respectively. Following repeat 200 mg lefamulin (CBNS), the arithmetic mean (SD) $AUC_{0\text{-}12}$, C_{max}, and $T_{1/2}$ were 9202 (1701) $ng{\cdot}hr{\cdot}mL^{-1}$, 3027 (437) $ng{\cdot}mL^{-1}$, and 13.1 (1.07) hr, respectively.

Accumulation, as assessed by the ratio of $AUC_{0\text{-}12,\ last\ dose}/AUC_{0\text{-}12,\ first\ dose}$, was approximately 1.4 and 1.3 for the 150- and 200-mg doses, respectively. Steady-state was reached after the second dose. Statistical analyses suggested that the increases in $AUC_{0\text{-}12}$ and C_{max} were subproportional to dose.

Study 1009: A randomized, double-blind, placebo-controlled, single center, parallel group, safety, tolerability, and PK study with subjects receiving either placebo or two different formulations of lefamulin under single and repeat 150 mg IV.

A total of 60 subjects (35 females) were enrolled. Plasma lefamulin PK samples were obtained up to 12 hrs postdose (Day 1 and Day 8).

- Group 1: 150 mg lefamulin IV q12 hr infused over 1 hr in NS (n=25) for 7.5 days
- Group 2: 150 mg lefamulin IV q12 hr infused over 1 hr in CBNS (n=25) for 7.5 days
- Group 3: NS IV q12 hr infused over 1 hr in saline (n=10)

Pain and erythema occurred more often and with higher intensity when given with NS compared with CBNS. The diluent for XENLETA injection is CBNS to reduce the incidence of administration-site reactions.

Table 112. LEF PK Parameters Following Repeat IV Administration of Lefamulin in CBNS

	AUC_{0-12} (ng·hr·mL^{-1})		C_{max} (ng·mL^{-1})	
Dose (mg)	**Day 1**	**Day 8**	**Day 1**	**Day 8**
150	5078.5 (1339)	6929.1 (1972.1)	2259.3 (484.9)	2383.9 (568.0)

LEF = lefamulin; AUC_{0-12} = area under the concentration-time curve from time 0 to 12 hours after drug administration; C_{max} = maximum plasma concentration of drug; PK = pharmacokinetic; CBNS = citrate buffered normal saline; IV = intravenous; SD = standard deviation
¥Data presented as arithmetic mean (SD)
¥¥Lefamulin in NS demonstrated near identical PK exposures (data not shown).

Accumulation ratio of AUC and C_{max} was 1.4- and 1.1-fold, respectively (for both formulations).

Oral

Study 1102 was a randomized, double-blind, placebo-controlled, repeat oral dose, parallel 3-treatment, safety, tolerability, and PK study of 200 mg to 600 mg lefamulin (Phase 1 capsules).

The study enrolled a total of 24 males, 20 to 45 years of age, with 8 per cohort (2 placebo). Oral medication was given with 250 mL water. The morning dose after an overnight fast of at least 8 hr with breakfast served 1-hr postdose. The evening dose was given 2 hr after dinner.

- Treatment 1: Lefamulin 200 mg (1x 200 mg capsule) PO BID or placebo for 9.5 days.
- Treatment 2: Lefamulin 400 mg (2x 200 mg capsule) PO BID or placebo for 9.5 days.
- Treatment 3: Lefamulin 600 mg (3x 200 mg capsule) PO BID or placebo for 9.5 days.

Table 113. LEF PK Parameters Following Repeat Oral Administration

	AUC_{0-12} (ng·hr·mL^{-1})		C_{max} (ng·mL^{-1})	
Dose (mg)	**Day 1**	**Day 10**	**Day 1**	**Day 10**
200	1605.2 (791.6)	2975.4 (1100.3)	542.7 (218.9)	781.0 (216.8)
400	NC[a]	5848.9 (835.0)	NC[a]	1184.8 (234.6)
600	6519.6 (2145.6)	11939.5 (4044.0)	1552.7 (232.7)	2081.2 (185.2)

LEF = lefamulin; AUC_{0-12} = area under the concentration-time curve from time 0 to 12 hours after drug administration; C_{max} = maximum plasma concentration of drug; PK = pharmacokinetic; SD = standard deviation
[a]Human error in dosing. Subjects received a single dose of 200 mg instead of 400 mg.
¥Data presented as arithmetic mean (SD)

Accumulation as assessed by AUC ($AUC_{0-12,\ last\ dose}$ / $AUC_{0-12,\ first\ dose}$) and C_{max} were similar across dose levels and approximately 1.8- and 1.3-fold, respectively, for 600 mg PO BID. Urine PK was consistent with other studies.

Effect of Food Intake on Bioavailability of Lefamulin Tablets

Study 1106: A randomized, open-label, 3-period, 3-treatment, crossover, comparative fed and fasted bioavailability study of a 600 mg lefamulin (Phase 1 IR tablet) dose.

The study enrolled 13 males, 22 to 54 years of age.

- Treatment A: Fasted state with no breakfast.
- Treatment B: Fasted state with breakfast 1 hr postdose.
- Treatment C: Fed state with dosing 1 hr postbreakfast

The washout period between drug administrations was 4 days. The total kcal with fat, carbohydrate, and protein content were not specified.

Table 114. Effect of Food and Timing of Meal on LEF PK Following Oral Administration; Ratio (90% CI) (N=12)

Parameter	B/A (%)	C/A (%)
$AUC_{0-\infty}$	0.91 (0.82–0.99)	0.75 (0.68–0.82)
C_{max}	0.91 (0.74–1.10)	0.63 (0.52–0.77)

LEF = lefamulin; $AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; PK = pharmacokinetic; CI = confidence interval
Ratio = adjusted geometric means for treatment X/ treatment Y; *p<0.05

T_{max} (median; [range]): Treatment A – 1.0 [0.3–4.0] hr; Treatment B – 0.75 [0.3–3.0] hr; Treatment C – 4.5 [2.0–6.0] hr.

There does not appear to be a food-effect when given 1-hr before a meal.

Study 1107: An open-label, randomized, single dose, 4-period, 4-treatment, crossover, comparative fed and fasted bioavailability study.

The study enrolled 12 males and 8 females, 22 to 55 years of age.

- Treatment A: 1 x 600 mg lefamulin (Phase 3 IR tablets) PO after overnight fast ≥8 hr.
- Treatment B: 3 x 200 mg lefamulin capsules PO after overnight fast ≥8 hr.
- Treatment C: 150 mg lefamulin diluted in 250 mL CBNS infused over 1 hr.
- Treatment D: 1 x 600 mg lefamulin (Phase 3 IR tablets) PO 1-hr after a high-fat, high-calorie breakfast

The washout period between drug administrations was 4 days. The total kcal with fat, carbohydrate, and protein content were not specified.

Table 115. Effect of Food on Oral Lefamulin Relative Bioavailability; Geomean Ratio (90% CI) (n=20)

Parameter	Oral Fed/Oral Fasted (%)	Oral Fasted/IV (%)	Oral Fed/IV (%)
$AUC_{0\text{-}inf}$	0.82 (0.75–0.88)	1.03 (0.95–1.13)	0.84 (0.77–0.92)
$AUC_{0\text{-}12}$	0.72 (0.66–0.80)	0.99 (0.92–1.07)	0.72 (0.65–0.79)
C_{max}	0.77 (0.68–0.88)	0.49 (0.45–0.54)	0.38 (0.34–0.42)

$AUC_{0\text{-}\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; $AUC_{0\text{-}12}$ = area under the concentration-time curve from time 0 to 12 hours after drug administration; C_{max} = maximum plasma concentration of drug; CI = confidence interval; IV = intravenous
Geomean = geometric means; relative = not dose corrected.

Table 116. Effect of Food on Oral Lefamulin Absolute Bioavailability; Ratio (90% CI) (n=20)

Parameter	Fasted (%)	Fed (%)
$AUC_{0\text{-}inf}$	0.26 (0.24–0.28)	0.22 (0.19–0.23)
$AUC_{0\text{-}12}$	0.25 (0.23–0.28)	0.18 (0.16–0.20)
C_{max}	0.12 (0.11–0.13)	0.09 (0.08–0.11)

$AUC_{0\text{-}\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; $AUC_{0\text{-}12}$ = area under the concentration-time curve from time 0 to 12 hours after drug administration; C_{max} = maximum plasma concentration of drug; CI = confidence interval
Ratio = adjusted geometric means for Treatment A/ treatment C.; absolute = dose corrected.

Food appears to affect the oral bioavailability rate of lefamulin which results in a lower extent of oral bioavailability if given every 12 hours compared to a one time dose.

Adverse events were reduced when LEF IR tablet was taken under fed compared to fasted conditions (5% versus 45%). Symptoms were nausea and abdominal pain.

16.3.2.2. Drug-drug interactions

Effect of Intravenous Lefamulin on Midazolam Exposure

Study 1004 was a single-center, randomized, cross-over study in 16 healthy subjects (8 males) 25 to 52 years of age. Lefamulin injection was administered as a 500 mL infusion over 120 min.

- Session 1: A single 2 mg oral midazolam dose alone
- Session 2: A single IV dose of 150 mg lefamulin at 1 hr after administration of a single 2 mg oral midazolam dose.

Subjects were fasted for at least 8 hours before study drug administration. Fasting continued ca. 4 hr after the start of the lefamulin infusion (3 hr post midazolam). The washout period between sessions was at least 2 days. Midazolam plasma PK samples were collected up to 24 hr.

Table 117. Midazolam (MID) PK Parameters After a Single Oral Administration of 2 mg MID With and Without 150 mg LEF Injection

Parameter	LEF+MID (T) Mean (SD)	MID Alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0-\infty}$ (ng·hr·mL^{-1})	35.99 (21.87)[a]	31.23 (18.47)[b]	1.17 (0.82–1.67)
C_{max} (ng·mL^{-1})	10.84 (4.09)	10.39 (3.19)	1.03 (0.82–1.30)
$T_{1/2}$ (hr)	5.41 (2.30)[a]	4.90 (2.76)[b]	1.20 (0.82–1.75)

Geo = geometric; arithmetic mean unless stated otherwise.
[a]n =15; R2<0.8 or unable to define terminal slope (3 or more points)
[b]n =15; R2<0.8 or unable to define terminal slope (3 or more points)
$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; CI = confidence interval; $T_{1/2}$ = half-life; LEF = lefamulin; MID = midazolam; SD = standard deviation; PK = pharmacokinetic

Effect of Oral Lefamulin on Midazolam Exposure

Study 1110 was an open-label, multiple-dose, fixed-sequence, 2-treatment cross-over study in healthy subjects (2-females) 22 to 55 years of age. Fourteen subjects were enrolled and 13 completed the study.

- Days 1 and 5: single 2 mg midazolam PO dose.
- Days 2–5: 600 mg lefamulin (Phase 3 IR tablets) PO q12 hr

Lefamulin and midazolam were coadministered in the morning of Day 5. Subjects were fasted for at least 10 hours before morning dosing on Days 1 and 5. Lefamulin tablets were administered at least 1 hour before and 2 hours after a meal on Days 2 to 4 and evening of Day 5. Midazolam plasma PK samples were collected up to 24 hr on Days 1 and 5.

Table 118. Midazolam PK Parameters After a Single Oral Administration of 2 mg With and Without 600 mg LEF Tablet

Parameter	LEF+MID (T) Mean (SD)	MID alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0-\infty}$ (ng*hr/mL)	119.3 (47.7)	37.56 (14.22)	3.23 (2.90–3.61)
C_{max} (ng/mL)	24.72 (5.50)	12.36 (2.96)	2.03 (1.84–2.23)

$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; MID = midazolam; LEF = lefamulin
Geo = geometric; Arithmetic mean unless stated otherwise.

Study 1111, was an open-label, randomized, 3-sequence, 4-period, 2-treatment, cross-over study in 18 healthy subjects (5-females) 20 to 53 years of age.

Midazolam plasma PK samples collected up to 24 hr.

- Day 1: Single 2 mg midazolam PO dose (Treatment A)
- Day 2: 600 mg lefamulin (Phase 3 IR tablet) PO dosing q12 hr
- Day 3: 600 mg lefamulin (Phase 3 IR tablet) PO dosing q12 hr
- Day 4: 600 mg lefamulin (Phase 3 IR tablet) PO dosing q12 hr
- Day 5: Co-administration of a single 2 mg midazolam PO dose and 600 mg lefamulin PO dose then 600 mg lefamulin PO 12 hrs later (Treatment B)
- Day 6: 600 mg lefamulin PO dosing q12 hr
- Day 7: A single 2 mg midazolam PO dose administered 2 hr after a 600 mg lefamulin PO dose then 600 mg lefamulin PO 12 hrs after the last lefamulin dose (Treatment C)
- Day 8: 600 mg lefamulin PO dosing q12 hr
- Day 9: A single 2 mg midazolam PO dose administered 4 hr after a 600 mg lefamulin PO dose then 600 mg lefamulin PO 12 hrs after the last lefamulin dose (Treatment D)
- Day 10: 600 mg lefamulin PO dosing q12 hr

*All patients received each treatment. Treatment B, C, and D sequences were randomized.

Table 119. Midazolam (MID) PK Parameters Following Single Oral Administration of 2 mg With or Without 600 mg LEF Tablet

Parameter	T/R (1) GeoMean Ratio (90% CI)	T/R (2) GeoMean Ratio (90% CI)	T/R (3) GeoMean Ratio (90% CI)
$AUC_{0\text{-}inf}$ (ng*hr/mL)	2.74 (2.54–2.97)[a]	3.02 (2.79–3.26)[a]	2.74 (2.53–2.96)[a]
C_{max} (ng/mL)	1.76 (1.57–1.97)[b]	2.21 (2.79–3.26)	1.92 (1.72–2.15)[b]

(1) = MID+LEF/MID alone; (2) = MID 2hr post LEF/MID alone; (3) MID 4 hr post LEF/MID alone
n=16-18 ; exclusion due to R^2<0.8 or unable to define terminal slope (3 or more points). Two exclusions due to the same subject having a predose MID >5% of C_{max} (Treatment B, D)
$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; Geo = geometric;

The increase of midazolam exposure due to oral lefamulin holds even when midazolam was administered up to 4 hours after administration of oral lefamulin.

Effect of Oral Lefamulin on Digoxin Exposure

Study 1109 was an open-label, multiple-dose, fixed-sequence, 2-treatment cross-over study in 19 healthy subjects (1-female) 20 to 52 years of age.

- Days1 and 8: single 0.5 mg digoxin PO dose
- Days 5–10: 600 mg lefamulin (Phase 3 IR tablets) PO q12 hr

Subjects were fasted for at least 10 hours before morning dosing on Days 1 and 8; with fasting continued for ca. 4 hours postdose. Lefamulin tablets was administered at least 1 hr before and 2 hr after a meal. Digoxin plasma PK samples were collected up to 96 hr on Days 1 and 8.

Table 120. Digoxin (DIG) PK Parameters Following Single Oral Administration of 0.5 mg DIG With or Without 600 mg LEF Tablet

Parameter	DIG+LEF (T) Mean (SD)	DIG alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0\text{-}inf}$ (ng*hr/mL)	38.59 (11.4)	34.3 (8.42)	1.11 (0.98–1.27)
C_{max} (ng/mL)	2.18 (0.68)	2.07 (0.70)	1.05 (0.88–1.26)
$T_{1/2}$ (hr)	52.18 (12.24)	37.41 (5.25)	NR

$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; Geo = geometric; NR = not reported; Arithmetic mean unless stated otherwise.

Interactions Between Intravenous Lefamulin and Ketoconazole

Study 1006 was a single-center, randomized, double-blind, cross-over study in 12 healthy males 25 to 53 years of age. Lefamulin and ketoconazole plasma PK samples collected up to 24 and 12 hr, respectively.

- Days1–2: single IV dose of 150 mg lefamulin or placebo
- Days 4–7: 200 mg ketoconazole BID
- Days 7: single IV dose of 150 mg lefamulin at 1 hr post morning ketoconazole dose.

Table 121. LEF PK Parameters Following a Single 150 mg LEF Injection With or Without Multiple Oral Administration of 200 mg KET BID

Parameter	LEF+KET (T) Mean (SD)	LEF alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0\text{-}inf}$ (ng*hr/mL)	9934 (1791)	7561 (821)	1.30 (1.16–1.45)
C_{max} (ng/mL)	2708 (383)	2551 (307)	1.06 (0.96–1.16)
$T_{1/2}$ (hr)	8.91 (1.74)	7.91 (0.80)	1.11 (1.0–1.24)

$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; KET = ketoconazole; BID = twice a day; Geo = geometric; Arithmetic mean unless stated otherwise

Table 122. Ketoconazole (KET) PK Parameters Following Multiple Oral Administration of 200 mg BID With or Without Single 150 mg LEF Injection

Parameter	KET+LEF (T) Mean (SD)	KET alone (R) Mean (SD)	T/RGeoMean Ratio (90% CI)
$AUC_{0\text{-}inf}$ (ng*hr/mL)	22783 (9775)	24204 (12171)	0.96 (0.67–1.37)
C_{max} (ng/mL)	4065 (1809)	4356 (1982)	0.93 (0.65–1.32)
$T_{1/2}$ (hr)	2.89 (0.74)	2.95 (0.82)	0.98 (0.82–1.19)

$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; KET = ketoconazole; BID = twice a day; Geo = geometric; Arithmetic mean unless stated otherwise

Interactions between Oral Lefamulin and Ketoconazole

Study 1103 was a single-center, open-label study in healthy males aged 21 to 54 years of age. A total of 17 males entered the study, with 16 males completing all assessments. Lefamulin, BC-8041, and ketoconazole plasma PK samples were collected to 24-, 24-and 12 hr, respectively.

- Days 1 and 6: single morning dose of 400 mg lefamulin (2x200 mg Phase 1 capsules). On Day 6, lefamulin and ketoconazole were administered together.
- Days 3–6: 200 mg ketoconazole BID

Table 123 LEF PK Parameters Following a Single Oral Dose of 400 mg LEF With or Without Multiple Oral Administration of 200 mg KET BID

Parameter	LEF+KET (T) Mean (SD)	LEF Alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0\text{-}inf}$ (ng*hr/mL)	10948.5 (25223.1)	4182.3 (1184.8)[a]	2.65 (2.43–2.90)
C_{max} (ng/mL)	1548.6 (278.3)	1037.5 (469.2)	1.58 (1.38–1.81)
$T_{1/2}$ (hr)	6.59 (0.76)	6.05 (0.51)[a]	1.06 (1.0–1.1)

$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; KET = ketoconazole; BID = twice a day; Geo = geometric; Arithmetic mean unless stated otherwise
[a]n =15; R2<0.8 or unable to define terminal slope (3 or more points)

Table 124. BC-8041 PK Parameters Following a Single Oral Dose of 400 mg LEF With or Without Multiple Oral Administration of 200 mg KET BID

Parameter	LEF+KET (T) Mean (SD)	LEF Alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0\text{-}\infty}$ (ng*hr/mL)	2011.6 (1043.7)[a]	895.4 (316.7)[a]	2.13 (1.95–2.34)
C_{max} (ng/mL)	196.2 (72.4)	170.7 (55.4)	1.12 (1.02–1.24)
$T_{1/2}$ (hr)	8.05 (1.81)	5.38 (0.67)[a]	1.45 (1.35–1.56)

$AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; KET = ketoconazole; BID = twice a day; Geo = geometric; Arithmetic mean unless stated otherwise
[a]n =14; R2<0.8 or unable to define terminal slope (3 or more points)

Table 125. KET PK Parameters Following Multiple Administration Of 200 mg KET BID With or Without 400 mg Oral LEF

Parameter	KET+LEF (T) Mean (SD)	KET Alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
$AUC_{0-\infty}$ (ng*hr/mL)	28041.3 (8869.0)	23056.7 (9978.7)	1.25 (1.09–1.43)
C_{max} (ng/mL)	4733.2 (1187.4)	4101.0 (1371.1)	1.17 (1.0–1.37)
$T_{1/2}$ (hr)	3.25 (1.48)	2.79 (1.02)	1.15 (1.10–1.2)

AUC_{0-inf} = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; KET = ketoconazole; BID = twice a day; Geo = geometric; Arithmetic mean unless stated otherwise

Effect of Rifampin on Oral and IV Lefamulin

Study 1108 was an open-label, fixed-sequence, 2-parellel part, 2-period, 2-treatment study in healthy subjects 19 to 54 year of age. A total of 28 subjects (3-female) participated. There was a 2-day washout between Period 1 and Period 2. Lefamulin and BC-8041 plasma PK samples were collected to 36 hr.

Part1:

- Treatment A: Single 600 mg lefamulin (Phase 3 tablet) PO on Day 1 of Period 1
- Treatment B: Multiple doses 600 mg rifampin (2x300 mg caps) QD on Days 1 to 12 of Period 2 with a single 600 mg lefamulin PO coadministered on Day 11 of Period 2.

Part2:

- Treatment A: Single 150 mg IV lefamulin infused over 60 min on Day 1 of Period 1
- Treatment B: Multiple doses 600 mg rifampin (2x300 mg caps) QD on Days 1 to 12 of Period 2 with a single 150 mg IV lefamulin infused over 60 min coadministered on Day 11 of Period 2.

Table 126. LEF PK Parameters Following a Single 600 mg LEF Tablet With or Without Multiple Oral Administration of 600 mg Rifampin (RIF) QD

Study Drug/ Parameter	RIF+LEF (T) Mean (SD)	LEF alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
Lefamulin			
$AUC_{0-\infty}$ (ng*hr/mL)	3037 (927.82)	10850 (2565.5)	0.28 (0.25–0.31)
C_{max} (ng/mL)	705.5 (204.96)	1686 (585.92)	0.43 (0.37–0.51)
$T_{1/2}$ (hr)	7.71 (0.62)	8.24 (0.0.92)	NR
BC-8041			
$AUC_{0-\infty}$ (ng*hr/mL)	1304 (550.67)	2033 (700.56)	0.62 (0.54–0.72)
C_{max} (ng/mL)	309.3 (117.66)	276.2 (103.28)	1.12 (0.93–1.34)
$T_{1/2}$ (hr)	6.25 (1.42)	8.23 (0.80)	NR

AUC_{0-inf} = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; QD = once a day; Geo = geometric; NR = not reported; Arithmetic mean unless stated otherwise

Table 127. LEF PK Parameters Following a Single 150 mg LEF Injection With or Without Multiple Oral Administration of 600 mg Rifampin QD

Study Drug/ Parameter	RIF+LEF (T) Mean (SD)	LEF alone (R) Mean (SD)	T/R GeoMean Ratio (90% CI)
Lefamulin			
$AUC_{0-\infty}$ (ng*hr/mL)	6581 (888.59)	9067 (1397.7)	0.73 (0.70–0.76)
C_{max} (ng/mL)	2433 (340.10)	2656 (381.80)	0.92 (0.87–0.97)
$T_{1/2}$ (hr)	8.23 (0.78)	8.62 (0.73)	NR
BC-8041			
$AUC_{0-\infty}$ (ng*hr/mL)	44.16 (10.61	367.8 (134.54)	0.12 (0.11–0.14)
C_{max} (ng/mL)	5.85 (1.30)	40.77 (17.10)	0.12 (0.13–0.17)
$T_{1/2}$ (hr)	5.47 (0.83)	9.86 (1.55)	NR

AUC_{0-inf} = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; PK = pharmacokinetic; SD = standard deviation; LEF = lefamulin; QD = once a day; Geo = geometric; NR = not reported; Arithmetic mean unless stated otherwise

Lefamulin PO T_{max} (median; [range]): Lefamulin alone – 2.0 [0.33, 4.0] hr; Lefamulin + Rifampin – 1.0 [0.5, 5.0] hr after single doses. The BC-8041 T_{max} values after lefamulin without or with rifampin are similar, respectively.

16.3.2.3. Intrinsic factors

Renal Impairment

Study 1011 was a nonrandomized, multicenter single-dose (150 mg IV infused over 1 hr) study. In this study, the lefamulin and BC-8041 PK in subjects with severe renal impairment (eGFR $\leq$ 30 mL/min/1.73 m^2 not on dialysis: n=8; MDRD equation) and subjects on hemodialysis (HD: n=8) were compared with age-, gender-, and weight-matched subjects with normal renal function (n=7). Plasma, urine, and dialysate samples were collected up to 36 hr for LEF and BC-8041 PK.

Table 128. Lefamulin and BC-8041 PK Parameters [Arithmetic Mean (SD)] After Single Dose Administration in Subjects With Different Renal Function

		Hemodialysis		Normal Renal
Study Drug/Parameter	**Severe Impairment**	**On Dialysis**	**Off Dialysis**	**Function**
Lefamulin				
$AUC_{0-\infty}$ ($hr \cdot ng \cdot mL^{-1}$)	12262 (7798)[a]	8955 (3103)	8606 (2815)	9004 (2591)
C_{max} ($ng \cdot mL^{-1}$)	3138 (990)	3341 (916)	2893 (653)	3182 (697)
CL ($L \cdot hr^{-1}$)	15.7 (7.15)	18.6 (6.40)	19.0 (5.60)	17.9 (5.37)
$T_{1/2}$ (hr)	9.40 (0.935)	9.27 (1.42)	9.27 (1.42)	10.1 (1.85)
A_e (mg)	3.90 (1.57)	1.67 (1.95)[b]	1.86 (2.23)[b]	11.1 (5.02)
BC-8041				
$AUC_{0-\infty}$ ($hrs \cdot ng \cdot mL^{-1}$)	695 (448)	734 (716)	643 (408)	413 (134)
C_{max} ($ng \cdot mL^{-1}$)	56.1 (15.7)	60.0 (40.0)	51.2 (21.9)	48.7 (12.8)
$T_{1/2}$ (hr)	11.4 (2.17)	15.1 (4.38)	12.8 (1.97)	13.5 (4.5)
A_e (mg)	0.162 (0.104)	0.0965 (0.115)[b]	0.0809 (0.0905)[b]	0.417 (0.171)

$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plas concentration of drug; CL = total body clearance of the drug from plasma; $T_{1/2}$ = half-life; A_e = cumulative amount of unchanged drug excreted into the urine; PK = pharmacokinetic; SD = standard deviation; "on dialysis"= dialysis started within 1 hr postinfusion dose; "off dialysis" = no dialysis day. On and Off periods were separated by ≥7 days.
[a]1 outlier AUC >2-fold mean AUC
[b]n =2

Table 129. Statistical Comparisons of Lefamulin and BC-8041 Exposure Measures

	Severe Renal/Healthy	Dialysis On/OFF
Study Drug/Parameter	**Geo Mean Ratio (90% CI)**	**Geo Mean Ratio (90% CI)**
Lefamulin		
$AUC_{0-\infty}$	1.23 (0.82, 1.84)[a]	1.03 (0.96, 1.10)
C_{max}	0.96 (0.73, 1.24)	1.14 (0.96, 1.35)
BC-8041		
$AUC_{0-\infty}$	1.48 (0.94, 2.33)	1.02 (0.89, 1.17)
C_{max}	1.14 (0.88, 1.47)	1.08 (0.91, 1.28)

[a]1 outlier AUC >2-fold mean AUC. Excluding outlier Lefamulin AUC was 106.24 (77.44, 145.73) and BC-8041 AUC was 128.26 (87.21, 188.63)
$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; CI = confidence interval

Lefamulin protein binding was comparable across all groups with mean bound drug greater than 94%.

Lefamulin concentrations in 35/38 dialysate samples were below the lower limit of quantification (LLOQ <10 ng/mL). The highest concentration was 12.5 ng/mL.

Lefamulin and BC-8041 concentrations did not change in subjects with severe renal impairment or on dialysis versus subjects with normal renal function. Lefamulin and BC-8041 removal by dialysis filtration appears to be negligible.

Gender and Age

Study 1003 was a randomized, placebo-controlled, two-treatment, two-period, two-group cross-over study in healthy subjects ≥65 years of age (n=12) and healthy subjects 18 to 55 years

of age (n=26). The total age range was 24 to 78 with 18 males and 20 females. A single 150 mg lefamulin dose was administered IV by a 1 hr infusion.

Table 130. Summary of LEF PK After Single Dose Administration

Parameter	18–55 Years of Age Geo Mean (CV%)	≥65 Years of Age Geo Mean (CV%)	Male Geo Mean (CV%)	Female Geo Mean (CV%)
$AUC_{0\text{-}inf}$ ($hr \cdot ng \cdot mL^{-1}$)	7660 (24.5)	7500 (33.6)	7250 (26.5)	7950 (27.7)
C_{max} ($ng \cdot mL^{-1}$)	2590 (23.9)	2440 (22.8)	2450 (16.8)	2620 (28.2)
$T_{1/2}$ (hr)	8.88 (12.2)	10.4 (15.8)	9.17 (15.9)	9.47 (14.7)
V_{ss} (L)	140 (22.3)	166 (26.2)	155 (24.3)	141 (24.6)
A_e (mg)	10.7 (43.1)	8.70 (60.6)	11.2 (3.69)	10.4 (3.03)

$AUC_{0\text{-}\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; V_{ss} = Volume of distribution at steady state; A_e = cumulative amount of unchanged drug excreted into the urine; CV = coefficient of variation; LEF = lefamulin; PK = pharmacokinetic; Geo = geometric

Table 131. Statistical Comparisons of LEF Exposure Measures by Age and Gender

Parameter	Age ≥65 Years/18–55 Years Geo Mean Ratio (90% CI)	Gender Female/Male Geo Mean Ratio (90% CI)
Clearance	1.02 (0.87, 1.20)	0.91 (0.79, 1.05)
V_{ss}	1.18 (1.03, 1.35)	0.91 (0.91, 1.04)

V_{ss} = volume of distribution at steady state; CI = confidence interval; LEF = lefamulin

Total body weight, height, and BMI had no/minimal influence on lefamulin clearance. There is no clinically meaningful difference in lefamulin plasma exposure measures (<10%) between males and females. The clinical relevance of the lefamulin exposure change by gender is not considered to be significant. No age-dependent effects on PK parameters or plasma exposure measures were observed.

Hepatic Impairment

Study 1010 was a nonrandomized, multicenter single-dose study. Eight subjects with moderate hepatic impairment (Child-Pugh 7 to 9) and eight subjects with severe hepatic insufficiency (Child-Pugh ≥10) were enrolled together with the age-, gender-, and weight-matched subjects with normal hepatic function (n=11). Subjects received a single 150 mg lefamulin dose given IV as a 1 hr infusion. Plasma and urine lefamulin and BC-8041 PK samples were collected up to 48 hr after the start of infusion. Plasma protein binding of lefamulin was determined from plasma samples collected at 1, 3, and 8 hr after the start of infusion.

Table 132. Lefamulin and BC-8041 PK Parameters [Arithmetic Mean (SD)] After Single Dose Administration in Subjects With Different Hepatic Function

Parameter	Severe Impairment	Moderate Impairment	Normal Function
Lefamulin			
$AUC_{0\text{-}\infty}$ ($hr \cdot ng \cdot mL^{-1}$)	8938 (1640)	8233 (2286)	7615 (1554)
C_{max} ($ng \cdot mL$)	1468 (328)	1746 (524)	2463 (403)
$T_{1/2}$ (hr)	17.5 (3.35)	13.6 (3.06)	11.5 (1.75)
A_e (mg)	24.5 (6.88)	21.0 (6.45)	9.74 (2.47)

Parameter	Severe Impairment	Moderate Impairment	Normal Function
BC-8041			
$AUC_{0-\infty}$ ($hr \cdot ng \cdot mL^{-1}$)	647 (441)	499 (463)	303 (116)
C_{max} (ng·mL)	20.4 (12.3)	37.9 (41.2)	33.3 (9.69)
$T_{1/2}$ (hr)	33.8 (14.8)	24.4 (20.0)	14.4 (4.51)
A_e (mg)	0.968 (0.646)	0.691 (0.441)	0.326 (0.099

$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; A_e = cumulative amount of unchanged drug excreted into the urine; SD = standard deviation; PK = pharmacokinetic

Table 133. Statistical Comparisons of LEF and BC-8041 Exposure Measures

Parameter	Moderate/Healthy Control Geo Mean Ratio (90% CI)	Severe/Healthy Geo Mean Ratio (90% CI)
Lefamulin		
$AUC_{0-\infty}$	1.06 (0.88, 1.28)	1.18 (0.98, 1.42)
C_{max}	0.69 (0.58, 0.82)	0.59 (0.50, 0.70)
$T_{1/2}$ (hr)	1.16 (1.0, 1.36)	1.51 (1.29, 1.76)
BC-8041		
$AUC_{0-\infty}$	1.43 (0.90, 2.25)	1.92 (1.22, 3.04)
C_{max}	0.75 (0.44, 1.27)	0.55 (0.33, 0.94)
$T_{1/2}$ (hr)	1.47 (0.97, 2.08)	2.29 (1.57, 3.36)

$AUC_{0-\infty}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; $T_{1/2}$ = half-life; CI = confidence interval; LEF = lefamulin

Table 134. LEF Plasma Protein Binding as a Function of Time After the Beginning of Infusion

Time (h)	Norm (CV%) N=11	Mod (CV%) N=8	Sev (CV%) N=8
1	94.8 (1.4)	89.2 (3.6)	86.5 (3.8)
3	97.0 (0.6)	91.8 (3.1)	89.6 (2.5)
8	97.1 (0.6)	92.8 (3.1)	90.8 (3.1)

The arithmetic mean and coefficient of variation expressed as a percent (%CV) for subjects with normal hepatic function (Norm) and hepatic impairment (Mod = Child-Pugh B, Sev = Child-Pugh C).
CV = coefficient of variation; LEF = lefamulin
Source: Study Report NAB-BC-3781-1010-pharmacokinetic, Table 9, pg 36.

Table 135. Lefamulin Exposure Across Hepatic Stages

Parameter	Normal	Moderate	Severe		
Single IV dose (mg)	150	150	150		
Total (Bound + Unbound) LEF Exposure					
AUC_{0-inf} (ng*h/mL)	7,615	8,233	8,938		
C_{max} (ng/mL)	2,463	1,746	1,468		
CL (L/h)	20.5	19.6	17.4		
$t_{1/2}$ (h)	11.5	13.6	17.5	**Fold Change**	
Unbound LEF Exposure				**Mod/Norm**	**Sev/Norm**
AUC_{0-inf} (ng*h/mL)	294	693	903	2.3	3
C_{max} (ng/mL)	128	180	194	1.4	1.5

The arithmetic means for subjects without pneumonia with normal hepatic function (NORMAL) or hepatic impairment (MODERATE, SEVERE) following administration of LEF injection. Unbound LEF concentrations for the NORMAL, MODERATE, and SEVERE groups were approximated by multiplying the total LEF concentrations by the plasma protein binding estimate from the time interval which the concentration fell within (0–2, 3–6, >8 hr; Table 10). Average exposures were compared to subjects with normal hepatic function (fold-change).
AUC_{0-inf} = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; CL = total body clearance of the drug from plasma; $t_{1/2}$ = half-life; LEF = lefamulin
Source: Adopted with modification from NAB-BC-3781-1010-pharmacokinetic report

Unbound Lefamulin PPB increased approximately 2- to 3-fold in subjects with moderate or severe hepatic impairment compared to subjects with normal hepatic function. This results in higher unbound (biologically active) lefamulin concentrations and overall exposure. Dose adjustment needs to be considered. Lefamulin PPB values in subjects with normal hepatic function are in line with the values observed from Study XS-1103, but not EVT-00756-3781 (see Section 16.3.1.1).

