CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214916Orig1s000

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)	214916
Priority or Standard	Priority
Submit Date(s)	December 23, 2020
Received Date(s)	December 23, 2020
PDUFA Goal Date	August 23, 2021
Division/Office	Division of Dermatology and Dentistry
Review Completion Date	August 20, 2021
Established/Proper Name	difelikefalin
(Proposed) Trade Name	KORSUVA
Pharmacologic Class	Kappa opioid agonist
Code name	CR845
Applicant	Cara Therapeutics, Inc.
Dosage form	Intravenous solution
Applicant proposed Dosing Regimen	(b) (4) /mL solution in sterile isotonic acetate buffer for intravenous (IV) injection to be given at the end of each dialysis session (0.5mcg/kg), generally 3 times a week.
Applicant Proposed Indication(s)/Population(s)	For the treatment of moderate-to-severe pruritus associated with chronic kidney disease (CKD-aP) in adult patients undergoing hemodialysis (HD).
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	707151000, Uremic Pruritus
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	KORSUVA is indicated for the treatment of moderate-to-severe pruritus associated with chronic kidney disease (CKD-aP) in adults undergoing hemodialysis (HD). Limitations of Use KORSUVA has not been studied in patients on peritoneal dialysis and is not recommended for use in this population.
Recommended SNOMED CT Indication Disease Term for each Indication (if applicable)	707151000, Uremic Pruritus
Recommended Dosing Regimen	(b) (4) /mL solution in sterile isotonic acetate buffer for intravenous (IV) injection to be given at the end of each dialysis session (0.5mcg/kg), generally 3 times a week.

Table of Contents

Table of Tables

Table of Figures

Reviewers of Multi-Disciplinary Review and Evaluation

Regulatory Project Manager	Jennifer Harmon, PharmD
Nonclinical Reviewer	Yongcheng Huang, PhD
Nonclinical Supervisor	Barbara Hill, PhD
Nonclinical Division Director	Andrew Goodwin
Office of Clinical Pharmacology Reviewer(s)	Dipak Pisal, MS, PhD
Office of Clinical Pharmacology Team Leader(s)	Chinmay Shukla, PhD
Clinical Reviewer	Gary Chiang, MD, MPH
Clinical Team Leader	Amy Woitach, DO, MS
Statistical Reviewer	Matthew Guerra, PhD
Statistical Team Leader	Mohamed Alosh, PhD
Cross-Disciplinary Team Leader	Amy Woitach, DO, MS
Division Director (DDD)	Kendall Marcus, MD
Division Director (OCP)	Suresh Doddapaneni, PhD
Division Director (OB)	Laura Lee Johnson, PhD
Office Director	Julie Beitz, MD

Additional Reviewers of Application

OPQ	Application Technical Lead: Hamid Shafiei, PhD Regulatory Business Process Manager: Melinda Bauerlien, MS Drug Substance: Katharine Duncan, PhD/Donna Christner, PhD Drug Product: Mark Seggel, PhD/Wendy Wilson-Lee, PhD Manufacturing: Kumar Janoria, PhD/ Renee Marcsisin, PhD/ Elizabeth Bearr, PhD Microbiology: Renee Marcsisin, PhD/ Elizabeth Bearr, PhD Environmental Assessment: Mark Seggel, PhD/Wendy Wilson-Lee, PhD
OPDP Reviewer	Laurie Buonaccorsi, PharmD
OPDP Team Leader	Matthew Falter, PharmD
OSI Reviewer	Phuc Nguyen, MD
OSI Team Leader	Karen Bleich, MD
OSE/DEPI	Catherine Lerro, PhD
OSE/DMEPA Reviewer	Madhuri Patel, PharmD
OSE/DMEPA Team Leader	Sevan Kolejian, PharmD
OSE/DRISK	Donella Fitzgerald, PharmD
OSE/DRISK Team Leader	Jacqueline Sheppard, PharmD

OSE/DPV Reviewer	Melissa Reyes, MD
OSE/DPV Team Leader	Vicky Chan, PharmD
CSS Reviewer	Katherine Bonson, PhD

OPQ=Office of Pharmaceutical Quality
OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DEPI= Division of Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
DRISK=Division of Risk Management
CSS=Clinical Substance Staff

Signatures

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Office of Pharmaceutical Quality Technical Lead	Hamid Shafiei, PhD	OPQ/DNDP II/NDPB V	Sections: 4.2	**Select one:** __ Authored _X__ Approved
	Signature: *{See appended electronic signature page}*			
Nonclinical Reviewer	Yongcheng Huang, PhD	OII/DPT-II	Sections: 5, 16.3	**Select one:** _X_ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			
Nonclinical Supervisor	Barbara Hill, PhD	OII/DPT-II	Sections: 5, 16.3	**Select one:** ___ Authored _X_ Approved
	Signature: *{See appended electronic signature page}*			
Nonclinical Division Director	Andrew Goodwin, PhD	OII/DPT-II	Sections: 5, 16.3	**Select one:** ___ Authored _X_ Approved
	Signature: *{See appended electronic signature page}*			
Clinical Pharmacology Reviewer	Dipak Pisal, MS, PhD	OCP/DIIP	Sections 6, 16.4	**Select one:** _X__ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Pharmacology Team Leader	Chinmay Shukla, PhD	OCP/DIIP	Section: 6, 16.4	**Select one:** ___ Authored _X__ Approved
	Signature: *{See appended electronic signature page}*			
Pharmacometrics Reviewer	Yangbing Li, PhD	OCP/DPM	Section: 16.4	**Select one:** _X__ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			
Pharmacometrics Team Leader	Jiang Liu, PhD	OCP/DPM	Section: 16.4	**Select one:** ___ Authored _X__ Approved
	Signature: *{See appended electronic signature page}*			
Clinical Pharmacology Division Director	Suresh Doddapaneni, PhD	OCP/DIIP	Section: 6, 16.4	**Select one:** ___ Authored __X_ Approved
	Signature: *{See appended electronic signature page}*			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Reviewer	Gary Chiang MD, MPH	OII/DDD	Sections:1, 2, 3, 4.1, 7, 8, 9, 10, 11, 12, 13, 16	**Select one:** _X__ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			
Clinical Team Leader	Amy Woitach DO, MS	OII/DDD	Sections: 1, 2, 3, 4.1, 7, 8, 9, 10, 11, 12, 13, 16	**Select one:** ___ Authored _X__ Approved
	Signature: *{See appended electronic signature page}*			
Division Director (Clinical)	Kendall Marcus, MD	OII/DDD	Sections: 14	**Select one:** _X_ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			
Office Director	Julie Beitz, MD	OII	Sections: 15	**Select one:** _X_ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			
Statistical Reviewer	Matthew Guerra, PhD	OTS/OB/DBIII	Sections: 8.1, 8.3	**Select one:** _X_ Authored ___ Approved
	Signature: *{See appended electronic signature page}*			
Statistical Team Leader	Mohamed Alosh, PhD	OTS/OB/DBIII	Sections: 8.1, 8.3	**Select one:** ___ Authored _X_ Approved
	Signature: *{See appended electronic signature page}*			
Division Director (OB)	Laura Lee Johnson, PhD	OTS/OB/DBIII	Sections: 8.1, 8.3	**Select one:** ___ Authored _X_ Approved

	Signature: *{See appended electronic signature page}*

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
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
CKD-aP	Chronic kidney disease – associated pruritus
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
eCTD	electronic common technical document
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
HD	Hemodialysis
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
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
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
PT	Preferred term
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event
WI-NRS	Worst Itching Intensity Numeric Rating Scale

1 Executive Summary

1.1. Product Introduction

Difelikefalin is a selective and full agonist at the kappa opioid receptors (KORs), with minimal identified off-target activity. In the evaluation of difelikefalin, it does not seem to have binding or functional activity at mu opioid receptors (MORs), the main target of opioid analgesics. The selective activity of difelikefalin at KORs mostly avoids mu opioid associated side effects, such as respiratory depression, dependence, and euphoria. The physiochemical properties of difelikefalin (e.g., hydrophilic, synthetic D-amino acid peptide with high polar surface area and charge at physiological pH) minimize passive diffusion or active transport across membranes, to restrict penetration into the brain.

The pathophysiology of chronic kidney disease associated pruritus (CKD-aP) is not fully understood but is thought to be multifactorial, including systemic inflammation and an imbalance in the endogenous opioid system (e.g., overexpression of MORs and concomitant downregulation of KORs). Opioid receptors are known to modulate itch signals and inflammation, with KOR activation reducing itch and producing immunomodulatory effects.

The pharmacological actions of KOR agonists on peripheral sensory neurons and immune cells are considered mechanistically responsible for the antipruritic and anti-inflammatory effects and were the basis for the development of difelikefalin for the proposed indication. Difelikefalin preferentially activates KORs expressed outside of the CNS, mitigating side effects such as dysphoria and psychomimetic effects associated with the activation of centrally located KORs.

1.2. Conclusions on the Substantial Evidence of Effectiveness

Cara Therapeutics Inc. submitted an NDA for KORSUVA (difelikefalin) intravenous drug product for the treatment of moderate-to-severe pruritus in hemodialysis patients. KORSUVA was granted breakthrough therapy designation (BTD) on 21-JUNE-2017 by the Agency and was granted a priority review by the Division of Dermatology and Dentistry (DDD). The applicant has provided substantial evidence of effectiveness and tolerable safety in the clinical development program for KORSUVA. The evidence consists of 18 completed clinical studies, with two placebo-controlled, randomized, 12-week, double-blind studies with up to 52-week open-label extensions and two open-label safety studies in subjects with chronic kidney disease-associated pruritus (CKD-aP). The safety database includes 848 subjects in the pivotal double-blind Phase 3 clinical trials and 1306 subjects in the extension and open-label Phase 3 clinical trials. These subjects all had moderate-to-severe pruritus associated with hemodialysis.

The primary endpoint for the pivotal Phase 3 clinical trials was based on achieving ≥ 4 (and ≥ 3)-point improvement from baseline with respect to the weekly mean of the daily 24-hour worse-

itch numeric rating scale (WI-NRS). These clinical trials met their primary endpoint and demonstrated efficacy compared to placebo (*diff.* 22% and 11% for ≥ 3 WI-NRS; 19% and 12% for ≥ 4 WI-NRS, CLIN3102 and CLIN3103, respectively). Safety for KORSUVA was based on the adverse events profiles in the clinical trials. The main concerns are dizziness, somnolence, mental status changes, gait disturbances/falls, and hyperkalemia. Other events including strokes, cardiac events, and infections are seen at baseline with the hemodialysis patients and are no higher between KORSUVA and placebo.

Cara Therapeutics Inc. has provided adequate evidence of safety and efficacy of KORSUVA (difelikefalin) IV for the treatment of moderate-to-severe pruritus in hemodialysis patients. The benefits of KORSUVA outweigh the risks associated with treatment and approval for this drug product for marketing is recommended.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Difelikefalin, a selective, kappa opioid receptor (KOR) agonist, is a new molecular entity (NME) proposed for the treatment of moderate-to-severe chronic kidney disease-associated pruritus (CKD-aP) in adult patients undergoing hemodialysis (HD).

Approximately 20% to 40% of patients undergoing hemodialysis (HD) suffer from moderate-to-severe chronic kidney disease-associated pruritus (CKD-aP). CKD-aP is a medical condition characterized by a generalized and intractable itch. This systemic pruritus does not originate from skin lesions, but rather is a persistent itch sensation that often leads to considerable mechanical skin damage due to a continuous and uncontrollable urge to scratch. Patients with CKD-aP suffer from severely impaired physical and mental health. In addition, subjects who are undergoing HD and have severe itching are reported to have a higher rate of all-cause mortality, including higher rates of cardiovascular-related mortality and infection-related mortality, relative to patients without pruritus.

No treatment has been approved for CKD-aP in the United States. Several treatments have been used off-label, such as antihistamines, corticosteroids, gabapentin, and pregabalin; however, these drugs are limited by a lack of proven antipruritic efficacy and poor tolerability. Thus, there remains an unmet medical need for safe and effective treatments for CKD-aP in a susceptible population of patients who present with significant comorbid conditions.

Difelikefalin demonstrated effectiveness in two adequate and well-controlled trials (i.e., CLIN3102 and CLIN3103) in subjects with moderate-to-severe CKD-aP undergoing HD. These two replicate trials showed difelikefalin to be statistically superior to placebo for the protocol-specified primary efficacy endpoint (≥3-point improvement in Worst Itching Intensity Numerical Rating Scale [WI-NRS] at Week 12) and the Agency's recommended primary efficacy endpoint (≥4-point improvement in WI-NRS at Week 12) in the target population. For the proportion of subjects achieving a ≥3-point improvement (reduction) in WI-NRS at Week 12, the treatment effect (i.e., difference between difelikefalin and placebo) was 22% and 11% for Trials CLIN3102 and CLIN3103, respectively. For the ≥4-point improvement (reduction) threshold at Week 12, the treatment effect was 19% and 12% for Trials CLIN3102 and CLIN3103, respectively. The odds ratios for both endpoints ranged from 1.6 to 2.9 across the two trials.

The safety profile of difelikefalin was characterized for this hemodialysis population through analyses of 1306 subjects in the Phase 3 clinical trials, the open-label extensions, and the open-label clinical trials. This analysis was focused on the Primary Safety Pool, the two Phase 3 clinical trials CLIN3102 and CLIN3103 (N=848). Difelikefalin had an acceptable safety profile and was well tolerated. The most common treatment-

emergent adverse events (TEAEs) in the Primary Safety Pool (≥ 2% in difelikefalin and ≥ 1% than placebo) included diarrhea, gait disturbances/falls, dizziness, nausea, hyperkalemia, headache, somnolence, and back pain. These events were mostly mild-to-moderate in severity and few events lead to discontinuations. The incidence of fatal TEAEs and serious TEAEs were similar to placebo and deaths were considered unrelated to study drug. The incidence of serious TEAEs in the cardiac disorders system organ class (SOC) was low, but with treatment group imbalances noted between the difelikefalin and placebo group. However, the incidence rates for serious cardiac events did not increase over time as the Difelikefalin Exposure Safety Pool did not show a notable increase over the rates observed in the Primary Safety Pool. The rates were generally consistent with cardiac event rates reported for patients undergoing HD, a patient population highly burdened by cardiac comorbidities. There were no unexpected safety signals that emerged during the long-term use of difelikefalin, with the nature of the reported safety events aligned with the morbidity and mortality in patients with CKD-aP undergoing hemodialysis. Difelikefalin is expected to have no meaningful abuse potential and no physical dependence. Product labeling and routine pharmacovigilance monitoring should serve as adequate risk mitigation strategies.

The safety and effectiveness of difelikefalin in pediatric patients have not been established. Assessments in special populations included geriatric subjects and subjects with hepatic impairment. Of the 848 subjects in the placebo-controlled studies who received difelikefalin, 278 subjects (32.8%) were 65 years of age and older and 98 subjects (11.6%) were 75 years of age and older. No overall differences in safety or effectiveness of difelikefalin have been observed between patients 65 years of age and older and younger adult subjects, with the exception of the incidence of somnolence which was higher in difelikefalin -treated subjects 65 years of age and older (7.0%) than in difelikefalin -treated subjects less than 65 years of age (2.8%), and was comparable in both placebo age groups (3.0% and 2.1%, respectively). No differences in plasma concentrations of difelikefalin were observed between subjects 65 years of age and older and younger adult subjects. The influence of mild-to-moderate hepatic impairment on the pharmacokinetics of difelikefalin was evaluated in a population pharmacokinetic analysis which concluded that no difelikefalin dosage adjustments are needed in these populations. The influence of severe hepatic impairment on the pharmacokinetics of difelikefalin in subjects undergoing HD has not been evaluated; therefore, use of difelikefalin in this population is not recommended. The evaluation conducted in special populations is adequate to support the applicability of the overall clinical trial data to the majority of patients with End Stage Renal Disease (ESRD) on hemodialysis in the United States.

Overall, the data provided by the applicant for difelikefalin intravenous administration of 0.5 mcg/kg after each dialysis for the treatment of moderate-to-severe CKD-aP in hemodialysis patients appear to show that the benefits of treatment outweigh the potential risks. This application provides evidential support for the approval of difelikefalin.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Chronic kidney disease-associated pruritus (CKD-aP) is a common, distressing, and underrecognized condition that affects more than 60% of patients undergoing HD, with 20 to 40% of patients reporting moderate-to-severe pruritus. • This systemic pruritus does not originate from skin lesions, but rather is a persistent itch sensation that often leads to considerable mechanical skin damage due to a continuous and uncontrollable urge to scratch. • Patients with CKD-aP suffer from severely impaired physical and mental health, including sleep disturbance, insomnia, chronic fatigue, shame, social isolation, and increased depression. • Scratching often leads to an increase in infections (e.g., cellulitis, sepsis, bacteremia, and infections of the dialysis access). • Severe itching is also independently associated with an increased risk of mortality, including higher rates of cardiovascular-related mortality and infection-related mortality, relative to patients undergoing HD without CKD-aP. • The pathophysiology of CKD-aP is not fully understood but is thought to be multifactorial, including metabolic abnormalities, a dysregulated immune system, and dysregulation of the endogenous opioid system (e.g., over expression of mu opioid receptors [MORs] and concomitant under expression of KORs). • Activation of peripherally located KORs produces anti-itch and anti-inflammatory effects.	CKD-aP is a serious, distressing medical condition characterized by a generalized and intractable itch. The intense pruritus may disrupt sleep, cause chronic fatigue, and severely impair mental health. In addition, patients are at an increased risk of infections and those with severe itching have a higher rate of all-cause mortality, including cardiovascular related and infection related mortality compared to patients not bothered by pruritus. CKD-aP is thought to be multifactorial, including involvement of KORs in mediating pruritus.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current Treatment Options	• There are currently no FDA-approved therapies for CKD-aP in adult patients undergoing HD. • Medications have been used as off-label treatments for CKD-aP, e.g., antihistamines, corticosteroids, gabapentin, and pregabalin. These off-label therapies are not always well tolerated by patients, and the evidence for their antipruritic efficacy is limited and lacking support from randomized, well-controlled studies.	The lack of FDA-approved therapies and limitations of available off-label medications to treat CKD-aP in adult patients undergoing HD indicate that there is an unmet medical need for safe and effective treatments.
Benefit	• Evidence for the efficacy of 0.5 mcg/kg intravenous (IV) difelikefalin administered 3 times a week for the treatment of moderate-to-severe CKD-aP in patients undergoing HD is provided in two, well-controlled pivotal studies (CR845-CLIN3102 and CR845-CLIN3103). • Difelikefalin showed statistical improvement to placebo for the primary endpoint (3-point improvement in daily 24-hour WI-NRS scores at Week 12) and key secondary endpoint or Agency recommended primary endpoint (4-point improvement) in both studies. • Antipruritic effect was maintained for at least 1 year based on open-label extensions of placebo-controlled pivotal studies. • Difelikefalin was effective across a broad range of subjects independent of age, race, sex, and prior use of anti-itch medications. • Difelikefalin is administered 0.5 mcg/kg by intravenous bolus injection into the venous line of the dialysis circuit at the end of each HD , 3 times per week in the dialysis clinic	In two adequate and well-controlled studies, difelikefalin provided evidence of effectiveness in the target population. Treatment with difelikefalin resulted in sustained itch reduction that was both statistically significant (as measured by worst itch scores) and clinically meaningful (≥3-point and ≥4-point improvement in WI-NRS score). Difelikefalin provides a convenient dosing regimen with a painless route of administration for patients with CKD-aP undergoing HD.
Risk and Risk Management	• The difelikefalin clinical development program included an adequate safety database which allowed characterization of the safety profile and risks associated with difelikefalin treatment in the targeted patient population.	The overall size of the safety database and number of long-term exposures presented in this NDA meet ICH E1 recommendations for a drug intended for long-term treatment.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• The Primary Safety Pool consisted of 848 subjects (424 in difelikefalin and 424 in placebo). • The risks included dizziness, gait disturbances/falls, somnolence, mental status changes and hyperkalemia as the main concerns to treatment. • Assessments in special populations were conducted.	Labeling to inform use in special populations are included. The characterized safety profile can be managed with professional labeling. Post-approval studies may be required to further evaluate risks.

1.4. Patient Experience Data

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

				Section of review where discussed, if applicable
☐	**The patient experience data that were submitted as part of the application include:**			
	☐	Clinical outcome assessment (COA) data, such as:		
		X	Patient reported outcome (PRO)	8.11, 8.12, 8.13
		☐	Observer reported outcome (ObsRO)	
		☐	Clinician reported outcome (ClinRO)	
		☐	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

Dialysis-dependent end-stage renal disease (ESRD) is a serious illness with high disease burden, poor quality of life, and an increased all-cause mortality relative to the general population (United States Renal Data System [USRDS] 2019 unadjusted incidence rate of death in HD patients: 167 events per 1000 person-years [PY]).[1] Diabetes and hypertension are highly prevalent in this population, and are the primary causes of chronic kidney disease (CKD). [2] The most common comorbidity in patients undergoing HD is cardiovascular disease, occurring in up to 70% of patients with the most prevalent (>20%) cardiac conditions being heart failure, coronary artery disease, peripheral arterial disease, and atrial fibrillation.[3] The incidence rate of hospitalizations due to cardiovascular events in patients undergoing HD is 460 events per 1000 PY. In the United States (US), cardiac events are the leading cause of death for patients undergoing HD, with cardiac arrest being the most common reason (incidence rate of 53.5 events per 1000 PY).[3]

Approximately 20% to 40% of patients undergoing HD suffer from moderate-to-severe chronic kidney disease-associated pruritus (CKD-aP) , a generalized, persistent, and intractable itch [4], which further impairs their quality of life and severely impacts their physical and mental health, including experiencing sleep disturbance, chronic fatigue, and increased incidence of depression[5]. Increasing severity of CKD-aP is associated with an increased risk of infection. The mechanical damage to the skin from scratching leads to an increased risk of infections, such as cellulitis, sepsis, bacteremia, and infections of the dialysis access. Patients with CKD-aP also have higher medication utilization (antibiotics, erythropoiesis-stimulating agents, and iron) and miss dialysis sessions more often as pruritus worsens.[6] In addition, patients who are extremely bothered by itchy skin who are undergoing HD have a higher rate of all-cause mortality than HD patients who are not bothered by

[1] United States Renal Data System. 2018 USRDS Annual Data Report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 2019.
[2] Atkins RC. The epidemiology of chronic kidney disease. *Kidney Int Suppl*. 2005;94: S14-18.
[3] United States Renal Data System. 2018 USRDS Annual Data Report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 2018.
[4] Shirazian S, Aina O, Park Y, Chowdhury N, Leger K, Hou L, et al. Chronic kidney disease-associated pruritus: impact on quality of life and current management challenges. *Int J Nephrol Renovasc Dis*. 2017; 10: 11-26.
[5] Patel TS, Freedman BI, Yosipovitch G. An update on pruritus associated with CKD. *Am J Kidney Dis*. 2007;50(1): 11-20.
[6] Ramakrishnan K, Bond TC, Claxton A, Sood VC, Koorsikas M, Agnese W, et al. Clinical Characteristics and Outcomes of End-Stage Renal Disease Patients with Self-Reported Pruritus Symptoms. *Int J Nephrol Renovac Dis*. 2013;7: 1-12.

itchy skin, including higher rates of cardiovascular-related mortality and infection-related mortality.[7]

The pathophysiology of uremic pruritus is not completely understood. Several hypotheses implicating immune function, opioidergic systems and other contributing factors have been proposed. The immunohypothesis proposes that uremic pruritus is the result of systemic inflammation rather than a local skin disorder.[8,9] The opioid hypothesis proposes that imbalances in the expression of mu and kappa opioid receptors cause pruritus. Thus, the pruritus is increased by mu-receptor activation and kappa-receptor blockade and decreased by kappa-receptor activation and mu-receptor blockade.[10] This hypothesis is supported by the observation that the ratio of the mu-receptor agonist (beta-endorphin) to the kappa-receptor agonist (dynorphin-A) is increased in hemodialysis patients compared with healthy controls, and this ratio increased with severity of pruritus. Other contributing factors such as mast cell release of histamine and other pruritogens and xerosis may all contribute to the pathogenesis of uremic pruritus.

Clinical characteristics of uremic pruritus mostly involves the arms, head and abdomen. Generalized pruritus is also frequently reported. Symptoms tend to be worse at night and can result in sleep disruption. Most patients report increased pruritus with heat and stress. Physical findings are limited, as repetitive scratching is most commonly seen with lichen simplex, pruritus nodularis, kerotoic papules and follicular hyperkeratosis.[11] Xerosis (dry skin), which is present in most patients with uremic pruritus, may not be apparent unless the skin is inspected closely when scaling and epidermal cracking may be visible.

Uremic pruritus is extremely common in dialysis patients. A significant number of patients will have markedly elevated calcium/phosphate, parathyroid hormone (PTH), and/or blood urea nitrogen (BUN) levels.[12] Diagnosis will require elimination of non-uremic causes of pruritus and use of topical emollients and analgesic agents and oral antihistamines or gabapentin. The initial therapy includes optimization of dialysis, treatment of lab abnormalities, and regular use of emollients and/or topical analgesics. The use of oral antihistamines also can be tried. Gabapentin and pregabalin may

---

[7] Pisoni RL, Wikstrom B, Elder SJ, Akizawa T, Asano Y, Keen ML, et al. Pruritus in hemodialysis patients: International results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrol Dial Transplant*. 2006;21(12): 3495-505.
[8] Mettang T, Pauli-Magnus C, Alscher DM. Uremic pruritus – new perspectives and insights from recent trials. *Nephrol Dial Transplant*. 2002; 17(9): 1558.
[9] Kimmel M, Alscher DM, Dunst R, Braun N, Machleidt C, Keifer T, Stulten C, van der Kuip H, Pauli-Magnus C, Raub U, Kuhlmann U, Mettang T. The role of micro-inflammation in the pathogenesis of uremic pruritus in hemodialysis patients. *Nephrol Dial Transplant*. 2006; 21(3): 749.
[10] Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. The neurobiology of itch. *Nat Rev Neurosci.* 2006; 7(7): 535.
[11] Bencini PL, Montagnino G, Citterio A, Braziani G, Crosti C, Ponticelli C. Cutaneous abnormatlities in uremic patients. *Nephron.* 1985; 40(3): 316.
[12] Zucker I, Yosipovitch G, David M, Bafter U, Boner G. Prevelence and characterization of uremic pruritus in patients undergoing hemodialysis: uremic pruritus is still a major problem for patients with end-stage renal disease. *J Am Acad Dermatol*. 2003;49(5): 842.

improve the neurological pain of uremic pruritus.

2.2. Analysis of Current Treatment Options

No treatment has been approved for uremic pruritus or chronic kidney disease-associated pruritus (CKD-aP) in the US or European Union. Management of the condition is limited. Most recommendations are based on anecdotal reports and small, uncontrolled clinical trials. Other complicating factors regarding therapies for uremic pruritus include the high placebo effects noted in studies.

Clinical Management:

- Dialysis modification/Optimal dialysis
- Correction of parathyroid, calcium, and phosphate abnormalities
- Topical treatments with emollients and/or topical analgesic agents
- Oral cromolyn sodium or topical cromolyn sodium
- Oral antihistamines
- Gabapentin and pregabalin
- Sertraline antidepressants
- Montelukast
- Phototherapy has been used with some benefit

Various other treatments have been studied, but the management of uremic pruritus remains challenging. There is an unmet medical need for proven therapies in the treatment of uremic pruritus.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Difelikefalin is not marketed in the United States. Nalfurafine (REMITCH) is an antipruritic that is marketed in Japan for the treatment of uremic pruritus in individuals with chronic kidney disease undergoing hemodialysis. It is a potent, selective, centrally-penetrant Kappa-opioid receptor (KOR) agonist. Nalfurafine is dosed by oral capsules 2.5 µg once daily and was approved on January 21, 2009. Nalfurafine hydrochloride is indicated to suppress itch when other existing antihistamine or antiallergic medications are ineffectual. The label states it can be used in dialysis and/or chronic liver disease when other existing treatment is insufficient.

3.2. Summary of Presubmission/Submission Regulatory Activity

The IND was opened in JUN-2014 with a 30-day protocol with IV CR845 for the treatment of uremic pruritus in patients on hemodialysis. (b) (4) Cara Therapeutic then submitted a meeting request for a type B meeting to discuss the development of CR845. This meeting was held DEC-2015. The briefing document reported a completed Phase 2 study in hemodialysis patients with moderate-to-severe pruritus. Further details were discussed regarding an 11-point NRS primary endpoint with a success defined as ≥ 4-point improvement from baseline. On 14-JUNE-2016, Cara Therapeutics requested Breakthrough Therapy Designation. DDD evaluated the request and the data from the Phase 2 clinical study. On 21-JUNE-2017, the Breakthrough Therapy Designation was granted for IV CR845 in the treatment of moderate-to-severe uremic pruritus for patients on hemodialysis. Subsequently, the sponsor requested an End-of-Phase 2 (EOP2) meeting on 6-SEP-2017 and a post BTD meeting on 6-DEC-2017. In these meetings, the Division was able to reach agreements on the endpoints and development plans for IV CR845. The sponsor then submitted an initial pediatric protocol (iPSP), which the Division deemed inadequate (8-NOV-2018). The sponsor submitted three Phase 3 protocols for review (20-DEC-2018):

- CLIN3102 (US double-blind (DB) placebo-controlled pivotal study with a 52-week open-label extension phase); randomized 378 subjects in DB phase; 313 entered into the open-label extension
- CLIN3103 (Global DB placebo-controlled pivotal study with a 52-week open-label extension phase); randomized 473 subjects in DB phase; 399 subjects entered in the open-label extension.

- CLIN3101 (US long-term 52-week, open-label safety study); 288 subjects exposed

The final iPSP was agreed to by the Division on 11-MARCH-2020. A full waiver was agreed upon with consent from PeRC. The Pre-NDA meeting was completed on 12-MAY-2020. The NDA was received by the Agency on 23-DECEMBER-2020.

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

4.1. Office of Scientific Investigations (OSI)

See OSI review for issues regarding inspections. A number of study conduct issues were identified for clinical investigator Jayant Kumar, MD (Site 116 and Site 840023). The enrollment for this investigator encompassed 22 subjects in study CLIN3102 and 13 subjects in CLIN3103. Analysis conducted with the Agency's recommended primary efficacy endpoint (i.e., ≥4-point improvement in WI-NRS) at Week 12 comparing all sites and sites with the Kumar site removed found that the impact on the results was minimal (see Table 39 in Section 16.5). The conclusion remains the same (i.e., difelikefalin is statistically superior to placebo in both trials).

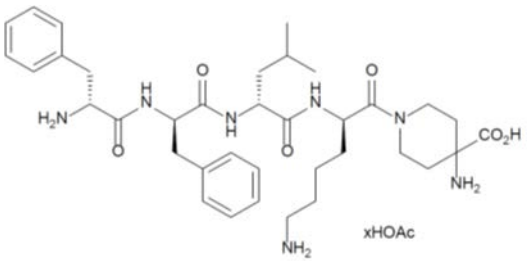
4.2. Product Quality

1) Drug Substance

The drug substance, difelikefalin is a small synthetic peptide and kappa opioid receptor (KOR) agonist. Difelikefalin has not been previously approved as a drug or marketed in the United States and therefore, it is classified as a new molecular entity (NME). Difelikefalin has been developed and formulated as an injection drug product for intravenous bolus administration for treatment of moderate-to-severe pruritus associated with chronic kidney disease.

Difelikefalin is manufactured by (b) (4) .

Difelikefalin acetate is a white to off-white lyophilized (b) (4) powder. It is highly hygroscopic and is freely soluble in water with pKa values of (b) (4) Difelikefalin acetate has the chemical name, D-phenylalanyl-D-phenylalanyl-D-leucyl-D-lysyl-□-(4-N-piperidinyl)-amino-carboxylic acid, acetate salt, a molecular formula of $C_{36}H_{53}N_7O_6$ (as free base), a molecular mass of 679 (b) (4) g/mole (as free base), and the molecular structure below:



Difelikefalin acetate for this application is manufactured in accordance with the current Good Manufacturing Practices (cGMP) requirements, packaged in (b) (4) stored at (b) (4)C. It is tested, released, and accepted according to a specification that assures the identity, strength, purity, and quality of the drug substance at release and through its proposed retest date of (b) (4) months. Sufficient stability data that support the proposed retest date of (b) (4) months at the storage condition of (b) (4)C have been submitted to the application.

2) Drug Product

The drug product KORSUVA (difelikefalin) Injection is a sterile preservative-free clear colorless solution formulated in an isotonic 40mM acetate buffer for intravenous administration and is indicated for the treatment of moderate to severe pruritus in adult patients with chronic kidney disease undergoing hemodialysis. KORSUVA is packaged in a single-dose vial containing 65 mcg of difelikefalin in 1.3 mL. It should be administered without dilution at the recommended dose of 0.5 mcg/kg into the venous line of the dialysis circuit at the end of each hemodialysis treatment. The remaining drug product in the single-dose vial after the patient administration should be discarded.

Each milliliter of KORSUVA injection contains 50 mcg/mL of difelikefalin equivalent to an average of 58.3 mcg/mL of difelikefalin acetate as the active ingredients and 1.3 mg of acetic acid, 2.5 mg of sodium acetate trihydrate, 7.2 mg of sodium chloride (to adjust tonicity), and water for injection as inactive ingredients. Inactive ingredients used in the formulation of KORSUVA are all compendial materials.

KORSUVA injection is manufactured at (b) (4) in accordance with current Good Manufacturing Practices (cGMP) and is tested against a specification that assures the identity, strength, purity, and quality of the drug product at release and throughout its proposed expiration dating period of 36 months. Sufficient stability data that supports the proposed expiration dating period of 36 months has been submitted to the application.

This drug product should be stored at 20°C to 25°C (68°F to 77°F), excursions permitted to 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature]. Do not freeze.

3) The OPQ Recommendation

- The applicant of this 505(b)(1) new drug application has provided sufficient CMC information to assure the identity, purity, strength, and quality of the drug substances, difelikefalin, and the drug product, KORSUVA (difelikefalin) Injection, 65mcg/1.3mL intended for intravenous (IV bolus) administration.
- Labels/labeling issues have been satisfactorily addressed.

- The Office of Pharmaceutical Manufacturing Assessment has made an overall "Acceptable" recommendation regarding the facilities involved in this NDA.
- The claim for categorical exclusion of the environmental assessment has been granted.

Therefore, from the OPQ perspective, this NDA is recommended for approval with the expiration dating period of 36 months.

4.3. Devices and Companion Diagnostic Issues

No devices or diagnostic devices were used in this application.

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Difelikefalin is a new-molecular-entity small synthetic peptide that, when injected intravenously, activates kappa opioid receptors on peripheral sensory neurons and immune cells. Currently, there is no kappa opioid receptor agonist approved in the US. The drug product under NDA 214916, i.e., difelikefalin injection, (b) (4) mL, has been proposed to relieve moderate-to-severe pruritus associated with chronic kidney disease in adult patients undergoing hemodialysis.

Difelikefalin has been evaluated in a battery of nonclinical studies that included evaluation of ADME, pharmacology, safety pharmacology, repeat-dose toxicity (via the intravenous route), genetic toxicology, carcinogenicity, and reproductive and developmental toxicology.

Difelikefalin was evaluated in safety pharmacology studies, which included an in vitro assessment of the effects on hERG potassium channel current, neurobehavioral assessments in rats, an in vivo assessment for respiratory effects in rats, an in vivo assessment for cardiovascular effects in monkeys, and in vivo assessments for gastrointestinal transit in rats. Difelikefalin did not inhibit hERG potassium channel current at clinically relevant concentrations. During the neurobehavioral, cardiovascular, and respiratory safety assessments, intravenous administration of difelikefalin produced: (1) an overall decrease of activity in rats at ≥ 1 mg/kg/day; (2) decrease of blood pressure, heart rate, and body temperature in monkeys at ≥ 0.25 mg/kg/day with no effects on ECG parameters; and (3) decrease of respiratory rates and slight increase of tidal volumes with no overall effect on minute volume in rats at ≥ 1 mg/kg/day. These pharmacological effects seemed to be correlated with the general state of arousal. Further, intravenous administration of difelikefalin had no effect on gastric emptying or gastrointestinal transit time in rats under normal conditions or rats with post-operative ileus. The findings in the safety pharmacology studies are deemed monitorable in clinical practice and therefore acceptable from a nonclinical perspective.

Difelikefalin was evaluated in three pivotal intravenous repeat-dose general toxicity studies, one in rats (6 months) and the other two in monkeys (9 months). In the 6-month rat toxicity study, difelikefalin was administered intravenously at the doses of 0, 0.25, 2.5, and 25 mg/kg/day once daily. Difelikefalin caused abnormalities in the testis at the highest dose 25 mg/kg/day, including bilateral atrophy in the seminiferous tubules, decrease in sperm in the epididymis, and cell debris in the lumen of the epididymis. The NOAEL for the 6-month rat toxicity study was 2.5 mg/kg/day for males based on findings in the testis and 25 mg/kg/day in females, corresponding to $AUC_{0\text{-last}}$ of 8640 and 53870 ng·hr/mL, respectively. In the first 9-month monkey toxicity study, difelikefalin was administered intravenously at the doses of 0, 0.06, 0.25, and 1.0 mg/kg/day once daily. Difelikefalin caused very slight deposits of brown

pigment in the pars recta of the proximal tubule in the kidney in all males and two females at the highest dose 1.0 mg/kg/day. The NOAEL for the 9-month monkey toxicity study was 1 mg/kg/day, corresponding to $AUC_{0\text{-last}}$ of 105500 and 134600 ng·hr/mL in males and females, respectively. In the second 9-month monkey toxicity study, difelikefalin was administered intravenously at the doses of 0, 0.06, 0.25, and 1.0 mg/kg/day once daily. Difelikefalin at the highest dose 1.0 mg/kg/day caused a decrease in white blood cell (WBC) counts, which was associated with decreases in neutrophils, monocytes, basophils, and lymphocytes. The NOAEL was 0.25 mg/kg/day, corresponding to $AUC_{0\text{-last}}$ of 21000 and 17200 ng·hr/mL in males and females, respectively. In summary, at clinically relevant doses, difelikefalin-related toxicities were generally not severe and recoverable.

Difelikefalin tested negative in a battery of genotoxicity studies, i.e., an Ames assay, an in vitro mammalian chromosome aberration test, and an in vivo mammalian (mouse) erythrocyte micronucleus test.

Difelikefalin did not cause increases of tumor incidences in a 6-month subcutaneous carcinogenicity study in transgenic rasH2 mice and a 2-year subcutaneous carcinogenicity study in rats. In the mouse carcinogenicity study, difelikefalin (0 [saline], 3, 10, and 30 mg/kg/day in both males and females) was subcutaneously administered once daily to transgenic rasH2 mice for 6 months. Three intraperitoneal doses of urethane (1000 mg/kg/day on Days 1, 3, and 5) were administered to positive controls. No groups were terminated early. Positive controls produced expected results. Difelikefalin treatment did not result in a significantly increased incidence of tumors in either male or female mice. In the rat carcinogenicity study, difelikefalin [0 (saline), 0.25, 0.5, and 1.0 mg/kg/day in both males and females] was subcutaneously administered once daily to Sprague-Dawley rats for two years. All female rats were terminated early in Week 92, because the number of surviving females in the saline control group declined to 20. Males survived ≥ 103 weeks of treatment. Difelikefalin treatment for a lifetime did not result in a significantly increased incidence of tumors in either male or female rats.

In a rat intravenous fertility and early embryonic development study, difelikefalin (0, 0.25, 2.5, and 25 mg/kg/day) demonstrated an effect on estrous cyclicity (prolonged diestrus) that was associated with a slight increase in days to mating at doses of 2.5 mg/kg/day and higher. There was, however, no effect on female fertility, implantation, or early embryonic development at any dose. There was no effect on male fertility at any dose. The NOAEL for mating in females was 0.25 mg/kg/day, based on alterations in estrous cyclicity. The NOAEL for mating and fertility in males and the NOAEL for fertility and early embryonic development in females were both 25 mg/kg/day.

In a rat intravenous embryofetal study, maternal toxicity, including transient behavioral signs, reduced body weight gain or weight loss, and decreased food consumption, was observed when difelikefalin was administered during organogenesis (gestation days 7 – 17) at doses of 0.25, 2.5, and 25 mg/kg/day. There were no difelikefalin-related effects on ovarian or uterine parameters, embryofetal survival, embryofetal growth, or malformations. A low incidence of

nonadverse skeletal variations (wavy ribs and incompletely ossified ribs) was observed at 25 mg/kg/day. The developmental NOAEL was 25 mg/kg/day. The maternal NOAEL could not be determined due to effects on maternal body weight and food consumptions at all doses.

In a rabbit intravenous embryofetal study, difelikefalin had no effects on embryofetal development or teratogenicity when administered at doses of 0.025, 0.05, and 0.1 mg/kg/day during the period of organogenesis (gestation days 7-19). However, there was a decrease in total number of pregnancies and a single abortion at 0.1 mg/kg/day. Maternal toxicity, including transient behavioral signs, decreases in body weight gain or weight loss, and decreases in food consumption, was observed across all doses. The developmental NOAEL was 0.1 mg/kg/day. The maternal NOAEL could not be determined due to reduction in maternal body weight and food consumption at all doses evaluated in this study.

In a rat intravenous pre- and postnatal development study, difelikefalin had no effects on maternal reproductive function and no effects on growth, survival, sexual maturation, neurobehavioral, or reproductive function of the offspring, when administered at doses of 0.6, 2.5, and 10 mg/kg/day from gestation day 7 to lactation day 21. Maternal toxicity, including transient behavioral signs and reduced body weight, weight gain, and food consumption, were observed across all doses. The maternal NOAEL was 0.6 mg/kg/day due to decreases in maternal body weights and food consumption at MD and HD. The maternal reproductive NOAEL and developmental NOAEL were both 10 mg/kg/day.

The difelikefalin impurities are specified at not more than (NMT) (b) (4) % for each in the drug product. Therefore, the maximum daily intake of each impurity is (b) (4) µg at the drug's recommended human dose 0.5 µg/kg/day. No further qualification is needed for the difelikefalin impurities. In addition, there is no safety concern on organic leachables and elemental impurities that are potentially present in the final drug product.

Difelikefalin injection does not contain novel excipients. All excipients are present at the same or lower levels when compared to levels in previously approved injection drug products.

This NDA is approvable from a nonclinical perspective. There are no recommended nonclinical postmarketing commitments or postmarketing requirements for this NDA.

5.2. Referenced NDAs, BLAs, DMFs

None

5.3. Pharmacology

Difelikefalin is a new molecular entity kappa opioid receptor (KOR) agonist. In vitro, difelikefalin selectively binds to and activates the human KOR with a Ki of 0.32 nM and an EC_{50} of 0.16 nM, with no agonist activity for human mu or delta opioid receptors at concentrations up to 10 µM.

Difelikefalin had no activity at a panel of known receptors, ion channels, transporters, or enzymes at clinically relevant concentrations in vitro.

Safety pharmacology

- Neurological effects:

Sprague-Dawley rats (10/sex/group) received a single intravenous dose of difelikefalin [doses: 0 (0.9% sodium chloride), 1, 5, and 25 mg/kg]. Two and 24 hours after dosing, body temperature was recorded and a functional observation battery (FOB) was performed. At ≥ 1 mg/kg, the following effects were observed at 2 hours post-dose with improvement by 24 hours: (1) decreased activity and changes in posture; (2) increase in palpebral closure, pupil response (miosis), lacrimation, and urination; (3) minimal or no response to approach or tactile and auditory stimuli; (4) prolonged tail flick response; (5) decrease in body tone, slight to severe gait abnormalities, and slight impairments in surface or air righting reflexes; and (6) decrease of rectal body temperature. The observed effects were generally consistent with sedation.

Sprague-Dawley male rats (6/group) received a single intravenous dose of difelikefalin [doses: 0 (0.9% sodium chloride), 0.3, 1, 3, 10, and 30 mg/kg], and then tested on the rotarod for motor coordination at 5, 15, 30, 60, 90, 180, and 360 minutes post-dose. At 0.3 and 1 mg/kg, motor coordination of rats was not affected. Doses ≥ 3 mg/kg produced motor coordination impairment starting at 5 minutes post-dose.

- Respiratory effects:

Male Sprague-Dawley rats (8/group) received a single intravenous dose of difelikefalin [doses: 0 (0.9% sodium chloride), 1, 5, and 25 mg/kg]. Pulmonary parameters (respiratory rate, tidal volume, and minute volume) were assessed using whole body plethysmography for 24 hours post-dose. At ≥ 1 mg/kg, difelikefalin caused decrease of respiratory rates and slight compensatory increase of tidal volumes at post-dose time points. However, there was no overall effect on minute volume in rats.

- Cardiovascular effects:

Difelikefalin did not inhibit hERG potassium channel current at 10 and 100 μM in vitro.

Four telemetered male cynomolgus monkeys received single intravenous escalating doses of difelikefalin (doses: 0, 0.25, 1, and 4 mg/kg) on Days 1, 7, 14, and 21. Cardiovascular parameters were monitored from 2 hours pre-dose through 24 hours post-dose, including arterial blood pressure, heart rate, and ECG (including PR, QRS, QT, and QTc intervals). Lethargy or hunched posture was observed in most animals following each dose of difelikefalin. Emesis was observed at ≥ 0.25 mg/kg. Blood pressure (systolic, diastolic, and mean arterial pressure) was reduced at all doses with maximal decreases (up to 43%) occurring within 10-60 minutes post-dose, persisting for 4-18 hours, with an inverse relationship to dose. Body temperature was decreased with maximal effect at 75-105 minutes post-dose. Heart rate was

initially increased for the first 15 minutes at all dose levels, but then decreased steadily in a pattern similar to controls for approximately 1 hour. There were no biologically significant or test article-related changes in quantitative (PR, QRS, QT, and QTc intervals) or qualitative ECG parameters.

- Gastrointestinal effects:

Male Sprague-Dawley rats (10/group) received a single intravenous dose of difelikefalin [doses: 0 (saline), 1, 3, and 10 mg/kg], and then were given charcoal slurry for measurement of gastrointestinal (GI) transit. Difelikefalin had no effect on gastric emptying, but produced a small decrease in charcoal transit at all doses.

Male Sprague-Dawley rats (9-12/group) that had post-surgical ileus received a single intravenous dose of difelikefalin [doses: 0 (saline), 0.1, 0.3, 1, 3, and 10 mg/kg], and then were given charcoal slurry for measurement of GI transit. Difelikefalin had no effect on gastric emptying or charcoal transit at any dose.

5.4. ADME/PK

Type of Study	Major Findings
Distribution	
In vitro protein binding of ^{14}C-CR845 in rat, monkey, and human plasma/ PBC069-124	In vitro, protein binding of ^{14}C- CR845 at 1 µM was 20.9%, 18.7%, and 18.3% and in rat, monkey, and human plasma, respectively. At 10 µM, protein binding was 21.3% , 17.7%, and 16.7% in rat, monkey, and human plasma, respectively.
In vitro protein binding of CR845 in rat, dog, monkey, and human plasma/ CR845-DMPK022	In vitro, protein binding of CR845 at 1 µM was 28.1%, 24.4%, 15.8%, and 26.8% and in rat, dog, monkey, and human plasma, respectively. At 10 µM, protein binding was 49.1% , 40.4%, 25.3%, and 43.2% in rat, dog, monkey, and human plasma, respectively.
Tissue distribution of total radioactivity after a single intravenous dose of ^{14}C-CR845 in male and female rats/ PBC069-094	Crl:CD(SD) rats received a single intravenous dose of ^{14}C-CR845 at 1 mg/kg. Maximum concentration in most tissues was reached within 5 minutes. The radioactivity was specifically distributed in the kidney cortex and kidney medulla, and poorly distributed in the central nervous system.
Distribution of CR845 into brain, spinal cord, sciatic nerve, CSF, and plasma following a single intravenous dose in male SD rats/ CR845-DMPK212	Male Sprague Dawley rats received a single intravenous dose of CR845 at 1 mg/kg. At C_{max}, < 1% plasma concentration of CR845 was detected in the brain, and the level in the brain remained below quantifiable levels over time. Further, CR845 distributed preferentially into the sciatic nerve and spinal cord, with the highest level in sciatic nerve tissue relative to brain, spinal cord, or CSF.

Type of Study	Major Findings
Metabolism	
In vitro biotransformation of CR845 in cryopreserved hepatocytes/ CR845-DMPK023	CR845 (10 μM) was incubated for 2 hours with pooled male and female cryopreserved hepatocytes from rat, dog, monkey, or human. Putative metabolites of CR845 formed from oxidation, conjugation, and hydrolysis were searched, and none of such metabolites was detected.
In vitro biotransformation of [^{3}H]CR845 in whole blood, hepatocytes, intestinal S9 fraction, and kidney S9 fraction from rat, monkey, and human/ CR845-DMPK216	[^{3}H]CR845 (3.0 and 30 μM) was incubated with whole blood, hepatocytes, intestinal S9 fraction, and kidney S9 fraction from rat, monkey, and human. Samples were analyzed to assess the disappearance of parent molecule and appearance of any metabolites. There was no evidence of CR845 metabolism.
Excretion	
Excretion of in radioactivity in urine and feces following a single intravenous dose of ^{14}C-CR845 in male monkeys/ PBC069-089	Male cynomolgus monkeys received a single intravenous dose of ^{14}C-CR845 at 1 mg/kg. Radioactivity was excreted predominantly in urine, with the remainder being excreted in feces.
Biliary excretion of ^{14}C-CR845 following a single intravenous dose in male rats/ PBC069-086	Male Crl:CD(SD) rats received a single intravenous dose of ^{14}C-CR845 at 1 mg/kg. Radioactivity was excreted predominantly in urine, with the remainder being excreted in bile and feces. 48 hours post-dose levels were: 64.9%, 13.0%, and 2.6% in urine, bile, and feces, respectively, amounting to 80.5% of the dose.
Lacteal excretion of CR845 following repeated intravenous dosing in pregnant Sprague Dawley rats/ CR845-TOX077	CR845 was intravenously administered to pregnant Sprague Dawley rats once daily. CR845 crossed the placenta and into the milk following intravenous dosing daily.

Type of Study	Major Findings
TK data from general toxicology studies	
A 26-week repeated intravenous dose toxicity study of CR845 in rats followed by a 9-week recovery period/ SBL069-104	Rat at the NOAEL of 2.5 mg/kg/day (male, MD) and 25 mg/kg/day (female, HD) on Day 181 t_{max}: 0.08 hr (males and females) $AUC_{0\text{-last}}$: 53870 (female, HD) and 8640 ng·hr/mL (male, MD) C_{max}: 104100 (female, HD) and 16410 ng/mL (male, MD) Accumulation: Yes, at all doses. Dose proportionality: Exposure generally increased dose proportionally on Days 1 and 181.
A 39-week study of CR845 by intravenous bolus Injection in cynomolgus monkeys with a 4-week recovery period/ CR845-TOX085	Monkey at the NOAEL of 0.25 mg/kg/day on Day 271 t_{max}: 0.12 hr (males and females) $AUC_{0\text{-last}}$: 17200 (female) and 21000 ng·hr/mL (male) C_{max}: 2670 (female) and 2460 ng/mL (male) Accumulation: Yes, at all tested doses. Dose proportionality: Exposure generally increased greater than dose proportionally on Days 1 and 271.
A 39-week repeated intravenous dose toxicity study of CR845 in cynomolgus monkeys followed by a 4-week recovery period/ SBL069-103	Monkey at the NOAEL of 1 mg/kg/day on Day 272 t_{max}: 0.08 hr (males and females) $AUC_{0\text{-last}}$: 134600 (female) and 105500 ng·hr/mL (male) C_{max}: 10890 (female) and 8893 ng/mL (male) Accumulation: Yes, at all tested doses. Dose proportionality: Exposure generally increased dose proportionally on Days 1 and 272.

Type of Study	Major Findings
TK data from reproductive toxicology studies Effect of intravenously administered CR845 on fertility and early embryonic development to implantation in rats/ CR845-TOX073	<u>Rat at the NOAEL of 25 mg/kg/day for mating and fertility in males and early embryonic development in females, and at the NOAEL of 0.25 mg/kg/day for mating in females on Day 1</u> $AUC_{0\text{-}24h}$ for early embryonic development NOAEL of 25 mg/kg/day: 49900 (female) and 76300 (male) ng·hr/mL $AUC_{0\text{-}24h}$ for female mating NOAEL of 0.25 mg/kg/day: 401 ng·hr/mL C_{max} for early embryonic development NOAEL of 25 mg/kg/day: 84400 (female) and 105000 (male) ng/mL C_{max} for female mating NOAEL of 0.25 mg/kg/day: 803 ng/mL
Effect of intravenously administered CR845 on embryo-fetal development in rats/ CR845-TOX075	<u>Rat at the developmental NOAEL of 25 mg/kg/day on GD 17</u> Note: Maternal NOAEL could not be determined based on the magnitude and persistence of the effects on maternal body weight gain and food consumption $AUC_{0\text{-}24h}$: 55900 ng·hr/mL C_{max}: 90400 ng/mL
Effect of intravenously administered CR845 on embryo-fetal development in rabbits/ CR845-TOX076	<u>Rabbit at the developmental NOAEL of 0.1 mg/kg/day on GD 19</u> Note: Maternal NOAEL could not be determined based on the magnitude and persistence of the effects on maternal body weight gain and food consumption $AUC_{0\text{-}inf}$: 679 ng·hr/mL C_{max}: 906 ng/mL
TK data from Carcinogenicity studies A 26-week repeated dose subcutaneous carcinogenicity study in Tg.rasH2 mice/ CR845-CARC086	<u>Tg.rasH2 mouse at the NOAEL of 30 mg/kg/day on Day 170</u> $AUC_{0\text{-}24h}$: 42800 (male) and 34700 (female) ng·hr/mL C_{max}: 41700 (male) and 33300 (female) ng/mL
A 2-year carcinogenicity study of CR845 by subcutaneous injection in rats/ CR845-CARC088	<u>Rat at the NOAEL of 1 mg/kg/day on Day 183</u> $AUC_{0\text{-}last}$: 46900 (male) and 161000 (female) ng·hr/mL C_{max}: 5080 (male) and 15300 (female) ng/mL

5.5. Toxicology

5.5.1. General Toxicology

Study title/ number: A 26-Week Repeated Intravenous Dose Toxicity Study of CR845 in Rats followed by a 9-Week Recovery Period/ SBL069-104

Key Study Findings

- Difelikefalin caused abnormalities in the testis at the highest dose 25 mg/kg/day, including bilateral atrophy in the seminiferous tubules, decrease in sperm in the epididymis, and cell debris in the lumen of the epididymis.
- The NOAEL for 26-week intravenous dosing in rats was 2.5 mg/kg/day for males based on findings in the testis and 25 mg/kg/day in females, corresponding to $AUC_{0\text{-last}}$ of 8640 and 53870 ng·hr/mL, respectively.

Conducting laboratory and location (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 0.25, 2.5, and 25 mg/kg, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	Sprague Dawley rat
Number/Sex/Group:	12/sex/group
Age:	5-6 weeks
Satellite groups/ unique design:	6/sex/group in vehicle and HD group for recovery, 4/sex/group for vehicle toxicokinetics and 8/sex/group for LD, MD and HD toxicokinetics
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	Major findings
Mortality	No difelikefalin-related findings.
Clinical Signs	Decrease in spontaneous activity, ataxic gait, abduction of the foreleg, and/or reddish eye mucus on the first two days of dosing. Resolved afterwards.
Body Weights	Decreases in body weights at all dose levels in the first three weeks. Recovered afterwards.
Ophthalmoscopy	No difelikefalin-related findings.
Hematology	No difelikefalin-related findings.
Clinical Chemistry	No difelikefalin-related findings.
Urinalysis	No difelikefalin-related findings.
Gross Pathology	No difelikefalin-related findings.

Organ Weights	Decreases in prostate and lung weights in males, and increase in liver weights and decreases in ovary and uterus weights in females. However, all individual data were within or only slightly deviated from the historical control values.
Histopathology Adequate battery: Yes/No	Yes. Testicular bilateral atrophy in the seminiferous tubules at HD, associated with unilateral (2 males) or bilateral (2 males) cell debris in the lumen of the epididymis.

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

Study title/ number: A 39-Week Repeated Intravenous Dose Toxicity Study of CR845 in Cynomolgus Monkeys Followed by a 4-Week Recovery Period/ SBL069-103

Key Study Findings

- Difelikefalin caused very slight deposits of brown pigment in the pars recta of the proximal tubule in the kidney in all HD males and two HD females. This was not resolved by the end of the recovery period.
- The NOAEL for this 39-week repeat intravenous dose toxicity study in monkeys was 1 mg/kg/day the highest dose tested, corresponding to $AUC_{0\text{-last}}$ of 105500 and 134600 ng·hr/mL in males and females, respectively.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 0.06, 0.25 and 1.0 mg/kg, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	2/sex/group for vehicle and HD groups, 4/sex/group for LD and MD groups
Age:	3-6 years
Satellite groups/ unique design:	4/sex/group for vehicle and HD recovery groups
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	Major findings
Mortality	None.
Clinical Signs	Transient signs at MD and HD in the first two weeks of dosing, including sedation, somnolence, decrease in spontaneous activity, and emesis. Resolved afterwards.
Body Weights	Decreases in body weights at HD in the first three days. Recovered afterwards.
Ophthalmoscopy	No difelikefalin-related findings.
ECG	No difelikefalin-related findings.