16.3.2.4. Population pharmacokinetics

16.3.2.4.1. General population

Plasma PK Model

The Applicant refined a previously developed population PK model using concentration-time data pooled from four Phase 1 studies (Studies 1010, 1011, 1107, and 1108), one Phase 2 study in patients with ABSSSI (Study 2001), and two Phase 3 studies in patients with CABP (Studies 3101 and 3102). The demographic and clinical characteristics of the subjects included in the population PK analysis are summarized in Table 136.

Table 136. Demographics and Clinical Characteristics of Subjects in the Pooled Pharmacokinetic Analysis

Variable	Phase 1 (N=98) Median (Min. – Max.)	Phase 2 (N=129) Median (Min. – Max.)	Phase 3 (N=622) Median (Min. – Max.)	Total (N=849) Median (Min. – Max.)
Age (yr)	50 (19 - 77)	41 (18 - 73)	61 (19 - 97)	57 (18 - 97)
Height (cm)	173 (146 - 191)	173 (150 - 196)	168 (133 - 200)	170 (133 - 200)
Weight (kg)	83.4 (54 - 124)	87.5 (43.8 - 161)	75 (31 - 175)	78 (31 - 174.6)
BSA (m^2)	1.99 (1.53 - 2.44)	2.02 (1.4 - 2.68)	1.85 (1.13 - 2.73)	1.89 (1.13 - 2.73)
BMI (kg/m^2)	27.6 (19.7 - 38.5)	30.3 (12.1 - 55.5)	26 (13 - 56.8)	26.6 (12.1 - 56.8)
CL_{CR} ($mL/min/1.73\ m^2$)	87.8 (5.4 - 130)	87.6 (24.1 - 171)	69 (14.1 - 192)	73.4 (5.40 - 192)
Albumin (g/L)	4.5 (2.8 - 5.6)	4.2 (2.8 - 5.2)	4.0 (2.0 - 5.3)	4.1 (2.0 - 5.6)
Gender				
Male	74/98 (75.5%)	86/129 (66.7%)	360/622 (57.9%)	520/849 (61.2%)
Female	24/98 (24.5%)	43/129 (33.3%)	262/622 (42.1%)	329/849 (38.8%)
Race				
White	74/98 (75.5%)	97/129 (75.2%)	493/622 (79.3%)	664/849 (78.2%)
Black	20/98 (20.4%)	20/129 (15.5%)	29/622 (4.66%)	69/849 (8.13%)
Asian	1/98 (1.02%)	1/129 (0.775%)	70/622 (11.3%)	72/849 (8.48%)
American Indian/Alaskan Native	2/98 (2.04%)	6/129 (4.65%)	23/622 (3.7%)	31/849 (3.65%)
Native-Hawaiian/Other Pacific Islander	0	4/129 (3.1%)	0	4/849 (0.471%)
Other	1/98 (1.02%)	1/129 (0.775%)	7/622 (1.13%)	9/849 (1.06%)

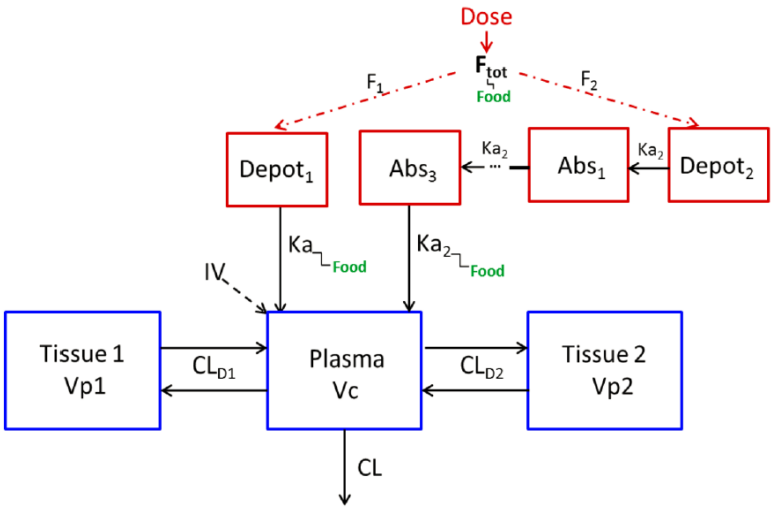
CL_{CR} = creatinine clearance; BSA = body surface area; BMI = body mass index
Source: Applicant's population PK report (b) (4) 00488-1), Table 4, Page 41. Creatinine clearance (CLcr) was determined by the Cockcroft and Gault equation normalized by body surface area (BSA). BSA was determined using the DuBois and DuBois equation.

The Phase 1 studies included in the pooled population PK analysis were a single dose bioavailability and food-effect study (Study 1107, N=20), a DDI study with rifampin (Study 1108, N=28), a hepatic impairment study (Study 1010, N=20), and a renal impairment study (Study 1011, N=28). Intensive blood sampling for PK analysis was done in all Phase 1 studies. The Phase 2 study (Study 2001, N=129) included subjects with ABSSSI receiving IV lefamulin for 5 to 14 days who provided up to 9 blood samples over 3 visits for determination of lefamulin concentrations in plasma. The Phase 3 studies (Studies 3101 and 3102), consisted of subjects with CABP who received either IV-only, IV-to-oral switch, or oral-only therapy. In Study 3101 (N=375), the regimens included multiple IV 150-mg doses over 1 hr q12h with optional switch to 600 mg PO q12h for total treatment duration of 7 days (10 days if confirmed/suspected MRSA). Following IV dosing, plasma sampling was scheduled at predose on morning of Day 3 and within 10 minutes of end of infusion, 1 to 3 hours and 7 to 11 hours postdose. Following PO dosing, plasma sampling was scheduled at predose the morning of switch, 1 to 3 hours and 4 to 8 hours postdose. In Study 3102, subjects received multiple 600 mg PO doses q12h for up to 7 days (10 days if confirmed/suspected MRSA) and provided up to four blood samples for determination of lefamulin concentration in plasma samples on Day 3 of therapy.

Population PK Model Development

A total of 6,205 plasma concentration records from 849 subjects were available from the 7 studies used for the development of the lefamulin population PK model. The Applicant used a prior structural model — a 3-compartment model with linear clearance, nonlinear protein binding, and first-order disposition into and out of ELF — for further refinement. The structural model is shown in Figure 9. The population PK model caters for lefamulin administration via IV infusion, using a zero-order input, and oral IR tablets, using a biphasic absorption model to account for rapid and slow absorption phases.

Figure 9. Structural Representation of Lefamulin Base Population PK Model



F_{tot} - total PO bioavailability; F1, F2 - fraction of administered dose going to the fast and slow absorption processes, respectively; Abs1, Abs3 - transit compartments used for slow absorption process; Ka, ka_2- absorption rate constant through the immediate process, and the delayed process, respectively; Vp1 and Vp2 - volume of distribution for peripheral compartment 1 and compartment 2, respectively. Vc - volume of distribution of the central compartment. CL_{D1} and CL_{D2} - distributional clearance to peripheral compartment 1 and compartment 2, respectively. Source: Applicant's population PK report (b) (4) 00488-1), Figure 5, Page 46.

After confirmation of appropriateness of the model, the Applicant performed comprehensive covariate analysis to identify subject descriptors associated with the interindividual variability in lefamulin plasma pharmacokinetics. Key covariate effects that were identified in Applicant's previous analyses including the effects of food and the effect of concomitant rifampin therapy. Covariates assessed included various measures of body size, renal function, age, gender, and potentially other demographic characteristics such as PORT risk stratification. Those covariates which passed the initial statistical screen were incorporated into the population PK model. The final population PK model for this analysis was qualified by examining the distribution of normalized prediction distribution errors (NPDE) and using a prediction-corrected visual predictive check (PC-VPC), which graphically examines the agreement between the 5th, 50th, and 95th percentiles of the observed and the individual simulated (N=500) lefamulin concentrations across time intervals.

Incorporation of nonlinearity on protein binding

The Applicant previously developed a model accounting for nonlinear plasma protein binding of lefamulin relating the total plasma concentration (CtP) to unbound plasma concentration (CuP) as follows:

$$CtP = CuP\ (1 + \frac{Bmax}{Kd + CuP})$$

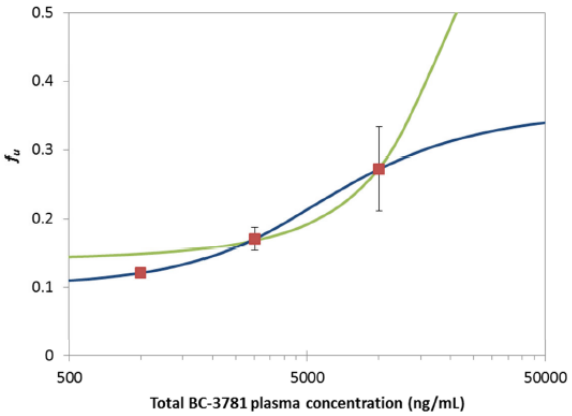
In this equation, the parameters B_{max} and K_d were not estimated based on the clinical observations but were set to estimates based on the in vitro data alone.

The Applicant developed an E_{max} model based on in vitro data to account for nonlinearity in protein-binding which was better in model fitting. It was parameterized as shown below:

$$Fu = Fu_{min} + Fu_{\max}(\frac{CuP}{Fu50 + CuP})$$

where Fu is the fraction unbound of lefamulin in plasma with minimum value of Fu_{min} and maximum value of $Fu_{\max}$. $Fu50$ is the concentration at $Fu_{\max}$. The Estimates of Fu_{min} , $Fu_{\max}$ and $Fu50$ were fixed based on in vitro data. The Applicant also reported that due to the close to perfect fit of this new Fu model to the in vitro Fu data (as expected with only 3 observations) no residual error could be estimated for these observations and hence no reliable parameter precision could be presented for the protein binding parameters of the final model. Figure 10 shows how the model performed in fitting in vitro data. We note that this PPB model could not predict the observed unbound lefamulin fractions in subjects with and without hepatic impairment (see 16.3.2.4.2 for more details).

Figure 10. Mean f_u Versus Total Lefamulin Plasma Concentration From In Vitro Experiment.



(Red squares, SD error bars), model fit with a B_{max} model utilized in a previous population PK analysis (green) and the protein binding model utilized in the final model (blue).
Source: Frx-bc3781-pmt-1; BC-3781, Fig. 2, Pg 31.

Results

The Applicant's final population PK model for lefamulin was a 3-compartment model with linear clearance, nonlinear protein binding, and first-order disposition into and out of ELF. Intravenous infusions were modeled as zero-order input rates and oral absorption was modeled using parallel immediate and delayed absorption processes, with the delayed absorption described using transit compartments. Interindividual variability was estimated for total plasma clearance (CL), volume of distribution of the central compartment (V_c), distributional clearance to peripheral compartment 1 (CLd1), and volume of distribution for peripheral compartment 1 (Vp1) using exponential error models. Residual variability was described using a combined

additive and proportional error model. The population PK parameter estimates and their associated precision (%SEM) for the fit of the 3-compartment model are provided inTable 137.

Table 137. Final Lefamulin Population Pharmacokinetic Model Parameter Estimates and Associated Standard Errors

Parameter[a]	Final Estimate	%SEM	Shrinkage
CL (L/hr)	79.4	2.13	—
Vc (L)	46.3	6.87	—
CLd1 (L/hr)	40.6	7.76	—
Vp1 (L)	249	9.13	—
CLd2 (L/hr)	199	—	—
Vp2 (L)	259	—	—
Ka (hr^{-1})	1.2	—	—
Ka2 (hr^{-1})	2.12	—	—
F_{tot}	0.244	—	—
FS	0.802	—	—
ALAG (hr)	0.15	—	—
$f_{u,\ min}$	0.0997	—	—
$f_{u,\ max}$	0.259	—	—
f_{u50} (mg/L)	1.35	—	—
Ka_{fed} (hr^{-1})	0.0541	6.15	—
$Ka2_{fed}$ (hr^{-1})	0.445	1.55	—
$F_{tot,\ fed}$	0.763	3.23	—
CL:Albumin[b]	1.214	9.21	—
CL:Phase 2[c]	1.827	12.5	—
CL:Phase 1[c]	1.766	20.5	—
CLd1:Phase 2[c]	1.44	32.8	—
CLd1:Phase 1[c]	2.12	20.5	—
Vp1:WTKG[d]	1.0129	24.4	—
Vp1:Phase 2[c]	1.985	32.8	—
Vp1:Phase 1[c]	2.75	43.6	—
ω^2_{CL}	0.171 (41.4% CV)	5.12	9.4%
ω^2_{Vc}	0.39 (62.4% CV)	25.1	63.5%
ω^2_{CLd1}	0.119 (34.5% CV)	29.8	65.4%
ω^2_{Vp1}	0.623 (78.9% CV)	—	55.2%
ω^2_{Ka}	0.800 (89.4% CV)	—	70.65%
ω^2_{Ka2}	0.400 (63.2% CV)	—	47.3%
ω^2_{Ftot}	0.100 (31.6% CV)	—	39.8%
ω^2_{FS}	0.170 (41.2% CV)	—	77.2%
$\sigma^2_{Proportional}$	0.103 (32.0% CV)	1.37	11.5%
$\sigma^2_{Additive}$	0.0000343 (0.00586 mg/L)	17.6	11.5%

ALAG=lag time; CL=total plasma clearance; CLd1=distributional clearance to peripheral compartment 1; CLd2=distributional clearance to peripheral compartment 2; FS=fraction of dose absorbed through slow pathway; F_{tot}=total oral bioavailability; $F_{tot,\ fed}$=total oral bioavailability under fed conditions; f_{u50}=concentration at which fraction unbound is half-maximal; $f_{u,\ max}$=maximum fraction unbound; $f_{u,\ min}$=minimum fraction unbound; Ka=absorption rate constant through the immediate process; Ka2=absorption rate constant through the delayed process; Ka_{fed}= Ka under fed conditions; $Ka2_{fed}$=Ka2 under fed conditions; ω^2_{CL}=intersubject variance of clearance; ω^2_{CLd1}=intersubject variance of distributional clearance to peripheral compartment 1; ω^2_{FS}=intersubject variance of fraction of dose absorbed through slow pathway; ω^2_{Ftot}=intersubject variance of total oral bioavailability; ω^2_{Ka}=intersubject variance of absorption rate constant through the immediate process; ω^2_{Ka2}=intersubject variance of absorption rate constant through the delayed process; ω^2_{Vc}=intersubject variance of volume of distribution of the central compartment; ω^2_{Vp1}=intersubject variance of volume of distribution for peripheral compartment 1; $\sigma^2_{Additive}$=additive residual error variance; $\sigma^2_{Proportional}$=proportional residual error variance; %SEM=percent standard error of the mean; V_c=volume of distribution of the central compartment; Vp1=volume of distribution for peripheral compartment 1; Vp2=volume of distribution for peripheral compartment 2; WTKG=body weight

[a] Parameters represent population mean values for a typical CABP patient and are in terms of unbound lefamulin disposition.

[b] Fold change in lefamulin CL per every 1 g/dL deviation in albumin from the population median value of 4.1 g/dL.

[c] Fold-increase in PK parameter due to study phase.

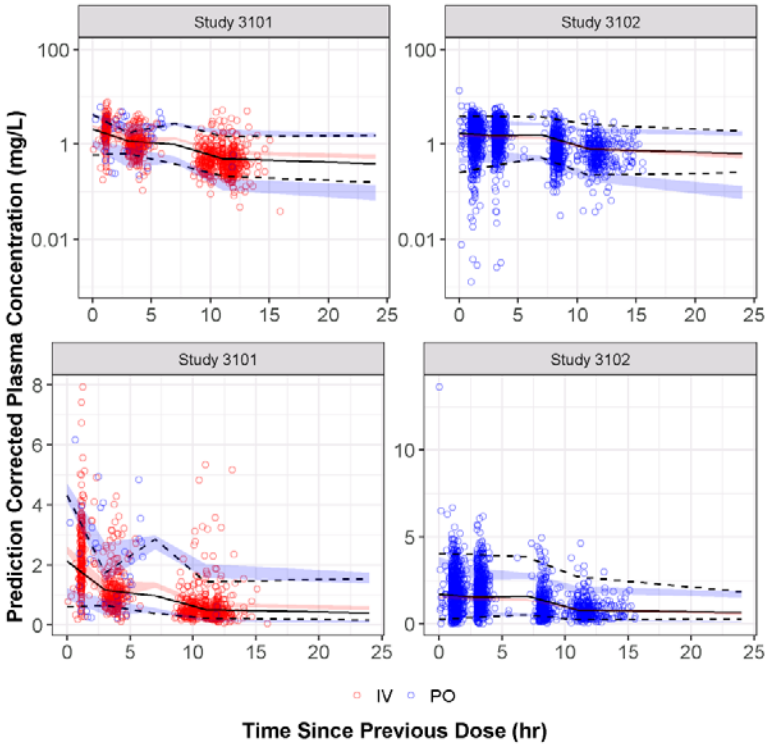
[d] Fold change in lefamulin Vp1 per every 1 kg deviation in WTKG from the population median value of 78 kg.

Source: Applicant's population PK report (b) (4) 00488-1), Table 8, Page 55.

Using the full, pooled PK dataset, The Applicant identified 5 statistically significant relationships: serum albumin was significantly related to the interindividual variability (IIV) in CL; total body

weight was significantly related to the IIV in Vp1; and study phase was significantly related to the IIV in CL, CLd1, and Vp1. The overall distribution of NPDE appeared to be symmetrical around a value of 0 and did not appear to deviate from a normal distribution. In addition, there did not appear to be any noticeable differences in the distribution of NPDE between healthy subjects and infected subjects. The PC-VPC (Figure 11) revealed that there was reasonable agreement between the median and 5th and 95th percentiles of the observed and simulated data over time following lefamulin dosing in ABSSSI and CABP subjects.

Figure 11. Semi-Log (Top) and Linear (Bottom) Scale Prediction-Corrected Visual Predictive Checks for the Final Lefamulin Population Pharmacokinetic Model Using Pooled Data (Phase 3 Studies Only)



Open circles are observed concentrations, black solid lines are the median observed concentrations, black dashed lines are the 5th and 95th percentiles of the observed concentrations. Red and blue shaded regions are the 90% confidence intervals for the median, 5th, and 95th percentiles from the simulations.
Source: Applicant's population PK report (b) (4) 00488-1), Figure 10, Page 59.

Comparison of exposures

The Applicant performed post hoc analysis to obtain Day 1 and steady-state lefamulin pharmacokinetic exposure indices for Phase 2 and Phase 3 trials. The comparisons are shown in Table 138.

Table 138. Summary of Lefamulin Plasma Pharmacokinetic Exposure Parameters for Patients Enrolled in Phase 2 and Phase 3 Trials

Parameter	Study 2001 (IV)[a]		Study 3101 (IV)		Study 3102 (PO)	
	Day 1 (n=65)	Steady State (n=62)	Day 1 (n=258)	Steady State (n=252)	Day 1 (n=364)	Steady State (n=230)
AUC_{0-24} (mg•h/L)[b]						
Arithmetic Mean (%CV)	15.3 (25.8)	16.0 (27.5)	27.0 (31.8)	28.4 (45.1)	30.7 (45.0)	32.7 (49.2)
Geometric Mean (Geo %CV)	14.8 (24.7)	15.4 (28.0)	25.8 (29.6)	26.3 (38.0)	28.0 (43.8)	29.4 (45.3)
Median (Min-Max)	14.7 (8.38 - 33.5)	15.7 (7.07 - 34.5)	25.1 (13.5 - 57.5)	24.5 (10.3 - 84.0)	28.1 (7.39 - 115)	28.6 (7.97 - 97.9)
CL (L/h)[c]						
Arithmetic Mean (%CV)	157 (30.8)	157 (29.0)	90.1 (36.2)	90.3 (36.3)	79.3 (40.1)	80.2 (38.5)
Geometric Mean (Geo %CV)	150 (29.6)	151 (27.1)	83.2 (43.1)	83.3 (43.3)	73.1 (41.5)	74.4 (39.7)
Median (Min-Max)	154 (63.3 - 354)	154 (63.3 - 354)	91.8 (18.8 - 227)	91.9 (18.8 - 227)	76.9 (16.1 - 232)	77.2 (23.0 - 232)
C_{max} (mg/L)[d]						
Arithmetic Mean (%CV)	1.95 (12.5)	2.01 (12.8)	3.50 (11.7)	3.03 (28.9)	2.24 (36.4)	2.24 (37.1)
Geometric Mean (Geo %CV)	1.94 (12.7)	1.99 (12.9)	3.48 (11.2)	2.90 (30.9)	2.10 (37.0)	2.09 (37.6)
Median (Min-Max)	1.94 (1.26 - 2.82)	1.98 (1.30 - 2.96)	3.42 (2.55 - 5.15)	3.20 (1.18 - 5.56)	2.15 (0.616 - 6.72)	2.15 (0.731 - 5.91)
C_{min} (mg/L)[e]						
Arithmetic Mean (%CV)	0.140 (60.1)	0.213 (48.6)	0.398 (68.1)	0.571 (86.2)	0.593 (67.3)	0.765 (75.7)
Geometric Mean (Geo %CV)	0.122 (51.0)	0.192 (46.6)	0.325 (63.5)	0.439 (69.8)	0.470 (73.2)	0.598 (71.6)
Median (Min-Max)	0.118 (0.0422 - 0.613)	0.190 (0.0505 - 0.719)	0.330 (0.0539 - 1.41)	0.409 (0.0595 - 2.91)	0.490 (0.0258 - 2.46)	0.623 (0.0541 - 3.16)

AUC_{0-24} = area under the concentration-time curve from time 0 to 24 hours after drug administration; CL = total body clearance of the drug from plasma; C_{max} = maximum plasma concentration of drug; C_{min} = minimum plasma drug concentration; CV = coefficient of variation; IV = intravenous; PO = by mouth
Source: Applicant's population PK report (b) (4) 00488-1), Table 10, Page 64

The Day 1 geometric mean AUC_{0-24} is demonstrably (1.74-fold) higher in CABP patients enrolled in Study 3101 relative to those who received a lefamulin dosing regimen of 150 mg IV q12h in Study 2001 (ABSSSI patients), suggesting pharmacokinetic differences between patient populations. The exposure following PO and IV dosing were comparable, though oral dosing had numerically higher AUC_{0-24}.

Food effect

Total- and free-drug plasma exposure is predicted to be 15% to 43% higher at steady-state, depending on the route of administration and concomitant food intake. Subjects who were fed were predicted to have 24% lower bioavailability compared to fasting subjects (taking lefamulin at least 1 hr before food or 2 hours after a meal). The meal consisted of high fat/high calories.

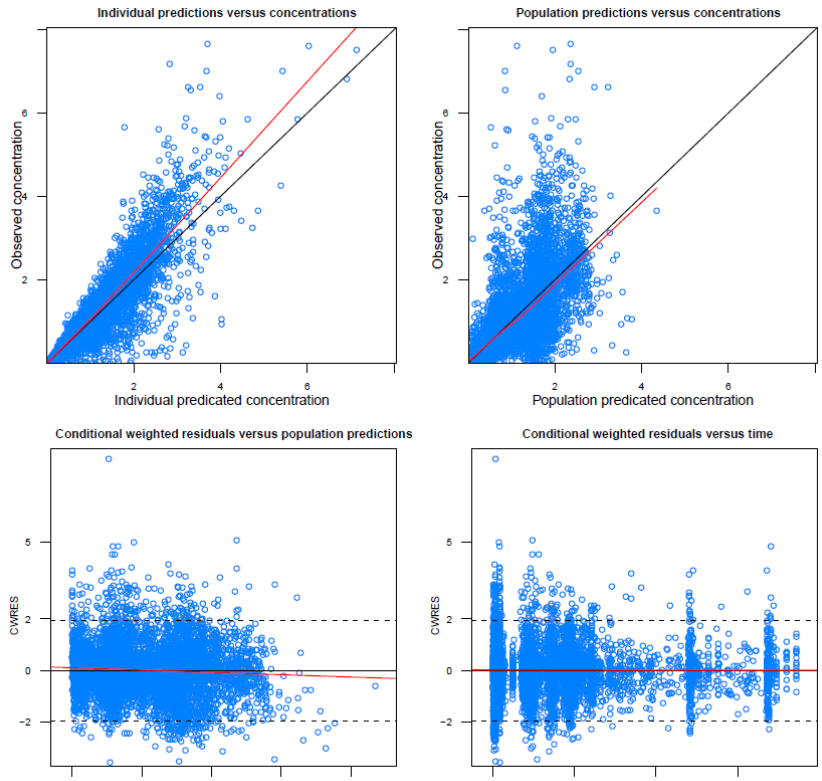
Applicant's Conclusions

A 3-compartment model with linear clearance, nonlinear protein binding provided a robust fit to the pooled lefamulin plasma concentration-time data from Phase 1, 2, and 3 studies. Three subject specific covariates were associated with the interindividual variability in lefamulin pharmacokinetics: albumin, body weight, and study phase. The inclusion of these covariates into the final population PK model resulted in an improvement in the overall model fit. However, none of the covariate relationships were deemed to be clinically relevant as they were of insufficient magnitude to warrant lefamulin dose adjustments. The model was fully qualified for both the estimation of lefamulin exposure in individual subjects and the conduct of model-based simulations.

Reviewer's comments*: The Applicant's population PK analysis reasonably described the population pharmacokinetics of lefamulin as shown in the visual predictive checks, based on the*

ability of the model's simulated 90% PI to accommodate the 5th, 50th and 95th percentiles of observed data. The submitted final population PK parameter model is reproducible. The Applicant did not evaluate the robustness of their model used to describe nonlinearity in protein binding using clinical PK samples which were collected from the dedicated renal and hepatic impairment studies. The impact of missing a dose of lefamulin no more than 4 hours needs further evaluation. FDA Reviewer performed independent analysis to address these issues.

Figure 12. Goodness-of-Fit Plot for Final Population PK Model for Lefamulin



CWRES = conditional weight residuals. The black solid line is the line of identity or the zero line, and the red solid line is the trend line. The blue circles represent observed data (FDA analysis)
PK = pharmacokinetic

Reviewer's Independent Analysis

The objectives were to:

- Assess the adequacy of Applicant's population PK model data to adequately describe PK data and nonlinearity in protein binding for hepatic impaired subjects — See Section 16.3.2.4.2
- Assess the impact of missed dose instructions — See Section 16.3.2.4.3
- Evaluate the performance of model with concentration dependent change in ELF based on changes in lung penetration ratio (LPR) — See ELF PK Model

- Evaluate the PTA based on protein binding of 96% and PK-PD targets which are either medians, randomly assigned log-normally distributed, median or 3rd quartile of distribution tied to food effects — see Reviewers Analysis.

Dataset

The data sets and Applicant's model files used in the analysis are in the EDR.

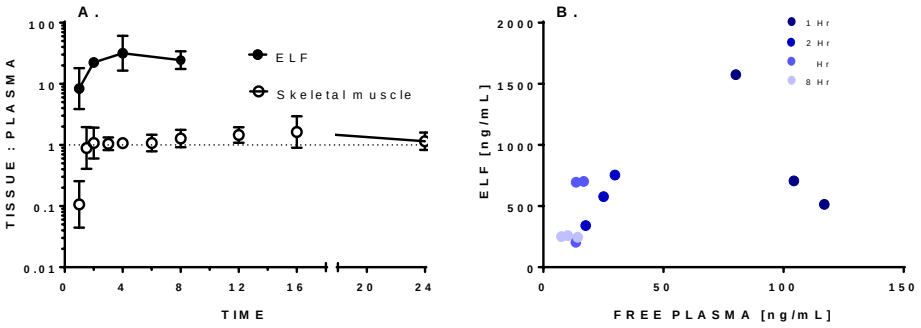
Methods

NONMEM 7 and R were used for the Reviewer's analysis.

ELF PK Model

During the review, we considered the impact of a greater fraction of bound lefamulin on the lung penetration estimate determined in Study 1005. Assuming a free lefamulin fraction of 0.0379 resulted in a time averaged lung penetration ratio (by total ELF AUC_{0-24} / free plasma AUC_{0-24}) of approximately 20 (time averaged PPB estimate of lefamulin determined from clinical studies NAB-BC-3781-1010, 1011). Interestingly, data suggest the lefamulin plasma-ELF relationship is not linear but rather a saturable lung penetration process (Figure 13). Therefore, the noncompartment AUC method is a conservative estimate of lung penetration. Importantly, lung penetration (by ELF AUC_{0-24} / free plasma AUC_{0-24}) was dependent on route of administration in mice (Table 139). The reason for this is not clear, but given this result, we cannot confirm that total ELF lefamulin concentrations will be independent of administration route (i.e., IV versus PO) given the lack of clinical data (no ELF concentrations with PO lefamulin).

Figure 13. Lefamulin Ratio in Tissue to Free-Drug Plasma Over Time (A) and Free Lefamulin Plasma Concentration- Total ELF Relationship (B)



ELF = epithelial lining fluid

Table 139. PK Parameters and Lung Penetration of Single Doses of Lefamulin by Different Administration Routes

PPB =0.8	BioMatrix	C_{max} (ng/mL)	$tAUC_{0\text{-}inf}$ (ng*h/mL)	$fAUC_{0\text{-}inf}$ (ng*h/mL)	ELF: *f*Plasma
35 mg/kg IV	Plasma	7,082	5,443	1,088.6	----
	ELF	22,810	25,440	25,440	23
35 mg/kg SC	Plasma	1,946	5,795	1,159	----
	ELF	2,911	14,160	14,160	12
100 mg/kg PO	Plasma	1,279	6,171	1,234.2	----
	ELF	1,954	12,310	12,310	10

[a]Data from noninfected mice. Applicant report NABRIVA 2010-27 PKPD; Table 2, pg 11.
LEF = epithelial lining fluid; PK = pharmacokinetic; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity after drug administration; C_{max} = maximum plasma concentration of drug; PPB = plasma protein binding; *t* = total lefamulin; *f* = free or unbound lefamulin

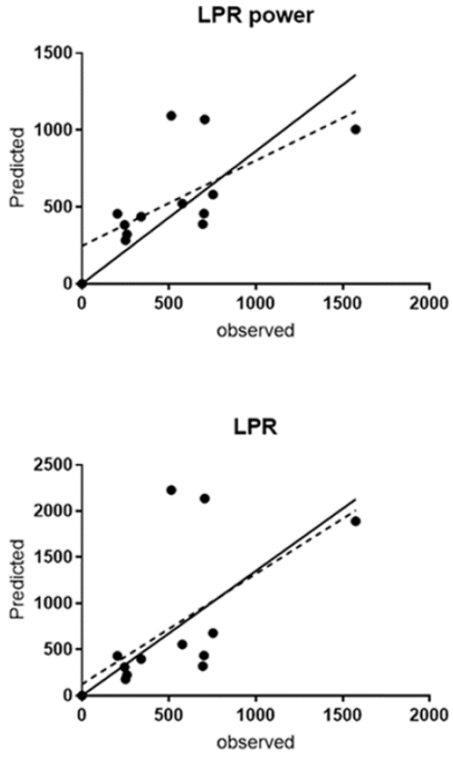
Given that that lefamulin ELF lung penetration ratio (LPR) is plasma concentration dependent and thus varies with time due to plasma concentration effect (Figure 13), the Reviewer used the following plasma-ELF link function to assess the need for adjusting for this effect:

$$C_{ELF} = LPR\left(\frac{1\ mg}{L}\right) * [C_P(t) * 0.0379\ ]^{power}$$

where LPR (1 mg/L) is the LPR at a plasma concentration of 1 mg/L, and the power parameter allows the penetration ratio to change with plasma concentration. The plasma concentration was adjusted by 0.0379, fraction unbound of lefamulin in plasma. If power =1 the model is identical to a proportional constant between ELF and plasma concentrations (LPR model), whereas for values <1, the penetration ratio decreases as concentration increases. The model where power was estimated is referred to as (LPR power).

The results of the assessment showed that the LPR power model (r^2=0.45) was better that LPR model (r^2=0.37) (Figure 14). The model predictions are not as good as Figure 13 because of the differences in input data. Figure 14 is based on post hoc estimates (predicted concentrations) from the final population PK model which are highly variable and the observed ELF concentrations from BAL (NAB-BC-3781-1005). The Applicant's ELF model did not account for concentration-dependent changes in ELF tied to changes in lung penetration ratio. The Reviewer notes a great uncertainty on the predicted ELF concentrations. The population PK model is not robust enough to describe the PK of lefamulin in ELF, hence plasma concentration should be used for PTA analysis.

Figure 14. Assessments of Adequacy of LPR and LPR Power Model in Estimating Lefamulin Concentrations in ELF



LPR = lung penetration ratio; ELF = epithelial lining fluid
Source: Applicant report frx-bx-3781-pmt-1, dataset ppkin.xpt.

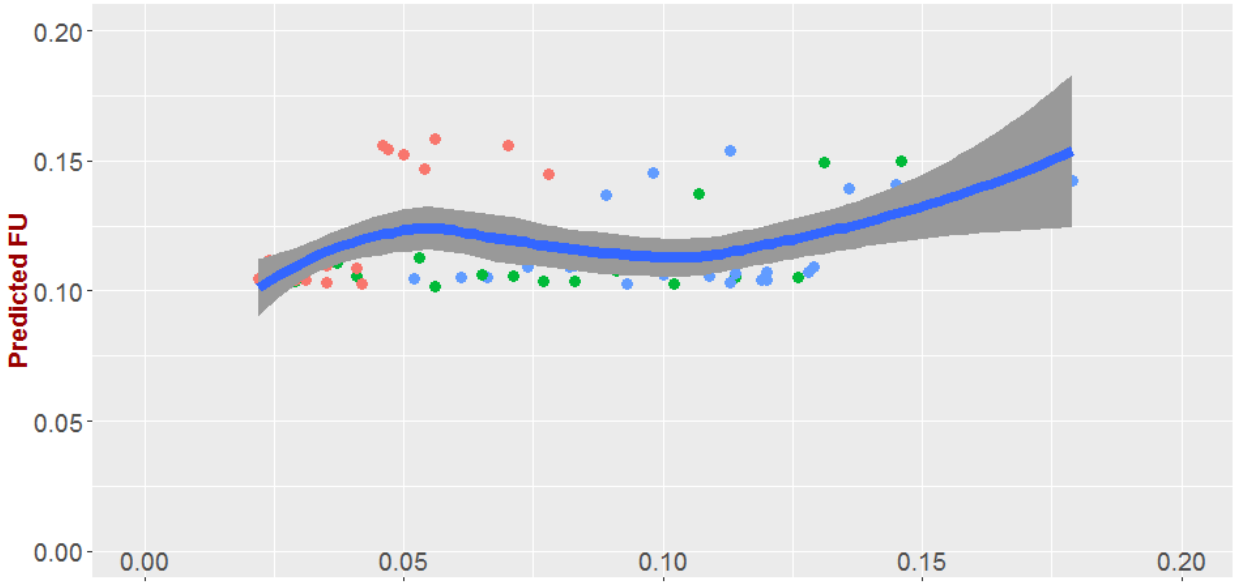
16.3.2.4.2. Hepatic impairment scenario

Using the Applicant's final population PK, the Reviewer performed a sensitivity test to assess the robustness of the model in describing nonlinear protein binding using data from the dedicated hepatic impairment study (NAB-BC-3781-1010), This study investigated the pharmacokinetics of lefamulin in subjects with moderate to severe hepatic impairment (Child-Pugh classification) compared with age-, gender-and weight-matched healthy subjects with normal hepatic function after a single IV dose. Plasma protein binding of lefamulin was measured at the end of infusion, and 3- and 8-h after the start of infusion for all subjects enrolled. Total (bound and unbound) plasma concentrations of lefamulin were also captured at these times.

The Reviewer also conducted a sensitivity analysis by fitting the model to the data from the dedicated hepatic impairment study where protein binding was measured. The results of the analysis showed that the model cannot adequately describe protein binding in subjects from this study based on predicted and observed lefamulin unbound fractions in plasma (Figure 15).

Overall, the overprediction of the unbound lefamulin fraction may results in falsely higher susceptibility breakpoints. The Reviewer recommends that the model not be used for any assessment in hepatically impaired subjects.

Figure 15. Predicated Versus Observed Fraction Unbound of Lefamulin in Plasma.



The red, green and blue dots represent, normal, moderate and severely hepatic function, respectively.
Source: FDA analysis

16.3.2.4.3. Missed dose scenario

The Applicant proposes that, lefamulin can be taken at most four hours after missing the dose. The effect of missed doses under fed or fasting conditions was evaluated by comparing the magnitude of change in AUCs and lefamulin plasma-concentrations for a typical CABP patient (78 kg) after taking the first dose, then taking the next dose of lefamulin at 16-hours compared to taking the first dose, then the next dose 12-hours later. Starting at 24 hr lefamulin was given every 12-hours for both scenarios. The AUC and concentrations of lefamulin in plasma were assessed before taking the next dose at 24-hour.

The Reviewer's analysis showed that taking the dose at 16-hours (4 hrs past scheduled dose) compared to 12 hrs, results in approximately 7.2% lower free-drug AUC at 24-hour under the fasted condition. The Reviewer agrees that missing the dose up to 4 hours is not expected to compromise safety or efficacy.

16.3.2.5. Dose/Exposure Response Relationships

16.3.2.5.1. Probability of Target Attainment (Exposure site, PD Variability, Protein binding)

Efficacy

The Applicant used a modeling and simulation approach to evaluate a clinical PK-PD efficacy relationship and nonclinical PK-PD efficacy relationship for *S. pneumoniae* and *S. aureus* lung infections (report (b) (4) 00488-2).

Clinical Exposure-Efficacy Relationship.

The Applicant used the population PK model for lefamulin and data from lefamulin-treated subjects with CABP enrolled in Studies 3101 and 3102. The analyses were undertaken to evaluate PK/PD relationships for efficacy. The analysis populations included lefamulin-treated subjects with pharmacokinetics from among the microbiologically evaluable population and subsets of subjects with pathogens of interest. Analysis populations consisting of subsets of these subjects with pathogens isolated from baseline cultures other than nasopharyngeal (NP) cultures were also evaluated.

Efficacy endpoints evaluated included early clinical response (ECR) assessed at 96±24 hours, investigator's assessment of clinical response (IACR) at the end-of-treatment (EOT), test-of-cure (TOC), and late follow-up (LFU) visits, and microbiological response at EOT, TOC and LFU. The AUC/MIC ratio was used to portend lefamulin efficacy, which has been identified to be the PK/PD index most closely associated with bactericidal activity in murine studies. PK-PD analyses were performed by the Applicant using R Version 3.3.1 and the free-drug plasma AUC/MIC ratio was evaluated as an independent variable. Univariate analysis of relationships for dichotomous efficacy endpoints were examined using chi-square or Fisher's exact tests for categorical independent variables and logistic regression for continuous independent variables. In addition to the evaluation of the influence of the lefamulin AUC/MIC ratio, AUC, MIC, patient demographics, disease-related characteristics, underlying comorbidities, and other potential predictors of response were considered.

The Applicant also performed multivariate analyses for any efficacy endpoint for which a biologically plausible univariable relationship was identified at a 0.10 significance level ($p \leq 0.10$). Biologically plausible univariate relationships were those for which increased AUC/MIC or AUC or decreased MIC was associated with improved response. Those univariable relationships lacking in biological plausibility were those for which decreased free-drug plasma AUC/MIC, total-drug ELF AUC/MIC, free-drug plasma AUC, total-drug ELF, or increased MIC were associated with improved response. Multivariate analyses were carried out using multiple logistic regression and were developed using the forward inclusion of independent variables with an entry criterion of largest improvement of Akaike's Information Criterion AIC.

Results

A total of 92 lefamulin-treated subjects with CABP from Studies 3101 and 3102 had an appropriate source pathogen and MIC data and were evaluable for ECR at 96±24 hours and IACR at EOT, TOC, or LFU. Fifty-four out of 92 subjects had *S. pneumoniae* isolated at baseline. High percentages of successful response were achieved for all efficacy endpoints evaluated among all subjects (n=92; 87.5% to 93.5%) and among subjects with *S. pneumoniae* at baseline (n=54; 85.4% to 88.9%). If one excludes subjects with NP cultures, 60 of the 92 subjects were available for analysis and 22/60 had *S. pneumoniae* isolated at baseline. A high percentage of successful response was achieved for all efficacy endpoints evaluated among all subjects (84.3% to 95.0%). However, successful responses among subjects with *S. pneumoniae* at baseline (n=22) were lower ranging from 73.7% to 86.4%. Therefore, a lower likelihood of successful response was observed in this group compared to the larger subset of patients. However, because of the limited sample size the upper confidence bound crossed 90% limiting any definitive conclusion.

The Applicant determined that none of the univariable relationships evaluated were both statistically significant at the 0.05 level and in the direction of increased efficacy with increased free-drug plasma AUC:MIC ratio or free-drug plasma AUC. It is important to note that, as shown in Table 140, 100% of subjects with *S. pneumoniae* or *S. aureus* at baseline, irrespective of culture type, achieved nonclinical free-drug plasma AUC:MIC ratio targets for efficacy against *S. pneumoniae* (1.37 hrs) and *S. aureus* (2.13 hrs). Based on the data for *S. pneumoniae* and *S. aureus*, free-drug plasma AUC:MIC ratios achieved in subjects with these pathogens appear to have been associated with the upper plateau of the nonclinical PK-PD relationships for efficacy (Table 140).

Table 140. Summary of the Percentage of Patients With *S. pneumoniae* or *S. aureus* at Baseline Achieving Non-Clinical Free-Drug Plasma or Total-Drug ELF AUC/MIC Targets

Endpoint for free-drug plasma or total-drug ELF AUC:MIC ratio targets	% (n/N)			
	Patients with all baseline cultures[a]		Patients with all baseline cultures excluding NP cultures[a]	
	Patients with *S. pneumoniae* at baseline	Patients with *S. aureus* at baseline	Patients with *S. pneumoniae* at baseline	Patients with *S. aureus* at baseline
1-$\log_{10}$ CFU reduction from baseline[b]	100 (54/54)	100 (15/15)	100 (22/22)	100 (15/15)
2-$\log_{10}$ CFU reduction from baseline[c]	100 (54/54)	100 (15/15)	100 (22/22)	100 (15/15)

[a]. Patient counts by baseline pathogen group and overall are shown in Applicant's PKPD report (b) (4) 00488-2), Table 9, Page 52.
[b]. Based on the assessment of median free-drug plasma and total-drug ELF AUC:MIC ratio targets associated with a 1-log10 CFU reduction from baseline of 1.37 and 14.0, respectively, for *S. pneumoniae* and 2.13 and 21.7, respectively for *S. aureus*.
[c]. Based on the assessment of median free-drug plasma and total-drug ELF AUC:MIC ratio targets associated with a 2-log10 CFU reduction from baseline of 2.15 and 22.0, respectively, for *S. pneumoniae* and 6.24 and 63.9, respectively for *S. aureus*.
AUC = area under the concentration-time curve; MIC = minimum inhibitory concentration; ELF = epithelial lining fluid; NP = nasopharyngeal
Source: Applicant's PKPD report (b) (4) 00488-2), Table 14, Page 62.

Applicant's conclusions

The results of the PK-PD analyses for efficacy based on data from subjects with CABP enrolled in Studies 3101 and 3102 herein failed to demonstrate statistically significant and biologically plausible relationships between free-drug plasma AUC:MIC and response. These data indicate that lefamulin exposures were efficacious because all subjects achieved free-drug plasma AUC:/MIC that were above nonclinical PK-PD targets. Thus, results of these analyses provide support for the lefamulin dosing regimens: 150 mg IV q12h or 600 mg orally q12h evaluated for subjects with CABP in Studies 3101 and 3102.

Reviewer's Comment*: The exposure-response analysis performed by the Applicant is acceptable. The Reviewer agrees with conclusions that the high response rate* (see Section *8.1.6*) *limited the power to detect statistically significant relationships between free-drug plasma AUC:MIC and response.* The distribution of the total lefamulin $AUC_{0\text{-}24}$ was similar between responders *and nonresponders as assessed by the early clinical response endpoint and do not suggest a trend.*

16.3.2.5.2. Probability of Target Attainment in CABP Patients Using PKPD Targets Derived From Murine Models of *S. pneumoniae* and *S. aureus* Pneumonia

The Applicant used the final population PK model to generate individual PK parameters, lefamulin free-drug plasma and total-drug ELF concentration-time profiles for 5000 simulated subjects with CABP after administration of lefamulin 150 mg IV q12h, 600 mg orally q12h for 5 days, under fasting conditions (fasted), and 600 mg orally q12h for 5 days, under fed conditions (fed). Using numerical integration, the free-drug plasma and total-drug ELF AUC for the 24-hour period (AUC) corresponding to Days 1 and 3 were calculated by the Applicant.

The Non-clinical PK-PD targets for efficacy used for evaluation by the Applicant were based on the PK-PD relationships for lefamulin against *S. pneumoniae* and *S. aureus*, which were derived using data from a neutropenic murine-lung infection model. The Applicant based the selection of the PK-PD target on the results of previous dose-fractionation studies conducted using a neutropenic murine-thigh infection model which showed the AUC:MIC to be most predictive of lefamulin efficacy. Total-drug ELF and free-drug plasma AUC:MIC targets associated with 1- and 2-$\log_{10}$ CFU reductions from baseline for *S. pneumoniae* and *S. aureus* can be found in Table 105. Total-drug ELF AUC:MIC targets were based on plasma and ELF PK data from uninfected mice, which (according to the Applicant) demonstrated approximately a 2-fold higher total-drug ELF compared to total-drug plasma AUC values.

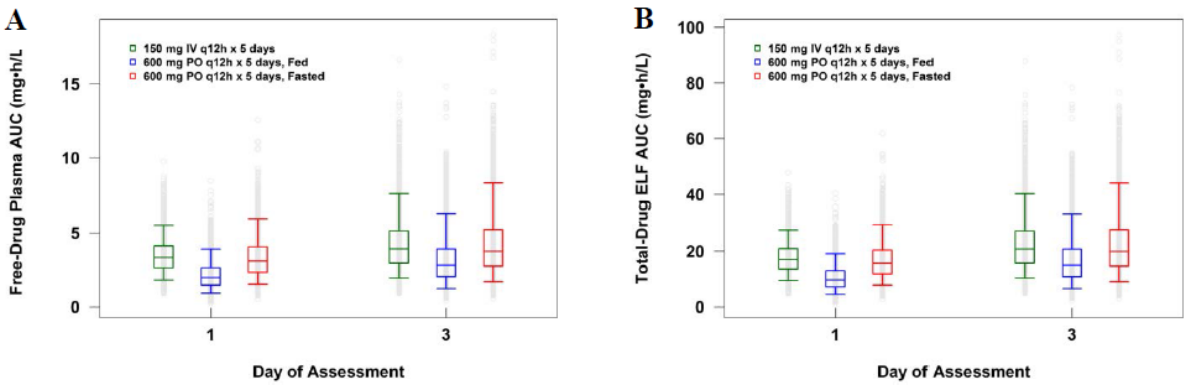
The bacterial reduction endpoint of interest for studies evaluating the PK-PD of lefamulin against *S. pneumoniae* and *S. aureus* using the neutropenic murine-lung infection model was a 1-$\log_{10}$ CFU reduction from baseline. Free-drug plasma and total-drug ELF AUC values for each lefamulin dosing regimen and for each simulated human subjects were divided by MIC values doubled over a discrete range. The free-drug plasma and total-drug ELF AUC:MIC ratios were assessed to determine the percent probability of attaining median and randomly assigned free-drug plasma and total-drug ELF AUC:MIC targets associated with 1- and 2-$\log_{10}$ CFU reductions from baseline by MIC value.

Results

The Applicant evaluated the exposure differences between fasted and fed free-drug plasma and total-drug ELF AUC values on Days 1 and 3 among simulated subjects following administration of IV or PO lefamulin dosing (Figure 16). The lefamulin oral dosing regimen under fasted conditions gave mean and median free-drug plasma AUC values on Day 1 that were 3.76% and 7.49%, respectively, higher compared with the IV dosing regimen. Based on simulations, the Applicant also determined that the lefamulin oral dosing regimen under fed conditions yielded mean and median free-drug plasma AUC values on Day 1 that were 38.2 and 41.0% lower, respectively, compared with the IV dosing regimen. Relative to Day 1, mean and median free-drug plasma AUC values on Day 3 were 22.3 and 17.4% higher for the IV dosing regimen, 26.7 and 21.7% higher for the PO dosing regimen under fasted conditions, and 48.1 and 42.6%

higher for the PO dosing regimen under fed conditions. The PO regimen under fed conditions yielded lower mean and median AUC values on Days 1 and 3 compared to the other 2 regimens which had comparable mean and median values to one another (Figure 16).

Figure 16. Box-and-Whisker Plots Showing Distributions of Free-Drug Plasma (a) and Total-Drug Epithelial Lining Fluid (b) Area Under the Concentration Versus Time Curve on Days 1 and 3 Among Simulated Subjects After Administration of Lefamulin Intravenous and Oral Dosing Regimens



AUC=area under the concentration versus time curve; ELF=epithelial lining fluid

Source: Applicant's summary of Clinical Pharmacology, Figure 13, Page 94.

The Applicant's percent probabilities of PK-PD target attainment by MIC on Day 1 based on median total-drug ELF and free-drug plasma AUC:MIC targets after administration of lefamulin 150 mg IV and 600 mg oral q12h are shown in Table 141. To cater for interspecies variability of nonclinical AUC:MIC targets (uncertainty), the Applicant used a randomly assigned nonclinical AUC:MIC target based on an estimated log normal distribution of AUC:MIC targets associated with a given endpoint for each pathogen (Table 105).

Table 141. Applicant's Day 1 Lefamulin Exposure Measures and Target Attainment Analysis[a] by Dosing Regimens and by MIC for *S. pneumoniae* and *S. aureus*

			S. pneumoniae MIC [mcg/mL]				*S. aureus* MIC [mcg/mL]			
			0.12	0.25[d]	0.5	1	0.12	0.25	0.5[e]	1
Median PD Target	ELF	IV	1	1	0.993	0.722	1	0.998	0.903	0.212
		PO-Fast	1	1	0.971	0.605	1	0.993	0.817	0.203
		PO-Fed	1	0.983	0.766	0.191	0.997	0.892	0.397	0.024
	Plasma	IV	1	1	1	0.992	1	1	0.998	0.894
		PO-Fast	1	1	1	0.970	1	1	0.992	0.818
		PO-Fed	1	1	0.988	0.793	1	0.997	0.908	0.429
Random PD Target	ELF	IV	0.996	0.941	0.75	0.448	0.989	0.893	0.655	0.334
		PO-Fast	0.992	0.914	0.720	0.418	0.983	0.862	0.612	0.308
		PO-Fed	0.952	0.785	0.513	0.227	0.915	0.701	0.399	0.140
	Plasma	IV	1	0.995	0.940	0.751	1	0.987	0.891	0.653
		PO-Fast	1	0.991	0.915	0.724	1	0.98	0.865	0.618
		PO-Fed	0.997	0.953	0.800	0.532	0.992	0.916	0.720	0.418

[a]Probability of target attainment (PTA) based on median or randomly assigned AUC/MIC targets associated with a 1-$\log_{10}$ CFU reduction from baseline. PPB was modeling as $f_u = f_{u,\,min} = f_{u,\,max} * C_{u,\,plasma} / (C_{u,\,plasma\,50} + C_{u,\,plasma})$ where f_u = unbound fraction; $f_{u,\,min}$ = population minimum unbound fraction fixed at 0.0997; $f_{u,\,max}$ = population maximum unbound fraction fixed at 0.259; $C_{u,\,plasma}$ = unbound plasma concentration; $C_{u,\,plasma\,50}$ = population $C_{u,plasma}$ where f_u is increased by half.
[b]Units $mg \cdot L^{-1}$
[c] Units $mg \cdot hrs \cdot L^{-1}$
[d]*S. pneumoniae* MIC_{90} from Pooled Phase 3 microITT analysis (Summary Clinical Pharmacology microbiology, Table 98, pg. 239)
[e]*S. aureus* MIC_{90} from Pooled Phase 3 microITT analysis (Summary Clinical Pharmacology microbiology, Table 98, pg. 239)
[¥]Blue box denotes largest MIC in which early clinical response (ECR) by pathogen is >10 in pooled Phase 3 microITT population (Summary Clinical Pharmacology microbiology, Table 102 and 103, pg. 249, 251.
[¥¥]Gray box denotes PTA ≥0.9
[¥¥¥]Food-effect results were derived from 20 healthy subjects (Study 1107).
ELF = epithelial lining fluid; MIC = minimum inhibitory concentration; IV = intravenous; PO = by mouth; PD = pharmacodynamic

Applicant's Conclusions

The results of the PK-PD target attainment analyses provide support for the dose selection of lefamulin 150 mg IV q12h and 600 mg orally q12h for subjects with CABP. Percent probabilities of attaining median total-drug ELF or free-drug plasma AUC/MIC targets associated with a 1-$\log_{10}$ CFU reduction from baseline for *S. pneumoniae* or *S. aureus* on Day 1 exceeded 90% at the MIC_{90} values for each pathogen after administration of IV or oral dosing regimens, irrespective of fed or fasting conditions.

Reviewer's Conclusions: *The plasma protein binding of lefamulin (73% to 88%) appears to be underestimated in Study EVT-00756-3781 since plasma protein binding was determined using pooled blank plasma diluted to 85% (v/v) following the addition of lefamulin solution. Lefamulin plasma protein binding should be 94% to 97%, as estimated in Studies 1010 and 1011, where plasma protein binding was determined directly from plasma collected from subjects administered intravenous lefamulin. Importantly, the plasma protein binding values from Studies 1010 and 1011 are comparable to estimates obtained in Study XS-1103, where pooled blank adult plasma was used without dilution.*

The Applicant wanted to choose a randomly assigned target that is lognormally distributed; However, a review of the code submitted with this application showed a randomly assigned target based on a normal distribution.

Other potential ELF models to account for concentration-dependent changes in lung penetration ratio were not explored.

Based on simulations, the Applicant found a slightly higher food-effect estimate than what was determined by noncompartmental analysis of the dedicated food-effect study (NAB-BC-3781-1107). From the food-effect study, the geometric mean ratios (GMRs) for Fed/Fasted after a high calorie/high fat meal 1 hour before dosing was 0.66 to 0.80 for AUC_{0-12}. The population PK estimate suggests a 41% lower AUC_{0-12} for oral lefamulin in the fed state compared with IV dosing. While broadly in agreement the difference could be attributed to study heterogeneity incorporated in the population PK model. Population PK findings suggest that food reduces the absorption rate constant of oral lefamulin. The PTA analysis using 41% lower AUC for fed state compared to IV dosing are preferred for clinical decision making; specifically managing risks to efficacy.

Reviewer's Analysis

During the review, we considered the impact of a greater fraction of bound lefamulin on target attainment analyses based on our interpretation of the plasma protein binding data (See Section 16.3.1.1) and reevaluated the nonclinical PK-PD relationship (Table 142). Additionally, when simulating $\log_{10}$ normal data (for the random target[10]) the arithmetic mean (m) and standard deviation (sd) were used to derive the corresponding parameters for the underlying normal distribution of $\log_{10}$ data. Consequently, the following formulas were used:

$$mu = \log\left(\frac{m^2}{\sqrt{sd^2 + m^2}}\right)$$

$$sigma = \sqrt{\log\left(1 + \left(\frac{sd^2}{m^2}\right)\right)}$$

[10] PD target variability (i.e., AUC_{0-24}/MIC) incorporated by randomly estimating a target value based upon an observed mean and standard deviation (murine lung infection PKPD studies) and truncated (2 SD) $\log_{10}$ normal distribution.

Table 142. Reviewer's Day 1 Lefamulin Exposure Measures and Target Attainment Analysis[a] by Dosing Regimens and by MIC for *S. pneumoniae* and *S. aureus*

			S. pneumoniae MIC [mcg/mL]				*S. aureus* MIC [mcg/mL]			
			0.12	**0.25[d]**	**0.5**	**1**	**0.12**	**0.25**	**0.5[e]**	**1**
Median PD Target	ELF	IV	1.0	1.0	1.0	0.96	1.0	1.0	0.99	0.61
		PO-Fast	1.0	1.0	0.99	0.86	1.0	1.0	0.94	0.45
		PO-Fed	1.0	1.0	0.93	0.46	0.99	0.98	0.68	0.09
	Plasma	IV	1.0	1.0	0.96	0.29	1.0	0.99	0.63	0.0
		PO-Fast	1.0	0.99	0.87	0.20	1.0	0.95	0.48	0.01
		PO-Fed	1.0	0.93	0.47	0.02	0.98	0.69	0.10	0.0
Random PD Target	ELF	IV	1.0	0.99	0.90	0.63	1.0	0.98	0.84	0.46
		PO-Fast	1.0	0.97	0.84	0.55	1.0	0.96	0.77	0.42
		PO-Fed	0.9	0.90	0.66	0.35	0.98	0.85	0.55	0.20
	Plasma	IV	0.99	0.89	0.64	0.27	0.99	0.84	0.48	0.13
		PO-Fast	0.98	0.84	0.56	0.27	0.97	0.78	0.43	0.11
		PO-Fed	0.91	0.67	0.36	0.10	0.86	0.56	0.21	0.03

[a]Probability of target attainment (PTA) based on median or randomly assigned AUC/MIC targets associated with a 1-$\log_{10}$ CFU reduction from baseline. PPB was assumed linear and fixed at 0.0379. Consequently, a lung penetration ratio (LPR) of 20 found and a proportional model (Concertation ELF (t)= LPR * Concentration plasma (t) used to estimate ELF $AUC_{0\text{-}24}$. Drawing from 3101 patients we ran 1032 virtual patients. Drawing from 3102 patients we ran 1452 virtual patients.
[b]Units $mg{\cdot}L^{-1}$
[c] Units $mg{\cdot}hrs{\cdot}L^{-1}$
[d]*S. pneumoniae* MIC_{90} from Pooled Phase 3 microITT analysis (Summary Clinical Pharmacology microbiology, Table 98, pg. 239)
[e]*S. aureus* MIC_{90} from Pooled Phase 3 microITT analysis (Summary Clinical Pharmacology microbiology, Table 98, pg. 239)
[¥]Blue box denotes largest MIC in which early clinical response (ECR) by pathogen is >10 in pooled Phase 3 microITT population (Summary Clinical Pharmacology microbiology, Table 102 and 103, pg. 249, 251.
[¥¥]Gray box denotes PTA ≥0.9
[¥¥¥]Food-effect results were derived from 20 healthy subjects (Study 1107).
ELF = epithelial lining fluid; MIC = minimum inhibitory concentration; IV = intravenous; PO = by mouth; PD = pharmacodynamic

16.3.2.6. Physiologic Based Pharmacokinetic Modeling

16.3.2.6.1. Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's PBPK study report, entitled "PBPK Model Development Report - Study Report" to support the intended uses. Specifically, the PBPK analyses were used to evaluate the effects of CPY3A/P-gp inhibitors (ketoconazole, fluconazole, and fluvoxamine) and inducers (rifampin and efavirenz) on the PK of IV and oral lefamulin; the effect of IV and oral lefamulin on the PK of CYP3A, P-gp, OATP/BCRP, $OAT_{1/2}$/MATE substrates; and the effect of elevated gastric pH on the PK of oral lefamulin.

The Division of Pharmacometrics has reviewed the PBPK report, supporting modeling files, and the Applicant's responses to FDA's information request (IR) submitted on Mar. 18, 2019, and concluded the following:

- Due to the uncertainties associated with the lefamulin (substrate) model structure, the parameter value estimation and the noninclusion of liver secretion clearance in the model, along with the possibility of underestimating P-gp substrate sensitivity of lefamulin, the Applicant's model is inadequate for the Drug-Drug Interaction (DDI) assessment for lefamulin as a victim with CYP3A and P-gp modulators.
- Due to the uncertainties associated with the lefamulin (substrate) model structure, the parameter value estimation and the noninclusion of liver secretion clearance in the model, the Applicant's model is inadequate to predict the effect of lefamulin on the PK of digoxin. However, based on the observed clinical DDI results between lefamulin and digoxin, the effect of lefamulin on a drug PK, which is a P-gp substrate, is expected to be low.
- The Applicant's perfusion rate-limited PBPK model may not be adequate to characterize the liver disposition of lefamulin. The estimated effects of lefamulin on the PK of CYP3A substrates, which is driven by the unbound intrahepatic lefamulin concentration, may be biased.
- The Applicant's perfusion rate-limited PBPK model may not be adequate to characterize the liver disposition of lefamulin. The estimated effects of lefamulin on the PK of OATP and BCRP substrate, which is driven by the unbound plasma and intrahepatic lefamulin concentration, may be biased.
- The Applicant's perfusion rate-limited PBPK model may not be adequate to characterize the kidney disposition of lefamulin. The estimated effects of lefamulin on the systemic or kidney PK of metformin, which is driven by the unbound plasma and intracellular renal lefamulin concentration, may be biased.
- The Applicant's lefamulin model using in vitro dissolution profiles as model input was inappropriate to assess the effect of elevated gastric pH on the PK of oral lefamulin because the model was not able to describe the observed lefamulin PK following oral administration.

16.3.2.6.2. Pharmacokinetics

Lefamulin is formulated as an acetate salt in both IV and oral formulations. The absorption of orally administered lefamulin was rapid with a bimodal peak, starting with an initial plasma concentration peak (C_{max1}) occurring 20 minutes to 1 hour after dosing followed by a second concentration peak (C_{max2}) occurring between 1 and 4 hours after dosing. The absolute bioavailability (F_a) of lefamulin was reduced from 25.8% under fasted condition to 21% under fed condition in healthy subjects. (Summary of Clinical Studies)

Lefamulin is proposed to be approximately 73% to 88% bound to plasma protein, demonstrating saturable, nonlinear binding as a function of lefamulin concentrations ranging from 1 μg/mL to 10 μg/mL. Lefamulin distributes rapidly into tissues with the volume of distribution at steady state (V_{ss}) of 116 L to 160 L. The V_{ss} of lefamulin showed a nonlinear increase with the dose. After repeated dosing, independent of the route of administration (IV or oral), steady-state was reached after 2 days of every 12 hours (q12h) treatment and trough levels (C_{min}) remained constant throughout the duration of the treatment. (Summary of Clinical Studies)

In plasma, unchanged lefamulin accounts for the majority of the circulating total drug related material (total radioactivity) (IV: 76%; oral: 58%). The remaining 24% and 42% of lefamulin, respectively, are metabolized, primarily driven by CYP450 phase I reactions, leading mainly to hydroxylated metabolites. BC-8041 is the main metabolite and showed no relevant antibacterial activity. BC-8041 is the only metabolite in plasma accounting for more than 10% (13.6% to 17.3%) of total drug related material (total radioactivity) after oral dosing. After IV dosing, all metabolites were well below 10% (≤6.7%) compared with total radioactivity. (Summary of Clinical Studies)

Lefamulin and its metabolites are predominantly eliminated via the fecal route. A total of 77.3% and 88.5% of the administered radioactivity were recovered in feces following IV and oral administration, respectively; 7.8% to 24.8% and 4.2% to 9.1% of the dose were excreted in feces as unchanged lefamulin after oral and IV dosing, respectively. In urine, 15.5% (9.6% to 14.1% as unchanged lefamulin) and 5.3% (4.2% to 9.1%) of the total radioactivity were recovered after IV and oral dosing, respectively. (Summary of Clinical Studies)

16.3.2.6.3. Drug Interaction

In Vitro Studies

In vitro studies showed that lefamulin is a CYP3A, P-gp and OCT-1 substrate, a competitive inhibitor for CYP3A, an inhibitor for efflux transporters BCRP and P-gp, uptake transporter OCT1 and efflux transporters MATE1 and MATE2-K and a very weak inhibitor for uptake transporters OATP1B1 and OATP1B3.

Table 143. Identification of CYP Enzymes Involved in Lefamulin Primary Oxidative Metabolism in Recombinant Human CYP Enzymes and Hepatocytes, and Transporters Involved in Lefamulin Transport in the Intestine and Liver

Enzymes/ Transporters	In Vitro System	Parameters	Sources
CYP3A5	Recombinant human CYP3A5	CL_{int} =4.43 µL/min/pmol	Study 15570
CYP3A4	Recombinant human CYP3A4	CL_{int} =11.47 µL/min/pmol	Study 15570
Pooled enzymes	Pooled human hepatocytes	CL_{int} =12.5 µL/min/million cells	PBPK report (in house data, study report was not provided)
P-gp	Caco-2 cells	Efflux ratio (ER)=68 (10µM)	Study 8NABRP3
P-gp	Caco-2 cells	K_m =110µM J_{max} =188 pmol/cm^2/min	Study 18NABRP1
		K_m =75.7µM J_{max} =74.2 pmol/cm^2/min	Study 18NABRP6
P-gp	SIVA v2.0 toolkit	K_m =0.1µM J_{max} =403.8 pmol/min	PBPK Report
OCT1	OCT-1 transfected HEK293 cells	K_m =18.7µM J_{max} =417 pmol/cm^2/min	Study 12FOREP4R1-85737

CL_{int} = apparent intrinsic clearance

Table 144. Evaluation of Lefamulin as an Inhibitor of Drug Metabolizing Enzymes in Human Liver Microsomes, or Inhibitor of Transporters in in Vitro Cell Systems

Enzymes/ Transporters	Probe Substrate/ Metabolite	In Vitro System	Mechanism	Parameters	Sources
CYP3A4/5	Midazolam/1'-hydroxymidazolam	Human liver microsomes	Competitive inhibition	K_I =0.86µM	Study XT125055
BCRP	Estrone-3-sulfate	BCRP M membrane	Efflux transporter inhibition	IC_{50}=128.6µM	Study Nabriva-03a-23Jun2015
		Caco-2 cells	Efflux transporter inhibition	IC_{50}=42.18µM	Study VV-NAB-NC-000350
P-gp	N-methyl quinidine	MDR1-K membrane	Efflux transporter inhibition	IC_{50}=13.76µM	Study Nabriva-03a-23Jun2015
	Digoxin	Caco-2 cells	Efflux transporter inhibition	IC_{50}=34.1µM	Study 8NABRP5P2-3781
OCT-1	MPP^+	OCT-1 transfected HEK293 cells	Uptake transporter inhibition	IC_{50}=20.3µM	Study 12FOREP4R1-85736
MATE-1	Metformin	MATE-1 transfected MDCKII cells	Efflux transporter inhibition	IC_{50}=0.297µM	Study Nabriva-03c-23Jun2015
MATE2-K	Metformin	MATE2-K transfected MDCKII cells	Efflux transporter inhibition	IC_{50}=76.4µM	Study Nabriva-03c-23Jun2015

Enzymes/ Transporters	Probe Substrate/ Metabolite	In Vitro System	Mechanism	Parameters	Sources
OATP1B1	Atorvastatin	OATP1B1 transfected HEK293 cells	Uptake transporter inhibition	IC_{50}=122µM	Study 12FOREP4R1-85736
OATP1B3	Atorvastatin	OATP1B3 transfected HEK293 cells	Uptake transporter inhibition	IC_{50}=122µM	Study 12FOREP4R1-85736

Relevant Clinical DDI Studies

Table 145. Results of Clinical DDI Studies Conducted by Applicant Between Lefamulin and CYP Enzyme Substrates, CYP Enzyme and P-gp Modulators or P-gp Substrate

Modulator/Substrate		Dosing Regimen	Observed Parent C_{max}R and AUCtR	Sources
Lefamulin (IV) as a victim with CYP3A/P-gp modulators				
Ketoconazole	CYP3A/P-gp inhibitor	Lefamulin: IV infusion, 1hr, 150 mg, day 1 and 1 hour after the morning dose of ketoconazole on day 7 Ketoconazole: oral, 200 mg, bid, day 4 to day 7	C_{max}R: 1.06 AUCtR: 1.26	Study 1006
Rifampin	CYP3A/P-gp inducer	Lefamulin: IV infusion, 1 hr, 150 mg, day1 and 11 Rifampin: oral, 600 mg, qd, day 3 to day 12	C_{max}R: 0.92 AUCtR: 0.73	Study 1108
Lefamulin (IV) as a perpetrator with CYP enzyme substrates				
Midazolam	CYP3A substrate	Lefamulin: IV infusion, 2 hrs, 150 mg Midazolam: oral, 2 mg, dosed 1 hr after the start of dosing with lefamulin	C_{max}R: 1.03 AUCtR: 1.15	Study 1004
Lefamulin (Oral) as a victim with CYP3A/P-gp modulators				
Ketoconazole	CYP3A/P-gp inhibitor	Lefamulin: oral, 400 mg, day 1 and day 6 administered with the morning dose of ketoconazole Ketoconazole: oral, 200 mg, bid, day 3 to day 6	C_{max}R: 1.58 AUCtR: 2.44	Study 1103
Rifampin	CYP3A/P-gp inducer	Lefamulin: Oral, 600 mg, day 1 and day 11 Rifampin: oral, 600 mg, qd, day 3 to day 12	C_{max}R: 0.43 AUCtR: 0.28	Study 1108
Lefamulin (Oral) as a perpetrator with CYP enzyme and P-gp substrates				
Midazolam	CYP3A substrate	Lefamulin: Oral, 600 mg, bid, day 2 to day 5 Midazolam: Oral, 2 mg, day 1 and day 5, on day 5 dosed at the same time as the morning dose of lefamulin under fasting condition	C_{max}R: 2.03 AUCtR: 3.07	Study 1110
Midazolam	CYP3A substrate	Lefamulin: Oral, 600 mg, bid, day 2 to day 9 Midazolam: Oral, 2 mg, day 1 and day 3, on day 3 dosed at the same time as the morning dose of lefamulin under fasting condition	C_{max}R: 1.76 AUCtR: 2.62	Study 1111
Midazolam	CYP3A substrate	Lefamulin: Oral, 600 mg, bid, day 2 to day 9 Midazolam: Oral, 2 mg, day 5, dosed 2 hr after the morning dose of lefamulin under fasting condition	C_{max}R: 2.21 AUCtR: 2.88	Study 1111

Modulator/Substrate		Dosing Regimen	Observed Parent $C_{max}R$ and AUCtR	Sources
Midazolam	CYP3A substrate	Lefamulin: Oral, 600 mg, bid, day 2 to day 9 Midazolam: Oral, 2 mg, day 7, dosed 4 hr after the morning dose of lefamulin under fasting condition	$C_{max}R$: 1.92 AUCtR: 2.55	Study 1111
Digoxin	P-gp substrate	Lefamulin: Oral, 600 mg, bid, day 5 to day 10 Digoxin: Oral, 0.5 mg, day 1 and day 8	$C_{max}R$: 1.05 AUCtR: 1.00	Study 1109

R: ratio of test over reference product; C_{max} = maximum plasma concentration of drug; AUC = area under the concentration-time curve

16.3.2.6.4. Part A: DDI assessment

Applicant's PBPK Modeling Effort

PBPK Software

Simcyp V16 (Simcyp Ltd, UK) was used to develop the PBPK models and predict the effects of lefamulin on the PK of midazolam, ethinyl estradiol, zolpidem, repaglinide, rosuvastatin, metformin, and digoxin, and the effects of ketoconazole, fluconazole, fluvoxamine, rifampin and efavirenz on the PK of lefamulin.