Hematology	No difelikefalin-related findings.
Clinical Chemistry	No difelikefalin-related findings.
Urinalysis	Decrease in urine volume at MD and HD. No effects at the end of the recovery period.
Gross Pathology	No difelikefalin-related findings.
Organ Weights	No difelikefalin-related findings.
Histopathology Adequate battery: Yes/No	Yes. Very slight deposits of brown pigment in the pars recta of the proximal tubule in the kidney in all HD males and two HD females. This was not resolved by the end of the recovery period.

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

Study title/ number: A 39-Week Study of CR845 by Intravenous Bolus Injection in Cynomolgus Monkeys with a 4-Week Recovery Period/ CR845-TOX085

Key Study Findings

- Difelikefalin at the dose 1.0 mg/kg caused a decrease in WBC counts, which was associated with decreases in neutrophils, monocytes, basophils, and lymphocytes in individual animals when compared to baseline. The changes persisted after recovery.
- The NOAEL for the 39-week repeat intravenous dose toxicity study in monkeys was 0.25 mg/kg/day, because not all effects seen at 1 mg/kg were recoverable. The NOAEL corresponded to $AUC_{0\text{-last}}$ of 21000 and 17200 ng·hr/mL in males and females, respectively.

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

Methods

Dose and frequency of dosing:	0, 0.06, 0.25 and 1.0 mg/kg, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group
Age:	2.2 to 5.1 years old
Satellite groups/ unique design:	3/sex/group for interim sacrifice on Day 182; 2/sex/group in vehicle and HD recovery groups
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	**Major findings**
Mortality	No difelikefalin-related findings.
Clinical Signs	Transient signs in the first 10 days of dosing, including decreased activity, hunched posture, uncoordinated movement, eyes partly closed, and dilated pupils at HD. All signs resolved afterwards.
Body Weights	No difelikefalin-related findings.

Ophthalmoscopy	No difelikefalin-related findings.
ECG	No difelikefalin-related findings.
Hematology	Decreases in WBC counts (↓44%), associated with decreases in neutrophils, monocytes, basophils, and lymphocytes (for individual animals) in most HD animals when compared to baseline. These changes persisted after recovery. All values were still within historical range.
Clinical Chemistry	No difelikefalin-related findings.
Urinalysis	No difelikefalin-related findings.
Gross Pathology	No difelikefalin-related findings.
Organ Weights	No difelikefalin-related findings.
Histopathology Adequate battery: Yes/No	Yes. Minimal to mild pigmented macrophage aggregates in the hepatic sinusoids and/or spleen red pulp in MD and HD animals. No changes were observed after recovery.

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

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title/ number: Bacterial Reverse Mutation Assay/ CR845-TOX029

Key Study Findings:

- Difelikefalin exhibited no mutagenic effects under the conditions of this Ames assay at concentrations up to 5000 µg/plate.

GLP compliance: Yes
Test system: *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA; ±S9
Study is valid: Yes

In Vitro Assays in Mammalian Cells

Study title/ number: In Vitro Mammalian Chromosome Aberration Test/ CR845-TOX031

Key Study Findings:

- Difelikefalin exhibited no in vitro clastogenic effects at concentrations up to 5000 µg/mL under the conditions of this assay.

GLP compliance: Yes
Test system: Human peripheral blood lymphocytes, up to 5000 µg/mL (-S9: 4-hour and 20-hour incubation; +S9: 4-hour incubation)
Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title/ number: Mouse Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Administration of CR845/ CR845-TOX054

Key Study Findings:

- Difelikefalin exhibited no in vivo clastogenic effects in the mouse bone marrow micronucleus assay when administered intravenously at doses up to 100 mg/kg/day

GLP compliance: Yes
Test system: Mouse, bone marrow cell micronuclei; single intravenous dose of 0 (vehicle), 25, 50, or 100 mg/kg/day; assessments at 24 and 48 hours post-dose.
Study is valid: Yes

5.5.3. Carcinogenicity

In a 6-month carcinogenicity study in transgenic rasH2 mice, difelikefalin was administered by subcutaneous injection once daily at the doses of 0 (saline control), 3, 10, and 30 mg/kg/day. Difelikefalin did not increase mortality at any dose. Difelikefalin caused transient clinical signs such as ataxia and decreased motor activity at all doses, and prostration and labored breathing at MD and HD in early days of dosing. Difelikefalin increased overall body weight gains compared to controls at all doses with no dose relationship. Difelikefalin-related non-neoplastic microscopic findings, i.e., increases in incidence of kidney infarction, were noted in the kidney. No difelikefalin-related tumor findings were noted.

In a 2-year carcinogenicity study in rats, difelikefalin was administered by subcutaneous injection once daily at the doses of 0 (saline control), 0.25, 0.5, and 1 mg/kg/day. Difelikefalin did not increase mortality at any dose. Difelikefalin caused transient decrease in activity at all doses. Difelikefalin did not have effect on body weight gains. Difelikefalin did not cause non-neoplastic changes. No difelikefalin-related tumor findings were noted.

Refer to Appendix 16.3.3 for full carcinogenicity study reviews.

5.5.4. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development
Study title/ number: Study of Fertility and Early Embryonic Development to Implantation of CR845 Administered by Intravenous Injection in Rats/ CR845-TOX073
Key Study Findings

- Difelikefalin had no effects on the number of days to mating, mating index, or fertility index. There were no difelikefalin-related effects on any ovarian or uterine parameters in the treated females mated with untreated males at any dose.
- Difelikefalin caused a significant decrease in the number of estrous cycles per 14 days (2.6, 1.8, and 1.3 cycles at LD, MD, and HD, respectively vs. 3.3 cycles in controls) during the premating dose period in all treated females. The number at LD was within the range of historical controls.
- The NOAEL for mating and fertility in males and the NOAEL for fertility and early embryonic development in females were both 25 mg/kg/day, corresponding to $AUC_{0\text{-}24h}$ of 49900 (female) and 76300 (male) ng·hr/mL. The NOAEL for mating in females was

0.25 mg/kg/day, based on alterations in estrous cyclicity, corresponding to $AUC_{0\text{-}24h}$ of 401 ng·hr/mL (female).

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

Methods

Dose and frequency of dosing:	0, 0.25, 2.5 and 25 mg/kg, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	25/sex/group
Satellite groups:	6/sex/group for toxicokinetic analysis
Study design:	Males were dosed beginning 28 days before cohabitation (with untreated females), during cohabitation, and through the day before euthanasia. Females were dosed beginning 15 days before cohabitation (with untreated male breeders), during cohabitation, and continued until Day 7 of presumed gestation (GD 7).
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	No difelikefalin-related effects.
Clinical Signs	Males: hunched posture, decreased motor, activity, ataxia, straub tail, mild dehydration, hyperreactivity to touch, chromodacryorrhea, vocalization to touch, ptosis, lacrimation, bradypnea, tachypnea, and ungroomed coat at all doses; chromorhinorrhea, low carriage, and increased motor activity at MD and HD; and splayed limbs, slight excess salivation, and hyperreactivity in the home cage at HD. Females: mild dehydration and decreased motor activity at all doses; moderate dehydration, hunched posture, chromodacryorhea, ungroomed coat, lacrimation, ptosis, urine-stained abdominal fur, and vocalization to touch at MD and HD; and ataxia at HD.
Body Weights	Males: reduced mean body weight gain on GD 78 at LD (↓16%) and HD (↓12%). Females: reduced mean body weight gain on GD 15 at LD (↓28%) and MD (↓21%).
Necropsy findings	No difelikefalin-related effects.

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

Embryo-Fetal Development

Study title/ number: An Embryo-Fetal Development Study of CR845 by Intravenous Injection in Rats/ CR845-TOX075

Key Study Findings

- Difelikefalin caused a dose-dependent increase in the incidence of wavy ribs (1, 2, and 4 fetuses in the LD, MD, and HD groups, respectively), and a dose-dependent increase in the incidence of incompletely ossified ribs (1, 2, and 5 fetuses in the LD, MD, and HD groups, respectively).
- Difelikefalin had no adverse effects on embryo-fetal survival or fetal body weights.
- Difelikefalin had no adverse effects in fetal morphology (i.e., malformations) at any dose. The developmental NOAEL was 25 mg/kg/day, corresponding to a maternal $AUC_{0\text{-}24h}$ of 55900 ng·hr/mL and C_{max} of 90400 ng/mL. The maternal NOAEL could not be determined due to effects on maternal body weight and food consumptions at all doses.

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

Methods

Dose and frequency of dosing:	0, 0.25, 2.5 and 25 mg/kg/day, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	25/group
Satellite groups:	3 in control group and 10/group in all difelikefalin dose groups for toxicokinetics.
Study design:	Animals were dosed from GD 7 to GD 17 and euthanized on GD 21.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	No difelikefalin-related effects.
Clinical Signs	Decreased motor activity, mild dehydration, hunched posture, ptosis, and urine-stained abdominal fur at all doses; moderate dehydration, chromodacryorrhea, chromorhinorrhea, tip toe walking, and vocalization to touch at MD and HD; ungroomed coat, thin body condition, and hyperreactivity to sound at HD.
Body Weights	Significantly reduced mean maternal body weight gain from GD 7 to GD 21 across all dose groups (83% to 86% of controls).
Necropsy findings Cesarean Section Data	No difelikefalin-related effects.
Necropsy findings Offspring	A dose-dependent increase in the incidence of wavy ribs (1, 2, and 4 fetuses in the LD, MD, and HD groups, respectively); A dose-dependent increase in the incidence of incompletely ossified ribs (1, 2, and 5 fetuses in the LD, MD, and HD groups, respectively); The skeletal variations listed above in the LD and MD are within the range of historical control. No difelikefalin-related effects on malformations were noted in this study.

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

Study title/ number: An Embryo-Fetal Development Study of CR845 by Intravenous Injection in Rabbits/ CR845-TOX076

Key Study Findings

- Maternal mortality occurred at the highest dose 0.1 mg/kg/day. Further, difelikefalin caused reduction of maternal food intake and maternal body weight gain, with increased incidence of clinical signs at all doses.
- Difelikefalin had no adverse effects on embryo-fetal survival or fetal body weights.
- No difelikefalin related effects on malformations were noted in this study.
- The maternal NOAEL could not be determined due to reduction in maternal body weight and food consumption. The developmental NOAEL was 0.1 mg/kg/day, corresponding to maternal $AUC_{0\text{-}inf}$ of 679 ng·hr/mL and C_{max} of 90400 ng/mL.

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

Methods

Dose and frequency of dosing:	0 (vehicle control), 0.025, 0.05 and 0.1 mg/kg/day, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	20/group
Satellite groups:	3/group for toxicokinetic analysis
Study design:	Animals were dosed between GD 7 and GD 19 and euthanized on GD 29
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	At HD, one pregnant rabbit died on GD 16 and another rabbit aborted on GD 23 and was subsequently euthanized.
Clinical Signs	Thin body condition and mild dehydration at MD, and scant feces, tachypnea, and red substance in the cage pan at HD.
Body Weights	Reduced maternal body weight gain between GD 7 and GD 29 at all doses (↓22%, ↓22%, and ↓35% in LD, MD, and HD, respectively), associated with reduced food intake.
Necropsy findings Cesarean Section Data	No difelikefalin-related effects.
Necropsy findings Offspring	No difelikefalin-related effects.

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

Prenatal and Postnatal Development

Study title/ number: A Developmental and Perinatal/Postnatal Reproduction Study of CR845 by Intravenous Injection in Rats, Including a Postnatal Behavioral/Functional Evaluation/ CR845-TOX077

Key Study Findings

- Difelikefalin had no adverse effects on maternal (F0) reproductive function at any dose. Difelikefalin had persisting effects on overall maternal body weight and/or maternal body weight gain as well as food consumption at MD and HD for the duration of the F0 generation in-life phase.
- Difelikefalin had no effects on growth, sexual maturation, neurobehavioral, or reproductive function in the F1 generation rats at any dose.
- The maternal NOAEL was 0.6 mg/kg/day due to decreases in maternal body weights and food consumption at MD and HD. The maternal reproductive NOAEL and developmental NOAEL were both 10 mg/kg/day, the highest dose tested.

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

Methods

Dose and frequency of dosing:	0, 0.6, 2.5 and 10 mg/kg/day, once daily
Route of administration:	Intravenous
Formulation/Vehicle:	Physiological saline
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	25/group
Satellite groups:	7/group for toxicokinetic analysis
Study design:	Animals were dosed from GD 7 to LD 20. Animals that had not delivered by GD20 were dosed until GD24.
Deviation from study protocol affecting interpretation of results:	None

Observations and Results

Generation	Major Findings
F0 Dams	No difelikefalin-related effects on F0 maternal reproductive function. Reduced maternal body weight (↓6% and ↓9% in MD and HD, respectively) on LD17, and reduced maternal body weight gain (↓17% in HD) from LD1 to LD 17.
F1 Generation	No difelikefalin-related effects on F1 growth, sexual maturation, neurobehavioral, or reproductive function.
F2 Generation	No difelikefalin-related effects.

5.5.5. Other Toxicology Studies

Impurity Qualification

The difelikefalin impurities are specified at not more than (NMT (b) (4) % for each in the drug product. Therefore, at the drug's recommended human dose 0.5 μg/kg/day (30 μg/day based on a 60-kg body weight), the maximum daily intake of each impurity is (b) (4) μg, lower than the ICH Q3B qualification threshold and the ICH M7 TTC of 1.5 μg/day. No further qualification is needed for the difelikefalin impurities.

The sponsor conducted extractables and leachables (E/Ls) studies on the container closure system (CCS). Further, the sponsor conducted an extractables study on a (b) (4) used in the manufacturing process for (b) (4) In either study, there were no organic leachables that had maximum daily exposure above the threshold of (b) (4) μg/day. Further, elemental impurities were all below the acceptable values recommended in ICH Q3D. Therefore, no further qualification is needed for CCS and (b) (4) E/Ls present in the final drug product.

Excipient Qualification

Difelikefalin injection does not contain novel excipients. All excipients are present at the same or lower levels when compared to levels in previously approved injection drug products.

6 Clinical Pharmacology

6.1. Executive Summary

The Applicant is seeking the approval of KORSUVA, a small synthetic peptide CR845 (generic name: difelikefalin) for the treatment of moderate-to-severe pruritus associated with chronic kidney disease (CKD-aP) in adult patients undergoing hemodialysis (HD). The clinical pharmacology of IV CR845 has been evaluated in a total of 15 phase 1 and phase 2 studies, and efficacy and safety was evaluated in 2 phase 3 studies.

The pharmacokinetics (PK) of difelikefalin is dose proportional over a single intravenous dosage range from 1 to 3 mcg/kg and multiple intravenous dosage range from 0.5 to 2.5 mcg/kg in chronic kidney disease patients undergoing HD. Steady-state was reached after the second administered dosage and mean accumulation ratio was up to 1.6. The mean apparent volume of distribution after IV administration of difelikefalin 0.5 mcg/kg is approximately 238 mL/kg.

The half-life of difelikefalin in HD subjects prior to dialysis ranged between 23 and 31 hours. Following administration of radiolabeled difelikefalin, >99% of circulating radioactivity was present in plasma as a parent moiety. Hemodialysis reduced the difelikefalin plasma concentrations by 70% to 80% and difelikefalin was not detectable in plasma after 2 dialysis cycles. Following administration of difelikefalin to HD patients, 11% of the dosage was excreted in urine, 59% in feces, and 20% in dialysate fluid.

No clinically significant differences in the pharmacokinetics of difelikefalin were observed based on age (25 to 80 years of age), sex, race/ethnicity, or mild to moderate hepatic impairment. The effect of severe hepatic impairment on the pharmacokinetics of difelikefalin is unknown.

No clinical studies evaluating the drug interaction potential of difelikefalin was conducted.

The results of in-vitro studies which evaluated the distribution, metabolism and excretion of difelikefalin indicate that it is not an inhibitor or substrate of clinically relevant enzymes or transporters. Difelikefalin is not metabolized by cytochrome P450 (CYP) enzymes CYP1A2, CYP2C19, CYP2C8, CYP2C9, CYP2D6 or CYP3A observed in human hepatic microsomes or hepatocytes, in vitro.

Difelikefalin did not inhibit CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A, or CYP2D6), or induce CYP enzymes (CYP1A2, CYP2B6, or CYP3A).

Difelikefalin is not an inhibitor of UGT1A3, UGT1A9, or UGT2B7.

Difelikefalin did not inhibit BCRP, Pgp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, or, MATE2-K human transporters and is not a substrate of OAT1, OAT2, OAT3, OATP1A2, OCT2,

OCT3, LAT1, PEPT1, PEPT2, ASBT, BSEP, MRP2, OATP1B1, OATP1B3, OATP2B1, OCT1, OCTN1, OCTN2, Pgp, BCRP, OSTα/β, MATE1, or, MATE2-K.

The results from a thorough QT/corrected QT interval (QTc) study (Study 100201) showed that at a dose 6 times the maximum approved recommended dose, KORSUVA does not prolong the QT interval to any clinically relevant extent.

No apparent dose-response in antipruritic efficacy was observed among the 3 doses (0.5, 1 and 1.5 mcg/kg) assessed in study CLIN2101. Based on the similar efficacy across dose groups and dose response trends observed in the safety results, a difelikefalin dose of 0.5 mcg/kg appeared to achieve the most favorable benefit-risk profile and was thus selected as the dose to be further evaluated in the phase 3 studies.

The results of the two phase 3 clinical studies, CR845-CLIN3102 and CR845-CLIN3103, demonstrated that difelikefalin, at the proposed dose of 0.5 mcg/kg, 3 times a week, was efficacious in the treatment of moderate-to-severe CKD-aP in adult subjects undergoing HD.

Recommendations
The Office of Clinical Pharmacology/Division of Inflammation and Immune Pharmacology (OCP/DIIP) has reviewed the clinical pharmacology information submitted under NDA 214916 and finds the NDA approvable.

Post marketing requirement/Post marketing commitment
None.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

The pharmacokinetics of difelikefalin is dose proportional over a single intravenous dosage range from 1 to 3 mcg/kg, (2 to 6 times the recommended dosage) and multiple intravenous dosage range from 0.5 to 2.5 mcg/kg (1 to 5 times the recommended dosage) in chronic kidney disease patients undergoing HD. Steady-state was reached after the second administered dosage and the mean accumulation ratio was up to 1.6.

Distribution
The mean volume of distribution of difelikefalin is approximately 238 mL/kg. Difelikefalin binding to human plasma protein in dialysis patients is 23% - 28%.

Elimination
The half-life of difelikefalin in HD subjects prior to dialysis ranged between 23 and 31 hours. Following administration of radiolabeled difelikefalin, >99% of circulating radioactivity was

present in plasma as parent. Hemodialysis reduced the difelikefalin plasma concentrations by 70% to 80% and difelikefalin was not detectable in plasma after 2 dialysis cycles.

Metabolism
Difelikefalin is not metabolized by cytochrome P450 (CYP) enzymes CYP 1A2, CYP2C19, CYP2C8, CYP2C9, CYP2D6 or CYP3A observed inhuman hepatic microsomes or hepatocytes, in-vitro.

Excretion
Following administration of difelikefalin to HD patients, 11% of the dosage was excreted in urine, 59% in feces, and 20% in dialysate fluid.

Specific Populations
No clinically significant differences in the pharmacokinetics of difelikefalin were observed based on age (25 to 80 years of age), sex, race/ethnicity, or mild to moderate hepatic impairment. The effect of severe hepatic impairment on the pharmacokinetics of difelikefalin is unknown.

Drug Interaction Studies

Clinical Studies
No clinical studies evaluating the drug interaction potential of difelikefalin was conducted.

***In-Vitro* Studies**
Cytochrome P450 (CYP) Enzymes: Difelikefalin did not inhibit CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP 2C19, CYP3A, or CYP2D6), or induce CYP enzymes (CYP1A2, CYP2B6, or CYP3A) and is not a substrate of CYP450 enzymes (CYP1A2, CYP2C19, CYP2C8, CYP2C9, CYP2D6 or CYP3A).

Uridine diphosphate (UDP)-glucuronosyl transferase (UGT) Enzymes: Difelikefalin is not an inhibitor of UGT1A3, UGT1A9, or UGT2B7.

Transporter Systems: Difelikefalin did not inhibit BCRP, Pgp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, or, MATE2-K human transporters and is not a substrate of OAT1, OAT2, OAT3, OATP1A2, OCT2, OCT3, LAT1, PEPT1, PEPT2, ASBT, BSEP, MRP2, OATP1B1, OATP1B3, OATP2B1, OCT1, OCTN1, OCTN2, Pgp, BCRP, OSTα/β, MATE1, or, MATE2-K.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

Difelikefalin is intended for IV bolus administration at a dose of 0.5 mcg/kg injection, 3 times per week at the end of each hemodialysis treatment.

Therapeutic Individualization

Not Applicable.

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Review Issues	**Recommendations and Comments**
Mechanism of Action	Difelikefalin is a kappa opioid receptor (KOR) agonist. The relevance of KOR activation to therapeutic effectiveness is not known.
Active Moieties	Difelikefalin is a peptide and it is the active moiety.
QT Prolongation	A TQT study (Study 100201) was performed, and at a dose 6 times the recommended dosage, difelikefalin does not prolong the QTc interval to any clinically relevant extent.
General Information	
Bioanalysis	Difelikefalin plasma and urine concentrations were measured using validated liquid chromatography (LC) with tandem mass spectrometric detection (MS).
Healthy Volunteers vs. Patients	Compared with healthy volunteers, HD patients had reduced plasma clearance (CL) of DIFELIKEFALIN , resulting in prolonged exposure with at least 10-fold increase in terminal half-life (Studies CLIN1301, CLIN1003, CLIN2005, and PR-13A9-P1-B).
Drug exposure at steady state following the therapeutic dosing regimen	Study CLIN2005 demonstrated that the steady state of C_{trough} was reached by the second dose administration.
Minimal effective dose or exposure	A Phase 2 dose ranging Study CLIN2101 of difelikefalin in HD patients with moderate to severe pruritus demonstrated that lower dose of difelikefalin (0.5 mcg/kg) was equally effective and superior to placebo treatment on primary endpoint [change from baseline in Worst Itching Intensity Numerical Rating Scale (WI-NRS) score at the end of week 8] compared to higher doses of BD (1.0 and 1.5 mcg/kg).
Maximal tolerated dose or exposure	40 mcg/kg is the highest dose of difelikefalin investigated under this NDA.
Dose Proportionality	The pharmacokinetics of difelikefalin is dose proportional over a single dosage range from 1 to 3 mcg/kg, and multiple intravenous dosage range from 0.5 to 2.5 mcg/kg in chronic kidney disease patients undergoing HD (Study CLIN1003 and Study CLIN2005).

Accumulation	Multiple-dose studies (Study CLIN2005 & PR-13A9-P1B) with doses 0.5 mcg/kg to 2.5 mcg/kg, demonstrated that following 0.5 mcg/kg, 3 times a week for 1 week treatment in patients with HD showed minimal accumulation. The accumulation ratio was up to 1.6 on Day 5.
Absorption	
Bioavailability	The proposed formulation is an IV dosage form and hence oral PK data are not described in this NDA. Bioavailability following IV administration is 100%.
t_{max}	Median t_{max} of difelikefalin was around 0.083 hours following IV dose of difelikefalin in HD patients (Studies CLIN2005, CLIN2101).
Food effect	Food effect was not evaluated in this NDA.
Distribution	
Volume of Distribution	The mean volume of distribution of difelikefalin is approximately 238 mL/kg (Study CLIN2005).
Plasma protein binding	Difelikefalin binding to human plasma protein in dialysis patients is 23% - 28% (Study CLIN1003).
Substrate transporter systems [in-vitro]	Difelikefalin showed limited membrane permeability by passive diffusion in-vitro (Study CR845-DMPK028) and is not a substrate or inhibitor for P-glycoprotein (Studies CR845-DMPK028, CR845-DMPK061), or any other major efflux and uptake transporters.
Elimination	
Mean Terminal Elimination half-life	The half-life of difelikefalin in HD subjects prior to dialysis ranged between 23 and 31 hours.
Metabolism	
Primary metabolic pathway(s) [*in-vitro*]	Difelikefalin is not metabolized by cytochrome P450 (CYP) enzymes CYP 1A2, CYP2C19, CYP2C8, CYP2C9, CYP2D6 or CYP3A observed in human hepatic microsomes or hepatocytes, in vitro.
Inhibitor/Inducer	Difelikefalin did not inhibit CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP 2C19, CYP3A, or CYP2D6), or induce CYP enzymes (CYP1A2, CYP2B6, or CYP3A) and is not a substrate of CYP450 enzymes (CYP1A2, CYP2C19, CYP2C8, CYP2C9, CYP2D6 or CYP3A). Difelikefalin is not an inhibitor of UGT1A3, UGT1A9, or UGT2B7 (Studies CR845-DMPK026, CR845-DMPK087, PBC069-123, and CR845-DMPK211). Difelikefalin is not an inducer of CYP1A2, CYP2B6, or CYP3A. (Study CR845-DMPK077)
Excretion	
Primary excretion pathway	Following administration of difelikefalin to HD patients, 11% of the dosage was excreted in urine, 59% in feces, and 20% in dialysate fluid. (Study 100302)

6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

Yes. The results of the two phase 3 clinical studies, CR845-CLIN3102 and CR845-CLIN3103, demonstrated that difelikefalin, at the proposed dose of 0.5 mcg/kg three times a week, was efficacious in the treatment of moderate-to-severe CKD-aP in adult subjects undergoing HD.
The primary endpoint for the pivotal Phase 3 clinical trials was based on achieving ≥ 4 (and ≥ 3)-point improvement from baseline with respect to weekly mean of the daily 24-hour worse-itch numeric rating scale (WI-NRS). These clinical trials met their primary endpoint and demonstrated efficacy compared to placebo (diff. 21.6% and 10.6% for ≥ 3 WI-NRS; 18.6% and 11.5% for ≥ 4 WI-NRS, CLIN3102 and CLIN3103, respectively). Safety for difelikefalin was based on the adverse event profile in the clinical trials. The main concerns are dizziness, somnolence, mental status changes, gait disturbances/falls, and hyperkalemia. Other events including strokes, cardiac events, and infections are seen at baseline with the hemodialysis patients and are no higher between difelikefalin and placebo. Please refer to the section 7 and 8 for more details on efficacy and safety.

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

Yes. No apparent dose-response in antipruritic efficacy was observed among the 3 doses of difelikefalin (0.5, 1 and 1.5 mcg/kg) studied in phase 2 dose ranging study CLIN2101 (Table 1 below). Based on the similar efficacy across dose groups and dose response trends observed in the safety results, a difelikefalin dose of 0.5 mcg/kg appeared to achieve the most favorable benefit-risk profile and was thus selected as the dose to be further evaluated in the phase 3 studies. Due to the low clearance in HD subjects, difelikefalin does not need to be administered more than once after each dialysis session. Based on the PK profile in HD subjects, IV difelikefalin is administered after each HD session (i.e., 3 times a week). The proposed dosing regimen is acceptable for this patient population.

Table 1: Percentage of Subjects Achieving ≥3-Point and ≥4-Point Reduction in WI-NRS Scores at Week 8

	Difelikefalin				
	Placebo (N=45)	**0.5 mcg/kg (N=44)**	**1.0 mcg/kg (N=41)**	**1.5 mcg/kg (N=44)**	**All Doses (N=129)**
Percentage Achieving ≥3-Point Reduction	29.5%	**62.4%**	**44.4%**	**57.3%**	54.9%
P value vs placebo		0.003	0.159	0.013	0.005
Percentage Achieving ≥4-Point Reduction	23.6%	**48.0%**	**32.9%**	**36.0%**	39.1%
P value vs placebo		0.019	0.344	0.216	0.072

Note: P-values were based on logistic regression run within each week, improvement criteria, and imputation run with effects for treatment and covariates for prior anti-itch medication usage. These results were then combined by week and improvement criteria to produce P-values.
WI-NRS: Worst Itching Intensity Numerical Rating Scale.
(Source: Clinical Study Report (Study CLIN 2101 Part A), Tables 14.2.5.3,Page 75.)

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

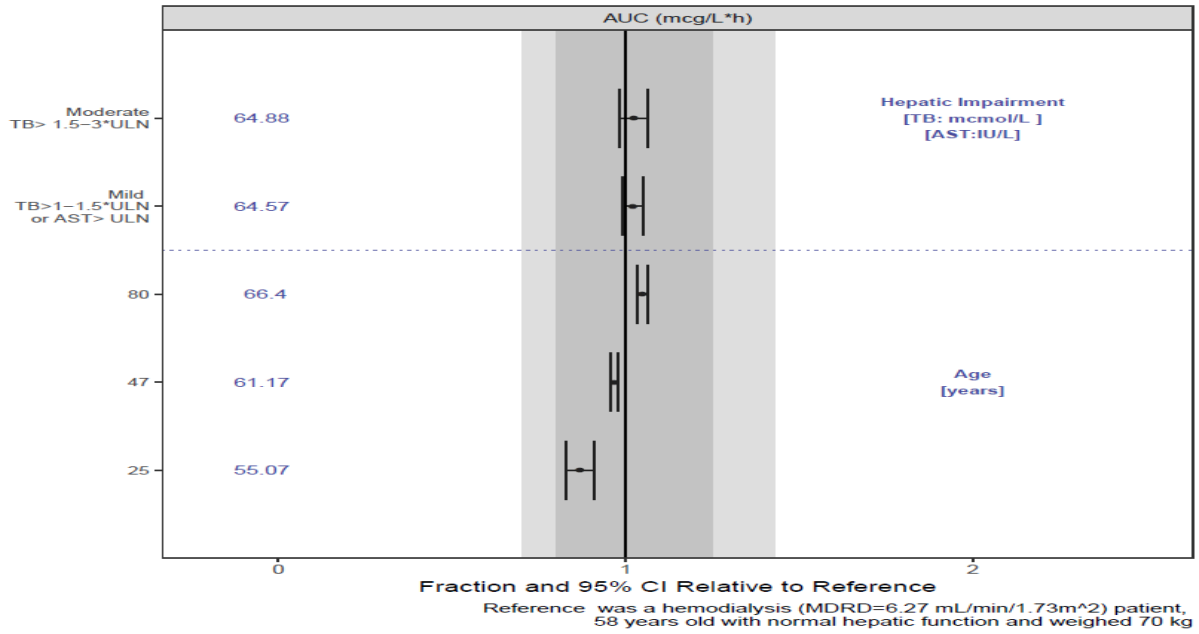
No. Difelikefalin is intended for IV administration at a dose of 0.5 mcg/kg, 3 times per week at the end of each hemodialysis treatment. A population PK analysis showed no effect of sex and race on difelikefalin exposure. Difelikefalin exposure [i.e., area under the curve (AUC)] in mild and moderate hepatic impairment status were within the reference range when compared to those with normal hepatic function (Figure 1 below); therefore, dose adjustments for this patient population are not warranted. Hemodialysis subjects between 25 and 80 years of age present generally similar CR845 exposure relative to a HD subject of 58 years of age; thus, no dose adjustments for age are recommended. Please refer to pharmacometrics review in Appendix 16.4.2, for more information.

Renal Impairment Patients:
Difelikefalin is eliminated primarily through the kidney. Consequently, total body clearance of difelikefalin in subjects with severe renal impairment is reduced relative to healthy, matched, control subjects (Study CLIN1005). Plasma concentrations of difelikefalin in HD subjects remain relatively constant until cleared during dialysis. Hemodialysis reduced the difelikefalin plasma concentrations by 70% to 80% and difelikefalin was not detectable in plasma after 2 dialysis cycles. (Studies CLIN1003, 100303 and CLIN2005 Part A). Half-life ranges between 23 and 31 hours in HD subjects compared with a typical range of 2 to 3 hours in subjects with normal renal function. The [^{14}C]-ADME/metabolism study conducted in HD subjects and healthy volunteer (Study CLIN100302) subjects demonstrated that difelikefalin was eliminated primarily in the urine in healthy volunteers and primarily in feces and dialysate in HD subjects. As the intended population for the relief of moderate-to-severe pruritus associated with CKD-aP are subjects maintained on HD, no additional dose adjustment is needed.

Elderly:
As CKD-aP affects adult HD patients of any age and elderly subjects were included in Phase 3, a separate Phase 1 study in geriatric patients has not been conducted. There is no reason to expect the PK profile in geriatric HD subjects to be different from that of non-geriatric HD subjects. Difelikefalin is a small peptide drug and it is neither a substrate nor inhibitor of CYP isozymes or transporters. Thus, age-dependent changes in these clearance mechanisms in the HD geriatric population have little bearing on total difelikefalin clearance, which depends primarily on kidney function.

The potential effect of age on difelikefalin elimination was evaluated in the pooled population PK model. The point estimates and 95% CI for AUC of hemodialysis patients older than 80 and as young as 25 years of age were comparable relative to the reference patient (58-year-old hemodialysis patient), see Figure 1 below:

Figure 1: Covariate Effects of Age and Hepatic Function on Difelikefalin Area Under the Curve (AUC)



(Source: Summary of Clinical Pharmacology, Figure 4, page 49, & Population PK report, (CTX0201F-Report-v1.0-Final), October 15, 2020, Figure 2, page 61)

Reviewer's Comment:
It is important to note that patients with renal failure in this analysis had an age distribution with a median value much older than subjects with normal renal function (58 versus 27 years of age). There were only three patients with renal failure younger than 25 years, and the remaining subjects were older than 37 with almost half the patients older than 60 years of age. However, for normal renal function subjects, the median age was 27 years old with only three subjects above 60 years (i.e. 62, 63 and 68 years). Inferences about elimination in hemodialysis patients that are 25 years and younger should be made with caution, as the data was not well balanced or informative enough to support claims regarding age in the renal failure population without also discussing the unbalanced age distribution in the dataset.

<u>Hepatically Impaired Patients:</u>
The study 100302 demonstrated that parent difelikefalin was the most abundant analyte in systemic circulation accounting for >99% of total systemic exposure. The most prevalent metabolite (MP1) accounted for 0.48% and 0.10% of total plasma exposure in healthy volunteers

and HD subjects respectively. This finding verified *in-vitro* data demonstrating no metabolism and establishes that difelikefalin is not metabolized in neither HD subjects nor subjects with normal renal function. As the target population, HD patients already have one route of elimination impaired. A separate PK study in hepatically impaired subjects was not conducted.

The influence of mild to moderate hepatic impairment on the pharmacokinetics of difelikefalin was evaluated in a population pharmacokinetic PK analysis which concluded that no difelikefalin dosage adjustments were needed in these populations. Please refer to pharmacometrics review in Appendix 16.4.2 for more details. Briefly, in the IV only analysis, most subjects had normal hepatic function (95.9%), while 17 subjects had mild hepatic impairment (3.9%), 1 had moderate impairment (0.23%) and 0 had severe hepatic dysfunction. The point estimates and 95% CI for AUC of mild and moderate hepatic impairment subjects were compared relative to the reference patient (58-year-old normal hepatic impairment subject), see Figure 1 above.

Reviewer's Comment:
The influence of severe hepatic impairment on the pharmacokinetics of difelikefalin in subjects undergoing HD has not been evaluated; therefore, use of difelikefalin in this population is not recommended. Within the same population PK analysis, the pooled IV + oral analysis described in Report Population PK Modeling and Simulation for CR845 (CTX0201F-Report-v1.0-Final, October 15, 2020), which contained an enriched number of subjects with mild (n=50) and moderate (n=9) hepatic impairment, also demonstrated that point estimates and 95% CI were contained within the region on practical equivalence. Therefore, no dose adjustments are recommended for those with mild or moderate hepatic impairment. Please refer to the pharmacometrics review in Appendix 16.4.2 for more information.

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

No. The effects of other drugs on difelikefalin PK have not been evaluated in any clinical study, but the modest degree of plasma protein binding and lack of interaction of CR845 with common drug transporters and metabolic enzymes *in-vitro* suggest a low likelihood of drug-drug interactions that would impact the safety or efficacy of difelikefalin.

Difelikefalin's major clearance pathway is excretion of the unchanged parent compound into urine dialysate, and/or feces. Results of *in-vitro* studies indicate that difelikefalin is metabolically stable. The data also suggests that it is not an inhibitor or substrate for clinically relevant enzymes and transporters (Table 2 below). In addition, difelikefalin appears to have minimal to no potential for induction of human CYP1A2, CYP2B6, or CYP3A4; together these suggest a negligible potential for drug-drug interaction based on P450 metabolism. These data indicate that difelikefalin should not significantly affect the clearance and/or metabolism of any co-administered drug and also the clearance and/or metabolism of difelikefalin should not be impacted by co-administration of other drugs.

Table 2: In Vitro Metabolism Studies

Report #	Aims	Conclusion
***In Vitro* Metabolism Studies**		
Metabolic Stability of CR845 in Cryopreserved Hepatocytes (CR845-DMPK025 and CR845-DMPK216)	To evaluate the metabolic stability of CR845 after incubation with rat, dog, monkey, and human (pooled male and female) whole blood, hepatocytes, and kidney and intestinal subcellular fraction containing drug metabolizing enzymes, (S9) fractions.	CR845 was present in all species. No metabolites formed via oxidation, conjugation, or hydrolysis were detected from hepatocytes in any of the species tested. This suggests a lack of hepatic metabolism as major clearance mechanism.
In Vitro Biotransformation of CR845 in Cryopreserved Hepatocytes (CR845-DMPK023)	Incubation of CR845 with hepatocytes to identify the potential metabolites and/or the transformation products using Sprague-Dawley rat, beagle dog, cynomolgus monkey and human cryopreserved hepatocytes.	No potential metabolites formed by oxidation, conjugation, or hydrolysis were identified.
In vitro Assessment of Metabolism (PBC069-127)	To evaluate the metabolism of [^{14}C]-CR845 by hepatocytes from rats, and humans.	Radio-high performance liquid chromatography (HPLC) of samples obtained when rat, monkey, and human hepatocytes were incubated with [^{14}C]-difelikefalin revealed trace levels of a single peak in rats and humans that was not identified at Time 0 or in control samples
Pharmacokinetic Drug Interactions In Vitro P450 Studies		
CYP Reaction Phenotyping (CR845-DMPK027)	To evaluate which CYP isoforms are potentially involved in CR845 oxidative metabolism by evaluating CR845 in Bactosome ™ expressing human recombinant CYP450s.	95 to 122 % of CR845 remain after 0 to 45 minutes. CR845 does not appear to be a substrate for CYP1A2, CYP2C19, CYP2C8, CYP2C9, CYP2D6, or CYP3A4.
Cytochrome P450 Inhibition		
In Vitro Cytochromes Inhibition Assessment (CR845-DMPK026 and CR845-DMPK087)	To evaluate potential of CR845 to inhibit cytochrome P450 activity by evaluating CR845 in concentrations up to 10 µM in human liver microsomes.	CR845 showed no inhibitory effect on CYP2B6, CYP2C8, CYP2C9, or CYP2D6. CR845 minimally inhibited CYP3A-, CYP1A-, and CYP2C19-mediated metabolism by 20%, 16%, and 5%, respectively, at 10 µM Negligible likelihood of any CYP enzyme-based drug-drug interaction in humans.
P450 Enzyme Inhibition Study of CR845 in Human Liver Microsomes (PBC069-123)	To evaluate the potential for CR845 to inhibit probe substrates when incubated with human liver microsomes.	In human liver microsomes with or without NADPH regenerating system pre-incubation, the percent inhibition of CYP2C9, CYP1A2, and CYP3A (midazolam) was ≤3.8% and of CYP3A (testosterone), with and without pre-incubation, was 36% and 17.3%, respectively, indicating that difelikefalin is an unlikely inhibitor of the CYP enzymes.

Cytochrome P450 Induction		
Potential for Induction of Cytochrome P450 Enzymes in Human Hepatocytes (CR845-DMPK077)	The potential induction of cytochrome P450 (CYP) mRNA expression and enzyme activity in human hepatocytes by CR845 was evaluated.	CR845 is not an inducer of CYP1A2, CYP2B6, or CYP3A.
Inhibition of Glucuronidation		
Potential to inhibit UDP glucuronidation (CR845-DMPK211)	The potential for CR845 to inhibit human recombinant UDP glucuronosyl-transferases	CR845 does not cause direct inhibition of recombinant UGT1A3, UGT1A9 or UGT2B7.
Substrate and Inhibitor Transporters		
Substrate Potential (CR845-DMPK061 and DMPK095)	To evaluate the substrate potential of CR845 for P-gp, BCRP, OAT1, OAT3, OATP1A2, OCT2, OCT3 and LAT1 in human transfected MDCK-II, MDCK-MDR1 and Caco-2 cells.	CR845was not a substrate of OAT1, OAT3, OATP1A2, OCT2, OCT3, LAT1, P-gp or BCRP and was not an inhibitor of P-gp or BCRP.
Inhibitor Potential (CR845-DMPK210)	To characterize the inhibitory potential of CR845 on BCRP, OAT1, OAT3, OCT1, OCT2, OATP1B1, or OATP1B3 transporters in human transfected MDCK-II, MDCK-MDR1 and Caco-2 cells.	Difelikefalin did not act as an inhibitor of BCRP, OAT1, OAT3, OCT1, OCT2, OATP1B1 or OATP1B3.
In-Vitro Interaction Potential MRP2 Efflux Transporter and with PEPT1 and PEPT 2 Uptake Transporters (CR845-DMPK060 and DMPK093)	To determine the effect of CR845 on human MRP2 vesicular transport and PEPT1 and PEPT2 uptake.	CR845 was not a substrate or inhibitor of multidrug resistance associated protein 2 (MRP2). Difelikefalin at concentrations up to 30 µM (approximately 20 mcg/mL) was not a substrate or inhibitor of PEPT1 or PEPT2.
Renal Efflux Transporter Interactions (CR845-DMPK078)	To determine if CR845 is a potential substrate or inhibitor for renal efflux transporters MATE1 and MATE2-K in human transfected HEK293 cells.	CR845 does not appear to be a substrate for and does not inhibit activity for human MATE1 or MATE2-K.
Biliary Transporter interactions (CR845-DMPK093)	To determine if CR845 is a potential substrate for biliary transporters.	CR845 is not a substrate for ASBT, BSEP, MRP2, peptide transporter (PEPT) 1, PEPT2, OAT2, OATP1B1, OATP1B3, OATP2B1, OCT1, OCTN1, OCTN2, OSTα/β. CR845 did not inhibit BSEP.

BCRP = breast cancer resistance protein; Conc. = concentration; CYP = cytochrome P450; MATE = multidrug and toxin extrusion protein; MRP = multidrug resistance-associated protein; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT = organic cation transporter; PEPT = peptide transporter; P-gp = P-glycoprotein; UDP = uridine diphosphate; UGT = uridine diphosphate glucuronosyl transferase.

(Source: Summary of Clinical Pharmacology Studies, Table 5, pages 55 to 58)

What is the impact of physicochemical properties of the drug and different mechanisms to support that the Difelikefalin (IV CR845) is unlikely cross the Blood Brain Barrier?

The blood brain barrier (BBB) is a specialized system of capillary endothelial cells that prevents

both the transcellular and paracellular passage of substances from the systemic circulation into the brain. The physiochemical properties of difelikefalin were therefore selected by the applicant to be the opposite of drugs targeting the central nervous system (CNS). The experimental evidence for the peripheral restriction of difelikefalin is based on a totality of evidence not only from in-vitro studies but also in-vivo nonclinical studies summarized below:

(b) (4)

1 Page has been Withheld in Full as b4 (CCI/TS) immediately following this page

3. Difelikefalin's restricted BBB penetration is further supported by a series of in vivo rat and monkey tissue distribution studies showing that the distribution of difelikefalin to the central nervous system (CNS) tissue protected by the BBB was very low with no increase in distribution over time at plasma concentrations >386 to 1600-fold above the Cmax achieved at the proposed clinical dose of 0.5 mcg/kg in hemodialysis patients.

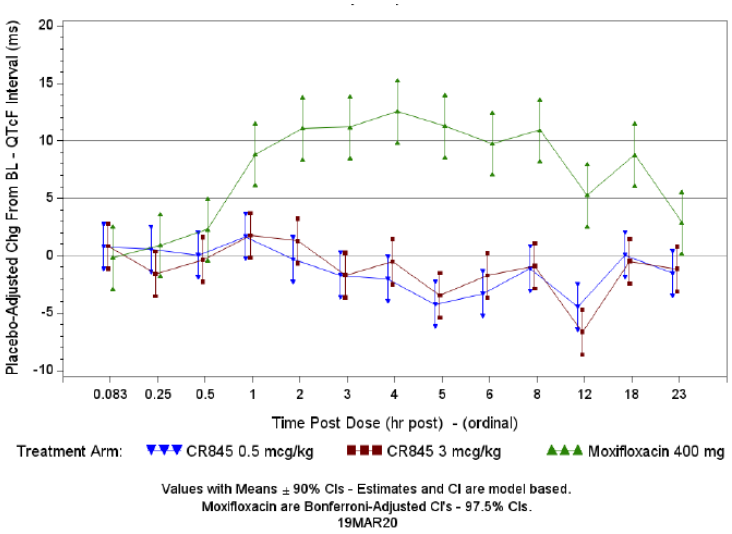
Taken together, these data show negligible passive diffusion or active transport of difelikefalin across membranes with no detection in or poor distribution to the brain based on whole-body autoradiography and tissue distribution studies in rats and monkeys. Although the evidence at hand suggests that the permeation of difelikefalin into BBB is unlikely based on the known mechanisms; the reason why CNS related AEs were observed cannot be explained.

(**Source:** Response to Clinical Pharmacology Information Request, dated March, 30,2021).

Does difelikefalin shows any clinically relevant prolongation of the corrected QT interval (QTc) in healthy subjects?

No. The results from a thorough QT/corrected QT interval (QTc) study (Study 100201) showed no signal of any clinically significant effect of difelikefalin on heart rate, atrioventricular conduction as measured by the PR interval, or cardiac depolarization as measured by the QRS duration, and there was no signal of clinically significant effects on cardiac repolarization as evidenced by the results of the time-averaged, time-matched, outlier, and PK-PD analyses.

Figure 2: Placebo-Adjusted Mean Change from Baseline-QTcF Interval (ΔΔQTcF)



(Source: Summary of Clinical Pharmacology Studies, Figure 2, page 37)

Are the Bioanalytical Methods Properly Validated to Measure PK in Plasma & Urine Samples?

Well validated bioanalytical methods were developed to quantify CR845 (difelikefalin) plasma protein binding and the concentration of CR845 in plasma and urine. In clinical studies, human K2- Ethylenediaminetetraacetic acid plasma samples were extracted by solid phase extraction method and analyzed using liquid chromatography-tandem mass spectrometry assays. A sensitive and specific liquid chromatography-tandem mass spectrometry assay was also developed for determination of CR845 in human urine. The bioanalytical reports describe the assay performance and sample assay results. Incurred Sample Reanalysis (ISR) was conducted for both plasma and urine samples from appropriate phase 1 and phase 2 studies and the results were within ± 20%. The additional details of bioanalytical methods are provided in Appendix 16.4.1.

7 Sources of Clinical Data and Review Strategy

Refer to table of clinical studies covered in this application.

7.1. Table of Clinical Studies

Table 3: Table of Clinical Studies Pertinent to the Claimed Indication

Study	Phase	Country	Description	Design	Dosing	Population	Duration	Number of Subjects
Controlled Studies pertinent to the Claimed Indication								
CR845-CLIN2005	2	US	Safety, PK, and efficacy in HD patients with and without pruritus	Single-center (Part A) or multicenter (Part B), randomized, DB, PC, 2-part	Part A: Single IV bolus of 0.5, 1, and 2.5 mcg/kg Part B: IV bolus of 1 mcg.kg IV 3 times a week for 2 weeks	Part A: patients undergoing hemodialysis Part B: hemodialysis patients with moderate-to-severe CKD-aP	2 weeks	Part A: 19 CR845 5 placebo Part B: 33 CR845 32 placebo
CR845-CLIN2101	2	US	Efficacy and safety in HD patients with pruritus	Multicenter, randomized, DB, PC	0.5, 1.0, and 1.5 mcg/kg IV bolus 3 times a week for 8 weeks	HD patients with moderate-to-severe CKD-aP	8 weeks	129 CR845 45 placebo
PR-13A0-P2-A	2	JAPAN	Efficacy and safety in HD patients with pruritus	Multicenter, randomized, DB, PC, parallel-group	0.25, 0.5, 1.0, and 1.5 mcg/kb IV bolus 3 times a week for 2 weeks	HD patients with moderate-to-severe CKD-aP	2 weeks	84 CR845 21 placebo
CR845-CLIN3102DB	3	US	Safety and efficacy in HD patients with pruritus	Multicenter, randomized, DB, PC with an open-label extension	0.5 mcg/kg IV bolus 3 times a weeks for 12 weeks	HD patients with moderate-to-severe CKD-aP	12wk (DB)	189 CR845 189 placebo
CR845-CLIN3103DB	3	GLOBAL	Safety and efficacy in HD patients with pruritus	Multicenter, randomized, DB, PC with an open-label extension	0.5 mcg/kg IV bolus 3 times a weeks for 12 weeks	HD patients with moderate-to-severe CKD-aP	12wk (DB)	235 CR845 236 placebo
Uncontrolled Studies pertinent to claimed indication								
CR845-CLIN3101	3	US	Long-term safety in HD	Multicenter, open-label	0.5 mcg/kg IV bolus 3 times a week for	HD patients with moderate-to-severe	Up to 52-weeks	288

Study	Phase	Country	Description	Design	Dosing	Population	Duration	Number of Subjects
			patients with pruritus		up to 52 weeks	CKD-aP who participated in either CR845-CLIN2005 (PartB) or CR845-CLIN2101 and de novo patients		
CR845-CLIN3105	3	GLOBAL	Safety and efficacy in HD patients with pruritus	Multicenter, open-label	0.5 mcg/kg IV bolus 3 times a week for up to 12 weeks	HD patients with moderate-to-severe CKD-aP	12 weeks	222
CR845-CLIN3102 OLE	3	US	Safety and efficacy in HD patients with pruritus	Multicenter, open-label extension	0.5 mcg/kg IV bolus 3 times a week for up to 52 weeks	HD patients with moderate-to-severe CKD-aP	52wk OLE	313
CR-845-CLIN3103 OLE	3	GLOBAL	Safety and efficacy in HD patients with pruritus	Multicenter, open-label extension	0.5 mcg/kg IV bolus 3 times a week for up to 52 weeks	HD patients with moderate-to-severe CKD-aP	52wk OLE	399

Source: adapted from Module 5.2, Tabular listing of All Clinical Studies
HD-hemodialysis, US-United States, PK-pharmacokinetics, DB-double-blind, PC-placebo-controlled, CKD-aP: chronic kidney disease associated pruritus, CR845 is difelikefalin

7.2. Review Strategy

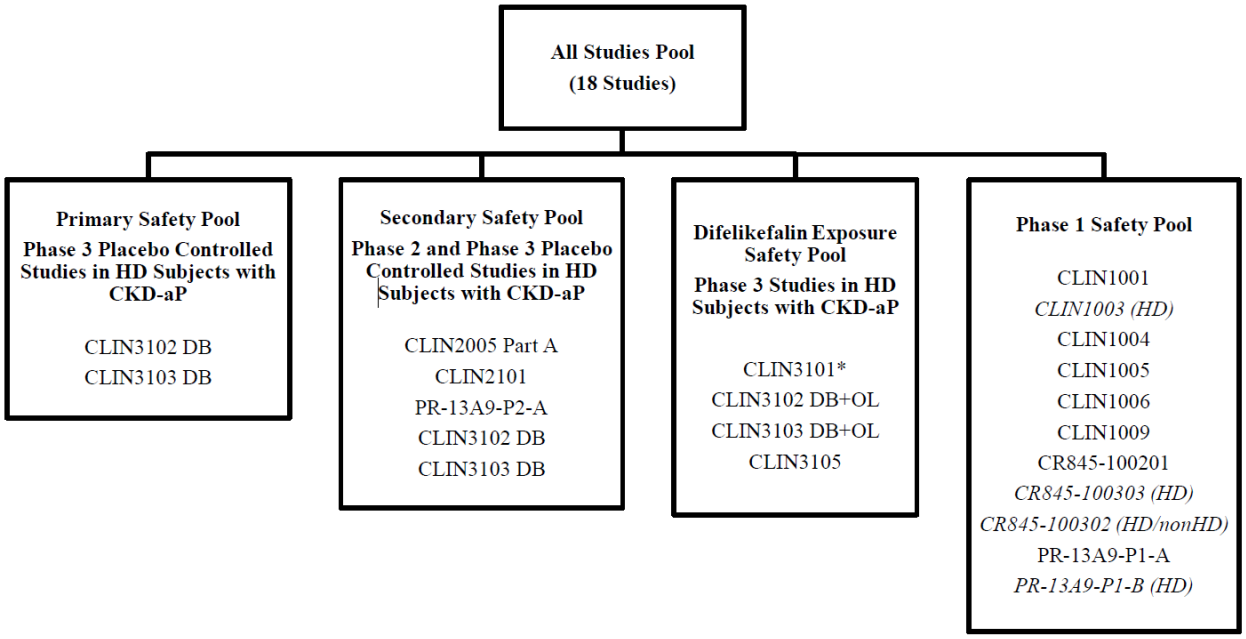
The applicant conducted two randomized, multicenter, double-blind, placebo-controlled, parallel-group, Phase 3 clinical trials (845-CLIN3102 & 845-CLIIN3103). These studies enrolled ≥ 18 years of age (CLIN3102) or 18-85 years of age (CLIN3103), ESRD on hemodialysis 3 times per week for at least 3 months prior to screening, and a body weight between 40 kg and 135 kg, inclusive. All eligible subjects completed a 7-day run-in period during the week prior to randomization, starting on the first dialysis session of that week (i.e., Mon for a Mon-Wed-Fri dialysis schedule or Tue for a Tue-Thr-Sat dialysis schedule). Subjects were trained to complete the 24-hour Worst Itching Intensity Numeric Rating Scale (WI-NRS) and were required to record their WI-NRS score each day (at a similar time of day) of the run-in period. Efficacy analysis will focus on the two 12-week clinical trials.

Eighteen (18) studies provided safety data on difelikefalin for the integrated safety database. In the Primary Safety Pool (3102 and 3103) double-blind treatment period (DB) for up to 12 weeks: 424 exposed to difelikefalin; 424 in the placebo. The mean age of subjects was approximately 59 years (range 22 to 88 years), and 59% of the subjects were male. Of the total subjects, 60.7% were White, 29.2% were Black or African American, and 5.3% were Asian. In these 2 trials, 43% of subjects in both treatment groups were treated concomitantly with antipruritic medications, including most commonly diphenhydramine and hydroxyzine.

In the placebo-controlled (secondary) safety pool, there were 1306 subjects on HD, with moderate-to-severe pruritus, exposed to ≥ 1 dose of difelikefalin. In the Long-Term Difelikefalin Exposure Safety Pool (4 studies: 2 open label safety and 2 PC clinical DB-OL) included:

- 1093 (83.7%) exposure duration of ≥3 months, and
- 718 (55%), continuous exposure ≥6 months
- 541 (41.4%), continuous exposure ≥9 months
- 412 subjects (31.5%) continuous exposure ≥12 months (P53 Table 9 [5.3.5.3] ISS)

Figure 3: Summary of Safety Analysis Pooling Strategy



Source: Applicant's Figure 1 in Integrated Summary of Safety

The safety analysis will focus on the primary and secondary safety pools. The remaining safety data will be evaluated for supplemental safety.

Compliance with Good Clinical Practices

The applicant reports the studies were conducted in accordance with the protocol, legal and regulatory requirements, and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), International Council on Harmonisation (ICH) Guideline for Good Clinical Practice (GCP), and the Declaration of Helsinki.

Financial Disclosure

The applicant submitted FDA form 3454 for financial disclosures. The applicant certified that for the covered studies CR845-CLIN3102 and CR845-CLIN3103, none of the clinical investigators entered into any financial arrangement.

8 Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study Design

The Applicant conducted two Phase 3 trials (CLIN3102 and CLIN3103). Both were randomized, multicenter, double-blind, placebo-controlled, parallel-group trials to evaluate the safety and efficacy of difelikefalin 0.5 mcg/kg for the treatment of moderate to severe pruritus in hemodialysis subjects. The trials consisted of a Double-blind Phase and an Open-label Phase.

Double-blind Phase:
This phase consisted of a screening visit (Days -28 to -7), a 7-day run-in period, and a 12-week double-blind treatment period.

All eligible subjects completed a 7-day run-in period during the week prior to randomization, starting on the first dialysis session of that week (i.e., Monday for subjects on a Monday-Wednesday-Friday dialysis schedule or Tuesday for subjects on a Tuesday-Thursday-Saturday dialysis schedule). During the first visit of the run-in period, subjects were trained on completion of the 24-hour Worst Itching Intensity Numeric Rating Scale (WI-NRS) and were required to record their WI-NRS score each day of the run-in period. Subjects were requested to complete the WI-NRS worksheets at a similar time of day around the normal start of their dialysis. To be eligible for inclusion in the trial, subjects were to meet the following protocol-specified inclusion criteria:

- Male or female
- 18 years of age and older (Trial CLIN3102) / 18 to 85 years of age (Trial CLIN3103)
- End stage renal disease (ESRD) and on hemodialysis 3 times per week for at least 3 months prior to the start of screening
- Body weight between 40.0 and 135.0 kg, inclusive

After the 7-day run-in period, the protocols specified that approximately 350 subjects from up to 80 sites in the United States (Trial CLIN3102) or up to 95 sites globally (Trial CLIN3103) will be randomized in a 1:1 ratio to receive either difelikefalin 0.5 mcg/kg (N=175) or placebo (N=175). To be eligible for randomization on Day 1, subjects were required to:

- Have completed at least four WI-NRS worksheets from the start of the run-in period up to and including the pre-randomization assessment on Day 1
- Have a mean baseline WI-NRS score >4 (Trial CLIN3102) or ≥5 (Trial CLIN3103), defined as the average of all non-missing scores reported from the start of the run-in period up to and including the pre-randomization assessment on Day 1

The randomization was stratified by use of anti-itch medication at baseline (Yes/No) and history of specific medical conditions (Yes/No). These medical conditions included history of fall or

fracture (related to fall), confusional state or mental status change or altered mental status or disorientation, and gait disturbance or movement disorder. Subjects were administered difelikefalin or matched placebo (0.04 M isotonic acetate buffer, pH 4.5) as an IV bolus after the end of their dialysis session during the 12-week treatment period so that each subject received difelikefalin or placebo 3 times weekly. Subjects were instructed to report their WI-NRS score over the last 24 hours daily during the entire 12-week treatment period. In addition, the protocols specified that other PROs will be recorded on selected study visits.