Model Development

Lefamulin

The absolute oral bioavailability of a 600 mg IR tablet formulation of lefamulin were 25.8% and 21.0% under the fasted and fed condition, respectively, in healthy subjects. The calculated fh (fraction of administered drug passing the liver into the systemic circulation) from the value of CLIV is 0.70. The predicted fg (fraction of administered drug passing the gut wall into the portal vein) from the Qgut model is 0.93. Therefore, the calculated f_a (fraction of administered drug entering enterocytes) for the IR tablet is 0.40 (=0.258/0.70/0.93) and this was used in the model to optimize lefamulin intestinal permeability and K_m of intestine P-gp.

The Advanced Dissolution, Absorption & Metabolism (ADAM) module within the Simulator was applied to predict the absorption of lefamulin. A mechanistic effective permeability (MechPeff) model was used as the permeability input and a *Ptrans,0* value (=21400 × 10^{-6} cm/s) was used based on calibrating against the Caco-2 *Papp* value. The in vitro Jmax value (=403.8 pmol/min) of P-gp efflux transport was directly used in the model. The intestinal P-gp K_m was optimized from the clinical data based on the recovery of fa and C_{max}, as well as the observed AUC in the absence or presence of ketoconazole (Table 145, Study NAB-BC-3781-1103). The lefamulin P_{eff} in Jejunum I (the region where the majority of the absorption occurs) was also optimized to

improve the recovery of C_{max}. An intestinal P-gp K_m of 10µM and the P_{eff} in Jejunum I of 4×10^{-4} cm/s was used in the final model.

The lefamulin volume of distribution at steady state (V_{ss}) showed a nonlinear increase, with a value ranging from 85.8 L to 253 L following the intravenous administration of 25 mg to 400 mg lefamulin. The POP-PK analysis indicated that the observed dose-dependent increase in V_{ss} can be potentially attributed to the nonlinearity in the plasma protein binding. The fraction of unbound lefamulin in plasma (f_u) reported by the Applicant was 12.1%, 17.1% and 27.3% at lefamulin concentrations of 1, 3 and 10 µg/ml by equilibrium dialysis. The concentration-dependent plasma protein binding was not incorporated in the lefamulin PBPK model. The lowest f_u of 0.121 was used, as the predicted V_{ss} (1.8 L/kg) matched the estimated V_{ss} (1.81 L/kg) based on the clinical study data following a single or multiple intravenous administration of lefamulin (Table 145, Study-NAB-BC-3781-1001, 1002, 1003, and 1107).

The lefamulin hepatic intrinsic clearance (CL_{int}) was back-calculated from the observed total clearance (CL_{iv}) using a well-stirred liver model. Based on the clinical DDI study results between lefamulin and ketoconazole or rifampin, 31% of hepatic intrinsic clearance was assigned to CYP3A4 and the rest to $CL_{int,others}$ (additional systemic clearance). The P-gp mediated luminal efflux was included in the model to recover the observed DDI between oral administered lefamulin and ketoconazole. A value of 403.8 pmol/min (Table 143, SIVA v2.0 toolkit) was chosen for lefamulin J_{max} for P-gp in the model and the K_m was optimized based the observed DDI results between lefamulin and ketoconazole. Since the predicted total lefamulin CLiv of 18.7 L/hr based on the hepatic CL_{int} determined in human hepatocyte is comparable to the reported average CL_{iv} of 21.4 L/h from the clinical studies, the hepatic uptake transporter is thought to play a limited role in vivo and has not been incorporated in the lefamulin PBPK model. It was assumed that the DDI between lefamulin and ketoconazole was arising from the inhibitory effect of ketoconazole on CYP3A and intestinal P-gp. Renal clearance (CL_R =1.6 L/h) is a minor clearance pathway (fe =10%).

The CYP3A inhibitory parameter (K_i) was optimized based on the clinical interaction study results with midazolam (NAB-BC-3781- 1110 and 1111). A CYP3A4 K_I value of 0.86 and 0.2µM was used in the model to describe the lefamulin-mediated CYP3A4 inhibition kinetics following intravenous and oral administration of lefamulin, respectively. The in vitro K_i values ($K_i = IC_{50}$) for transporters BCRP (IC_{50}: 42.2µM), OATP1B1 (IC_{50}: 122µM) and OATP1B3 (IC_{50}: 122µM) were used in the lefamulin model. Simulations using a 10-fold lower K_i value for OATP1B1 and 1B3 were performed to account for the uncertainty in the in vitro K_i values for OATP1B1 and 1B3. The in vitro K_i values ($K_i = IC_{50}$) for transporters MATE1 (IC_{50}: 0.297µM), OCT2 (IC_{50}: 122µM) and OCT1 (IC_{50}:20.3µM) were used in the lefamulin model. Simulations using a 20-fold lower K_i value for MATE1 and $OCT_{1/2}$ were also performed to account for the uncertainty in the in vitro K_i values for MATE1 and $OCT_{1/2}$.

FDA's Assessment

(1) Fraction absorbed

The Applicant calculated fa is 0.4 and the fa value of 0.4 was used in lefamulin oral absorption model parameter optimization (lefamulin intestinal permeability and K_m of intestine P-gp) to better recover the clinical PK data. However, the estimated f_a based on the amount of parent drug excreted in feces should be greater than or equal to a value ranging from 0.75 to 0.92 (Mass balance study, NAB-BC-3781-1013). There was no adequate justification provided in the submitted PBPK report or the response to the FDA's Information Request with respect to the lower f_a value of 0.4 used in the model development.

(2) Intestinal permeability and K_m value of intestinal P-gp

The lefamulin intestinal permeability and K_m of intestine P-gp were optimized based on the recovery of f_a (0.4) and observed lefamulin C_{max} and AUC in the presence and absence of ketoconazole. As aforementioned, due to the inconsistency of f_a value used in the model compared to the clinical observed data in the mass balance study (NAB-BC-3781-1013), the uncertainty associated with the estimated lefamulin intestinal permeability and K_m value of intestinal P-gp cannot be excluded.

(3) Liver P-gp

In the in vitro study, lefamulin was characterized as a P-gp substrate with an efflux ratio (ER) of 68. A few different K_m values were reported in the different test systems, ranging from 0.1 μM to 110 μM (Table 143). The Applicant's model incorporated P-gp in the intestine to account for the potential interaction via intestine P-gp. However, the DDI between lefamulin and P-gp modulator in the liver needs to be evaluated given the uncertainty associated with the lefamulin K_m value for P-gp. On Mar. 18, 2019, an information request was issued requesting the evaluation of the potential DDI between lefamulin and a P-gp modulator in the liver. FDA's evaluation of the Applicant's response is provided in the result section.

(4) Permeability rate-limited liver model

a. The in vitro intrinsic clearance obtained from hepatocyte incubation was about 40-fold lower compared with that obtained using recombinant CYP3A4 and the predicted CLiv (18.7 L/hr) using in vitro intrinsic clearance obtained from hepatocyte incubation is comparable to the reported average CLiv of 21.4 L/h. This indicates that the overall hepatic clearance may be uptake rate-limited. Thus, it may be necessary to incorporate the permeability rate-limited liver and kidney in the PBPK model to describe the tissue disposition of lefamulin for the purpose of evaluating DDI driven by the intracellular unbound lefamulin concentration.

b. As a perfusion rate-limited instead of a permeability rate-limited liver model was used in the Applicant's model to describe the disposition of lefamulin, the estimated fmCYP3A4 (0.31) was likely underestimated and the calculated fh based on a perfusion rate-limited liver model maybe biased.

c. The Applicant's model did not account for the active uptake of lefamulin by OCT1 in the liver based on an assumption that OCT1 did not play an important role on the drug uptake in a perfusion limited liver model. However, the active uptake of lefamulin by OCT1 in the liver is likely rate-determining in hepatic clearance of lefamulin and needs to be considered in a permeability rate-limited liver model.

Perpetrator Drugs

Fluconazole, fluvoxamine, and efavirenz

The default PBPK models of fluconazole, fluvoxamine, and efavirenz in SimCYP V16 were used without any modification for DDI prediction.

Ketoconazole

The default PBPK model of ketoconazole in SimCYP V16 was used with one modification. An in vitro P-gp K_I of 0.028µM for ketoconazole was used in the simulation. This value was obtained by applying a 15-fold correction factor to the lowest reported in vitro P-gp K_I of 0.42µM (Kishimoto *et al*., 2014[11]) determined in Caco-2 cells using digoxin (1µM) as the probe substrate.

Rifampicin

The default rifampicin model within SimCYP V16 was used with one modification. To incorporate the induction effect on intestinal P-gp by rifampicin treatment (600 mg/d for 10 days), an intestinal P-gp relative expression factor (REF) value of 3.5 was used in the lefamulin model, assuming a 3.5-fold increase in P-gp activity following rifampicin treatment. The assumed 3.5-fold increase in intestinal P-gp activity was based on in vivo studies in which duodenal biopsies were obtained from subjects treated with multiple doses of rifampicin and P-gp expression was quantified by western blotting (Greiner *et al*., 1999[12]).

Victim Drugs

Midazolam, zolpidem, repaglinide, rosuvastatin, and digoxin

The default PBPK models of midazolam, zolpidem, repaglinide, rosuvastatin, and digoxin in SimCYP V16 were used without any modification for DDI prediction.

[11] Kishimoto W, Ishiguro N, Ludwig-Schwellinger E, Ebner T, Schaefer O. In vitro predictability of drug-drug interaction likelihood of P-glycoprotein-mediated efflux of dabigatran etexilate based on [I]2/IC_{50} threshold. Drug Metab Dispos. 2014 Feb;42(2):257-63.

[12] Greiner B1, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, Kroemer HK. The role of intestinal P-lycoprotein in the interaction of digoxin and rifampin. J Clin Invest. 1999 Jul;104(2):147-53.

Ethinyl estradiol

The ethinyl estradiol PBPK model is not available in SimCYP V16. A published ethinyl estradiol PBPK model was used (Ezuruike *et al*., 2018[13]). The parameter values for ethinyl estradiol physico-chemical property and ADME in the Applicant's ethinyl estradiol PBPK model are consistent with the default ethinyl estradiol PBPK model in SimCYP V17.

Metformin

The default PBPK models of metformin in SimCYP V16 was used for DDI prediction. The electrochemical gradient (EGD) model was applied for modeling of renal OCT2 transport.

FDA's Assessment:

(1) As the K_m (P-gp transport) of lefamulin was optimized based on the observed DDI between lefamulin and ketoconazole, the application of lowest ketoconazole K_i for P-gp in the model may artificially reduce the sensitivity of lefamulin acting as a P-gp substrate. Thus, the predicted DDI magnitude between lefamulin and other P-gp inhibitors tends to be underestimated.

(2) It appears reasonable to assume a 3.5-fold increase in P-gp activity following rifampicin treatment. Literature reported that a 3.5-fold increase in intestinal P-gp protein expression after coadministration of rifampin[14] and the predicted decreases in AUC and C_{max} of digoxin as a result of a 3.5-fold intestinal P-gp induction following administration of rifampicin (600 mg qd for 9 days) were broadly consistent with the clinically observed data.[15]

PBPK Model Verification

Fluconazole, fluvoxamine, and efavirenz, midazolam, zolpidem, repaglinide, rosuvastatin, and digoxin, ethinyl estradiol and metformin

The default PBPK models in SimCYP V16 for fluconazole, fluvoxamine, and efavirenz, midazolam, zolpidem, repaglinide, rosuvastatin, and digoxin, ethinyl estradiol and metformin were used for DDI predictions without further model verification. The model performance verification for these drugs conducted within the SimCYP was provided.

[13] Ezuruike U, Humphries H, Dickins M, Neuhoff S, Gardner I, Rowland Yeo K. Risk-Benefit Assessment of Ethinylestradiol Using a Physiologically Based Pharmacokinetic Modeling Approach. Clin Pharmacol Ther. 2018 Dec;104(6):1229-1239.

[14] J Clin Invest. 1999 Jul 15; 104(2): 147–153. Bernd Greiner,1Michel Eichelbaum, Peter Fritz, Hans-Peter Kreichgauer, Oliver von Richter, Johannes Zundler, and Heyo K. Kroemer. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest. 1999 Jul 15; 104(2): 147–153.

[15] Neuhoff S, Yeo KR, Barter Z, Jamei M, Turner DB, Rostami-Hodjegan A. J Pharm Sci. Application of permeability-limited physiologically-based pharmacokinetic models: part I-digoxin pharmacokinetics incorporating P-glycoprotein-mediated efflux. 2013 Sep;102(9):3145-60.

Ketoconazole

The modified ketoconazole PBPK model by assigning a P-gp K_i of 0.028µM in the model was not further verified.

Rifampicin

The verification of modified rifampicin PBPK model by assigning an intestinal P-gp REF value of 3.5 to account for the induction effect on intestinal P-gp by rifampicin treatment was reported in the literature.[16]

Lefamulin

The lefamulin model was verified against observed PK following single or multiple intravenous or oral administration of lefamulin in healthy subjects, and the DDI study results between lefamulin and ketoconazole, rifampin, or midazolam.

PBPK Model Application

The developed PBPK models were used to simulate the DDIs for lefamulin in the following scenarios.

(1) To predict the effect of IV and oral lefamulin on ethinyl estradiol (a CYP3A substrate), zolpidem (a CYP3A substrate) and repaglinide (a CYP3A and CYP2C8 substrate) PK at steady-state in healthy subjects.

(2) To predict the effect of IV and oral lefamulin on rosuvastatin (an OATP and BCRP substrate), metformin (an OCT1 and MATE substrate) PK at steady-state in healthy subjects.

(3) To predict the effect of efavirenz (a moderate CYP3A inducer), fluvoxamine (a moderate CYP3A4 inhibitor), and fluconazole (a moderate CYP3A inhibitor) on IV and oral lefamulin PK at steady-state in healthy subjects.

Results

Lefamulin Model Verification

Figure 17 shows the simulated lefamulin PK profiles following a single intravenous, oral or multiple oral administration of lefamulin in healthy subjects. The C_{max} and AUC values obtained from model simulation and clinical studies (Table 145, Study 1003, Study 1005, Study 1006 and

[16] Neuhoff S, Yeo KR, Barter Z, Jamei M, Turner DB, Rostami-Hodjegan A. J Pharm Sci. Application of permeability-limited physiologically-based pharmacokinetic models: part I-digoxin pharmacokinetics incorporating P-glycoprotein-mediated efflux. 2013 Sep;102(9):3145-60.

Study 1008) are presented in Table 146. The simulated C_{max} and AUC values are in line with the observed data following a single intravenous, oral or multiple oral administration of lefamulin.

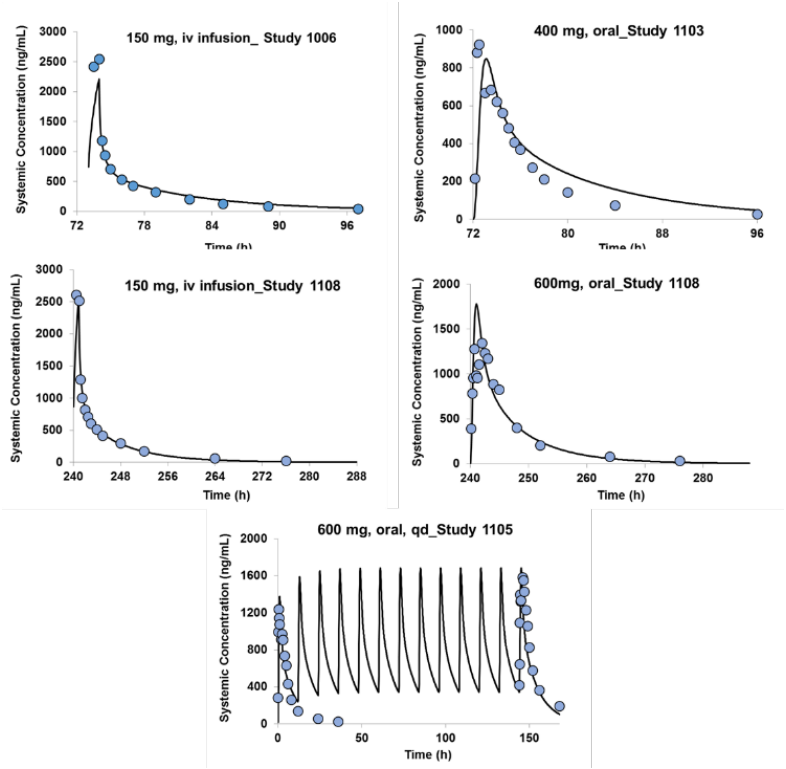
FDA's Assessment: As aforementioned in the 'lefamulin model development' section, due to the uncertainties associated with the lefamulin (substrate) model structure, the parameter value estimation, such as f_a, intestinal permeability, fmCYP3A, lefamulin K_m for P-gp, the noninclusion of liver secretion clearance in the model, and the possibility of underestimating the P-gp substrate sensitivity of lefamulin, the Applicant's lefamulin model is inadequate to predict the effect of enzyme or transporter modulators on lefamulin PK although the model was able to describe the observed PK.

Table 146. Observed and Simulated Lefamulin C_{max} and AUC and the C_{max} and AUC Ratios Following a Single Intravenous (150 mg IV Infused Over 1 hr), Oral (600[a] or 400[b] mg) or Multiple Oral Administration (600 mg BID for 6 Days) of Lefamulin in Healthy Subjects

Dose	C_{max} (ng/mL)[c] Obs./Pred./$R_{Pred/Obs}$	AUC (ng*h/mL)[c] Obs./Pred./$R_{Pred/Obs}$	Sources
Single dose IV	2551 / 2209 / 0.87	7044 / 7341 / 1.04	NAB-BC-3781-1006
Single dose IV	2630 / 2570 / 0.98	8960 / 7868 / 0.88	NAB-BC-3781-1108
Single dose oral[a]	1590 / 1675 / 1.05	10500 / 8579 / 0.82	NAB-BC-3781-1108
Single dose oral[b]	1037 / 883 / 0.85	4242 / 5359 / 1.26	NAB-BC-3781-1103
Multiple dose oral	Day 1: 1463 / 1433 / 0.98 Day 7: 1850 / 1739 / 0.94	Day 1[d]: 6350 / 6626 / 1.04 Day 7[e]: 10803 / 11422 / 1.06	NAB-BC-3781-1105

[c]: The data were presented as mean value for Study 1006, Study 1103, Study 1105, and Study 1108 and geometric mean value for Study 1109.
[d]: $AUC_{0\text{-}12h}$
[e]: $AUC_{0\text{-}24h}$
IV = intravenous; C_{max} = maximum plasma concentration of drug; AUC = area under the concentration-time curve; BID = twice a day
Source: predicted and observed data were obtained from Applicant PBPK report and the relative clinical studies, respectively.

Figure 17. Observed (Blue Dots) and Simulated (Black Lines) Lefamulin Concentration-Time Profiles Following a Single Intravenous, Oral or Multiple Oral Administration of Lefamulin in Healthy Subjects



Source: Applicant's PBPK submission package

Model DDI Predictive Performance Evaluation

Lefamulin as a victim drug

Assessment of the effects of ketoconazole (a dual inhibitor of CYP3A and P-gp) on lefamulin PK following a single intravenous or oral administration of lefamulin

FDA's assessment: The Applicant verified lefamulin PBPK model against the observed DDI between lefamulin (iv or oral) and ketoconazole and refined fmCYP3A, intestinal permeability, and intestinal lefamulin K_m for P-gp to better recover the interaction results. The correlation of model parameters (such as f_a, intestinal permeability, fmCYP3A4 and K_m values for P-gp) may cause the uncertainty in the estimation of these parameters, which was not addressed in the Applicant's PBPK report.

As the Applicant's model did not incorporate liver P-gp to account for lefamulin biliary clearance, per FDA's information request on March 28, 2019, the Applicant incorporated the liver P-gp in the model and reevaluated the DDI between lefamulin and ketoconazole. A value of 1.406 µL/min /million cells (7% of the CLiv) was assigned to describe P-gp mediated biliary clearance in the liver assuming that the fraction of the dose recovered (around 7%) in the feces

as unchanged lefamulin following an IV dose represents the dose that undergoes biliary clearance followed by enterohepatic recycling. However, the value of P-gp mediated biliary clearance (1.406 µL/min/million cells) is much smaller compared to the value assigned to the P-gp mediated efflux secretion clearance in the intestine (40.3 µL/min/cm^2).[17] There was no adequate justification provided in the response to the FDA's Information Request with respect to the different P-gp mediated secretion clearance estimates used in the model. In addition, the predicted fraction of the dose recovered in the feces as unchanged lefamulin following oral administration using Applicant's model is much higher than the observed value (Table 147). If the values of lefamulin V_{max} and J_{max} for intestinal P-gp used in the Applicant's model was also assigned to the liver P-gp, the predicted $C_{max}R$ and AUCR are much greater than the observed $C_{max}R$ and AUCR when intravenous or oral administration of lefamulin was given with ketoconazole (Table 147), indicating that lefamulin parameter values involved in the DDI between lefamulin and ketoconazole may not be appropriate. Therefore, the reevaluated DDI between lefamulin and ketoconazole was deemed inadequate to predict the effect of a P-gp inhibitor on the PK of lefamulin.

Table 147. Observed and Predicted $C_{max}R$, AUCR and Fraction of the Dose Recovered in the Feces as Unchanged Lefamulin Following Intravenous Infusion or Oral Administration of Lefamulin

	IV Lefamulin			Oral Lefamulin		
Parameter	$C_{max}R$	AUCR	Parent Drug in Feces (%)	$C_{max}R$	AUCR	Parent Drug in Feces (%)
Observed	1.06	1.26	4.2–9.1	1.58	2.44	7.8–24.8
Applicant's model[a]	1.06	1.29	4.9	1.90	2.41	60.4
FDA reviewer's analysis[b]	1.25	1.95	37.4	3.19	4.93	66.0

[a] Applicant's model: Hepatic efflux :1.406 µL/min/million cells; Intestinal efflux: J_{max} =403.8 pmol/min, K_m =10µM
[b] FDA Reviewer's analysis: Hepatic efflux :40.3 µL/min/million cells; Intestinal efflux: J_{max} =403 8 pmol/min, K_m =10µM
C_{max} = maximum plasma concentration of drug; AUC = area under the concentration-time curve; IV = intravenous
Source: observed data were from Study 1103; predicted results using applicant's model were from the response to the FDA's Information Request on March 28, 2019

Due to the uncertainties associated with the model structure, parameter value estimation, the noninclusion of liver secretion clearance in the lefamulin (substrate) model along with the possibility of underestimating P-gp substrate sensitivity of lefamulin, the Applicant's model verification based on the DDI between lefamulin and ketoconazole cannot be used as the basis for further DDI assessment between lefamulin and other CYP3A and P-gp inhibitors.

Assessment of the effects of rifampin (a dual inducer of CYP3A and P-gp) on lefamulin PK following a single intravenous or oral administration of lefamulin

FDA's Assessment: Due to the uncertainties associated with the lefamulin (substrate) model structure, the parameter value estimation and the noninclusion of liver secretion clearance in the model, the Applicant's model verification based on the DDI between lefamulin and

[17] assuming that P-gp in 1 million hepatocytes have the same P-gp activity as the P-gp available in 1 cm^2 of Caco-2 in the Transwell system

rifampicin cannot be used as the basis for further DDI assessment between lefamulin and other CYP3A and P-gp inducers.

Lefamulin as a Perpetrator Drug

DDI assessment of the perpetrator potential of IV and oral lefamulin on midazolam (a sensitive CYP3A substrate) PK

FDA's Assessment: To recover the observed midazolam AUCR and C_{max}R following the administration of lefamulin, different K_i values were used in the Applicant's model depending on the route of administration of lefamulin. A CYP3A4 K_i value of 0.86 and 0.2µM was used to predict the effect of lefamulin on oral midazolam PK following intravenous and oral administration of lefamulin, respectively. This may indicate that the Applicant's model prediction did not capture the lefamulin liver concentration appropriately. The unbound lefamulin liver concentration was highly likely overestimated by using the Applicant's perfusion rate-limited liver model. The same K_i value should be used for lefamulin in both IV and oral models.

By using a K_i value of 0.2µM, the observed DDI between lefamulin and midazolam following the oral administration of lefamulin was recovered, however, this may be attained by overestimating the DDI magnitude in the liver and underestimating the DDI magnitude in the intestine in a perfusion rate-limited model. Therefore, the predicted DDI between lefamulin and other CYP3A substrate with different f_h and f_g values than those of midazolam may be misleading. Therefore, the Applicant's model is inadequate to predict the effect of lefamulin on the PK of CYP3A substrates.

DDI assessment of the perpetrator potential of IV and oral lefamulin on digoxin (a P-gp substrate) PK

FDA's Assessment: Due to the uncertainties associated with the lefamulin (substrate) model structure, the parameter value estimation and the noninclusion of liver secretion clearance in the model, the Applicant's model was deemed inadequate to predict the effect of lefamulin on the PK of digoxin. However, based on the observed clinical DDI results between lefamulin and digoxin, the effect of lefamulin on a drug PK, which is a P-gp substrate, is expected to be low.

Model Application Evaluation

Model Application for Lefamulin as a Victim Drug DDI Evaluation

Assessment of the effects of efavirenz, fluconazole, or fluvoxamine on lefamulin PK following the intravenous or oral administration of lefamulin

FDA's Assessment: Due to the uncertainties associated with the lefamulin (substrate) model structure, the parameter value estimation and the noninclusion of liver secretion clearance in the model, along with the possibility of underestimating P-gp substrate sensitivity of lefamulin, the Applicant's model is inadequate for the DDI assessment for lefamulin as a victim with CYP3A and P-gp modulators.

Model Application for Lefamulin as a Perpetrator Drug DDI Evaluation

Assessment of the effects of lefamulin on ethinyl estradiol (CYP3A substrate), zolpidem (CYP3A substrate), and repaglinide (CYP3A and CYP2C8 substrate) PK following the intravenous or oral administration of lefamulin

FDA's Assessment: As aforementioned, the Applicant's perfusion rate-limited PBPK model may not be adequate to characterize the liver disposition of lefamulin. The estimated effects of lefamulin on the PK of CYP3A substrates, which is driven by the unbound intrahepatic lefamulin concentration, may be biased.

Assessment of the effects of lefamulin on rosuvastatin (OATP and BCRP substrate) PK following the intravenous or oral administration of lefamulin

FDA's Assessment: As aforementioned, the Applicant's perfusion rate-limited PBPK model may not be adequate to characterize the liver disposition of lefamulin. The estimated effects of lefamulin on the PK of OATP and BCRP substrate, which is driven by the unbound plasma and intrahepatic lefamulin concentration, may be biased. However, the effect of lefamulin on rosuvastatin (OATP and BCRP substrate) PK is expected to be low, given the possible low intrahepatic concentration (permeability rate-limited) and the weak in vitro inhibitory potencies of lefamulin on OATP and BCRP (Table 144).

***Assessment of the effects of lefamulin on metformin ($OCT_{1/2}$ and MATE substrate) PK following the intravenous or oral administration of* lefamulin**

FDA's Assessment: As aforementioned, the Applicant's perfusion rate-limited PBPK model may not be adequate to characterize the kidney disposition of lefamulin. The estimated effects of lefamulin on the systemic or kidney PK of metformin, which is driven by the unbound plasma and intracellular renal lefamulin concentration, may be biased. In addition, the in vitro study

showed that lefamulin inhibited MATE much stronger than OCT2 (Table 144), which may disrupt the balance between OCT-mediated uptake and MATE-mediated efflux of their common substrates. Hypothetically, this may lead to intracellular accumulation of $OCT_{1/2}$ and MATE substrates.

Conclusions

The Applicant's lefamulin PBPK model is not adequate to predict the effects of enzyme and transporter modulators on lefamulin PK and the effects of lefamulin on enzyme or transporter substrate PK due to the reasons described above. The effects of lefamulin on OATP, BCRP and P-gp substrate PK are expected to be low given the weak in vitro inhibitory potencies of lefamulin on OATP and BCRP and the clinically observed nonsignificant DDI between lefamulin and digoxin (a P-gp substrate). Hypothetically, the intracellular accumulation of $OCT_{1/2}$ and MATE substrates in the kidney could be increased due to the interaction between lefamulin and these transporters.

16.3.2.6.5. Part B: Assessment of the Effect of Gastric pH on the Absorption of Lefamulin

Applicant's PBPK Modeling Effort

PBPK Software

Simcyp V16 (Simcyp Ltd, UK) was used to develop the PBPK models and predict the effect of gastric pH on the absorption of lefamulin.

Model Development

The dissolution profile of an older, immediate-release (IR) 600-mg tablet at pH 1.0 and pH 6.8 was used as the dissolution inputs for stomach and small intestine, respectively, at the fasted state. The Applicant stated that the IR tablets and the current Phase 3 tablets showed comparable PK profiles in vivo (Studies 1105, 1107 and BC3-PK-02). Two sets of simulations were performed at gastric pH of 1.5 and 5.5, the latter to mimic pH-elevating effects from proton pump inhibitors.

PBPK Model Verification

The model prediction using in vitro dissolution profiles as input was not verified against the observed clinical PK data.

PBPK Model Application

The developed PBPK model using in vitro dissolution profiles at pH 1.0 and pH 6.8 as input was applied to assess the effects of gastric pH on lefamulin absorption.

Results

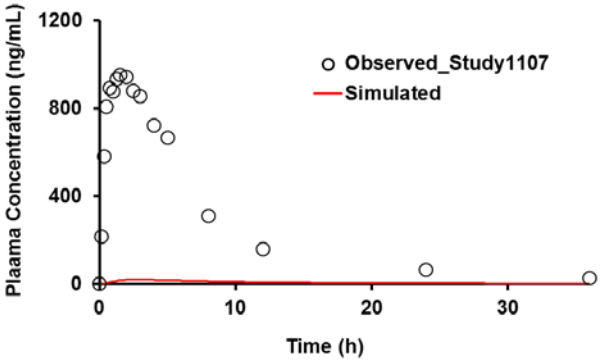
Assessment of Potential Gastric pH *Effect on the Absorption of Lefamulin*

FDA's Assessment: Lefamulin showed high aqueous solubility across physiological pH conditions. Dissolution rate of oral lefamulin tablets did not show pH dependent property. Therefore, it is not expected that changes in gastric pH would affect lefamulin oral absorption.

Nevertheless, the Applicant's model is inadequate to predict the effects of gastric pH on lefamulin absorption for the following reasons.

The Applicant did not verify the predictive performance of the model using in vitro dissolution profiles as input against the observed clinical PK data. The Reviewer compared the simulated and observed lefamulin plasma PK. **Figure 18** shows the comparison between simulated (using Applicant's model with in vitro dissolution profiles as input) and observed plasma concentration-time profiles following the oral administration of 600 mg lefamulin in healthy subjects. The model significantly underpredicted the observed PK. The reasons for underprediction could be that 1) the current available in vitro dissolution data are not sufficient to describe the in vivo drug disintegration or dissolution processes; 2) the drug permeation process is not appropriately described by the Applicant's model; or 3) both drug disintegration and dissolution and permeation processes are not appropriately captured by the Applicant's model.

Figure 18. Observed and Reviewer's Simulated (Using Applicant's Model With In Vitro Dissolution Profiles as Input) Lefamulin Plasma Concentration-Time Profiles Following a Single Oral Administration of 600 mg Lefamulin in Healthy Subjects



Conclusions

The Applicant developed a lefamulin model using in vitro dissolution profiles as model input. The Applicant further used this model to assess the effect of elevated gastric pH on lefamulin

PK. The model is inappropriate to assess the effect of elevated gastric pH on PK of oral lefamulin because the model is not able to describe the observed lefamulin PK.