Open-Label Phase:
The protocols specified that all subjects who received at least 30 doses of the planned 36 doses of study product during the 12-week treatment period are eligible for entry into the open-label phase. Subjects were specified to receive difelikefalin 0.5 mcg/kg after each dialysis session, 3 times per week for up to 52 weeks. Subjects were instructed to report their WI-NRS score during this phase.

Interim Analysis:
The protocols specified conducting an interim analysis for sample size re-estimation after 50% of the 350 randomized subjects either complete the 12-week treatment period or discontinue study drug prematurely. The re-estimation was based on the conditional power for the primary efficacy endpoint. The maximum total sample size specified in the protocol was 500 subjects (250 per arm). An unblinded statistician who was not part of the study team was specified to conduct the interim analysis and provide results to the (b) (4) The protocols specified that the (b) (4) will "only communicate the decision to either keep the original sample size or to increase it; no other results will be provided to blinded staff." The protocols specified the following decision rules:

- If the conditional power CP1 is ≥90%, then
  - If the conditional power CP2 is ≥80%, the (b) (4) will recommend to continue enrollment without any change to the sample size
  - If the conditional power CP2 is <80%, the (b) (4) will recommend to increase the sample size to 250 patients per group
- If the conditional power CP1 is <90% and ≥70% then (b) (4) will recommend the sample size will be increased to 215 subjects per group
- If the conditional power CP1 is <70% and ≥40% then the (b) (4) will recommend the sample size will be increased to 250 subjects per group
- If the conditional power is <40% the (b) (4) will recommend to continue enrollment without any change to the sample size

where CP1 is calculated with respect to the proportion of subjects achieving ≥3-point improvement from baseline to Week 12 on the WI-NRS; and CP2 is calculated with respect to the proportion of subject achieving ≥4-point improvement from baseline to Week 12 on the WI-NRS.

Based on the above specified decision rules, the (b) (4) recommended no change to the sample size for Trial CLIN3102; however, for Trial CLIN3103, the (b) (4) recommended the sample size be increased to 215 subjects per group.

8.1.2. Endpoints

For both trials, the protocol-specified primary efficacy endpoint was the proportion of subjects achieving at least a 3-point improvement in WI-NRS score from baseline to Week 12. The baseline value was defined as the average daily WI-NRS scores reported during the 7-day run-in period. The weekly mean score was defined as the sum of the daily scores reported during a specific week divided by the number of days with non-missing scores for that week. In several communications (e.g., EOP2 meeting on September 6, 2017, guidance meeting on December 6, 2017, and advice letter sent on January 10, 2019), the Agency recommended the primary efficacy endpoint to be the proportion of subjects achieving at least a 4-point improvement in WI-NRS score from baseline to Week 12.

The following are the secondary efficacy endpoints specified in the protocol for Trial CLIN3102:

- Change from baseline to Week 12 in the 5-D Itch Scale total score
- Change from baseline to Week 12 in total Skindex-10 Scale total score
- Proportion of subjects achieving at least a 4-point improvement in WI-NRS score from baseline to Week 12

The following are the secondary efficacy endpoints specified in the protocol for Trial CLIN3103:

- Proportion of subjects achieving at least a 4-point improvement in WI-NRS score from baseline to Week 12
- Proportion of subjects achieving at least a 3-point improvement in WI-NRS score from baseline to Week 8
- Proportion of subjects achieving at least a 3-point improvement in WI-NRS score from baseline to Week 4
- Proportion of subjects achieving at least a 4-point improvement in WI-NRS score from baseline to Week 8
- Proportion of subjects achieving at least a 4-point improvement in WI-NRS score from baseline to Week 4
- Change from baseline to Week 12 in Skindex-10 Scale total score
- Change from baseline to Week 12 in the 5-D Itch Scale total score

Figure 4: Worst Itching Intensity Numeric Rating Scale (WI-NRS)

Worst Itching Over the Past 24 Hours
Please indicate the intensity of the **WORST ITCHING** you experienced over the past 24 hours. 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ **NO ITCHING** — **WORST ITCHING IMAGINABLE**

Source: page 84 of the protocol for Trial CLIN3102.

Figure 5: 5-D Itch Scale

1.	DURATION:	During the last 2 weeks, how many hours a day have you been itching?				
		Less than 6 hrs/day ☐	6-12 hrs/day ☐	12-18 hrs/day ☐	18-23 hrs/day ☐	All day ☐
2.	DEGREE:	Please rate the intensity of your itching over the past 2 weeks				
		Not present ☐	Mild ☐	Moderate ☐	Severe ☐	Unbearable ☐
3.	DIRECTION:	Over the past 2 weeks has your itching gotten better or worse compared to the previous month?				
		Completely resolved ☐	Much better, but still present ☐	Little bit better, but still present ☐	Unchanged ☐	Getting Worse ☐
4.	DISABILITY:	Rate the impact of your itching on the following activities over the last 2 weeks				
	Sleep	Never affects sleep ☐	Occasionally delays falling asleep ☐	Frequently delays falling asleep ☐	Delays falling asleep and occasionally wakes me up at night ☐	Delays falling asleep and frequently wakes me up at night ☐
		N/A	Never affects this activity	Rarely affects this activity	Occasionally affects this activity	Frequently affects this activity	Always affects this activity
	Leisure/Social	☐	☐	☐	☐	☐	☐
	Housework/ Errands	☐	☐	☐	☐	☐	☐
	Work/School	☐	☐	☐	☐	☐	☐

5. DISTRIBUTION:	Mark whether itching has been present in the following parts of your body over the last 2 weeks. If a body part is not listed, choose the one that is closest anatomically.			
	Head/Scalp	☐	Soles	☐
	Face	☐	Palms	☐
	Chest	☐	Tops of Hands/Fingers	☐
	Abdomen	☐	Forearms	☐
	Back	☐	Upper Arms	☐
	Buttocks	☐	Points of Contact w/ Clothing (e.g waistband, undergarment)	☐
	Thighs	☐		
	Lower legs	☐	Groin	☐
	Tops of Feet/Toes	☐		

Source: page 86 of the protocol for Trial CLIN3102.

Figure 6: Skindex-10 Scale

INSTRUCTIONS: During the past **WEEK**, how often have you been bothered by:							
	0 (Never bothered)	**1**	**2**	**3**	**4**	**5**	**6** (Always bothered)
1. Your itching	☐	☐	☐	☐	☐	☐	☐
2. The persistence/reoccurrence of your itching	☐	☐	☐	☐	☐	☐	☐
3. The appearance of your skin from scratching	☐	☐	☐	☐	☐	☐	☐
4. Frustration about your itching	☐	☐	☐	☐	☐	☐	☐
5. Being annoyed about your itching	☐	☐	☐	☐	☐	☐	☐
6. Feeling depressed about your itching	☐	☐	☐	☐	☐	☐	☐
7. Feeling embarrassed about your itching	☐	☐	☐	☐	☐	☐	☐
8. The effects of your itching on your interactions with others (for example: interactions with family, friends, close relationships, etc.)	☐	☐	☐	☐	☐	☐	☐
9. The effects of your itching on your desire to be with people	☐	☐	☐	☐	☐	☐	☐
10. The effect of your itching making it hard to work or do what you enjoy	☐	☐	☐	☐	☐	☐	☐

Source: page 85 of the protocol for Trial CLIN3102.

8.1.3. Statistical Methodologies

The protocol-specified primary analysis population is the intent-to-treat (ITT) population, defined as all randomized subjects. The protocols also specified a per-protocol (PP) population, defined as the subset of subjects in the ITT population who do not have any major protocol deviations that could affect the efficacy analyses. The PP population was defined as subjects who:

- Received at least 80% of the planned study drug doses while in the study
- Received at least 1 study dose in each of Week 11 and 12 of the double-blind treatment period, if present through Week 12
- Did not receive a different treatment than the treatment to which they were randomized
- Had a mean baseline WI-NRS score >4.0 (Trial CLIN3102) or ≥5.0 (Trial CLIN3103)
- Had a non-missing average 24-hour weekly WI-NRS score available for at least 75% of study weeks while in the study (weeks with >3 missing daily values were considered missing)
- Did not have significant amounts of restricted and prohibited medications listed in the protocol, based on medical review
- Did not have other major protocol violations that would have impacted efficacy outcomes

For the analysis of the primary efficacy endpoint (i.e., the proportion of subjects achieving ≥3-point improvement from baseline to Week 12 in WI-NRS score), the protocols and statistical analysis plans (SAPs) specified using logistic regression with treatment, baseline NRS score, region (only Trial CLIN3103), and the factors used to stratify the randomization (i.e., prior use of anti-itch medication [yes/no] and presence of specific medical condition [yes/no]) as factors in the model. The protocols and SAPs specified that the final p-value will be calculated using the Cui, Hung, and Wang (CHW) approach where the z-score is a weighted average of the z-score at the interim and the z-score observed for data collected after the interim, following the formula below:

$$Z_{final} = Zi_{nterim} * \sqrt{(n/N)} + Z_{post\text{-}interim} * \sqrt{(1 - n/N)}$$

where n is the number of subjects at the interim and N is the initial number of subjects planned (i.e., 350). The SAPs also specified using the Lawrence and Hung approach to calculate the point estimates and confidence intervals.

For the analysis of the continuous secondary efficacy endpoints (i.e., Skindex-10 Scale total score and the 5-D Itch Scale total score), the protocol for Trial CLIN3102 specified using a mixed effect model for repeated measures (MMRM); however, the SAP specified using analysis of covariance (ANCOVA) with treatment, baseline value, the factors used to stratify the randomization as factors in the model. The clinical study report for Trial CLIN3102 followed the SAP. For Trial CLIN3103, the protocol and SAP specified using ANCOVA with the same factors as those specified for Trial CLIN3102 as well as region. For the analysis of the binary secondary

efficacy endpoints, the protocols and SAPs for both trials specified using "methodology similar to the one employed for the primary analysis of the primary endpoint."

The protocol and SAPs specified analyzing the secondary endpoint using a sequential gatekeeping approach to control the Type I error rate. The secondary endpoints were specified to be analyzed in the order specified in Section 8.1.2.

For WI-NRS, the protocols and SAPs specified that subjects must report at least 4 daily values for a week for the weekly mean to be non-missing. For missing NRS data at Week 12, the protocols and SAPs specified using the following multiple imputation (MI) approach:

- Intermittent missing NRS scores will first be imputed using the Markov Chain Monte Carlo (MCMC) method
- The monotone missing NRS values will then be imputed using the regression method.

The protocols and SAPs specified that MI will be performed within treatment groups with covariates for baseline NRS score, use of prior anti-itch medication, and history of select medical conditions. In addition, MI was specified to be performed separately for the interim analysis cohort and the post-interim analysis cohort. The missing data was specified to be imputed 20 times.
For the primary efficacy endpoint, the protocols and SAPs specified the following sensitivity analyses for the handling of missing data:

- Non-Responder Imputation (NRI) with MI: subjects who discontinue study drug early are imputed as non-responders (including subjects that discontinue study drug but continue to report NRS scores). Subjects that do not discontinue but have missing Week 12 data are imputed using MI as done in the primary analysis.
- Multiple Imputation – Missing Not at Random (MI-MNAR): pattern mixture model that draws from different population based on reason for withdrawal.
    - Intermittent missing NRS score are first be imputed using MCMC method
    - For subjects who discontinue due to adverse events, NRS score missing after discontinuation are imputed using the distribution of the baseline value of all subjects' daily worst itching score assuming a trimmed normal (from 4 to 10).
    - For subjects who discontinue due to reasons other than adverse event, missing NRS scores after subject discontinue are multiply imputed using data from subjects within the same treatment group that have complete data at that time.
- Tipping point analysis:
  "Multiple imputation with mixed missing data mechanisms (MNAR for a missing data pattern and MAR for others) will be used to assess the robustness of the MAR assumption. This sensitivity analysis is to investigate the departure from MAR assumption by progressively decreasing the treatment differences with respect to the NRS scores over the missing visits in active treatment group until conclusion from the primary analysis is overturned.
    - Uses MCMC methodology from PROC MI by treatment group to impute the intermittent missing data to a monotone missing pattern

- Uses a standard MAR-based MI approach from PROC MI to impute data from monotone missing data
- For patients in the active treatment group, shift parameter from PROC MI will be progressively applied to impute the missing data, until the p-value >0.05"

The SAPs specified imputing missing data for the continuous secondary efficacy endpoints using MI. Intermittent missing data will first be imputed using the MCMC method. The monotone missing data will then be imputed using the regression method. The SAPs specified that MI will be performed within treatment groups with covariates for baseline value, use of prior anti-itch medication, and history of select medical conditions. The missing data was specified to be imputed 20 times.

8.1.4. Subject Disposition, Demographics, and Baseline Disease Characteristics

Trial CLIN3102 enrolled and randomized a total of 378 subjects (189 to difelikefalin and 189 to placebo) from 56 centers in the United States. Trial CLIN3103 enrolled and randomized a total of 473 subjects (237 to difelikefalin and 236 to placebo) from 75 centers worldwide (i.e., Australia, Canada, Czech Republic, Germany, Great Britain, Hungary, South Korea, New Zealand, Poland, Taiwan, and United States). Table 4 presents the disposition of subjects for Trials CLIN3102 and CLIN3103. In both trials, the discontinuation rate was higher in the difelikefalin group compared to the placebo group.

Table 4: Disposition of Subjects (ITT[1])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin	**Placebo**	**Difelikefalin**	**Placebo**
Randomized Subjects	189	189	237	236
Treated Subjects	189	188	235	236
Discontinued, n (%)[2]	27 (14)	18 (10)	29 (12)	13 (6)
Adverse events	14 (7)	9 (5)	13 (6)	7 (3)
Eligibility	1 (1)	2 (1)	2 (1)	0
Lack of efficacy	0	0	1 (<1)	0
Lost to follow-up	0	0	1 (<1)	0
Noncompliance	1 (1)	1 (1)	1 (<1)	2 (1)
Withdrew consent	8 (4)	6 (3)	5 (2)	1 (<1)
Other	3 (2)	0	6 (3)	3 (1)

[1] Intent-to-Treat (ITT) population: all randomized subjects.
[2] The percentages were calculated based on the number of subjects that were randomized and treated.
Source: Statistical Reviewer's Analysis (same results as Applicant's Analysis); ADSL.xpt

The demographics and baseline disease characteristics for Trials CLIN3102 and CLIN3103 are presented in Table 5. The demographics were generally balanced across the treatment groups within each trial and were similar in terms of age and sex between the two trials. Trial CLIN3102 had a higher proportion of subjects identify as Black or African American compared to Trial CLIN3103. The baseline disease characteristics were generally balanced across the treatment groups within each trial and were similar between the two trials.

Table 5: Demographics and Baseline Disease Characteristics (ITT[1])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
Age (years)				
Mean (SD)	58 (11)	57 (14)	60 (13)	60 (13)
Median	59	57	61	60
Min, Max	22, 85	24, 88	23, 90	24, 85
Categories				
< 65	135 (71)	137 (72)	147 (62)	153 (65)
≥ 65	54 (29)	52 (28)	90 (38)	83 (35)
Sex, n (%)				
Male	112 (59)	119 (63)	137 (58)	139 (59)
Female	77 (41)	70 (37)	100 (42)	97 (41)
Race, n (%)				
White	91 (48)	93 (49)	164 (69)	169 (72)
Black or African American	82 (43)	76 (40)	53 (22)	38 (16)
Asian	6 (3)	7 (4)	12 (5)	20 (8)
Other	10 (5)	13 (7)	8 (3)	9 (4)
Weight (kg)				
Mean (SD)	86 (20)	85 (22)	81 (20)	80 (19)
Median	84	82	79	77
Min, Max	47, 135	42, 152	42, 130	44, 135
Region, n (%)				
United States	189 (100)	189 (100)	146 (62)	133 (56)
Eastern Europe	0	0	54 (23)	60 (25)
Western Europe	0	0	29 (12)	31 (13)
Asia	0	0	8 (3)	12 (5)
Years since diagnosis of ESRD				
Mean (SD)	4.7 (3.9)	5.6 (5.2)	5.2 (4.7)	5.5 (4.5)
Median	3.7	4.1	4.0	4.1
Min, Max	0.3, 26.5	0.3, 28.7	0.3, 30.2	0.3, 27.9
Years since diagnosis of CKD				
n	189	188	236	232
Mean (SD)	6.9 (5.9)	7.0 (5.7)	9.2 (7.6)	9.8 (7.0)
Median	5.5	5.3	7.2	7.8
Min, Max	0.5, 42.9	0.3, 28.9	0.3, 46.3	0.6, 48.3
Years on chronic hemodialysis				
Mean (SD)	4.4 (4.0)	4.7 (4.2)	4.8 (4.6)	5.1 (4.3)
Median	3.3	3.6	3.7	4.0
Min, Max	0.2, 26.5	0.0, 22.9	0.3, 30.2	0.3, 27.9
WI-NRS				
Mean (SD)	7.1 (1.4)	7.2 (1.6)	7.3 (1.4)	7.1 (1.4)
Median	7.0	7.4	7.1	7.0
Min, Max	4.2, 10.0	4.1, 10.0	4.5, 10.0	4.8, 10.0
Prior anti-itch medication use, n (%)				
Yes	72 (38)	78 (41)	87 (37)	85 (36)
No	117 (62)	111 (59)	150 (63)	151 (64)
Specific medical conditions, n (%)				
Yes	25 (13)	28 (15)	42 (18)	37 (16)
No	164 (87)	161 (85)	195 (82)	199 (84)

[1] Intent-to-Treat (ITT) population: all randomized subjects.
Source: Statistical Reviewer's Analysis (Applicant's tables included only treated subjects); ADSL.xpt

8.1.5. Results of the Primary Efficacy Endpoint

As noted in Section 8.1.2, the protocol-specified primary efficacy endpoint was the proportion of subjects achieving a ≥3-point improvement in WI-NRS score from baseline to Week 12; however, the Agency recommended in several communications the primary efficacy endpoint to be the proportion of subjects achieving a ≥4-point improvement in WI-NRS score from baseline to Week 12. The 4-point threshold at Week 12 was specified as a key secondary efficacy endpoint in both trials.

Table 6 presents the results for both a ≥3-point improvement and a ≥4-point improvement in WI-NRS from baseline to Week 12. In both trials, difelikefalin was statistically superior to placebo for both thresholds at Week 12 (p-values ≤ 0.020). The results based on the PP population (not shown) were similar to those based on the ITT population (i.e., Table 6). The results presented in Table 6 were adjusted for conducting the interim analysis. The results without adjusting for conducting the interim analysis (see Table 38 in Appendix 16.5) were similar to those presented in Table 6.

Table 6: Results for WI-NRS at Week 12 (ITT[1,2])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
≥3-point Improvement in WI-NRS				
Proportion	52%	31%	49%	38%
Difference (95% CI)	22% (12%, 32%)		11% (1%, 20%)	
Adjusted Proportion[3]	51%	28%	54%	42%
Odds Ratio (95% CI)[3]	2.7 (1.7, 4.3)		1.6 (1.1, 2.4)	
P-value[3]	<0.001		0.020	
≥4-point Improvement in WI-NRS				
Proportion	40%	21%	37%	26%
Difference (95% CI)	19% (9%, 28%)		12% (3%, 20%)	
Adjusted Proportion[3]	39%	18%	41%	28%
Odds Ratio (95% CI)[3]	2.9 (1.8, 4.8)		1.8 (1.1, 2.7)	
P-value[3]	<0.001		0.010	

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate and p-value using the Cui, Hung, and Wang (CHW) methodology.
[3] Adjusted proportion, odds ratio (95% CI), and p-value are based on a logistic regression with treatment, baseline NRS score, region (only Trial CLIN3103), prior use of anti-itch medication (yes/no), and presence of specific medical condition (yes/no) as factors in the model.
Source: Statistical Reviewer's Analysis (same results as Applicant's Analysis); ADEF.xpt

Table 7 presents the number of subjects with missing weekly mean WI-NRS score by week. As noted in Section 8.1.3, subjects must have reported at least 4 daily values for a week in order for the weekly mean score to be non-missing. In both trials, the proportion of subjects with missing Week 12 scores was higher in the difelikefalin group compared to the placebo group.

Table 7: Subjects with Missing Weekly Mean WI-NRS Score by Week (ITT[1])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
Week 1	6 (3%)	8 (4%)	6 (3%)	4 (2%)
Week 2	10 (5%)	11 (6%)	12 (5%)	4 (2%)
Week 3	11 (6%)	13 (7%)	16 (7%)	9 (4%)
Week 4	13 (7%)	11 (6%)	23 (10%)	11 (5%)
Week 5	17 (9%)	13 (7%)	21 (9%)	10 (4%)
Week 6	19 (10%)	13 (7%)	25 (11%)	12 (5%)
Week 7	23 (12%)	16 (8%)	24 (10%)	12 (5%)
Week 8	22 (12%)	16 (8%)	28 (12%)	15 (6%)
Week 9	24 (13%)	15 (8%)	30 (13%)	14 (6%)
Week 10	24 (13%)	16 (8%)	30 (13%)	16 (7%)
Week 11	32 (17%)	21 (11%)	35 (15%)	17 (7%)
Week 12	32 (17%)	24 (13%)	46 (19%)	29 (12%)

[1] Intent-to-Treat (ITT) population: all randomized subjects.
Source: Statistical Reviewer's Analysis (same results as Applicant's Analysis); ADWEEK.xpt

The results for a ≥3-point improvement and a ≥4-point improvement in WI-NRS from baseline to Week 12 by the various specified methods to handle missing data in both trials are presented in Table 8 and Table 9, respectively. The statistical reviewer included a pure non-responder imputation (NRI) approach for handling missing data (i.e., all missing data regardless of reason were imputed as non-responders). For both thresholds, the results were generally similar across the various methods. For the primary efficacy endpoint, the Applicant also conducted a tipping point analysis that investigated the departure from the missing-at-random assumption by progressively increasing the size of the missing value in the difelikefalin group by 0.25-point increments until the conclusion are overturn (i.e., p-value > 0.05). For Trial CLIN3102, difelikefalin remained statistically superior to placebo for the largest shift level (i.e., 6.5 points) used by the Applicant. For Trial CLIN3103, difelikefalin was not statistically superior to placebo when the shift value was ≥ 1.

Table 8: Results for ≥3-point Improvement in WI-NRS at Week 12 by Various Methods to Handle Missing Data (ITT[1,2])

	Difelikefalin (N=189)	Placebo (N=189)	Difference (95% CI)
Trial CLIN3102			
MI (Primary)	52%	31%	22% (12%, 32%)
NRI with MI[3]	44%	27%	17% (8%, 27%)
NRI	43%	27%	17% (7%, 26%)
MI-MNAR	48%	30%	18% (8%, 28%)
Trial CLIN3103			
MI (Primary)	49%	38%	11% (1%, 20%)
NRI with MI[3]	41%	35%	6% (-3%, 14%)
NRI	40%	33%	8% (-1%, 16%)
MI-MNAR	46%	37%	10% (1%, 19%)

[1] Intent-to-Treat (ITT) population: all randomized subjects.
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate and p-value using the Cui, Hung, and Wang (CHW) methodology.
[3] Subjects who discontinue study drug early are imputed as non-responders (including subjects that discontinue study drug but continue to report NRS scores). Subjects that do not discontinue but have missing Week 12 data are imputed using MI as done in the primary analysis.
Source: Statistical Reviewer's Analysis; ADEF.xpt, ADEFNR.xpt, ADWEEK.xpt, ADEFMNAR.xpt

Table 9: Results for ≥4-point Improvement in WI-NRS at Week 12 by Various Methods to Handle Missing Data (ITT[1,2])

	Difelikefalin (N=189)	Placebo (N=189)	Difference (95% CI)
Trial CLIN3102			
MI (Primary)	40%	21%	19% (9%, 28%)
NRI with MI[3]	34%	19%	16% (7%, 24%)
NRI	34%	19%	15% (6%, 24%)
MI-MNAR	37%	20%	16% (7%, 25%)
Trial CLIN3103			
MI (Primary)	37%	26%	12% (3%, 20%)
NRI with MI[3]	31%	24%	7% (-1%, 16%)
NRI	31%	22%	9% (1%, 17%)
MI-MNAR	35%	25%	10% (2%, 19%)

[1] Intent-to-Treat (ITT) population: all randomized subjects.
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate and p-value using the Cui, Hung, and Wang (CHW) methodology.
[3] Subjects who discontinue study drug early are imputed as non-responders (including subjects that discontinue study drug but continue to report NRS scores). Subjects that do not discontinue but have missing Week 12 data are imputed using MI as done in the primary analysis.
Source: Statistical Reviewer's Analysis; ADEF.xpt, ADEFNR.xpt, ADWEEK.xpt, ADEFMNAR.xpt

8.1.6. Results of the Secondary Efficacy Endpoints

Table 10 presents the results for a ≥3-point improvement and a ≥4-point improvement in WI-NRS from baseline to Weeks 4 and 8. As noted in Section 8.1.2, only Trial CLIN3103 specified these endpoints as secondary efficacy endpoints. In Trial CLIN3103, difelikefalin was statistically superior to placebo for both thresholds at Weeks 4 and 8 (p-values ≤ 0.036).

Table 10: Results for WI-NRS at Weeks 4 and 8 (ITT[1,2])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
≥3-point Improvement in WI-NRS at Week 8				
Proportion	44%	27%	45%	34%
Difference (95% CI)	16% (6%, 26%)		11% (2%, 20%)	
Adjusted Proportion[3]	43%	25%	49%	36%
Odds Ratio (95% CI)[3]	2.2 (1.4, 3.6)		1.7 (1.1, 2.5)	
P-value[3]	Not Specified		0.010	
≥3-point Improvement in WI-NRS at Week 4				
Proportion	34%	18%	36%	22%
Difference (95% CI)	15% (6%, 24%)		14% (5%, 22%)	
Adjusted Proportion[3]	33%	17%	38%	24%
Odds Ratio (95% CI)[3]	2.5 (1.5, 4.2)		2.0 (1.3, 3.1)	
P-value[3]	Not Specified		0.002	
≥4-point Improvement in WI-NRS at Week 8				
Proportion	31%	19%	31%	21%

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
Difference (95% CI)	12% (3%, 21%)		10% (2%, 19%)	
Adjusted Proportion[3]	27%	15%	36%	24%
Odds Ratio (95% CI)[3]	2.1 (1.3, 3.5)		1.8 (1.2, 2.9)	
P-value[3]	Not Specified		0.010	
≥4-point Improvement in WI-NRS at Week 4				
Proportion	19%	9%	21%	13%
Difference (95% CI)	10% (3%, 16%)		7% (0%, 14%)	
Adjusted Proportion[3]	16%	7%	26%	17%
Odds Ratio (95% CI)[3]	2.8 (1.4, 5.4)		1.8 (1.0, 3.0)	
P-value[3]	Not Specified		0.036	

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate and p-value using the Cui, Hung, and Wang (CHW) methodology.
[3] Adjusted proportion, odds ratio (95% CI), and p-value are based on a logistic regression with treatment, baseline NRS score, region (only Trial CLIN3103), prior use of anti-itch medication (yes/no), and presence of specific medical condition (yes/no) as factors in the model.
Source: Statistical Reviewer's Analysis (same results as Applicant's Analysis); ADEF.xpt

Table 11 presents the results for the change from baseline in 5-D itch total score at Week 12 and the change from baseline in Skindex-10 total score at Week 12. In Trial CLIN3102, difelikefalin was statistically superior to placebo (p-values < 0.001). In Trial CLIN3103, difelikefalin was not statistically superior to placebo for Skindex-10 total score at Week 12 (p-value = 0.171). In addition, statistical superiority cannot be claimed for 5-D itch total score at Week 12 per the multiplicity testing procedure (i.e., sequential gatekeeping approach [see Section 8.1.3]).

Table 11: Results for 5-D Itch Total Score and Skindex-10 Total Score at Week 12 (ITT[1])

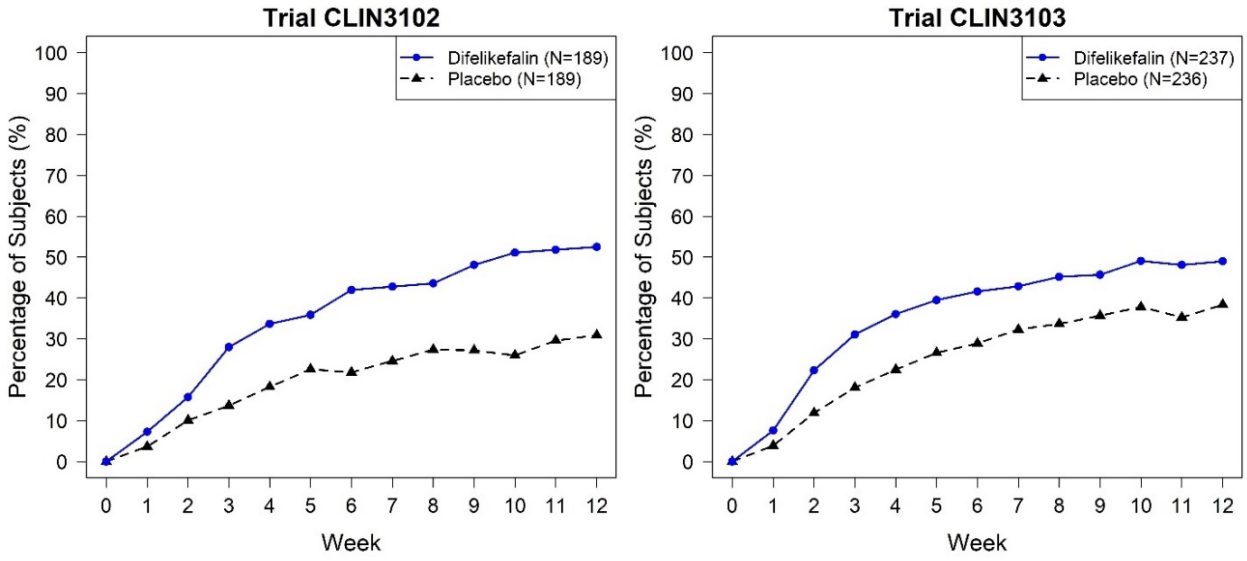
	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
Change from Baseline in 5-D Itch Total Score at Week 12				
Mean[2]	-4.7	-4.1	-4.7	-3.4
LS Mean[3]	-5.0	-3.7	-4.9	-3.8
Difference (95% CI)[3]	-1.3 (-2.0, -0.5)		-1.1 (-1.7, -0.4)	
P-value[3]	<0.001		0.002 (Not Significant[4])	
Change from baseline in Skindex-10 Total Score at Week 12				
Mean[2]	-17.2	-13.0	-15.2	-13.0
LS Mean[3]	-17.2	-12.0	-16.6	-14.8
Difference (95% CI)[3]	-5.1 (-8.0, -2.3)		-1.8 (-4.3, 0.8)	
P-value[3]	<0.001		0.171	

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Average over the 20 imputed datasets.
[3] LS mean, difference (95% CI), and p-value are based on ANCOVA with treatment, baseline score, region (only Trial CLIN3103), prior use of anti-itch medication (yes/no), and presence of specific medical condition (yes/no) as factors in the model.
[4] The SAP specified a sequential gatekeeping approach to control the Type I error rate. For Trial CLIN3103, the SAP specified testing change from baseline in Skindex-10 total score at Week 12 before testing change from baseline in 5-D total score at Week 12.
Source: Statistical Reviewer's Analysis (same results as Applicant's Analysis); ADFIVDMI.xpt, ADSKINMI.xpt

8.1.7. Efficacy Over Time

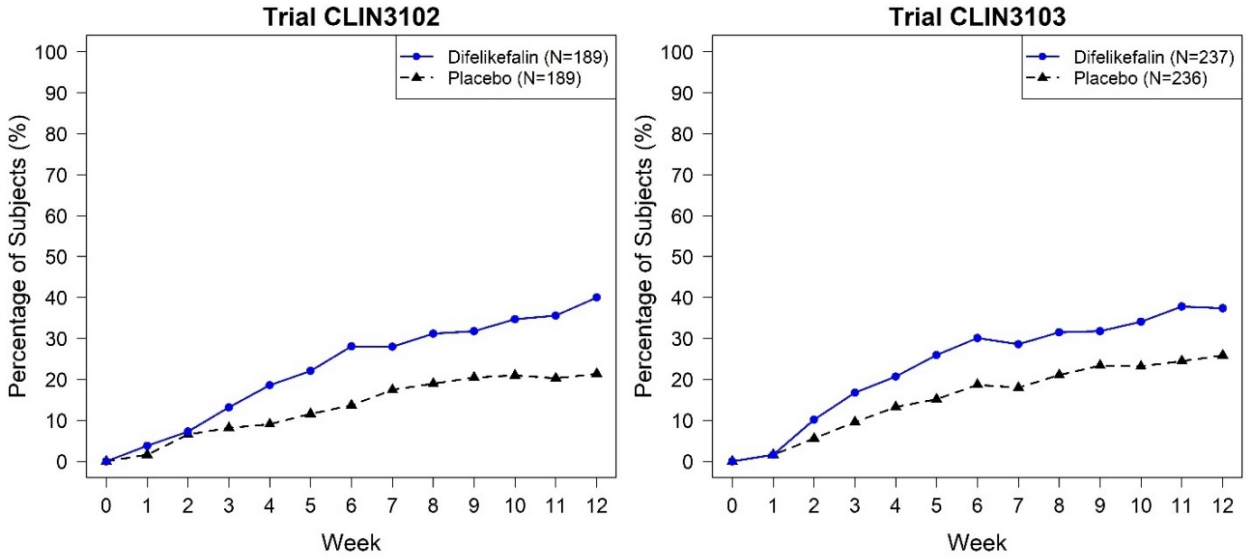
Figure 7 presents the results for the proportion of subjects that achieve a ≥3-point improvement in WI-NRS from baseline by week. Figure 8 presents the results for the proportion of subjects that achieve a ≥4-point improvement in WI-NRS from baseline by week.

Figure 7: Results for ≥3-point Improvement in WI-NRS from Baseline by Week (ITT[1,2])



[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate using the Cui, Hung, and Wang (CHW) methodology.
Source: Statistical Reviewer's Analysis; ADEF.xpt

Figure 8: Results for ≥4-point Improvement in WI-NRS from Baseline by Week (ITT[1,2])



[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).

[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate using the Cui, Hung, and Wang (CHW) methodology.
Source: Statistical Reviewer's Analysis; ADEF.xpt

8.1.8. Findings in Special/Subgroup Populations

The results for a ≥3-point improvement in WI-NRS from baseline at Week 12 by age (< 65 and ≥ 65), sex, race (white, black or African American, and other), prior anti-itch medication use, specific medical conditions, and country (United States and outside United States) for Trial CLIN3102 and CLIN3103 are presented in Table 12 and Table 13, respectively. The results for a ≥4-point improvement in WI-NRS from baseline at Week 12 by age (< 65 and ≥ 65), sex, race (white, black or African American, and other), prior anti-itch medication use, specific medical conditions, and country (United States and outside United States) for Trial CLIN3102 and CLIN3103 are presented in Table 14 and Table 15, respectively. The sample size in the subgroup that did not identify as White or Black/African American was small in both trials; therefore, it would be difficult to detect any differences in efficacy between this subgroup and its complements. For both thresholds, treatment effects were generally consistent across subgroups.

Table 12: Results for ≥3-point Improvement in WI-NRS at Week 12 by Age, Sex, Race, Prior Anti-itch Medication Use, Specific Medication Condition, and Country – Trial CLIN3102 (ITT[1,2])

	Difelikefalin (N=189)	Placebo (N=189)	Difference (95% CI)
Age			
< 65 (135, 137)	57%	29%	27% (16%, 39%)
≥ 65 (54, 52)	42%	33%	9% (-10%, 27%)
Sex			
Male (112, 119)	52%	27%	25% (12%, 37%)
Female (77, 70)	54%	38%	16% (0%, 33%)
Race			
White (91, 93)	52%	30%	22% (8%, 36%)
Black or African American (82, 76)	58%	32%	25% (10%, 40%)
Other (16, 20)	29%	34%	-5% (-44%, 33%)
Prior anti-itch medication use			
Yes (72, 78)	52%	30%	23% (7%, 39%)
No (117, 111)	52%	32%	20% (8%, 33%)
Specific medical conditions			
Yes (25, 28)	62%	18%	43% (20%, 67%)
No (164, 161)	51%	33%	17% (7%, 28%)
Country			
United States (189, 189)	52%	31%	22% (12%, 32%)
Outside United States (0, 0)	-	-	-

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate using the Cui, Hung, and Wang (CHW) methodology.
Source: Statistical Reviewer's Analysis; ADEF.xpt

Table 13: Results for ≥3-point Improvement in WI-NRS at Week 12 by Age, Sex, Race, Prior Anti-itch Medication Use, Specific Medication Condition, and Country – Trial CLIN3103 (ITT[1,2])

	Difelikefalin (N=237)	Placebo (N=236)	Difference (95% CI)
Age			
< 65 (147, 153)	50%	39%	12% (0%, 23%)
≥ 65 (90, 83)	47%	38%	9% (-6%, 25%)
Sex			
Male (137, 139)	43%	40%	3% (-8%, 13%)
Female (100, 97)	58%	38%	20% (6%, 34%)
Race			
White (164, 169)	49%	37%	12% (1%, 23%)
Black or African American (53, 38)	54%	34%	20% (-2%, 42%)
Other (20, 29)	40%	48%	-7% (-27%, 13%)
Prior anti-itch medication use			
Yes (87, 85)	41%	26%	15% (0%, 30%)
No (150, 151)	39%	32%	7% (-4%, 17%)
Specific medical conditions			
Yes (42, 37)	54%	41%	13% (-10%, 36%)
No (198, 199)	48%	38%	10% (0%, 20%)
Country			
United States (146, 133)	43%	36%	7% (-6%, 20%)
Outside United States (91, 103)	59%	40%	19% (4%, 33%)

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate using the Cui, Hung, and Wang (CHW) methodology.
Source: Statistical Reviewer's Analysis; ADEF.xpt

Table 14: Results for ≥4-point Improvement in WI-NRS at Week 12 by Age, Sex, Race, Prior Anti-itch Medication Use, Specific Medication Condition, and Country – Trial CLIN3102 (ITT[1,2])

	Difelikefalin (N=189)	Placebo (N=189)	Difference (95% CI)
Age			
< 65 (135, 137)	41%	19%	22% (12%, 33%)
≥ 65 (54, 52)	36%	26%	10% (-8%, 28%)
Sex			
Male (112, 119)	40%	19%	21% (9%, 33%)
Female (77, 70)	39%	25%	14% (-1%, 29%)
Race			
White (91, 93)	41%	20%	21% (8%, 34%)
Black or African American (82, 76)	43%	22%	21% (6%, 36%)
Other (16, 20)	22%	26%	-4% (-22%, 13%)
Prior anti-itch medication use			
Yes (72, 78)	40%	21%	19% (4%, 34%)
No (117, 111)	41%	22%	19% (7%, 30%)
Specific medical conditions			
Yes (25, 28)	52%	11%	41% (18%, 65%)
No (164, 161)	38%	23%	15% (5%, 25%)
Country			
United States (189, 189)	40%	21%	19% (9%, 28%)
Outside United States (0, 0)	-	-	-

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate using the Cui, Hung, and Wang (CHW) methodology.
Source: Statistical Reviewer's Analysis; ADEF.xpt

Table 15: Results for ≥4-point Improvement in WI-NRS at Week 12 by Age, Sex, Race, Prior Anti-itch Medication Use, Specific Medication Condition, and Country – Trial CLIN3103 (ITT[1,2])

	Difelikefalin (N=237)	Placebo (N=236)	Difference (95% CI)
Age			
< 65 (147, 153)	38%	27%	11% (0%, 22%)
≥ 65 (90, 83)	37%	24%	12% (-2%, 26%)
Sex			
Male (137, 139)	28%	24%	4% (-7%, 15%)
Female (100, 97)	50%	29%	21% (7%, 35%)
Race			
White (164, 169)	39%	26%	12% (2%, 23%)
Black or African American (53, 38)	40%	27%	12% (-9%, 34%)
Other (20, 29)	23%	21%	2% (-44%, 48%)
Prior anti-itch medication use			
Yes (87, 85)	42%	22%	20% (6%, 34%)
No (150, 151)	35%	29%	6% (-5%, 17%)
Specific medical conditions			
Yes (42, 37)	39%	33%	6% (-20%, 32%)
No (198, 199)	37%	25%	13% (3%, 22%)
Country			
United States (146, 133)	33%	23%	9% (-2%, 21%)
Outside United States (91, 103)	44%	29%	15% (2%, 29%)

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate using the Cui, Hung, and Wang (CHW) methodology.
Source: Statistical Reviewer's Analysis; ADEF.xpt

8.2. Review of Safety

8.2.1. Safety Review Approach

Reference to section 7.2 (REVIEW STRATEGY) for a complete discussion of the safety review approach and the safety pooling strategy. Analyses for each pooling group were completed using the Safety Population, which in each case consisted of all subjects who received at least 1 dose of any study drug (difelikefalin, placebo, or other control if applicable). Subjects were analyzed according to the actual treatment received. The focus of the safety review will be for the Primary Safety Pool (Phase 3, placebo-controlled studies in subjects with CKD-aP undergoing HD) and the Difelikefalin Exposure Safety Pool (double-blind studies + OL extensions and the open-label long-term studies), only subjects who received at least 1 dose of difelikefalin in the phase 3 studies were included.

8.2.2. Review of the Safety Database

Table 16: Relevant Safety Pooling for Review

Pooling Group	Studies Included	Purpose
Primary Safety Pool	CR845-CIN3102 DB CR845-CLIN3103 DB	Primary placebo-controlled pool in hemodialysis population with difelikefalin 0.5 mcg/kg IV 3 times a week
Difelikefalin Exposure Safety Pool (Phase 3 PC and Open-labeled studies in subject with CKD-aP undergoing HD)	CR845-CLIN3101 CR845-CIN3102 DB + OLE CR845-CLIN3103 DB +OLE CR845-CLIN3105	Includes safety data collected during exposure to difelikefalin 0.5 mcg/kg IV in the double-blind or open-label phase of the PC Phase 3 studies, or in the open-label phase of the Phase 3 studies

Source: Module 5.3.5.3 Integrated Summary of Safety
DB= Double-blind, PC= placebo-controlled, OLE= open-label extension, CKD-aP= chronic kidney disease associated pruritus
HD= hemodialysis

Overall Exposure

Overall, the ISS All Studies Pool was composed of 1879 subjects who received at least 1 dose of difelikefalin, 1592 were undergoing HD, and 287 not undergoing HD. Of the 1454 subjects who received the 0.5 mcg/kg dose, 1400 were undergoing HD and 54 were not.

Table 17: Safety Pool Population

Safety Database for the Study Drug[1] Individuals exposed to Difelikefalin in this development program for Uremic Pruritus in Hemodialysis Patients N=1879

Clinical Trial Pooled Safety Groups	**Difelikefalin 0.5 mcg/kg IV**	**Placebo**	**Overall**
ALL Studies Pool [4]	1454	714	1879
Primary Safety Pool[2]	424	424	848
Secondary Safety Pool[3]	496	495	991
Difelikefalin Exposure Safety Pool	1306	NA	1306
Phase 1 Safety Pool	95	187	360

[1] *study drug* means the drug being considered for approval.
[2] includes studies CR845-CLIN3102DB, CR845-CLIN3103DB
[3] if placebo arm patients switch to study drug in open label extension, the n should include their number; do not count twice patients who go into extension from randomized study drug arm
[4] include all studies from Phase 1 to Phase 3 and open-label studies identified in Section 7.2 of this review

In this review, the focus will be on subjects who received the to-be-marketed dose (0.5 mcg/kg) and are on hemodialysis. The primary safety pool was composed of 424 subjects who received at least 1 dose of 0.5 mcg/kg difelikefalin and 424 subjects who received ≥ 1 dose of placebo. Overall, 86.8% of subjects treated with difelikefalin and 92.7% of subjects treated with placebo completed study treatment.

Subjects who discontinued treatment early was 13.2% for difelikefalin and 7.3% for placebo. The most common reason for early discontinuation was AE (6.4% and 3.8% of subjects treated with difelikefalin and placebo, respectively). Other reasons for discontinuation that were reported in at least 2% of subjects in either treatment group were withdrawal by subject (3.1% and 1.7% for difelikefalin and placebo, respectively) and other (2.1% and 0.7%, respectively).

The Safety Population of the Difelikefalin Exposure Safety Pool was composed of 1306 subjects, 26.4% of whom (345 subjects) discontinued treatment early. The most common reason for discontinuation was AE (122 subjects, 9.3%). Other reasons for discontinuation that were reported by more than 2% of subjects included other (89 subjects, 6.8%), withdrawal by subject (66 subjects, 5.1%), and administrative (49 subjects, 3.8%). In addition to the subjects who discontinued from the study early, 407 subjects (31.2%) could not complete treatment due to the sponsor's decision to stop the study for reasons unrelated to safety or lack of drug effect.

The high early discontinuation rates are noted for the safety population. In addition to the adverse events as noted for dropouts, the population of hemodialysis patients are heavily burdened with prior medical conditions which is an issue for tolerability to additional medications. In all, a sufficient number of subjects enrolled and completed the studies to allow for safety analyses.

Adequacy of the safety database:

The safety database, for the intended treatment of hemodialysis patients, appear adequate to evaluate safety of this drug product.

In the Primary Safety Pool, including 424 pooled difelikefalin and 424 pooled placebo subjects who received ≥1 dose of their assigned treatment, with a median duration of treatment (and duration of cumulative exposure) of 85.0 days for both treatment groups (range of 3 to 93 days for subjects treated with difelikefalin and range of 3 to 94 days for subjects treated with placebo). The majority of subjects (79.7% and 85.1% of 0.5 mcg/kg difelikefalin and placebo subjects, respectively) had a treatment duration of ≥3 months.

In the Difelikefalin Exposure Safety Pool, duration of treatment for subjects who participated in CR845-CLIN2005 (Part B) or CR845-CLIN2101 and then went on to participate in CR845-CLIN3101 was based on only the subject's duration of treatment in CR845-CLIN3101, since a primary objective of this pool was to evaluate the long-term safety of difelikefalin. All 1306 subjects in the Difelikefalin Exposure Safety Pool received ≥1 dose of study treatment. The median duration of continuous exposure was 6.9 months (range 0 to 17 months). The majority of subjects (1089 subjects, 83.4%) had a continuous exposure duration of ≥3 months, and 711 subjects (54.4%), 533 subjects (40.8%), and 400 subjects (30.6%) had continuous exposure durations of ≥6, ≥9, and ≥12 months, respectively. The median duration of cumulative exposure was 6.7 months (range 0 to 16 months).

8.2.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

There were no issues regarding the integrity of the submitted data for safety.

Categorization of Adverse Events

Adverse events were coded using MedDRA *version 22.0*. SAEs were defined as described in *ICH Harmonised Tripartitie Guideline Clinical Safety Data Management: Definitions and Standards for Expedited Reporting E2A*.

Routine Clinical Tests

Clinical laboratory testing was done in the clinical studies including:

- Serum chemistry: albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, phosphorus, potassium, and sodium
- Hematology: basophils (percentage and absolute count), eosinophils (percentage and absolute count), hematocrit, hemoglobin, lymphocytes (percentage and absolute count), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, monocytes (percentage and absolute count), neutrophils (percentage and absolute count), platelet count, red cell distribution width, red blood cell count, white blood cell count

The full evaluation of laboratory results is presented in Section 8.2.4, Safety Results.

8.2.4. Safety Results

Deaths

Primary Safety Pool

In the primary safety pool, 3 subjects (0.7%) in the pooled difelikefalin group and 5 subjects (1.2%) in the pooled placebo group died as a result of a TEAE; the incidence rates for fatal TEAEs were 30.6 and 49.5 events per 1000PY, respectively.

Table 18: Incidence of TEAE Leading to Death by SOC and PT – Primary Safety Pool

	CLIN3102 DB		CLIN3103DB		Pooled	
	Placebo (N = 188) n (%)	Difelikefalin 0.5 mcg/kg (N = 189) n (%)	Placebo (N = 236) n (%)	Difelikefalin 0.5 mcg/kg (N = 235) n (%)	Placebo (N = 424) n (%) IR	Difelikefalin 0.5 mcg/kg (N = 424) n (%) IR
System Organ Class Preferred Term						
Subjects with any event	3 (1.6%)	1 (0.5%)	2 (0.8%)	2 (0.9%)	5 (1.2%) 49.5	3 (0.7%) 30.6
Blood and lymphatic system disorders	0	0	0	1 (0.4%)	0	1 (0.2%)
Anemia	0	0	0	1 (0.4%)	0	1 (0.2%)
Cardiac disorders	0	0	1 (0.4%)	2 (0.9%)	1 (0.2%)	2 (0.5%) 20.4
Cardiac arrest	0	0	1 (0.4%)	1 (0.4%)	1 (0.2%)	1 (0.2%)
Cardiac failure	0	0	1 (0.4%)	1 (0.4%)	1 (0.2%)	1 (0.2%)
General disorders and admin site conditions	1 (0.5%)	0	0	0	1 (0.2%)	0
Death	1 (0.5%)	0	0	0	1 (0.2%)	0
Infections and infestation	2 (1.1%)	1 (0.5%)	1 (0.4%)	0	3 (0.7%) 29.7	1 (0.2%) 10.2
Staphylococcal sepsis	0	1 (0.5%)	0	0	0	1 (0.2%)
Sepsis	0	0	1 (0.4%)	0	1 (0.2%)	0
Septic shock	2 (1.1%)	0	0	0	2 (0.5%) 19.8	0

Source: Module 2.6.1 Integrated Summary of Safety; Table 9: 45

DB= double-blind
Incidence rate is calculated as 1000 times the number of events (n) divided by the total person-years. Person-years is the sum across all subjects in the column of the individual subject risk times: the number of days from their first dose to the last day of the period when an event would be deemed to be treatment-emergent.
Note: One additional subject who participated in CR845-CLIN3102 DB, Subject [redacted] on CR845, experienced an adverse event leading to death. This death is not included in this summary table because it was not treatment emergent, having occurred outside the treatment-emergent definition timeframe for the Integrated Summary of Safety. In addition, Subject [redacted] on placebo had an event leading to death during the Discontinuation Period of CR845-CLIN3102 DB; this subject is included in deaths in this summary table because the event met the treatment-emergent definition timeframe for the Integrated Summary of Safety.

Preferred terms of the three fatal TEAEs in the pooled difelikefalin group included anemia due to gastrointestinal bleeding leading to cardiac failure, cardiac arrest, and staphylococcal sepsis complicated by cerebral infarction (0.2% [1 subject] each). The five fatal TEAEs reported in the placebo group were septic shock (0.5% [2 subjects]), cardiac arrest following myocardial infarction, death of unknown cause, and sepsis with cardiac failure (0.2% [1 subject] each). All fatal TEAEs were considered not related to study drug by the investigator.

Subjects on Difelikefalin With a TEAE Leading to Death, N=3

CR845-CLIN3103 Double-blind Phase Subject [redacted], 75-year-old white female, received the first dose of difelikefalin on [redacted]. The subject's relevant medical history included hypertension, atrial fibrillation, and anemia of chronic pulmonary disease. On [redacted] (Study Day 37), the subject experienced the serious TEAE of anemia, manifested by a hemoglobin of 83 g/L (reference range 120 to 155 g/L) and hematocrit of 0.25 (reference range 0.36 to 0.49) and resulting in the subject's hospitalization. The cause of anemia was later identified as gastrointestinal bleeding. On [redacted] (Study Day 50), the subject experienced the nonserious TEAE of cardiopulmonary failure, which led to study drug discontinuation, and the serious TEAE of cardiac failure, which resulted in death on the same day. Dosing with study drug was not changed due to the events of anemia and cardiac failure. The subject received a total of 22 doses of difelikefalin prior to death on [redacted]. The investigator considered the events of anemia and cardiac failure to be unrelated to study drug.

CR845-CLIN3103 Double-blind Phase Subject [redacted], a 58-year-old white male, received the first dose of difelikefalin on [redacted] The subject's relevant medical history included diabetes, hypertension, arrhythmia, hyperlipidemia, and hypoalbuminemia. On [redacted] (Study Day 52), 4 days after the most recent and final dose of study drug, the subject experienced the serious TEAE of cardiac arrest at home, which resulted in death on the same day, no further information was provided. The subject received a total of 21 doses of difelikefalin during participation in the study. The investigator considered the serious TEAE of cardiac arrest and subsequent death as not related to study drug.

CR845-CLIN3102 Double-blind Phase Subject [redacted], a 52-year-old black or African American male, received the first dose of difelikefalin on [redacted]. The subject's relevant medical history included diabetes, peripheral vascular disease and pulmonary embolism. On [redacted] (Study Day 28), 2 days after receiving the most recent dose of study drug on [redacted] the

subject presented to the emergency room and was subsequently admitted to hospital with weakness, inability to walk, and diarrhea. Study drug was interrupted due to the asthenia and gait inability. Stool and blood cultures were positive for Clostridium difficile (toxin A and B) and Staphylococcus aureus, respectively. The subject was treated with antibiotic (oral vancomycin), dexamethasone, and acetaminophen/hydrocodone and received hemodialysis treatment. Subsequently the subject developed septic shock and was transferred to the intensive care unit. Despite treatment, the sepsis progressed and the subject had a cerebral infarction due to embolism of bilateral cerebellar arteries. He was transferred to another hospital for additional treatment and further testing. The subject was reported to have died on (b) (6) The SAE of staphylococcal sepsis (due to MRSA) was reported as having a fatal outcome. The subject received a total of 12 doses of difelikefalin during participation in the study. The investigator assessed the fatal SAE of staphylococcal sepsis as not related to study drug.

Subjects on Placebo With a TEAE Leading to Death, N=5

CR845-CLIN3102 Double-blind Phase Subject (b) (6), a 40-year-old white male, received the first dose of placebo on (b) (6). Relevant medical history included hypertension, congestive heart failure, anemia of CKD, and malnutrition. On (b) (6) (Study Day 46), the subject suffered complications from an elective colectomy (for sessile polyps) that included the nonserious TEAEs of gastric outlet obstruction leading to discontinuation of study drug and pneumonia and the serious TEAE of septic shock considered secondary to "intra-abdominal pathology." On (b) (6) the subject was transferred to the intensive care unit for a gastrointestinal bleed. The subject died on (b) (6) due to septic shock. The subject received a total of 19 doses of placebo during participation in the study. The investigator considered the serious TEAE of septic shock and subsequent death to be not related to study drug.

CR845-CLIN3102 Double-blind Phase Subject (b) (6) a 64-year-old black or African American male, received the first dose of placebo on (b) (6). Relevant medical history included coronary artery disease, diabetes, hypertension, myocardial infarction, chronic obstructive pulmonary disease, and atrial fibrillation. On (b) (6) (Study Day 90), the subject experienced the serious TEAE of septic shock, which was graded as severe and required hospitalization. This event was preceded by the following serious TEAEs: dehydration, electrolyte imbalance, hypoglycemia, metabolic encephalopathy, and azotemia (b) (6) [Study Day 77] through (b) (6) [Study Day 82]); diabetes mellitus inadequate control (b) (6) [Study Day 86] through day of death on (b) (6) [Study Day 90]); and abdominal pain and diarrhea (b) (6) [Study Day 87] through day of death on (b) (6) [Study Day 90]). Study drug was withdrawn as a result of the event of septic shock. The subject died on (b) (6) due to septic shock. The subject received a total of 34 doses of placebo during participation in the study. The investigator considered the serious TEAE of septic shock and subsequent death to be not related to study drug.

CR845-CLIN3102 Double-blind Phase Subject (b) (6), a 59-year-old white female who received

a total of 36 doses of placebo during study participation, received the first dose of placebo on (b) (6). Relevant medical history included diabetes, hypertension, and hypocalcemia. It was reported that on (b) (6) (Study Day 93) the subject had laboratory testing in preparation for early termination of study that showed critically high blood glucose (527; reference range 60-115), critically low hemoglobin (6.7; 12-16), and alkaline phosphatase 937 (37-116) (units not provided). An attempt by the site to locate the subject for immediate medical attention for the critical laboratory results was unsuccessful. On (b) (6), the family notified the study site that the subject had died on (b) (6) (Study Day 93; during the Discontinuation Period). The death was assessed by the investigator as not related to study drug. The subject did not experience any additional TEAEs during the study.

CR845-CLIN3103 Double-blind Phase Subject (b) (6), a 71-year-old white male, received the first dose of placebo on (b) (6). Relevant medical history included hypertension, myocardial infarction, diastolic heart failure, anemia, and atrial fibrillation. On (b) (6) (Study Day 71), the subject experienced the serious TEAE of dyspnea, which was graded as severe and resulted in hospitalization. The event of dyspnea resolved as of (b) (6). On (b) (6) (Study Day 72), the subject experienced the serious TEAEs of hypotension and sepsis, which were graded as severe. The subject died due to hypotension and cardiac failure on (b) (6); the primary cause of death was reported as sepsis. The subject received a total of 31 doses of placebo during participation in the study. The investigator assessed the serious adverse events of dyspnea, hypotension, and sepsis as not related to study drug, attributable to multiple underlying morbidities.

CR845-CLIN3103 Double-blind Phase Subject (b) (6), a 60-year-old white, Hispanic or Latino male, received the first dose of placebo on (b) (6). The subject's relevant medical history included diabetes, hypertension, 2 prior myocardial infarctions, and cardiac failure. On (b) (6) the subject experienced the serious TEAE of cardiac arrest, which was graded as severe. On (b) (6) (Study day 21), the subject died due to the event of cardiac arrest. Cardiac arrest and death were attributed to myocardial infarction. The subject received a total of 9 doses of placebo during participation in the study. The investigator considered the serious TEAE of cardiac arrest and subsequent death as not related to study drug.

In the Difelikefalin Exposure Safety Pool, 56 subjects (4.3%) treated with difelikefalin, including long-term open-label treatment, experienced a TEAE leading to death. None of the fatal TEAEs were considered related to study drug by the investigator. The incidence rate for fatal TEAEs in the Difelikefalin Exposure Safety Pool was 69.0 events per 1000 PY.

The most common system organ class of TEAEs leading to death in the pooled difelikefalin group was cardiac disorders (2.0%), with other system organ classes reported in less than 1% of subjects. The most common (≥0.3% of subjects) preferred terms of fatal TEAEs in the pooled difelikefalin group were cardiac arrest (0.8%), death (0.5%), cardiac failure congestive (0.3%), myocardial infarction (0.3%), and sepsis (0.3%). Additional preferred terms synonymous with cardiac arrest (cardiorespiratory arrest [0.2%]), cardiac failure congestive (cardiac failure

[0.2%]), and myocardial infarction (acute myocardial infarction [<0.1%]) were reported. The incidence rate for fatal TEAEs classified as cardiac disorders in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool was 32.0 events per 1000 PY, and the rate for fatal TEAEs classified as vascular disorders was 3.7 events per 1000 PY.

The incidence rates for the most common (≥0.3% of subjects) preferred terms of fatal TEAEs in the Difelikefalin Exposure Safety Pool were as follows: cardiac arrest, with low events in the trials (13.6 events per 1000 PY) as compared to 53.5 events per 1000 PY described by the USRDS database. In addition, the Difelikefalin Exposure Safety Pool also described death (7.4 events per 1000 PY), cardiac failure congestive, myocardial infarction, and sepsis (4.9 events per 1000 PY each). Although the population studied in the difelikefalin clinical trials may not fully compare to the US ESRD database (USRDS 2019 report[13]), we can use it as a source of descriptive epidemiology of ESRD patients covering areas such as incidence, prevalence, modality of renal replacement and treatment history, along with deaths, and mortality issues. The rates we've reviewed in the difelikefalin submission appear to align with those expected in the US patient population undergoing HD.

Nonfatal Serious Treatment Emergent Adverse Events (TEAE)

Serious TEAE in the Primary Safety Pool

In the Primary Safety Pool, 107 subjects (25.2%) in the pooled difelikefalin group reported at least 1 nonfatal serious TEAE with the incidence being similar (less than 1.5x difference) to that in the pooled placebo group (96 subjects [22.6%]). The most common (≥4% of subjects) system organ classes of nonfatal serious TEAEs in subjects treated with difelikefalin were infections and infestations (8.3% for difelikefalin and 7.1% for placebo); cardiac disorders (4.5% and 1.9%, respectively); and respiratory, thoracic, and mediastinal disorders (4.5% and 3.8%). Events classified as cardiac disorders were reported more frequently (by a factor of 1.5 or more) in the pooled difelikefalin group than in the pooled placebo group and had an incidence rate of 244.8 events per 1000 PY (128.6 events per 1000 PY for placebo).

Table 19: Incidence of Nonfatal Serious TEAE by SOC and PT for PT with ≥ 2 subjects in the Primary Safety Population

	CLIN3102 DB		CLIN3103 DB		Pooled	
	Placebo (N = 188) n (%)	Difelikefalin 0.5 mcg/kg (N = 189) n (%)	Placebo (N = 236) n (%)	Difelikefalin 0.5 mcg/kg (N = 235) n (%)	Placebo (N = 424) n (%)	Difelikefalin 0.5 mcg/kg (N = 424) n (%)
System Organ Class Preferred Term						

[13] US Renal Data System 2019 Annual Data Report: Epidemiology of Kidney Disease in the United States

Subjects with any event	46 (24.3%)	52 (27.5%)	50 (21.2%)	55 (23.4%)	96 (22.6%)	107 (25.2%)
Cardiac disorders	**7 (3.72%)**	**11 (5.82%)**	**5 (2.11%)**	**16 (6.8%)**	**12 (2.83%)**	**27 (6.36%)**
Atrial fibrillation	1 (0.5%)	2 (1.1%)	0	1 (0.4%)	1 (0.2%)	3 (0.7%)
Cardiac failure congestive	1 (0.5%)	2 (1.1%)	2 (0.8%)	1 (0.4%)	3 (0.7%)	3 (0.7%)
Acute coronary syndrome	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Acute MI	1 (0.5%)	2 (1.1%)	2 (0.8%)	1 (0.4%)	3 (0.7%)	3 (0.7%)
Angina pectoris	0	2 (1.1%)	0	0	0	2 (0.5%)
General disorders and administration stie conditions	3 (1.6%)	4 (2.1%)	1 (0.4%)	9+ (3.8%)	4 (0.9%)	13 (3.1%)
Chest pain[1]	3 (1.6%)	1 (0.5%)	1 (0.4%)	7 (3.0%)	4 (0.9%)	8 (1.9%)
Pyrexia	0	1 (0.5%)	0	2 (0.9%)	0	3 (0.7%)
Asthenia	0	2 (1.1%)	0	0	0	2 (0.5%)
Gastrointestinal disorders	**8 (4.3%)**	**9 (4.8%)**	**6 (2.5%)**	**6 (2.6%)**	**14 (3.3%)**	**15 (3.5%)**
GI hemorrhage	0	3 (1.6%)	1 (0.4%)	1 (0.4%)	1 (0.2%)	4 (0.9%)
Abdominal pain	2 (1.1%)	1 (0.5%)	1 (0.4%)	2 (0.9%)	3 (0.7%)	3 (0.7%)
Diarrhea	1 (0.5%)	2 (1.1%)	2 (0.8%)	1 (0.4%)	3 (0.7%)	3 (0.7%)
Impaired gastric emptying	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Hepatobiliary disorders	0	2 (1.1%)	1 (0.4%)	1 (0.4%)	1 (0.2%)	3 (0.7%)
Cholelithiasis	0	2 (1.1%)	1 (0.4%)	1 (0.4%)	1 (0.2%)	3 (0.7%)
Infections and infestation	**16 (8.5%)**	**14 (7.4%)**	**14 (5.9%)**	**21 (8.9%)**	**30 (7.1%)**	**35 (8.3%)**
Pneumonia	7 (3.7%)	3 (1.6)	0	3 (1.3%)	7 (1.7%)	6 (1.4%)
Sepsis	7 (3.7%)	3 (1.6%)	0	3 (1.3%)	7 (1.7%)	6 (1.4%)
Abscess limb	4 (2.1%)	2 (1.1%)	3 (1.3%)	3 (1.3%)	7 (1.7%)	5 (1.2%)
Cellulitis	0	0	0	2 (0.9%)	0	2 (0.5%)
Endocarditis	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Influenza	0	0	0	2 (0.9%)	0	2 (0.5%)
Localized infection	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Osteomyelitis	1 (0.5%)	0	1 (0.4%)	2 (0.9%)	2 (0.5%)	2 (0.5%)
Septic shock	1 (0.5%)	1 (0.5%)	0	1 (0.4%)	1 (0.2%)	2 (0.5%)
UTI	2 (1.1%)	0	1 (0.4%)	2 (0.9%)	3 (0.7%)	2 (0.5%)
Injury, procedural	6 (3.2%)	3 (1.6%)	10 (4.2%)	7 (3.0%)	16 (3.8%)	10 (2.4%)

complications						
Falls	0	1 (0.5%)	1 (0.4%)	2 (0.9%)	1 (0.2%)	3 (0.7%)
Vascular access malfunction	0	1 (0.5%)	2 (0.8%)	1 (0.4%)	2 (0.5%)	2 (0.5%)
Metabolism and nutritional disorders	14 (7.4%)	6 (3.2%)	10 (4.2%)	7 (3.0%)	16 (3.8%)	10 (2.4%)
Hyperkalemia	5 (2.7%)	4 (2.1%)	3 (1.3%)	4 (1.7%)	8 (1.9%)	8 (1.9%)
Fluid overload	5 (2.7%)	2 (1.1%)	2 (0.8%)	0	7 (1.7%)	2 (0.5%)
Nervous system disorders	**5 (2.7%)**	**6 (3.2%)**	**3 (1.3%)**	**6 (2.6%)**	**8 (1.9%)**	**12 (2.8%)**
Syncope	0	1 (0.5%)	1 (0.4%)	1 (0.4%)	1 (0.2%)	2 (0.5%)
Psychiatric disorders	**2 (1.1%)**	**3 (1.6%)**	**0**	**4 (1.7%)**	**2 (0.5%)**	**7 (1.7%)**
Mental status changes	2 (1.1%)	2 (1.1%)	0	3 (1.3%)	2 (0.5%)	5 (1.2%)
Respiratory, thoracic and mediastinal disorder	**11 (5.9%)**	**10 (5.3%)**	**5 (2.1%)**	**9 (3.8%)**	**16 (3.8%)**	**19 (4.5%)**
COPD	1 (0.5%)	3 (1.6%)	1 (0.4%)	2 (0.9%)	2 (0.5%)	5 (1.2%)
Dyspnea	1 (0.5%)	3 (1.6%)	1 (0.4%)	2 (0.9%)	2 (0.5%)	5 (1.2%)
Respiratory failure	3 (1.6%)	1 (0.5%)	2 (0.8%)	3 (1.3%)	5 (1.2%)	4 (0.9%)
Pleural effusion	1 (0.5%)	1 (0.5%)	1 (0.4%)	1 (0.4%)	2 (0.5%)	2 (0.5%)
Vascular disorders	**4 (2.1%)**	**5 (2.6%)**	**13 (5.5%)**	**5 (2.1%)**	**17 (4.0%)**	**10 (2.4%)**
Hypotension	2 (1.1%)	3 (1.6%)	5 (2.1%)	0	7 (1.7%)	3 (0.7%)
DVT	0	0	1 (0.4%)	2 (0.9%)	1 (0.2%)	2 (0.5%)
Peripheral ischemia	0	0	1 (0.4%)	2 (0.9%)	1 (0.2%)	2 (0.5%)

Source: 5.3.5.3 Integrated Summary of Safety: p.149. Table adapted from source.
DB=double blind phase; MI= myocardial infarction; GI= gastrointestinal; UTI= urinary tract infection; COPD= chronic obstructive pulmonary disorder; DVT= deep vein thrombosis
[1]Chest Pain under general disorders and administration site conditions was reviewed as non-cardiac chest pain in origin (i.e., GERD, cough, atypical thoracic pain)

Analysis of Cross Over

An analysis was completed to assess the incidence of nonfatal serious TEAEs in subjects who received placebo during the double-blind (DB) phase and then crossed over to difelikefalin treatment in the open-label extension phase. The first 3 months of the Open-label Extension Phase was chosen for this analysis to try to mimic the Double-blind Treatment Period and determine whether the incidence of events increased when subjects were newly exposed to difelikefalin. During the 3 months after subjects crossed over from placebo to difelikefalin treatment in the Open-label Extension Phase, the incidence of nonfatal serious TEAEs classified as cardiac disorders remained low: cardiac disorders was 1.1% in placebo and 4.1% of subjects originally on difelikefalin in the Double-blind Phase compared to 2.4% during the first 3 months of the Open-label Extension Phase.

The most common (≥1% of subjects) preferred terms of nonfatal serious TEAEs in the pooled difelikefalin group of the Primary Safety Pool were chest pain (1.9% for difelikefalin and 0.9% for placebo), hyperkalemia (1.9% and 1.9%, respectively), pneumonia (1.4% and 1.7%), sepsis (1.2% and 1.7%), mental status changes (1.2% and 0.5%), and chronic obstructive pulmonary disease (1.2% and 0.5%). The incidences of nonfatal serious events of chest pain, mental status changes, and chronic obstructive pulmonary disease in the pooled difelikefalin group were at least twice those in the pooled placebo group; however, the number of subjects reporting any of these events was generally small (n ≤8 in either pooled treatment group). Upon review of the non-cardiac causes of chest pain cases, there did not appear to be drug attribution.

Serious TEAE in the Difelikefalin Exposure Safety Pool

Five hundred forty-two subjects (41.5%) treated with difelikefalin, including long-term open-label treatment, experienced at least 1 nonfatal serious TEAE. The incidence rate for experiencing as least 1 nonfatal serious TEAE in the pooled difelikefalin group was 1824.3 events per 1000 PY, showing no increase over the rate of 2040.0 events per 1000 PY for the pooled difelikefalin group during shorter-term (12 weeks) exposure in the Primary Safety Pool. The Difelikefalin Exposure Safety Pool has an observation period of up to 15 months, whereas the Primary Safety Pool, has an observation period of 12 weeks.

The most common (≥8% of subjects) system organ class of nonfatal serious TEAEs in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool included infections and infestations (16.3%); cardiac disorders (8.2%); and respiratory, thoracic, and mediastinal disorders (8.0%). The incidence rates for these system organ classes were as follows: infections and infestations (393.2 events per 1000 PY); cardiac disorders (176.3 events per 1000 PY); and respiratory, thoracic, and mediastinal disorders (192.3 events per 1000 PY). These rates did not show any appreciable increase over the Primary Safety Pool, which had shorter exposure to study drug.

The most common (≥2% of subjects) preferred terms of nonfatal serious TEAEs in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool were pneumonia (4.7%), fluid overload (3.3%), hyperkalemia (3.2%), sepsis (2.8%), respiratory failure (2.3%), acute myocardial infarction (2.0%), and mental status changes (2.0%). The incidence rates for these events were as follows: pneumonia (83.8 events per 1000 PY), fluid overload (65.3 events per 1000 PY), hyperkalemia (61.6 events per 1000 PY), sepsis (46.8 events per 1000 PY), acute myocardial infarction (34.5 events per 1000 PY), respiratory failure (39.4 events per 1000 PY), and mental status changes (35.7 events per 1000 PY). Compared with the Primary Safety Pool, the Difelikefalin Exposure Safety Pool had a higher (by factor of 1.5 or more) incidence rate of fluid overload (65.3 versus 20.4 events per 1000 PY) and acute myocardial infarction (34.5 versus 20.4 events per 1000 PY); the rates for the other common preferred terms showed no notable increase over those in the those in the Primary Safety Pool.

The meaning of the observed increase in the incidence rates of events for preferred terms fluid overload and acute myocardial infarction seen in the Difelikefalin Exposure Safety Pool is

difficult to ascertain in a population that is at-risk for such events. In this safety pool, long-term exposure occurred primarily in difelikefalin-treated subjects. Thus, additional events may be related to a progression of comorbidities associated with ESRD and increased time on dialysis. Dialysis patients become fluid overloaded due to diet, changes in medications, missed dialysis, or other factors that are not well controlled, even during clinical trials. Hemodialysis itself exposes patients to hemodynamic stress which is likely to contribute to cardiovascular risk. There was no clear association with difelikefalin and acute myocardial infarction or congestive heart failure seen in the primary safety pool comparison with placebo. Given the multiple comorbidities seen in the hemodialysis patient population, the absence of signal in the controlled trial and the lack of biologic plausibility for a relation with such events, , a causal association between difelikefalin and events of myocardial infarction and fluid overload does not appear to be supported by the totality of the data.

The serious TEAE of acute myocardial infarction (26 subjects [2.0%]) in the Difelikefalin Exposure Safety Pool was driven by 13 subjects in CR845-CLIN3101 (4.5% of the study Safety Population). It should be noted that subjects in CR845-CLIN3101 had a longer period on dialysis, which may have contributed to increased adverse cardiac outcomes. The cross-over analysis did not indicate any higher non-fatal serious TEAE for those that switched to difelikefalin from placebo.

Dropouts and/or Discontinuations Due to Adverse Effects

In the Primary Safety Pool

Twenty-nine subjects (6.8%) in the pooled difelikefalin group and 17 subjects (4.0%) in the pooled placebo group reported at least 1 TEAE leading to study drug discontinuation. The most common (≥1% of subjects) system organ classes of these events in subjects treated with difelikefalin were gastrointestinal disorders (1.2% for difelikefalin and 0.5% for placebo), psychiatric disorders (1.7% and 0.5%, respectively), and nervous system disorders (1.4% and 0.2%). The incidence of TEAEs in each of these system organ classes was at least twice as high in the pooled difelikefalin group as in the pooled placebo group.

The most common (≥0.5% of subjects) preferred terms of TEAEs leading to study drug discontinuation in the pooled difelikefalin group of the Primary Safety Pool were dizziness (0.9% for difelikefalin and 0.2% for placebo), nausea (0.5% and 0%, respectively), headache (0.5% and 0%), anxiety (0.5% and 0%), insomnia (0.5% and 0%), and mental status changes (0.5% and 0.2%).

Table 20: Incidence of TEAE Leading to Study Drug Discontinuation by SOC and PT for PT with ≥ 2 Subjects in the Pooled Difelikefalin Treatment Group – Primary Safety Pool

	CLIN3102 DB		CLIN3103DB		Pooled	
	Placebo (N = 188)	Difelikefalin 0.5 mcg/kg	Placebo (N = 236)	Difelikefalin 0.5 mcg/kg	Placebo (N = 424)	Difelikefalin 0.5 mcg/kg

	n (%)	(N = 189) n (%)	n (%)	(N = 235) n (%)	n (%)	(N = 424) n (%)
System Organ Class Preferred Term						
Subjects with any event	9 (4.8%)	16 (8.5%)	8 (3.4%)	13 (5.5%)	17 (4.0%)	29 (6.8%)
Gastrointestinal disorders	**1 (0.5%)**	**2 (1.1%)**	**1 (0.4%)**	**3 (1.3%)**	**2 (0.5%)**	**5 (1.2%)**
Nausea	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Nervous system disorders	**0**	**4 (2.1%)**	**1 (0.4%)**	**2 (0.9%)**	**1 (0.2%)**	**6 (1.4%)**
Dizziness	0	3 (1.6%)	1 (0.4%)	1 (0.4%)	1 (0.2%)	4 (0.9%)
Headache	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Psychiatric disorders	**1 (0.5%)**	**4 (2.1%)**	**1 (0.4%)**	**3 (01.3%)**	**2 (0.5%)**	**7 (1.7%)**
Anxiety	0	0	0	2 (0.9%)	0	2 (0.5%)
Insomnia	0	0	0	2 (0.9%)	0	2 (0.5%)
Mental status changes	1 (0.5%)	1 (0.5%)	0	1 (0.4%)	1 (0.2%)	2 (0.5%)

Source: Module 5.3.5.3 Integrated Summary of Safety; Table 43: p160
DB= double-blind

Hyperkalemia and somnolence were not a cause of discontinuation. The events that lead to discontinuation was dizziness, nausea, headache, and diarrhea.

In the Difelikefalin Exposure Safety Pool

A total of 121 subjects (9.3%) treated with difelikefalin, including treatment in long-term open-label studies, reported at least 1 TEAE leading to study drug discontinuation. The most common (≥1% of subjects) system organ classes of these events in the pooled difelikefalin group were cardiac disorders (1.9%), nervous system disorders (1.6%), gastrointestinal disorders (1.2%), infections and infestations (1.2%), and general disorders and administration site conditions (1.1%).

The most common (≥0.3% of subjects) preferred terms of TEAEs leading to study drug discontinuation in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool were cardiac arrest (0.8%), death (0.5%), dizziness (0.5%), cardiac failure congestive (0.3%), cardio-respiratory arrest (0.3%), myocardial infarction (0.3%), nausea (0.3%), somnolence (0.3%), and respiratory failure (0.3%). Fewer than 7 subjects in any of the most common preferred term of all TEAEs discontinued study drug (diarrhea, nausea, fall, hypotension, abdominal pain, vomiting, hyperkalemia, dizziness pneumonia, dyspnea, and headache).

The causes of discontinuation were not significantly different for the Primary Safety Pool (12 weeks) or the Difelikefalin Exposure Safety Pool (15 months). The longer exposure period in hemodialysis patients do appear to have slightly higher TEAEs causing discontinuation; however, the longer the patients are on dialysis, the higher the risk of adverse events and co-morbidities leading to discontinuation. It is difficult to make a full comparison between the two safety pools. Hyperkalemia was not a significant cause of discontinuation. Subjects who were on concomitant opioids (28%) experienced increased risk of hyperkalemia (RR 1.78) and somnolence (RR 2.7). The incidence of hyperkalemia was higher in subjects who took concomitant opioids regardless of treatment and was almost doubled in the pooled difelikefalin group (11.7%) compared to the pooled placebo group (6.2%). The incidence of somnolence was higher in pooled difelikefalin subjects (5.8%) who took opioids compared to pooled placebo in the same subgroup (1.6%).

Significant Adverse Events

Treatment Emergent Adverse Events and Adverse Reactions

In the Primary Safety Pool, the incidences of related TEAEs in the pooled difelikefalin (N = 424) and placebo (N = 424) groups were 8.0% and 6.4%, respectively. The most common (≥1% of subjects) preferred terms of related TEAEs in the pooled difelikefalin group were somnolence (1.9% for difelikefalin and 0.9% for placebo) and dizziness (1.4% and 0.2%, respectively). The incidence of both of these related TEAEs in the pooled difelikefalin group was at least twice that in the placebo group.

The methodology used for selecting adverse events for inclusion in the adverse reactions section of the difelikefalin prescribing information was based on the 21 CFR 201.57 (c) (7) and FDA Guidance for Industry: Adverse Reactions Section of Labeling for Human Prescription Drug and Biological Products — Content and Format (January 2006). The factors used: 1. Frequency of reporting (≥2%), 2. Whether AE rate for drug exceeds placebo rate (≥1%), 3. Extent of dose-response (based on CLIN2101 Part A), 4. Extent to which AE is consistent with pharmacology of drug, 5. Timing of the AE relative to time of drug exposure, 6. Whether the AE is known to be caused by related drug (Nalfurafine or REMITCH only in Japan).

The adverse reactions that occurred at a rate of ≥ 2% in the difelikefalin group and ≥ 1% higher than that of the placebo group during the 12-week placebo-controlled period of the pooled Phase 3 clinical studies (CLIN3102 and CLIN3103). The percentage of subjects who discontinued treatment due to any adverse reaction was 2.6% for subjects taking KORSUVA and 0.7% for subjects taking placebo. The most common adverse reactions (≥0.5% of subjects) leading to discontinuation were falls combined with gait disturbance (7.1% for difelikefalin and 5.7% for placebo), dizziness (0.9% for difelikefalin and 0.2% for placebo), mental status change (0.7% and 0.2%, respectively), nausea (0.5% and 0%, respectively), and headache (0.5% and 0%,

respectively). The percentage of subjects who developed serious adverse reactions was 4.5% in the difelikefalin group and 2.8% in the placebo group.

Table 21: Adverse Reaction Reported in ≥ 2% of Subjects on Difelikefalin and ≥ 1% Higher than Placebo; During 12-Week Double-Blind Treatment in Subjects with CKD-aP Undergoing HD

Adverse Reactions	Placebo (N=424) n (%)	Difelikefalin (N=424) n (%)
Diarrhea	24 (5.7)	38 (9.0)
Falls [a]	24 (5.7)	30 (7.1%)
Dizziness	16 (3.8)	29 (6.8)
Nausea	19 (4.5)	28 (6.6)
Hyperkalemia	15 (3.5)	20 (4.7)
Headache	11 (2.6)	19 (4.5)
Somnolence	10 (2.4)	18 (4.2)
Back pain	4 (0.9%)	11 (2.6%)
Mental Status Change [b]	6 (1.4)	14 (3.3)

Source: Module 5.3.5.3 Integrated Summary of Safety
a. Falls is combined with gait disturbances
b. Mental Status Change included preferred terms of confusional state and mental status change.

(b) (4) . We disagree with the applicant's analyses that (b) (4) When combining the preferred terms, the relationship was clear and some central characteristics of unsteadiness is clearly seen. Most gait unsteadiness events were mild or moderate and concomitant medications could have been attributed to the unsteadiness. These events have been updated to the adverse reaction table and will be labeled in the physicians insert.

A total of 86 subjects (6.6%) in the Difelikefalin Exposure Safety Pool, which includes only subjects treated with difelikefalin (N = 1306), experienced at least 1 TEAE considered related to study drug. The most common (≥0.5% of subjects) preferred terms of related events were somnolence (1.1%), dizziness (0.9%), nausea (0.7%), headache (0.6%), vomiting (0.5%), and paresthesia (0.5%).

For TEAEs in the Primary Safety Pool that appear related to the difelikefalin study drug, somnolence (1.9% difelikefalin and 0.9% placebo) and dizziness (1.4% and 0.2% respectively) is suspected. This was also evidenced in the Difelikefalin Exposure Safety Pool, which includes only subjects treated with difelikefalin (N = 1306); the most common (≥0.5% of subjects) preferred terms of related events were somnolence (1.1%), dizziness (0.9%), nausea (0.7%), headache (0.6%), vomiting (0.5%), and paresthesia (0.5%).

Laboratory Findings

Laboratory assessments were collected in all clinical studies. This review will focus on the primary safety pool and the difelikefalin exposure safety pool, rather than present all the changes in laboratory values are examined. Parameters to focus on evaluation of subjects in the safety pools are in the table below.

Table 22: Treatment-Emergent Laboratory Parameters of Clinical Interest to Identify

Parameter	Low	High
Hemoglobin (g/dL)	<7	>14
Calcium (mg/dL)	<7	>10.5
Serum albumin (mg.dL)	<3	>5.5
Phosphate (mg/dL)	<2.5	>8
Potassium (mmol/L)	<2.5	>7
Calcium x phosphate (mg^2/dL^2)		≥55

Source: Agency Clinical Reviewer

In the primary safety pool, TEAE laboratory of clinical interest in summarized in the table below. Among treatment-emergent abnormalities reported in at least 0.5% of difelikefalin subjects, a greater (by a factor of 1.5 or more) percentage of subjects in the pooled difelikefalin group than in the pooled placebo group experienced a treatment-emergent hemoglobin value >14g/dL (1.7% versus 0.8%) and a treatment-emergent potassium value >7 mmol/L (2.8% and 1.0%).

Table 23: Treatment-Emergent Laboratory Abnormalities of Clinical Interest – Primary Safety Pool

	Pooled			
Lab Test Category Parameter (unit)	**Placebo (N = 424)**		**Difelikefalin 0.5 mcg/kg (N = 424)**	
	Low n/nn (%)	High n/nn (%)	Low n/nn (%)	High n/nn (%)
Hemoglobin (g/dL) Low: <7; High >14	4/404 (1%)	3/398 (0.8%)	2/404 (0.5%)	7/402 (1.7%)
Calcium (mg/dL) Low: <7; High >10.5	5/397 (1.3%)	2/401 (0.5%)	4/399 (1.0%)	1/401 (0.2%)
Serum albumin (mg.dL) Low: <3; High >5.5	8/397 (2.0%)	0/406	8/400 (2.0%)	0/404
Phosphate (mg/dL) Low: <2.5; High >8	19/389 (4.9%)	19/356 (5.3%)	12/385 (3.1%)	24/357 (6.7%)
Potassium (mmol/L) Low: <2.5; High >7	0/403	4/398 (1.0%)	1/403 (0.2%)	11/396 (2.8%)
Calcium x phosphate (mg^2/dL^2) High ≥ 55		47/266 (17.7%)		51/276 (18.5%)

Source: Module 5.3.5.3 Integrated Summary of Safety Table 62: page 225

No subjects with a TEAE in hemoglobin of > 14 g/dL experienced a thromboembolic event during the double-blind phase. No subjects with a TEAE potassium of > 7 mmol/L reported a cardiac disorder at the time of increase potassium.

In the hemodialysis subjects who received difelikefalin versus placebo had increased potassium levels difference of 1.8% in the primary safety pool.

Table 24: Treatment-Emergent Laboratory Abnormalities of Clinical Interest – Difelikefalin Exposure Safety Pool

	Pooled Difelikefalin 0.5 mcg/kg (N = 1306)	
Lab Test Category Parameter (unit)	Low n/nn (%)	High n/nn (%)
Hemoglobin (g/dL) Low: <7; High >14	10/1263 (0.8%)	39/1240 (3.1%)
Calcium (mg/dL) Low: <7; High >10.5	49/1250 (3.5%)	34/1259 (2.7%)
Serum albumin (mg.dL) Low: <3; High >5.5	44/1250 (3.5%)	0/1267
Phosphate (mg/dL) Low: <2.5; High >8	93/1219 (7.6%)	171/1137 (15.0%)
Potassium (mmol/L) Low: <2.5; High >7	3/1257 (0.2%)	54/1234 (4.4%)
Calcium x phosphate (mg^2/dL^2) High ≥ 55		312/873 (35.7%)

Source: Module 5.3.5.3 Integrated Summary of Safety; Table 63: page 227

In the Difelikefalin Exposure Safety Pool, 39 subjects (3.1%) experienced a treatment-emergent hemoglobin value >14 g/dL, and 54 subjects (4.4%) experienced a treatment-emergent potassium value >7 mmol/L. Among subjects with a treatment-emergent potassium >7 mmol/L, there were 2 subjects ; Subject (b) (6) in CR845-CLIN3101 and Subject (b) (6) in CR845-CLIN3103OLE, who had a mild, concomitant TEAE of atrioventricular block first degree reported at the time of the increased potassium. Both TEAEs were considered not related to study drug. The investigator assessed the TEAE for subject (b) (6) attributable to the use of a beta-blocker.

For high hemoglobin levels, thrombosis was seen in subjects with hemoglobin >14 g/dL.

- A moderate event of acute myocardial infarction reported 3 weeks after hemoglobin >14 g/dL was observed in Subject (b) (6) in CR845-CLIN3101 (Subject (b) (6) in

CR845-CLIN2005 Part B)

- A severe event of arteriovenous fistula thrombosis reported 9 days after hemoglobin acute >14 g/dL was observed; severe events of acute myocardial infarction, venous thrombosis limb, and deep vein thrombosis reported 2 months after hemoglobin >14 g/dL was observed; and a fatal myocardial infarction reported approximately 5 months after hemoglobin acute >14 g/dL was observed in Subject (b) (6) in CR845-CLIN3101 (Subject (b) (6) in CR845-CLIN2101)

Non-serious thrombic events were also reported in subjects with TEAE hemoglobin >14.

- A mild event of vascular access site thrombosis occurred 4 days after hemoglobin >14 g/dL was reported in Subject (b) (6) in CR845-CLIN3101 (Subject (b) (6) in CR845-CLIN2101)

The laboratory abnormalities are noted in both the Primary Safety Pool and the Difelikefalin Exposure Safety Pool. Noted is the hyperkalemia (diff 1.8%) and concentration of hemoglobin (diff 0.9%) in difelikefalin group versus the placebo group. It is not clear that the changes in laboratory value can be fully attributed to the drug product. Hemodialysis subjects are dependent on dialysis for electrolyte balance. In addition, free fluid removal can cause hemoconcentration.

Liver functions were also examined in subjects on hemodialysis and receiving difelikefalin for uremic pruritus. In the primary safety pool, the most frequent abnormality was an alkaline phosphatase value >1.5 × upper limit of normal (ULN), which was reported in similar proportions of subjects in the pooled difelikefalin and pooled placebo groups (54 subjects [12.7%] and 51 subjects [12.0%], respectively). An AST value >3 × ULN was reported in 1 subject (0.2%) in the pooled difelikefalin group and no subjects (0%) in the placebo group. No subject in the Primary Safety Pool had an ALT value >3 × ULN, or an ALT or AST value >5 × ULN. Three subjects (0.7%) in each of the pooled treatment groups had a bilirubin value >2 × ULN. No subject in the Primary Safety Pool met laboratory criteria for a Hy's law case.

The most frequent reported clinically significant liver function test abnormality in the Difelikefalin Exposure Safety Pool was an alkaline phosphatase value >1.5 × ULN, which was reported in 207 subjects (15.8%) in the pooled difelikefalin group (Table 65). One subject (<0.1%) reported an ALT value >3 × ULN, and 2 subjects (0.2%) reported an AST value >3 × ULN. Values for ALT and AST >5 × ULN were reported in 2 subjects (0.2%) and 1 subject (<0.1%), respectively. A total of 11 subjects (0.8%) had a bilirubin value >2 × ULN.

One subject died due to pneumonia. This subject had multiple laboratory abnormalities.

- This subject, in the Difelikefalin Exposure Safety Pool, Subject (b) (6) in CR845-CLIN3103, met the laboratory criteria AST or ALT >3 x ULN and total bilirubin >2 × ULN. He was a 74-year-old white male subject with the relevant medical history of multiple episodes of gastrointestinal hemorrhage, congestive heart failure, atrial fibrillation, myocardial infarction, endocarditis, and anemia. On (b) (6), Study Day 358, the subject was hospitalized and diagnosed with pneumonia. White blood cell count was

18,400/mL (reference range: 4,000-10,000). Serum C-reactive protein was 102.3 mg/L (reference range: <5). Blood culture grew Enterococcus faecium and Enterobacter cloacae. The subject was treated with antibiotics, and the pneumonia resolved after 17 days. Dosing with study drug was not changed in response to this event. On (b) (6), while still hospitalized, the subject developed lower gastrointestinal hemorrhage. Blood hemoglobin was 7.3 g/dL (reference range: 10-12). The subject developed thrombocytopenia (platelet count not specified). Treatment included platelet transfusion. On (b) (6), hypotension and multiorgan failure ensued. Treatment included continuous ultrafiltration. The subject subsequently developed respiratory failure and exacerbation of heart failure secondary to the multiorgan failure. On (b) (6), the subject died due to lower gastrointestinal hemorrhage and multiorgan failure. The subject had abnormal liver function tests at the open-label discontinuation (end of treatment) visit, 3 days (b) (6) after receiving the most recent dose of the study drug, and during his hospitalization for lower gastrointestinal hemorrhage and multiorgan failure. The subject had normal range AST, ALT, and bilirubin values at all preceding visits throughout the study. No AEs were noted due to this abnormality. The investigator assessed the serious TEAEs of gastrointestinal hemorrhage, diverticulitis, pneumonia, lower gastrointestinal hemorrhage, and multiple organ dysfunction syndrome as not related to study drug.

In conclusion, the laboratory parameters were consistent within the hemodialysis patient population. A greater percentage of subjects in the pooled difelikefalin group than in the pooled placebo group of the Primary Safety Pool experienced treatment-emergent laboratory abnormalities of hemoglobin >14g/dL (1.7% versus 0.8%) and potassium >7 mmol/L (2.8% and 1.0%). This was also consistent in the Difelikefalin All Exposure Safety Pool. Of note, no subject in the Primary Safety Pool experienced a thromboembolic TEAE concomitantly with elevated hemoglobin, and no subject in the Primary Safety Pool or Difelikefalin Exposure Safety Pool reported TEAEs in the system organ class of cardiac disorders concomitantly with elevated potassium.

Vital Signs

The applicant provided box plots of mean and median values of all vitals for the Primary Safety Pool and the Difelikefalin Exposure Safety Pool. The values were reviewed and will not be presented in this report. Specific vital signs will be presented if they are outside of the parameters:

- Systolic blood pressure (SBP) ≥ 180 or ≤ 90 mmHg
- Diastolic blood pressure (DBP) ≥ 100 or ≤ 60 mmHg
- Heart rate (HR) > 130 beats per minute or < 55 beats per minute

The vital signs were evaluated by time points of Weeks 2, 4, 6, 8, 10 and at the end of treatment period. In the pooled difelikefalin group, the subject incidence of a postbaseline systolic blood pressure ≥180 mmHg fluctuated over the course of the treatment period, with

percentages ranging from 8.8% at the end of Week 2 to 13.5% at the end of Week 8, and a baseline incidence of 10.8%. The postbaseline incidence of a systolic blood pressure ≥180 mmHg in the placebo group also fluctuated, ranging from 6.4% at the end of Week 2 to 9.3% at the end of Weeks 4 and 12, with a baseline incidence of 9.7%. The pooled difelikefalin and pooled placebo groups showed similar postbaseline incidences of systolic blood pressure ≥180 mmHg at all time points except the end of Week 6 and end of Week 8, when the incidence was higher (by a factor of 1.5 or more) in the pooled difelikefalin group than in the pooled placebo group (11.2% versus 7.3% at end of Week 6; 13.5% versus 8.0% at end of Week 8). At Week 12, the incidence was 11.4% for the difelikefalin group and 9.3% for the placebo group. It is noted that for approximately half of the measured timepoints, there was only a modest increase (<6% total subjects) in the percentage of difelikefalin-treated subjects with a reported systolic blood pressure ≥180 mmHg as compared to baseline. Other timepoints showed a similarly modest decrease in the percentage of difelikefalin-treated subjects with a reported systolic blood pressure ≥180 mmHg as compared to baseline. The pattern of elevated systolic blood pressure was inconsistent and did not appear to be related to drug attribution but more likely due to fluctuating volume and sodium status experienced by this population.

The incidence of a postbaseline diastolic blood pressure ≥100 mmHg in the pooled difelikefalin group fluctuated during the treatment period, ranging from 9.3% at the end of Week 4 to 5.4% at the end of Week 12, with a baseline incidence of 6.4%. The incidence in the pooled placebo group also fluctuated, ranging from 4.9% at the end of Week 2 to 8.5% at the end of Week 12, with a baseline value of 7.3%. Except for the end of Week 2, when 9.1% of subjects in the pooled difelikefalin group and 4.9% of subjects in the pooled placebo group had a diastolic blood pressure ≥100 mmHg, the incidence of this vital sign abnormality at any time point was comparable between the 2 treatment groups. At Week 12 the incidence was 5.4% for the difelikefalin group and 8.5% for the placebo group.

The incidence of any postbaseline systolic blood pressure ≤90 mmHg in the pooled difelikefalin and pooled placebo groups was ≤1.3%, with baseline incidences of 0.5% and 0%, respectively.

The subject incidence of a postbaseline diastolic blood pressure ≤60 mmHg in the pooled difelikefalin group fluctuated, ranging from 13.5% at the end of Week 2 to 9.3% at the end of Week 8, with a baseline incidence of 11.1%. The postbaseline incidence of this vital sign abnormality in the placebo group also fluctuated, ranging from 9.5% at the end of Weeks 4 and 10 to 13.6% at the end of Week 8, with a baseline incidence of 13.0%. The incidence of a diastolic blood pressure ≤60 mmHg was similar between the pooled difelikefalin and placebo groups at all time points.

The incidences of any postbaseline heart rate >130 bpm in the pooled difelikefalin and pooled placebo groups were ≤0.5% and 0%, respectively, with a baseline incidence of 0% in each treatment group. The incidences of any postbaseline heart rate <55 bpm were ≤2.8% and ≤2.3% for difelikefalin and placebo, respectively, with baseline incidences of 2.1% and 0.9%, respectively.

In the Primary Safety Pool, the vital signs of interest discussed above were comparable between the pooled difelikefalin and pooled placebo groups. No trends were seen over the time points evaluated. This was also seen in the Difelikefalin Exposure Safety Pool.

Electrocardiograms (ECGs)

Electrocardiograms were completed in the Phase 3 clinical studies. The ECGs were submitted to a central ECG laboratory, (b) (4) which was blinded to treatment. The results of the ECG analysis showed no signal of any clinically significant effect of difelikefalin on heart rate, atrioventricular conduction as measured by the PR interval, or cardiac depolarization as measured by the QRS duration. There were no new clinically relevant morphological changes. In addition, there was no signal of a clinically significant effect of difelikefalin on cardiac repolarization as evidenced by the results of the time-averaged, time-matched, outlier, and PK-pharmacodynamic analyses.

Reported cardiac adverse events were low and generally balanced across both treatment groups. The number and percentage of patients who developed new, clinically significant ECG morphologic findings was relatively evenly distributed between the difelikefalin and placebo treatment groups. Noted exceptions were an imbalance in ECG findings of myocardial infarction (3 in the placebo group; 0 in the difelikefalin group) and in the number of subjects with new, potentially significant T wave inversion (4 in the placebo group; 9 in the difelikefalin group). The clinical significance of the T wave inversion is unclear, and the subjects with the changes were evaluated for myocardial infarctions. The population of hemodialysis subjects are at a high prevalence of pre-existing cardiovascular disease and cardiovascular risk factors. Hemodialysis can occasionally cause T and ST wave abnormalities on ECG with related fluid, potassium, magnesium, calcium, and other electrolyte imbalances. The applicant provided a thorough QT/QTc study to demonstrate that difelikefalin had no effects on heart rate, PR and QRS interval duration, cardiac repolarization, or other ECG parameters.

QT

A TQT study (CR845-100201) was conducted in Phase 1. The conclusions of the TQT study showed no signal of clinically significant effect of difelikefalin on heart rate, atrioventricular conduction as measured by the PR interval, or cardiac depolarization as measured by the QRS duration.

8.2.5. Analysis of Submission-Specific Safety Issues

Specific adverse events of interest were analyzed. Standardized MedDRA queries were conducted to examine categories of TEAEs based on the results of the pivotal phase 3 studies CR845-CLIN3102 and CR845-CLIN3103. The SMQs included cardiac arrhythmias, cardiac failure, acute central respiratory depression, and vestibular disorders. The analyses of SMQs were conducted for all safety pools. Events of special interest includes MACE and TEAE of special

interest (dizziness, mental status changes, gait disturbance/falls, and other central acting issues).

8.2.5.1. Major Adverse Cardiovascular Events (MACE)

In the Primary Safety Pool, 16 subjects (3.8%) had at least 1 MACE event in the difelikefalin group and 10 subjects (2.4%) in the placebo group. The most common (≥0.5%) preferred terms of MACEs in subjects treated with difelikefalin were cardiac failure congestive (0.7% for difelikefalin and 0.3% for placebo), myocardial infarction (0.5% and 0.2%), and cardiac failure (0.5% and 0%).

MACE (definitions):

- PT in myocardial infarction (SMQ)
- Stroke by PT:
    - Basal ganglia stroke
    - Brain stem stroke
    - Cerebellar stroke
    - Cerebrovascular accident
    - Hemorrhagic cerebral infarction
    - Hemorrhagic stroke
    - Hemorrhagic transformation stroke
    - Stroke in evolution
    - Vertebrobasilar stroke
    - Basal ganglia infarction
    - Brain stem infarction
    - Cerebellar infarction
    - Cerebral infarction
    - Embolic cerebral infarction
    - Embolic stroke
    - Ischemic cerebral infarction
    - Ischemic stroke
    - Lacunar infarction
    - Lacunar stroke
    - Thalamic infarction
    - Thrombotic cerebral infarction
    - Thrombotic stroke
- PT in cardiac failure SMQ (narrow) resulting in hospitalization
- Revascularization defined as one of the PT:
    - Carotid revascularization
    - Coronary revascularization
- PT of Percutaneous coronary intervention
- PT of coronary artery bypass
- PT:

- Cardiac arrest
- Cardiorespiratory arrest
- Cerebellar embolism
- Cerebral hemorrhage
- Circulatory collapse

- PT of "Sudden death"
- PT in Cardiac Disorders SOC that are fatal or have and outcome of death

Table 25: Incidence of MACE by Preferred Term – Primary Safety Pool

	CLIN3102 DB		CLIN3103DB		Pooled	
	Placebo (N = 188) n (%)	Difelikefalin 0.5 mcg/kg (N = 189) n (%)	Placebo (N = 236) n (%)	Difelikefalin 0.5 mcg/kg (N = 235) n (%)	Placebo (N = 424) n (%)	Difelikefalin 0.5 mcg/kg (N = 424) n (%)
Subjects with any event	5 (2.7%)	9 (4.8%)	5 (2.1%)	7 (3.0%)	10 (2.4%)	16 (3.8%)
Heart Failure Congestive	1 (0.5%)	2 (1.1%)	2 (0.8%)	1 (0.4%)	3 (0.7%)	3 (0.7%)
Acute Coronary Syndrome	0	1 (0.5%)	0	1 (0.4%)	0	2 (0.5%)
Acute Myocardial infarction	1 (0.5%)	0	0	2 (0.9%)	1 (0.2%)	2 (0.5%)
Cardiac failure	0	0	0	2 (0.9%)	0	2 (0.5%)
Acute Pulmonary edema	0	1 (0.5%)	0	0	0	1 (0.2%)
Angina unstable	0	1 (0.5%)	0	0	0	1 (0.2%)
Cardiac arrest	1 (0.5%)	0	1 (0.4%)	1 (0.4%)	2 (0.5%)	1 (0.2%)
Cardiac failure acute	0	1 (0.5%)	0	0	0	1 (0.2%)
Cardiogenic shock	0	0	0	1 (0.4%)	0	1 (0.2%)
Cerebellar embolism	0	1 (0.5%)	0	0	0	1 (0.2%)
Cerebral infarction	0	1 (0.5%)	0	0	0	1 (0.2%)
Cerebrovascular accident	1 (0.5%)	1 (0.5%)	0	0	1 (0.2%)	1 (0.2%)
Pulmonary edema	0	1 (0.5%)	1 (0.4%)	0	1 (0.2%)	1 (0.2%)
Troponin I increased	0	1 (0.5%)	0	0	0	1 (0.2%)
Myocardial infarction	0	0	1 (0.4%)	0	1 (0.2%)	0
Troponin increased	2 (1.1%)	0	1 (0.4%)	0	3 (0.7%)	0

Source: Module 5.3.5.3 Integrated Summary of Safety, Table was based on pooled analysis by Agency reviewer

In the larger Difelikefalin Exposure Safety Pool, the incidence rate for any MACE in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool was 199.7 events per 1000 PY, showing no notable increase over the rate of 193.8 events per 1000 PY in the pooled difelikefalin group of the Primary Safety Pool.

In terms of MACE, hemodialysis patients are at a high risk for all events. There were no significantly noticeable differences in the placebo versus the difelikefalin treated group. Significant MACE is likely related to the cardiovascular vulnerabilities and co-morbidities rather than drug related. Review of the events in MACE is complicated by multiple medical conditions of patients on hemodialysis. It does not appear that difelikefalin treatment has contributed to the experienced MACE adverse events in the study population.

8.2.5.2. TEAE of Special Interest

This section discusses the treatment-emergent adverse events of special interest in the Primary Safety Pool. Seventy-five subjects (17.7%) treated with difelikefalin and 55 subjects (13.0%) treated with placebo reported at least 1 TEAE of special interest. The most common (≥2% of subjects) categories of TEAEs of special interest in the pooled difelikefalin group were dizziness (6.8% for difelikefalin and 3.8% for placebo), falls (5.4% and 5.0%, respectively), and somnolence (4.2% and 2.4%). The incidences of dizziness and somnolence were higher (by a factor of 1.5 or more) in the pooled difelikefalin group than in the pooled placebo group. The table shows the categories of AESI from most to least. Note that the preferred term of gait disturbances and falls were combined.

Table 26: Incidence Rates of Adverse Events of Special Interest – Primary Safety Pool

	CLIN3102 DB		CLIN3103DB		Pooled	
AESI Category	Placebo (N = 188) n (IR)	Difelikefalin 0.5 mcg/kg (N = 189) n (IR)	Placebo (N = 236) n (IR)	Difelikefalin 0.5 mcg/kg (N = 235) n (IR)	Placebo (N = 424) n (IR)	Difelikefalin 0.5 mcg/kg (N = 424) n (IR)
Total Person-Years	46.55	45.66	54.51	52.38	101.07	98.04
Any Event	**24 (515.5)**	**41 (306.6)**	**16 (293.5)**	**17 (324.5)**	**72 (712.4)**	**106 (1081.2)**
Dizziness	3 (64.4)	14 (306.6)	16 (293.5)	17 (324.5)	19 (188.0)	31 (316.2)
Falls [a]	8 (171.9)	10 (219.0)	16 (293.5)	23 (439.0)	24 (237.5)	33 (336.6)
Somnolence	5 (107.4)	6 (131.4)	5 (91.7)	14 (267.3)	10 (98.9)	20 (204.0)
Mental Status Changes	3 (64.4)	3 (65.7)	0	3 (57.3)	3 (29.7)	6 (61.2)
Syncope	2 (21.5)	2 (43.8)	3 (55.0)	4 (76.4)	4 (39.6)	6 (61.2)
Palpitations	2 (43.0)	1 (21.9)	1 (18.3)	3 (57.3)	3 (29.7)	4 (40.8)
Tachycardia	1 (21.5)	3 (65.7)	6 (110.1)	1 (19.1)	7 (69.3)	4 (40.8)
Mood changes	0	1 (21.9)	1 (18.3)	0	1 (9.9)	1 (10.2)

Seizures	1 (21.5)	1 (21.9)	0	0	1 (9.9)	1 (10.2)

Source: Module 5.3.5.3 Integrated Summary of Safety Table 55, adapted by Agency Reviewer
[a] Falls: includes PT of gait disturbance and Falls
Tachycardia included the following PT: Tachycardia, sinus tachycardia and tachyarrhythmia

Combining the related events of falls and gait disturbance increased the incidence rates to 33 (336.6). This was second only to dizziness, which can also affect falls. It was common that concomitant centrally-acting medications combined with difelikefalin contributed to dizziness, somnolence, or gait disturbance/falls. In addition, diabetic neuropathy also contributed to the issues of gait disturbance in this vulnerable population. The combining of PT for falls and gait disturbance was justified by evaluating the data in the Japanese Phase 2 study (Section 8.2.8) submitted in the 120-day safety update and the events descriptions themselves. The combination of the PT events highlights the major safety issues that appears to limit this drug in the moderate-to-severe pruritus population.

A total of 317 subjects (24.3%) in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool reported at least 1 TEAE of special interest. The incidence rate of TEAEs of special interest in the pooled difelikefalin group was 641.0 events per 1000 PY, which showed no increase over the rate in the pooled difelikefalin group of the Primary Safety Pool (1081.2 events per 1000 PY).

Table 27: Incidence of TEAE of Special Interest – Difelikefalin Exposure Safety Pool

	CLIN3101	CLIN3102 DB+OLE	DLIN3103 DB+OLE	CLIN3015	Pooled
AESI Category	Difelikefalin 0.5mcg/kg (N=288) n %	Difelikefalin 0.5mcg/kg (N=351) n %	Difelikefalin 0.5mcg/kg (N=445) n %	Difelikefalin 0.5mcg/kg (N=222) n %	Difelikefalin 0.5mcg/kg (N=1306) n %
Subjects with Any Event	**80 (27.8%)**	**119 (33.9%)**	**96 (21.6%)**	**22 (9.9%)**	**317 (24.3%)**
Falls [a]	44 (15.2%)	56 (16.0%)	52 (11.6%)	4 (1.8%)	156 (12.0%)
Dizziness	24 (8.3%)	40 (11.4%)	32 (7.2%)	7 (3.2%)	103 (7.9%)
Mental Status Changes	13 (4.5%)	18 (5.1%)	10 (2.2%)	2 (0.9%)	43 (3.3%)
Syncope	14 (4.9%)	15 (4.3%)	10 (2.2%)	4 (1.8%)	43 (3.3%)
Somnolence	3 (1.0%)	7 (2.0%)	13 (2.9%)	6 (2.7%)	29 (2.2%)
Tachycardia	8 (2.8%)	16 (4.6%)	3 (0.7%)	0	11 (0.8%)
Seizures	5 (1.7%)	5 (1.4%)	1 (0.2%)	0	11 (0.8%)
Palpitations	2 (0.7%)	3 (0.9%)	4 (0.9%)	1 (0.5%)	10 (0.8%)
Mood changes	0	1 (0.3%)	0	0	1 (<0.1%)

Source: Module 5.3.3.5 Integrated Summary of Safety, Table 57, revised by Agency Reviewer

AESI = adverse event of special interest; DB = Double-blind Phase; MedDRA = Medical Dictionary for Regulatory Activities; OLE = Open-label Extension Phase
Note: Tachycardia included the following preferred terms: Tachycardia, Sinus tachycardia, and Tachyarrhythmia.
[a] Falls = the PT for Falls and Gait disturbance
The table is sorted by descending subject incidence in the Pooled CR845 0.5 mcg/kg column for AESI. A subject is counted only once for each AESI if he/she had multiple events of the same AESI.

The most common (≥2% of subjects) categories of TEAEs of special interest in the Difelikefalin Exposure Safety Pool were falls/gait disturbances (12%), dizziness (7.9%), mental status changes (3.3%), syncope (3.3%), somnolence (2.2%), and tachycardia (2.1%). Once again, the incidence rates for falls, dizziness, and somnolence were higher but was not notably increased over the rates in the pooled difelikefalin group of the Primary Safety Pool. However, combining the preferred terms of gait disturbances and falls generates this adverse event as the most common event due to drug for events of special interest.

The combined falls and gait disturbances for the Primary Safety Pool was an IR 33 (336.6) for difelikefalin compared with an IR 24 (237.5). When combined, the preferred term of falls and gait disturbances qualified for adverse reactions section. This was added to the labeling in section 6.1 and a new section with a discussion of the adverse reaction was added. in the Difelikefalin Exposure Pool (N=1306), the IR 217 (267.4) for pooled difelikefalin. There were not any notable differences in increases over the corresponding rates in the pooled difelikefalin group of the Primary Safety Pool. This held true to the Difelikefalin Exposure Safety Pool.

Centrally-acting adverse events commonly occurred in all safety pools. These events pose a potential risk for falls and accidents. For injury mitigation, warnings and precaution labeling should inform of this potential risk.

8.2.6. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

COA was not asked to weigh in on the safety of this product during the NDA review. COA previously weighed in on the utility of the primary and secondary endpoints during the IND phase of the drug product development.

8.2.7. Safety Analyses by Demographic Subgroups

Subgroup analyses for safety was done for age, sex, race, renal and hepatic impairment and pre-existing specific medical conditions for the Primary Safety Pool.

In the Primary Safety Pool, the majority of subjects (67.2%) were <65 years of age. Overall, there were 196 subjects (69.8%) and 180 subjects (62.3%) in the pooled difelikefalin and pooled placebo groups, respectively, aged <65 years who experienced at least 1 TEAE. There were 106 subjects (74.1%) and 97 subjects (71.9%) in the pooled difelikefalin and pooled placebo groups, respectively, aged ≥65 years who reported at least 1 TEAE.

On review of the subgroup analyses, sex and race had no effects on how the drug product

worked in comparison to placebo. In addition, hepatic functions had little impact on the PK of difelikefalin and is essentially not metabolized by the liver.

Generally, having a pre-existing medical condition does not appear to amplify or contribute to unexpected adverse events in subjects treated with difelikefalin. In the Primary Safety Pool, a majority of subjects (84.6%) had a prior specific medical condition. Adverse events were similar between those that received drug and those that received placebo. However, concomitant medications, in particular opioids and diphenhydramine, appear to be related to an increase of centrally-acting adverse events.

Additional factors were reviewed, including geographical regions for the safety analyses. The majority of subjects were from the United States (77.2%). The overall risk of having a TEAE associated with drug in the subgroups of non-US verses US-subjects were small and therefore not interpretable.

8.2.8. Specific Safety Studies/Clinical Trials

Supportive Clinical Study (Japan)

The applicant provided a completed Japanese Phase 2 study (MR13A9-4) at the 3-month safety update for NDA 214916. This study included 3 difelikefalin dose (0.25, 0.5, 1 mg/kg) given to hemodialysis patient with uremic pruritus. This study was sponsored by Kissei Pharmaceutical Company, Ltd. and was not conducted under the IND. When the enumeration data from Study MR13A9-4 are combined with the 18 ISS studies, the total number subjects who received at least 1 dose of difelikefalin is 2063; 1776 of these subjects were undergoing HD and 287 were not. Of the 1515 subjects who received the 0.5 mcg/kg dose of difelikefalin, 1461 were undergoing HD.

The incidence of AEs during the Treatment Period of Study MR13A9-4 increased in a dose-related manner, with 72.1%, 77.0%, and 85.5% of subjects in the difelikefalin 0.25, 0.5, and 1 mcg/kg groups, respectively, and 66.7% of subjects in the placebo group reporting at least 1 AE. The incidence of adverse drug reactions (AEs occurring after the start of administration of the study drug for which causal relationship with study drug was assessed as related) also increased in a dose-related manner: 14.8%, 14.8%, and 27.4% for difelikefalin 0.25, 0.5, and 1 mcg/kg, respectively, and 11.1% for placebo.