16.4. Clinical Appendices

16.4.1. Treatment-Emergent Adverse Events Occurring in <1% of Subjects

Table 148. Treatment-Emergent Adverse Events Occurring in <1% of Subjects by Preferred Term

Preferred Term	LEF N=641 n (%)	MOX N=641 n (%)
Aspartate aminotransferase increased	6 (0.9)	6 (0.9)
Gamma-glutamyltransferase increased	6 (0.9)	2 (0.3)
Infusion site phlebitis	6 (0.9)	3 (0.5)
Respiratory tract infection viral	5 (0.8)	1 (0.2)
Urinary tract infection	5 (0.8)	10 (1.6)
Anxiety	4 (0.6)	1 (0.2)
Blood alkaline phosphatase increased	4 (0.6)	0 (0.0)
Diabetes mellitus	4 (0.6)	3 (0.5)
Electrocardiogram QT prolonged	4 (0.6)	5 (0.8)
Gastritis	4 (0.6)	2 (0.3)
Pleurisy	4 (0.6)	0 (0.0)
Anaemia	3 (0.5)	4 (0.6)
Atrial fibrillation	3 (0.5)	4 (0.6)
Blood creatine phosphokinase increased	3 (0.5)	1 (0.2)
Blood pressure increased	3 (0.5)	2 (0.3)
Dyspepsia	3 (0.5)	4 (0.6)
Sepsis	3 (0.5)	0 (0.0)
Acute respiratory distress syndrome	2 (0.3)	0 (0.0)
Asthma	2 (0.3)	2 (0.3)
Bronchospasm	2 (0.3)	1 (0.2)
Constipation	2 (0.3)	6 (0.9)
Dizziness	2 (0.3)	6 (0.9)
Gastritis erosive	2 (0.3)	0 (0.0)
HIV infection	2 (0.3)	0 (0.0)
Hyperthermia	2 (0.3)	0 (0.0)
Infectious pleural effusion	2 (0.3)	2 (0.3)
Infusion site erythema	2 (0.3)	2 (0.3)
Infusion site reaction	2 (0.3)	1 (0.2)
Injection site pain	2 (0.3)	0 (0.0)
Injection site reaction	2 (0.3)	0 (0.0)
Liver function test increased	2 (0.3)	0 (0.0)
Myocardial infarction	2 (0.3)	0 (0.0)
Non-cardiac chest pain	2 (0.3)	1 (0.2)
Oral candidiasis	2 (0.3)	3 (0.5)
Oral fungal infection	2 (0.3)	0 (0.0)
Oropharyngeal candidiasis	2 (0.3)	0 (0.0)
Palpitations	2 (0.3)	3 (0.5)

Preferred Term	LEF N=641 n (%)	MOX N=641 n (%)
Pharyngitis	2 (0.3)	3 (0.5)
Pulmonary embolism	2 (0.3)	1 (0.2)
Pulmonary hypertension	2 (0.3)	1 (0.2)
Somnolence	2 (0.3)	0 (0.0)
Thrombocytopenia	2 (0.3)	0 (0.0)
Transaminases increased	2 (0.3)	1 (0.2)
Type 2 diabetes mellitus	2 (0.3)	1 (0.2)
Urinary retention	2 (0.3)	0 (0.0)
Abdominal wall haematoma	1 (0.2)	0 (0.0)
Acute myeloid leukaemia	1 (0.2)	0 (0.0)
Acute myocardial infarction	1 (0.2)	3 (0.5)
Acute respiratory failure	1 (0.2)	1 (0.2)
Acute sinusitis	1 (0.2)	1 (0.2)
Arrhythmia supraventricular	1 (0.2)	0 (0.0)
Arthralgia	1 (0.2)	0 (0.0)
Arthritis	1 (0.2)	0 (0.0)
Bacteriuria	1 (0.2)	1 (0.2)
Basophil count increased	1 (0.2)	0 (0.0)
Benign prostatic hyperplasia	1 (0.2)	1 (0.2)
Blister	1 (0.2)	0 (0.0)
Blood creatine phosphokinase decreased	1 (0.2)	0 (0.0)
Blood creatinine increased	1 (0.2)	1 (0.2)
Blood potassium increased	1 (0.2)	0 (0.0)
Bradycardia	1 (0.2)	0 (0.0)
Bronchial disorder	1 (0.2)	0 (0.0)
Bronchitis	1 (0.2)	0 (0.0)
Cardiac failure chronic	1 (0.2)	2 (0.3)
Cardiac failure congestive	1 (0.2)	0 (0.0)
Catheter site inflammation	1 (0.2)	0 (0.0)
Catheter site pain	1 (0.2)	0 (0.0)
Cholecystitis	1 (0.2)	0 (0.0)
Cholecystitis chronic	1 (0.2)	1 (0.2)
Cholelithiasis	1 (0.2)	1 (0.2)
Chronic sinusitis	1 (0.2)	0 (0.0)
Clostridium difficile colitis	1 (0.2)	0 (0.0)
Creatinine renal clearance decreased	1 (0.2)	0 (0.0)
Cystitis	1 (0.2)	1 (0.2)
Deafness	1 (0.2)	0 (0.0)
Delirium	1 (0.2)	0 (0.0)
Drug-induced liver injury	1 (0.2)	0 (0.0)
Duodenitis	1 (0.2)	0 (0.0)
Dysgeusia	1 (0.2)	0 (0.0)
Empyema	1 (0.2)	0 (0.0)
Encephalopathy	1 (0.2)	0 (0.0)
Endocarditis	1 (0.2)	0 (0.0)
Eosinophil count increased	1 (0.2)	0 (0.0)
Epigastric discomfort	1 (0.2)	1 (0.2)

Preferred Term	LEF N=641 n (%)	MOX N=641 n (%)
Epistaxis	1 (0.2)	1 (0.2)
Gastroenteritis	1 (0.2)	1 (0.2)
Gastrooesophageal reflux disease	1 (0.2)	0 (0.0)
Glucose tolerance impaired	1 (0.2)	0 (0.0)
Gouty arthritis	1 (0.2)	0 (0.0)
Haemangioma of liver	1 (0.2)	1 (0.2)
Haematoma	1 (0.2)	0 (0.0)
Haematuria	1 (0.2)	3 (0.5)
Haemoptysis	1 (0.2)	2 (0.3)
Hepatic cyst	1 (0.2)	0 (0.0)
Hepatic steatosis	1 (0.2)	1 (0.2)
Hepatitis C	1 (0.2)	0 (0.0)
Hepatitis toxic	1 (0.2)	0 (0.0)
Hyperglycaemia	1 (0.2)	3 (0.5)
Hypertensive crisis	1 (0.2)	0 (0.0)
Hypotension	1 (0.2)	1 (0.2)
Infusion site coldness	1 (0.2)	0 (0.0)
Injection site bruising	1 (0.2)	0 (0.0)
Injection site erythema	1 (0.2)	1 (0.2)
Intervertebral disc degeneration	1 (0.2)	0 (0.0)
Iron deficiency anaemia	1 (0.2)	1 (0.2)
Leukaemoid reaction	1 (0.2)	0 (0.0)
Leukocytosis	1 (0.2)	2 (0.3)
Leukocyturia	1 (0.2)	4 (0.6)
Leukopenia	1 (0.2)	2 (0.3)
Liver disorder	1 (0.2)	0 (0.0)
Lung abscess	1 (0.2)	4 (0.6)
Lung neoplasm	1 (0.2)	0 (0.0)
Lymphocyte count decreased	1 (0.2)	2 (0.3)
Mitral valve incompetence	1 (0.2)	0 (0.0)
Mouth haemorrhage	1 (0.2)	0 (0.0)
Muscle spasms	1 (0.2)	0 (0.0)
Musculoskeletal chest pain	1 (0.2)	0 (0.0)
Musculoskeletal pain	1 (0.2)	2 (0.3)
Myalgia	1 (0.2)	0 (0.0)
Neutropenia	1 (0.2)	0 (0.0)
Nuclear magnetic resonance imaging brain abnormal	1 (0.2)	0 (0.0)
Oedema peripheral	1 (0.2)	2 (0.3)
Orchitis	1 (0.2)	0 (0.0)
Oropharyngeal pain	1 (0.2)	0 (0.0)
Osteoarthritis	1 (0.2)	1 (0.2)
Otitis media	1 (0.2)	0 (0.0)
Phlebitis	1 (0.2)	0 (0.0)
Pneumonia bacterial	1 (0.2)	0 (0.0)
Pneumonitis	1 (0.2)	0 (0.0)
PO2 decreased	1 (0.2)	0 (0.0)
Poor quality sleep	1 (0.2)	0 (0.0)

Preferred Term	LEF N=641 n (%)	MOX N=641 n (%)
Postoperative wound infection	1 (0.2)	0 (0.0)
Procalcitonin increased	1 (0.2)	1 (0.2)
Prostatitis	1 (0.2)	1 (0.2)
Prothrombin time prolonged	1 (0.2)	0 (0.0)
Pulmonary microemboli	1 (0.2)	0 (0.0)
Pulmonary oedema	1 (0.2)	0 (0.0)
Pulmonary tuberculosis	1 (0.2)	2 (0.3)
Pyelonephritis chronic	1 (0.2)	0 (0.0)
Pyrexia	1 (0.2)	0 (0.0)
Pyuria	1 (0.2)	0 (0.0)
Rash	1 (0.2)	3 (0.5)
Renal cancer	1 (0.2)	0 (0.0)
Renal cyst	1 (0.2)	0 (0.0)
Respiratory rate increased	1 (0.2)	0 (0.0)
Sinus bradycardia	1 (0.2)	0 (0.0)
Sinusitis	1 (0.2)	0 (0.0)
Skin lesion	1 (0.2)	0 (0.0)
Spinal osteoarthritis	1 (0.2)	0 (0.0)
Squamous cell carcinoma of lung	1 (0.2)	1 (0.2)
Steatohepatitis	1 (0.2)	0 (0.0)
Tachycardia	1 (0.2)	0 (0.0)
Thrombocytosis	1 (0.2)	0 (0.0)
Upper gastrointestinal haemorrhage	1 (0.2)	0 (0.0)
Upper respiratory tract infection	1 (0.2)	0 (0.0)
Ventricular arrhythmia	1 (0.2)	0 (0.0)
Ventricular extrasystoles	1 (0.2)	1 (0.2)
Vertigo	1 (0.2)	0 (0.0)
Vessel puncture site erythema	1 (0.2)	1 (0.2)
Vessel puncture site haematoma	1 (0.2)	1 (0.2)
Viral infection	1 (0.2)	0 (0.0)
Viral pharyngitis	1 (0.2)	0 (0.0)
Vulvovaginal mycotic infection	1 (0.2)	0 (0.0)
White blood cell count increased	1 (0.2)	3 (0.5)

LEF = lefamulin; MOX = moxifloxacin

***M.O. Comment**: Table 91 and Table 148 list all adverse events in the Phase 3 safety population. All the selected adverse reactions listed in section 6.1 of the product label are accounted for in at least one of these two tables. Most of the adverse reactions listed in the product label occurred more commonly among LEF subjects compared to MOX subjects either overall or in one of the two Phase 3 trials. Some of the gastrointestinal reactions (for example, epigastric discomfort) did not occur more frequently among LEF subjects but their mention in the product label could be justified because LEF is known to cause other GI reactions such as nausea and diarrhea. However, the preferred term of (b) (4) occurred less frequently in LEF subjects and was removed as an adverse reaction.*

16.4.2. Treatment-Emergent Adverse Events Occurring in >2% of Subjects in Study 3101 and Study 3102

Table 149. Treatment-Emergent Adverse Events Occurring in >2% of Lefamulin-Treated Subjects by Preferred Term in Study 3101

Preferred Term	LEF N=273 n (%)	MOX N=273 n (%)
Administration site reactions[1]	20 (7.3)	7 (2.6)
Hepatic enzyme elevation[2]	9 (3.3)	8 (2.9)
Hypokalemia	8 (2.9)	6 (2.2)
Insomnia	8 (2.9)	5 (1.8)
Nausea	8 (2.9)	6 (2.2)
Headache	5 (1.8)	5 (1.8)

[1] See Section 8.2.5.1 for preferred terms included in administration site reactions
[2] Includes alanine aminotransferase increased, aspartate aminotransferase increased, and liver function test increased.
LEF = lefamulin; MOX = moxifloxacin

Table 150. Treatment-Emergent Adverse Events Occurring in >2% of Lefamulin-Treated Subjects by Preferred Term in Study 3102

Preferred Term	LEF N=368 n (%)	MOX N=368 n (%)
Diarrhea	45 (12.2)	4 (1.1)
Nausea	19 (5.2)	7 (1.9)
Vomiting	12 (3.3)	3 (0.8)
Hepatic enzyme elevation[1]	6 (1.6)	8 (2.2)

[1] Includes alanine aminotransferase increased, aspartate aminotransferase increased, and liver function test increased.
LEF = lefamulin; MOX = moxifloxacin

16.4.3. Review of Respiratory Treatment-Emergent Adverse Events from Study 2001

In Study 2001, subjects with ABSSSI were randomized to receive LEF 100 mg, LEF 150 mg, or vancomycin 1 g. In the respiratory disorders SOC, eight subjects had TEAEs in the LEF 150 mg arm (11.3%) compared to four subjects each in the LEF 100 mg and vancomycin arms (5.7% and 6.1% respectively). Of the TEAEs in the respiratory SOC, there was only one SAE; a subject receiving LEF 150 mg developed severe respiratory failure on Day 5 that was also associated with aspiration pneumonia on Day 8. Another LEF 150 mg patient developed severe hemothorax on Day 1. The other TEAEs were mostly mild. In the infections and infestations SOC, there was no imbalance between the treatment arms for PTs related to lung infection.

M.O. Comment*: There were more respiratory TEAEs in the 150 mg LEF arm compared to the 100 mg LEF and vancomycin arms. However, most of the AEs were mild and nonserious.*

16.4.4. Investigator Assessment of Clinical Response at Test of Cure in Subjects in the Micro-ITT-2 Population with a Baseline Pathogen of *S. Pneumoniae*

In Study 3101, there were 42 subjects in the LEF arm and 44 subjects in the MOX arm who were in the micro-ITT-2 population with a baseline pathogen of *S. pneumoniae*. Of note, the micro-ITT-2 population consists of subjects with at least 1 baseline pathogen, but excluding those pathogens diagnosed using PCR methods. At the TOC timepoint, the IACR success rates were: 34/42 (81.0%) in the LEF arm and 38/44 (86.4%) in the MOX arm. Among the *S. pneumoniae* subjects in the micro-ITT-2 population, those with PORT Risk Class IV and V were 18/42 (42.9%) in the LEF arm and 11/44 (25.0%) in the MOX arm.

M.O. Comment*: The higher proportion of PORT Risk Class IV and V in the LEF arm may explain the higher rates of failure at TOC compared to the MOX arm. However, the difference in success rates in the two arms is not great and is similar to the difference in success rates in the overall population. The remainder of this section will focus on the results from Study 3102.*

In Study 3102, there were 45 subjects in the LEF arm and 56 subjects in the MOX arm who were in the micro-ITT-2 population with a baseline pathogen of *S. pneumoniae*. At the TOC timepoint, the IACR success rates were: 36/45 (80.0%) in the LEF arm and 53/56 (94.6%) in the MOX arm. Among the *S. pneumoniae* subjects in the micro-ITT-2 population, those with PORT Risk Class III or higher CABP numbered 30/45 (66.7%) in the LEF arm and 23/56 (41.1%) in the MOX arm.

M.O. Comment*: The higher proportion of PORT Risk Class III and higher in the LEF arm may explain the higher rates of failure at TOC compared to the MOX arm.*

Regarding subjects in Study 3102 in the micro-ITT-2 population who were not successes at the TOC, the following LEF subjects were noteworthy:

- Subject (b) (6) had a pre-existing lung abscess that was not recognized until after one dose of lefamulin was administered. The study drug was stopped, and alternative antibacterial therapy was started.

M.O. Comment*: Had the lung abscess been identified earlier, the subject likely would have been excluded from the study. With receipt of only one dose, this failure cannot be totally attributed to lack of efficacy of lefamulin.*

- Subject (b) (6) presented with high fever (40.5°C), dyspnea, productive cough, and chest pain. His oxygen saturation was 90%, HR was 131 beats/min, and BP was 90/60 mmHg. Notable laboratory findings included a WBC count of 36.3 x 10^9 /L. He was started on oral study drug one day after admission to the hospital. On day 2, he developed ARDS requiring intubation and mechanical ventilation. Despite these interventions, the patient had cardiac arrest and died.

***M.O. Comment**: In retrospect, this patient may have been managed inappropriately as initiation of antibacterial therapy was delayed and oral therapy was started instead of IV. It is possible these factors could have contributed to the failure and death of the subject.*

- Subject (b) (6) was found to have *S. pneumoniae* infection by urine antigen. However, he also had *K. pneumoniae* isolated from sputum culture on day 5. He was deemed a failure at EOT because nonstudy antibacterial drugs were required to treat elevated "measures of inflammation" including a WBC count of 14.31 x 10^9/L.

***M.O. Comment**: This subject had a copathogen which was not sensitive to lefamulin. As a result, this failure may not represent failure to treat the S. pneumoniae.*

- Subject (b) (6) was found to have *S. pneumoniae* from blood culture, sputum culture, NP swab PCR, sputum PCR, and urine antigen testing. A baseline arterial blood gas showed: pH 7.52, pCO2 28 mmHg, and pO2 57 mmHg. Oral lefamulin was stopped after 4 days due to lack of efficacy and nonstudy antibacterial drugs were started.

***M.O. Comment**: In retrospect, this patient may have been managed inappropriately as oral therapy was started instead of IV in an ill patient with hypoxemia and respiratory alkalosis. It is possible this could have contributed to the failure.*

- Subject (b) (6) had bacteremia with *S. pneumoniae* and also had *K. variicola* identified by sputum culture. The subject withdrew informed content after one dose of oral lefamulin on the advice of a relative.

***M.O. Comment**: With receipt of only one dose, this failure cannot be totally attributed to lack of efficacy of lefamulin. In addition, there likely was a copathogen which was not sensitive to lefamulin.*

Regarding subjects in Study 3102 in the micro-ITT-2 population who were not successes at the TOC, the following MOX subject was noteworthy:

- Subject (b) (6) had PORT Risk Class V CABP and received 4 days of oral moxifloxacin before experiencing respiratory failure resulting in death.

***M.O. Comment**: This subject should not have been enrolled in Study 3102 as she had PORT Risk Class V CABP and was not likely a candidate for oral antibacterial therapy.*

Overall, regarding subjects in Study 3102 in the micro-ITT-2 population with a baseline pathogen of *S. pneumoniae*, there was a lower rate of success at the TOC for lefamulin subjects [36/45 (80.0%)] versus moxifloxacin subjects [53/56 (94.6%)]. However, there are several factors to consider when interpreting these data. First, subjects in the micro-ITT-2 population with a baseline pathogen of *S. pneumoniae* constitute a subgroup of the overall study (101 out of 736 total subjects). Second, lefamulin subjects in this subgroup may have been more ill

compared to moxifloxacin subjects based on a higher proportion of them having PORT Risk Class III or higher CABP. Lastly, five of the lefamulin subjects had possible alternative reasons for failure including the presence of copathogens not covered by lefamulin and inappropriate clinical management.

***M.O. Comment**: After taking these factors into account, I am less concerned that lefamulin may have decreased efficacy in subjects with a baseline pathogen of S. pneumoniae. However, it should be noted that lefamulin lacks activity against Enterobacteriaceae which may contribute to treatment failure in some patients.*

16.4.5. Clinical Success in Subjects with a Baseline Pathogen of *S. pneumoniae* Without a Positive Nasopharyngeal (NP) Swab

The following table lists the clinical success at different timepoints in those subjects in the micro-ITT population with a baseline pathogen of *S. pneumoniae* who were not included based on a positive NP swab.

Table 151. Clinical Success in Subjects with a Baseline Pathogen of *S. pneumoniae* Without a Positive Nasopharyngeal Swab

	Study 3101		Study 3102		Pooled	
Endpoint	**LEF**	**MOX**	**LEF**	**MOX**	**LEF**	**MOX**
ECR	73/84 (86.9%)	79/85 (92.9%)	94/106 (88.7%)	99/107 (92.5%)	167/190 (87.9%)	178/192 (92.7%)
IACR at TOC	70/84 (83.3%)	73/85 (85.9%)	90/106 (84.9%)	93/107 (86.9%)	160/190 (84.2%)	166/192 (86.5%)
IACR at LFU	67/84 (79.8%)	72/85 (84.7%)	90/106 (84.9%)	93/107 (86.9%)	157/190 (82.6%)	165/192 (85.9%)

LEF = lefamulin; MOX = moxifloxacin; IACR = Investigator's Assessment of Clinical Response; TOC = test of cure; LFU = late follow-up; ECR = early clinical response

***M.O. Comment**: Subjects with* S. pneumoniae *as a baseline pathogen were included in the micro-ITT population based on a positive blood culture, BAL culture, NP swab culture or PCR, sputum culture or PCR, or urinary antigen. There has been concern expressed about the relevance of NP swab specimens in the diagnosis of pneumonia as the microbiology of the nasopharynx may not reflect the lower respiratory tract. As a result, this subgroup analysis was conducted which excluded subjects who had been included based solely on a positive NP swab. The results show that the clinical success rates at ECR, TOC, and LFU did not differ greatly between the treatment arms in either study. Therefore, we can conclude that the subjects with a positive NP swab did not have an outsized role in influencing the overall results in the S. pneumoniae micro-ITT population.*

16.4.6. Clinical Success in Subjects with Bacteremia

In the two Phase 3 studies, there were 25 subjects with bacteremia (Table 152). For all of the patients, follow-up blood cultures were either not obtained or negative.

In Study 3101 (IV), there were 10 subjects with bacteremia, seven in the lefamulin (LEF) arm and 3 in the moxifloxacin (MOX) arm. Of these subjects, six subjects in the LEF arm and none in the MOX arm had *S. pneumoniae* bacteremia. The other subjects had bacteremia with *S. aureus* or Gram-negative organisms. Of the six subjects in the LEF arm with *S. pneumoniae* bacteremia, 1 was a clinical success at TOC and 5 were failures (3 clinical failures, 1 failure due to AE of bradycardia leading to withdrawal though responding clinically, and 1 failure with *Enterobacter cloacae* empyema).

In Study 3102 (oral), there were 15 subjects with bacteremia, 6 in the LEF arm and 9 in the MOX arm. Of these subjects, three subjects in the LEF arm and 5 in the MOX arm had *S. pneumoniae* bacteremia. The other subjects had bacteremia with *S. aureus* or Gram-negative organisms. Of the three subjects in the LEF arm with *S. pneumoniae* bacteremia, one was a clinical success at TOC and 2 were failures (1 clinical failure and 1 withdrew consent after 1 day of treatment on the advice of a relative). Of the five subjects in the MOX arm with *S. pneumoniae* bacteremia, all were clinical successes at TOC. Of note, three of the five MOX patients were PORT Risk Class II.

M.O. Comment: *Given the small numbers of subjects with bacteremia, the uneven distributions of* S. pneumoniae *and subjects with low PORT Risk Class among the treatment arms of the two studies, and the alternative reasons for failure for some subjects outlined above, I do not think there is sufficient information to adequately assess the efficacy of lefamulin in the treatment of CABP patients with bacteremia.*

Additional information related to this analysis follows.

Table 152. Subjects with Bacteremia in the Two Phase 3 Trials

Subject ID	PORT Risk Class	Baseline Blood Culture	IACR at TOC/LFU	Reason for Failure/Notes
			Study 3101 (IV)	
Lefamulin				
(b) (6)	Class III	*Streptococcus pneumoniae*	Failure	Empyema requiring nonstudy antibacterial drugs after 8 days
(b) (6)	Class III	*Staphylococcus aureus*	Failure	Found to have endobronchial diverticulosis as cause of ongoing pulmonary symptoms (chest pain, cough, hemoptysis) which likely preceded the study
(b) (6)	Class IV	*Streptococcus pneumoniae*	Failure	Patient was an ECR responder with signs of clinical improvement including lower white blood cell count and resolved fever, but discontinued study drug due to the AE of bradycardia.

Subject ID	PORT Risk Class	Baseline Blood Culture	IACR at TOC/LFU	Reason for Failure/Notes
(b) (6)	Class III	*Streptococcus pneumoniae*	Failure	Death from respiratory failure
(b) (6)	Class IV	*Streptococcus pneumoniae*	Success	-
(b) (6)	Class III	*Streptococcus pneumoniae*	Failure	Continued fever
(b) (6)	Class IV	*Streptococcus pneumoniae*	Failure	Continued fever; found to have *Enterobacter cloacae* empyema
Moxifloxacin				
(b) (6)	Class III	*Burkholderia cepacia*	Success	-
(b) (6)	Class III	*Escherichia coli*	Success	-
(b) (6)	Class IV	*Escherichia coli*	Failure	Empyema requiring nonstudy antibacterial drugs after 4 days; continued fever
Study 3102 (Oral)				
Lefamulin				
(b) (6)	Class III	*Klebsiella pneumoniae*	Failure	Signs and symptoms of CABP not resolved; *K. pneumoniae* not covered by lefamulin
(b) (6)	Class II	*Streptococcus pneumoniae*	Success	-
(b) (6)	Class II	*Streptococcus pneumoniae*	Failure	Continued fever; acute respiratory failure; blood cultures on Day 17 were no growth
(b) (6)	Class III	*Acinetobacter ursingii*	Success	-
(b) (6)	Class III	*Staphylococcus aureus* (MRSA)	Success	-
(b) (6)	Class III	*Streptococcus pneumoniae*	Failure	Received only one day of study drug; subject withdrew consent on the advice of a relative
Moxifloxacin				
(b) (6)	Class II	*Pasteurella pneumotropica*	Failure	Discontinued study drug because of an adverse event of elevated liver enzymes
(b) (6)	Class IV	*Acinetobacter calcoaceticus*	Success	-
(b) (6)	Class III	*Staphylococcus aureus* (MRSA)	Failure	Continued fever after 4 days of study drug; also, study drug discontinued per protocol because of *S. aureus* bacteremia
(b) (6)	Class II	*Streptococcus pneumoniae*	Success	-
(b) (6)	Class II	*Streptococcus pneumoniae*	Success	-
(b) (6)	Class IV	*Streptococcus pneumoniae*	Success	Blood cultures on Day 5 were no growth
(b) (6)	Class II	*Streptococcus pneumoniae*	Success	-
(b) (6)	Class III	*Streptococcus pneumoniae*	Success	Blood cultures on Days 6 and 8 were no growth
(b) (6)	Class II	*Staphylococcus aureus* (MSSA)	Success	Blood cultures on Days 7 and 12 were negative for MSSA

PORT = Pneumonia Outcomes Research Team; IACR = Investigator's Assessment of Clinical Response; CABP = community-acquired bacterial pneumonia; TOC = test of cure; LFU = late follow-up; IV = intravenous; ECR = early clinical response; AE = adverse event; MSSA = methicillin-sensitive *S. aureus*; MRSA = methicillin-resistant *S. aureus*

M.O. Comment:

- *There were no subjects in the MOX arm in Study 3101 who had* S. pneumoniae *bacteremia. As a result, all the MOX subjects with* S. pneumoniae *bacteremia came from Study 3102 which generally enrolled subjects with a lower severity of illness. For example, of the 5 MOX subjects with* S. pneumoniae *bacteremia, 3 were PORT Risk Class II which is associated with a low risk of mortality.*
- *The finding of Burkholderia, Acinetobacter, and Pasteurella bacteremia is unusual in subjects with CABP. I suspect these organisms may not be related to the CABP diagnosis as most of these subjects had evidence of CABP caused by* S. pneumoniae *using other diagnostic methods.*
- *In Study 3102, the finding of* S. aureus *bacteremia required subjects to discontinue study drug, but this was not done uniformly.*

The following lefamulin subjects with bacteremia had alternative reasons for clinical failure unrelated to the efficacy of the study drug in CABP.

- Subject (b) (6) was deemed a failure because signs and symptoms had not resolved requiring nonstudy antibacterial drugs. However, further clinical studies revealed endobronchial diverticulosis to be the cause of the symptoms.

M.O. Comment*: This condition likely preceded the study and would not be expected to improve with antibacterial drug therapy.*

- Subject (b) (6) was deemed a failure because of an adverse event of bradycardia requiring nonstudy antibacterial drugs. However, at the time of lefamulin discontinuation, there were signs of clinical improvement including lower white blood cell count and resolved fever. In addition, the subject was a responder at the early clinical response timepoint (ECR).

M.O. Comment*: The subject was deemed a failure because of an adverse event unrelated to the efficacy of lefamulin.*

- Subject (b) (6) was deemed a failure because of continued fever requiring nonstudy antibacterial drugs. However, in addition to *Streptococcus pneumoniae* bacteremia, the subject had an empyema caused by *E. cloacae* which is not covered by lefamulin.

M.O. Comment*: One would not expect an infection caused by* E. cloacae *to improve with only lefamulin treatment.*

- Subject (b) (6) was deemed a failure because of signs and symptoms of CABP had not resolved requiring nonstudy antibacterial drugs. However, this subject had bacteremia with *Klebsiella pneumoniae* which is not covered by lefamulin.

M.O. Comment*: One would not expect* K. pneumoniae *bacteremia to resolve with only lefamulin treatment.*

- Subject (b) (6) was deemed a failure because the subject withdrew informed consent on the advice of a relative after one day and nonstudy drugs were initiated.

M.O. Comment*: There was insufficient time available to determine the efficacy of lefamulin in the treatment of this subject.*

The following moxifloxacin subject with bacteremia had an alternative reason for clinical failure unrelated to the efficacy of the study drug in CABP.

- Subject (b) (6) was deemed a failure because of an adverse event of elevated liver enzymes requiring nonstudy antibacterial drugs. However, at the time of moxifloxacin discontinuation, there were signs of clinical improvement including lower white blood cell count and resolved fever.

M.O. Comment*: The subject was deemed a failure because of an adverse event unrelated to the efficacy of moxifloxacin.*

17 Clinical Microbiology Review

17.1. Activity In Vitro

Antibacterial Activity

The assessment of lefamulin activity came from individual study collections, clinical trials and the SENTRY global surveillance programs (2015-2017). The tables below summarize the in vitro activity (MIC_{90} and MIC range) of lefamulin against a number of organisms associated with community acquired bacterial pneumonia (CABP). Information on pathogens was pooled from surveillance and the combined Phase 3 studies.

Table 153. In Vitro Activity of Lefamulin Against Indicated Pathogens Listed in the Agency's First List

Pathogen	N	MIC_{90} (mcg/mL)	MIC Range (mcg/mL)
Streptococcus pneumoniae	7753	0.25	≤0.008–1
Staphylococcus aureus (MSSA)	6492	0.12	≤0.008–32
Haemophilus influenzae	2198	2	0.015–8
Mycoplasma pneumoniae	61	0.002	≤0.00025–0.008
Chlamydophilia pneumoniae	50	0.04	0.02–0.08
Legionella pneumophila	44	1	0.12–1

MIC = minimium inhibitory concentration; MSSA = methicillin-sensitive *S. aureus*
Source: Reviewer's table adapted from sources

Table 154. In Vitro Activity of Lefamulin Against Indicated Pathogens in the Agency's Second List

Pathogen	N	MIC_{90} (mcg/mL)	MIC Range (mcg/mL)
Staphylococcus aureus (MRSA)	4545	0.12	≤0.008–32
Streptococcus agalactiae	683	0.03	≤0.008–32
Streptococcus anginosus	108	0.5	≤0.008–1
Streptococcus mitis	282	0.5	≤0.015–1
Streptococcus pyogenes	652	0.03	≤0.008–0.12
Streptococcus salivarius	81	0.25	≤0.008–1
Haemophilus parainfluenzae	505	4	≤0.008–8
Moraxella catarrhalis	1306	0.12	≤0.008–1

MIC = minimum inhibitory concentration; MRSA = methicillin-resistant *S. aureus*
Source: Reviewer's table adapted from sources

Lefamulin demonstrated in vitro antibacterial activity against the Agency's proposed first list bacteria: *S. pneumoniae* (MIC_{90} of 0.25 mcg/mL), *H. influenzae* (MIC_{90} of 2 µg/mL), *S. aureus* MSSA (MIC_{90} of 0.12 mcg/mL), *L. pneumophila* (MIC_{90} of 1 mcg/mL), *M. pneumoniae* (MIC_{90} of 0.002 mcg/mL), and *C. pneumoniae* (MIC_{90} of 0.04 mcg/mL).

Lefamulin demonstrated in vitro activity against the the Agancy's proposed second list organisms: *S. aureus* MRSA, *S. agalactiae*, *S. anginosus*, *S. mitis*, *S. pyogenes*, *S. salivarius*, *H. parainfluenzae*, *M. catarrhalis*. The MIC_{90}s are shown in the table above.

***Reviewer's Comment**: A discussion of the adequacy of the organisms for the first and second lists of bacteria is provided at the end of this clinical microbiology review. We note that inclusion in the first list is based on clinical experience. All second list organisms were evaluated for activity in vitro. The Applicant included an analysis of lefamulin activity against S. pneumoniae that are penicillin-intermediate non-meningitis, penicillin-resistant non-meningitis, macrolide resistant, tetracycline resistant, or trimethoprim-sulfamethoxazole resistant.* (b) (4) *Those considered multidrug resistant are shown in the tables below:*

Table 155. Activity of Lefamulin and Comparator Antimicrobial Agents When Tested Against 518 Multidrug-Resistant Isolates of *S. pneumoniae* Collected During 2015, 2016, and 2017

Antimicrobial Agent	N	MIC Values (µg/mL)			CLSI[a,b]			EUCAST[a,b]		
		MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Lefamulin	518	0.06	0.12	≤0.008 to 0.5	-	-	-	-	-	-
Amoxicillin-clavulanic acid[c]	517	4	>4	≤0.03 to >4	48.7	21.1	30.2	-	-	-
Azithromycin	518	>4	>4	1 to >4	0.0	1.2	98.8	0.0	0.0	100
Ceftaroline	518	0.12	0.25	≤0.008 to >1	99.0	-	-	95.8	-	4.2
Ceftriaxone	518	1	2	≤0.015 to >2	30.1 62.9	32.8 28.4	37.1[d] 8.7[e]	30.1	61.2	8.7
Clindamycin	518	>1	>1	≤0.25 to >1	21.0	1.7	77.2	22.8	-	77.2
Erythromycin	518	>2	>2	1 to >2	0.0	0.0	100	0.0	0.0	100
Imipenem[c]	166	0.25	0.5	≤0.015 to >2	38.0	57.2	4.8	99.4	-	0.6
Levofloxacin	518	1	2	0.25 to >4	95.0	0.2	4.8	95.0	-	5.0
Linezolid	518	1	1	0.25 to 2	100	-	-	100	0.0	0.0
Meropenem[c]	352	0.5	1	≤0.008 to >1	27.3	23.6	49.1	27.3 100	70.7	2.0[d] 0.0[e]
Moxifloxacin[c]	352	0.12	0.25	≤0.03 to >4	96.0	2.3	1.7	96.0	-	4.0
Penicillin	518	2	4	≤0.06 to >4	9.3 9.3 55.4	21.6 40.0	69.1[f] 90.7[g] 4.6[h]	9.3 9.3	46.1	90.7[d] 4.6[e]
Tetracycline	518	>4	>4	4 to >4	0.0	0.0	100	0.0	0.0	100
Tigecycline	518	0.06	0.06	≤0.008 to 0.25	95.2[i]	-	-	-	-	-
Trimethoprim-sulfamethoxazole	518	>4	>4	4 to >4	0.0	0.0	100	0.0	0.0	100
Vancomycin	518	0.25	0.5	≤0.06 to 0.5	100	-	-	100	-	0.0

CLSI=Clinical and laboratory Standards Institute; EUCAST=The European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC=minimum inhibitory concentration; MIC_{50}=concentration required to inhibit isolates by 50%; MIC_{90}=concentration required to inhibit isolates by 90%; R=resistant; S=susceptible.

[a] Isolates were resistant to either erythromycin, tetracycline, or folate-pathway inhibitors (trimethoprim-sulfamethoxazole).

[b] Criteria as published by CLSI (Clinical and Laboratory Standards Institute 2018c) and EUCAST (European Committee on Antimicrobial Susceptibility Testing 2018).

[c] Sample sizes are as follows: amoxicillin-clavulanic acid=517, imipenem=166, meropenem=352, and moxifloxacin=352.

[d] Using meningitis breakpoints.

[e] Using non-meningitis breakpoints.

[f] Using oral breakpoints.

[g] Using parenteral, meningitis breakpoints.

[h] Using parenteral, non-meningitis breakpoints.