The most common (≥5% of subjects in any treatment group) preferred terms of AEs during the Treatment Period of Study MR13A9-4 were epipharyngitis (13.1%, 9,8%, and 4.8% for difelikefalin 0.25, 0.5, and 1 mcg/kg, respectively; 11.1% for placebo), somnolence (3.3%, 4.9%, and 9.7%; 4.8%), dizziness (4.9%, 4.9%, and 8.1%; 4.8%), constipation (8.2%, 4.9%, and 11.3%; 0%), vomiting (1.6%, 1.6%, and 6.5%; 4.8%), nausea (0%, 1.6%, and 6.5%; 1.6%), joint pain (4.9%, 8.2%, and 3.2%; 3.2%), malaise (3.3%, 3.3%, and 6.5%; 0%), fever (3.3%, 6.6%, and 0%; 0%), hypotension (0%, 6.6%, and 4.8%; 3.2%), and hypotension due to treatment (6.6%, 4.9%, and 3.2%; 4.8%). Of these events, somnolence, dizziness, nausea, vomiting, and malaise showed

an increased incidence with difelikefalin dose; however, except for malaise, the incidence of these events in the difelikefalin 0.5 mcg/kg group was similar to or less than that in the placebo group.

The incidences of falls and elevated blood potassium were low across all the treatment groups. Falls were reported in 1.6%, 3.3%, and 1.6% of subjects in the difelikefalin 0.25, 0.5, and 1 mcg/kg groups, respectively, and in 1.6% of subjects in the placebo group. The incidence of elevated blood potassium was 1.6%, 0%, and 0% in the difelikefalin 0.25, 0.5, and 1 mcg/kg groups, respectively, and 0% in the placebo group.

8.2.9. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

There is no indication that difelikefalin causes carcinogenicity or tumor development in human subjects.

Human Reproduction and Pregnancy

No studies in human reproduction or pregnancy are expected at this time. A limited number of female patients on hemodialysis would be expected to become pregnant due to underlying disease. Thus, there is limited utility in a pregnancy registry or a lactation study in the use of difelikefalin in the hemodialysis population.

Pediatrics and Assessment of Effects on Growth

There are no uses for this drug product beyond the indication as described. This application will receive a full waiver for patients < 17 years and 11 months (See Pediatric Waiver).

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Particular interest was placed on evaluation of drug abuse potential, overdose, and withdrawal and rebound was placed on this first in class kappa opioid treatment for uremic pruritus. The Agency CSS reviewed the studies related to drug abuse potential. Difelikefalin will not be labeled as a scheduled drug. (b) (4)

In the dose-finding Phase 2 study CR845-CLIN2101 Part A, subjects with CKD-aP undergoing HD were randomized to receive 1 of 3 doses of difelikefalin (0.5, 1.0, or 1.5 mcg/kg) or placebo as an IV bolus 3 times a week after each HD session for 8 weeks. Overall, 68.4% of subjects reported at least 1 TEAE, with the incidence being similar (less than a factor of 1.5 difference) across the difelikefalin 0.5, 1.0, and 1.5 mcg/kg groups (84.1%, 70.7%, and 77.3%, respectively), and higher in the difelikefalin groups than in the placebo group (42.2%)

Common preferred terms with possible dose-related increases in incidence included the following:

- **Somnolence** (4.5%, 4.9%, and 11.4% for difelikefalin 0.5, 1.0, and 1.5 mcg/kg, respectively; 2.2% for placebo)
- **Mental status change** (0%, 2.4%, and 11.4% for difelikefalin 0.5, 1.0, and 1.5 mcg/kg, respectively; 0% for placebo)
- Paresthesia (2.3%, 4.9%, and 6.8% for difelikefalin 0.5, 1.0, and 1.5 mcg/kg, respectively; 0% for placebo)
- Fatigue (2.3%, 2.4%, and 6.8% for difelikefalin 0.5, 1.0, and 1.5 mcg/kg, respectively; 0% for placebo)
- Hypertension (0%, 2.4%, and 6.8% for difelikefalin 0.5, 1.0, and 1.5 mcg/kg, respectively; 0% for placebo)

The incidence of at least 1 TEAE leading to study drug discontinuation showed a relationship to difelikefalin dose, with the incidence increasing with increasing dose: 9.1%, 9.8%, and 15.9% for difelikefalin 0.5, 1.0, and 1.5 mcg/kg, respectively; 2.2% for placebo

Table 28: Common (>5% of Subjects in Any Treatment Group) TEAE by PT in CR845-CLIN2101 Part A (Safety Population)

		CR845		
Preferred Term	Placebo (N = 45) n (%)	0.5 mcg/kg (N = 44) n (%)	1.0 mcg/kg (N = 41) n (%)	1.5 mcg/kg (N = 44) n (%)
At least 1 TEAE	19 (42.2%)	37 (84.1%)	29 (70.7%)	34 (77.3%)
Diarrhoea	0	7 (15.9%)	4 (9.8%)	5 (11.4%)
Dizziness	2 (4.4%)	6 (13.6%)	4 (9.8%)	2 (4.5%)
Nausea	0	5 (11.4%)	2 (4.9%)	3 (6.8%)
Somnolence	1 (2.2%)	2 (4.5%)	2 (4.9%)	5 (11.4%)
Fall	0	3 (6.8%)	2 (4.9%)	2 (4.5%)
Abdominal pain	0	4 (9.1%)	1 (2.4%)	1 (2.3%)
Headache	1 (2.2%)	0	5 (12.2%)	0
Hyperglycaemia	0	3 (6.8%)	1 (2.4%)	2 (4.5%)
Mental status changes	0	0	1 (2.4%)	5 (11.4%)
Paraesthesia	0	1 (2.3%)	2 (4.9%)	3 (6.8%)
Fatigue	0	1 (2.3%)	1 (2.4%)	3 (6.8%)
Hyperkalaemia	0	3 (6.8%)	1 (2.4%)	1 (2.3%)
Pruritus	0	3 (6.8%)	1 (2.4%)	1 (2.3%)
Anaemia	3 (6.7%)	0	1 (2.4%)	0
Hypertension	0	0	1 (2.4%)	3 (6.8%)
Pulmonary oedema	0	1 (2.3%)	0	3 (6.8%)

TEAE = treatment-emergent adverse event

Note: Adverse events were coded using the Medical Dictionary for Regulatory Activities Version 19.0. The table is sorted by descending subject incidence in the total column for preferred term. A subject was counted only once for each preferred term if he/she had multiple events of the same preferred term.

Source: Module 5.3.5.3 Integrated Summary of Safety, applicant table 31

This Phase 2 study demonstrated that somnolence and mental status changes are dose-related adverse events. While the phase 2 adverse event data appear to demonstrate a dose-dependent relationship between difelikefalin and hypertension, significant hypertensive events were infrequent. Only 1 subject with a history of hypertension discontinued the study due to experiencing hypertension after receiving the first infusion of difelikefalin 1.5 mg/kg. Overall, the percentage of difelikefalin-treated subjects with a systolic blood pressure ≥ 180 mm Hg was generally similar or lower at the end of the study as compared to the percentage of subjects at baseline.

Based on the phase 2 findings, the relationship between hypertension and the 0.5mcg/kg dose administered in the phase 3 trials was assessed. Across Phase 3 clinical studies CLIN3102 and CLIN3103 the incidences of systolic blood pressure (SBP) ≥ 180 mm Hg fluctuated. For difelikefalin-treated subjects, the baseline incidence of a SBP ≥ 180 mm Hg was 9%. The incidence of post-baseline systolic blood pressure ≥ 180 mm Hg ranged from 12% at the end of Week 24 to 8% at the end of Week 36. There were no discontinuations due to hypertension in either the Primary Safety Pool or the Difelikefalin Exposure Safety Pool. Serious adverse events related to hypertension (PTs of hypertension, hypertensive crisis hypertensive urgency, accelerated hypertension) were reported in the primary safety pool for 6 (1.4%) subjects and 1 (0.2%) subject in the placebo and difelikefalin arms, respectively. In addition, a total of 5 (0.4%) subjects reported a serious hypertensive event in the long-term difelikefalin exposure safety pool.

No relationship between hypertension and difelikefalin was identified in animal studies, exposed healthy subjects or in diseased subjects in the Phase 3 studies. There does not appear to be a correlation between difelikefalin treatment and an increase in blood pressure measurements based on the totality of the data.

Naloxone and Naltrexone
The Agency requested information on the use of opioid antagonist for the event of overdosage with difelikefalin from the applicant. In vitro, there is one binding study that used naloxone at a concentration of 10 uM to compete for the binding of [3H]-difelikefalin to the human kappa opioid receptor. The data show that naloxone does compete for difelikefalin on human KORs.

In vivo, there were two instances where naloxone was used to attenuate the severity of clinical signs following single and multiple doses of intravenous (IV) difelikefalin in a monkey maximum tolerated dose (MTD) toxicology study.

- At the highest single IV dose tested of 16 mg/kg, one female animal required veterinary intervention due to the severity of clinical signs, mainly ataxia, unresponsiveness and decrease in body temperature, where IV naloxone (0.2 mg/kg) was initially administered and appeared to improve the clinical signs but was followed a few hours later with another dose of 0.2 mg/kg naloxone administered subcutaneously due to the short-lived effects observed following the initial IV dose of naloxone to aid in the gradual recovery

of the animal. Although body temperature had increased, lethargy or decreased activity was present through 7 days post dose, at which point the animal appeared to recover fully.

- On Day 1 of the 7-day repeat dose phase of the monkey MTD study, one male animal administered an IV dose of 8 mg/kg difelikefalin initially exhibited stupor and decreased body temperature and at 5 hours post dose was unresponsive. The animal was given 0.2 mg/kg IV of naloxone at 5 hours post dose which transiently improved the clinical signs. At 6 hours after dosing, the clinical signs continued but the body temperature had increased so the animal was given a SC dose of 0.2 mg/kg naloxone and then another SC dose of naloxone 0.2 mg/kg at 12 hours post dose for the same clinical signs. By Day 2, unresponsiveness and low body temperature were no longer present and the animal continued to be dosed on each subsequent day of the study and the clinical signs continued to improve. By Day 3 or 4 through the remainder of the study, the animal showed signs of lethargy, decreased activity, intermittent tremors and/or decreased feces, but stupor, low body temperature and unresponsiveness were no longer observed.

In human clinical studies, there are no cases where naloxone was used to reverse the adverse effects of difelikefalin. In the investigator's brochure, the applicant suggested use of opioid antagonist for acute management of IV overdose and in extreme cases, suggested dialysis may be considered for treatment of overdose.

Insufficient information exists for the use of opioid antagonist such as naloxone in the treatment of difelikefalin overdosage. We know that difelikefalin is mostly excreted by the kidneys and is removed by dialysis. This reviewer recommends the addition of language in SECTION 10 OVERDOSAGE of the label with specific instructions on using dialysis in the event of severe adverse reactions due to difelikefalin.

Additional Safety Evaluation for Concomitant Medications
In addition to what is described in other sections of this review, further descriptions of concomitant opioid and CNS anti-depressant use is described and broken down for safety.

Table 29: Concomitant opioid use OR CNS depressant Medications – Primary Safety Pool

	CLIN3102 DB		CLIN 3103 DB		Pooled		
	Placebo (N=188) n (%)	CR845 0.5mcg/kg (N=189) n (%)	Placebo (N=236) n (%)	CR845 0.5mcg/kg (N=235) n (%)	Placebo (N=424) n (%)	CR845 0.5mcg/kg (N=424) n (%)	Overall (N=848) n (%)
Concomitant Opioid use							

YES	60 (31.9%)	48 (25.4%)	69 (29.2%)	55 (23.4%)	129 (30.4%)	103 (24.3%)	232 (27.4%)
NO	128 (68.1%)	141 (74.6%)	167 (70.8%)	180 (76.6%)	295 (69.6%)	321 (75.7%)	616 (72.6%)
Concomitant CNS – Depressant Medication Use							
YES	166 (88.3%)	154 (81.5%)	197 (83.5%)	187 (79.6%)	363 (85.4%)	341 (80.4%)	704 (83%)
NO	22 (11.7%)	35 (18.5%)	39 (16.5%)	48 (20.4%)	61 (14.4%)	83 (19.6%)	144 (17.0%)

Source: Module 5.3.5.3 ISS Table 12

Opioid Use:

- Tramadol (5.7%), hydrocodone/paracetamol (4.7%), oxycodone (3.1%)
- In the 12-Week Primary Safety Pool, 24.3% in the difelikefalin arms used opioid products.
- difelikefalin: 87 subjects (84.5%); Placebo: 105 subjects (81.4%) who did have (YES) concomitant opioid use (24.3%) experienced at least 1 TEAE
    - hyperkalemia (drug 11.7% vs placebo 6.2% RR 1.7887)
    - somnolence (drug 5.8% vs placebo 1.6% RR 2.7256)
- Overall TEAE Risk Ratio for difelikefalin vs placebo was 0.903

CNS Depressants

- Diphenhydramine (26.7%), clonidine (23.6%), gabapentin (17.7%), ondansetron (14.6%), hydroxyzine (11.3%)
- In the 12-Week Primary Safety Pool, 83% of subjects in the difelikefalin arms used some CNS depressant medication (YES)
- Difelikefalin: 254 (74.5%); Placebo 249 (68.6%) who did have (YES) concomitant CNS depressant use (83%) experienced at least 1 TEAE
    - Hyperkaliemia (RR 1.9657)
    - Dizziness (RR 2.0891)

Based on the Primary Safety Pool, subjects had a high percentage of CNS depressants use during the clinical trial. In addition, it was brought to this reviewer's attention that standard dialysis clinics will have diphenhydramine iv as a PRN (as needed) drug during hemodialysis. It is not clear if administration of PRN drugs was recorded at all investigation sites. Subanalysis by biostatistics do not show a difference in the efficacy results with positive use of CNS depressants or anti-itch medications. Furthermore, safety review of this product was not affected by the liberal use of CNS depressants or anti-itch medications.

Hemodialysis for Treatment of Overdosage

In the Primary Safety Pool, a similar number of subjects missed dialysis visits across the 2

groups; 202 (47.6%) subjects in the difelikefalin pooled group and 198 (46.7%) subjects in the placebo pooled group. Of the subjects who missed dialysis, most subjects (across both groups) missed 1-3 dialysis visits: 165 (38.9%) in the difelikefalin pooled group and 167 (39.4%) in the placebo pooled group, with very few subjects (less than 9%) across both groups missing more than 3 dialysis sessions.

In the Primary Safety Pool, the incidence of Treatment-Emergent Adverse Events (TEAEs) for subjects who missed at least one dialysis visit was 75.2% in the difelikefalin pooled group and 69.2% in the placebo pooled group. There was a similar increase in TEAEs in each treatment group (7.6% for difelikefalin and 7.3% for placebo), in subjects who missed dialysis, compared to subjects who did not miss dialysis. In subjects who missed dialysis, the most common (≥15% of subjects in the difelikefalin group) system organ classes (SOCs) of TEAEs in the pooled difelikefalin group versus the pooled placebo group were gastrointestinal disorders (31.2% difelikefalin and 19.2% placebo), infections and infestations (24.3% and 23.7%), nervous system (18.8% and 11.6%), and general disorders and administration site conditions (17.8% and 12.1%). Among them, gastrointestinal disorders were the only SOC where TEAEs were reported more frequently (by a factor of 1.5 or more) in the pooled difelikefalin group than in the pooled placebo group in subjects who missed dialysis and were reported more frequently (by a factor of 1.5 or more) in difelikefalin subjects who missed dialysis (31.2%) compared to those that did not miss dialysis (21.2%). In addition, cardiac disorders (9.9% and 6.6%) and ear and labyrinth disorders (2.5% and 0%) for drug vs placebo, respectively, was more frequent.

Additional analyses were conducted looking at any TEAEs that occurred during the missed dialysis period i.e., from the time of the missed dialysis visit up until the next dialysis visit. This analysis may provide a more accurate reflection of TEAEs that may have occurred due to missed dialysis, especially since the majority of subjects who missed dialysis missed only 1-3 dialysis visits.

Overall, there were 37.9% subjects in the difelikefalin group and 32% of subjects in the placebo group that experienced a TEAE during a missed dialysis period. There was only 1 SOC (hepatobiliary disorders) for which TEAEs were reported more frequently (by a factor of 1.5x or more) in the pooled difelikefalin group than in the pooled placebo group (2.3% and 0.6%, respectively) and were reported more frequently (by a factor of 1.5 or more) in the subjects who missed dialysis (2.3%) compared to those that did not miss dialysis (0%). There were 3 events of cholelithiasis and 1 event of acute hepatic failure.

In the Difelikefalin Exposure Safety Pool, there were 987 (75.6%) subjects who missed dialysis visits. Of the subjects who missed dialysis, 534 (40.9%) subjects missed 1-3 dialysis visits and 453 (34.7%) subjects missed more than 3 dialysis sessions. Subjects in the Difelikefalin Exposure Safety Pool continued in the extension studies and were treated for up to 52 weeks. The increase in TEAEs in subjects who missed dialysis compare to those that did not was similar to the Primary Safety Pool.

The conclusions obtained from this analysis is that both safety pools showed the same trends based on adverse events profile of those that missed dialysis and those that did not miss dialysis and received the investigational drug, despite the duration of exposure. Subjects in the Primary Safety Pool who missed dialysis reported a greater frequency of gastrointestinal events, namely diarrhea and abdominal pain, and several other less commonly reported TEAEs (back pain, upper respiratory tract infection, atrial fibrillation, gastroenteritis, contusion, syncope, and chronic obstructive pulmonary disease). In the Difelikefalin Exposure Safety Pool, the gastrointestinal TEAEs of diarrhea and abdominal pain were the only events that were reported at a greater frequency, which is consistent with the TEAEs of the Primary Safety Pool in subjects who missed dialysis.

Across both safety pools, the TEAEs during the missed dialysis period, increase in gastrointestinal events and the other less commonly reported events were not evident, suggesting these events are likely not due to missed dialysis. The label suggests that if missed dialysis and dosing, difelikefalin may be given at the next interval dialysis. This reviewer would agree with that assessment. In addition, hemodialysis may be used to treat overdosage of difelikefalin as it is dialyzed out in renal failure patients. See draft labeling.

8.2.10. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

Difelikefalin is not approved in the U.S. or outside of the U.S. A different kappa agonist product is approved in Japan and was discussed earlier in the review. The Japanese product, nalfurafine (REMITCH) oral capsules is a kappa agonist for the treatment of uremic pruritus in hemodialysis and/or chronic liver disease when other existing treatment is insufficient. REMITCH capsules is distributed in the 2.5mcg dose. The label (translated to English) is shown in Figure 9.

Figure 9: Japanese Label for REMITCH CAPSULES

Kusuri-no-Shiori

Internal
Revised: 5/2020

The information on this sheet is based on approvals granted by the Japanese regulatory authority. Approval details may vary by country. Medicines have adverse reactions (risks) as well as efficacies (benefits). It is important to minimize adverse reactions and maximize efficacy. To obtain a better therapeutic response, patients should understand their medication and cooperate with the treatment.

Brand name :REMITCH CAPSULES 2.5 mcg
Active ingredient: Nalfurafine hydrochloride
Dosage form:slightly pale yellow to pale yellow capsule (major axis: approx. 9.7 mm, minor axis: approx. 6.6 mm)
Print on wrapping:レミッチカプセル2.5μg, TR11, REMITCH

Effects of this medicine

This medicine is a selective κ (kappa) opioid receptor agonist to suppress itch. It controls itch when other existing antihistamine or antiallergic medicines are ineffectual.
It is usually used for improvement of itch in dialysis and/or chronic liver disease when other existing treatment is insufficient.

Before using this medicine, be sure to tell your doctor and pharmacist

- If you have previously experienced any allergic reactions (itch, rash, etc.) to any medicines.
- If you are pregnant or breastfeeding.
- If you are taking any other medicinal products. (Some medicines may interact to enhance or diminish medicinal effects. Beware of over-the-counter medicines and dietary supplements as well as other prescription medicines.)

Dosing schedule (How to take this medicine)

- **Your dosing schedule prescribed by your doctor is ((to be written by a healthcare professional))**
- In general, for adults, take 1 capsule (2.5 mcg of the active ingredient) at a time, once daily, after supper or before bedtime. The dosage may be increased according to the symptoms, however, the dosage is limited to 2 capsules (5 mcg) once daily. Strictly follow the instructions.
- If you are taking this medicine for improvement of pruritus caused by hemodialysis, leave a sufficient interval between taking this medicine and receiving hemodialysis.
- If you are taking this medicine for improvement of pruritus caused by peritoneal dialysis, leave a sufficient interval between taking this medicine and exchanging dialysate.
- If you miss a dose, in case you are instructed to take the medicine after supper and remember it before bedtime on the same day, take a dose as soon as possible. In other cases, skip the missed dose and continue your regular dosing schedule. You should never take two doses at one time.
- If you accidentally take more than your prescribed dose, consult with your doctor or pharmacist.
- Do not stop taking this medicine unless your doctor instructs you to do so.

Precautions while taking this medicine

- The medicine may cause sleepiness or dizziness. Do not drive a car or operate dangerous machinery.
- Grapefruit juice may raise the plasma concentration and enhance the effect of the medicine. Never take the medicine with grapefruit juice.

Possible adverse reactions to this medicine

The most commonly reported adverse reactions include sleeplessness, constipation and sleepiness in hemodialysis; sleeplessness, sleepiness and vomiting in peritoneal dialysis; constipation, pollakiuria, nocturia, sleeplessness and sleepiness in chronic liver disease. If any of these symptoms occur, consult with your doctor or pharmacist.

The symptoms described below are rarely seen as initial symptoms of the adverse reactions indicated in brackets. If any of these symptoms occur, stop taking this medicine and see your doctor immediately.

- generalized fatigability, loss of appetite, yellowness of the skin or conjunctiva [liver dysfunction, jaundice]

The above symptoms do not describe all the adverse reactions to this medicine. Consult with your doctor or pharmacist if you notice any symptoms of concern other than those listed above.

Storage conditions and other information

- Keep out of reach of children. Store away from light, heat and moisture. Remove from press-through package (PTP) sheet just before taking.
- Discard the remainder. Do not store them. Ask your pharmacist or medical institution how to discard them.

For healthcare professional use only: Day Month Year

Reference: Online search

In addition to obtaining the label for REMITCH, DEPI conducted a search of FAERS and VigiBASE, the World Health Organization-Uppsala Monitoring Centre's database, for AE reports associated with nalfurafine (REMITCH).

FAERS

In the FAERS search for Product Active Ingredient: Nalfurafine hydrochloride, DEPI used all time through April 27, 2021.

The results showed that N=9 with no deaths attributed to REMITCH. All PTs coded in the 9 reports are displayed below, followed by a brief summary of each report with the PTs italicized.

FAERS Case # 12275604: 80-year-old woman with chronic hep C experienced Generalized edema 3 weeks after starting ombitasvir/paritaprevir/ritonavir (discontinued spironolactone and amlodipine at the same time), then Cardiac failure congestive and Anemia occurred approximately 1 month later. Nalfurafine was started 12 days prior to initiating ombitasvir/paritaprevir/ritonavir. She was hospitalized for cardiac failure and improved upon discontinuing ombitasvir/paritaprevir/ritonavir and nalfurafine.

FAERS Case # 14923236: 64-year-old man with multiple cardiovascular and hepatic medical histories developed Tremor on the same day he started nalfurafine and an unspecified time after starting ferric citrate hydrate to treat iron deficiency anemia. Nalfurafine was discontinued on the same day and the tremor resolved 4 days later. No information was provided regarding the action taken for ferric citrate hydrate. He was taking 14 other concomitant medications.

FAERS Case # 15920368: 58-year-old man with compensated cirrhosis, end-stage renal failure on dialysis, and hypertension experienced Ascites, Blood albumin decreased, and Blood creatinine increased, possibly related to glecaprevir/pibrentasvir use. He was also taking 6 other concomitant medications, including nalfurafine; no therapy dates were provided for these drugs.

FAERS Case # 7063175: 67-year-old man with diabetic nephropathy, thrombocytopenia, and severe anemia (requiring transfusions) developed Eosinophilia and Rash about 1 month after starting lanthanum carbonate for hyperphosphatemia and nalfurafine. Both drugs were discontinued and the patient recovered on an unspecified date.

FAERS Case # 8394021: 83-year-old woman with a history of Dementia that was aggravated due to a possible drug interaction with itraconazole. The patient had 19 concomitant medications, including nalfurafine.

FAERS Case # 8468753: 59-year-old man with restless leg syndrome, and multiple cardiovascular and renal disorders developed somatic hallucination (description not provided) ~2 months after starting nalfurafine. The patient had 4 concomitant medications, including pramipexole, but nalfurafine was most recently initiated prior to developing somatic hallucination. He also experienced Supraventricular tachycardia, Diabetes mellitus, and Pruritus prior to the initiation of nalfurafine.

FAERS Case # 8770962: 70-year-old man with a history of dialysis experienced Delirium, Hallucination, and Hallucination, visual (described as “cats came out or ghosts came out when the patient stayed in bed at night”) ~1 year after starting nalfurafine. He was also taking hydroxyzine and zolpidem. AEs resolved but unknown action taken with nalfurafine and

zolpidem; continued hydroxyzine.

FAERS Case # 9033510: 66-year-old man with a history of chronic renal failure on dialysis and Parkinson's disease experienced Sudden onset of sleep and Road traffic accident was attributed to pramipexole. His concomitant medications that were continued were nalfurafine, epinastine hydrochloride (antihistamine), droxidopa; unknown action taken were lafutidine, verapamil, warfarin, and allopurinol.

FAERS Case # 9319041: 56-year-old man with a history of restless leg syndrome and chronic renal failure on dialysis experienced Hepatic function abnormal while on treatment with rotigotine patch and nalfurafine. Therapy dates for both drugs were not reported. The hepatic function tests were taken one week apart, presumably, he discontinued rotigotine patch in between labs:

AST: 134 IU/L -> 29 IU/L
ALT: 70 IU/L -> 36 IU/L
GGT: 330 IU/L -> 352 IU/L
ALP: 370 IU/L -> 406 IU/L
LDH: 279 IU/L -> 231 IU/L

VigiBase

A search for Drugs: Nalfurafine (Active ingredient), Remitch (Trade name) in the VigiBase database for Dataset Date: April 28, 2021.

(b) (4)

1 Page has been Withheld in Full as b4 (CCI/TS) immediately following this page

Given that the Japanese product REMITCH has been on the market since 2009 and has limited uptake and use, it is likewise that difelikefalin will have limited population penetration in the United States. Post-marketing evaluation of a product within the same drug class did not identify additional safety concerns that would lead to a targeted risk mitigation strategy. REMS is not recommended for this product as an IV only treatment in hemodialysis patients. Labeling should be sufficient to prompt safe use of IV difelikefalin. In addition, standard post-marketing safety monitoring is sufficient for this drug product in the marketplace.

8.2.11. Integrated Assessment of Safety

The safety profile of difelikefalin was characterized for the adults-only, hemodialysis population through analyses of 1306 subjects in the Phase 3 clinical trials, the open-label extensions, and the open-label clinical trials. This safety analysis was focused on the Primary Safety Pool, the

two Phase 3 clinical trials CLIN3102 and CLIN3103 (N=848). Difelikefalin had an acceptable safety profile and was well tolerated. The most common treatment-emergent adverse events (TEAEs) in the Primary Safety Pool (≥ 2% in difelikefalin and ≥ 1% than placebo) included diarrhea, gait disturbances/falls, dizziness, nausea, hyperkalemia, headache, somnolence, and back pain. These events were mostly mild-to-moderate in severity and few events lead to discontinuations.

The incidence of fatal TEAEs and serious TEAEs were similar to placebo and deaths were considered unrelated to study drug. Serious adverse events are expected to occur in this population due to underlying disease as mortality remains high and high rates of comorbidities are often present in hemodialysis patients. The incidence of serious TEAEs in the cardiac disorders system organ class (SOC) was low, but with treatment group imbalances noted between the difelikefalin and placebo group. However, the incidence rates for serious cardiac events in the Difelikefalin Exposure Safety Pool did not show a notable increase over the rates observed in the Primary Safety Pool, with the rates generally consistent with cardiac event rates reported for patients undergoing HD, a patient population highly burdened by cardiac comorbidities.

There were no unexpected safety signals that emerged during the long-term use of difelikefalin, with the nature of the reported safety events aligned with the morbidity and mortality in patients with moderate-to-severe CKD-aP undergoing hemodialysis. We also examined subjects who missed dialysis for any adverse events. There was a similar increase in TEAEs in each treatment group (7.6% for difelikefalin and 7.3% for placebo), in subjects who missed dialysis, compared to subjects who did not miss dialysis. The TEAEs were not significantly different in those who missed dialysis and received the drug, the drug may be given at the next interval dialysis after a missing session.

Difelikefalin is expected to have no meaningful abuse potential and no physical dependence. The applicant conducted appropriate clinical studies meeting the Agency CSS criteria. In addition, this Reviewer asked the applicant to provide information on the use of reversal agents for opioids in the event of overdosage. The data provided were limited and a consult to the Agency Division of Analgesic (DAAP) recommended no PMC/PMR for this novel kappa opioid agonist. Product labeling and routine pharmacovigilance monitoring should serve as adequate risk mitigation strategies.

8.3. Summary and Conclusions

8.3.1. Statistical Issues

There were no major statistical issues affecting the overall conclusions. For both Phase 3 trials (i.e., CLIN3102 and CLIN3103), the Applicant conducted an interim analysis for sample size re-estimation after 50% of the planned 350 randomized subjects either completed the 12-week treatment period of discontinued study drug. The interim analyses were prespecified and appropriate statistical approaches to control the Type I error rate and adjust the point estimates were prespecified in the protocols. The results with adjusting for the interim analysis (see Table 6) were similar to those without adjusting for the interim analysis (see Table 38). In terms of handling missing data, results were generally similar irrespective of the method used to impute the missing data (see Table 8 and Table 9). In addition, there were no substantial differences in efficacy among subgroups (see Table 12 through Table 15).

In addition, based on the Agency's recommendation that the proportion of subjects achieving a ≥ 4-point improvement in WI-NRS score from baseline to Week 12 as the primary efficacy endpoint, the Applicant evaluated this endpoint as the first key secondary efficacy endpoint in both Phase 3 trials. With the efficacy results, the Agency intends to use the ≥ 4-point improvement in WI-NRS as the primary endpoint in labeling.

8.3.2. Conclusions and Recommendations

Difelikefalin, a selective, kappa opioid receptor (KOR) agonist, is a new molecular entity (NME) proposed for the treatment of moderate-to-severe chronic kidney disease-associated pruritus (CKD-aP) in adult patients undergoing hemodialysis (HD).

Approximately 20% to 40% of patients undergoing hemodialysis (HD) suffer from moderate-to-severe chronic kidney disease associated pruritus (CKD-aP). CKD-aP is a medical condition characterized by a generalized and intractable itch. This systemic pruritus does not originate from skin lesions, but rather is a persistent itch sensation that often leads to considerable mechanical skin damage due to a continuous and uncontrollable urge to scratch. Patients with CKD-aP suffer from severely impaired physical and mental health. In addition, subjects who are undergoing HD and have severe itching have a higher rate of all-cause mortality, including higher rates of cardiovascular-related mortality and infection-related mortality, relative to patients without pruritus.

No treatment has been approved for CKD-aP in the United States. Several treatments have been used off-label, such as antihistamines, corticosteroids, gabapentin, and pregabalin; however, these drugs are limited by a lack of proven antipruritic efficacy and poor tolerability. Thus, there remains an unmet medical need for safe and effective treatments for CKD-aP in a susceptible population of patients who present with significant comorbid conditions.

Difelikefalin demonstrated effectiveness in two adequate and well-controlled trials in subjects with moderate-to-severe CKD-aP undergoing HD. These two replicate trials showed difelikefalin to be statistically improved to placebo for the protocol-specified primary efficacy endpoint (≥3-point improvement in WI-NRS at Week 12) and the Agency's recommended primary efficacy endpoint (≥4-point improvement in WI-NRS at Week 12) in the target population, see Table 6 in Section 8.1.5. For the proportion of subjects achieving a ≥3-point improvement (reduction) in WI-NRS at Week 12, the treatment effect (i.e., difference between difelikefalin and placebo) was 22% and 11% for Trials CLIN3102 and CLIN3103, respectively. For the ≥4-point improvement (reduction) threshold at Week 12, the treatment effect was 19% and 12% for Trials CLIN3102 and CLIN3103, respectively. The odds ratios for both endpoints ranged from 1.6 to 2.9 across the two trials.

The safety profile of difelikefalin was characterized for this hemodialysis population through analyses of 1306 subjects in the Phase 3 clinical trials, the open-label extensions, and the open-label clinical trials. This analyses was focused on the Primary Safety Pool, the two Phase 3 clinical trials CLIN3102 and CLIN3103 (N=848). Difelikefalin had an acceptable safety profile and was well tolerated. The most common treatment-emergent adverse events (TEAEs) in the Primary Safety Pool (≥ 2% in difelikefalin and ≥ 1% than placebo) included diarrhea, gait disturbances/falls, dizziness, nausea, hyperkalemia, headache, somnolence, and back pain. These events were mostly mild-to-moderate in severity and few events lead to discontinuations. The incidence of fatal TEAEs and serious TEAEs were similar to placebo and deaths were considered unrelated to study drug.

The incidence of serious TEAEs in the cardiac disorders system organ class (SOC) was low, but with treatment group imbalances noted between the difelikefalin and placebo group. However, the incidence rates for serious cardiac events in the Difelikefalin Exposure Safety Pool did not show a notable increase over the rates observed in the Primary Safety Pool, with the rates generally consistent with cardiac event rates reported for patients undergoing HD, a patient population highly burdened by cardiac comorbidities. There were no unexpected safety signals that emerged during the long-term use of difelikefalin, with the nature of the reported safety events aligned with the morbidity and mortality in patients with CKD-aP undergoing hemodialysis. Difelikefalin is expected to have no meaningful abuse potential and no physical dependence. Product labeling and routine pharmacovigilance monitoring should serve as adequate risk mitigation strategies.

Overall, the data provided by the applicant for difelikefalin intravenous administration of 0.5 mcg/kg after each dialysis for the treatment of moderate-to-severe CKD-aP in hemodialysis patients appear to show that the benefits of treatment outweigh the potential risk associated with difelikefalin treatment. The application provides evidential support for the approval of difelikefalin.

9 Advisory Committee Meeting and Other External Consultations

An advisory committee was not convened for this product.

10 Pediatrics

The initial pediatric study plan (iPSP) was submitted in NOV 2017. At the time, the Agency review of the iPSP found it to be insufficient and lacking supportive data to demonstrate that pruritus in children on hemodialysis was not a disease that was rare. The sponsor was informed of the insufficient iPSP and resubmitted in AUG 2019. The iPSP was discussed at PeRC and the Division recommended against a full pediatric waiver for subjects <17 year of age. The Division and PeRC recommended a partial waiver down to 8 years of age and the conduct of a PK/PD/safety trial in adolescent patients on hemodialysis with end stage renal disease. The applicant collected further data to submit to the Agency with references from a survey of (b) (4)

The survey concludes:

"The current rate of uremic pruritus in pediatric patients undergoing hemodialysis reported as by the PNRC members is very low; 1.2% with half of the patients requiring medications to manage their pruritus."

The Division has not been able to find any literature to confirm or deny this and are inclined to accept this rationale. Further discussion with the Chairperson of Agency PeRC agrees with the conclusion of the consortium information.

Reviewer's comment: *The Agency accepts the iPSP as submitted. Difelikefalin will be granted a full waiver for studies in pediatric patients 17 years and younger.*

13 Pages of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

12 Risk Evaluation and Mitigation Strategies (REMS)

No REMS are recommended for this approval.

13 Postmarketing Requirements and Commitment

No PMR/PMCs are recommended for this drug product approval.

14 Division Director (Clinical) Comments

NDA 214916 was submitted through the 505(b)(1) regulatory pathway by the applicant for KORSUVA (difelikefalin) intravenous drug product in support of an indication for the treatment of moderate-to-severe pruritus associated with chronic kidney disease (CKD-aP) in adults undergoing hemodialysis (HD). No approved treatments for CKD-aP are currently available in the United States, although several treatments are used off-label.

Difelikefalin is a small synthetic peptide that acts as a peripheral kappa opioid receptor (KOR) agonist. It has no binding activity at mu opioid receptors (MORs), the main target of opioid analgesics. The physiochemical properties of difelikefalin were selected by the applicant to prevent/minimize central nervous system (CNS) penetration. It has high hydrophilicity and a synthetic peptidic structure composed entirely of non-natural D-amino acids with high polar surface areas and charge at physiological pH. Minimal penetration of difelikefalin through the BBB is supported by in vitro and in vivo non-clinical studies.

Approximately 500,000 patients are currently undergoing HD in the United States (US; 2019, USRDS), and a subset of these patients will report moderate to severe pruritus. The pathophysiology of CKD-aP is not fully understood but is thought to be multifactorial and include effects of systemic inflammation and an imbalance in the endogenous opioid system. Opioid receptors are known to modulate itch signals and inflammation, with KOR activation reducing itch and producing immunomodulatory effects.

In support of this application the sponsor conducted 18 clinical studies, including two phase 3, placebo-controlled, randomized, 12-week, double-blind studies with up to 52-week open-label extensions and two open-label safety studies in subjects with CKD-aP. In the phase 3 clinical trials, difelikefalin was administered as a dose of 0.5 mcg/kg by intravenous bolus injection into the venous line of the dialysis circuit at the end of each HD, 3 times per week. Key inclusion criteria included having a mean baseline WI-NRS score >4 (Trial CLIN3102) or ≥5 (Trial

CLIN3103) after a 7-day run-in period. Randomization was stratified by use of anti-itch medication at baseline and history of fall, confusional state or mental status change, and gait disturbance or movement disorder.

The protocol-specified primary efficacy endpoint for both phase 3 trials was the proportion of subjects achieving at least a 3-point improvement on the 11-point Worst Itch Numeric Rating Scale (WI-NRS) score from baseline to Week 12. In several communications to the sponsor the Agency recommended the primary efficacy endpoint to be the proportion of subjects achieving at least a 4-point improvement in WI-NRS score from baseline to Week 12. In both trials, difelikefalin was statistically superior to placebo for both thresholds at Week 12 (p-values ≤ 0.020). In a sponsor-conducted analysis pooling the 2 phase 3 clinical trials, 38.7% of difelikefalin-treated subjects were responders as compared to 23.4% of placebo-treated subjects at Week 12 on the 4-point improvement in WI-NRS score. Efficacy findings were generally consistent across subgroup analyses, including baseline h/o of use of anti-itch medication.

The safety profile of difelikefalin in CKD patients undergoing hemodialysis appears to be adequately characterized. A total of 1879 subjects received at least one dose of difelikefalin, including 1400 subjects undergoing HD who received the to-be-marketed dose. The primary safety pool comprised the 12-week placebo-controlled period of the 2 phase 3 studies and included 424 pooled difelikefalin and 424 pooled placebo subjects who received ≥1 dose of their assigned treatment, with a median duration of treatment of 85.0 days. The difelikefalin exposure safety pool included 1306 subjects who received at least one dose of difelikefalin; 1089 were exposed for at least 3 months, 711 subjects for at least 6 months, and 400 subjects were exposed for at least 12 months.

HD patients have a high incidence of life-threatening comorbid conditions such as cardiovascular disease and are at risk for life-threatening infections, including vascular-access related infections and sepsis. Consequently, they have a relatively high mortality rate as compared to the general population. The mortality rates observed in the difelikefalin development program fall withing the range of mortality rates reported for US HD patients, with the exception of cardiac failure. When the terms of cardiac failure and cardiac failure congestive were combined, the mortality incidence rate in the long-term safety pool was 7.4 events per 1000 patient years (PY), as compared to 3.7 events per 1000 PY reported in the USRDS 2019 report. The sponsor referenced literature supporting that HD patients with more severe pruritus have higher rates of cardiac failure. No imbalance in deaths was reported during the randomized, double-blind, placebo-controlled portions of the 2 phase 3 trials, when 3 subjects receiving difelikefalin and 5 subjects receiving placebo died. The causes of death included gastrointestinal bleed leading to cardiac failure, myocardial infarction, cardiac arrest, staphylococcal sepsis, and septic shock. No deaths during treatment with difelikefalin were considered related to treatment.

The most common serious adverse events reported were in the system organ class of infections and infestations, cardiac disorders, and respiratory, thoracic, and mediastinal disorders. The incidence of serious adverse events in the 12-week, blinded, placebo-controlled period of the phase 3 studies was generally similar between difelikefalin and placebo, with the exception of cardiac disorders. Notably, during this placebo-controlled period, the incidence rate of cardiac disorders in difelikefalin-treated subjects fell within the range of reported incidence rate of cardiac disorders in this patient population.

Although non-clinical data indicated minimal CNS penetration, imbalances in nervous system and psychiatric disorders were observed between difelikefalin-treated and placebo-treated subjects, including the incidence of discontinuations due to these events. The incidence of falls (falls combined with gait disturbances), dizziness, headache, somnolence, and mental status changes (mental status change combined with confusional state) were reported more frequently in difelikefalin-treated subjects. Somnolence was reported more frequently in subjects at least 65 years of age (7.0%) than in subjects under 65 years of age (2.8%). The most common adverse events reported as related to treatment were somnolence and dizziness. As a result, product labeling includes warnings about dizziness, somnolence, gait disturbances and risk of driving and operating machinery. Despite these findings, clinical evaluation did not demonstrate potential for abuse or physical dependence and difelikefalin will not be a scheduled drug.

In summary, the applicant has provided substantial evidence of efficacy for difelikefalin for the treatment of moderate to severe pruritus in patients on hemodialysis. The safety profile has been well characterized and product labeling appears adequate to convey adverse reactions associated with its use.

15 Office Director Comments

I concur with the recommendation from the Division of Dermatology and Dentistry to approve NDA 214916, difelikefalin, for the treatment of moderate-to-severe pruritus associated with chronic kidney disease (CKD-aP) in adults undergoing hemodialysis. Hemodialysis patients can experience intractable generalized pruritus. There are no approved therapies in this setting, and available anti-pruritus medications used off-label have limited efficacy.

Difelikefalin is a kappa opioid receptor (KOR) agonist and a new molecular entity. Its action on peripheral sensory neurons and immune cells is believed to result in anti-pruritic and anti-inflammatory effects. Preferential activation of KORs outside of the CNS is thought to mitigate against adverse events associated with centrally-acting KOR agonists, such as dysphoria. Difelikefalin is administered intravenously at the end of each dialysis session.

Substantial evidence of effectiveness comes from two replicate placebo-controlled, randomized trials conducted in hemodialysis patients with moderate-to-severe CKD-aP showing that difelikefalin statistically improved pruritus relative to placebo.

The Difelikefalin Exposure Safety Pool included 1306 patients with a median duration of continuous exposure to active treatment of 6.9 months. Difelikefalin was generally well tolerated with dizziness, somnolence, mental status changes, gait disturbances, and falls reported more frequently in difelikefalin-treated patients compared to placebo-treated patients. Concomitant use of centrally-acting depressant medications, antihistamines and opioids may increase the likelihood of these adverse reactions and should be avoided during treatment. Product labeling will adequately warn about these risks. Difelikefalin is expected to have no meaningful abuse potential and no potential for physical dependence.

Serious adverse events included infections, fluid overload, myocardial infarction, and respiratory, thoracic, and mediastinal disorders. These events are consistent with the clinical hallmarks of chronic kidney disease in patients undergoing hemodialysis and are not directly attributable to the use of difelikefalin.

16 Appendices

16.1. References

References are in foot notes in text.

16.2. Financial Disclosure

Financial disclosure FDA form 3454 was submitted and a list of the covered clinical studies and investigators were provided.

Covered Clinical Study (Name and/or Number): CR-CLIN3102 AND CR845-CLIN3103

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 142		
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: NA Significant payments of other sorts: NA Proprietary interest in the product tested held by investigator: NA Significant equity interest held by investigator in S Sponsor of covered study: NA		
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) NA		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation

		from Applicant)

16.3. Nonclinical Pharmacology/Toxicology

16.3.1. Calculations for multiples of exposures

At the recommended human dose (0.5 μg/kg/day) in adult subjects, $AUC_{0\text{-}72h}$ and C_{max} were 78.6 ng·hr/mL and 5.90 ng/mL, respectively (study # CR845-CLIN2005). Even though some of the nonclinical study reports (fertility, rat EFD, and mouse carcinogenicity studies) only provided $AUC_{0\text{-}24h}$ values, such values were used nevertheless in place of $AUC_{0\text{-}72h}$ in calculating multiples of exposure. The rationale is that $AUC_{0\text{-}24h}$ is not expected to differ much from $AUC_{0\text{-}72h}$ given the short T_{max} (several minutes) and short half-life (<6 hr) of difelikefalin in the blood in animal studies. For the prenatal and postnatal development (PPND) study in rats, the sponsor did not provide AUC data. However, because systemic exposure (gestation day 17) of difelikefalin in the rat EFD study was dose-proportional in the dose range of 0.25-25 mg/kg/day, the reviewer was able to estimate the $AUC_{0\text{-}72h}$ values at the doses of 0.6 and 10 mg/kg/day for the PPND study. The following table summarizes the multiples of exposure based on AUC comparisons between the MRHD and AUC values from nonclinical studies referenced in the label.

Study Type	Dose of Interest (NOAEL or dose that resulted in toxicity as described in label) (mg/kg/day)	Estimated $AUC_{0\text{-}72h}$ (ng·hr/mL)	Multiples of exposure
Fertility	Female NOAEL for mating: 0.25 mg/kg/day	401	5
	Minimal dose that caused prolonged diestrus: 2.5 mg/kg/day	4410	56
	Female NOAEL for fertility and early embryonic development: 25 mg/kg/day	49900	635
	Male NOAEL: 25 mg/kg/day	76300	971
Embryofetal development (Female Rats)	NOAEL for fetal effects: 25 mg/kg/day*	55900	711
Embryofetal development (Female Rabbits)	NOAEL for fetal effects: 0.1 mg/kg/day	679	9
Prenatal and postnatal development (Dams plus pups)	NOAEL for maternal effects: 0.6 mg/kg/day Minimal dose that caused decreases of food consumption,	1065	14

	maternal body weight and/or maternal body weight gain: 2.5 mg/kg/day NOAEL for F1 Generational effects: 10 mg/kg/day	5333 22181	68 282
Carcinogenicity (Rats)	NOAEL: 1 mg/kg/day	46900 (male) 161000 (female)	597 2048

*: Skeletal variations only were seen at this dose.

16.3.2. Nonclinical labeling

Recommended changes to nonclinical information in sections 8.1, 8.2, 8.3, 12.1, and 13.1 of the applicant's proposed labeling are provided below. The pharmacologic class for difelikefalin is kappa opioid receptor agonist. Although the applicant provided nonclinical data to factually support statements made in section 12.1, several portions are of unclear relevance to the mechanism of action and should be removed. Section [redacted] should be removed from the label [redacted]. Reviewer-recommended deletions and additions are indicated by ~~strike through~~ and underlined text, respectively.

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE
KORSUVA is a kappa opioid receptor agonist indicated for the treatment of...

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Risk Summary
The limited human data on use of KORSUVA in pregnant women are not sufficient to evaluate a drug-associated risk for major birth defects or miscarriage. In animal reproduction studies, intravenous injection of difelikefalin to pregnant rats and rabbits during the period of organogenesis at doses 711 and 9 times the maximum recommended human dose (MRHD), respectively, resulted in no adverse effects in either rats or rabbits *(see Data)*.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data
Animal Data
In an embryofetal development study, difelikefalin was administered by intravenous injection to pregnant rats at doses of 0.25, 2.5, and 25 mg/kg/day during the period of organogenesis.

Difelikefalin was not associated with embryofetal lethality or fetal malformations. Difelikefalin increased the incidences of skeletal variations (wavy ribs and incompletely ossified ribs) at the dose of 25 mg/kg/day (711 times the MRHD based on AUC comparison).

In an embryofetal development study, difelikefalin was administered by intravenous injection to pregnant rabbits at doses of 0.025, 0.05, and 0.1 mg/kg/day during the period of organogenesis. Maternal toxicity evidenced by decreased maternal body weight gain was noted in all dose groups. Difelikefalin was not associated with embryofetal lethality or fetal malformations at doses up to 0.1 mg/kg/day (b) (4) times the MRHD based on AUC comparison).

In a prenatal and postnatal development study, difelikefalin was administered by intravenous injection to pregnant rats at doses of 0.6, 2.5, and 10 mg/kg/day beginning on gestation day 7 and continuing through lactation day 20. Persisting effects on decreased maternal body weight and/or maternal body weight gain as well as food consumption were noted at doses greater than or equal to 2.5 mg/kg/day (68 times the MRHD based on AUC comparison). No maternal effects were observed at 0.6 mg/kg/day (14 times the MRHD based on AUC comparison). No difelikefalin-related effects on postnatal developmental, neurobehavioral, or reproductive performance of offspring were noted at doses up to 10 mg/kg/day (282 times the MRHD based on AUC comparison).

8.2 Lactation

Risk Summary

There are no data regarding the presence of KORSUVA in human milk or effects on the breastfed infant or on milk production.

Studies in rats showed difelikefalin was transferred into the milk in lactating rats (b) (4) When a drug is present in animal milk, it is likely that the drug will be present in human milk. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for KORSUVA and any potential adverse effects on the breastfed child from KORSUVA or from the underlying maternal condition.

Data

Animal Data

Difelikefalin was administered to lactating rats by intravenous injection at doses of 0.6, 2.5, or 10 mg/kg/day from gestation day 7 through lactation day 14. Difelikefalin was detected in the milk of the lactating rats with the concentration ratio for milk:plasma of 0.04 to 0.05 across the doses. There was no measurable difelikefalin in the plasma of nursing pups.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

KORSUVA is a kappa opioid receptor (KOR) agonist. The relevance of KOR activation to therapeutic effectiveness is not known.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 2-year carcinogenicity study in rats, difelikefalin was not carcinogenic when administered via subcutaneous injection at doses up to 1.0 mg/kg/day (597 times the MRHD based on AUC comparison). Difelikefalin was not carcinogenic in a 6-month carcinogenicity study in transgenic rasH2 mice at subcutaneous doses up to 30 mg/kg/day.

Difelikefalin was negative for genotoxicity in a bacterial reverse mutation assay, an in vitro mammalian chromosomal aberration assay, and an in vivo mouse micronucleus assay.

Difelikefalin administered via intravenous injection caused a significant decrease in the number of estrous cycles per 14 days (i.e., prolonged diestrus) in female rats at doses greater than or equal to 2.5 mg/kg/day (56 times the MRHD based on AUC comparison). Difelikefalin had no effects on mating index, fertility index, or any ovarian or uterine parameters in female rats at doses up to 25 mg/kg/day (635 times the MRHD based on AUC comparison). Difelikefalin did not impair male fertility at doses up to 25 mg/kg/day (971 times the MRHD based on AUC comparison).

16.3.3. Review of Carcinogenicity Studies Conducted with difelikefalin

Study Title: CR845: 26-Week Repeated Dose Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

Study no.:	CR845-CARC086, study report No. AC06EH.7S8R.BTL
Study report location:	SDN 1, Module 4.2.3.4.1
Conducting laboratory and location:	[redacted]
GLP compliance:	Yes
Drug, lot #, and % purity:	Difelikefalin (CR845), lot # MZ77268, purity 99.7%
Prior Exec CAC Dose Concurrence:	Y
Basis for Dose Selection:	MTD

Reviewer Carcinogenicity Conclusion: Negative
ECAC Carcinogenicity Conclusion: Negative

Tumor Findings: No statistically significant differences in tumor incidences were observed in mice of either sex in this study, according to the statistical criteria used by the Executive Carcinogenicity Assessment Committee (ECAC). The NOAEL was the highest dose 30 mg/kg/day.

Methods

Doses:	0 (vehicle control), 3, 10, and 30 mg/kg/day difelikefalin
Frequency of dosing:	Once daily
Number/Sex/Group:	25
Dose volume:	10 mL/kg
Formulation/Vehicle:	Sterile saline
Route of administration:	SUBCUTANEOUS
Species:	MOUSE
Strain:	CB6F1-TgN (RasH2)
Age:	Approx. 8 weeks at initiation of treatment.
Comment on Study Design and Conduct:	The intended route of administration to humans is intravenous. Since daily intravenous injections in mice was not feasible for the 6-month study, subcutaneous dosing was selected. This route could result in exposure in mice that is comparable to or exceeds the expected exposure in humans. The main study was conducted in hemizygous Tg.rasH2 mice; however, the TK study was conducted in wild-type homozygous Tg.rasH2 mice. The study design is summarized below:

Group	Dose Levels (mg/kg/day)	Number of Animals			
		Main Cohort (Tg.rasH2)		TK Cohort** (CByB6F1)	
		Male	Female	Male	Female
Group 1	0	25	25	5	5
Group 2	3	25	25	20	20
Group 3	10	25	25	20	20
Group 4	30	25	25	20	20
Group 5*	1000 (urethane)	10	10	-	-
Total***		110	110	65	65

*The positive control animals were administered a total of 3 i.p. injections, one each on Days 1, 3, and 5 (or Days 5, 7, and 9 for the replacement animal).
**Extra TK animals (2/sex/group) were used ensure adequate animal numbers for TK bleeding.
***At the discretion of the Study Director, animals that were found dead or moribund sacrificed within the first week of the study were allowed to be discarded without necropsy and were replaced with an extra animal to keep the same total number of animals on study.

Dosing Comments (Dose Adjustments or Early Termination):	None
Dosing Solution Analysis:	Adequate. The concentrations of all doses were within ± 10% of their nominal values.

Observations and Results

Mortality

No difelikefalin related deaths were observed.

Clinical Signs

Ataxia was noted in all difelikefalin-treated groups (Groups 2-4) for both sexes with no dose-dependence. At ≥ 3 mg/kg/day, decreased motor activity was noted in both sexes. At ≥ 10 mg/kg/day, prostration and labored breathing were noted in the males only, and the incidence increased with the dose. Rapid and shallow breathing was noted in 21/25 animals at both 10 mg/kg/day and 30 mg/kg/day in the males and in 2/25 animals in the 10 mg/kg/day females. These observations listed above were transient and were only noted in the first week of doing.

During weekly detailed hands-on observations, rapid and shallow breathing was noted in 10/25, 9/25 and 13/25 males in all difelikefalin treated groups (Groups 2, 3, and 4, respectively), from Days 141 through 148.

Body Weights

Mean body weights (BW) were increased at all difelikefalin dose levels as compared to controls. In males, total body weight gains from Day 1 to Day 183 increased 68.3%, 35.7%, and 40.2% at 3, 10, and 30 mg/kg/day, respectively. In females, body weight gains increased 45.7%, 26.5%, and 27.4% at 3, 10, and 30 mg/kg/day, respectively. There was no dose relationship for increased body weight gains. Body weight gains are summarized in the following table.

Table X: Body weight gains of transgenic mice treated with difelikefalin

Group		Dose levels (mg/kg/day)	Total Body Weight Gain (mean±SD, g)	% Increase of Body Weight Gain Compared to Controls
1	M	0	8.16 ± 3.11	-
	F		6.34 ± 1.66	-
2	M	3	13.73 ± 3.31*	↑68.3
	F		9.24 ± 1.44*	↑45.7
3	M	10	11.07 ± 2.97*	↑35.7
	F		8.02 ± 1.34*	↑26.5
4	M	30	11.4 ± 2.63*	↑40.2
	F		8.08 ± 1.55*	↑27.4

*: p< 0.05

Feed Consumption

No treatment-related effects on mean food consumption were observed.

Gross Pathology
No treatment-related effects on gross pathology were observed

Histopathology
Peer Review Conducted: Yes
Historical Control Provided for Tumor Incidence: Yes

Histopathology was performed on a standard list of tissues, plus any gross lesions, from all animals, including both premature decedents and animals killed at terminal sacrifice.

Neoplastic
No statistically significant differences in tumor incidence were observed in mice of either sex in this study, according to the statistical criteria used by the ECAC.

Non-Neoplastic
Treatment-related non-neoplastic microscopic findings were noted in the kidney. Incidence of kidney infarction was increased at all difelikefalin doses, in both males and females. However, the incidence was not dose-related. The infarcts were not associated with any evidence of thrombosis or any indication of vasculitis in the kidney. In addition, no acute changes associated with renal infarction were observed.

Table X: Kidney infarcts in difelikefalin-treated transgenic mice

	Males				Females			
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Dose Level (mg/kg/day)	0	3	10	30	0	3	10	30
Number Examined	25	25	25	25	25	25	25	25
Minimal	0	4	2	1	1	1	2	0
Mild	0	3	5	3	0	3	2	3
Moderate	0	0	0	0	1	0	0	2
Total number of animals affected	0	7	7	4	2	4	4	5

Toxicokinetics
Toxicokinetic data obtained after 170 days (24 weeks) of dosing are summarized below. Difelikefalin was rapidly absorbed after subcutaneous dosing, and T_{max} was 0.25-0.5 hour post-dose in both sexes across all dose levels. Systemic difelikefalin exposures, as indicated by C_{max} and $AUC_{0\text{-}24h}$ values, increased dose-proportionally in both sexes. Difelikefalin exposures were generally similar in female and male mice.

Table x: Toxicokinetic parameters of difelikefalin in transgenic mice in Week 25 after repeated daily dosing

Group	Dose (mg/kg)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (hr*ng/mL)	λ_z (1/hr)	$t_{1/2}$ (hr)
2	3	M	4140	0.250	4200	NC	NC
		F	4260	0.250	3810	0.118	5.89
3	10	M	10500	0.500	12300	NC	NC
		F	12700	0.250	11300	NC	NC
4	30	M	41700	0.250	42800	NC	NC
		F	33300	0.250	34700	NC	NC

AUC_{0-24}: area under the concentration-time curve from time 0 to 24 hours post-dose; C_{max}: maximum concentration; F: female; M: male; NC: could not be calculated; $t_{1/2}$: terminal half-life; t_{max}: time of maximum concentration; λ_z: terminal rate constant

Study Title+: A 2-year Carcinogenicity Study of CR845 by Subcutaneous Injection in Rats

Study no.: CR845-CARC088, study report No. 20094527
Study report location: SDN 1, Module 4.2.3.4.1
Conducting laboratory and location: (b) (4)
GLP compliance: Yes
Drug, lot #, and % purity: difelikefalin (CR845), batch # SP070543N/MZ77348, purity 99.8%
Prior Exec CAC Dose Concurrence: Y
Basis for Dose Selection: AUC ratio

Reviewer Carcinogenicity Conclusion: Negative
ECAC Carcinogenicity Conclusion: Negative

Tumor Findings: No statistically significant differences in tumor incidences were observed in rats of either sex in this study, according to the statistical criteria used by the ECAC. The NOAEL was the highest dose 1 mg/kg/day.

Methods

Doses:	0 (vehicle control), 0.25, 0.5, and 1 mg/kg/day
Frequency of dosing:	Once daily
Number/Sex/Group:	60
Dose volume:	1 mL/kg/day
Formulation/Vehicle:	Sterile saline
Route of administration:	ORAL GAVAGE
Species:	RAT
Strain:	SPRAGUE-DAWLEY
Age:	8 weeks at initiation of treatment
Comment on Study Design and Conduct:	The intended route of administration to humans is intravenous. Since daily intravenous injections in rats was not feasible for the 2-year study, subcutaneous dosing was selected. The study design is summarized below:

Experimental Design – Carcinogenicity

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Adjusted Dose Concentration (mg/mL)[a]	Animal Numbers Carcinogenicity Study	
					Males	Females
1	0.9% Sodium Chloride, Injection	0	1	0	41-100	101-160
2	CR845	0.25	1	0.30	161-220	221-280
3	CR845	0.5	1	0.59	281-340	341-400
4	CR845	1	1	1.18	401-460	461-520

[a] Dose concentrations were adjusted using a correction factor for free base peptide content based on the current Certificate of Analysis (correction factor of (b) (4) was applied during the formulation process).

Experimental Design – Toxicokinetic

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Adjusted Dose Concentration (mg/mL)[a]	Animal Numbers Toxicokinetic Study	
					Males	Females
1	0.9% Sodium Chloride, Injection	0	1	0	521-523	524-526
2	CR845	0.25	1	0.30	527-535	536-544
3	CR845	0.5	1	0.59	545-553	554-562
4	CR845	1	1	1.18	563-571	572-580

[a] Dose concentrations were adjusted using a correction factor for free base peptide content based on the current Certificate of Analysis (correction factor of (b) (4) was applied during the formulation process).

Dosing Comments (Dose Adjustments or Early Termination):	Due to decreased survival in the female saline control animals, female rats in all groups were euthanized in 93 weeks. Males were euthanized in Weeks 103-106.
Dosing Solution Analysis:	Adequate. All study samples analyzed had mean concentrations within ± 10% of the nominal values except very few samples that had concentrations 80-90% of the nominal values. The limited occurrences and small variation from the targeted concentrations are not expected to have a significant impact on the study outcomes.

Observations and Results

Mortality

There were no difelikefalin-related effects on survival.

Clinical Signs

All difelikefalin-treated groups had transient decreased activity beginning on Day 1, which resolved in the first week of dosing. No other difelikefalin-related clinical signs were observed.

Body Weights
There were no statistically significant difelikefalin-related effects on total body weight gains in either sex.

Feed Consumption
Food consumption was initially lower (≤ 19% less) in difelikefalin-treated rats during the first dosing week when compared to controls. The magnitude of the difference decreased during the following three weeks of dosing and resolved to generally ≤ 8% less than controls following the first 30 days, throughout the first year. In the second year of the study, food consumption in females receiving difelikefalin was generally greater than in controls.

Gross Pathology
There were no difelikefalin-related gross pathology findings.

Histopathology
Peer Review Conducted: Yes
Historical Control Provided for Tumor Incidence: No

Histopathology was performed on a standard list of tissues from all treated animals killed at scheduled sacrifice, plus all main-study premature decedents from all groups. Histopathological examination was performed for all gross lesions sampled at necropsy from animals in all groups.

Neoplastic
No statistically significant differences in tumor incidence were observed in rats of either sex in this study, according to the statistical criteria used by the exec-CAC.

Non-Neoplastic
There were no difelikefalin-related non-neoplastic findings.

Toxicokinetics
Difelikefalin plasma exposures increased dose proportionally in both sexes on Day 1. After repeated dosing for 183 days, difelikefalin plasma exposures were greater than dose proportional in males, and slightly less than dose proportional in females. Difelikefalin exposure generally increased in plasma after repeated dosing. There were no sex differences in difelikefalin exposures after the first dose; however, after 183 days of dosing, females had notably higher exposures than males. The TK parameters are summarized in the following table.

Table x: Toxicokinetic parameters of difelikefalin in Sprague Dawley rats on Day 1 and Day 183 after repeated daily dosing

Day	Group	Dose (mg/kg)	Sex	C_{max} (ng/mL)	t_{max} (hr)	λ_z (1/hr)	$t_{1/2}$ (hr)	t_{last} (hr)	$AUC_{0\text{-}last}$ (hr*ng/mL)
1	2	0.25	M	404	0.250	1.90	0.366	4.00	696
			F	320	0.500	NC	NC	4.00	602
	3	0.50	M	818	0.500	NC	NC	4.00	1380
			F	667	0.500	NC	NC	4.00	1410
	4	1.0	M	1520	0.500	NC	NC	4.00	2730
			F	1500	0.500	NC	NC	4.00	2900
183	2	0.25	M	372	0.500	NC	NC	8.00	763
			F	4890	0.500	NC	NC	24.0	59000
	3	0.50	M	2350	1.00	NC	NC	24.0	18500
			F	7460	1.00	NC	NC	24.0	88100
	4	1.0	M	5080	0.500	NC	NC	24.0	46900
			F	15300	0.250	NC	NC	24.0	161000

Note: $AUC_{0\text{-}last}$ = area under the concentration-time curve from time 0 to the time of the last measurable concentration; C_{max} = maximum concentration; F = female; M = male; NC = could not be calculated; $t_{1/2}$ = terminal half-life; t_{last} = time of the last measurable concentration; t_{max} = time of maximum concentration; λ_z = terminal rate constant.

Overall, difelikefalin injection did not appear to be carcinogenic in mice and rats as described in the two carcinogenicity studies.

16.4. OCP Appendices (Technical documents supporting OCP recommendations).

16.4.1. Bioanalytical Method Validation

Bioanalytical methods were developed to quantify CR845 plasma protein binding and the concentration of CR845 in plasma and urine. The bioanalytical reports describe the assay performance and sample assay results. Incurred Sample Reanalysis (ISR) was conducted for both plasma and urine samples from appropriate phase 1 and phase 2 studies and the results were within ± 20%. The human K2-EDTA plasma samples were extracted by solid phase extraction method and analyzed using liquid chromatography – tandem mass spectrometry (LC-MS-MS) assays.

Bioanalytical Methods in Plasma:

The initial bioanalytical assay was developed by (b) (4) and only used for the first-in-human CLIN1001 study. The method was then transferred to (b) (4) and utilized for subsequent clinical pharmacology studies (CLIN1003, CLIN1004, CLIN2001). A new bioanalytical assay was then developed by (b) (4) who have completed bioanalytical work on the other studies, this includes CLIN1005, CLIN1006, CLIN2003, CLIN2005, CLIN2101, CLIN1301 CLIN100201 and 100303. The pharmacokinetic samples collected in the Japanese studies (PR-13A9-P1-A and PR-13A9-P1-B) were assayed by (b) (4)

Table 30: Clinical Studies with PK Evaluating IV Difelikefalin.

Study	Phase	Abbreviated Study Description	Study Design	Dosing Regimen	Study Population/ PK Population
CR845-CLIN1001	1	Safety/PK/PD in healthy volunteers	Single-center, randomized, DB, PC, ascending single-dose	2 to 40 mcg/kg (15-minute IV infusion)	Healthy volunteers/ 35 males and 2 females (N= 37)
CR845-CLIN1003	1	Safety/PK in patients with CKD on HD	Single-center, randomized, DB, PC ascending single-dose	1, 3, and 6 mcg/kg (15-minute IV infusion)	Patients with CKD on HD/ 11 males and 7 females (N = 18)
CR845-CLIN1004	1	Safety/PK in healthy Volunteers	Multicenter, randomized, DB, PC, ascending multiple-dose	5 to 15 mcg/kg (15-minute IV infusions every 3 hours over a 24-hour period after a loading dose)	Healthy Volunteers/ N = 24 males
CR845-CLIN2001	2	Efficacy and safety in subjects undergoing laparoscopic assisted hysterectomy	Cohort 1: single-dose, proof of concept	8, 24, and 40 mcg/kg (15-minute IV infusion)	Post-surgical subjects (laparoscopic hysterectomy) N=40 females
CR845-CLIN1005	1	Safety/PK in healthy volunteers and mild, moderate, or severe renal impairment not on dialysis	Multicenter, single-dose, open-label	3 mcg/kg (IV bolus)	Healthy volunteers and patients with mild, moderate, or severe renal impairment not on dialysis. / 15 males and 9 females (N = 24 renally- compromised) 8 males and 4 females (N = 12 healthy volunteers) (N = 36)
CR845-CLIN1006	1	Human abuse potential study	Single-center, randomized, single-dose, DB, PC, 4-way crossover	4 Treatment Periods; a single IV bolus of pentazocine (0.5 mg/kg, IV), placebo or CR845 (5 or 15 mcg/kg, IV) followed by a 48-hour washout period	Healthy recreational polydrug users/ 34 males and 9 females (n = 43*) *this includes one subject who only received pentazocine.
CR845-CLIN2003	2	Efficacy and safety in patients undergoing bunionectomy	Single-center, randomized, DB, PC, parallel-group	5 mcg/kg (every 8 hours repeated up to 6 times over 48 hours; IV bolus)	Postsurgical patients (bunionectomy)/ 5 males and 29 females

					(N = 34)
CR845-CLIN2005	2	Safety and PK in HD patients with and without uremic pruritus	Single-center (Part A) or multicenter (Part B), randomized,	Part A: 0.5, 1, and 2.5 mcg/kg IV 3 times a week for 1 week	Part A: patients undergoing HD Part B: patients undergoing HD/ N=19 Part A
CR845-CLIN2101	2	Efficacy and safety in HD patients with moderate to severe pruritus	Multicenter, randomized, DB, PC	0.5, 1.0, and 1.5 mcg/kg IV bolus 3 times a week for 8 weeks	HD patients with uremic pruritus/ N=26
CR845-CLIN1301	1	Safety/PK/ bioavailability relative to IV dose in patients with ESRD on HD	Single-center, randomized (all parts); DB, PC (Parts A and B) or open-label, crossover (Part C)	Part C: 1 mg oral tablet and 1.0 mcg/kg IV as single doses	HD patients/ N=7 Part C
CR845-100201	1	A single IV dose QTc Study	A single IV dose, randomized, DB, PC, four- way crossover study	Placebo IV as a bolus injection (Placebo: P) 1 tablet moxifloxacin 400 mg orally (Positive Control: PC) CR845 0.5 mcg/kg IV as a bolus injection (Therapeutic Dose: DT) CR845 3 mcg/kg IV as a bolus injection (Supratherapeutic Dose: DS)	Healthy volunteers/ 26 males and 32 females (N=58)
CR845-100303	1	Physical withdrawal of IV CR845 in dialysis patients	Multicenter, randomized, DB, PC, multiple dose	0.5 mcg/kg IV bolus 3 times a week for 3 weeks then: Placebo or 0.5 mcg/kg IV bolus 3 times a week for 2 weeks	HD patients/ Open label: 20 males and 15 females Double blind: 9 males and 7 females
PR-13A9-P1-A	1	Safety/PK/PD (biomarkers) in healthy volunteers	Single-center, randomized, DB, PC, ascending single- and multiple-dose	1 to 40 mcg/kg (IV bolus as single dose) or 1 to 20 mcg/kg every 3 hours for 21 hours	Healthy Volunteers/ N = 65 males.
PR-13A9- P1-B	1	Safety/PK in HD patients	Single-center, randomized, DB, PC, ascending, multiple-dose	0.5, 1, and 2.5 mcg/kg IV bolus (3 times a week for 1 week)	HD patients/ N=10

BID = twice daily; CKD = chronic kidney disease; CSR = clinical study report; DB = double-blind; ESRD = end-stage renal disease; HD = hemodialysis; IV = intravenous; PC = placebo-controlled; PD = pharmacodynamics; PK = pharmacokinetics; UK = United Kingdom; US = United States.

Briefly, for the assay developed by Worldwide, human plasma containing CR845 and the internal standard (IS), CR839, were extracted by 96-well solid phase extraction (SPE). After evaporation and reconstitution, an aliquot was injected on a (b) (4) LC-MS-MS equipped with a high -

performance liquid chromatography (HPLC) column. The peak area of the (b) (4) CR845 product ion was measured against the peak area of the (b) (4) internal standard product ion. Quantitation was performed using a weighted ($1/x^2$) linear least squares regression analysis generated from calibration standards prepared immediately prior to each run.

The summary from validation reports of the analytical methods used in the clinical pharmacokinetic studies for the determination of CR845 in human plasma is described in following Table 31.

Table 31: Difelikefalin (CR845) in plasma Bioanalytical Assay Details

CRO/Assay	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Method #	No. 247	ATM-1502	BAM.0137.01 BAM.0137.02	BAM.0137.03 BAM.0137.04 BAM.0137.05	13CR845VAL01
Lower limit of quantification	1.02 ng/mL	0.0500 ng/mL	0.05 ng/mL	0.100 ng/mL	0.05 ng/mL
Upper limit of quantification	996.07 ng/mL	50.0 ng/mL	50 ng/mL	50 ng/mL	200 ng/mL
Calibration Standards	1.02, 5.09, 10.18, 50.91, 99.61, 199.21, 348.63, 498.04, 752.59 and 996.07 ng/mL	0.0500, 0.100, 0.500, 1.00, 5.00, 25.0, 45.0, and 50.0 ng/mL	0.0500, 0.100, 0.500, 2.500, 5.000, 20.000, 40.000, and 50.000 ng/mL	0.100, 0.500, 2.500, 5.000, 20.000, 40.000, and 50.000 ng/mL	0.05, 0.2, 0.5, 2, 5, 20, 50, and 200 ng/mL
QC Concentration	1.03, 2.89, 500.94, and 969.56 ng/mL Diluted QC- 5049.79 and 12624.48 ng/ml	0.150, 5.00, and 40.0 ng/mL Diluted QC- 250 ng/mL	0.150, 3.750, and 37.500 ng/mL Diluted QC- 100.000 ng/mL	0.100, 0.300, 3.750, and 37.500 ng/mL Diluted QC- 100.000 ng/mL BAM.0137.05: QC added:18.8 ng/ml Diluted QC increased to 500.000 ng/mL	0.05, 0.1, 5 and 160 ng/mL Diluted QC- 50-and 250- fold dilution
Average within and between run precision (CV)	Intra-run 2.91% to 13.48% Inter-run 2.86% to 11.44% Diluted QC 1.83 to 12.67%	Intra-run 0.8% to 14.8% Inter-run 3.3% to 12.4% Diluted QC 11.2%	Intra-run 0.9% to 5.5% Inter-run 1.8% to 4.8 % Diluted QC 1.3%	Intra-run 0.6% to 2.4% Diluted QC 1.3%	Intraday 0.4% to 6.3% Inter-day 1.3% to 10.2% Diluted QC 1.0-1.3%
Average Accuracy Bias	Intra-run -1% to 2% Inter-run 0.11% to 5.16% Diluted QC -7% to 2%	Intra-run -9.0% to 6.2% Inter-run -3.0to 2.4% Diluted QC -5.2%	Intra-run -1.8% to 7.3% Inter-run 0.2% to 2.7% Diluted QC 1.0%	Intra-run -6.7% to 0.8% Diluted QC 1.0%	Intraday -3.5% to 2.6%, Inter-day -2.0% to 2.7% Diluted QC -2.2 and 4 2%
Short Term Stability: Benchtop stability at Room temperature	4 h	50 h	39 h	28.4 h (BAM.0137.05)	24 h
Short Term Stability: Freeze -thaw cycles	Three cycles at -20°C	Seven cycles	Three cycles at -20°C and -80°C (BAM.0137.01) Five cycles at -20°C and -80°C (BAM.0137.02)	Five cycles at -20°C and -80°C (BAM.0137.05)	Three cycles at -30° and -70° C

Long Term Stability	14 days stored at -20°C (±10°C)	144 days stored at -20 ºC and -70 ºC	22 days at -20°C and -80°C (BAM.0137.01) 194 days at -20°C and -80°C (BAM.0137.02)	194 days at -20°C and -80°C (BAM.0137.03) 271 days at 20°C or -80 °C (BAM.0137.04) 427 days at -20°C and -80°C (BAM.0137.04)	94 days at −30°C and −70°C

(Source: Summary of Biopharmaceutic Studies and Associated Analytical Methods (section 2.7.1) Table 5 & 6., pages 20 to23)

Bioanalytical Methods in Urine:

Similar to the bioanalytical methods in human plasma, sensitive and specific LC-MS-MS assays for the determination of CR845 in human urine were developed, validated and also modified over a period of time by various bioanalytical service providers.

The summary from validation reports of the analytical methods used in the clinical pharmacokinetic studies for the determination of CR845 in human urine is described in following Table 32.

Table 32: Difelikefalin (CR845) in urine Bioanalytical Assay Details

CRO/Assay	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Method #	No. 253	ATM-1659	BAM.0140.01 BAM 0140.02 BAM 0140.03 BAM 0140.04	13CR845VAL01
Lower limit of quantification	9.96 ng/mL	10.0 ng/mL 30.0 ng/mL (DCN02820H2 am2)	1.00 ng/mL	1 ng/mL
Upper limit of quantification	10,010.45 ng/mL	10,000 ng/mL	1000 ng/mL	100 ng/mL
Calibration Standards	9.96, 49.79, 99.58, 500.52, 995 80, 1991.61, 3511.52, 5031.43, 7494.73, 10010.45 ng/mL	10.0, 20.0, 50.0, 200, 1000, 5000, 9000, and 10,000 ng/mL	1.00, 2.00, 10.0, 50.0, 100, 400, 800, 1000 ng/mL	1, 2, 5, 10, 20, 80 and 100 ng/mL
QC Concentration	10.33, 29.95, 9967.60 ng/mL Diluted QC- 5009.62 ng/ml	8000, 1000, and 30.0 ng/mL Diluted QC- 50,000 ng/mL	1.00, 3.00, 75.0, 750 ng/mL Diluted QC- 10000 ng/mL	1, 2, 20 and 80 ng/mL Diluted QC- 50-and 250-fold dilution
Average within and between run precision (CV)	Intra-run 4.86% to 9.31% Inter-run 1.43 to 4.8%	Intra-run 1.1% to 3.4% Inter-run 3.9% to 4.5% Diluted QC 3.2%	Intra-run 0.7% to 5.4% Inter-run 6.4% to 8.7% Diluted QC 1.7%	Intraday 16.5% for LLOQ and 4.3-5 9% for others Inter day 4.3% to 12.7%
Average within and between run Accuracy (Bias)	-7% to -1%	Intra-run -1.0% to 15% Inter-run 2.5% to 10.0% Diluted QC 4.0%	Intra-run -10.8% to 6.7% Inter-run -6.5% to-3.0% Diluted QC -8.7%	Intraday -3.7 to 7.4% Inter-day -2.8% to 2.2%
Short Term Stability: Freeze-thaw cycles	Three cycles at -20°C	Five cycles	Four cycles at -20°C and -80°C	Four cycles at -20°C and -80°C
Long Term Stability	112 days stored at -20°C (±10°C)	116 days stored at -20 ºC and -70 ºC	8 days at -20°C and -80°C (BAM.0140.01)	95 days at -30 and -70°C

	(Bioanalytical Method 253 [Version 2])	230 days stored at -20 °C (DCN02820H2_am4) 257 days stored at -70 °C (DCN02820H2_am5)	168 days at -20°C and -80°C (BAM.0140.02) 243 days at -20°C and -80°C (BAM.0140.03)	
Stability of Stock solutions)	Stored at 5ºC 56 day (CR845) 70 day (IS) Stored at Room temperature 6 hours	In Methanol/water (1:9) Stored at 4 ºC 138 days (CR845) 102 days (CR839)	In 40mM sodium acetate, pH 4.5 stored at -10°C to -30°C 18 days (CR845) 45 days (CR138192)	Room temperature: 24 hours (CR845 and IS) Refrigeration 106 days (CR845 and IS)

(Source: Summary of Biopharmaceutic Studies and Associated Analytical Methods (section 2.7.1) Table 8 & 9., pages 25 to27)

16.4.2. Population PK Analysis

Population PK analysis was conducted by the applicant to characterize the PK profile of difelikefalin and identify covariate factors that could affect difelikefalin disposition. Data was collected from studies including oral and IV administration of difelikefalin CLIN1001, CLIN1003, CLIN1004, CLIN1005, PR-13A9-P1-A, PR-13A9-P1-B, CLIN2001-PO, CLIN2005, CLIN2101, CLIN1002-PO, CLIN1006, CLIN1301, CLIN1303, CLIN1401, CLIN2001, CLIN2003, and CLIN100201. These studies were summarized in Table 33.

Table 33: Summary of studies included in the population PK analysis.

Study	# of subjects	Subjects	Dose levels	Sampling times
CLIN1001	37	Healthy Subjects	2, 4, 6, 8, 10, 16, 24 and 40 mcg/kg for IV doses	0, 8, 15, 20, 25, 30 and 45 minutes, plus 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12 and 24 hours after the start of infusion.
CLIN100201	57	Healthy Subjects	0.5 and 3 mcg/kg for IV doses	Pre-dose, 5 minutes post-dose and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18 and 23 hours after dose administration
CLIN1003	18	Hemodialysis Patients	1, 3 and 6 mcg/kg for IV doses	Pre-dose, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after the start of infusion. Additional pre-dialysis, post-dialysis or discharge blood samples were taken from the predialyzer (arterial) and postdialyzer (venous) lines at the start of dialysis (t=0) and at 1- and 3-hours during dialysis if measurable concentrations of difelikefalin were detected in the pre-dialysis samples from cohort A1. For part B, only one blood sample was to be collected around the T_{max} determined from Part A.
CLIN1004	28	Healthy Subjects	Loading dose: 0.0067, 0.0325, 0.013, 0.02, 0.017 mg/kg. Maintenance dose: 0.005, 0.01, 0.015, 0.013 mg/kg	Pre-dose, 4, 8, 12, 16, 20, 24, 28, 32, 36, 48 and approximately 57 hours (at discharge) after the start of the infusion.
CLIN1005	36	Healthy Subjects	3 mcg/kg single dose for IV doses	Pre-dose, 1, 2, 5 and 10 minutes after dosing plus 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 hours after dosing.
CLIN1006	42	Healthy Subjects	5 and 15 mcg/kg for IV doses	Pre-dose, 5 minutes, and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 8 hours postdose on each treatment day
PR-13A9-P1-A	66	Healthy Subjects	1, 3, 5, 10, 20 and 40 mcg/kg for IV doses	Step 1 (single dose): Pre-dose, 1, 5 and 10 minutes, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6,

Study	# of subjects	Subjects	Dose levels	Sampling times
				8, 12 and 24 hours after administration of the single dose. Step 2 (repeated dose): Pre-dose, 1 minute, 5 minutes, 0.25, 0.5, 1, 2, 3, 3.083 (3 hour, 5 minutes), 6, 6.083 (6 hour 5 minutes), 9, 9.083 (9 hours, 5 minutes), 12, 15, 18, 21, 21.0167 (21 hours, 1 minute), 21.083 (21 hours, 5 minutes), 21.25, 21.5, 22, 23, 24, 25, 29, 37, 45, 57 and 69 hours after the first dose. Samples taken at 3, 6, 9, 12, 15, 18 and 21 hours were collected pre-dose for the 2nd, 3rd, 4th, 5th, 6th, 7th and 8th administrations respectively.
PR-13A9-P1-B	14	Hemodialysis Patients	0.5, 1, and 2.5 mcg/kg for IV doses	Pre- and post-dialysis, plus 5 minutes, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post dose on days 1 and 5. Additional samples were taken 5 minutes prior to dialysis on days 3 and 8 and 10 minutes after dialysis on day 8.
CLIN2001	63	Pain Patients	8 and 24 mcg/kg for IV doses	15 and 30 minutes and 1, 2, 4, 6, 9 and 12 hours after the start of infusion
CLIN2003	34	Pain Patients	5 mcg/kg for IV doses	0.16, 0.33, 0.66 and 1.25 hours after the first dose, then 10 minutes following the dose at 8 hours (or when difelikefalin is next administered after that time) and 10 minutes following the dose at 24 hours (or when difelikefalin is next administered after that time). If an additional dose of difelikefalin is administered during the first hour, blood samples were collected at 10 and 30 minutes after the 2nd dose rather than 40 and 75 minutes after the first dose.
CLIN2005	19	Hemodialysis Patients with Uremic Pruritus	0.5, 1, or 2.5 mcg/kg Q3W for IV doses	Pre-dose/pre-dialysis, 5 minutes, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post dose and post-dialysis.
CLIN2101	174	Hemodialysis Patients with Moderate-to-Severe Pruritus.	0.5, 1.0, or 1.5 mcg/kg Q3W for IV doses	Pre- and post-dialysis, 5 minutes, 0.5, 1, 2, 4, and 24 hours post-dialysis on days 1 and 50 (week 8). Additional pre-dialysis and 5-minute post-dialysis samples were also collected on days 3, 8, 22, 36, and 52 (weeks 1, 2, 4, 6 and 8 respectively)
CLIN1002-PO	120	Healthy Subjects	0.1, 0.25, 0.5, 1, 5, and 10 mg for Oral doses	Predose, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9, 10, 12, 16, and 24 hours post-dose. Subjects in Cohorts A1-3, A1-5, and A1-6 had 3 additional samples collected at 11, 13, and 14 hours post-dose for evaluation of food effect on oral dosing of difelikefalin.
CLIN1301	69	Hemodialysis Patients	0.25, 0.5, 1.0, and 2.5 mg QD for oral doses. 1 mg oral and 1 mcg/kg IV as single doses	Pre-dialysis, pre-dose (within 10 minutes following the end of dialysis and prior to study drug administration), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 20, 24, and 32 hours after the day 1 and day 5 doses. Additional samples were collected: (1) 44 hours post-dose on day 5; and (2) pre-dialysis and pre-dose on days 3 and 8.
CLIN1303	90	Patients with chronic kidney disease	0.25, 0.5 or 1.0 mg and up to 3 optional groups with 2.5 mg QD or dose previously evaluated BID for oral doses	pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11, 12, 14, 16, 20 and 24 hours post dose.

Study	# of subjects	Subjects	Dose levels	Sampling times
CLIN1401	72	Patients with Chronic Liver Disease or Health Subjects	1, 2.5 and 5 mg QD for oral doses	Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11, 12, 14, 16, 20 and 24 hours post dose on days 1 and 8. Pre-dose samples only were collected on days 2 to 7.
CLIN2001-PO	87	Patients with Osteoarthritis of the Hip or Knee	0.25, 0.5, 1, and 5 mg BID for oral doses	Pre-dose (1st dose only), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 12 hours (just prior to the next dose) after the first dose on days 1 and 2.

Source: Reviewer's summary based on CTX0201F-Report-v1.0-Final.

There was a total of 22332 serum concentration samples from 811 subjects including difelikefalin given either IV and/or orally. Of these, 18844 samples had quantifiable difelikefalin concentrations, and 3488 samples (15.6%) were BLQ. Approximately 24% of the total observations (5484/22,332) were collected from 261 subjects receiving a single difelikefalin dose, while approximately 76% of total observations (17221/22332) were from the 550 subjects receiving multiple difelikefalin doses. The IV dosing dataset for the base model included 436 subjects and 7,641 observations: 312 subjects received a bolus dose (5,979 PK observations), and 124 subjects received a continuous infusion (1,662 PK observations). 597 observations (7.8%) were BLQ.

Table 34: Summary of PK samples in population PK analysis.

Study	Subjects	Total PK samples	PK samples above LLQ	BLQ samples: n[%]
CR845-CLIN1001	37	626	572	54.0 [8.63]
CR845-CLIN100201	57	1436	1130	306 [21.3]
CR845-CLIN1002-PO	84	4442	3264	1178 [26.5]
CR845-CLIN1003	18	171	171	0.00 [0.00]
CR845-CLIN1004	28	566	566	0.00 [0.00]
CR845-CLIN1005	36	602	482	120 [19.9]
CR845-CLIN1006	42	848	808	40.0 [4.72]
CR845-CLIN1301	68	2097	1674	423 [20.2]
CR845-CLIN1303	90	4219	3658	561 [13.3]
CR845-CLIN1401	72	3600	3051	549 [15.2]
PR-13A9-P1-A	66	1444	1396	48.0 [3.32]
PR-13A9-P1-B	14	230	230	0.00 [0.00]
CR845-CLIN2001	41	288	288	0.00 [0.00]
CR845-CLIN2001-PO	80	747	556	191 [25.6]
CR845-CLIN2003	33	169	169	0.00 [0.00]
CR845-CLIN2005	19	473	462	11.0 [2.33]
CR845-CLIN2101	26	374	367	7.00 [1.87]
Total				
All studies	811	22332	18844	3488 [15.6]

Source: CTX0201F-Report-v1.0-Final, Page 78, Table S7.

In the IV dosing dataset, the majority of subjects were White (214, 9%), Black (126, 28.7%) and Asian (87, 20.4%). The median age of all subjects was 36 years old (range: 18 - 86), 36.2% were

female and the median baseline body weight was 73.1 kg (range: 40 - 129 kg). The median value of the calculated creatinine clearance, albumin, alanine aminotransaminase, aspartate aminotransaminase and total bilirubin were 111 mL/min (range: 4.4 - 249), 4.4 g/dL (range: 3.2, 5.4), 16 IU/L (range: 4, 87), 18 IU/L (range: 4, 68) and 8.6 mcmol/L (range: 1.7 - 38.6). The majority (418; 95.9%) of the 436 subjects were of normal liver function, 17 (3.9%) were of mild hepatic dysfunction and only 1 (0.2%) was of moderate hepatic dysfunction based on NCI hepatic impairment classification. No subjects with severe hepatic function were involved in the analysis. The majority of the subjects had normal (238; 54.6%) and mildly impaired (94; 21.6%) renal function. 81 (18.6%) subjects were kidney failure. In total dataset, which subjects received difelikefalin by IV and/or oral doses, most of the demographics and baseline covariates were similar as the primary analysis dataset. In total dataset, the majority (750; 92.5%) of the 811 subjects were of normal liver function, 50 (6.2%) were of mild hepatic dysfunction and 9 (1.1%) were of moderate hepatic dysfunction. Only 2 (0.2%) subjects were severe hepatic dysfunction based on NCI hepatic impairment classification. The baseline covariates for population PK analysis were summarized in Table 35.

Table 35: Summary of Demographics and Baseline Covariates in the Analysis Dataset

Variable		Median (Range)		
Administration		IV	Oral	Total
n		436	394	811
age (year)		36 (18, 86)	58 (18, 82)	47 (18, 86)
sex (Female)		158 (36.2%)	137 (34.7%)	289 (35.6%)
race	White	214 (49%)	239 (61%)	447 (55%)
	Black	126 (29%)	131 (33%)	244 (30%)
	Asian	89 (20%)	6 (2%)	95 (12%)
	Other	7 (2%)	18 (4%)	25 (3%)
baseline weight (kg)		73.1 (40, 129)	83.7 (45.1, 144.5)	78 (40, 144.5)
baseline hemoglobin (g/dL)		13.7 (7.1, 18.5)	13.3 (7.3, 18.4)	13.5 (7.1, 18.5)
baseline platelets (E+9/L)		233 (93, 483)	214 (36, 427)	244 (36, 483)
baseline red blood cell count (E+12/L)		4.66 (2.6, 6.11)	4.46 (2.49, 6.17)	4.56 (2.49, 6.17)
baseline white blood cell count (E+9/L)		6.1 (2.9, 21.8)	6.6 (1.9, 13.4)	6.3 (1.9, 21.8)
baseline albumin (g/dL)		4.4 (3.2, 5.4)	4.1 (2, 4.9)	4.2 (2, 5.4)
baseline alkaline phosphatase (IU/L)		71 (28, 400)	71 (26, 369)	71 (26, 400)
baseline alanine aminotransaminase (IU/L)		16 (4, 87)	17 (3, 141)	16 (3, 141)
baseline aspartate aminotransaminase (IU/L)		18 (4, 68)	20.5 (3, 162)	19 (3, 162)
baseline total bilirubin (mcmol/L)		8.6 (1.7, 37.6)	8.55 (3.4, 72.7)	8.6 (1.7, 72.7)
baseline serum creatinine (mg/dL)		0.9 (0.5, 20.4)	1.1 (0.45, 18.6)	0.95 (0.45, 20.4)
creatinine clearance (mL/min)		111 (4.4, 249)	86.8 (4.3, 218)	103 (4.3, 249)
Glomerular filtration (mL/min/ 1.73m^2)		93 (2.9, 170)	70 (3.3, 176)	84 (2.9, 176)
Chronic kidney disease stage	Normal	238 (54.6%)	128 (32.5%)	356 (43.9%)
	Mildly decreased	94 (21.6%)	98 (24.9%)	190 (23.4%)
	Mildly to moderately decreased	6 (1.4%)	33 (8.4%)	39 (4.8%)
	Moderately to severely decreased,	6 (1.4%)	38 (9.6%)	44 (5.4%)
	Severely decreased	11 (2.5%)	24 (6.1%)	35 (4.3%)
	Kidney failure	81 (18.6%)	73 (18.5%)	147 (18.1%)

NCI ODWG Hepatic Function	Normal	418 (95.9%)	351 (89%)	750 (92.5%)
	Mild	17 (3.9%)	33 (8.4%)	50 (6.2%)
	Moderate	1 (0.2%)	8 (2%)	9 (1.1%)
	Severe	0	2 (0.5%)	2 (0.2%)

Source: Reviewer's analysis.

The population PK analysis was conducted via nonlinear mixed-effects modeling with the NONMEM software, version 7.44 using SAEM and IMP methods. The final model was re-estimated with the BAYES estimation method to obtain the full posterior distribution with more accurate standard errors of parameter estimates and individual estimates. Data management, exploratory analyses, diagnostic graphics, and post-processing of the data and NONMEM outputs were performed using statistical software R (version 3.6.2 or later).

The plasma concentrations of difelikefalin data with IV dosing were described by a three-compartment disposition model with linear clearance. Several covariates including body weight on all CL and V terms were included in the model. Additionally, the effects of age, AST and BILI were estimated on drug CL, and the MDRD effect was estimated on CL and V. This full covariate modeling approach emphasized parameter estimation rather than stepwise hypothesis testing.

The final population PK parameters for difelikefalin are presented in Table 36. The typical patient was 36 years old, 73.2 kg, with MDRD value of 93.27 and an AST value of 19. The typical values of CL (95% CI), V 1, Q2, V2, Q3, and V3, for the typical patient, were 4.33 (4.14, 4.53), 5.77 (5.46, 6.08), 10.1 (8.97, 11.3), 5.09 (4.75, 5.43), 0.201 (0.174, 0.233) and 1.99 (1.82, 2.19), respectively. Residual random effects were described with a proportional error model. Fixed and random effect parameters in the full model were well estimated, as judged by the width of the 95% CI.

Table 36: Parameter estimates of final population PK model with IV dosing only.

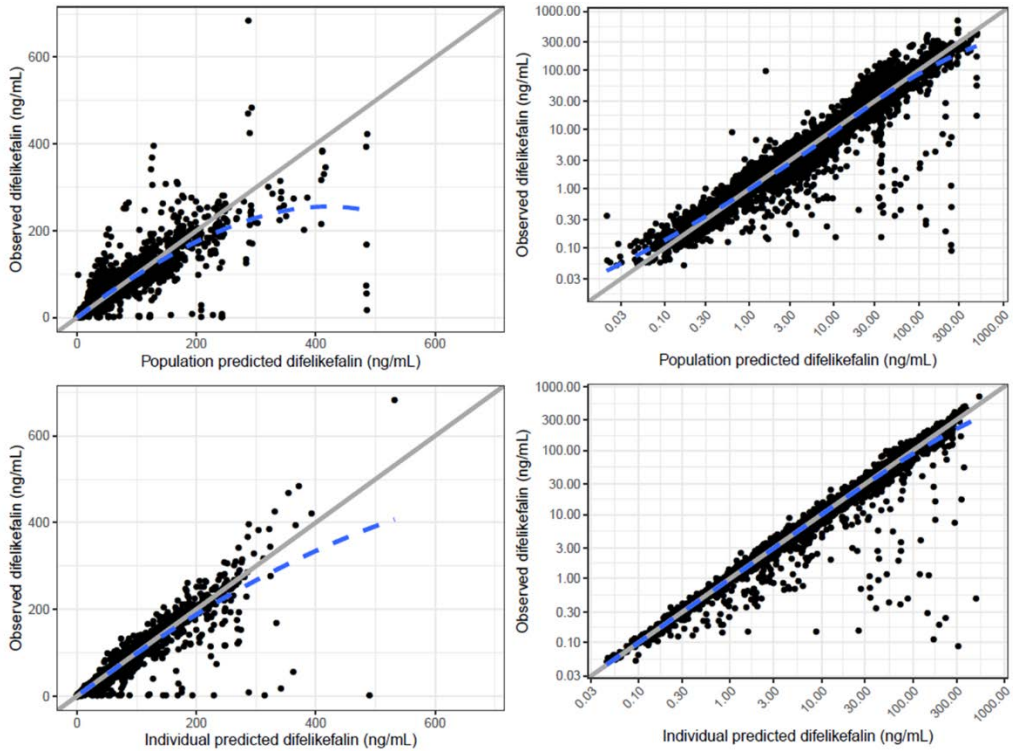
			Estimate	95% CI
Structural model parameters				
CL (L/h)	$\exp(\theta_1)$	Clearance	4.33	(4.14, 4.53)
V1 (L)	$\exp(\theta_2)$	Central volume	5.77	(5.46, 6.08)
Q2 (L/h)	$\exp(\theta_3)$	Intercompartmental clearance	10.1	(8.97, 11.3)
V2 (L)	$\exp(\theta_4)$	Peripheral volume 1	5.09	(4.75, 5.43)
Q3 (L/h)	$\exp(\theta_5)$	Intercompartmental clearance	0.201	(0.174, 0.233)
V3 (L)	$\exp(\theta_6)$	Peripheral volume 2	1.99	(1.82, 2.19)
Covariate effect parameters				
$MDRD_{CL}$	θ_7	Glomerular filtration effect on CL	0.821	(0.783, 0.858)
AST_{CL}	θ_8	Aspartate aminotransaminase effect on CL	-0.0215	(-0.0965, 0.0536)
$BILI_{CL}$	θ_9	Total bilirubin effect on CL	-0.0281	(-0.0758, 0.0183)
AGE_{CL}	θ_{10}	Age effect on CL	-0.259	(-0.338, -0.178)
$MDRD_{V1}$	θ_{11}	Glomerular filtration effect on V1	-0.0624	(-0.103, -0.0211)
Interindividual variability parameters				
IIV-CL	$\Omega_{(1,1)}$	Variance of clearance	0.173 [CV%=43.5]	(0.148, 0.206)
IIV-V1	$\Omega_{(2,2)}$	Variance of central volume	0.17 [CV%=43.0]	(0.142, 0.204)
IIV-Q2	$\Omega_{(3,3)}$	Variance of intercompartmental clearance	0.616 [CV%=92.3]	(0.467, 0.822)
IIV-V2	$\Omega_{(4,4)}$	Variance of peripheral volume 1	0.325 [CV%=62.0]	(0.264, 0.402)
IIV-Q3	$\Omega_{(5,5)}$	Variance of intercompartmental clearance	0.53 [CV%=83.6]	(0.248, 0.79)
IIV-V3	$\Omega_{(6,6)}$	Variance of peripheral volume 2	0.306 [CV%=59.8]	(0.152, 0.443)
Residual variability				
PropErr	$\Sigma_{(1,1)}$	Variance of porportional error	0.0337 [CV%=18.4]	(0.0324, 0.035)

Abbreviations: CI = confidence intervals; CV = coefficient of variation
CV% of omegas and proportional error = sqrt(exp(estimate) - 1) * 100 ; CV% of sigma = sqrt(estimate) * 100
All clearance and volume terms were allometrically scaled (full parameter equations given in main text)

Source: CTX0201F-Report-v1.0-Final, Page 50, Table 5.

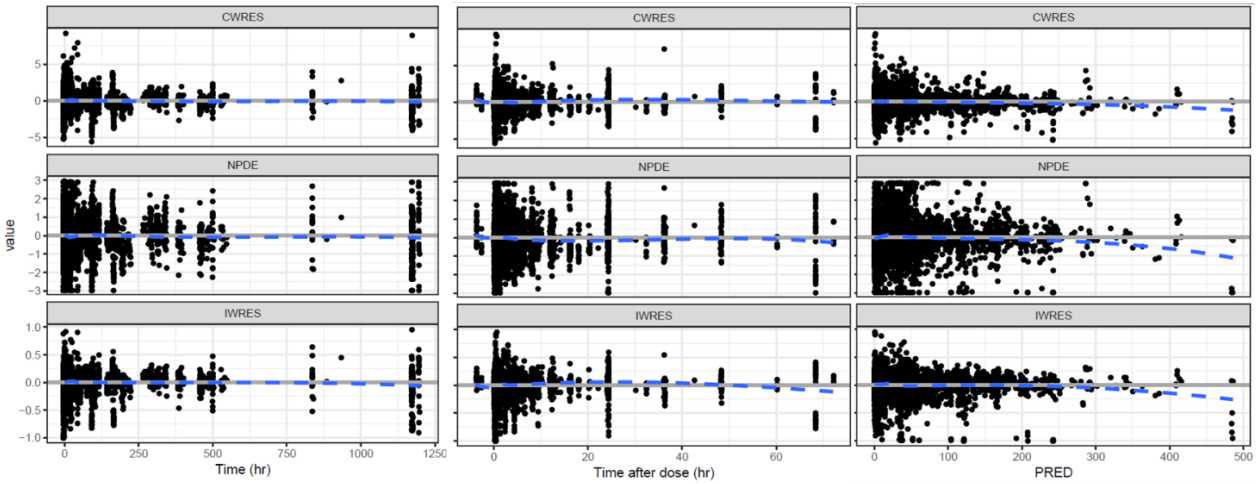
The diagnostic plots for the IV dosing final population PK model from one single chain of Bayesian model are shown in Figure 10 and Figure 11. The VPC (visual predictive check) stratified by renal function is shown in Figure 12. The dose normalized VPCs showed acceptable performance of the model in subjects with different renal functions. The population PK model appeared to adequately capture the central tendency and the variability of the data, as the general agreement between the observed 5th, 50th, and 95th percentiles of the data and the respective 95% CIs obtained from the simulation. No apparent bias was observed in the overall model fit for the data.

Figure 10: Observed versus population and individual predicted difelikefalin concentration for IV dosing final population PK model.



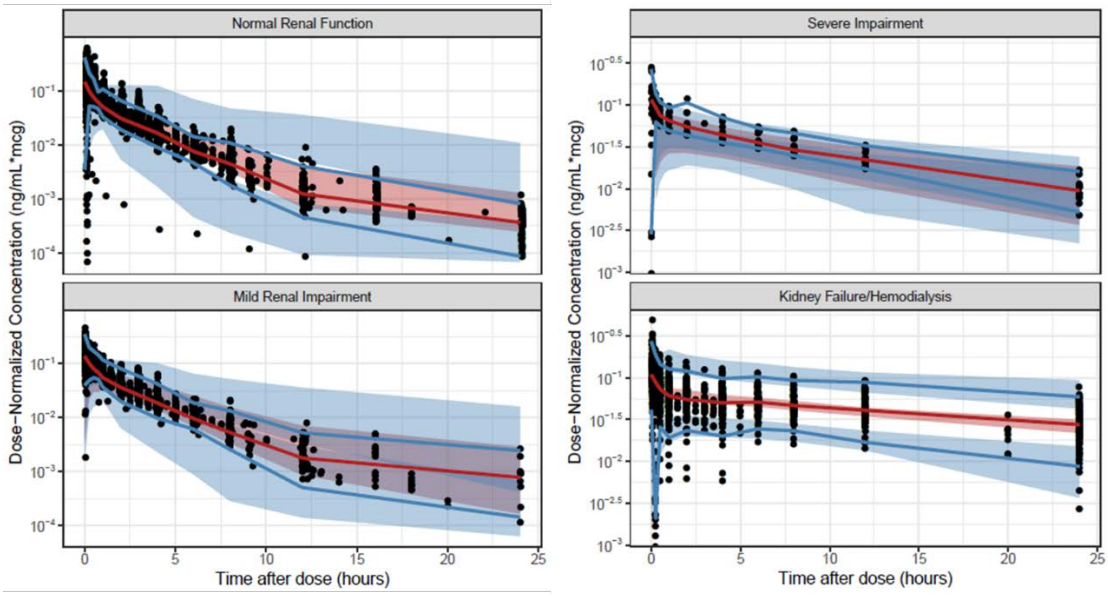
Source: CTX0201F-Report-v1.0-Final, Page 136-137, Figure S37-S38.

Figure 11: CWRES, NPDE and IWRES over Time, Time After Dose or PRED for IV dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 140-142, Figure S41-S43.

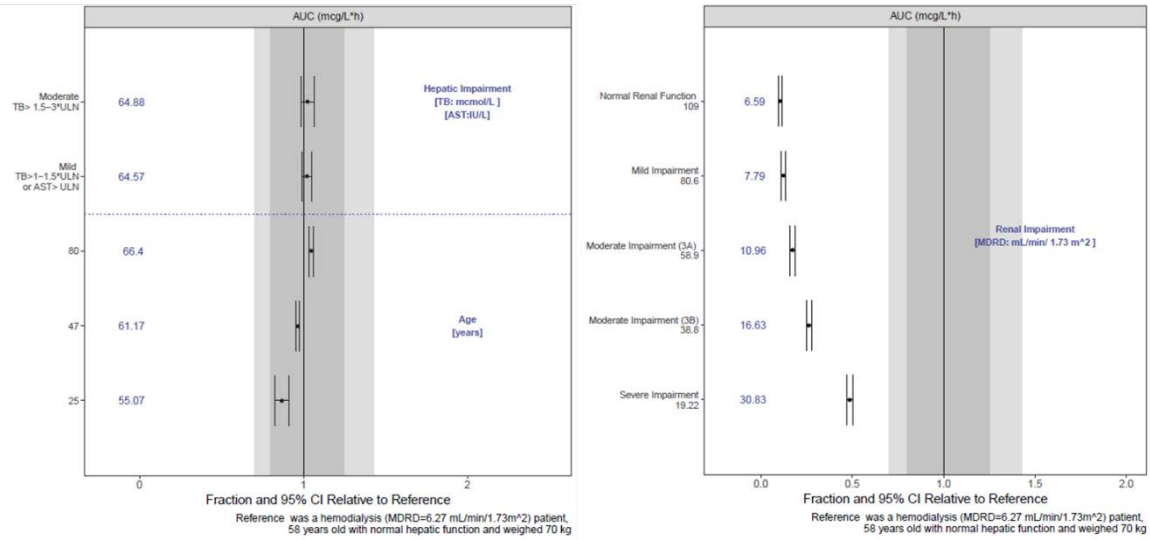
Figure 12: Visual Predictive Check (VPC) of the Dose Normalized difelikefalin Concentrations versus Time After Dose, Stratified by Renal Impairment for IV dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 158-159, Figure S59-S60.

Based on the IV dosing population PK model, covariate effects on difelikefalin exposure (AUC) were shown in the following forest plots. (Figure 13) The reference patient receives IV administration of 0.5 µg/kg difelikefalin. Subjects with normal, mild, moderate, and severe renal impairment had significantly different difelikefalin AUC compared to the reference subject. Difelikefalin AUC of subjects ranging from 25 to 80 years of age and those with mild and moderate hepatic impairment were within the reference range.

Figure 13: Covariate effects hepatic and renal function on the difelikefalin AUC based on IV dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 61, Figure 2 and Page 212, Figure S113.

The IV-oral dosing population PK model was developed based on the IV dosing model, to describe the PK of difelikefalin when administered orally or/and via IV. This model included absorption parameters, specific to oral dosing, and associated covariate effects. The final IV-oral model was determined based on a combination of factors including the parameter identifiability, model diagnostics and the objective function value. As with the IV dosing population PK model, the IV-oral model included fixed effects of body weight on all CL and V terms, the effects of BILI and AST estimated on drug CL and bioavailability (F1), the effect of age estimated on drug CL, and the MDRD effect estimated on CL and V. The oral absorption phase was characterized with a sequential zero-order, first order absorption model parameterized in terms of the zero-order release into an absorption compartment (D1), the first order absorption rate constant (k_a) and F1. The development of the IV-oral dosing model and initial estimation of the final model parameters were done with similar method as the IV dosing model.

The final population PK parameters for difelikefalin are presented in Table 37. The typical patient was 47 years old, weighed 78 kg with MDRD value of 83.502, BILI value of 8.552 and AST value of 19. The typical values of CL (95% CI), V 1, Q2, V2, Q3, V3, k_a, F1 and D1 for the typical patient, were 3.45 (3.25, 3.66), 6.03 (5.64, 6.42), 9.9 (8.55, 11.5), 5.76 (5.08, 6.49), 0.175 (0.128, 0.237), 1.92 (1.7, 2.16), 1.82 (1.45, 2.44), 0.0868 (0.0789, 0.0955) and 2.2 (1.79, 2.68), respectively. Fixed and random effect parameters in the IV-oral model were generally well estimated, as judged by the width of the 95% CI. The CV% for the IIV on Q3 was large, which might be due to the relatively high proportion of BLQ data observed during the later time points.

Table 37: Parameter estimates of final population PK model with IV and/or oral dosing.

			Estimate	95% CI
Structural model parameters				
CL (L/h)	$\exp(\theta_1)$	Clearance	3.45	(3.25, 3.66)
V1 (L)	$\exp(\theta_2)$	Central volume	6.03	(5.64, 6.42)
Q2 (L/h)	$\exp(\theta_3)$	Intercompartmental clearance	9.9	(8.55, 11.5)
V2 (L)	$\exp(\theta_4)$	Peripheral volume 1	5.76	(5.08, 6.49)
Q3 (L/h)	$\exp(\theta_5)$	Intercompartmental clearance	0.175	(0.128, 0.237)
V3 (L)	$\exp(\theta_6)$	Peripheral volume 2	1.92	(1.7, 2.16)
KA (/h)	$\exp(\theta_7)$	First order absorption rate constant	1.82	(1.45, 2.44)
$F1_{logTrans}$	$\exp(\theta_8)$	Logistic transformation for F1	0.0868	(0.0789, 0.0955)
D1 (h)	$\exp(\theta_9)$	Duration of zero order absorption	2.2	(1.79, 2.68)
Covariate effect parameters				
AST_{F1}	θ_{10}	Aspartate aminotransaminase effect on logistic transformation	0.0815	(-0.0862, 0.248)
$BILI_{F1}$	θ_{11}	Total bilirubin effect on logistic transformation	-0.0199	(-0.193, 0.157)
$MDRD_{CL}$	θ_{12}	Glomerular filtration effect on CL	0.809	(0.761, 0.854)
AST_{CL}	θ_{13}	Aspartate aminotransaminase effect on CL	0.00886	(-0.0573, 0.073)
$BILI_{CL}$	θ_{14}	Total bilirubin effect on CL	0.00165	(-0.0476, 0.0499)
AGE_{CL}	θ_{15}	Age effect on CL	-0.345	(-0.416, -0.277)
$MDRD_{V1}$	θ_{16}	Glomerular filtration effect on V1	-0.0267	(-0.0821, 0.0278)
Residual variability				
$PropErr_{IV}$	$\Sigma_{(1,1)}$	Variance of porportional error (IV)	0.0333 [CV%=18.2]	(0.0321, 0.0347)
$PropErr_{ORAL}$	$\Sigma_{(2,2)}$	Variance of porportional error (Oral)	0.308 [CV%=55.5]	(0.297, 0.319)

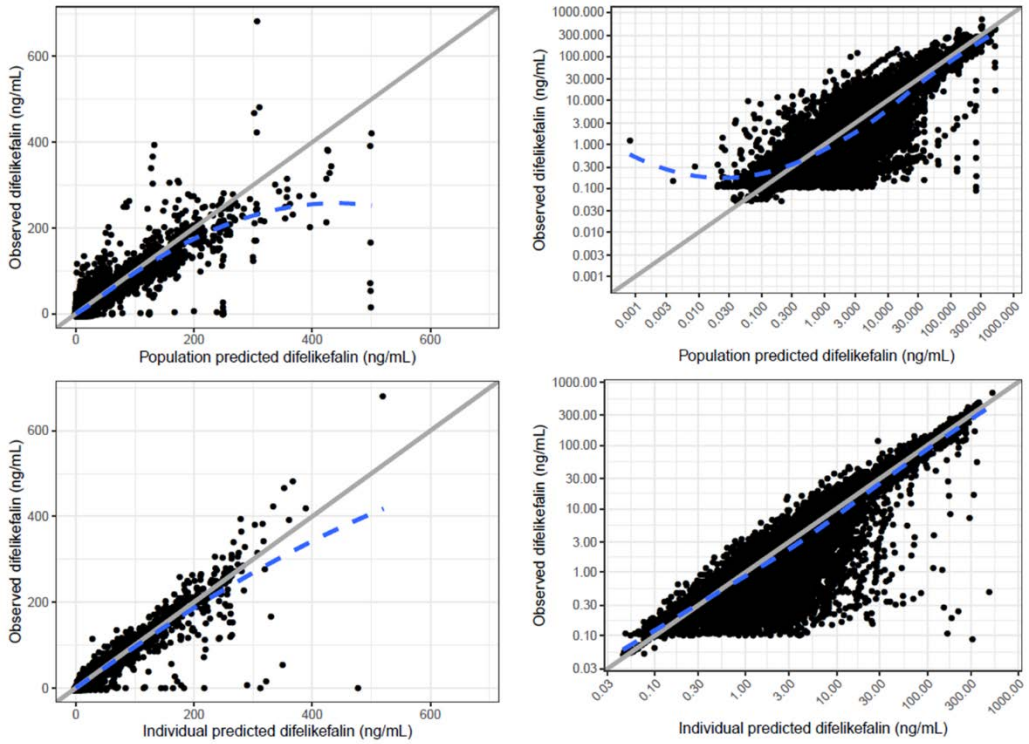
			Estimate	95% CI
Interindividual variability parameters				
IIV-CL	$\Omega_{(1,1)}$	Variance of clearance	0.331 [CV%=62.6]	(0.27, 0.403)
IIV-V1	$\Omega_{(2,2)}$	Variance of central volume	0.314 [CV%=60.7]	(0.253, 0.388)
IIV-Q2	$\Omega_{(3,3)}$	Variance of Q2	1.03 [CV%=134]	(0.808, 1.29)
IIV-V2	$\Omega_{(4,4)}$	Variance of peripheral volume 1	1.33 [CV%=167]	(1.04, 1.7)
IIV-Q3	$\Omega_{(5,5)}$	Variance of Q3	8.63 [CV%=7480]	(6.73, 10.4)
IIV-V3	$\Omega_{(6,6)}$	Variance of peripheral volume 2	1.01 [CV%=132]	(0.758, 1.29)
IIV-KA	$\Omega_{(7,7)}$	Variance of first order absorption rate	0.663 [CV%=97.0]	(0.272, 1.49)
IIV-F1	$\Omega_{(8,8)}$	Variance of logistic transformation for F1	0.376 [CV%=67.6]	(0.266, 0.528)
IIV-D1	$\Omega_{(9,9)}$	Variance of D1	1.47 [CV%=183]	(0.979, 1.96)
Covariance parameters				
CL-V1	$\Omega_{(2,1)}$	Covariance CL-V1	0.216 [SD=0.0284]	(0.164, 0.276)
CL-Q2	$\Omega_{(3,1)}$	Covariance CL-Q2	0.249 [SD=0.0579]	(0.148, 0.383)
V1-Q2	$\Omega_{(3,2)}$	Covariance V1-Q2	-0.0935 [SD=0.0475]	(-0.188, -0.000134)
CL-V2	$\Omega_{(4,1)}$	Covariance CL-V2	-0.411 [SD=0.0474]	(-0.507, -0.32)
V1-V2	$\Omega_{(4,2)}$	Covariance V1-V2	-0.311 [SD=0.05]	(-0.412, -0.215)
Q2-V2	$\Omega_{(4,3)}$	Covariance Q2-V2	-0.427 [SD=0.0956]	(-0.618, -0.239)
CL-Q3	$\Omega_{(5,1)}$	Covariance CL-Q3	1.36 [SD=0.166]	(1.04, 1.67)
V1-Q3	$\Omega_{(5,2)}$	Covariance V1-Q3	0.739 [SD=0.133]	(0.475, 1.01)
Q2-Q3	$\Omega_{(5,3)}$	Covariance Q2-Q3	1.96 [SD=0.307]	(1.35, 2.55)
V2-Q3	$\Omega_{(5,4)}$	Covariance V2-Q3	-2.97 [SD=0.344]	(-3.59, -2.35)
CL-V3	$\Omega_{(6,1)}$	Covariance CL-V3	0.503 [SD=0.0617]	(0.387, 0.629)
V1-V3	$\Omega_{(6,2)}$	Covariance V1-V3	0.277 [SD=0.047]	(0.188, 0.374)
Q2-V3	$\Omega_{(6,3)}$	Covariance Q2-V3	0.657 [SD=0.111]	(0.45, 0.886)
V2-V3	$\Omega_{(6,4)}$	Covariance V2-V3	-0.917 [SD=0.117]	(-1.14, -0.711)
Q3-V3	$\Omega_{(6,5)}$	Covariance Q3-V3	2.84 [SD=0.364]	(2.17, 3.49)

Abbreviations: CI = confidence intervals; CV = coefficient of variation
CV% of sigma = sqrt(estimate) * 100
Absolute bioavailability (F) was calculated using Equation 27: median (95*% CI) = 0.0752 (0.0687, 0.0826)
All clearance and volume terms were allometrically scaled (full parameter equations given in main text)

Source: CTX0201F-Report-v1.0-Final, Page 55-56, Table 7-8.