[i] FDA breakpoints published 2017-DEC-13

Source: Report NABRIVA 2018-06 MIB

Table 156. Activity of Lefamulin and Comparator Antimicrobial Agents When Tested Against 20 Extremely Multidrug-Resistant Isolates of *S. pneumoniae* Collected During 2015, 2016, and 2017

Antimicrobial Agent	N	MIC Values (µg/mL)			CLSI[a,b]			EUCAST[a, b]		
		MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%I	%R
Lefamulin	20	0.06	0.12	0.03 to 0.25	-	-	-	-	-	-
Amoxicillin-clavulanic acid	20	4	>4	1 to >4	25.0	30.0	45.0	-	-	-
Azithromycin	20	>4	>4	2 to >4	0.0	0.0	100	0.0	0.0	100
Ceftaroline	20	0.12	>1	0.06 to >1	80.0	-	-	65.0	-	35.0
Ceftriaxone	20	2	>2	0.5 to >2	15.0 40.0	25.0 15.0	60.0[c] 45.0[d]	15.0	40.0	45.0
Clindamycin	20	>1	>1	≤0.25 to >1	10.0	0.0	90.0	10.0	-	90.0
Erythromycin	20	>2	>2	1 to >2	0.0	0.0	100	0.0	0.0	100
Imipenem	8	0.5		0.25 to >2	0.0	87.5	12.5	87.5	-	12.5
Levofloxacin	20	>4	>4	>4 to >4	0.0	0.0	100	0.0	-	100
Linezolid	20	1	1	0.5 to 1	100	-	-	100	0.0	0.0
Meropenem	12	1	>1	0.25 to >1	16.7	25.0	58.3	16.7	58.3	25.0[c]
Moxifloxacin	12	2	4	0.25 to >4	8.3	50.0	41.7	8.3	-	91.7
Penicillin	20	4	>4	2 to >4	0.0 0.0 40.0	0.0 35.0	100[e] 100[f] 25.0[g]	0.0 0.0	 40.0	100[c] 60.0[d]
Tetracycline	20	>4	>4	>4 to >4	0.0	0.0	100	0.0	0.0	100
Tigecycline	20	0.06	0.06	0.015 to 0.12	95.0[h]	-	-	-	-	-
Trimethoprim-sulfamethoxazole	20	>4	>4	4 to >4	0.0	0.0	100	0.0	0.0	100
Vancomycin	20	0.25	0.25	0.12 to 0.5	100	-	-	100	-	0.0

CLSI=Clinical and laboratory Standards Institute; EUCAST=European Committee on Antimicrobial Susceptibility Testing; I=intermediate; MIC=minimum inhibitory concentration; MIC_{50}=concentration required to inhibit isolates by 50%; MIC_{90}=concentration required to inhibit isolates by 90%; R=resistant; S=susceptible.

[a] Isolates were resistant to erythromycin, tetracycline, folate-pathway inhibitors (trimethoprim-sulfamethoxazole), fluoroquinolones (levofloxacin), and oral penicillin.

[b] Criteria as published by CLSI (Clinical and Laboratory Standards Institute 2018c) and EUCAST (European Committee on Antimicrobial Susceptibility Testing 2018).

[c] Using meningitis breakpoints.

[d] Using non-meningitis breakpoints.

[e] Using oral breakpoints.

[f] Using parenteral, meningitis breakpoints.

[g] Using parenteral, non-meningitis breakpoints.

[h] FDA breakpoints published 2017-DEC-13.

Source: Report NABRIVA 2018-06 MIB

Lefamulin's in vitro activity against all *S. pneumoniae* serotypes ranged from 0.12 mcg/mL to 0.25 mcg/mL and does not appear to be different from the surveillance isolates.

Table 157. In Vitro Activity of Lefamulin and Comparators Against Selected Serotypes and Resistance Subsets of *S. pneumoniae* Collected During the SENTRY 2010 Surveillance Program

Serotype[a] (Total N tested /%)	MIC_{50} and MIC_{90} Results (µg/mL)															
	LEF		PEN		CRO		ERY		CLI		LEV		TET		TMP-SXT	
All (822)	**0.12**	**0.25**	**≤0.03**	**4**	**≤0.06**	**1**	**≤0.06**	**>8**	**≤0.25**	**>1**	**1**	**1**	**0.5**	**>8**	**≤0.5**	**4**
19A (123 / 15.0)	0.12	0.25	4	4	1	2	>8	>8	>1	>1	1	1	>8	>8	4	>4
3 (70 / 8.5)	0.06	0.12	≤0.03	≤0.03	≤0.06	≤0.06	≤0.06	0.12	≤0.25	≤0.25	1	1	0.5	>8	≤0.5	≤0.5
35B (54 / 6.6)	0.12	0.25	2	2	1	1	8	>8	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	>4
6C/6D (53 / 6.4)	0.12	0.25	0.12	1	0.25	0.5	≤0.06	8	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	>4
22A/22F (48 / 5.8)	0.12	0.25	≤0.03	≤0.03	≤0.06	≤0.06	≤0.06	8	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	≤0.5
11A/11D (47 / 5.7)	0.25	0.5	≤0.03	≤0.03	≤0.06	≤0.06	≤0.06	8	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	≤0.5
15A/15F (45 / 5.5)	0.12	0.25	0.25	0.25	0.12	0.5	>8	>8	>1	>1	1	1	>8	>8	≤0.5	4
7F (45 / 5.5)	0.12	0.25	≤0.03	≤0.03	≤0.06	≤0.06	≤0.06	≤0.06	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	≤0.5
15B/15C (39 / 4.7)	0.12	0.25	≤0.03	0.5	≤0.06	0.12	≤0.06	>8	≤0.25	≤0.25	1	1	0.5	>8	≤0.5	4
19F (24 / 2.9)	0.12	0.25	≤0.03	4	0.25	4	≤0.06	>8	≤0.25	>1	1	1	0.5	>8	≤0.5	>4
Other[b] (274 / 33.3)	0.12	0.25	≤0.03	0.25	≤0.06	0.12	≤0.06	8	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	2
MDR[c] (180 / 21.9)	0.12	0.25	2	4	1	2	>8	>8	>1	>1	1	1	>8	>8	4	>4
Non-MDR (642 / 78.1)	0.12	0.25	≤0.03	1	≤0.06	0.5	≤0.06	8	≤0.25	≤0.25	1	1	0.5	0.5	≤0.5	2

CLI=clindamycin; CRO=ceftriaxone; ERY=erythromycin; LEF=lefamulin; LEV=levofloxacin; MIC_{50}=concentration required to inhibit isolates by 50%; MIC_{90}=concentration required to inhibit isolates by 90%; PEN=penicillin; TET=tetracycline; and TMP-SXT=trimethoprim-sulfamethoxazole.

[a] The 13-valent conjugate vaccine contains coverage against serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

[b] Comprises 28 serotypes including nontypeable.

[c] MDR=multidrug-resistant (i.e. isolates displaying a resistance phenotype to at least 3 drug classes). Includes serotypes/serogroups (n): 19A (81), 15A/F (42), 15B/15C (10), 19F (11), nontypeable (7), 6C/6D (5), 23A (5), 3 (3), 9N/9L (3), 23F (3), 14 (3), 35B (2), 21 (1), 22F/22A (1), 6A (1), 7F (1), and 34 (1).

Source: (Mendes, Farrell et al 2016).

Table 158 shows the in vitro activity against *S. aureus*, methicillin-resistant (MRSA) and -sensitive (MSSA) surveillance isolates:

Table 158. MIC Distribution of Lefamulin Evaluated Against 11,037 *S. aureus* (MSSA and MRSA) Isolates by Study and Year

Organism Sources[a] (Year)	Panel Type	Number of Isolates at Specified Lefamulin Concentrations in µg/mL (Cumulative%)													Total N	MIC Value (µg/mL)	
		≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32		MIC_{50}	MIC_{90}
Total N		5	25	993	5397	4100	442	33	8	15	6	4	2	7	11,037	0.06	0.12
Total, cumulative%		0.05	0.27	9.3	58.2	95.3	99.3	99.6	99.7	99.8	99.9	99.9	99.9	100			
SENTRY (2015-2017)[b]	frozen	5	18	938	2,889	559	32	5	8	6	0	0	1	2	4,463	0.06	0.12
SENTRY (2017)[c]	frozen	0	8	193	1,053	259	20	3	5	2	0	0	0	1	1,544	0.06	0.12
SENTRY (2016)[d]	frozen	5	10	417	1,037	163	7	2	2	1	0	0	1	1	1,646	0.06	0.12
SENTRY (2015)[e]	frozen	0	0	328	799	137	5	0	1	3	0	0	0	0	1,273	0.06	0.12
SENTRY (2010)[f]	dried[g]	0	7	46	2,329	2,845	271	19	0	5	1	1	1	2	5,527	0.12	0.12
Report (b) (4) 2004 (2013)	frozen	0	0	0	2	88	9	0	0	3	3	1	0	0	106	0.12	0.25
Report 10-NAB-02B (2010)	frozen	0	0	0	46	112	48	6	0	0	0	0	0	0	212	0.12	0.25
Report 09-NAB-09 (2009)	frozen	0	0	0	6	62	36	0	0	0	0	0	0	0	104	0.12	0.25
Report 09-NAB-08 (2009)	frozen	0	0	7	71	29	1	0	0	0	0	0	0	0	108	0.06	0.12
Report 09-NAB-02 (2009)	frozen	0	0	2	13	171	20	0	0	1	2	2	0	3	214	0.12	0.25
Report 07-NAB-02 (2007)	frozen	0	0	0	41	234	25	3	0	0	0	0	0	0	303	0.12	0.12

MIC=minimum inhibitory concentration; MIC_{50}=concentration required to inhibit isolates 50%; MIC_{90}=concentration required to inhibit isolates 90%.
[a] All data from individual reports is also summarized for this table in Report NABRIVA 2018-06 MIB.
[b] Data from Report 18-NAB-06.
[c] Data from Report 17-NAB-01.
[d] Data from Report 16-NAB-01.
[e] Data from Report 16-NAB-07.
[f] Data from Report 10-NAB-01
[g] Dried Sensititre panels manufactured by Thermofisher (Trek).

Reviewer's Comment: *Of the 11037 MRSA and MSSA isolates tested by the Applicant, all had MIC_{90}s below the Agency's proposed susceptible breakpoint for MSSA of ≤0.25 mcg/mL. The Applicant reported only one isolate of S. aureus tested with an MIC greater than 2 and this was a MRSA isolate from a patient with a bloodstream infection.*

Lefamulin's in vitro activity against additional *S. aureus* populations was tested as follows:

MIC_{90} 0.12 mcg/mL for *S. aureus* vancomycin intermediate (VISA), MIC_{90} 0.25mcg/mL for hetero-resistant vancomycin intermediate (hVISA) and MIC_{90} 0.12 mcg/mL for vancomycin resistant (VRSA) *S. aureus*.

Lefamulin was tested against 149 beta-lactamase producing *H. influenzae* with an MIC_{90} of 2 mcg/mL.

Reviewer's Comment: *The MIC_{90} for lefamulin at 2 mcg/mL was at the Agency's proposed susceptible breakpoint for H. influenzae, a pathogen which can sometimes be found intracellularly.*

Lefamulin was tested against 223 beta-lactamase positive *M. catarrhalis* with an MIC_{90} of 0.06 mcg/mL.

For *L. pneumophila*, lefamulin had an MIC_{90} of 0.5 mcg/mL to 1 mcg/mL for the serotypes 1, 2, 3, 5, 6, and 10. Serogroups other than 1 were slightly more susceptible with MIC_{90} of 0.5 mcg/mL. The testing of *L. pneumophila* by serogroup is shown in the table below.

Table 159. Values for MIC_{50}, MIC_{90} and MIC Range for *L. pneumophila* Tested with Lefamulin and Comparator Antibiotics

Antimicrobial Agent	*L. pneumophila*, all serogroups (n=44)			*L. pneumophila*, serogroup 1 (n=32)			*L. pneumophila*, serogroups other than 1 (n=12)			*Legionella* spp. other than *L. pneumophila* (n=3)[a,b]
	MIC Values (µg/mL)			MIC Values (µg/mL)			MIC Values (µg/mL)			MIC Values (µg/mL)
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	Range
Lefamulin	0.5	1	0.125-1	0.5	1	0.125-1	0.25	0.5	0.125-1	0.06-2
Moxifloxacin	0.03	0.03	0.015-0.125	0.03	0.03	0.015-0.125	0.03	0.03	0.015-0.06	0.015-0.03
Azithromycin	0.06	0.5	0.03-1	0.06	0.5	0.03-1	0.06	0.125	0.03-0.125	0.06-0.125
Doxycycline	2	2	1-4	1	2	1-2	2	2	1-4	0.5-8
Solithromycin	0.03	0.03	0.008-0.06	0.03	0.03	0.015-0.06	0.03	0.03	0.015-0.03	0.06-0.125
Rifampin	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002-0.004
Erythromycin	0.25	0.5	0.06-1	0.25	0.5	0.06-1	0.25	0.25	0.125-0.5	0.125

MIC=minimum inhibitory concentration in µg/mL; MIC_{50}=concentration required to inhibit isolates by 50%; MIC_{90}=concentration required to inhibit isolates by 90%
[a] included *L. longbeacheae* (n=2) and *L. dumoffii*.
[,b] MIC_{50}/MIC_{90} values by agar dilution were significantly higher than broth dilution for azithromycin, levofloxacin and moxifloxacin.
Source: Report 4686-2, Table 2 and Table 3; also summarized in Report NABRIVA 2018-06 MIB.

For *M. pneumophila*, macrolide-resistant, the MIC_{90} was ≤0.002 mcg/mL. Against moxifloxacin-resistant *M. pneumoniae*, the MIC_{90} was 0.002 mcg/mL. Minimum Bactericidal Concentrations (MBC) were also tested against 2 macrolide-susceptible and 6 macrolide-resistant isolates. The MBCs were 2 to 4 times the MIC suggesting a bactericidal effect.

C. pneumoniae, an intracellular organism, had a lefamulin MIC_{90} of 0.04 mcg/mL.

Lefamulin's in vitro activity against respiratory pathogens in pediatric patients was found to be similar to its in vitro activity against respiratory pathogens in adult patients.

Reviewer's Comment: *Lefamulin's activity was provided by the Applicant against isolates from different regions of the world, and in comparison, to other antibacterial agents such as azithromycin, ceftaroline, clindamycin, daptomycin, doxycycline, erythromycin, gentamycin, levofloxacin, linezolid, moxifloxacin, oxacillin, teicoplanin, tigecycline, trimethoprim-sulfamethoxazole, vancomycin. Lefamulin's activity was favorable in comparison. For example,*

of 241 MRSA from 2017, the MIC_{90} for lefamulin was 0.12 mcg/mL which was the lowest MIC_{90} of the comparators.

Bactericidal Activity

Bactericidal activity of lefamulin was evaluated and defined as having a ≥3 $\log_{10}$ reduction in CFU/mL relative to baseline. *S. pneumoniae*, *H. influenzae* and *M. pneumoniae* were evaluated. For *S. aureus* (MRSA and MSSA) and beta-hemolytic *Streptococcus* spp., the effect of lefamulin was bacteriostatic.

The Applicant described the results as follows:

Lefamulin was bacteriostatic against *S. aureus* (MSSA and MRSA) at concentrations ranging from 1- to 16-fold MIC, reducing bacterial cell counts by 1 $\log_{10}$ to 2 $\log_{10}$. Against *S. pneumoniae* and *H. influenzae*, lefamulin was bactericidal (≥3 log10 reduction in CFU/mL) at concentrations of ≥1-times and ≥4-times MIC, respectively. Lefamulin was bacteriostatic against *S. agalactiae* at concentrations up to 8-times MIC, but bactericidal at concentrations of ≥16-times MIC at 24 hours. Against the *S. pyogenes* isolates tested, lefamulin was bacteriostatic at concentrations up to 32-times MIC. When tested against macrolide-susceptible and macrolide-resistant *M. pneumoniae* (n=8), lefamulin was bactericidal, with an MBC against *Mycoplasma* spp. of 0.002 mcg/mL to 0.008 mcg/mL, corresponding to 2-times to 4-times MIC.

Intracellular Antimicrobial Activity

In Report NABRIVA 2013-05 MIB, the intracellular concentration and accumulation of lefamulin was investigated in murine macrophages using strain J774. Azithromycin and penicillin G served as positive and negative controls, respectively. The intracellular concentrations (C_i) and extracellular concentrations (C_e) of lefamulin in cell lysate were determined in triplicate by LC-MS/MS. Lefamulin at C_e of 1 mcg/mL and 5 mcg/mL exhibited approximately 50-times accumulation in macrophages after 5 hours of incubation (See figure below).

Figure 19. Ratios of Intracellular and Extracellular Concentration for Lefamulin, Azithromycin, and Penicillin G at Nominal Extracellular Concentrations

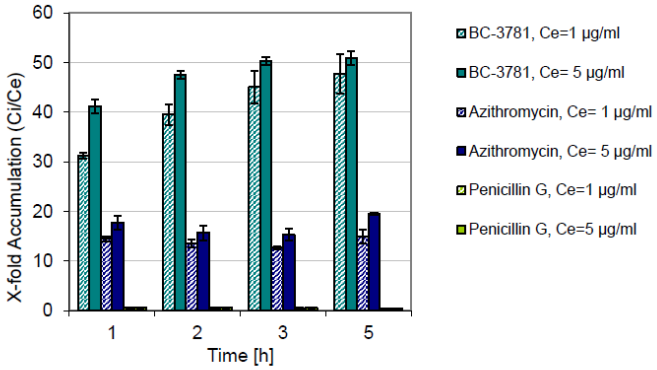


Figure 19. Lefamulin (BC-3781) effectively and rapidly accumulated within the murine macrophages as demonstrated by the ratio of intracellular to extracellular drug concentration (C_i/C_e) at both tested concentrations of 1 and 5 µg/mL. The positive control azithromycin also showed high intracellular concentrations under the same conditions though the C_i/Ce ratio was lower than that of lefamulin. The negative control Penicillin did not accumulate within the macrophages as expected. Source: Report NABRIVA 2013-05 MIB, Figure 2.

Reviewer's Comment: *The intracellular and extracellular concentrations of lefamulin were important to determine because CABP pathogens such as C. pneumoniae and sometimes H. influenzae are found intracellularly. Lefamulin's penetration ratio was 30- to 40-times (C_i/C_e) at 1 hour (h) and 50-times after 5 h. Confirmation of intracellular activity of lefamulin was also demonstrated by activity against the intracellular pathogen C. pneumoniae in human HEp-2 cells.*

Postantibiotic Effect

In Report NABRIVA 2008-14 MIB, the postantibiotic effects (PAE) of lefamulin were determined against *S. aureus* B9 (MSSA), *S. aureus* B29 (MRSA) and *S. pneumoniae* B415 (ATCC 6303) after exposure to concentrations ranging from 0.05 mcg/mL to 10 mcg/mL (0.5-, 1-, 4-, 8-, 10-, and 100-times MIC) for 1 and 3 h. Lefamulin exhibited an in vitro PAE against tested *S. aureus* (MSSA and MRSA) and *S. pneumoniae* isolates at 1-times MIC corresponding to 0.1 mcg/mL (MSSA) and 0.16 mcg/mL (MRSA). The PAE duration ranged from 2.5 h to 4.5 h and was longer for lefamulin than for the tested reference antibacterial drugs (azithromycin, moxifloxin and linezolid). The PAE of lefamulin was dependent on concentration and time of exposure, with longer exposure times (up to 3 hours) and higher concentrations (up to 100-times MIC) leading to a PAE prolongation of ≥22 hours. Even at sub-MIC concentrations (0.5-times MIC) a PAE was observed. Results are shown in the table below.

Table 160. Lefamulin Postantibiotic Effects Against *S. aureus* B9 (MSSA, ATCC 49951), *S. aureus* B29 (MRSA, Clinical Isolate), and *S. pneumoniae* B415 (ATCC 6303) in Comparison to Azithromycin, Moxifloxacin, and Linezolid

Organism		*S. aureus* B9 (MSSA)			*S. aureus* B29 (MRSA)			*S. pneumoniae* B415		
Exposure Time			1h	3h		1h	3h		1h	3h
Drug Treatment	MIC Value	Conc. (µg/mL)	PAE (h)	PAE (h)	Conc. (µg/mL)	PAE (h)	PAE (h)	Conc. (µg/mL)	PAE (h)	PAE (h)
Lefamulin	0.5-fold	0.05	2.5	3	0.05	2	1.5	0.08	2.5	3.5
	1-fold	0.1	3	4	0.1	2.5	3	0.16	3	4.5 (4)
	4-fold	0.4	5.5	7.5	0.4	4	6	0.64	5	8.5
	8-fold	0.8	9	22	0.8	5.5	8	1.28	7.5	>9
	10-fold	1	10.5	>22	1	6.5	>9.5	1.6	9.5	>21.5
	100-fold	10	>22	>22	10	>21.5	>21.5	16	>21.5	>21.5
Azithromycin	1-fold	1.6	1	1.5	ND[a]	ND[a]	ND[a]	0.08	1.5	2.5
Moxifloxacin	1-fold	0.1	1	0	3.2	3	2.5	0.16	0	2
Linezolid	1-fold	1.6	0.5	1	1.6	1.5	2	1.28	1	1.5

Conc=concentration; MIC=minimum inhibitory concentration; ND=not done, PAE=postantibiotic effect
[a] PAE not determined as *S. aureus* B29 is resistant against azithromycin.
Source: Report NABRIVA 2008-14 MIB, Table 5

Reviewer's Comment: *The postantibiotic effect (PAE) is the ability of an antimicrobial agent to suppress growth of target pathogens after a brief in vitro exposure period to supra-inhibitory concentrations of the agent followed by its subsequent removal.*

Effect on Gut Flora

The *in vitro* gut flora study of the working group of (b) (6) [Report VV-NAB-NC-000420] investigated the effect of lefamulin on the human gut microbiome and propensity to induce *Clostridioides difficile* (formerly *Clostridium difficile*) infection (CDI) using an in vitro model. Lefamulin, as with comparators levofloxacin and ceftriaxone, was found to induce *C. difficile* infection. The Applicant has proposed a warning statement in the product label to communicate this risk. In the Phase 3 clinical trials of lefamulin, one CDI case was observed in the oral lefamulin arm. Diarrhea and loose stool were less evident in the IV versus the oral formulation of lefamulin.

17.2. Mechanism of Action

Lefamulin is a novel derivative of the pleuromutilin class of antibacterial drugs. It is the first compound of the pleuromutilin class to be developed for systemic use. Changes to the pleuromutilin core, including modification to the C-14 extension, is said to contribute to the antibacterial activity of lefamulin. Lefamulin inhibits prokaryotic ribosomal protein synthesis by binding to the peptidyl transferase center (PTC) at the 50S subunit of bacterial ribosome, while mammalian protein synthesis appears unaffected. The selectivity is reportedly due to the different orientation of the pleuromutilin core binding nucleotides in eukaryotic versus bacterial ribosomes. The interaction of lefamulin with the central part of domain V at the 23S rRNA subsequently prevents the correct positioning of the CCA-ends of tRNAs for peptide

transfer. Notably, this specific type of interaction is unique to the pleuromutilin antibacterial drugs and is described in the literature (Poulsen, Karlsson et al. 2001; Bosling, Poulsen et al. 2003; Davidovich, Bashan et al. 2007).

A macromolecular biosynthesis inhibition study measuring the incorporation of radiolabeled substrates confirmed the inhibition of protein synthesis by lefamulin. An initial inhibition of DNA synthesis at high lefamulin concentrations was not confirmed in further experiments. No inhibition was observed for RNA, cell wall, or lipid synthesis for lefamulin or retapamulin [Report 12-29-2016-Nabriva3v3]. The proposed mechanism of action for lefamulin is shown in the figure below:

Figure 20. Lefamulin in the Bacterial PTC and the Overlaid Bacterial and Eukaryotic Binding Pocket of Lefamulin

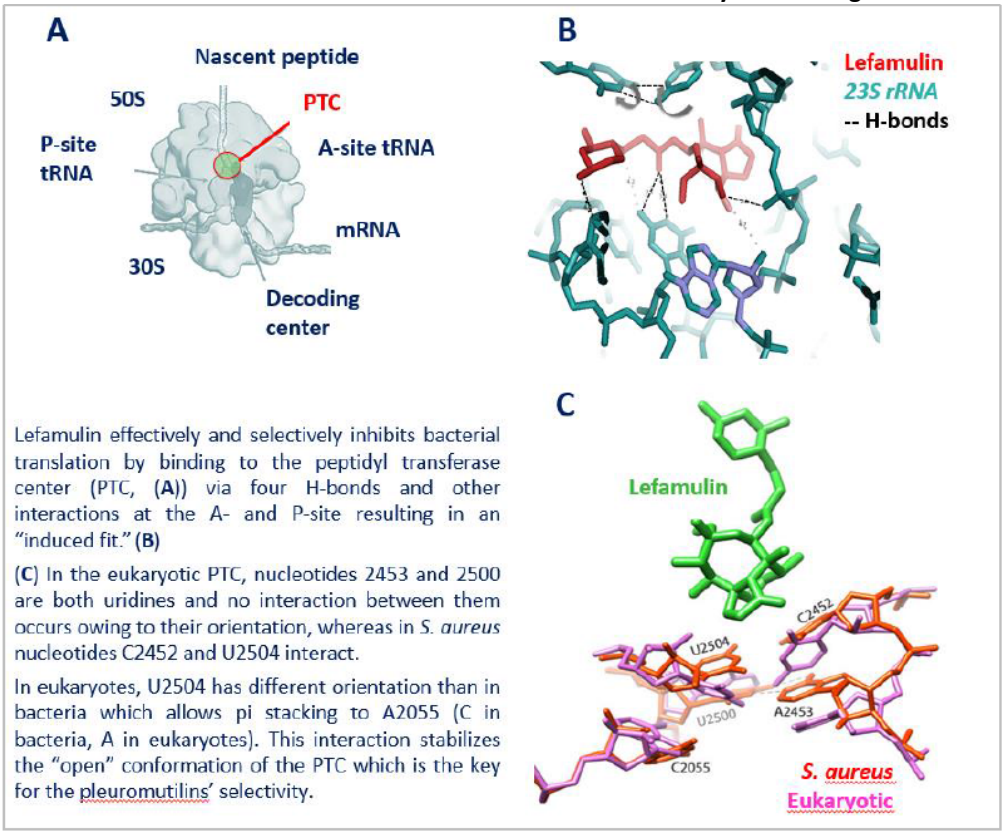


Figure 2. Pleuromutilin antibiotics inhibit prokaryotic ribosomal protein synthesis by binding to the peptidyl transferase center at the 50S subunit of the bacterial ribosome.
Source: (Yan, Madden et al 2006; Eyal, Matzov et al 2016).

The in vitro transcription-translation assay (TT assay) using ribosomes from rabbit reticulocytes (Paukner and Riedl 2017) was used to demonstrate that lefamulin selectively inhibits bacterial protein synthesis.

Table 161. IC_{50} Values for Lefamulin, Comparators, and Control in In Vitro Bacterial and Eukaryotic TT Assay

Pleuromutilin Analog or Comparator	IC_{50} [µM] (95% CI) for Ribosome Type		
	E. coli	*S. aureus*	Eukaryotic[a]
Lefamulin	0.58 (0.52-0.64)	0.29 (0.26-0.32)	952 (732-1238)
Retapamulin	0.69 (0.64-0.76)	0.35 (0.32-0.39)	850 (562-1287)
Puromycin	0.39 (0.34-0.46)	0.19 (0.16-0.23)	0.31 (0.27-0.36)
Cycloheximide	>100	>100	0.44 (0.29-0.68)

CI=confidence interval; IC_{50}=concentration inhibiting activity by 50%;
[a] The eukaryotic model was a rabbit reticulocyte lysate system.
Source: Report NABRIVA 2018-13 MIB.

17.3. Resistance

Cross-resistance with most antibacterial drug classes has not been observed for lefamulin, especially with regard to protein synthesis inhibitors such as macrolides, ketolides, or fusidic acid (Yan, Madden et al. 2006). The binding sites and mode of action of pleuromutilins can be differentiated from those of oxazolidinones, lincosamides, phenicols, and streptogramins; however, pleuromutilins also have partly overlapping interaction sites with these antibacterial drugs (Schlunzen, Pyetan et al. 2004). Therefore, resistance mechanisms exist which can mediate cross-resistance with these antibacterials.

The Applicant's cross-susceptibility analysis of lefamulin compared to azithromycin, clindamycin, and linezolid showed no correlation between lefamulin MIC values and those of the comparator agents. [Report 09-NAB-02B]. The collection tested did not include *cfr*-positive strains that are resistant to linezolid and lefamulin.

Lefamulin, as with other pleuromutilin antibacterials, reportedly binds to the pocket formed between the nucleotides G2576 with U2506 and G2505 in domain V of the 23S rRNA (Eyal, Matzov et al. 2015). G2576 is a nucleotide also critical for the activity of oxazolidinones. The single point mutation G2576T has been reported as one of the most common mechanisms for linezolid resistance (Gu, Kelesidis et al. 2013). Since the nucleotide G2576 is relevant to lefamulin, the effect of the G2576T point mutation on the lefamulin activity was evaluated. A subset of *S. aureus*, *S. epidermidis*, and *E. faecium* strains resistant to linezolid, characterized by the point mutation G2576T in the 23S rRNA, were tested against a series of antibacterial drugs including lefamulin and linezolid.

While linezolid MIC levels increased 4-times to 128-times (to 256 mcg/mL) by the single-point mutation G2576T, MIC values of lefamulin were elevated 2-times to 16-times when compared with the MIC_{90} of clinical wild-type isolates in the same study and reached MIC values of 0.2 mcg/mL to 1.6 mg/mL [Report NABRIVA 2008-11 MIB].

The data are shown in the tables below:

Table 162. In Vitro Antibacterial Activity of Lefamulin Against Linezolid-Resistant Bacterial Isolates Carrying the Point Mutation G2576T in the 23S rRNA Conferring Resistance to Linezolid

Species	Strain	MIC Values (µg/mL)							
		LEF	LZD	FUS	ERY	AZI	DOX	MOX	MUP
***S. aureus* (wt)**	**MIC_{90}**	**0.1**	**4**	**ND**	**6.4**	**6.4**	**0.2**	**0.1**	**ND**
S. aureus (G2576T)	B 440	0.8	128	0.5	-	-	-	-	≤0.125
S. aureus (G2576T)	B 1144	0.8	16	0.25	>256	>256	0.5	8	0.25
S. aureus (G2576T)	B 1145	0.4	32	0.25	64	128	0.25	≤0.125	0.25
***S. epidermidis* (wt)**	**MIC_{90}**	**0.1**	**2**	**ND**	**>256**	**>256**	**4**	**32**	**ND**
S. epidermidis (G2576T)	B 1141	0.8	256	0.25	16	32	2	32	>64
S. epidermidis (G2576T)	B 1142	1.6	32	0.25	1	1	2	64	64
S. epidermidis (G2576T)	B 1143	0.8	128	0.25	8	64	2	16	>64
***E. faecium* (wt)**	**MIC_{90}**	**0.1**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
E. faecium (G2576T)	B 1138	0.4	64	4	≤0.125	0.5	0.25	128	1
E. faecium (G2576T)	B 1139	0.4	32	4	≤0.125	0.5	0.25	128	0.5
E. faecium (G2576T)	B 1140	0.2	16	4	>256	>256	0.25	64	0.5

AZI=azithromycin; DOX=doxycycline; ERY=erythromycin; FUS=fusidic acid; LEF=lefamulin; LZD=linezolid; MOX=moxifloxacin; MUP=mupirocin; wt=wild-type strain.
Note: Underlined MIC values indicate resistance based on CLSI breakpoints.
Source: Report NABRIVA 2008-11 MIB.

Reviewer's Comment: *Some of the point mutations shown in the table above were above the Agency's proposed lefamulin susceptible breakpoint (≤0.25 mcg/mL for MSSA), such as S. aureus G2576T at 0.8 mcg/mL.*

Overview of Potential Mechanisms of Resistance

Potential acquired lefamulin resistance mechanisms identified to date included the following which the Applicant sorted by epidemiological relevance as follows:

Target protection by ABC-F proteins (formerly erroneously reported as putative efflux pumps**):**

- *vga*(A-E) of *Staphylococcus* spp.
- *lsa*(E) of *S. agalactiae*, *Enterococcus* spp. and *S. aureus*
- *sal*(A) of coagulase-negative *Staphylococcus* spp.

Modification of the target**:**

- Mutations in *rplC* and *rplD* genes encoding ribosomal proteins located outside of peptidyl transferase center (PTC)

- Mutations in domain V of the 23S rRNA
- Cfr methyl transferase methylating A2503 in the PTC

ABC-F proteins bind to the ribosome to affect the release of the ribosome-targeted antibacterial drugs, thereby rescuing the translation apparatus from antibacterial drug-mediated inhibition (Sharkey and O'Neill; 2018).

Methyltransferase Cfr, methylating the nucleotide A2503 of 23S rRNA, can confer resistance to lefamulin. Due to steric hindrance, binding of phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramins (PhLOPS antibiotics) is prohibited, which results in the PhLOPS-resistance phenotype.

Information on lefamulin activity in the presence of some of the resistance factors described are below:

Table 163. In Vitro Antibacterial Activity of Lefamulin and Reference Antibiotics Against Cfr-Producing *Staphylococcus* spp. Displaying the PhLOPS$_A$ Resistance Phenotype

Species	Strain	MIC Value (µg/mL)					
		Lefamulin	RET	LZD	CLI	CHL	QDA
S. epidermidis	426-3147	64	64	>256	>256	128	2
S. epidermidis	086-4303	128	256	64	>256	128	8
S. epidermidis	065-2363	256	>256	64	32	256	4
S. epidermidis	075-3831	32	32	16	>256	128	2
S. aureus	131-6952	128	256	16	16	128	4
S. aureus	004-737	16	32	8	>256	128	8
S. aureus	075-3827	64	64	16	>256	128	16

CHL=chloramphenicol; CLI=clindamycin; LEF=lefamulin; LZD=linezolid; MIC=minimum inhibitory concentration; RET=retapamulin; QDA=quinupristin-dalfopristin
Note: Underlined MIC values indicate resistance based on CLSI breakpoints.
Source: Report 08-NAB-06, Table 3

Reviewer's Comment: *The Applicant and the literature describe that mutations in the cfr gene have the potential to mediate cross-resistance between lefamulin and other antibacterials such as lincosamindes, oxazolidinones, streptogramin A and phenicols. This phenotype is called PhLOPS-resistance, and this reviewer recommends that the potential cross-resistance be described in lefamulin labeling under the "Resistance" subsection of Microbiology 12.4. The resistance frequency to lefamulin due to spontaneous mutations in vitro at 2 to 4 times MIC was 2 x 10^{-9} to 3 x 10^{-11} for S. aureus, 1 x 10^{-9} to 7 x 10^{-10} for S. pneumoniae, and 4 x 10^{-8} to 8 x 10^{-10} for S. pyogenes. Resistance development at sub-MIC concentrations, if observed, took several steps. This also should be reported in the lefamulin labeling.*

Table 164. Activity of Lefamulin and Comparators Against *S. aureus* Clinical Isolates Positive for *cfr* and *vga*(A)

Site	Isolate	Year	Country	MIC[a] [µg/mL]			Molecular results	
				Lefamulin	LZD	CLI	RM[b]	MLST[c]
004	272	2009	USA	>16	8	>16	*cfr*	5
027	1 687	2009	USA	>16	16	>16	*cfr*	5
131	6 952	2006	Belgium	>16	>16	>16	*cfr*	45
078	2 643	2008	France	8	2	0.5	*vga*(A)	8
300	1 203	2008	France	4	2	1	*vga*(A)	239
061	1 564	2008	France	8	2	>16[d]	*vga*(A)	8
078	5 092	2008	France	4	2	0.5	*vga*(A)	8
091	4 370	2008	France	2	1	0.25	*vga*(A)	8

CLI=clindamycin; LZD=linezolid; MIC=minimum inhibitory concentration; RM=resistance mechanism; MLST=multilocus sequence typing.
[a] linezolid and clindamycin MIC values are shown for comparison purposes.
[b] Molecular resistance mechanisms; *cfr* encodes for an S-adenosylmethionine enzyme; *vga*(A) encodes for an efflux pump protein, which belongs to the antimicrobial resistance (ARE) subfamily of ATP-binding cassette (ABC) transporter systems
[c] MLST, multilocus sequence typing; isolates associated with ST-8 belong to the Lyon clone
[d] Clindamycin resistance phenotype caused by *erm*A.
Source: Report 09-NAB-02B, Table 14

Resistance Mechanisms Observed During Surveillance

Possible resistance determinants have been characterized for all gram-positive cocci collected from the SENTRY surveillance studies 2010, 2015 and 2016 [Report 17-NAB-03 and Report 17-NAB-01] and display lefamulin MIC values of ≥1 mcg/mL or ≥0.5 mcg/mL, respectively.