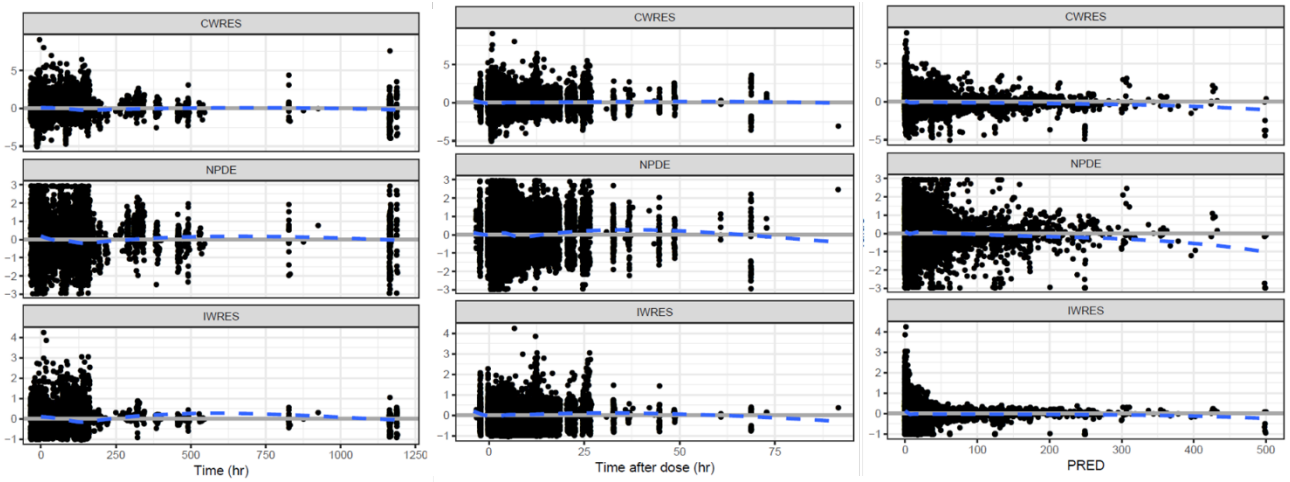
The diagnostic plots for the IV-oral dosing model from one single chain of Bayesian model are shown in Figure 14 and Figure 15. VPC plots stratified by renal function are shown in Figure 16. Similar as the IV dosing final population PK model, no significant bias was observed in the diagnostic plots. The dose normalized VPCs showed the median, 2.5th and 97.5th percentiles of the observed data were approximately centered within the within the 95% CI of the corresponding centile. The median of predicted difelikefalin concentration at approximately 12-hours post dose was slightly over-estimate in subjects with normal renal function. This might be due to the high proportion of the BLQ data.

Figure 14: Observed versus Population and Individual Predicted difelikefalin Concentration for IV-oral dosing final population PK model.



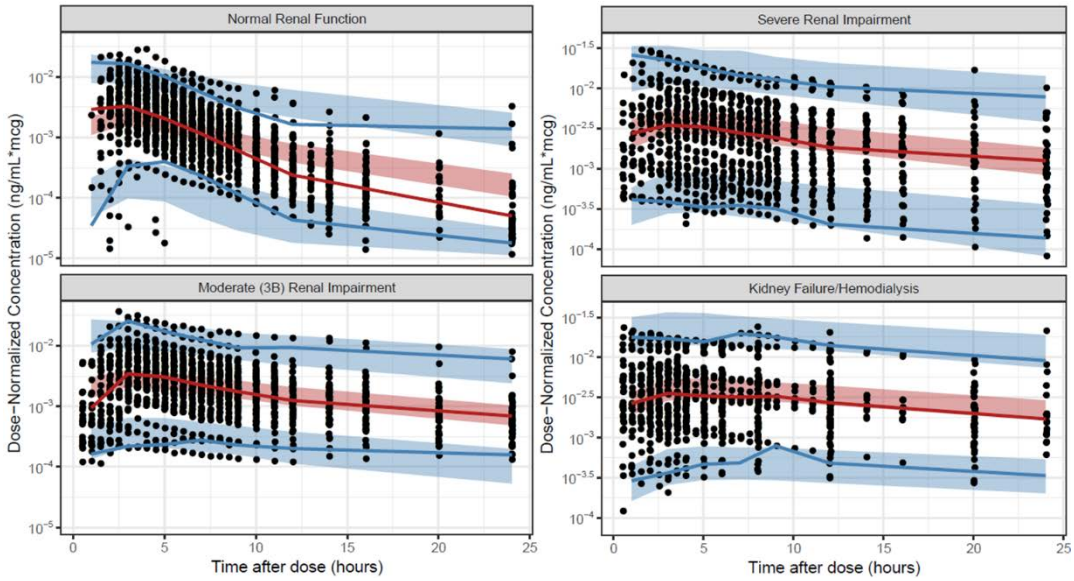
Source: CTX0201F-Report-v1.0-Final, Page 178-179, Figure S79-S80.

Figure 15: CWRES, NPDE and IWRES over Time, Time After Dose or PRED for IV-oral dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 182-184, Figure S83-S85.

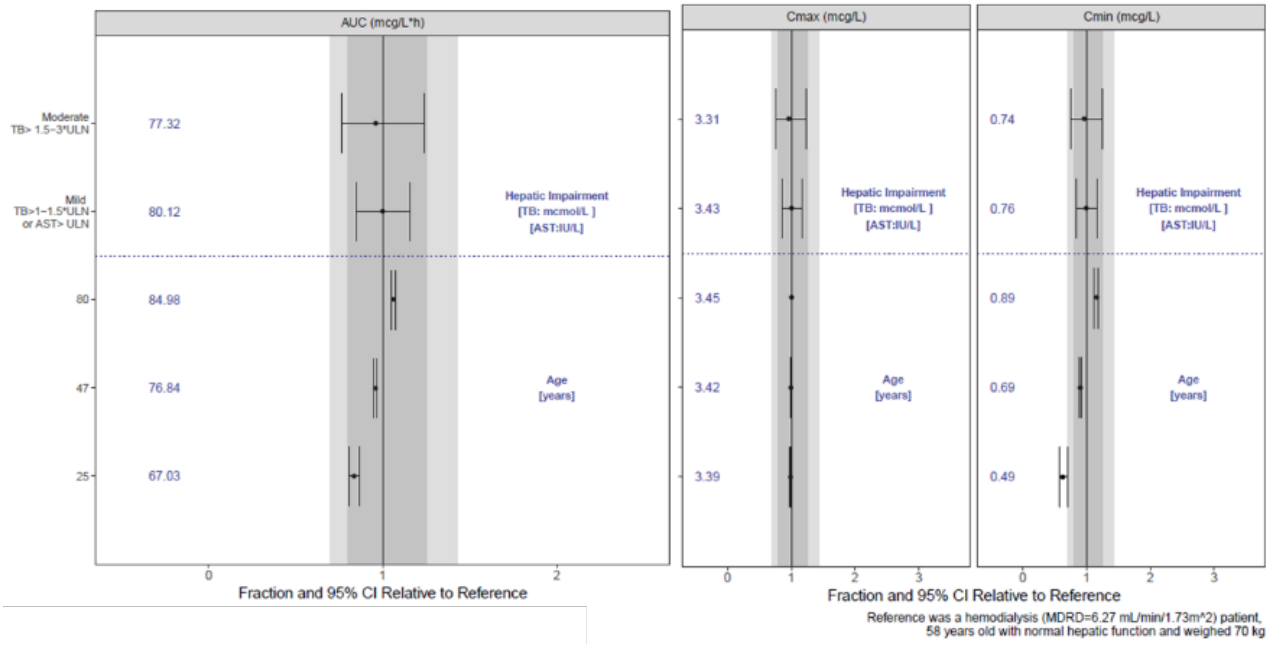
Figure 16: Visual Predictive Check (VPC) of the Dose Normalized difelikefalin Concentrations versus Time After Dose, Stratified by Renal Impairment for IV-oral dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 158-159, Figure S59-S60.

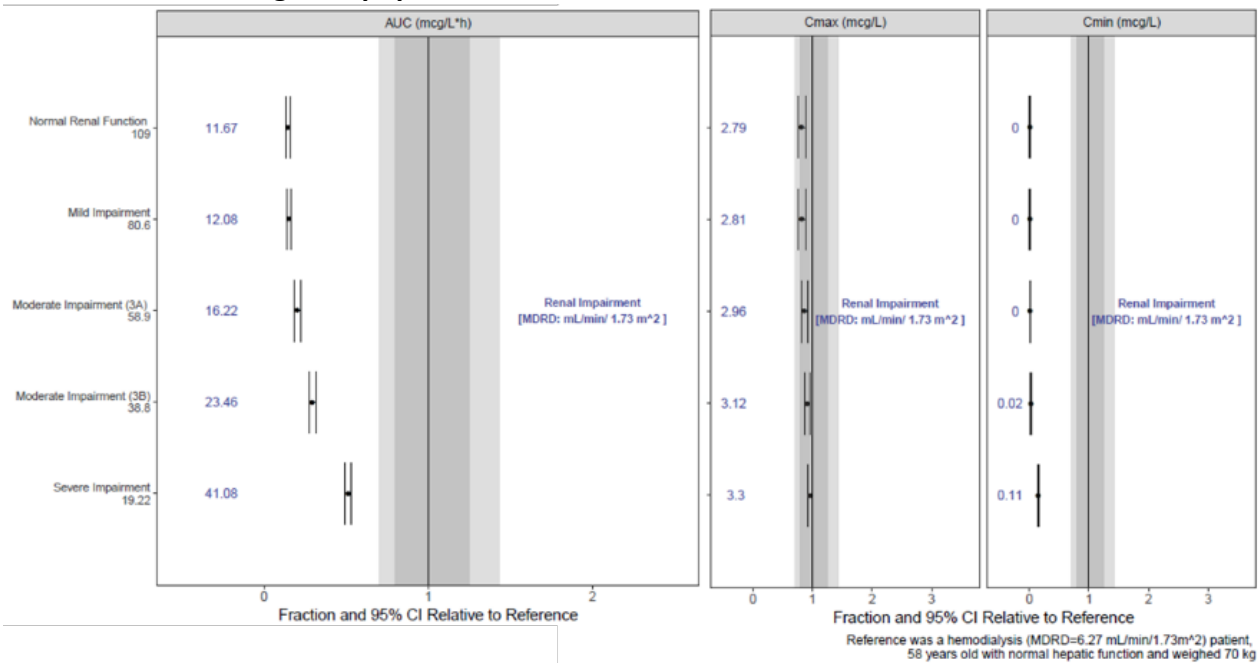
Based on the IV-oral dosing model, covariate effects on difelikefalin exposures (AUC, C_{max} and C_{min}) were shown in the following forest plots. (Figure 17 and Figure 18) The Reference patient receives oral administration of 0.5 mg difelikefalin. Subjects with normal renal function, mild, moderate, and severe renal impairment had significantly different exposures compared to the reference subject. Subjects ranging from 25 to 80 years of age and those with mild and moderate hepatic impairment had difelikefalin exposures values fully contained within the reference range. The results were similar as the IV dosing model.

Figure 17: Covariate Effects of Age and Hepatic Function on the difelikefalin AUC_{48h}, C_{max} and C_{min} based on IV and Oral dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 63, Figure 3 and Page 214, Figure S115.

Figure 18: Covariate Effects of renal Function on the difelikefalin AUC_{48h}, C_{max} and C_{min} based on IV and Oral dosing final population PK model.



Source: CTX0201F-Report-v1.0-Final, Page 213, Figure S114 and Page 215, Figure S116.

Reviewer's comments:

The population PK models developed by the applicant were checked by the reviewer with SAEM and IMP methods. Similar estimation results were obtained, and the models appear to be reasonable in general because of the good agreement between observations and predictions. The 95% CI of the covariates AST on CL and BILI on CL in the IV dosing model were (-0.0965, 0.0183) and (-0.0758, 0.0183). The 95% CI of the covariates AST on F1, BILI on F1, AST on CL and BILI on CL in the IV-oral dosing model were (-0.0862, 0.248), (-0.193, 0.157), (-0.0573, 0.073) and (-0.0476, 0.0499). The estimated 95% CI of these parameters indicated the covariate effects might not be significant.

Simulation results based on the population PK analysis showed that difelikefalin exposures in subjects with mild and moderate hepatic impairment were similar to those with normal hepatic function. No sufficient data were obtained to evaluate the PK profile for subjects with severe hepatic impairment as there were only 2 subjects with severe hepatic impairment involved in the studies. The results were acceptable. No effect of subject race or gender on CR845 exposure was identified in population PK analysis. Although subjects with younger age appear to have lower exposures than older subjects, the difference of difelikefalin exposure was within 80%-125% range. It's expected that age (25 to 80 years of age), race and gender do not affect the PK of difelikefalin. The exposure of difelikefalin were significant different in subjects with normal, mild, moderate and severe renal impairment compared to the reference hemodialysis patient. (Figure 18) The exposure of difelikefalin also decreased with improved renal function. The result is in line with the study CLIN1005 that subjects with moderate and severe renal impairment had higher exposure of difelikefalin than in subjects with mild renal impairment and subjects with severe renal impairment had higher exposure than subjects with moderate renal impairment.

16.5. Efficacy: Additional Information and Assessment

Table 38 presents the results for both a ≥3-point improvement and a ≥4-point improvement in WI-NRS from baseline to Week 12 without adjusting for conducting the interim analysis.

Table 38: Results for WI-NRS at Week 12 Without Adjusting for Conducting the Interim Analysis (ITT[1])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin (N=189)	Placebo (N=189)	Difelikefalin (N=237)	Placebo (N=236)
≥3-point Improvement in WI-NRS				
Proportion	52%	31%	49%	38%
Difference (95% CI)	21% (11%, 31%)		10% (1%, 20%)	
Adjusted Proportion[2]	51%	28%	53%	43%
Odd Ratio (95% CI)	2.6 (1.7, 4.1)		1.5 (1.1, 2.3)	
P-value[2]	<0.001		0.027	
≥4-point Improvement in WI-NRS				
Proportion	40%	21%	37%	26%
Difference (95% CI)	19% (9%, 28%)		11% (2%, 19%)	
Adjusted Proportion[2]	38%	18%	41%	29%
Odds Ratio (95% CI)	2.9 (1.7, 4.7)		1.7 (1.1, 2.5)	
P-value[2]	<0.001		0.016	

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Adjusted proportion and p-value are based on a logistic regression with treatment, baseline NRS score, region (only Trial CLIN3103), prior use of anti-itch medication (yes/no), and presence of specific medical condition (yes/no) as factors in the model.
Source: Statistical Reviewer's Analysis; ADEF.xpt

Table 39 presents the results for the Agency's recommended primary efficacy endpoint (i.e., proportion of subjects with a 4-point improvement in WI-NRS from baseline to Week 12) with and without the Kumar sites (#116 for Trial CLIN3102 and #840007 for Trial CLIN3103). For both trials, the results were similar with and without Kumar's site.

Table 39: Results for ≥4-point Improvement in WI-NRS at Week 12 With and Without Kumar Sites (ITT[1,2])

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin	Placebo	Difelikefalin	Placebo
All Sites	**N=189**	**N=189**	**N=237**	**N=236**
Proportion	40%	21%	37%	26%
Difference (95% CI)	19% (9%, 28%)		12% (3%, 20%)	
Adjusted Proportion[3]	39%	18%	41%	28%
Odds Ratio (95% CI)[3]	2.9 (1.8, 4.8)		1.8 (1.1, 2.7)	
P-value[3]	<0.001		0.010	
Without Kumar Site (#116 and #840007)	**N=180**	**N=176**	**N=215**	**N=224**
Proportion	39%	20%	38%	26%

	Trial CLIN3102		Trial CLIN3103	
	Difelikefalin	**Placebo**	**Difelikefalin**	**Placebo**
Difference (95% CI)	19% (10%, 29%)		11% (2%, 20%)	
Adjusted Proportion[3]	36%	15%	42%	29%
Odds Ratio (95% CI)[3]	3.0 (1.8, 5.1)		1.7 (1.1, 2.7)	
P-value[3]	<0.001		0.017	

[1] Intent-to-Treat (ITT) population: all randomized subjects. Missing data were imputed using multiple imputation (MI).
[2] Combined analysis used the separate interim and post-interim results to generate an adjusted overall estimate and p-value using the Cui, Hung, and Wang (CHW) methodology.
[3] Adjusted proportion, odds ratio (95% CI), and p-value are based on a logistic regression with treatment, baseline NRS score, region (only Trial CLIN3103), prior use of anti-itch medication (yes/no), and presence of specific medical condition (yes/no) as factors in the model.
Source: Statistical Reviewer's Analysis (same results as Applicant's Analysis); ADEF.xpt

---

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

JENNIFER L HARMON
08/20/2021 10:51:56 AM

HAMID R SHAFIEI
08/20/2021 10:57:29 AM

YONGCHENG HUANG
08/20/2021 11:19:06 AM

BARBARA A HILL
08/20/2021 11:22:46 AM

ANDREW C GOODWIN
08/20/2021 11:23:33 AM

DIPAK PISAL
08/20/2021 11:51:08 AM

CHINMAY SHUKLA
08/20/2021 11:54:23 AM

YANGBING LI
08/20/2021 12:00:12 PM

JIANG LIU
08/20/2021 12:11:43 PM

SURESH DODDAPANENI
08/20/2021 12:19:04 PM

MATTHEW W GUERRA
08/20/2021 12:22:01 PM

MOHAMED A ALOSH
08/20/2021 12:23:43 PM

LAURA L JOHNSON
08/20/2021 12:37:18 PM

GARY T CHIANG
08/20/2021 12:52:43 PM

AMY S WOITACH
08/20/2021 01:00:03 PM

KENDALL A MARCUS
08/20/2021 01:46:21 PM

JULIE G BEITZ
08/20/2021 01:49:46 PM

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

JENNIFER L HARMON
08/20/2021 10:51:56 AM

HAMID R SHAFIEI
08/20/2021 10:57:29 AM

YONGCHENG HUANG
08/20/2021 11:19:06 AM

BARBARA A HILL
08/20/2021 11:22:46 AM

ANDREW C GOODWIN
08/20/2021 11:23:33 AM

DIPAK PISAL
08/20/2021 11:51:08 AM

CHINMAY SHUKLA
08/20/2021 11:54:23 AM

YANGBING LI
08/20/2021 12:00:12 PM

JIANG LIU
08/20/2021 12:11:43 PM

SURESH DODDAPANENI
08/20/2021 12:19:04 PM

MATTHEW W GUERRA
08/20/2021 12:22:01 PM

MOHAMED A ALOSH
08/20/2021 12:23:43 PM

LAURA L JOHNSON
08/20/2021 12:37:18 PM

GARY T CHIANG
08/20/2021 12:52:43 PM

AMY S WOITACH
08/20/2021 01:00:03 PM

KENDALL A MARCUS
08/20/2021 01:46:21 PM

JULIE G BEITZ
08/20/2021 01:49:46 PM

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

Statistical Review and Evaluation

CLINICAL STUDIES

NDA/Serial Number:	214916
Drug Name:	Difelikefalin (intravenous CR845)
Indication:	Treatment of pruritus in patients undergoing hemodialysis
Study number:	CR845-100303
Applicant:	Cara Therapeutics, Inc.
Date(s):	Date of Document: 12/23/2020 Consult received date: 02/18/2021 Completion date: 06/30/2021
Review Priority:	Standard
Biometrics Division:	DBVI
Statistical Reviewer:	Anna Sun, Ph.D., Mathematical Statistician, OB/DBVI
Concurring Reviewers:	Qianyu Dang, Ph.D., Lead Mathematical Statistician, OB/DBVI Atiar Mohammad Rahman, Ph.D., Deputy Division Director, OB/DBVI
Medical Division:	Control Substance Staff
The CSS Team:	Katherine Bonson, Ph.D., Pharmacologist, OCD/CSS
Project Manager:	Sandra Saltz, Project Manager, CSS

Keywords: *placebo-controlled study, treatment discontinuation, physical withdrawal, Clinical Opiate Withdrawal Scale (COWS)*

Table of Contents

List of Tables

1. Executive Summary

Study CR845-100303 was a multicenter, randomized, double-blind, placebo-controlled study to assess the potential of physical withdrawal from difelikefalin upon treatment discontinuation after 3 weeks of treatment with 0.5 mcg/kg, administered as an IV bolus 3 times a week, in subjects undergoing hemodialysis.

The primary objective of this study was to assess the potential of physical withdrawal from difelikefalin at a dose of 0.5 mcg/kg upon treatment discontinuation after 3 weeks of administration in subjects undergoing hemodialysis.

The study consisted of a Screening Phase; a 3-week Open-label Phase; a 2-week randomized, placebo-controlled Double-blind Phase; and a Follow-up Visit.

The reviewer analyzed the primary endpoint: the treatment difference between difelikefalin and placebo for the maximum COWS total score during the Double-blind Phase, and the secondary endpoints included the treatment difference between difelikefalin and placebo for the following measures: The COWS total score at Week 4 and Week 5, Maximum SOWS total score during the Double-blind Phase, The SOWS total score at Week 4 and Week 5.

The reviewer's statistical analysis results

1. In the primary endpoint analysis using the Per-protocol Population, for determination of noninferiority (i.e., withdrawal scores in subjects switched to placebo were not clinically worse than withdrawal scores in subjects continuing to receive difelikefalin), the null hypothesis was defined as a median or mean difference between placebo and difelikefalin in maximum COWS total score of $\geqslant$ 4 points. The analysis results showed that:

    - The Hodges-Lehmann estimate of the median difference in maximum COWS total score was 1.00 (CI: -Infty, 2.00), with an upper limit of the CI being less than 4.

    - The LS mean difference between placebo and difelikefalin was 0.49 (CI: -Infty, 1.32), the mean difference was not statistically significant (P-value <0.0001).

    The results support the noninferiority of withdrawal symptoms for subjects switched to placebo versus subjects continued on difelikefalin.

2. In the sensitivity analysis of the primary endpoint analysis using Full Analysis Population, noninferiority for placebo was also demonstrated: The Hodges-Lehmann estimate of the median difference in maximum COWS total score was 1.00 (CI: -Infty, 2.00), with an upper limit of the CI being less than 4. The mean difference between placebo and difelikefalin was not statistically significant (P-value <0.0001).

3. The secondary endpoint of the treatment difference in COWS total scores at Week 4 and Week 5 also showed noninferiority for clinical withdrawal signs and symptoms in subjects switched from difelikefalin to placebo compared with subjects continuing to receive difelikefalin. The LS mean treatment group differences in COWS total score at Week 4 and Week 5 were 0.66 (CI: -Infty, 1.47) and 0.37 (CI: -Infty, 1.15),

respectively. The differences between placebo and difelikefalin for both week 4 and week 5 were not statistically significant (P-values <0.0001).

4. For the secondary endpoint of the treatment difference in maximum SOWS total scores, the results showed that:

   - The Hodges-Lehmann estimate of the median difference in maximum SOWS total score for the Per-protocol Population was 1.00 (CI: -Infty, 4.00), with an upper limit of the CI equals 4.

   - The LS mean values for maximum SOWS Total scores during the Double-blind Phase between the difelikefalin and placebo groups were: 3.24 and 4.05, respectively, with a LS mean difference of 0.81 (CI: -Infty, 2.63).

   NOTE: The margin used to test noninferiority with respect to maximum SOWS total score is not specified.

5. For the SOWS total scores at Week 4 and Week 5, the LS mean score was higher in the placebo group than in the difelikefalin group: 2.59 versus 1.52 at Week 4, with an LS mean difference of 1.07 (CI: -Infty, 2.10); and 2.38 versus 1.05 at Week 5, with an LS mean difference of 1.33 (CI: -Infty, 2.26).

Statistical Issues and Concerns:

1. The baseline value that the sponsor used was the average of the non-missing values during the Baseline Period (Day -7 to Day -1).

   The baseline value that the reviewer used for primary analysis, sensitivity analysis and secondary analysis was the Mean COWS Derived Total Score in Week 3.

   The way to measure withdrawal is to measure the symptoms of withdrawal relative to the baseline right before the discontinuation occurs which is derived from week 3 in this case.

2. Since the sample size of this study is small, comparing the median difference between Placebo and CR845 instead of mean difference may be more appropriate.

3. The study days that the sponsor used are shifted by one day from what table 3 (Study Schedule of Assessments) shows. For example, table 3 shows week 4 includes day 22, 24 and 26, while the sponsor used days 24, 26, and 29 for their analysis. This also applies to week 5. Furthermore, the double- blind treatment phase includes **7** days (days 22, 24, 26, 29, 31, 33 and 36) instead of 6 days.

   Since this is a physical dependence study, the withdraw syndrome may delay and last for a few days. The sponsor should explain why they used these days.

4. The sponsor's secondary analysis includes maximum SOWS total score during the Double-blind Phase. However, the margin used to test noninferiority with respect to maximum SOWS total score is not specified.

5. Is two weeks of treatment phase enough?

   The following table shows the number of subjects having the max COWS total score on each day. 18 out of 28 subjects had the max COWS total score at the last 3 visits. Especially for day 36, 11 out of 28 subjects had the max COWS total score on this day.

   It indicates that subjects had larger responses towards the end of the study. The reviewer is concerned if two weeks of treatment phase is enough to assess the potential of physical withdrawal from Intravenous CR845 (Difelikefalin) in Hemodialysis Patients.

Visit Day	Frequency
22	5
24	1
26	3
29	1
31	4
33	3
36	11

In conclusion, the reviewer's statistical analysis results support the sponsor's conclusion, that in subjects undergoing hemodialysis and treated with difelikefalin for 3 weeks, switching to placebo for 2 weeks did not elicit clinical signs or symptoms of withdrawal, as measured by maximum COWS total score, relative to subjects who continued treatment with difelikefalin.

However, we have noted some specific concerns listed above, we defer to Control Substance Staff (CSS) reviewers' decision.

2. Review Report on Study CR845-100303

2.1 Introduction

Chronic kidney disease-associated pruritus (CKD-aP; also known as uremic pruritus) is a chronic, unremitting, and highly bothersome condition in patients with chronic kidney disease, particularly those undergoing hemodialysis. Patients with CKD-aP frequently exhibit considerable mechanical skin damage, superimposed skin infections, and, in some instances, chronic lesions of prurigo nodularis or skin lichenification as a consequence of continual scratching and resultant excoriations. Currently no treatments are approved in the US for CKD-aP, and current off-label therapies do not adequately treat the condition.

Difelikefalin (intravenous [IV] CR845) is a selective and full agonist at the KOR, with no or negligible activity at mu or delta opioid receptors or other receptors, ion channels, or transporters. The unique peptidic structure of difelikefalin differs significantly from other small-molecule KOR agonists developed to date, which for the most part, are active in the central nervous system (CNS). Difelikefalin is a hydrophilic peptide with limited membrane permeability by passive diffusion, thereby limiting access of the drug to the CNS. Difelikefalin is expected to have an improved safety profile and be better tolerated than other opioid agonists, including centrally acting KOR agonists.

The antipruritic properties of difelikefalin have been demonstrated in nonclinical and clinical studies. In a phase 2 randomized, double-blind, placebo-controlled study in subjects with moderate-to-severe pruritus undergoing hemodialysis (CR845-CLIN2101), difelikefalin 0.5 mcg/kg, 1.0 mcg/kg, or 1.5 mcg/kg administered IV 3 times per week, demonstrated a significant decrease in itch intensity and improvement in measures of quality of life compared with placebo, as well as adequate safety and tolerability. Based on these results and the results of the Double-blind Phase of the phase 3 studies CR845-CLIN3102 and CR845-CLIN3103, difelikefalin is expected to reduce the intensity of itching for patients with CKD-aP who are undergoing hemodialysis.

Difelikefalin has been shown to have a low to no risk for abuse potential in humans in comparison to opioids in clinical use. In addition, there have been no clustering of adverse events (AEs) related to physical withdrawal symptoms in humans upon discontinuation of difelikefalin after IV administration up to a year of administration in patients undergoing hemodialysis. This clinical study was conducted to further assess the potential for the development of physical dependence with difelikefalin treatment as manifested by withdrawal symptoms upon cessation of treatment.

2.1.1 Objectives of the study

Primary objective: To assess the potential of physical withdrawal from difelikefalin at a dose of 0.5 mcg/kg upon treatment discontinuation after 3 weeks of administration in subjects undergoing hemodialysis.

Secondary objective: To assess the safety and plasma levels of difelikefalin at a dose of 0.5 mcg/kg in subjects undergoing hemodialysis

2.1.2 Study design

This was a multicenter, randomized, double-blind, placebo-controlled study to assess the potential of physical withdrawal from difelikefalin upon treatment discontinuation after 3 weeks of treatment with 0.5 mcg/kg, administered as an IV bolus 3 times a week, in subjects undergoing hemodialysis. The purpose of the study was to determine whether subjects undergoing hemodialysis and taking difelikefalin develop physical dependence and experience withdrawal symptoms upon cessation of treatment.

The study consisted of a Screening Phase; a 3-week Open-label Phase; a 2-week randomized, placebo-controlled Double-blind Phase; and a Follow-up Visit.
During the course of the study, signs of potential physical withdrawal were assessed using 1 observer-rated scale, several subject-reported scales, and vital signs and physiological measures. In addition, subjects completed the Worst Itching Intensity numerical rating scale (WI-NRS).

The Screening Phase had a duration of up to 28 days and consisted of a Screening Visit and a 1-week Baseline Period. The Screening Visit was performed to determine the subject'seligibility and was followed by a 1-week period during which the subject and trained study staffcompleted questionnaires at dialysis visits to establish a baseline prior to treatment and furtherconfirm eligibility. Subjects were required to complete the WI-NRS to determine whether theywere experiencing moderate-to-severe itch (defined as at least 1 WI-NRS score >4 during theBaseline Period prior to dosing).

Eligible subjects received open-label difelikefalin as an IV bolus administered at a dose of 0.5 mcg/kg at the end of each dialysis session (3 times/week) for 3 weeks. All scheduled study visits during the Open-label Phase were conducted on dialysis days. Clinical laboratory tests, vital signs, AEs, and concomitant medications were monitored throughout this phase. Subjects and staff also completed the following assessments for baseline purposes: Clinical Opiate Withdrawal Scale (COWS), Subjective Opiate Withdrawal Scale (SOWS), visual analog scales (VAS), and Leeds Sleep Evaluation Questionnaire (LSEQ).

On the first dialysis of Week 4 at the start of the Double-blind Phase, subjects were randomized 1:1 either to continue to receive difelikefalin or to receive placebo at the end of each dialysis session (3 times/week). Assessments for withdrawal symptoms were performed during the Double-blind Phase. Vital signs, AEs, and concomitant medications were monitored throughout this phase.

The Follow-up Visit occurred 7 to 10 days following the End of Treatment or Early Termination Visit. Adverse events, vital signs, and changes in concomitant medications were recorded.

Assessments of Physical Withdrawal:

The primary endpoint was the treatment difference between difelikefalin and placebo for the maximum COWS total score during the Double-blind Phase.

Secondary endpoints included the treatment difference between difelikefalin and placebo for the following measures:

- COWS total score at Week 4 and Week 5
- Maximum SOWS total score during the Double-blind Phase
- Subjective Opiate Withdrawal Scale (SOWS) total score at Week 4 and Week 5

Other withdrawal endpoints included the most extreme result of the Double-blind Phase and also results for Week 4 and Week 5 for the following:

- Four aspects of sleep using LSEQ (getting to sleep, quality of sleep, awakening from sleep, and
- Pain, feeling sick, bad effect, and hallucinating VASs
- Vital signs/physiological measures evaluated postdialysis

2.1.3 Number of subjects (Planned and Analyzed):

Planned: 40 enrolled subjects

Analyzed: 52 subjects enrolled; 35 subjects received treatment during the Open-label Phase;

30 subjects continued to the Double-blind Phase

Enrolled Population: 52 subjects

Full Analysis Population: 30 subjects

Per-protocol Population: 28 subjects

Open-label Safety Population: 35 subjects

Double-blind Safety Population: 30 subjects

1.1.4 Statistical and Analytical Plans

Analysis populations:

- Enrolled Population – all subjects who signed the informed consent form.
- Full Analysis Population – all subjects who completed the 3-week Open-label Phase and were randomized on Day 1 of Week 4 into the 2-week Double-blind Phase. This population was analyzed as randomized and used for the sensitivity analyses of the primary and secondary endpoints, as well as analyses of other withdrawal endpoints.
- Per-protocol Population – all subjects in the Full Analysis Population who received all double-blind doses during Weeks 4 and 5, did not have any missing COWS total scores during Weeks 4 and 5 (after estimation of individual missing item responses), and did not have significant protocol deviations that would have affected the evaluation of the primary endpoint. This population was analyzed as randomized and used for the primary

analysis of the primary and secondary endpoints, as well as analyses of other withdrawal endpoints.

- Open-label Safety Population – all subjects who received at least 1 dose of difelikefalin during the Open-label Phase. This population was used to analyze all Open-label Phase safety endpoints.

- Double-blind Safety Population – all subjects who received at least 1 dose of study drug during the Double-blind Phase. This population was analyzed as treated and used to analyze the Double-blind Phase safety endpoints.

Primary Endpoint Analyses

The primary endpoint was the treatment difference between difelikefalin and placebo for the maximum COWS total score during the Double-blind Phase.

Primary Analysis

The primary analysis of the primary endpoint was performed using the Per-protocol Population.

For determination of noninferiority, indicating that withdrawal scores for the placebo group were not clinically worse than withdrawal scores for the difelikefalin group, the mean maximum COWS total score for the placebo group should not have been greater than +4 points higher relative to the mean maximum COWS total score for the difelikefalin group. The null hypothesis H_0 below was tested against the alternative hypothesis H_A below:

H_0: The difference in mean maximum COWS total score, defined as (μ_{Pmax} - μ_{Dmax}) is $\geq$4.

H_A: The difference in mean maximum COWS total score, defined as (μ_{Pmax} - μ_{Dmax}) is <4.

Where μ_{Dmax} and μ_{Pmax} denote the mean maximum COWS total score during the Double-blind Phase in the difelikefalin and placebo groups, respectively.

The maximum COWS total scores over the Double-blind Phase were analyzed using an analysis of covariance (ANCOVA) model. The model contained treatment (difelikefalin versus placebo) and itching severity strata (none-mild or moderate-severe at randomization) as fixed effects and the baseline COWS score as a covariate. The least squares (LS) means and standard error (SE) were presented for each treatment group. The estimate for the difference between treatment groups was also presented along with SE and confidence interval (CI).

The testing of this 1-sided null hypothesis was conducted at the 2.5% error level.

The Hodges-Lehmann estimate and CI for the difference (placebo – difelikefalin) were also presented as a supportive analysis.

Sensitivity Analysis 1 (Full Analysis Population Assuming Missing at Random)

The same ANCOVA model and presentation used for the primary analysis were applied on the Full Analysis Population. The maximum COWS total score for each subject was calculated using observed data only.

Sensitivity Analysis 2 (Full Analysis Population Assuming Missing Not at Random – Worse Case Imputation)

This sensitivity analysis used a worse-case scenario for subjects with any missing Double-blind Phase COWS total scores in the Full Analysis Population. For subjects receiving placebo in the Double-blind Phase, the higher of 1) all the maximum COWS total scores reported by the remaining placebo subjects and 2) the maximum of the subject's observed Double-blind Phase scores were used. For subjects receiving difelikefalin in the Double-Blind Phase, only observed data (within a specific subject) were used to derive the maximum. This sensitivity analysis used the same ANCOVA model and presentation as used for the primary analysis.

Sensitivity Analysis 3 (Including Week 3 as a Baseline Covariate)

To investigate whether pre-randomization COWS scores collected during Week 3 of difelikefalin open-label treatment, in addition to baseline COWS scores, possibly affected scores post randomization, the primary analysis and sensitivity analysis 1 were repeated, but the alternate baseline COWS score (using Week 3 of the Open-label Phase) was added in the statistical model as a covariate alongside the baseline derived from the COWS collected during the Baseline Period.

Summary Tabulations Supporting the Primary Endpoint Analysis

The derived baseline, individual Double-blind Phase visit and maximum Double-blind Phase COWS total scores were summarized overall, by randomized itching severity strata (none-mild or moderate-severe) and by study site. In addition to continuous summary statistics, the COWS total score was summarized at each Double-blind Phase assessment using categories of 0-4 (no/minimal withdrawal symptoms), 5-12 (mild), 13-24 (moderate), 25-36 (moderately severe), and >36 (severe). Categories for the derived mean baseline COWS total scores were applied on the rounded means.

Secondary Endpoint Analyses

Secondary endpoints included the treatment difference between the difelikefalin and placebo groups for COWS total score at Week 4 and Week 5, maximum SOWS total score during the Double-blind Phase, and SOWS total score at Week 4 and Week 5. All the secondary endpoint analyses were performed using the Full Analysis and Per-protocol Populations and data collected on or prior to the date of the End of Treatment/Early Termination Visit in the Double-blind Phase.

Determination of Sample Size

The original primary endpoint for which the sample size was estimated was the comparison of the COWS as an objective clinical assessment of withdrawal symptom scores in the Double-blind Phase between the treatment and placebo groups during Week 4 (Days 24, 26, and 29). With the proposed sample size of 40 completed subjects (20 subjects per treatment

group), the coverage probability was greater than 80% that a two-sided 95% CI of the difference between treatment with difelikefalin and placebo during Week 4 would fall within ±4 points on the COWS scale (given alpha = 0.05 and a common SD of 4). A sample size of 36 subjects would be required for one-sided 95% CI for noninferiority.

However, because of site closures related to the COVID-19 pandemic, only 30 subjects could be randomized in the Double-blind Phase. Under the original sample size assumptions (common SD of 4), the power to show noninferiority from 30 subjects dropped to 75%. A common SD of 3.75 would ensure 80% power, and therefore 30 subjects was still deemed adequate to meet the objectives of the study.

2.1.5 Sponsor's Conclusions for Evaluation of withdrawal:

The potential for physical withdrawal from difelikefalin following treatment discontinuation after 3 weeks of treatment in subjects undergoing hemodialysis was evaluated by determining the difference in maximum COWS total scores during the Double-blind Phase between subjects continuing to receive difelikefalin and subjects switched from difelikefalin to placebo. In the primary endpoint analysis using the Per-protocol Population, the LS mean maximum COWS total scores for the difelikefalin and placebo were 2.42 and 2.94, respectively, with a LS mean treatment group difference of 0.52 (95% CI: -0.56, 1.59). The upper limit of the 95% CI for the LS mean difference in maximum scores (1.59) was less than 4, indicating that clinical withdrawal signs and symptoms, as measured by maximum COWS total score, in subjects switched from difelikefalin to placebo were noninferior to clinical withdrawal signs and symptoms in subjects continuing to receive difelikefalin. Noninferiority for placebo was also demonstrated in the following sensitivity analyses of the primary endpoint analysis:

- Sensitivity analysis 1 (Full Analysis Population, assuming data missing at random) – LS mean treatment group difference of 0.49 (95% CI: -0.53, 1.51)

- Sensitivity analysis 2 (Full Analysis Population, imputing missing data using a missing not atrandom approach) – LS mean treatment group difference of 0.49 (95% CI: -0.53, 1.51)

- Sensitivity analysis 3 of primary endpoint analysis (Per-protocol Population, includingpre-randomization [Week 3] COWS total scores as baseline) – LS mean treatment groupdifference of 0.47 (95% CI: -0.40, 1.34)

- Sensitivity analysis 3 of sensitivity analysis 1 (Full Analysis Population, includingpre-randomization [Week 3] COWS total scores as baseline) – LS mean treatment groupdifference of 0.42 (95% CI: -0.40, 1.24)

Further supporting the noninferiority of withdrawal symptoms for subjects switched to placebo versus subjects continued on difelikefalin, the Hodges-Lehmann estimate of the treatment group difference in maximum COWS total score for the Per-protocol Population was 1.00 (95% CI: -1.00, 2.00), with an upper limit of the 95% CI being less than 4.

The secondary endpoint of the treatment difference in COWS total scores at Week 4 and Week 5 also showed noninferiority for clinical withdrawal signs and symptoms in subjects

switched from difelikefalin to placebo compared with subjects continuing to receive difelikefalin. The LS mean treatment group difference in the Per-protocol Population was 0.59 (95% CI: -0.30, 1.48) at Week 4 and 0.30 (95% CI: -0.43, 1.03) at Week 5; findings were similar for the Full Analysis Population.

For the secondary endpoint of the treatment difference in maximum SOWS total scores, the difelikefalin and placebo groups of the Per-protocol Population showed no distinct differences: 3.29 and 4.11 for the difelikefalin group and placebo group, respectively, with a LS mean difference of 0.82 (95% CI: -1.05, 2.70). Sensitivity analysis 1 and sensitivity analysis 2 of this endpoint showed similar findings.

When SOWS total scores were analyzed at Week 4 and Week 5, the LS mean score was higher in the placebo group than in the difelikefalin group: 2.59 versus 1.52 at Week 4, with an LS mean difference of 1.07 (95% CI: 0.04, 2.10); and 2.38 versus 1.05 at Week 5, with an LS mean difference of 1.33 (95% CI: 0.40, 2.26). Sensitivity analysis 1 of this endpoint showed a similar treatment group difference. Regarding these findings, it should be noted that subjects randomized to placebo had a higher incidence of back pain than subjects randomized to difelikefalin (64.3% versus 31.3%), which may be indicative of greater ongoing pain in the placebo group. The findings for VAS pain scores noted are consistent with this observation.

Analyses of the secondary endpoints of LSEQ and VAS scores for pain, feeling sick, bad effect, and hallucinating showed no distinct differences between difelikefalin and placebo with respect to LS mean scores. These analyses included both ANCOVA for most extreme scores and MMRM for scores at Week 4 and Week 5. Although the 95% CI for the treatment group difference in maximum VAS pain score included 0 (LS mean difference 10.11 mm [95% CI: -8.76, 28.97]), the LS mean maximum VAS pain score in the placebo group was approximately 1.5 times higher than in the difelikefalin group (28.51 versus 18.41 mm). The LS mean scores at Weeks 4 and 5 differed by a factor of approximately 2, with the 95% CI again containing 0.

In the Per-protocol Population ANCOVA analysis of worst postdialysis vital sign/physiologic measures, the LS mean worst postdialysis diastolic blood pressure was higher in the placebo group than in the difelikefalin group, with an LS mean treatment group difference of 8.58 mmHg (95% CI: 2.18, 14.99). A similar treatment group difference in worst postdialysis diastolic blood pressure was observed in the Full Analysis Population (LS mean difference 8.27 mmHg [95% CI: 2.00, 14.55]), along with a higher LS mean worst postdialysis systolic pressure in the placebo group than in the difelikefalin group (LS mean difference 9.44 mmHg [95% CI: 0.26, 18.63]). In the MMRM analysis of postdialysis vital signs/physiologic measures at Week 4 and Week 5, the LS mean postdialysis diastolic blood pressure was higher in the placebo group than in the difelikefalin group at Week 5 (LS mean difference 6.20 mmHg [95% CI: 1.57, 10.84] for the Per-protocol Population and 6.31 mmHg [95% CI: 1.72, 10.91] for the Full Analysis Population). However, subjects who were randomized to placebo during the Double-blind Phase had higher mean postdialysis diastolic and systolic blood pressure values than the difelikefalin group at Baseline and at Week 3 (start of the Double-blind Phase). Relative to Week 3 values, no consistent increases in the mean postdialysis diastolic or systolic blood pressure values that would be indicative of withdrawal were observed in either the placebo or difelikefalin groups. Other postdialysis vital

signs/physiologic measures did not show any distinct treatment group differences in either the ANCOVA and MMRM analyses.

During Week 3 of the Open-label Phase (Days 15 to 19), mean CR845 predialysis plasma concentrations ranged from 380 to 671 pg/mL, and postdialysis concentrations (obtained 5 minutes after dosing) ranged from 5369 to 7203 pg/mL.

During the Double-blind Phase, for subjects randomized to placebo on Day 22, mean (SD) CR845 predialysis concentration was 331 (191) pg/mL. The mean postdialysis concentration was not estimated because 10 of 12 values were BLQ. The mean (SD) percentage of drug cleared by dialysis was 80.9% (8.7). After 1 dialysis cycle, CR845 concentrations in 83% (10 of 12) of the subjects were BLQ. After 2 dialysis cycles, all subjects had undetectable CR845plasma concentrations.

For subjects randomized to remain on difelikefalin during the Double-blind Phase, mean predialysis CR845 concentrations ranged from 583 to 875 pg/mL and concentrations obtained 5 minutes after dosing ranged from 5574 to 11653 pg/mL from Day 22 to 33, which demonstrates that CR845 plasma levels were maintained and comparable to the plasma concentrations observed in the Open-label Phase. On Day 36, the mean (SD) predialysis CR845 concentration was 502 (323.4) pg/mL. After dialysis, plasma CR845 concentration declined further to 90 (110.2) pg/mL. The mean (SD) percentage cleared by dialysis on Day 36 was 76.9% (7.6).

Across all subjects, the mean (SD) percentage cleared from plasma after the first dialysis after CR845 was stopped was 78.6% (8.19) and was undetectable in the plasma of subjects randomized to placebo following the second dialysis after CR845 was stopped, which occurred during Week 4. None of the Week 4 PROs described above indicated a worsening between baseline and this time point for placebo subjects.

In conclusion, in subjects undergoing hemodialysis and treated with difelikefalin for 3 weeks, switching to placebo for 2 weeks did not elicit clinical signs or symptoms of withdrawal, as measured by maximum COWS total score, relative to subjects who continued treatment with difelikefalin.

2.2 Data Location

The analysis datasets are located at

\\CDSESUB1\evsprod\NDA214916\0001\m5\datasets\cr845-100303\analysis\adam\datasets

2.3 Reviewer's Assessment

2.3.1 Primary Endpoint Analyses

The primary endpoint was the treatment difference between difelikefalin and placebo in the maximum COWS total score during the Double-blind Phase. The primary analysis of the primary endpoint was conducted using ANCOVA and the Per-protocol Population.

For determination of noninferiority, indicating that withdrawal scores for the placebo group were not clinically worse than withdrawal scores for the difelikefalin group, the mean or

median maximum COWS total score for the placebo group should not have been greater than +4 points higher relative to the difelikefalin group. The null hypothesis H_0 below was tested against the alternative hypothesis H_A below:

H_0: The difference in mean maximum COWS total score, defined as (μ_{Pmax} - μ_{Dmax}) is $\geq$4.

H_A: The difference in mean maximum COWS total score, defined as (μ_{Pmax} - μ_{Dmax}) is <4.

Where μ_{Dmax} and μ_{Pmax} denote the mean or median maximum COWS total score during the Double-blind Phase in the difelikefalin and placebo groups, respectively.

The maximum COWS total scores over the Double-blind Phase were analyzed using an analysis of covariance (ANCOVA) model. The model contained treatment (difelikefalin versus placebo) and itching severity strata (none-mild or moderate-severe at randomization) as fixed effects and the baseline COWS score as a covariate. The least squares (LS) means and standard error (SE) were presented for each treatment group.

The testing of this 1-sided null hypothesis was conducted at the 2.5% error level.

The Hodges-Lehmann estimate and CI for the difference (placebo – difelikefalin) were also presented.

Note: The baseline value that the sponsor used was the average of the non-missing values during the Baseline Period (Day -7 to Day -1). The reviewer thinks it is inappropriate to use this as the baseline value. The way to measure withdrawal is to measure the symptoms of withdrawal relative to the baseline right before the discontinuation occurs, which in this case is the Mean COWS Derived Total Score in Week 3.

The results of the primary endpoint analyses are summarized in Table 1. At baseline, mean (SD) COWS total scores were 1.87 (1.40) and 2.05 (1.47) for subjects in the Per-protocol Population randomized to difelikefalin and placebo, respectively. The LS mean maximum COWS total scores for each group during the Double-blind Phase were 2.47 and 2.96, respectively, with a LS mean difference of 0.49 (CI: -Infty, 1.32).

For determination of noninferiority (i.e., withdrawal scores in subjects switched to placebo were not clinically worse than withdrawal scores in subjects continuing to receive difelikefalin), the null hypothesis was defined as a mean or median difference between placebo and difelikefalin in maximum COWS total score of $\geqslant$ 4 points. The analysis results showed that:

- The Hodges-Lehmann estimate of the median difference in maximum COWS total score was 1.00 (CI: -Infty, 2.00), with an upper limit of the CI being less than 4.

- The LS mean difference between placebo and difelikefalin was 0.49 (CI: -Infty, 1.32), the mean difference was not statistically significant (P-value <0.0001).

The results support the noninferiority of withdrawal symptoms for subjects switched to placebo versus subjects continued on difelikefalin.

Note: Since the sample size of this study is small, comparing the median difference between Placebo and CR845 instead of mean difference may be more appropriate. Therefore, this reviewer used the Hodges-Lehmann Estimate for the comparison of median difference.

Table 1 Analysis of the Maximum COWS Total Score During the Double-blind Phase: ANCOVA Analysis (Per-protocol Population)

	Per-Protocol Population (N=28)	
	Placebo (N=13)	**CR845 0.5 mcg/kg** (N=15)
Baseline		
N	13	15
Mean	2.05	1.87
SD	1.47	1.40
Median	2.00	1.67
Min, Max	0, 4.33	0.33, 4.67
Hodges-Lehmann Estimate of median, α=0.025		
(Placebo - CR845)	1.00	
CI	-Infty, 2.00	
ANCOVA model		
LS Mean	2.96	2.47
SE	0.27	0.31
CI	2.31, 3.61	1.72, 3.23
Difference (Placebo - CR845), H_0: ($\mu_{Pmax} - \mu_{Dmax}$) ≥ 4, α=0.025		
LS Mean	0.49	
SE	0.40	
CI	-Infty, 1.32	
P- value	<.0001	

2.3.2 Sensitivity Analyses of the Primary Endpoint

Sensitivity Analysis

When the above analysis of the primary endpoint, which assumed data were missing at random, was repeated using the Full Analysis Population, the results were similar to those for the primary analysis using the Per-protocol Population (Table 1). The Hodges-Lehmann estimate of the median difference in maximum COWS total score was 1.00 (CI: -Infty, 2.00), with an upper limit of the CI being less than 4, the mean differences between placebo and difelikefalin were not statistically significant (P-value <0.0001), indicating that withdrawal signs and symptoms in subjects switched from difelikefalin to placebo were noninferior to withdrawal in signs and symptoms in subjects continuing to receive difelikefalin.

Note: The reviewer's sensitivity analysis is different from the sponsor. The sponsor's sensitivity analysis 1 (Full Analysis Population, assuming data missing at random) used the average of the non-missing values during the Baseline Period (Day -7 to Day -1) as baseline, and the sponsor's sensitivity analysis 3 included both baseline values: The Mean COWS Derived Total Score in Week 3 and the average of the non-missing values during the Baseline Period (Day -7 to Day -1). The reviewer disagree with the baseline value that the sponsor used. The way to measure withdrawal is to measure the symptoms of withdrawal relative to the baseline right before the discontinuation occurs which is derived from week 3 in this case.

Table 2 Analysis of the Maximum COWS Total Score in the DB phase: ANCOVA analysis (Primary Efficacy Analysis Including Sensitivity Analysis) Full Analysis Population

	Full Analysis Population (N=30)		**Per-Protocol Population (N=28)**	
	Placebo (N=14)	CR845 0.5mcg/kg (N=16)	Placebo (N=13)	CR845 0.5mcg/kg (N=15)
Baseline				
N	14	16	13	15
Mean	2.05	1.75	2.05	1.87
SD	1.41	1.43	1.47	1.40
Median	2.00	1.33	2.00	1.67
Min, Max	0, 4.33	0, 4.67	0, 4.33	0.33, 4.67
Hodges-Lehmann Estimate of median, α=0.025				
(Placebo - CR845)	1.00		1.00	
CI	-Infty, 2.00		-Infty, 2.00	
ANCOVA Model				
LS Mean	2.89	2.46	2.96	2.47
SE	0.26	0.29	0.27	0.31
CI	2.28, 3.51	1.77, 3.15	2.31, 3.61	1.72, 3.23
Difference (Placebo - CR845), H_0: (μ_{Pmax} - μ_{Dmax}) ≥ 4, α=0.025				
LS Mean	0.43		0.49	
SE	0.38		0.40	
CI	-Infty, 1.21		-Infty, 1.32	
P-Value	<0.0001		<0.0001	

2.3.3 Secondary Endpoint Analyses

2.3.3.1 Treatment Difference in COWS Total Scores at Week 4 and Week 5

A secondary endpoint for withdrawal was the treatment difference between placebo and difelikefalin for the COWS total score at Week 4 and Week 5, which was analyzed with an MMRM. Table 3 summarizes the results of the main analysis of this endpoint for the Per-protocol Population. The LS mean treatment group differences in COWS total score at Week

4 and Week 5 were 0.66 (CI: -Infty, 1.47) and 0.37 (CI: -Infty, 1.15), respectively. The differences between placebo and difelikefalin for both week 4 and week 5 were not statistically significant (P-values <0.0001), indicating noninferiority for clinical withdrawal signs and symptoms in subjects switched to placebo compared with subjects continuing to receive difelikefalin.

Table 3 Analysis of the COWS Total Score at Week 4 and Week 5 During the Double-blind Phase: MMRM Analysis (Per-protocol Population)

	Per-Protocol Population (N=28)	
	Placebo (N=13)	**CR845 0.5 mcg/kg** (N=15)
Week 4		
LS Mean	1.99	1.33
SE	0.30	0.28
Difference (Placebo - CR845), H_0: (μ_{Pmax} - μ_{Dmax}) ≥ 4, α=0.025		
LS Mean	0.66	
SE	0.39	
CI	-Infty, 1.47	
P-value	<0.0001	
Week 5		
LS Mean	1.84	1.46
SE	0.29	0.27
Difference (Placebo - CR845), H_0: (μ_{Pmax} - μ_{Dmax}) ≥ 4, α=0.025		
LS Mean	0.37	
SE	0.38	
CI	-Infty, 1.15	
P-value	<0.0001	

Sensitivity analysis was also performed for this secondary endpoint, using the Full Analysis Population and assuming data were missing at random (Table 4). The results were similar to those for the main analysis, with a LS mean treatment group difference in COWS total scores of 0.67 (CI: -Infty, 1.42) at Week 4 and 0.32 (-Infty, 1.06) at Week 5. The differences between placebo and difelikefalin for both week 4 and week 5 were not statistically significant (P-values <0.0001), indicating noninferiority for clinical withdrawal signs and symptoms in subjects switched to placebo compared with subjects continuing to receive difelikefalin.

Table 4 Analysis of the COWS Total Score in the DB phase: MMRM analysis (Main Analysis Including Sensitivity Analysis) Full Analysis Population

Week 4	**Full Analysis Population (N=30)**		**Per-Protocol Population (N=28)**	
	Placebo (N=14)	CR845 0.5 mcg/kg (N=16)	Placebo (N=13)	CR845 0.5 mcg/kg (N=15)
LS Mean	2	1.33	1.99	1.33
SE	0.28	0.26	0.30	0.28
Difference (Placebo - CR845), H_0: (μ_{Pmax} - μ_{Dmax}) ≥ 4, α=0.025				
LS Mean	0.67		0.66	
SE	0.37		0.39	
CI	-Infty, 1.42		-Infty, 1.47	
P-value	<0.0001		<0.0001	
Week 5	**Full Analysis Population (N=30)**		**Per-Protocol Population (N=28)**	
	Placebo (N=14)	CR845 0.5 mcg/kg (N=16)	Placebo (N=13)	CR845 0.5 mcg/kg (N=15)
LS Mean	1.74	1.42	1.84	1.46
SE	0.27	0.25	0.29	0.27
Difference (Placebo - CR845), H_0: (μ_{Pmax} - μ_{Dmax}) ≥ 4, α=0.025				
LS Mean	0.32		0.37	
SE	0.36		0.38	
CI	-Infty, 1.06		-Infty, 1.15	
P-value	<0.0001		<0.0001	

2.3.3.2 Treatment Difference in Maximum SOWS Total Scores During the Double-blind Phase

Another secondary endpoint for withdrawal was the treatment difference between difelikefalin and placebo in the maximum SOWS total score during the Double-blind Phase. Table 5 summarizes the main analysis for this endpoint using ANCOVA and the Per-protocol Population. At baseline (Week 3), mean (SD) SOWS total scores for the difelikefalin and placebo groups were 3.18 (3.61) and 3.62 (3.88), respectively.

The analysis results showed:

- The Hodges-Lehmann estimate of the median difference in maximum SOWS total score for the Per-protocol Population was 1.00 (CI: -Infty, 4.00), with an upper limit of the CI equals 4.

- The LS mean values for maximum SOWS Total scores during the Double-blind Phase between the difelikefalin and placebo groups were: 3.24 and 4.05, respectively, with a LS mean difference of 0.81 (CI: -Infty, 2.63).