In SENTRY 2010, 45 isolates (of 10,035 isolates in total) and in SENTRY 2015-2016, 33 isolates (of 4,090 isolates in total) were characterized by the Applicant. The most common resistance determinant among *S. aureus* collected in the SENTRY surveillance studies 2010, 2015 and 2016 was *vga*(A). Only one *cfr* positive *S. aureus* was collected in 2010, whereas during 2015 to 2016, none of the 2,919 isolates of *S. aureus* tested harbored *cfr*.
Isolates with elevated lefamulin MIC values of the most recent surveillance study are currently being analyzed but are not available at this time. Therefore, details are below for the SENTRY 2015-2016 surveillance:

The overall resistance to lefamulin was very low and a small number of isolates (25 of 7,684; 0.33%) had lefamulin MIC values ≥1 mcg/mL. Lefamulin resistance mechanisms identified in *S. aureus* isolates included *lsa*(E), *vga*(A), *vga*(E), and an alteration in L4 (E147K); *vga* was the most common determinant observed. None of the *S. aureus* isolates harbored *cfr*; however, *cfr* was identified for 2 coagulase-negative staphylococci from USA and Mexico. The most common mechanisms identified among coagulase-negative staphylococcal isolates were *vga*(A), and *vga*(B).

Two *S. sciuri* isolates exhibiting elevated lefamulin MIC values (16 mcg/mL to 32 mcg/mL) did not show any of the resistance mechanisms investigated. This species possesses an intrinsic resistance to pleuromutilins due to the presence of *sal*(A).

Among the ß-hemolytic streptococci, a *S. gallolyticus,* and a *S. lutetiensis* harbored *lsa*(E), while a *S. anginosus* isolate had alterations in L3. Five (5) *S. agalactiae* isolates from the 2010 SENTRY surveillance were additionally characterized and all harbored the *lsa*(E).

Results from this study indicated that *vga* and *lsa* genes were the most common pleuromutilin resistance mechanisms in staphylococcal and streptococcal clinical isolates, respectively, and global surveillance will be conducted to monitor changes over time. No isolates of *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* with lefamulin MICs higher than that of the wild-type population have been collected during any surveillance studies. The results of the characterization of resistance determinants during surveillance are shown in the table below:

Table 165. Resistance Determinants for Lefamulin Identified in SENTRY Surveillance Studies During 2010 and 2015-2016

Species Resistance Determinant	Number of Isolates (Incidence [%])		
	Year 2010	Year 2015	Year 2016
S. aureus	**5527**	**1273**	**1646**
cfr	1 (0.018%)	0	0
vga(A), *vga*(E)	6 (0.11%)[a]	3 (0.24%)[b]	5 (0.30%)[b]
Mutations in *rplC*, *rplD*	3 (0.05%)[a]	1 (0.08%)[b]	0
lsa(E)	ND	0	1 (0.06%)[b]
Coagulase-negative *Staphylococcus* spp.	**878**	**ND**	**276**
Cfr	2 (0.23%)[a]	-	2 (0.72%)[b]
vga(A), *vga*(B) or unspecified vga	22 (2.51%)[a]	-	8 (2.90%)[b]
Mutations in *rplC*, *rplD* only	2 (0.23%)	-	2 (0.72%)[b]
Unknown	4 (0.45%)	-	1 (0.36%)[b]
***Streptococcus* spp.**	**2654**	**1835**	**2654**
lsa(E)	5 (1.5%)[c]	0	5 (0.19%)[b, d]
Mutations in *rplC*, *rplD*, 23S rRNA		0	1 (0.04%)[b, e]

[a] Includes clonally related isolates from the same site
[b] Additional polymorphism in the 23S rRNA observed but with unknown relevance for resistance phenotype
[c] Incidence calculated based on total n of *S. agalactiae* tested; other *Streptococci* not taken into account
[d] Includes 3 *S. agalactiae* of 168 and 2 viridans group *Streptococcus* spp. of 177, incidences calculated based on n of total *Streptococcus* spp.
[e] Includes 1 viridans group *Streptococcus* spp.
Note that the Total surveillance reports for the entire collections are: Report 10-NAB-01, Report 16-NAB-07, and Report 16-NAB-01.
Source: Report 11-NAB-01 [for the 2010 strains, published as (Castanheira, Farrell et al 2013)] and Report 17-NAB-03.

Reviewer's Comment: *The Applicant has proposed to list the resistance determinants for lefamulin under the "Resistance" subsection of labeling in 12.4 Microbiology, however the Applicant did not include Isa(E), which has been identified in Staphylococcus and Streptococcus*

spp. This reviewer recommends that Isa(E) be included in the labeling. The clinical microbiology team also agreed to the addition of sal(A), as it is a lefamulin mechanism of resistance identified in Staphylococcus spp.

17.4. Susceptibility Test Methods and Interpretive Criteria

Effect of Laboratory Testing Conditions on Activity in Vitro

The ability to determine bacterial susceptibility to lefamulin using CLSI reference methods was evaluated in a series of studies. These studies included the determination of laboratory test method conditions for antimicrobial susceptibility testing, the appropriate lefamulin disk mass for disk diffusion assays, comparison of MICs determined by broth microdilution versus other methods and the quality control ranges for reference strains used to control test methods.

The effect of varying CLSI reference broth microdilution test conditions on the MIC results of lefamulin was evaluated against 12 bacterial isolates including 3 CLSI reference strains and clinical isolates of *S. aureus*, CoNS, *H. influenzae*, *S. pneumoniae*, and *E. faecium* [Report 07-NAB-01]. The following testing modifications were evaluated: incubation conditions (ambient air, 5% CO2 and anaerobic environment), inoculum concentrations (5×10^5, 5×10^3, and 5×10^7 CFU/mL), media (Mueller-Hinton Broth [MHB], Lysed Horse Blood [LHB], and *Haemophilus* Test Medium [HTM]), pH variations (pH 5.0, pH 6.0, pH 7.2 to 7.4, and pH 8.0), calcium ion content (<5, 25, and 50 mg/L) and polysorbate-80 supplementation (0.000002% to 2%). The Applicant reported that standard CLSI MIC assay conditions produced reproducible MIC results among the tested bacterial organisms, whereas in general the use of alternative (nonstandard) assay conditions resulted in either higher MIC values (anaerobic and CO2 environments, higher inocula, pH 5, pH 6, and elevated calcium content [coagulase-negative *Staphylococcus* only]) or lower MIC values (lower inocula and pH 8). Addition of polysorbate-80 (0.000002% to 0.02%) did not affect assay results (MIC value 0.12 µg/mL, data not shown) except at higher concentrations (0.2% and 2%), resulting in elevated MIC values of 0.5 µg/mL. The Applicant concluded that standard CLSI MIC assay conditions should be used for MIC determination of lefamulin by broth microdilution technique.

Reviewer's Comment: *Considering the variability seen with nonstandard test conditions for lefamulin, standard test conditions by CLSI methodology are recommended.*

Validation studies were done to determine the equivalency of MIC broth dilution tests using frozen and dried panels [Report (b) (4) 2004]. Dried Sensitire panels, and panels with 80% and 100% drug load were validated with a collection of 790 bacterial isolates including *S. aureus*, (MRSA and MSSA), coagulase-negative *Staphylococcus* spp., *E. faecium* (including VRE) *S. pneumoniae*, beta-hemolytic streptococcus species, viridans group streptococci, *H. influenzae*, and *M. catarrhalis*. Additionally, 7 nonwild type *S. aureus* and 17 resistant *E. faecium* were

selected for their elevated MICs for the testing. There were no very major or major errors reported when the following conditions were met by the Applicant:

- Susceptible MIC breakpoint of ≤1 µg/mL was used for *S. aureus*, coagulase-negative *Staphylococcus* spp., *Streptococcus pneumoniae*, ß-hemolytic and viridans group *Streptococcus* spp., *E. faecium* and *M. catarrhalis*
- Susceptible MIC breakpoint of ≤2 µg/mL was used for *Haemophilus influenzae*

Reviewer's Comment*: Differences in reading MICs at different laboratories and at 80% versus 100% growth were determined. No major effect was noted by the Applicant except for beta-hemolytic streptococci which had a 2-fold lower shift of MIC distribution compared to control when reading MICs at 80% growth inhibition.*

Agar Dilution Comparison to Microbroth Dilution

The MIC by agar dilution was evaluated and demonstrated equivalency of frozen and dried panels using a collection of 790 isolates as noted above. A minimum of 20 replicates was used for quality control.

The Applicant reported that equivalency of the agar dilution method and broth microdilution has been shown for *S. aureus* (MSSA and MRSA), while for coagulase-negative *Staphylococcus* spp., beta-hemolytic and viridans *Streptococcus* spp., broth microdilution using frozen panels resulted in approximately two-fold lower mode MIC values compared to agar dilution or broth microdilution using Sensititre® panels. For *E. faecium* the agar MIC distribution was lower by approximately a factor of two compared with broth MIC distribution. Despite the MIC shift for some organisms, no very major errors (false-susceptible) or major errors (false-resistant) were found when comparing agar dilution and broth dilution and applying a susceptibility cut-off value of ≤1 mcg/mL.

For scatterplots of MICs determined by broth microdilution versus MIC determined by agar dilution [Report (b) (4) 2004], the in vitro activity of lefamulin and comparators was evaluated by agar dilution in a surveillance study conducted in 2015/2016 by the British Society for Antimicrobial Chemotherapy (BSAC) against respiratory bacterial pathogens and gram-positive cocci collected from blood stream infections.

Disk Manufacturers

The disk content for disk diffusion (Kirby-Bauer) testing of lefamulin disks was evaluated in two studies (b) (4) [Report 07-NAB-05B and Report 09-NAB-06B]. The studies followed methods by CLSI M23. Five different disk contents were tested (1, 2, 5, 10, 20 mcg) against 30 bacterial isolates. The 20-mcg disk was selected based on the ability to discriminate best between wild-type and resistant strains including those from the SENTRY surveillance program. Scattergrams were used to compare lefamulin MIC and disk zone values and the

Applicant's proposed breakpoints were tested. Inter-method error was low at 0.4%. The disks used were manufactured at (b) (4) Provisional breakpoints determined were as follows:

Table 166. Tentative Breakpoints for Susceptibility by MIC and Disk Zone Diameters When Using Lefamulin 20 mcg Disks

Organism	Tentative Susceptible MIC [µg/mL]	Tentative Susceptible Zone Diameter [mm][a]		
	CLSI	CLSI	EUCAST	BSAC
Staphylococcus spp.	≤1	≥20	≥22	≥23
S. pneumoniae	≤1	≥19	≥18	≥21
β-hemolytic *Streptococcus* spp.	≤1	≥20 (≥21)[b]	≥21	≥26
Viridans *Streptococcus* spp.	≤1	≥15	≥15	≥21
E. faecium	≤1	≥20 (≥24)[b]	≥23	≥25
M. catarrhalis	≤1	≥20 (≥21)[b]	≥21	≥23
H. influenzae	≤2	≥20	≥16	≥18

BSAC= British Society of Antimicrobial Chemotherapy; CLSI=Clinical and laboratory Standards Institute; EUCAST=The European Committee on Antimicrobial Susceptibility Testing; MIC=minimum inhibitory concentration.

[a] Based on scatterplot data of Report (b) (4) 2004.

[b] Breakpoints in brackets are values reported in Report (b) (4) 2004. The lower susceptibility breakpoints of ≥ 20 mm represent breakpoints established in Report 09-NAB-02B. The application of the lower CLSI breakpoints (≥ 20 mm) did not result in any very major or major errors (false-susceptible or false-resistant).

Source: Report (b) (4) 2004

Disk Stability Studies

The stability of lefamulin disks of 3 batches (lot numbers 257108, 257109 and 257110) with a disk load of 20 mcg manufactured by (b) (4) was evaluated up to 18 months [Report VV-NAB-CMC-001844]. The Applicant reported that the results support a maximum shelf life of 18 months when stored at -20°C, 4°C and RT.

Reviewer's Comment: *The data on disk stability (Study Report Number 0907004-F) show that disk content remains within limits of the bioassay (90-125%) of label content through 12 months and possibly longer at at -20°C, 4°C and RTR (intended to simulate usage or transport and then return to refrigerated storage) with deterioration at elevated temperatures of RT, 37 °C and 56 °C.*

Quality Control for Susceptibility Testing

Studies conducted to establish QC ranges for the in vitro susceptibility testing of lefamulin were performed by the Applicant in accordance with guidelines established by CLSI (CLSI M7 and M23). Tier 2 multi-laboratory studies were used to establish quality control ranges QC ranges for microbroth dilution. Testing included three different lots of media, 10 replicates of each quality control strain and seven different laboratories. No variations by medium lot were observed against the three organisms, but a trailing effect was seen of the endpoint for *H. influenzae*.

Reviewer's Comment: *Quality control was presented and approved by the CLSI and is recommended by this reviewer.*

Proposed quality control is below:

Table 167. Proposed Lefamulin QC Ranges for Broth Microdilution

QC Organism	Proposed Lefamulin MIC QC Ranges		Reference
	MIC Range [µg/mL]	% in Range	
S. aureus ATCC 29213	0.06-0.25	100	Report 07-NAB-04B
S. pneumoniae ATCC 49619	0.06-0.5	98.6[a]	Report 07-NAB-04B
H. influenzae ATCC 49247[b]	0.5-2	94.3[a]	Report 07-NAB-04B
H. influenzae ATCC 49766	0.5-2	100	Report LMU-EDL-06

QC=quality control; MIC=minimum inhibitory concentration.
[a] QC range based on values obtained from 7 laboratories instead of 8.
[b] Trailing of the endpoint was observed.
Source: Report 07-NAB-04B and Report LMU-EDL-06

Table 168. CLSI-Approved QC Disk Diffusion Zone Diameters for Lefamulin According to CLSI Methodology

QC Organism	Disk Diffusion Zone Diameters for Lefamulin	
	Proposed Range [mm][a]	% in Range[a]
S. pneumoniae ATCC 49619	19–27 (19–28)	99.3 (100)
S. aureus ATCC 25923	26–32 (26–33)	97.4 (99.3)
H. influenzae ATCC 49247	22–28 (21–28)	96.0 (98.9)

QC=quality control.
[a] Proposed ranges calculated by the "Range Finder" method are shown in parentheses.
Source: Report 09-NAB-06B

Source: This submission.

Effect of Lung Surfactant and Serum on Lefamulin MIC Values

The antibacterial activity of lefamulin was evaluated in the presence of bovine lung surfactant (SurvantaTM) at concentrations ranging from 0.06% to 4% (v/v) against multidrug resistant and wild-type *S. pneumoniae* (n=3), *S. aureus* (MRSA and MSSA, n=2), *H. influenzae* (n=2) and beta-lactamase producing *E. coli* (n=1) by checkerboard broth microdilution technique [ReportNSR-BC3-ML-001]. None of the isolates tested had an increase in lefamulin MIC that was more than two-fold (within one dilution), whereas daptomycin MICs against *S. aureus* and *S. pneumoniae* increased by up to ≥160-fold with increasing concentrations of Survanta.

Lefamulin is known to exhibit protein binding in human serum. The effect of plasma protein binding on MIC values against *S. aureus* (MSSA and MRSA) isolates was investigated in three studies [Report NABRIVA2008-11, Report NABRIVA 2010-08 MIB and Report 10-NAB-03]

showing that the antibacterial activity of lefamulin was not significantly reduced (≤2.5 fold) when tested in the presence of mouse or human serum (20%, 50%, or 95%, v/v). Despite the observed moderate protein binding of lefamulin (78%) determined by equilibrium dialysis (Zeitlinger, Schwameis et al. 2016) (which is lower than the clinical pharmacology review team's assessment of protein binding as noted in other sections of this review), the in vitro antibacterial activity was maintained in the presence of human or mouse serum. Lefamulin is reported to have a low affinity for human serum albumin and alpha-acid glycoprotein.

Interaction (Synergy, Antagonism, Indifference) with Other Antibacterial Drugs (Report 01-08-2013-Nabriva1v3) evaluated the potential for synergy or antagonism of the antibacterial effects of lefamulin compared to various currently marketed antibacterial drugs against a panel of organisms. Organisms tested included *Staphylococcus aureus* (n=6), *Streptococcus pneumoniae* (n=6), *Streptococcus pyogenes* (n=3), *Streptococcus agalactiae* (n=3), *Haemophilus influenzae* (n=6), *Pseudomonas aeruginosa* (n=2) and Enterobacteriaceae (n=10). The tested antibacterial drugs included:

- For *S. aureus*: vancomycin, linezolid, levofloxacin, gentamicin, ceftriaxone, tigecycline, doxycycline, azithromycin, trimethoprim/sulfamethoxazole, clindamycin, chloramphenicol, quinupristin/dalfopristin, daptomycin, aztreonam, piperacillin/tazobactam, meropenem and amikacin.
- For *S. pneumoniae*: penicillin, ceftriaxone, levofloxacin, erythromycin, ampicillin, vancomycin, meropenem, aztreonam, piperacillin/tazobactam and amikacin.

The antibacterial susceptibility and synergy/antagonism were determined by checkerboard technique, using the broth microdilution technique according to CLSI (Clinical and Laboratory Standards Institute 2012c).

- For beta-hemolytic *Streptococcus* spp: penicillin, ceftriaxone, levofloxacin, erythromycin, ampicillin, and vancomycin.
- For *H. influenzae*: amoxicillin/clavulanic acid, ceftriaxone, trimethoprim/sulfamethoxazole, azithromycin and chloramphenicol.
- For Enterobacteriaceae and *P. aeruginosa*: aztreonam, piperacillin/tazobactam, meropenem and amikacin.

When combined with the antibacterial drugs tested, lefamulin exhibited no antagonistic effect. The effect was largely indifferent/additive with fractional inhibitory concentration indices (FICI) of 0.5 to 4 and mean FICI typically being close to 1. No apparent synergy was observed with the exception of a trend towards synergy observed across the tested *S. aureus* isolates when lefamulin was combined with doxycycline (in 5 of 6 tested isolates) and tigecycline (in 1 of 6 isolates) and a trend towards synergy observed for all *S. pneumoniae* (6 of 6 tested isolates) when lefamulin was combined with aztreonam. The Applicant used bactericidal analysis to confirm the synergy for lefamulin with doxycycline at 0.5 × MIC for five of six *S. aureus* strains evaluated at T =24 h. Synergy of lefamulin and aztreonam against *S. pneumoniae* could not be

evaluated by bactericidal curve, due to loss of activity of the growth control at T >6 h [Report 10-19-2016-Nabriva 2v3].

Activity of Lefamulin Metabolites

Analysis by the Applicant of metabolites following oral lefamulin dosing in humans showed one monohydroxylated metabolite (BC-8041) being present above the 10% level of the parent drug systemic exposure level at steady-state. The molecular structures of lefamulin, its main human metabolite BC-8041, and two chemical precursors for synthesis of lefamulin, BC-8042 (BC-8040 and 14-chloroacetyl motilin) and BC-8040, were tested. The MICs for BC-8042 were ≥4-fold higher than lefamulin. BC-8040 did not have activity (NABRIVIA 2011-06 MIB). Report NABRIVA 2018-15 MIB evaluated the in vitro antibacterial activity of BC-8041 and lefamulin against a panel of isolates including *S. aureus* (MSSA and MRSA), *S. epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *S. pneumoniae*, *M. catarrhalis*, *K. pneumoniae*, *A. baumannii*, and *H. influenzae*. This showed that the antibacterial activity of the main metabolite was reduced compared to lefamulin. BC-8041 displayed MIC values of 8-≥256 mcg/mL against all species tested in comparison to lefamulin, which had MIC values of ≤0.03 mcg/mL to 0.5 mcg/mL against *Staphylococcus* spp. and *Streptococcus* spp. and 0.06-4 mcg/mL against fastidious Gram-negative organisms.

Reviewer's Comment: *Based on the in vitro studies, the main human metabolite of lefamulin, BC-8041, does not appear to exhibit any relevant antibacterial activity.*

17.5. Animal Models of Infection

Murine Acute Systemic Infection with *S. aureus.*

The potential systemic therapeutic activity of lefamulin was assessed in the induced septicemic infection model in immunocompetent mice and compared to, linezolid and vancomycin [Report NABRIVA 2008-20 PKB]. Two clinically relevant *Staphylococcus aureus* strains were used: methicillin-susceptible ATCC 49951 and a methicillin-resistant *S. aureus* (clinical isolate, Austria). Drugs were administered subcutaneously (SC) and orally (PO). The ED50 values are shown in the table below:

Table 169. In Vivo Protective Efficacy (ED50) and MIC Values for Lefamulin, Linezolid, and Vancomycin Against MSSA and MRSA Strains in the Sepsis Model in Immunocompetent Mice

Compound	Organism	MIC (µg/mL)	Route	ED_{50} (95% CI) (mg/kg/day)
Lefamulin	*S. aureus* B9 (MSSA)	0.06	SC	1.77 (0.58–2.77)
	S. aureus B29 (MRSA)	0.125	SC	0.23 (0.12 – 0.36)
Linezolid	*S. aureus* B9 (MSSA)	2	SC	10.3 (7.40–15.07)
	S. aureus B29 (MRSA)	2	SC	2.05 (1.12 – 2.92)
Vancomycin	*S. aureus* B9 (MSSA)	1	SC	3.27 (1.48–4.66)
	S. aureus B29 (MRSA)	1	SC	6.16 (4.53 – 8.09)
Lefamulin	*S. aureus* B9 (MSSA)	0.06	PO	9.97 (8.00-12.07)
	S. aureus B29 (MRSA)	0.125	PO	ND
Linezolid	*S. aureus* B9 (MSSA)	2	PO	7.40 (4.71-12.11)
	S. aureus B29 (MRSA)	2	PO	ND

95% CI=95% confidence interval; ED_{50}=dose at 96 hours required to protect 50% of inoculated mice; MIC=minimum inhibitory concentration; ND=not done; PO=oral administration; SC=subcutaneous administration
Note: The inoculum was 3×10^7 CFU per mouse given intraperitoneally. The test compound and standard drugs were administered SC or PO 1 and 4 h after peritoneal infection to groups of 8 female NMRI mice per dose. Survival of mice was recorded daily until day 10 post-inoculation. The ED_{50} values (mg/kg/day) were determined by probit analysis using SYSTAT (SPSS Inc.).
Data sources: Report NABRIVA 2008-20 PKB, Table 1 and Table 2

S. aureus Bacteremia in Mice

The bacteremia model was used to compare the activity of therapeutic doses of lefamulin, daptomycin, vancomycin, linezolid, or tigecycline. *Staphylococcus aureus* (MSSA, strain ATCC 49951, B9) was used as the infective agent, administered IP to immunocompetent and neutropenic mice 1 hour before drug treatment [Report NABRIVA 2011-07 PKPD] using their predicted therapeutic human exposures reported for each drug. In immunocompetent mice, all antibacterial drugs showed a decrease in CFU/mL in blood, compared to the initial bacterial burden. In neutropenic animals all antibacterial drugs except linezolid showed a significant decrease in CFU/mL. Lefamulin induced a decrease in CFU in neutropenic mice that was very similar to that of daptomycin (a bactericidal drug) and vancomycin (modest bactericidal agent). Tigecycline was significantly less active than lefamulin, while linezolid barely achieved any bacterial killing in this model. In other in vitro kill curve studies, lefamulin was described as a predominantly bacteriostatic agent against *S. aureus*, but with bactericidal activity against *Streptococcus pneumoniae* and *Haemophilus influenzae*. However, this study demonstrated that lefamulin showed activity in vivo against *S. aureus* that was comparable to the bactericidal drugs daptomycin and vancomycin (approximately 4 log10 CFU/mL reduction). Lefamulin had more activity (4.5 log 10 CFU/mL reduction) in vivo in this model compared to linezolid and tigecycline (2 and 3 log reduction in CFU/mL, respectively).

Pulmonary Infection Model With *S. pneumoniae*

In the pulmonary infection murine model [NABRIVIA 2008-26 PKB]. Lefamulin was given subcutaneously in comparison to moxifloxacin and linezolid. The ED50±SE for lefamulin was 14.34±2.33 QD, and 44.06±16.75 TID. This was in comparison to moxifloxacin 31.14±7.98 QD and linezolid 63.05±30.85 QD. The bacteriostatic dose in mg/kg/day using a QD dosing regimen was 4.7 for lefamulin, 4.2 for moxifloxacin and 6.5 for linezolid.

Reviewer's Comment: *In the analysis above the SE refers to the standard error of the mean. QD is once daily dosing and TID is three times daily dosing. Some of these samples appeared to have a standard error that indicated variability in the testing. However, it does appear that lefamulin demonstrated activity in the animal models used by the Applicant versus approved comparator antibacterial drugs.*

Pulmonary Infection Model with *S. aureus*

Lefamulin, vancomycin and linezolid were tested in a severe necrotizing MRSA pneumonia model in immunocompromised BALB/c mice [NABRIVIA 2010-21 PKB]. Mice were inoculated with a lethal dose of *S. aureus* strain MRSA B29 or CA-MRSA, B118-USA300 into the lung. Two hours later the antibacterial drugs were given subcutaneously. Bacterial counts in lung tissue were measured. Lefamulin reached stasis at lower doses than linezolid and vancomycin. Maximum killing rates for MRSA B29 were -4.36 log10 CFU/lung for lefamulin, -5.33 for linezolid and -1.75 for vancomycin. For CA-MRSA lefamulin was -5.54 log10 CFU/lung, -4.79 for linezolid and -4.92 for vancomycin.

Reviewer's Comment: *The S. aureus strains used in the model had MIC values for lefamulin of 0.125 mcg/mL, linezolid of 2 mcg/mL and vancomycin of 0.5 mcg/mL*

Murine Thigh Infection Model with *S. aureus*

In report NABRIVIA 2009-27 PKB, the efficacy of lefamulin was evaluated in an immunocompetent and neutropenic murine thigh infection model with *S. aureus* B29 (MRSA). Subcutaneous and oral treatments of lefamulin were tested and showed activity in this model in comparison to linezolid and vancomycin. The results are shown in the table below:

Table 170. Therapeutic Efficacy of Lefamulin and Reference Antibiotics Against Murine Thigh Infection Caused by *S. aureus* B399 (MSSA) in Neutropenic Mice

Compound	BID Dose (mg/kg/day)	Route	MIC (µg/mL)	Viable Counts Mean±SD (Log_{10} CFU/Thigh)	Mean±SD (Δlog_{10} CFU/Thigh)
Pretreatment[a] (t=0 h)	-	-	-	6.38±0.10	±0.00
Untreated (t=48 h)	-	-	-	9.47±0.18[b,c]	+3.09
Lefamulin	100	SC	0.05	3.72±1.33[b]	-2.66
Vancomycin	100	SC	1	2.95±0.87[b]	-3.44
Linezolid	100	SC	2	6.42±2.48[b]	+0.04
Pretreatment[a] (t=0 h)	-	-	-	7.07±0.14	±0.00
Untreated (t=48 h)	-	-	-	8.86±0.30[b,c]	+1.79
Lefamulin	160	PO	0.05	3.31±0.34[b]	-3.76
Vancomycin	160	SC	1	2.65±0.76[b]	-4.42
Linezolid	160	PO	2	3.56±0.28[b]	-3.51

Δ=change from baseline; BID=twice daily; CFU=colony-forming unit; MIC=minimum inhibitory concentration; PO=oral administration; SC=subcutaneous administration.
Note: Infection with strain B399 (MSSA) was performed in neutropenic mice.
[a] indicates CFU before onset of treatment.
[b] $p < 0.05$ compared with early control (Dunnett's method).
[c] $p < 0.05$ compared with lefamulin (Bonferroni t-test).
Source: Report NABRIVA 2008-27 PKB, Table 1

Table 171. Therapeutic Efficacy of Lefamulin and Reference Antibiotics Against Murine Thigh Infection Caused by *S. aureus* B29 (MRSA) in Neutropenic Mice

Compound	Dose (mg/kg/day BID)	Route	MIC (µg/mL)	Viable Counts Mean±SD (Log_{10} CFU/Thigh)	Mean±SD (Δlog_{10} CFU/Thigh)
Pretreatment[a] (t=0 h)	-	-	-	6.76±0.26	±0.00
Untreated (t=48 h)	-	-	-	7.80±0.19[b]	+1.04
Lefamulin	100	SC	0.1	4.29±1.13[c]	-2.48
Vancomycin	100	SC	1	4.46±1.36[c]	-2.31
Linezolid	100	SC	2	4.12±1.04[c]	-2.65
Pretreatment[a] (t=0 h)	-	-	-	7.30±0.51	±0.00
Untreated (t=48 h)	-	-	-	7.60±0.41[b]	+0.30
Lefamulin	160	PO	0.1	5.99±0.72[c]	-1.31
Vancomycin	160	SC	1	5.19±0.81[c]	-2.12
Linezolid	160	PO	2	5.11±0.72[c]	-2.19

Δ=change from baseline; BID=twice daily; CFU=colony-forming unit; MIC=minimum inhibitory concentration; PO=oral administration; SC=subcutaneous administration.
[a] indicates CFU before onset of treatment.
[b] $p < 0.05$ compared to lefamulin (Bonferroni t-test).
[c] $p < 0.05$ compared with early control (Dunnett's method).
Source: Report NABRIVA 2008-27 PKB, Table 2

17.6. Pharmacokinetics and Pharmacodynamics

The PK parameters associated with different doses of lefamulin were determined in report NABRIVA 2009-28 PKPD. Exposure to lefamulin obtained in ELF and plasma was determined in study NABRIVA 2010-27 PKPD. A 2- to 4.7-fold higher exposure to lefamulin in ELF was reported by the Applicant. The pharmacokinetic parameters that the Applicant reported to best correlate with efficacy were C_{max}/MIC ratio and 24 h AUC/MIC. See the Agency's clinical pharmacology review for additional information on the effect of protein binding on PK/PD indices.

Postantibiotic Effect

A modest postantibiotic effect (PAE) was reported by the Applicant from in vivo studies of lefamulin. Report 03781A-PP04-001 included single doses of 10, 20, and 40 mcg/mL lefamulin to determine the in vivo killing rate for *S. pneumoniae* ATCC 10813. At 10 mg/kg regrowth was reported around 4 hours after dosing. At 40 mg/kg regrowth happened after 6 hours. Therefore, the post antibiotic effect was reported to be 3 to 3.5 hours for *S. pneumoniae*. Similar data were seen for *S. aureus* with a PAE of 1 to 1.5 hours.

17.7. Human Clinical Trials

Lefamulin efficacy in adult patients with CABP was established in two pivotal Phase 3 studies (Studies 3101 and 3102). Subjects in Study 3101 were treated with IV study drug for at least 3 days and then could be switched to oral therapy. Subjects in Study 3102 were treated with oral study drug only. See earlier sections of this review for additional details on the clinical trials. In both studies, diagnosis was made based on clinical signs and symptoms of CABP, laboratory abnormalities and pulmonary imaging. Pathogen identification included molecular and standard culture methods. Molecular methods were used because of poor diagnostic yield with traditional sputum cultures and to maximize the identification of baseline CABP pathogens in the Phase 3 studies. They were used to define the microITT analysis population. The diagnostic modalities used for the identification of baseline pathogens in the microITT and microITT2 populations are shown in the table below:

Table 172. Diagnostic Modalities Used for Identification of Baseline Pathogens (microITT and microITT-2 Analysis Populations)

	micro-ITT and microITT-2 Analysis Populations			microITT Analysis Population	
Test to be Performed	**Gram Stain and Culture[a]**	**Urinary Antigen Testing**	**Serological Testing**	**RQ- and RT-PCR from Sputum**	**RQ-PCR from NP Swab or OP Swab**
Timing of Assessment	**Baseline and repeat if clinically indicated**	**Baseline**	**Baseline and LFU**	**Baseline**	**Baseline**
S. pneumoniae	X[b]	X		X	X[b]
Beta-hemolytic *Streptococcus* spp.	X[c]				
S. aureus	X			X	
H. influenzae	X			X	
H. parainfluenzae	X[c]				
M. catarrhalis	X			X	
M. pneumoniae	X[d]		X	X[e]	X[d]
L. pneumophila	X[c,f]	X	X	X[e]	
C. pneumoniae	(X)[g]		X	X[e]	

LFU=Late Follow-up; microITT=microbiological intent-to-treat; microITT-2=microbiological intent-to-treat 2; PCR=polymerase chain reaction; RT-PCR=real-time (qualitative) PCR; RQ-PCR=real-time quantitative PCR.
[a] Specimens to include blood and sputum; BAL and/or pleural fluid were cultured only if clinically indicated.
[b] RQ-PCR for *S. pneumoniae* was done using sputum samples and additionally nasopharyngeal swabs. Note that culture for *S. pneumoniae* was also performed on the nasopharyngeal cultures.
[c] β-hemolytic *Streptococcus* spp. and *H. parainfluenzae* were not defined as target pathogens a priori in the SAP to be considered always as a pathogen. Inclusion as a baseline pathogen also required an appropriate morphology in the Gram-stain and must have met the criteria for adequate sputum.
[d] RT-PCR was done on oropharyngeal samples. If RT-PCR was positive, oropharyngeal samples were used for isolation of *M. pneumoniae* and subsequent susceptibility testing; on some occasions RT-PCR and culture were done in parallel.
[e] RT-PCR was performed for detection of *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila*.
[g] Culture of *C. pneumoniae* by the local laboratories was allowed per protocol though it was not cultured by any of the laboratories.
Note: If one of the indicated culture and/or non-culture tests (X) was positive (above the cut-off values for RQ-PCR and criteria for serology) the respective bacterial pathogen was included in the microITT Analysis Population.
Source: Study 3101, Section 9.5.1.3, and Study 3101, Section 9.5.1.3

Reviewer's Comment: *For the purposes of this review, decision-making focused primarily on culture, when available, for a particular pathogen. If no (or limited) culture data were available due to the fastidious nature of the organism, then emphasis was placed on FDA-cleared tests first, followed by serology. Sufficient numbers of pathogens were available that reliance on noncleared PCR-based tests was not necessary.*

Standard Culture and Gram Stain

Sputum samples were collected at screening for Gram staining and culture at local/regional laboratories. An adequate sputum sample was defined as a Gram stain with >25 polymorphonuclear lymphocytes, and <10 squamous epithelial cells per low power field. If an adequate sputum sample could not be obtained at screening, then a repeat sample was taken within 24 hours of the first dose of study drug. The Gram-stained slide read at the regional laboratory and a duplicate unstained slide were then sent to the central laboratory for confirmatory reading.

Standard culture methods were used for isolating CABP pathogens from respiratory samples or blood samples. The local/regional laboratory shipped isolates identified by culture of

respiratory or blood samples to the central laboratory for confirmatory pathogen identification at the genus and species level and for susceptibility testing. Organisms always to be sent to the central laboratory and those not to be sent were identified at the local laboratory to determine those reasonably considered an etiologic agent of CABP. The Gram stain also had to demonstrate an appropriate morphology.

The Applicant provided the following information on FDA-cleared molecular tests and exempt serological tests used during lefamulin clinical trials:

Rapid Urine Antigen Test for *L. pneumophila* and *S. pneumoniae*

Alere Binax NOW *S. pneumoniae* Urine Antigen Test: Urine Antigen test (UAT) for *S. pneumoniae* and *L. pneumophila*. This test is used in clinical practice and is FDA cleared for use in the diagnosis of pneumonia due to *S. pneumoniae* in conjunction with culture and/or other methods according to the manufacturer's instructions. A positive test result was considered predictive for *S. pneumoniae* as a causative pathogen in patients with CABP. Subjects with a positive pneumococcal UAT were included in the microITT and microITT-2. It was noted by the Applicant that only 12 subjects were vaccinated for *S. pneumoniae* in Trials 3101 and 3102. The Applicant stated that vaccination with polysaccharide is not thought to cause false-positive results 48 hours after vaccination.

Alere Binax NOW Legionella Urinary Antigen Test: Used widely in clinical practice and has been cleared by the FDA. It is deemed adequate, even in the absence of culture results, for the diagnosis and treatment of CABP caused by *L. pneumophila* according to the Infectious Disease Society of America. The specificity of UAT was greater than 99% and the UAT is used by physicians for diagnosis of *L. pneumophila*. Patients in Trials 3101 and 3102 with positive *Legionella* UAT were included in the microITT and micro-ITT-2 analysis populations. Legionella antigen can be detected in urine for up to one year following infection, therefore a patient's medical history is important. All sputum samples from subjects with a positive Legionella UAT were sent to a specialized laboratory (PA, USA) for culture, and if positive, then MIC testing was performed.

Serologic Tests for *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila*

The MBL BION *M. pneumoniae* serology test was used by the Applicant. This test was not cleared by the FDA but used because the FDA cleared test in use was discontinued by the manufacturer. The MBL BION *M. pneumoniae* antigen substrate slides and reagents were validated by the central laboratory. Inter-lab comparison of MBL BION results by the central laboratory when evaluating known negative (N=11) and positive (N=33) sera had an accuracy of 97% for titer determinations. Split sample testing was done with another laboratory and 88% percent inter-lab comparison for IgG and 97% for IgM was observed for 11 known negative and 33 known positive samples. Blood samples were collected at screening and Late Follow Up (LFU) and sent to the central laboratory by the Applicant for *M. pneumoniae* serology testing. A

positive test result was a 4-times or greater increase in *M. pneumoniae* IgG serum antibody titer to ≥1:160 between baseline and convalescent samples.

***C. pneumoniae* and *L. pneumophila* Serological Tests**

The Focus Diagnostics Chlamydia MIF IgG and IgM serologic test was used for identification of *C. pneumoniae* and Zeus *L. pneumophila* (group 1–6) indirect fluorescent antibody assay was used for *L. pneumophila* detection. Blood samples were collected at screening and LFU and sent to the central laboratory for *L. pneumophila* and *C. pneumoniae* serologic testing. A positive result was defined as a 4-fold or greater increase in *L. pneumophila* titer to >1:128 or a 4-times increase in *C. pneumoniae* IgG serum antibody titer between baseline and convalescent samples.

Reviewer's Comment: *The use of molecular tests for the purpose of use in the lefamulin clinical trials was reviewed at the IND stage (IND 106594 and IND 125546) in clinical microbiology reviews dated 1-25-16, 12-4-15 and 8-20-15 following consultation with the Center for Devices and Radiological Health (CDRH, FDA).*

In addition to the information above, information was also provided by the Applicant on tests that were not FDA-cleared including Real-time PCR of oropharyngeal swabs for M. pneumoniae, Real-Time PCR of Nasopharyngeal Swabs for S. pneumoniae, and Real-Time Qualitative/Quantitative PCR of sputum specimens. The amplified genes and cut-off values for RQ-PCR and RT-PCR were provided as well as the validation information on the molecular diagnostic methods for pathogen identification in Phase 3 clinical studies. The validation data included sensitivity, precision and reproducibility, and specificity and accuracy for the RT-PCR tests. Tests that were not FDA-cleared were not used as part of the analysis in this clinical microbiology review. Culture-based results were relied on for decision-making whenever possible.

Analysis Populations

The Microbiological Intent-to-Treat (microITT) Population included subjects from the ITT Population who had at least 1 baseline bacterial pathogen known to cause CABP, identified by at least one of the diagnostic modalities. Pathogens included *S. pneumoniae*, *H. influenzae*, *S. aureus*, *M. catarrhalis*, and *M. pneumoniae*. *L. pneumophila* regardless of Gram stain findings. For all other pathogens the Gram stain needed to also have demonstrated an appropriate morphology.

The microITT-2 Population was derived from the micro-ITT Population but excluded subjects with a baseline pathogen diagnosed by PCR methods, i.e., the microITT-2 comprised all subjects in the ITT Analysis Population who had at least 1 baseline bacterial pathogen known to cause CABP identified by a diagnostic method other than real-time PCR (i.e., culture, serology, or urine antigen).

Microbiological Assessments and Efficacy Endpoints

Selected pathogens were summarized by phenotypic susceptibility profile. *S. aureus* isolated at baseline were characterized for PVL and *mec*A status. By-pathogen microbiological responses were categorized as success (eradication, presumed eradication), failure (persistence, presumed persistence) or indeterminate. Subjects with superinfection and or colonization were determined as well as those with decreasing susceptibility. Decreasing susceptibility was defined as ≥4-times increase from baseline MIC or ≥6 mm decrease from baseline in disk inhibition zone.

In the microITT Analysis Population, the Applicant reported that the most frequently identified baseline pathogens were *S. pneumoniae* (59.3% lefamulin versus 64.6% moxifloxacin), *H. influenzae* (29.4% lefamulin versus 30.4% moxifloxacin) including a few beta-lactamase-positive isolates, *M. catarrhalis* (12.6% lefamulin versus 6.4% moxifloxacin), *M. pneumoniae* (10.7% lefamulin versus 9.9% moxifloxacin), and *L. pneumophila* (9.3% lefamulin versus 9.0% moxifloxacin). *S. aureus* was identified in 6.3% of lefamulin subjects and 2.9% of moxifloxacin subjects. Although excluded per protocol in Study 3102, three subjects had a baseline pathogen of MRSA (all resistant by cefoxitin disk test and confirmed to be *mecA* positive), all of which were enrolled in Study 3102. Baseline pathogens identified by any method were generally well-balanced between treatment groups, except for *M. catarrhalis* (12.6% lefamulin versus 6.4% moxifloxacin). Among the cultured *S. pneumoniae*, macrolide-resistant and MDR resistant *S. pneumoniae* were common (overall 31 and 32 subjects in the microITT Analysis Population, respectively; and 14 subjects each in the lefamulin treatment arm, respectively). Among the 14 subjects in the lefamulin treatment arm, 16 MDR *S. pneumoniae* isolates were collected from sputum (4) and nasopharyngeal cultures (10). The following information on resistance was provided by the Applicant:

- 6 subjects had an MDR *S. pneumoniae* resistant to 5 antibacterial drug classes (macrolides, doxycycline, clindamycin, trimethoprim-sulfamethoxazole and penicillin) with 4 being additionally, ceftriaxone intermediate
- 4 subjects had an MDR *S. pneumoniae* resistant to 3 classes (macrolides, doxycycline, clindamycin) with 3 being additionally penicillin intermediate
- The rest of the isolates were resistant to 4 classes (one subject) or to 2 classes (three subjects) and had an additional intermediate susceptibility to an additional class
- All isolates were susceptible to moxifloxacin and only 9 of 14 were susceptible to ceftriaxone

In the microITT-2 Analysis Population the percentages of subjects with *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were substantially lower compared with the microITT Analysis Population. The most frequently identified baseline pathogen by any method was *S. pneumoniae* (41.6% lefamulin versus 51.3% moxifloxacin); the next most frequently identified pathogens were *L. pneumophila* (15.3% lefamulin versus 15.9% moxifloxacin), and *M. pneumoniae* (13.9% lefamulin versus 11.8% moxifloxacin), followed by *H. influenzae* (11.0%

lefamulin versus 9.2% moxifloxacin) and *C. pneumoniae* (10.5% lefamulin versus 12.3% moxifloxacin). In the microITT-2 Analysis Population, baseline pathogens were generally well-balanced between treatment groups, except for *S. pneumoniae* (41.6% lefamulin versus 51.3% moxifloxacin) and *S. aureus* (9.1% lefamulin versus 3.1% moxifloxacin). Similar imbalances were observed in both of the individual clinical trials.

Reviewer's Comment: *The information above was provided based on any assessment for identification of the described pathogens including methods that were not FDA-cleared. The clinical microbiology review did not include assessments using non-FDA cleared methods. The information pertaining to specific diagnostic modalities is shown in the tables below.*

Serotype Distribution of *S. pneumoniae* Isolated at Baseline

All cultured *S. pneumoniae* collected from the sputum and nasopharynx were subject to serotyping. Overall, >30 different serotypes were observed in both Trial 3101 and 3102, with serotype 3 being the most common serotype identified and serotypes 19A and 19F being the second most common serotypes identified.

Baseline Pathogens by Diagnostic Modality

The Applicant evaluated how baseline pathogens were assessed by unique diagnostic modality and modality combinations, as well as how the modalities were concordant with each other.

The diagnostic modalities used by the Applicant for baseline pathogens are shown in the tables below:

Table 173. Baseline Pathogens by Diagnostic Modality (micro-ITT and micro-ITT-2 Analysis Populations)

Baseline Pathogen Positive Via Diagnostic Modality	microITT - Pooled 3101 and 3102			microITT-2 - Pooled 3101 and 3102		
	Lefamulin N=364 n (%)	Moxifloxacin N=345 n (%)	Overall N=709 n (%)	Lefamulin N=209 n (%)	Moxifloxacin N=195 n (%)	Overall N=404 n (%)
Gram-positive bacteria (aerobes) total	229 (62.9)	231 (67.0)	460 (64.9)	104 (49.8)	107 (54.9)	211 (52.2)
Any *Streptococcus pneumoniae*	216 (59.3)	223 (64.6)	439 (61.9)	87 (41.6)	100 (51.3)	187 (46.3)
Sputum culture	21 (5.8)	18 (5.2)	39 (5.5)	21 (10.0)	18 (9.2)	39 (9.7)
Blood culture	9 (2.5)	5 (1.4)	14 (2.0)	9 (4.3)	5 (2.6)	14 (3.5)
BAL culture	1 (0.3)	1 (0.3)	2 (0.3)	1 (0.5)	1 (0.5)	2 (0.5)
Pleural fluid culture	0	1 (0.3)	1 (0.1)	0	1 (0.5)	1 (0.2)
NP culture	50 (13.7)	60 (17.4)	110 (15.5)	50 (23.9)	60 (30.8)	110 (27.2)
Urinary antigen test	37 (10.2)	44 (12.8)	81 (11.4)	37 (17.7)	44 (22.6)	81 (20.0)
RQ-PCR from sputum	171 (47.0)	166 (48.1)	337 (47.5)	NI	NI	NI
NP RQ-PCR	80 (22.0)	92 (26.7)	172 (24.3)	NI	NI	NI
Any *Staphylococcus aureus*	23 (6.3)	10 (2.9)	33 (4.7)	19 (9.1)	6 (3.1)	25 (6.2)
Sputum culture	16 (4.4)	4 (1.2)	20 (2.8)	16 (7.7)	4 (2.1)	20 (5.0)
Blood culture	2 (0.5)	2 (0.6)	4 (0.6)	2 (1.0)	2 (1.0)	4 (1.0)
BAL culture	1 (0.3)	0	1 (0.1)	1 (0.5)	0	1 (0.2)
RQ-PCR from sputum	4 (1.1)	4 (1.2)	8 (1.1)	NI	NI	NI
Any beta hemolytic *streptococcus*	2 (0.5)	2 (0.6)	4 (0.6)	2 (1.0)	2 (1.0)	4 (1.0)
Any *Streptococcus pyogenes*	0	2 (0.6)	2 (0.3)	0	2 (1.0)	2 (0.5)
Sputum culture	0	2 (0.6)	2 (0.3)	0	2 (1.0)	2 (0.5)
Any *Streptococcus agalactiae*	2 (0.5)	0	2 (0.3)	2 (1.0)	0	2 (0.5)
Sputum culture	1 (0.3)	0	1 (0.1)	1 (0.5)	0	1 (0.2)
BAL culture	1 (0.3)	0	1 (0.1)	1 (0.5)	0	1 (0.2)
Gram-negative Fastidious Bacteria (aerobes)	138 (37.9)	118 (34.2)	256 (36.1)	34 (16.3)	23 (11.8)	57 (14.1)
Any *Haemophilus influenzae*	107 (29.4)	105 (30.4)	212 (29.9)	23 (11.0)	18 (9.2)	41 (10.1)
Sputum culture	23 (6.3)	17 (4.9)	40 (5.6)	23 (11.0)	17 (8.7)	40 (9.9)
BAL culture	0	1 (0.3)	1 (0.1)	0	1 (0.5)	1 (0.2)
RQ-PCR from sputum	98 (26.9)	95 (27.5)	193 (27.2)	NA	NA	NA
Any *Moraxella catarrhalis*	46 (12.6)	22 (6.4)	68 (9.6)	4 (1.9)	3 (1.5)	7 (1.7)
Sputum culture	4 (1.1)	3 (0.9)	7 (1.0)	4 (1.9)	3 (1.5)	7 (1.7)
RQ-PCR from sputum	43 (11.8)	19 (5.5)	62 (8.7)	NA	NA	NA
Any *Haemophilus parainfluenzae*	9 (2.5)	4 (1.2)	13 (1.8)	9 (4.3)	4 (2.1)	13 (3.2)
Sputum culture	9 (2.5)	4 (1.2)	13 (1.8)	9 (4.3)	4 (2.1)	13 (3.2)
Atypical pathogens	91 (25.0)	87 (25.2)	178 (25.1)	74 (35.4)	72 (36.9)	146 (36.1)
Any Mycoplasma pneumoniae	39 (10.7)	34 (9.9)	73 (10.3)	29 (13.9)	23 (11.8)	52 (12.9)
Oropharyngeal swab culture	10 (2.7)	7 (2.0)	17 (2.4)	10 (4.8)	7 (3.6)	17 (4.2)
Serology	24 (6.6)	20 (5.8)	44 (6.2)	24 (11.5)	20 (10.3)	44 (10.9)
RT-PCR from sputum	14 (3.8)	16 (4.6)	30 (4.2)	NA	NA	NA
Oropharyngeal swab PCR	16 (4.4)	12 (3.5)	28 (3.9)	NA	NA	NA
Any *Legionella pneumophila*	34 (9.3)	31 (9.0)	65 (9.2)	32 (15.3)	31 (15.9)	63 (15.6)
Legionella sputum culture	2 (0.5)	0	2 (0.3)	2 (1.0)	0	2 (0.5)
Urinary antigen test	16 (4.4)	8 (2.3)	24 (3.4)	16 (7.7)	8 (4.1)	24 (5.9)
Serology	23 (6.3)	23 (6.7)	46 (6.5)	23 (11.0)	23 (11.8)	46 (11.4)
RT-PCR from sputum	8 (2.2)	1 (0.3)	9 (1.3)	NA	NA	NA
Any *Chlamydophila pneumoniae*	27 (7.4)	31 (9.0)	58 (8.2)	22 (10.5)	24 (12.3)	46 (11.4)
Serology	22 (6.0)	24 (7.0)	46 (6.5)	22 (10.5)	24 (12.3)	46 (11.4)
RT-PCR from sputum	7 (1.9)	7 (2.0)	14 (2.0)	NA	NA	NA

BAL=bronchoalveolar lavage; microITT=Microbiological Intent-to-treat; PCR=polymerase chain reactions; RQ-PCR=Quantitative real-time PCR; RT-PCR=Qualitative real-time PCR; NP=nasopharyngeal; NA=not applicable.
Note: Percentages are based on the number of subjects in each treatment group. A subject could have had more than 1 pathogen identified in 1 or more testing modality. Multiple isolates of the same species from the same subject identified by the same testing modality were counted only once. Subjects with the same pathogen were counted only once in the "any" pathogen line. Subjects with more than 1 Gram-positive, Gram-negative fastidious, or atypical pathogen were counted only once in the overall tabulation of Gram-positive bacteria (aerobes), Gram-negative fastidious bacteria (aerobes), and atypical pathogens, respectively. Only proposed List 1 and List 2 pathogens are presented here.
Source: ISE Table 14.1.11.1. and ISE Table 14.1.11.2.

Susceptibility of Baseline Pathogens in Phase 3 Trials

Gram-positive and gram-negative pathogens were tested for susceptibility to lefamulin, moxifloxacin, and comparators (erythromycin, azithromycin, clindamycin, doxycycline, moxifloxacin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, ceftriaxone, linezolid, vancomycin, ceftaroline, and penicillin) by broth microdilution using CLSI methods. Disk susceptibility testing was done with 20 mcg lefamulin disks and comparators (moxifloxacin, ampicillin, erythromycin, cefoxitin). For *M. pneumoniae*, lefamulin, moxifloxacin, and comparators were tested by broth microdilution. For *L. pneumoniae*, agar dilution methods were used.

Correlation Between Phase 3 MIC Distributions and Surveillance Data

Overall, the MIC distributions for isolates from the pooled microITT analysis were similar with the MIC distribution from the global SENTRY Surveillance 2017. The mode values for *S. pneumoniae*, *S. aureus*, *H. influenzae* and *M. catarrhalis* were two-times higher than that observed in the surveillance study. The MIC distribution for *L. pneumophila* was not included as only 2 isolates were collected in the clinical program.

Efficacy Results

The primary efficacy outcome in Trials 3101 and 3102 was Early Clinical Response (ECR) in the ITT population. Early clinical response by pathogen and MIC is shown in the table below for pooled data from Trials 3101 and 3102. Clinical response was also evaluated among different serotypes of *S. pneumoniae* and *H. influenzae*, but the Applicant did not report any significant direct correlation between efficacy and serotypes.

Reviewer's Comment: *Clinical response rates by baseline pathogen and resistance phenotype were provided by the Applicant as pooled data from Trials 3101 and 3102. For all baseline pathogens and resistance phenotypes tested, the responder rate or clinical success was greater than 82%. See the clinical review for additional details related to the Agency's assessment of clinical response in the Phase 3 trials. Although molecular tests were used in the clinical trials, the clinical microbiology analysis focused primarily on the susceptibility testing results as shown in the table below. Culture was necessary for breakpoint analysis and determination.*

Table 174. Early Clinical Response by Pathogen and by Minimal Inhibitory Concentration (Pooled Data from Trial 3101 and Trial 3102-micro-ITT Analysis Population)

Pathogen	Subjects with Pathogen[a] N	No. of Pathogens Tested[b]	Lefamulin MIC (µg/mL)								
			≤0.03 n/N (%)	0.06 n/N (%)	0.12 n/N (%)	0.25 n/N (%)	0.5 n/N (%)	1 n/N (%)	2 n/N (%)	4 n/N (%)	>4 n/N (%)
Gram-Positive Bacteria (aerobes)											
Streptococcus pneumoniae	**64**	**61**	**0/0**	**0/1**	**9/10 (90.0)**	**35/41 (85.4)**	**7/9 (77.8)**	**0/0**	**0/0**	**0/0**	**0/0**
PSSP	47	47	0/0	0/1	8/9 (88.9)	22/28 (78.6)	7/9 (77.8)	0/0	0/0	0/0	0/0
PISP	9	9	0/0	0/0	1/1 (100)	8/8 (100)	0/0	0/0	0/0	0/0	0/0
PRSP	7	7	0/0	0/0	2/2 (100)	5/5 (100)	0/0	0/0	0/0	0/0	0/0
MDRSP	14	14	0/0	0/0	3/3 (100)	11/11 (100)	0/0	0/0	0/0	0/0	0/0
Macrolide resistant	14	14	0/0	0/0	2/2 (100)	11/12 (91.7)	0/0	0/0	0/0	0/0	0/0
Streptococcus pneumoniae (excluding NP culture)[c]	**28**	**22**	**0/0**	**0/0**	**0/0**	**13/16 (81.3)**	**5/6 (83.3)**	**0/0**	**0/0**	**0/0**	**0/0**
PSSP[c]	22	19	0/0	0/0	1/1 (100)	9/12 (75.0)	5/6 (83.3)	0/0	0/0	0/0	0/0
PISP[c]	4	4	0/0	0/0	0/0	4/4 (100)	0/0	0/0	0/0	0/0	0/0
MDRSP[c]	4	4	0/0	0/0	0/0	4/4 (100)	0/0	0/0	0/0	0/0	0/0
Macrolide resistant[c]	4	4	0/0	0/0	0/0	3/4 (75.0)	0/0	0/0	0/0	0/0	0/0
Staphylococcus aureus	**19**	**18**	**0/0**	**1/1 (100)**	**13/13 (100)**	**4/4 (100)**	**0/0**	**0/0**	**0/0**	**0/0**	**0/0**
MSSA (all *mecA* negative)	16	16	0/0	1/1 (100)	11/11 (100)	4/4 (100)	0/0	0/0	0/0	0/0	0/0
MRSA (all *mecA* positive)	2	2	0/0	0/0	2/2 (100)	0/0	0/0	0/0	0/0	0/0	0/0
PVL positive	1	1	0/0	0/0	0/0	1/1 (100)	0/0	0/0	0/0	0/0	0/0
PVL negative	17	17	0/0	1/1 (100)	13/13 (100)	3/3 (100)	0/0	0/0	0/0	0/0	0/0
Gram-Negative Fastidious Bacteria (aerobes)											
Haemophilus influenzae	23	20	0/0	0/0	0/0	0/0	2/2 (100)	12/13 (92.3)	4/4 (100)	1/1 (100)	0/0
β-lactamase positive	2	2	0/0	0/0	0/0	0/0	1/1 (100)	1/1 (100)	0/0	0/0	0/0
β-lactamase negative	18	18	0/0	0/0	0/0	0/0	1/1 (100)	11/12 (91.7)	4/4 (100)	1/1 (100)	0/0
Haemophilus parainfluenzae	9	8	0/0	0/0	0/0	0/0	1/1 (100)	1/1 (100)	2/2 (100)	2/2 (100)	2/2 (100)
Moraxella catarrhalis	4	4	0/0	1/1 (100)	2/3 (66.7)	0/0	0/0	0/0	0/0	0/0	0/0
Atypical Pathogens											
Mycoplasma pneumoniae	10	10	10/10 (100)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Legionella pneumophila	2	2	0/0	0/0	0/0	0/0	1/1 (100)	0/1	0/0	0/0	0/0

CLSI=Clinical and Laboratory Standards Institute; ECR=early clinical response; MIC=minimum inhibitory concentration; MDRSP=Multidrug-resistant *S. pneumoniae*; microITT=Microbiological Intent-to-Treat Analysis Population; MSSA=Methicillin-susceptible *S. aureus*; MRSA=Methicillin-resistant *S. aureus*; NP=Nasopharyngeal; PSSP=Penicillin-susceptible *S. pneumoniae*; PISP=Penicillin-intermediate *S. pneumoniae*; PRSP=Penicillin-resistant *S. pneumoniae*; PVL=Panton-Valentine Leukocidin.

[a] n=number of subjects with an ECR of Responder.

[b] N=number of subjects with the specified pathogen at the specified MIC.

[c] excluding NP Culture.

Note: Percentages are based on number of subjects with an ECR of Responder divided by number of subjects with the pathogen at the specified MIC (µg/mL) by CLSI methodology.

Note: A subject could have had more than 1 pathogen. Multiple isolates of the same species from the same subject were counted only once, regardless of source using the isolate with the highest MIC to study drug received.

Source: ISE Table 14.2.9.1

Reviewer's Comment: *It is noted that there are more pathogens in these trials than that shown in the table above as MICs were not obtained for some which were difficult to culture. This is particularly the case for the Phase 3 clinical trials in which molecular diagnostics were used.*

Emergent Infections and Decreasing Susceptibility

The Applicant reported that three subjects in the lefamulin arm had superinfections. All three included pathogens which were not thought to be part of the spectrum of activity for lefamulin. The pathogens included *C. koseri*, *P. aeruginosa*, *K. pneumoniae*, and *E. faecalis*. No pathogens were reported to have decreasing susceptibility in either Trial 3101 or Trial 3102. In terms of MIC, decreasing susceptibility was defined as a ≥4-times increase from baseline to the study drug received.

17.8. Interpretive Criteria

Susceptibility Testing Interpretive Criteria Breakpoint Proposal for MIC Dilution Testing (STIC)

The Applicant's STIC proposal was based on epidemiological cut-off values, clinical cut-off values, clinical exposure response cut-off values and clinical cut-off values. Disk diffusion correlations were proposed using the Error Rate Bounded method as stated in CLSI M23. Isolates used were from the pooled Phase 3 trials and nonclinical studies. The Applicant's STIC proposal is shown in the table below:

Table 175. Proposed Disk Diffusion Zone Diameter and MIC STIC (Breakpoints)

Organism	Proposed Susceptible MIC Value (µg/mL)				Tentative Susceptible Zone Diameter (mm)			
	S	I	R	NS	S	I	R	NS
S. pneumoniae	≤1	-	-	≥2	≥17	-	-	≤16
(b) (4)								
H. influenzae	≤4	-	-	≥8	≥15	-	-	≤14
H. parainfluenzae	≤8	-	-	≥16	-	-	-	-
M. catarrhalis	≤0.5	-	-	≥1	≥20	-	-	≤19
ß-hemolytic *Streptococcus* spp.	≤0.25	-	≥0.5	-	≥20	-	≤19	-
Viridans Group *Streptococcus* spp.	≤0.5	1	≥2	-	≥18	15-17	≤14	-

I=intermediate; MIC=minimum inhibitory concentration; NS=non-susceptible; R=resistant; S=susceptible.

Reviewer's Comment: *The Applicant's proposal was reevaluated by this reviewer and with concurrence from the clinical team.* (b) (4) *were not included in the Agency's proposed breakpoints. Specific beta-hemolytic Streptococcus spp. and Viridans group Streptococcus spp. (S. agalactiae, S. anginosis, S. pyogenes, S. salivarius, S. mitis) were included in the second list only due to lack of clinical experience for inclusion in the first list. See final clinical microbiology recommendations at the end of this document for additional details on the Agency's proposed breakpoints and labeling recommendations.*

Nonclinical PK/PD cutoff Value

Reviewer's Comment: *The Agency's clinical pharmacology team determined that there was a difference in the target attainment that was possible under fed versus fasting conditions. This*

difference was notable at a cut-off value of MIC 0.125 mcg/mL. Above that value, the exposures under fed conditions could not support the breakpoints. There was residual uncertainty in the cut-off values under fasting conditions and therefore reliance was on the clinical cut-off values and in vitro antimicrobial activity of lefamulin for determination of breakpoints. The Applicant's breakpoint proposal was different than the Agency's, and one reason is because of differences in determination of the nonclinical PK/PD cut-off values for susceptibility. The Agency's clinical pharmacology team further reevaluated probability of target attainment in epithelial lining fluid (ELF) versus plasma and the effect of protein binding. See the Agency's clinical pharmacology review for further details.

The Applicant also provided information on the activity of lefamulin against other species that are not relevant to the indication of CABP and stated that changes to the gut microbiome may occur with lefamulin if fecal lefamulin concentrations exceed the MIC of the organism as lefamulin has activity against organisms such as *Lactobacillus* spp., and *Bifidobacterium* spp. with MICs ≤1 mcg/mL.

Agency's Breakpoint Rationale

- Breakpoints were not provided for *H. parainfluenzae*, *M. catarrhalis*, Beta-hemolytic *Streptococcus* spp. or Viridans Group *Streptococcus* spp. due to insufficient clinical information. These organisms are included in the second list (i.e.; *H. parainfluenzae*, *M. catarrhalis* and *S. pyogenes*, *S. agalactiae, S. anginosus, S. salivarius, and S. mitis*).
- Breakpoints are provided for MSSA. MRSA is included in the second list. For MSSA, the susceptible only breakpoint was provided as shown in the table below. The susceptible breakpoint of ≤ (b) (4) mcg/mL (proposed by the Applicant) is not supported by the probability of PK-PD target attainment ((b) (4)) or by clinical data. The PTA was ~90% at MIC of 0.25 mcg/mL, supporting a susceptible breakpoint of ≤0.25 mcg/mL. Note that the susceptible breakpoint of (b) (4) mcg/mL is greater than MIC_{90} of 0.12 mcg/mL. At MIC ≤0.25 mcg/mL, the clinical success rate was 100% (16/16) in clinical trials (early clinical response in Trials 3101 and 3102); at MIC of 0.25 mcg/mL, the clinical success rate was 100% (4/4). No clinical data are available at MIC above 0.25 mcg/mL, so an intermediate breakpoint cannot be established.
- For *S. pneumoniae*, a susceptible only breakpoint was provided as shown in the table below. Similar to *S. aureus*, the PTA does not support the Applicant's proposed breakpoint of (b) (4) . The PTA was ~90% at MIC of 0.5 mcg/mL. Additionally, a susceptible breakpoint of 0.5 mcg/mL is above the MIC_{90} of 0.25mcg/mL for *S. pneumoniae*. At MICs ≤0.5 mcg/mL for *S. pneumoniae,* the clinical success rates were 51/60 (85%) overall and 18/22 (82%) for *S. pneumoniae* excluding those identified from a nasopharyngeal culture; clinical response rate at MIC 0.5 mcg/mL was 78% (7/9). No clinical data were available at MIC above 0.5 mcg/mL.
- For *H. influenzae*, a susceptible only breakpoint was provided as shown in the table below. At MIC of 2 mcg/mL, the susceptible breakpoint is at the MIC_{90} for *H. influenzae*

of 2 mcg/mL. The susceptible breakpoint of ≤2mcg/mL is supported by the clinical data with 18/19 (95%) clinical successes at or below an MIC of 2 mcg/mL. With only 1 isolate with MIC above 2 mcg/mL, there were not enough clinical data to propose a higher susceptible breakpoint.

Table 176. Agency's MIC Breakpoints for Lefamulin

	Minimum Inhibitory Concentrations (mcg/mL)		
Pathogen	**S**	**I**	**R**
S. aureus (MSSA)	≤0.25	---	---
S. pneumoniae	≤0.5	---	---
H. influenzae	≤2	---	---

S = Susceptible, I = Intermediate, R = Resistant
Note: The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC results other than "Susceptible" should be submitted to a reference laboratory for further testing.

MIC-Disk Correlation

The Agency's disk diffusion breakpoints were determined based on the correlation of the disk diffusion diameter to the Agency's MIC susceptible breakpoint for the proposed organisms. The rationale is in the clinical microbiology summary in section 4.3 of this review using re-analysis of the data submitted in the NDA and CLSI guidelines. The recommended susceptible disk diffusion zone diameter breakpoints were ≥23 mm for MSSA, ≥17 mm for *S. pneumoniae*, and ≥17 mm for *H. influenzae*.

The Agency is providing a disk diffusion breakpoint for MSSA and not MRSA for the following reason: although lefamulin has activity against MRSA both in vitro, and in vivo experimental models (murine bacteremia, thigh and pneumonia), without sufficient data from Phase 3 clinical trials, the Agency is unable to establish an MIC breakpoint for MRSA and to make a meaningful correlation between disk diffusion zone diameters and MIC values.

17.9. Final Clinical Microbiology Recommendations

From a clinical microbiology perspective, the information provided by the Applicant supports the efficacy of lefamulin for the treatment of susceptible bacteria listed in the product labeling for the indication of CABP. The following is a summary of the Agency's proposed clinical microbiology labeling changes and rationale:

- Subsection 12.4 has been updated in accordance with the FDA documents titled, "Microbiology Data for Systemic Antibacterial Drugs-Development, Analysis, and Presentation: Guidance for Industry" and "Systemic Antibacterial and Antifungal Drugs: Susceptibility Test Interpretive Criteria Labeling for NDAs and ANDAs: Guidance for Industry".

- Quality Control ranges used for susceptibility testing have been accepted by the Clinical and Laboratory Standards Institute (CLSI) and are recommended here as published in the current CLSI document M100.
- The mechanism of action subsection was revised for clarity, brevity and accuracy in comparison to current literature and submitted study reports.
- The resistance section was modified to describe the frequency of resistance for specific pathogens and the lefamulin concentration.
- The list of resistance mechanisms was updated to include *Isa*(E) which was identified among isolates with elevated lefamulin MICs (>32 mcg/mL) in *S. aureus* and beta-hemolytic *Streptococcus* spp. including *S. agalactiae*. A mechanism of resistance to lefamulin found in *Staphylococcus* spp., *sal*(A) was also added.
- A cross-resistance statement was added, "Cfr methyl transferase has the potential to mediate cross-resistance between lefamulin and phenicols, lincosamides, oxazolidinones, and streptogramin A antibacterials," based on the reference: Veve, et al.; Lefamulin: Review of a Promising Novel Pleuromutilin Antibiotic. Review of Therapeutics. 18, July 2018.
- The (b) (4) was removed from the first list of bacteria.
- The statement, "XENLETA has demonstrated synergy in vitro with doxycycline against *S. aureus* (b) (4) was revised, as Study Report: 10-19-2016-Nabrivia 2v3 FINAL Report stated that synergy betwee (b) (4)
- (b) (4) was removed from the first list of bacteria because there were less than 10 isolates (n=8) from the Phase 3 clinical trials. It was moved to the second list.
- (b) (4) was moved from the first list of bacteria to the second list because of lack of clinical data from culture and FDA cleared tests (4 isolates were obtained, 3 with a favorable clinical response at the ECR visit).
- Headings in the second list, (b) (4)" and " (b) (4) " were removed and specific species were listed, because not all species were relevant to the indications. The following were listed instead (*S. pyogenes, S. agalactiae*, *S. anginosus*, *S. salivarius, S. mitis*). " (b) (4)." was removed from the label because it was not relevant to CABP.
- The breakpoints are shown in the table below. The Applicant's proposal for breakpoints was revised based on the Agency's analysis of PK/PD taking fasting and fed states into consideration, use of standard culture-based tests, and lefamulin activity in vitro and in CABP clinical trials.

Table 177. Agency's Interpretive Criteria for Lefamulin

	Minimum Inhibitory Concentrations (mcg/mL)			Disk Diffusion (Zone Diameter in mm)		
Pathogen	**S**	**I**	**R**	**S**	**I**	**R**
Staphylococcus aureus (methicillin-susceptible isolates)	≤0.25	-	-	≥23	-	-
Streptococcus pneumoniae	≤0.5	-	-	≥17	-	-
Haemophilus influenzae	≤2	-	-	≥17	-	-

S = Susceptible; I = Intermediate; R = Resistant
Note: The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC results other than "Susceptible" should be submitted to a reference laboratory for further testing.

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