NOTE: The margin used to test noninferiority with respect to maximum SOWS total score is not specified.

Table 5 Analysis of the Maximum SOWS Total Score During the Double-blind Phase: ANCOVA Analysis (Per-protocol Population)

	Per-Protocol Population (N=28)	
	Placebo (N=13)	**CR845 0.5 mcg/kg** (N=15)
Baseline [1]		
N	13	15
Mean	3.62	3.18
SD	3.88	3.61
Median	2.67	1.67
Min, Max	0.0, 12.3	0.0, 12.3
Hodges-Lehmann Estimate of median, α=0.025		
(Placebo - CR845)	1.00	
CI	-Infty, 4.00	
ANCOVA model		
LS Mean	4.05	3.24
SE	0.55	0.72
CI	2.73, 5.37	1.51, 4.97
Difference (Placebo - CR845), H_0: (μ_{Pmax} - μ_{Dmax}) ≥ δ [1], α=0.025		
LS Mean	0.81	
SE	0.88	
CI	-Infty, 2.63	

[1] δ is not specified in this study.

2.3.3.3 Treatment Difference in SOWS Total Scores at Week 4 and Week 5

The third secondary endpoint for withdrawal was the treatment difference between difelikefalin and placebo in the SOWS total score at Week 4 and Week 5, which was analyzed using an MMRM. Table 6 summarizes the results of the main analysis for the Per-protocol Population. At Week 4, the LS mean SOWS total score was higher in the placebo group than in the difelikefalin group: 2.59 versus 1.52, with a LS mean difference of 1.07

(CI: -Infty, 2.10). Similar findings were observed at Week 5: 2.38 versus 1.05 for placebo vs difelikefalin, with a LS mean treatment group difference of 1.33 (CI: -Infty, 2.26).

NOTE: The margin used to test noninferiority with respect to maximum SOWS total score is not specified.

Table 6 Analysis of the SOWS Total Score at Week 4 and Week 5 During the Double-blind Phase: MMRM Analysis (Per-protocol Population)

	Per-Protocol Population (N=28)	
	Placebo (N=13)	**CR845 0.5 mcg/kg** (N=15)
Baseline		
N	13	15
Mean	3.62	3.18
SD	3.88	3.61
Median	2.67	1.67
Min, Max	0.0, 12.3	0.0, 12.3
Week 4		
LS Mean	2.59	1.52
SE	0.38	0.35
Difference (Placebo - CR845), H_0: ($\mu_{Pmax} - \mu_{Dmax}$) ≥ δ, α=0.025		
LS Mean	1.07	
SE	0.50	
CI	-Infty, 2.10	
Week 5		
LS Mean	2.38	1.05
SE	0.34	0.32
Difference (Placebo - CR845), H_0: ($\mu_{Pmax} - \mu_{Dmax}$) ≥ δ [1], α=0.025		
LS Mean	1.33	
SE	0.45	
CI	-Infty, 2.26	

[1] δ is not specified in this study

3. Conclusions

The primary objective of this study was to assess the potential of physical withdrawal from difelikefalin at a dose of 0.5 mcg/kg upon treatment discontinuation after 3 weeks of administration in subjects undergoing hemodialysis.

The reviewer analyzed the primary endpoint: the treatment difference between difelikefalin and placebo for the maximum COWS total score during the Double-blind Phase, and the

secondary endpoints included the treatment difference between difelikefalin and placebo for the following measures: The COWS total score at Week 4 and Week 5, Maximum SOWS total score during the Double-blind Phase, The SOWS total score at Week 4 and Week 5.

The reviewer's statistical analysis results

1. In the primary endpoint analysis using the Per-protocol Population, for determination of noninferiority (i.e., withdrawal scores in subjects switched to placebo were not clinically worse than withdrawal scores in subjects continuing to receive difelikefalin), the null hypothesis was defined as a median or mean difference between placebo and difelikefalin in maximum COWS total score of $\geqslant$ 4 points. The analysis results showed that:

    - The Hodges-Lehmann estimate of the median difference in maximum COWS total score was 1.00 (CI: -Infty, 2.00), with an upper limit of the CI being less than 4.

    - The LS mean difference between placebo and difelikefalin was 0.49 (CI: -Infty, 1.32), the mean difference was not statistically significant (P-value <0.0001).

    The results support the noninferiority of withdrawal symptoms for subjects switched to placebo versus subjects continued on difelikefalin.

2. In the sensitivity analysis of the primary endpoint analysis using Full Analysis Population, noninferiority for placebo was also demonstrated: The Hodges-Lehmann estimate of the median difference in maximum COWS total score was 1.00 (CI: -Infty, 2.00), with an upper limit of the CI being less than 4. The mean difference between placebo and difelikefalin was not statistically significant (P-value <0.0001).

3. The secondary endpoint of the treatment difference in COWS total scores at Week 4 and Week 5 also showed noninferiority for clinical withdrawal signs and symptoms in subjects switched from difelikefalin to placebo compared with subjects continuing to receive difelikefalin. The LS mean treatment group differences in COWS total score at Week 4 and Week 5 were 0.66 (CI: -Infty, 1.47) and 0.37 (CI: -Infty, 1.15), respectively. The differences between placebo and difelikefalin for both week 4 and week 5 were not statistically significant (P-values <0.0001).

4. For the secondary endpoint of the treatment difference in maximum SOWS total scores, the results showed that:

    - The Hodges-Lehmann estimate of the median difference in maximum SOWS total score for the Per-protocol Population was 1.00 (CI: -Infty, 4.00), with an upper limit of the CI equals 4.

    - The LS mean values for maximum SOWS Total scores during the Double-blind Phase between the difelikefalin and placebo groups were: 3.24 and 4.05, respectively, with a LS mean difference of 0.81 (CI: -Infty, 2.63).

    NOTE: The margin used to test noninferiority with respect to maximum SOWS total score is not specified.

5. For the SOWS total scores at Week 4 and Week 5, the LS mean score was higher in the placebo group than in the difelikefalin group: 2.59 versus 1.52 at Week 4, with an LS mean difference of 1.07 (CI: -Infty, 2.10); and 2.38 versus 1.05 at Week 5, with an LS mean difference of 1.33 (CI: -Infty, 2.26).

Statistical Issues and Concerns:

1. The baseline value that the sponsor used was the average of the non-missing values during the Baseline Period (Day -7 to Day -1).

   The baseline value that the reviewer used for primary analysis, sensitivity analysis and secondary analysis was the Mean COWS Derived Total Score in Week 3.

   The way to measure withdrawal is to measure the symptoms of withdrawal relative to the baseline right before the discontinuation occurs which is derived from week 3 in this case.

2. Since the sample size of this study is small, comparing the median difference between Placebo and CR845 instead of mean difference may be more appropriate.

3. The study days that the sponsor used are shifted by one day from what table 3 (Study Schedule of Assessments) shows. For example, table 3 shows week 4 includes day 22, 24 and 26, while the sponsor used days 24, 26, and 29 for their analysis. This also applies to week 5. Furthermore, the double- blind treatment phase includes **7** days (days 22, 24, 26, 29, 31, 33 and 36) instead of 6 days.

   Since this is a physical dependence study, the withdraw syndrome may delay and last for a few days. The sponsor should explain why they used these days.

4. The sponsor's secondary analysis includes maximum SOWS total score during the Double-blind Phase. However, the margin used to test noninferiority with respect to maximum SOWS total score is not specified.

5. Is two weeks of treatment phase enough?

   The following table shows the number of subjects having the max COWS total score on each day. 18 out of 28 subjects had the max COWS total score at the last 3 visits. Especially for day 36, 11 out of 28 subjects had the max COWS total score on this day.

   It indicates that subjects had larger responses towards the end of the study. The reviewer is concerned if two weeks of treatment phase is enough to assess the potential of physical withdrawal from Intravenous CR845 (Difelikefalin) in Hemodialysis Patients.

Visit Day	Frequency
22	5
24	1
26	3
29	1
31	4
33	3
36	11

In conclusion, the reviewer's statistical analysis results support the sponsor's conclusion, that in subjects undergoing hemodialysis and treated with difelikefalin for 3 weeks, switching to placebo for 2 weeks did not elicit clinical signs or symptoms of withdrawal, as measured by maximum COWS total score, relative to subjects who continued treatment with difelikefalin.

However, we have noted some specific concerns listed above, we defer to Control Substance Staff (CSS) reviewers' decision.

4. References

1) Guidance for Industry: Assessment of Abuse Potential for Drugs (January 2017) http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm198650.pdf

2) Guidance for Industry: Abuse Deterrent Opioids—Evaluation and Labeling (April 2015) http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm334743.pdf

---

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

ANNA SUN
07/07/2021 08:59:55 AM

QIANYU DANG
07/07/2021 09:46:20 AM

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number: NDA 214916

Drug Name: CR845

Indication: Treatment of moderate-to-severe pruritus associated with chronic kidney disease (CKD-aP) in adult patients undergoing hemodialysis (HD)

Applicant: Cara Therapeutics, Inc.
4 Stamford Plaza
107 Elm Street, 9th FL
Stamford, CT 06902

Test Facility for Rats Study: (b) (4)

Test Facility for Mice Study: (b) (4)

Documents Reviewed: Study reports (Rat Study CR845-CARC088and Mouse Study CR845-CARC086) and electronic data submitted on December 23, 2020 via

NDA214916/0001

Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Zhuang Miao, Ph.D.
Concurring Reviewer:	Karl Lin, Ph.D.
Reviewing Pharmacologist:	Yongcheng Huang, Ph.D.
Keywords:	Carcinogenicity, Dose response

Table of Contents

1. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in Sprague Dawley Crl:CD(SD) rats and one in Tg.rasH2 mice. These studies were intended to assess the carcinogenic potential of CR845, when administered via subcutaneous injection into the scapular and mid-dorsal areas at appropriate drug levels for 104 weeks in rats and 26 weeks in mice.

Rat Study: The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female rats. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female rats.

Tumor analysis: there were no statistically significant tumor findings among males or females.

Mouse Study: The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female mice. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female mice. The pairwise comparison between the vehicle control group and positive control group showed a statistically significant increase in mortality in male mice.

Tumor analysis:

1. For both male and female mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increase in incidence of alveolar-bronchiolar adenoma, lungs with bronchi (p-value<0.001) and combined tumors of alveolar-bronchiolar adenoma and carcinoma, lungs with bronchi (p-value<0.001).

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
Male mice			
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	3/25 (24)	10/10 (10) <0.001
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	4/25 (24)	10/10 (10) <0.001
Female Mice			
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	1/25 (25)	10/10 (10) <0.001
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	2/25 (25)	10/10 (10) <0.001

2. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in Sprague Dawley Crl:CD(SD) rats and one in Tg.rasH2 mice. These studies were intended to assess the carcinogenic potential of CR845, when administered via subcutaneous injection into the scapular and mid-dorsal areas at appropriate drug levels for 104 weeks in rats and 26 weeks in mice. Results of this review have been discussed with the reviewing pharmacologist Dr. Yongcheng Huang. This review analyzed the SAS data sets of these studies received from the sponsor on December 23, 2020 via NDA214916/0001.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as the dose increases.

3. Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one vehicle control group. Two hundred and forty Sprague Dawley Crl:CD(SD) rats of each sex were randomly assigned to the four groups in equal size of 60 rats per group. The dose levels for treated groups were 0.25, 0.5 and 1 mg/kg/day. The rats in the vehicle control group received the vehicle(0.9% Sodium Chloride). The study for the rats was designed to continue for up to 104 weeks. In accordance with study termination criteria, all surviving male rats were sacrificed during Week 105.

Table 1: Study Design in Rat Study

Protocol Group No.	Dose Levels (mg/kg/day)	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	60	60
2	0.25	CR845	60	60
3	0.5	CR845	60	60
4	1	CR845	60	60

3.1. Sponsor's analyses

3.1.1. Survival analysis

A log-rank test for survival was used to make the following comparisons: 1) pairwise comparisons of each treated group with the vehicle control group; and 2) trend test utilizing ordinal coefficients. All tests were 2-sided and conducted at the 0.05 significance level. Survival times in which the status of the animal's death was classified as an accidental death or terminal sacrifice were considered censored values for the purpose of the Kaplan-Meier estimates and survival rate analyses.

Sponsor's findings: Sponsor's analysis showed the numbers (percents) of survival were 20 (33%), 22 (37%), 19 (32%), and 22 (37%) in vehicle control, 0.25 mg/kg/day, 0.5 mg/kg/day, and 1 mg/kg/day dose groups, respectively in males and 20 (33%), 21 (35%), 22 (38%), and 25 (42%) in vehicle control, 0.25 mg/kg/day, 0.5 mg/kg/day, and 1 mg/kg/day dose groups, respectively in females.

The sponsor concluded that there were no statistically significant findings among males or females for survival rates.

3.1.2. Tumor data analysis

All analyses were conducted separately for each sex. For each tumor type, the following analyses were conducted: 1) 1-sided pairwise comparison of each treated group with control; and 2) 1-sided trend test with the treated groups and control group utilizing ordinal coefficients.

An exact permutation test was conducted for analyses with a tumor type with low tumor bearing animals across all the treatment groups. Statistical significance was determined according to the following guidelines: 0.01 and 0.05 significance levels for common and rare tumors, respectively. A rare tumor was defined as one in which the historical spontaneous tumor rate was less than 1%.

Sponsor's findings: The sponsor concluded that there were no statistically significant findings among males or females for tumor incidence.

3.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically on December 23, 2020 via NDA214916/0001.

3.2.1. Survival analysis

The survival distributions of animals in four groups were estimated by the Kaplan-Meier product limit method. The dose response relationship and homogeneity of survival distributions were tested for the vehicle controls, low, medium and high dose groups using the Likelihood Ratio test and the Log-Rank test. The intercurrent mortality data are given in Tables 6 and 6 in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1 and 2 in the appendix for males and females, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 8 and 9 in the appendix for males and females, respectively.

Reviewer's findings: This reviewer's analysis showed the numbers (percents) of survival were 20 (33%), 22 (37%), 19 (32%), and 22 (37%) in vehicle control, 0.25 mg/kg/day, 0.5 mg/kg/day, and 1 mg/kg/day dose groups, respectively in males and 20 (33%), 21 (35%), 22 (38%), and 25 (42%) in vehicle control, 0.25 mg/kg/day, 0.5 mg/kg/day, and 1 mg/kg/day dose groups, respectively in females.

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female rats. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female rats.

3.2.2. Tumor data analysis

The tumor data were analyzed for the positive dose response relationships and the positive pairwise comparison increases between each of the treated groups with control group. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-K method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ($w_{\max}$) or dies before the

terminal sacrifice but develops the tumor type being tested gets a score of s_h =1. An animal that dies at week w_h without a tumor before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}} \right)^k$ < 1. The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal with score s_h =1 can be considered as a whole animal while an animal with score s_h < 1 can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes were then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons are listed in Tables 10 and 11 in the appendix for male and female rats, respectively.

Adjustment for multiple testing: For the chronic study in rats, the adjustment of multiple testing of the dose response relationship for a submission with one chronic rat study and one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels α =0.005 for common tumors and $\alpha = 0.025$ for rare tumors for the chronic rat study. For pairwise comparisonsfor the chronic rat study in the above type of submission with one chronic rat study and one transgenic mouse study, the same guidance document suggests the use of test levels α =0.01 for common tumors and α =0.05 for rare tumors for the chronic rat study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer’s findings: The tumor types in Tables 2 below showed p-values less than or equal

to 0.05 in the trend tests and the tests for pairwise comparisons between the vehicle and the treated groups for male rats. Based on the above criterion for multiple testing adjustment, there were no statistically significant tumor findings among males or females.

Table 2: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Vehicle Control and Treated Groups-Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
GLAND, ADRENAL	PHEOCHROMOCYTOMA, MALIGNANT	1/60 (39) 0.2516	1/60 (41) 0.7655	7/60 (44) 0.0424	2/60 (40) 0.5096

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

4. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups, one vehicle control group and one positive control group. One hundred Tg.rasH2 mice of each sex were randomly assigned to the treated and vehicle control group in equal size of 25 mice per group. The dose levels for treated groups were 3, 10, and 30 mg/kg/day for males and females. The mice in the vehicle control group received the vehicle (sterile normal saline). The mice in the positive control group received three intraperitoneal (i.p.) injections of 1000 mg/kg/day of urethane in sterile
saline at a dose volume of 10 mL/kg body weight, one injection each on Days 1, 3 and 5.

Table 3: Study Design in Mouse Study

Protocol Group No.	Dose Levels (mg/kg/day)	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	25	25
2	3	CR845	25	25
3	10	CR845	25	25
4	30	CR845	25	25
5	1000	Positive	10	10

4.1. Sponsor's analyses

4.1.1. Survival analysis

The sponsor used the same survival analysis methods used for the rats study in this mouse study.

Sponsor’s findings: The sponsor’s analysis showed that the numbers (percents) of survival were 23 (92%), 22 (88%), 25 (100%), 25 (100%) and 8 (80%) in male mice, and 25 (100%), 24 (96%), 24 (96%), 24 (96%) and 10 (100%) in female mice in vehicol control, low, medium, high and positive dose groups, respectively.

The sponsor concluded that, among males, the survival rates among the positive control group were significantly lower when compared with the control group. There were no other statistically significant findings in survival rates.

4.1.2. Tumor data analysis

The sponsor used the same tumor data analysis methods used for the rat study in this mouse study

Sponsor’s findings: The sponsor concluded that there were no statistically significant tumor findings in the test article groups when compared to the vehicle control group.

4.2. Reviewer's analyses

To verify sponsor’s analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically on December 23, 2020 via NDA214916/0001.

4.2.1. Survival analysis

The survival distributions of three treated groups, one vehical control group, and one positive control group were estimated using the Kaplan-Meier product limit method. The dose response relationship in survival was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 3 and 4 in the appendix for male and female mice, respectively. The intercurrent mortality data are given in Tables 12 and 13 in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle control and three treated groups are given in Tables 14 and 15 in the appendix for male and female mice, respectively.

Reviewer’s findings: This reviewer’s analysis showed that the numbers (percents) of survival were 23 (92%), 22 (88%), 25 (100%), 25 (100%) and 8 (80%) in male mice, and 25 (100%), 24 (96%), 24 (96%), 24 (96%) and 10 (100%) in female mice in vehicol control, low, medium, high and positive dose groups, respectively.

The survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups in male or female mice. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups in male or female mice. The pairwise comparison between the vehicle control group and positive control group showed a statistically significant increase in mortality in male mice.

4.2.2. Tumor data analysis

The reviewer used the same tumor data analysis methods for the rat study in this mouse study.

The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons between vehicle control and three treated groups, and between vehicle control and positive control are listed in Tables 17, 18, 19, and 20 in the appendix for male and female mice, respectively.

Adjustment for multiple testing: For the chronic study in rats, the adjustment of multiple testing of the dose response relationship for a submission with one chronic rat study and one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels α =0.05 for both common tumors and rare tumors for the mouse study. For pairwise, the same guidance document suggests the use of test levels α =0.05 for both common tumors and rare tumors for the mouse study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings: The tumor types in Tables 4 and 5 below showed p-values less than or

equal to 0.05 in the tests for pairwise comparisons between vehicle and positive control groups for male mice and female mice, respectively.

Table 4: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Positive Control-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	3/25 (24)	10/10 (10) <0.001
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	4/25 (24)	10/10 (10) <0.001

Table 5: Tumor Types with P-Values ≤ 0.05 for Comparisons between Vehicle Control and Positive Control-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	1/25 (25)	10/10 (10) <0.001
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	2/25 (25)	10/10 (10) <0.001

Reviewer's findings: Based on the criteria of adjustment for multiple testing discussed in the mouse data analysis section, we make the following statistical conclusions:

1. For both male and female mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increase in incidence of alveolar-bronchiolar adenoma, lungs with bronchi (p-value<0.001) and combined tumors of alveolar-bronchiolar adenoma and carcinoma, lungs with bronchi (p-value<0.001),

Zhuang Miao, Ph.D.
Mathematical
Statistician
Concur:

Karl Lin, Ph.D.

Mathematical Statistician, Team Leader, Biometrics-6

cc:

Yi Tsong, Ph.D.

5. Appendix

Table 6: Intercurrent Mortality Rate -Male Rats

	Vehicle 0 mg|kg|day (N=60)		0.25 mg|kg|day (N=60)		0.5 mg|kg|day (N=60)		1 mg|kg|day (N=60)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**
0 - 52	4	6.67	5	8.33	3	5.00	4	6.67
53 - 78	17	35.00	12	28.33	9	20.00	16	33.33
79 - 91	9	50.00	12	48.33	13	41.67	10	50.00
92 - 104	10	66.67	9	63.33	16	68.33	8	63.33
Ter. Sac.	20	33.33	22	36.67	19	31.67	22	36.67

Cum. %: Cumulative percentage except for Ter. Sac.

Table 7: Intercurrent Mortality Rate -Female Rats

	Vehicle 0 mg|kg|day (N=60)		0.25 mg|kg|day (N=60)		0.5 mg|kg|day (N=60)		1 mg|kg|day (N=60)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**
0 - 52	4	6.67	1	1.67	1	1.67	4	6.67
53 - 78	22	43.33	25	43.33	23	40.00	19	38.33
79 - 91	13	65.00	13	65.00	14	63.33	9	53.33
92 - 104	1	66.67	.	.	.	.	3	58.33
Ter. Sac.	20	33.33	21	35.00	22	36.67	25	41.67

Cum. %: Cumulative percentage except for Ter. Sac.

Table 8: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Male Rats

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.8318	0.6532	0.6660	0.7875
Homogeneity	Log-Rank	0.9676	0.6495	0.6617	0.7852

Table 9: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Female Rats

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.3713	0.9359	0.6706	0.4239

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Homogeneity	Log-Rank	0.8382	0.9347	0.6656	0.4168

Table 10: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between the Vehicle Controls and the Treated Groups-Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
BONE MARROW	SARCOMA	1/60 (39) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
BRAIN	MICROGLIAL CELL TUMOR, MALIGNANT	3/60 (41) 0.6703	2/60 (42) 0.8277	2/60 (43) 0.8347	2/60 (39) 0.8045
EPIDIDYMIS	MESOTHELIOMA, MALIGNANT	0/60 (39) 0.6291	1/60 (41) 0.5125	1/60 (43) 0.5244	0/60 (39) NC
GLAND, ADRENAL	CORTICAL ADENOMA	2/60 (40) 0.7392	1/60 (41) 0.8842	1/60 (43) 0.8925	1/60 (39) 0.8751
	PHEOCHROMOCYTOMA, BENIGN	2/60 (39) 0.6390	3/60 (42) 0.5356	1/60 (43) 0.8968	2/60 (40) 0.7020
	PHEOCHROMOCYTOMA, MALIGNANT	1/60 (39) 0.2516	1/60 (41) 0.7655	7/60 (44) 0.0424	2/60 (40) 0.5096
GLAND, HARDERIAN	ADENOMA	1/60 (39) 0.6058	2/60 (41) 0.5190	2/60 (43) 0.5370	1/60 (40) 0.7595
GLAND, MAMMARY	ADENOCARCINOMA	0/52 (34) 0.7500	1/49 (33) 0.4925	0/49 (34) NC	0/52 (35) NC
	FIBROADENOMA	1/52 (34) 0.2170	0/49 (33) 1.0000	0/49 (34) 1.0000	2/52 (36) 0.5217
GLAND, PARATHYROID	ADENOMA	0/41 (28) 0.5175	0/39 (27) NC	1/42 (31) 0.5254	0/44 (28) NC
GLAND, PITUITARY	ADENOMA, PARS DISTALIS	36/59 (51) 0.7077	31/60 (52) 0.9156	37/60 (54) 0.6705	32/60 (51) 0.8532
	CARCINOMA, PARS DISTALIS	3/59 (39) 0.8996	2/60 (41) 0.8358	1/60 (43) 0.9530	1/60 (40) 0.9453
GLAND, SALIVARY	SCHWANNOMA, MALIGNANT	0/60 (39) 0.7578	1/60 (41) 0.5125	0/59 (42) NC	0/60 (39) NC
GLAND, THYROID	C-CELL ADENOMA	6/60 (40) 0.5254	8/60 (41) 0.4047	6/60 (44) 0.6883	7/60 (42) 0.5387
	C-CELL CARCINOMA	6/60 (40) 0.8930	1/60 (41) 0.9946	4/60 (44) 0.8796	2/60 (40) 0.9716
	FOLLICULAR CELL ADENOMA	1/60 (39) 0.7729	3/60 (41) 0.3269	5/60 (45) 0.1373	0/60 (39) 1.0000
	FOLLICULAR CELL CARCINOMA	2/60 (39) 0.1979	0/60 (41) 1.0000	0/60 (43) 1.0000	3/60 (39) 0.5000
GLAND, ZYMBALS	ADENOMA	0/60 (39) 0.5125	0/58 (39) NC	1/59 (43) 0.5244	0/58 (39) NC
	SQUAMOUS CELL CARCINOMA	0/60 (39) 0.5094	0/58 (39) NC	1/59 (42) 0.5185	0/58 (39) NC

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
HEART	SCHWANNOMA, MALIGNANT	1/60 (40) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
HEMOLYMPHORETICULA	HISTIOCYTIC SARCOMA	1/60 (39) 0.2721	3/60 (42) 0.3364	2/60 (43) 0.5370	3/60 (41) 0.3269
	LYMPHOMA, MALIGNANT	2/60 (40) 0.9861	1/60 (41) 0.8842	0/60 (43) 1.0000	0/60 (39) 1.0000
KIDNEY	AMPHOPHILIC VACUOLAR TUBULAR CARCINOMA	1/60 (39) 0.4590	0/60 (41) 1.0000	1/60 (44) 0.7823	1/60 (40) 0.7595
	LIPOMA	0/60 (39) 0.1854	0/60 (41) NC	1/60 (43) 0.5244	1/60 (39) 0.5000
	LIPOSARCOMA	1/60 (39) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
Kidney	C_lipomas+liposarcomas	1/60 (39) 0.4514	0/60 (41) 1.0000	1/60 (43) 0.7769	1/60 (39) 0.7532
LARGE INTESTINE, C	NEUROENDOCRINE CELL TUMOR, MALIGNANT	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
LIVER	CHOLANGIOCARCINOMA	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
	HEPATOCELLULAR ADENOMA	1/60 (39) 0.2884	1/60 (41) 0.7655	2/60 (44) 0.5457	2/60 (40) 0.5096
	HEPATOCELLULAR CARCINOMA	0/60 (39) 0.4941	0/60 (41) NC	2/60 (44) 0.2780	0/60 (39) NC
LUNG	BRONCHIOLOALVEOLAR ADENOMA	0/60 (39) 0.5062	0/60 (41) NC	1/60 (43) 0.5244	0/60 (39) NC
LYMPH NODE, MESENT	HEMANGIOSARCOMA	1/60 (39) 0.8531	1/60 (41) 0.7655	1/60 (44) 0.7823	0/60 (39) 1.0000
Liver	C_hepatocellular adenoma+carcinoma	1/60 (39) 0.2738	1/60 (41) 0.7655	4/60 (45) 0.2278	2/60 (40) 0.5096
MUSCLE, SKELETAL	LIPOSARCOMA	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
PANCREAS	ACINAR ADENOMA	1/60 (40) 0.1366	3/60 (41) 0.3172	1/60 (43) 0.7708	4/60 (40) 0.1794
	ISLET CELL ADENOMA	3/60 (39) 0.6840	4/60 (42) 0.5421	6/60 (44) 0.3058	2/60 (39) 0.8208
	ISLET CELL CARCINOMA	5/60 (39) 0.5062	1/60 (41) 0.9891	4/60 (44) 0.8154	4/60 (40) 0.7721
Pancreas	C_islet cell adenoma+carcinoma	8/60 (39) 0.6488	5/60 (42) 0.9131	9/60 (45) 0.6305	6/60 (40) 0.8252
SITE, INJECTION, 1	FIBROMA	0/60 (39) 0.3136	1/60 (41) 0.5125	0/60 (43) NC	1/60 (40) 0.5063
SITE, INJECTION, 2	FIBROMA	0/60 (39) 0.5062	0/60 (41) NC	1/60 (43) 0.5244	0/60 (39) NC

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
	FIBROSARCOMA	0/60 (39) 0.5062	0/60 (41) NC	1/60 (43) 0.5244	0/60 (39) NC
	HAIR FOLLICLE TUMOR, BENIGN	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
	KERATOACANTHOMA	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
	SEBACEOUS CELL ADENOMA	1/60 (39) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
SITE, INJECTION, 3	FIBROMA	1/60 (40) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
SITE, INJECTION, 4	FIBROSARCOMA	1/60 (40) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
SKIN	CARCINOMA	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
	FIBROMA	0/60 (39) 0.3758	2/60 (42) 0.2657	3/60 (44) 0.1441	1/60 (39) 0.5000
	FIBROSARCOMA	5/60 (40) 0.9892	1/60 (41) 0.9882	3/60 (44) 0.8961	0/60 (39) 1.0000
	HEMANGIOSARCOMA	0/60 (39) 0.2454	0/60 (41) NC	0/60 (43) NC	1/60 (40) 0.5063
	HIBERNOMA, BENIGN	0/60 (39) 0.5062	0/60 (41) NC	1/60 (43) 0.5244	0/60 (39) NC
	KERATOACANTHOMA	2/60 (40) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
	LEIOMYOSARCOMA	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
	LIPOMA	0/60 (39) 0.0676	0/60 (41) NC	2/60 (44) 0.2780	2/60 (39) 0.2468
	MYXOSARCOMA	1/60 (39) 0.5571	1/60 (41) 0.7655	1/60 (44) 0.7823	1/60 (40) 0.7595
	PAPILLOMA	2/60 (40) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
	PLEOMORPHIC FIBROSARCOMA	0/60 (39) 0.7593	1/60 (41) 0.5125	0/60 (43) NC	0/60 (39) NC
	SARCOMA	0/60 (39) 0.4941	0/60 (41) NC	2/60 (44) 0.2780	0/60 (39) NC
	SCHWANNOMA, MALIGNANT	1/60 (39) 0.5560	1/60 (41) 0.7655	1/60 (43) 0.7769	1/60 (40) 0.7595
	SEBACEOUS CELL ADENOMA	1/60 (39) 1.0000	0/60 (41) 1.0000	0/60 (43) 1.0000	0/60 (39) 1.0000
SMALL INTESTINE, J	ADENOCARCINOMA	0/60 (39) 0.5062	0/60 (41) NC	1/60 (43) 0.5244	0/60 (39) NC

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
STOMACH	PAPILLOMA	0/60 (39) 0.2407	0/60 (41) NC	0/60 (43) NC	1/60 (39) 0.5000
Skin	C_papillomas+carcinomas+keratoacanthomas	4/60 (41) 0.9992	1/60 (41) 0.9725	0/60 (43) 1.0000	0/60 (39) 1.0000
TESTIS	INTERSTITIAL (LEYDIG) CELL ADENOMA	0/60 (39) 0.5943	1/60 (41) 0.5125	2/60 (43) 0.2719	0/60 (39) NC
THYMUS	SQUAMOUS CELL CARCINOMA	0/57 (36) 0.7722	1/59 (40) 0.5263	0/59 (43) NC	0/59 (39) NC
Whold Body	C_Hemangiosarcoma	1/60 (39) 0.5571	1/60 (41) 0.7655	1/60 (44) 0.7823	1/60 (40) 0.7595

Table 11: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between the Vehicle Controls and the Treated Groups- Female Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
CERVIX	ENDOMETRIAL STROMAL SARCOMA	1/60 (30) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	GRANULAR CELL TUMOR, BENIGN	2/60 (30) 0.5567	2/60 (30) 0.6940	2/60 (32) 0.7180	2/60 (31) 0.7063
GLAND, ADRENAL	CORTICAL ADENOMA	1/60 (29) 0.3143	1/60 (29) 0.7544	1/60 (31) 0.7706	2/60 (31) 0.5254
	PHEOCHROMOCYTOMA, BENIGN	1/60 (30) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	PHEOCHROMOCYTOMA, MALIGNANT	0/60 (29) 0.2542	0/60 (29) NC	0/60 (30) NC	1/60 (30) 0.5085
GLAND, HARDERIAN	ADENOMA	0/60 (29) 0.2542	0/60 (29) NC	0/60 (30) NC	1/60 (30) 0.5085
	SARCOMA	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
GLAND, MAMMARY	ADENOCARCINOMA	17/60 (38) 0.8076	13/60 (35) 0.8150	18/60 (39) 0.5414	12/60 (36) 0.8932
	ADENOMA	9/60 (33) 0.9902	4/60 (31) 0.9606	8/60 (34) 0.7363	1/60 (30) 0.9993
	CARCINOSARCOMA	1/60 (29) 0.6033	1/60 (30) 0.7627	0/60 (30) 1.0000	1/60 (30) 0.7627
	FIBROADENOMA	38/60 (46) 0.9248	39/60 (48) 0.6689	35/60 (46) 0.8485	33/60 (46) 0.9325
GLAND, PITUITARY	ADENOMA, PARS DISTALIS	45/60 (52) 0.9535	41/60 (51) 0.8656	47/60 (54) 0.5827	34/60 (47) 0.9781
	ADENOMA, PARS INTERMEDIA	0/60 (29) 0.7542	1/60 (29) 0.5000	0/60 (30) NC	0/60 (30) NC
	CARCINOMA, PARS DISTALIS	5/60 (31) 0.7573	3/60 (31) 0.8723	4/60 (33) 0.7935	3/60 (31) 0.8723
GLAND, THYROID	C-CELL ADENOMA	6/60 (32) 0.8506	6/60 (33) 0.6472	7/60 (33) 0.5250	3/60 (31) 0.9189
	C-CELL CARCINOMA	1/60 (30) 0.7963	1/60 (29) 0.7458	2/60 (31) 0.5125	0/60 (30) 1.0000
GLAND, ZYMBALS	SQUAMOUS CELL CARCINOMA	1/58 (29) 0.7213	0/59 (29) 1.0000	2/55 (29) 0.5000	0/55 (27) 1.0000
Gland Thyroid	C cell Adenoma+Carcinoma	7/60 (33) 0.8893	7/60 (33) 0.6179	9/60 (34) 0.4142	3/60 (31) 0.9489
HEMOLYMPHORETICULA	HISTIOCYTIC SARCOMA	0/60 (29) 0.3111	1/60 (30) 0.5085	1/60 (31) 0.5167	1/60 (31) 0.5167

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
	LYMPHOMA, MALIGNANT	1/60 (30) 0.3125	1/60 (29) 0.7458	0/60 (30) 1.0000	2/60 (31) 0.5125
KIDNEY	AMPHOPHILIC VACUOLAR TUBULAR ADENOMA	1/60 (29) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	AMPHOPHILIC VACUOLAR TUBULAR CARCINOMA	1/60 (29) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	LIPOSARCOMA	0/60 (29) 0.2542	0/60 (29) NC	0/60 (30) NC	1/60 (30) 0.5085
LIVER	CHOLANGIOCARCINOMA	1/60 (29) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	HEPATOCELLULAR ADENOMA	0/60 (29) 0.2173	2/60 (30) 0.2542	0/60 (30) NC	2/60 (31) 0.2627
LUNG	BRONCHIOLOALVEOLAR CARCINOMA	0/60 (29) 0.2542	0/60 (29) NC	0/60 (30) NC	1/60 (30) 0.5085
OVARY	ADENOMA, SEX CORD STROMAL	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
	THECOMA, MALIGNANT	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
OVIDUCT	ADENOCARCINOMA	0/57 (28) 0.2586	0/59 (28) NC	0/60 (30) NC	1/60 (30) 0.5172
PANCREAS	ACINAR ADENOMA	0/60 (29) 0.2542	0/60 (29) NC	0/60 (30) NC	1/60 (30) 0.5085
	ACINAR CARCINOMA	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
	ISLET CELL ADENOMA	1/60 (29) 0.9472	3/60 (30) 0.3189	0/60 (30) 1.0000	0/60 (30) 1.0000
	ISLET CELL CARCINOMA	2/60 (30) 0.7383	0/60 (29) 1.0000	0/60 (30) 1.0000	1/60 (30) 0.8814
SITE, INJECTION, 3	KERATOACANTHOMA	0/60 (29) 0.2605	0/60 (29) NC	0/60 (30) NC	1/60 (31) 0.5167
SKIN	BASAL CELL TUMOR, BENIGN	0/60 (29) 0.7563	1/60 (30) 0.5085	0/60 (30) NC	0/60 (30) NC
	FIBROSARCOMA	1/60 (29) 0.6099	2/60 (30) 0.5129	2/60 (31) 0.5254	1/60 (30) 0.7627
	HEMANGIOPERICYTOMA	1/60 (29) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	LIPOMA	1/60 (30) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
	MYXOSARCOMA	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
	PAPILLOMA	1/60 (30) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	0.25 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	0.5 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	1 mg/kg/day High (N=60) P-value - Vehicle vs. High
	SARCOMA	0/60 (29) 0.7563	1/60 (30) 0.5085	0/60 (30) NC	0/60 (30) NC
	SQUAMOUS CELL CARCINOMA	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
SMALL INTESTINE, D	ADENOCARCINOMA	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
SPLEEN	HEMANGIOSARCOMA	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC
Skin	C_Papilloma+Carcinoma	1/60 (30) 0.8172	0/60 (29) 1.0000	1/60 (31) 0.7623	0/60 (30) 1.0000
THYMUS	THYMOMA, MALIGNANT	1/60 (29) 0.7945	1/60 (30) 0.7627	2/60 (31) 0.5254	0/58 (29) 1.0000
URINARY BLADDER	LEIOMYOMA	0/60 (29) 0.2542	0/60 (29) NC	0/59 (30) NC	1/60 (30) 0.5085
UTERUS	ADENOMA	0/60 (29) 0.2542	0/60 (29) NC	0/60 (30) NC	1/60 (30) 0.5085
	ENDOMETRIAL STROMAL POLYP	7/60 (32) 0.9956	5/60 (30) 0.7990	2/60 (31) 0.9850	1/60 (30) 0.9969
	GRANULAR CELL TUMOR, BENIGN	0/60 (29) 0.5608	1/60 (30) 0.5085	3/60 (32) 0.1378	0/60 (30) NC
	GRANULAR CELL TUMOR, MALIGNANT	0/60 (29) 0.1997	0/60 (29) NC	1/60 (31) 0.5167	1/60 (31) 0.5167
	LEIOMYOSARCOMA	1/60 (29) 1.0000	0/60 (29) 1.0000	0/60 (30) 1.0000	0/60 (30) 1.0000
VAGINA	GRANULAR CELL TUMOR, BENIGN	2/60 (30) 0.8424	2/60 (30) 0.6940	0/60 (30) 1.0000	1/60 (31) 0.8872
	GRANULAR CELL TUMOR, MALIGNANT	0/60 (29) 0.7542	1/60 (29) 0.5000	0/60 (30) NC	0/60 (30) NC
	SCHWANNOMA, MALIGNANT	0/60 (29) 0.2605	0/60 (29) NC	0/60 (30) NC	1/60 (31) 0.5167
Whold Body	C_Hemangiosarcoma	0/60 (29) 0.5126	0/60 (29) NC	1/60 (31) 0.5167	0/60 (30) NC

Table 12: Intercurrent Mortality Rate -Male Mice

	Vehicle 0 mg|kg|day (N=25)		3 mg|kg|day (N=25)		10 mg|kg|day (N=25)		30 mg|kg|day (N=25)		Positive 100 mg|kg|day (N=10)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**
0 - 13	.	.	1	4.00	.	.	.	.	2	100.00
14 - 26	2	8.00	2	12.00	.	.	.	.	.	.
Ter. Sac.	23	92.00	22	88.00	25	100.00	25	100.00	8	.

Cum. %: Cumulative percentage except for Ter. Sac.

Table 13: Intercurrent Mortality Rate -Female Mice

	Vehicle 0 mg|kg|day (N=25)		3 mg|kg|day (N=25)		10 mg|kg|day (N=25)		30 mg|kg|day (N=25)		Positive 100 mg|kg|day (N=10)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**	**Cum. %**
0 - 13	.	.	.	.	1	4.00	.	.	.	100.00
14 - 26	.	.	1	4.00	.	.	1	4.00	.	.
Ter. Sac.	25	100.00	24	96.00	24	96.00	24	96.00	10	.

Cum. %: Cumulative percentage except for Ter. Sac.

Table 14: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control-Male Mice

Test	**Statistic**	**P_Value Vehicle vs Treated Groups Dose Response**	**P_Value Vehicle vs. Low**	**P_Value Vehicle vs. Med**	**P_Value Vehicle vs. High**	**P_Value Vehicle vs. Positive**
Dose-Response	Likelihood Ratio	0.0234	0.6200	0.0935	0.0935	0.0231
Homogeneity	Log-Rank	0.1265	0.6194	0.1531	0.1531	0.0217

Table 15: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control-Female Mice

Test	**Statistic**	**P_Value Vehicle vs Treated Groups Dose Response**	**P_Value Vehicle vs. Low**	**P_Value Vehicle vs. Med**	**P_Value Vehicle vs. High**	**P_Value Vehicle vs. Positive**
Dose-Response	Likelihood Ratio	0.6025	0.2390	0.2390	0.2390	.

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Homogeneity	Log-Rank	0.7978	0.3173	0.3173	0.3173	.

Table 16: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	3 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	10 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	30 mg/kg/day High (N=25) P-value - Vehicle vs. High
HARDERIAN GLANDS	CARCINOMA	1/25 (24) 0.7255	3/25 (23) 0.2879	1/25 (25) 0.7653	1/25 (25) 0.7653
KIDNEYS	HEMANGIOSARCOMA	0/25 (24) 0.5155	0/25 (23) NC	1/25 (25) 0.5102	0/25 (25) NC
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	3/25 (24) 0.7786	1/25 (23) 0.9404	3/25 (25) 0.6864	1/25 (25) 0.9498
	ALVEOLAR-BRONCHIOLAR CARCINOMA	1/25 (24) 0.7099	3/25 (24) 0.3043	2/25 (25) 0.5156	1/25 (25) 0.7653
	HEMANGIOSARCOMA	0/25 (24) 0.5155	0/25 (23) NC	1/25 (25) 0.5102	0/25 (25) NC
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	4/25 (24) 0.8001	3/25 (24) 0.7921	5/25 (25) 0.5275	2/25 (25) 0.9144
MULTICENTRIC	LYMPHOMA	0/25 (24) 0.7526	1/25 (23) 0.4894	0/25 (25) NC	0/25 (25) NC
SEMINAL VESICLES	HEMANGIOSARCOMA	0/25 (24) 0.5155	0/25 (23) NC	1/25 (25) 0.5102	0/25 (25) NC
SPLEEN	HEMANGIOSARCOMA	1/25 (24) 0.5363	1/25 (23) 0.7447	1/25 (25) 0.7653	1/25 (25) 0.7653
STOMACH	HEMANGIOSARCOMA	0/25 (24) 0.5155	0/25 (23) NC	1/25 (25) 0.5102	0/25 (25) NC
THYROID GLANDS	FOLLICULAR CELL ADENOMA	0/25 (24) 0.2577	0/25 (23) NC	0/25 (25) NC	1/25 (25) 0.5102
Whold Body	C_Hemangiosarcoma	1/25 (24) 0.5645	1/25 (23) 0.7447	5/25 (25) 0.1039	1/25 (25) 0.7653

Table 17: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	3 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	10 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	30 mg/kg/day High (N=25) P-value - Vehicle vs. High
CAVITY, NASAL	HEMANGIOSARCOMA	0/25 (25) 0.2551	0/25 (24) NC	0/25 (24) NC	1/25 (25) 0.5000
HARDERIAN GLANDS	ADENOMA	2/25 (25) 0.9844	1/25 (24) 0.8752	0/25 (24) 1.0000	0/25 (24) 1.0000
	CARCINOMA	0/25 (25) 0.7423	1/25 (24) 0.4898	0/25 (24) NC	0/25 (24) NC
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	1/25 (25) 0.4356	0/25 (24) 1.0000	0/25 (24) 1.0000	1/25 (24) 0.7449
	ALVEOLAR-BRONCHIOLAR CARCINOMA	1/25 (25) 0.8262	1/25 (24) 0.7449	1/25 (24) 0.7449	0/25 (24) 1.0000
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	2/25 (25) 0.6721	1/25 (24) 0.8752	1/25 (24) 0.8752	1/25 (24) 0.8752
OVARIES	HEMANGIOSARCOMA	0/25 (25) 0.2474	0/25 (24) NC	0/25 (24) NC	1/25 (24) 0.4898
SPLEEN	HEMANGIOSARCOMA	0/25 (25) 0.2858	1/25 (25) 0.5000	1/25 (24) 0.4898	1/25 (24) 0.4898
UTERUS	HEMANGIOSARCOMA	0/25 (25) 0.7423	1/25 (24) 0.4898	0/25 (24) NC	0/25 (24) NC
Whold Body	C_Hemangiosarcoma	0/25 (25) 0.0814	2/25 (25) 0.2449	1/25 (24) 0.4898	3/25 (25) 0.1173

Table 18: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control- Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	3/25 (24)	10/10 (10) <0.001
	ALVEOLAR-BRONCHIOLAR CARCINOMA	1/25 (24)	0/10 (1) 1.0000
	HEMANGIOSARCOMA	0/25 (24)	0/10 (1) NC
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	4/25 (24)	10/10 (10) <0.001
MULTICENTRIC	LYMPHOMA	0/25 (24)	0/10 (1) NC
Whold Body	C_Hemangiosarcoma	1/25 (24)	0/10 (1) 1.0000

Table 19: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control- Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=10) P-value - Vehicle vs. Positive
LUNGS WITH BRONCHI	ALVEOLAR-BRONCHIOLAR ADENOMA	1/25 (25)	10/10 (10) <0.001
	ALVEOLAR-BRONCHIOLAR CARCINOMA	1/25 (25)	0/10 (1) 1.0000
Lungs with bronchi	C_ALVEOLAR-BRONCHIOLAR ADENOMA+CARCINOMA	2/25 (25)	10/10 (10) <0.001
Whold Body	C_Hemangiosarcoma	0/25 (25)	0/10 (1) NC

Figure 1: Kaplan-Meier Survival Functions for Male Rats

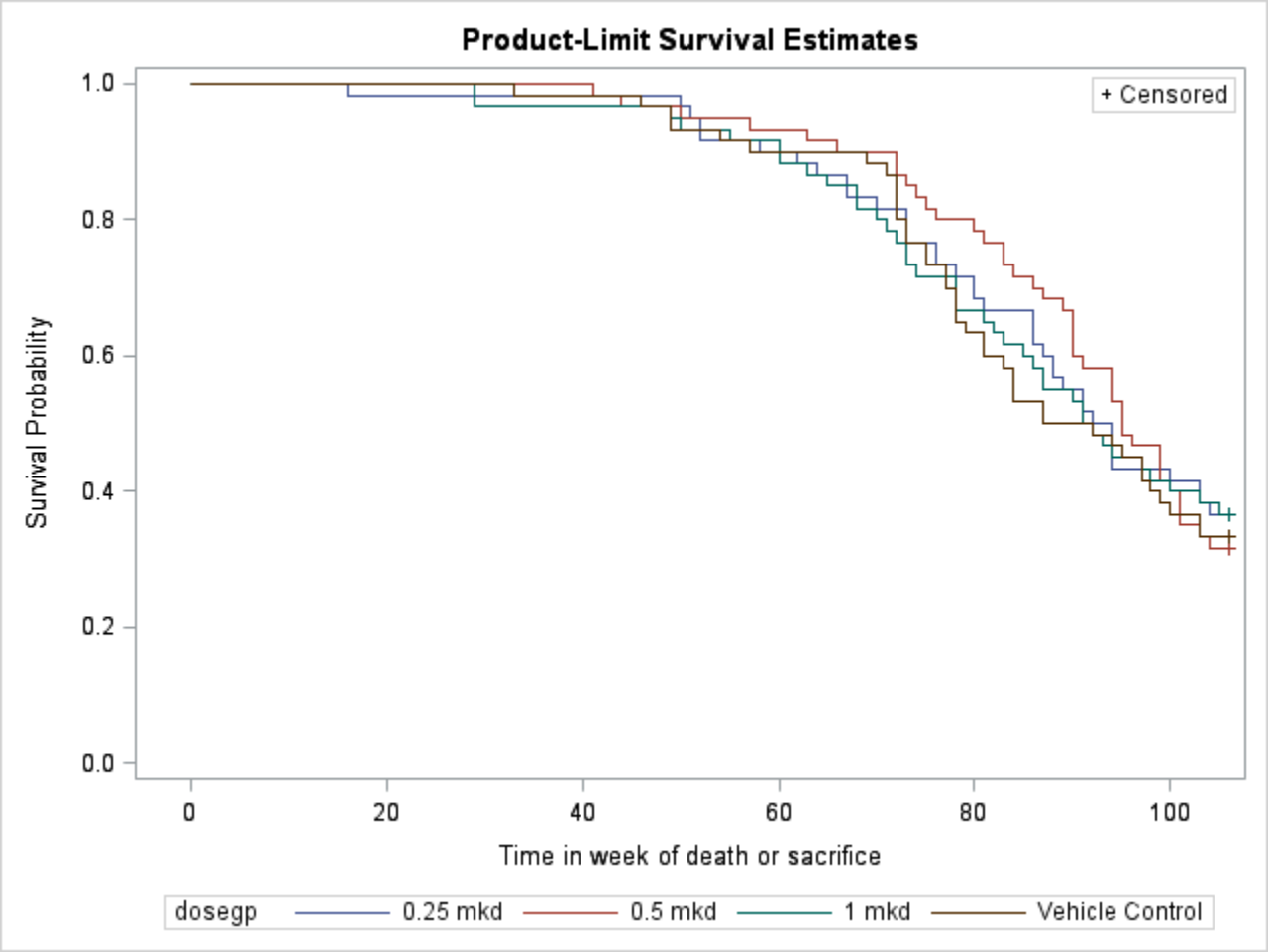


Figure 2: Kaplan-Meier Survival Functions for Female Rats

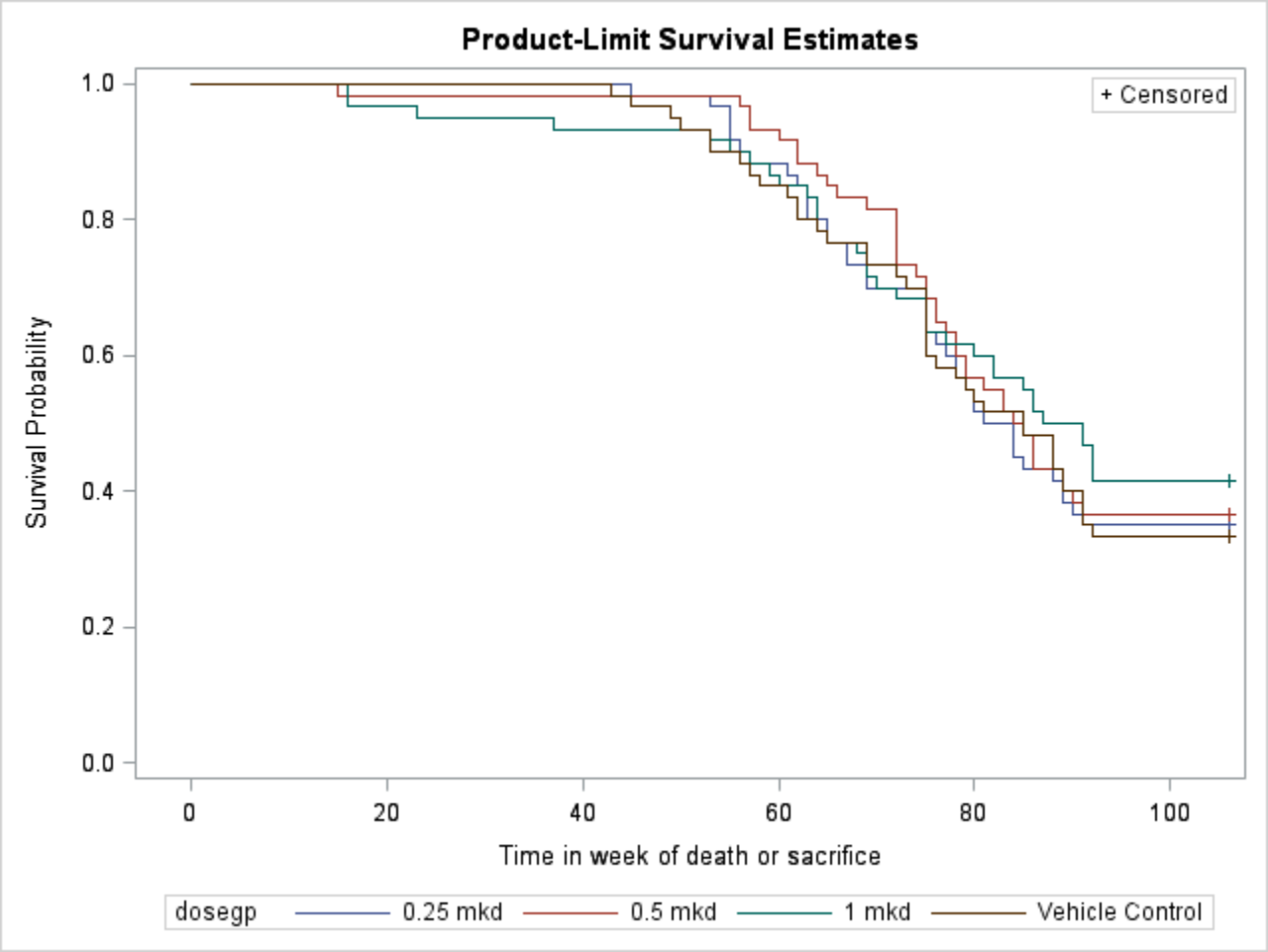


Figure 3: Kaplan-Meier Survival Functions for Male Mice

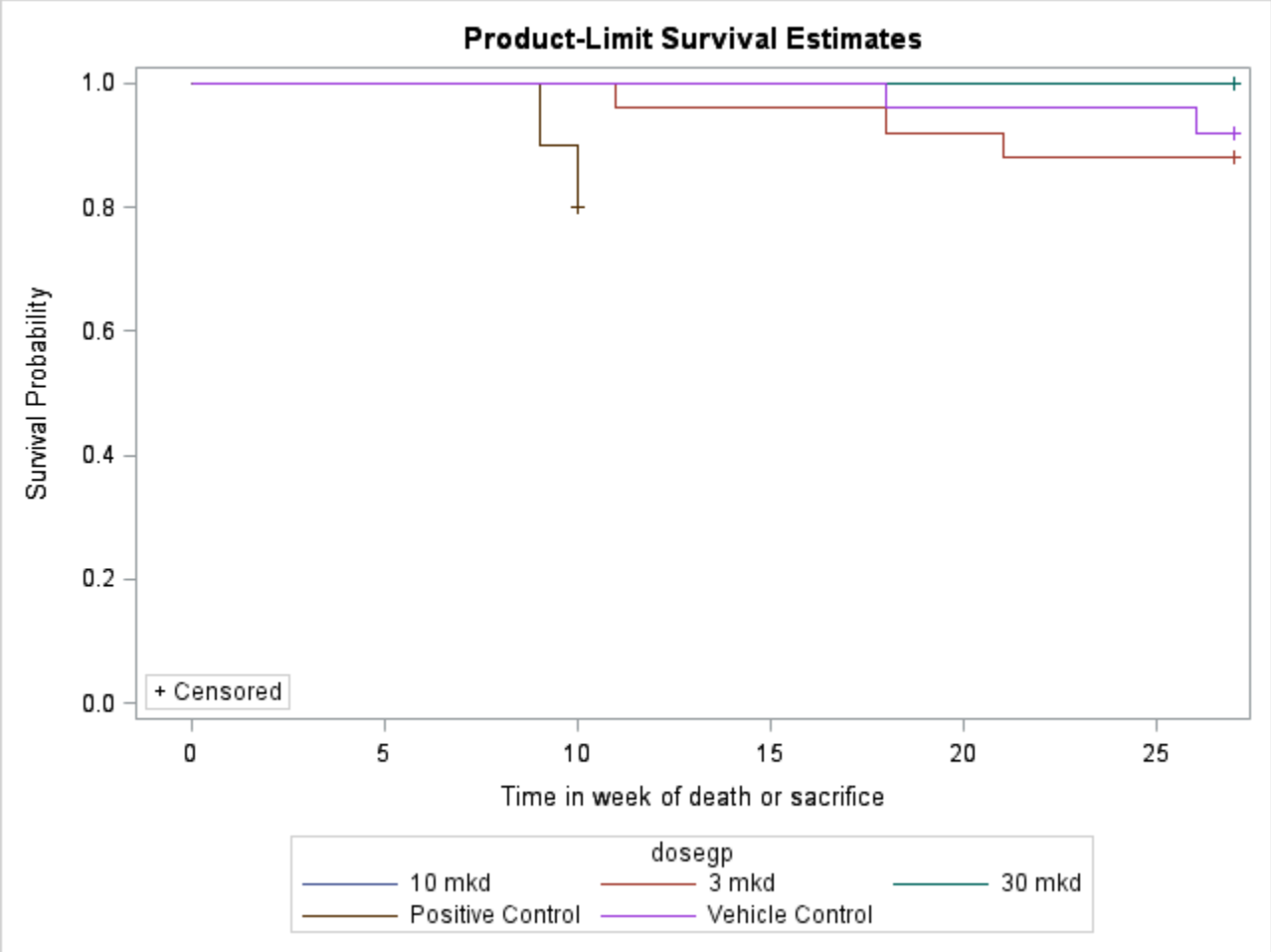
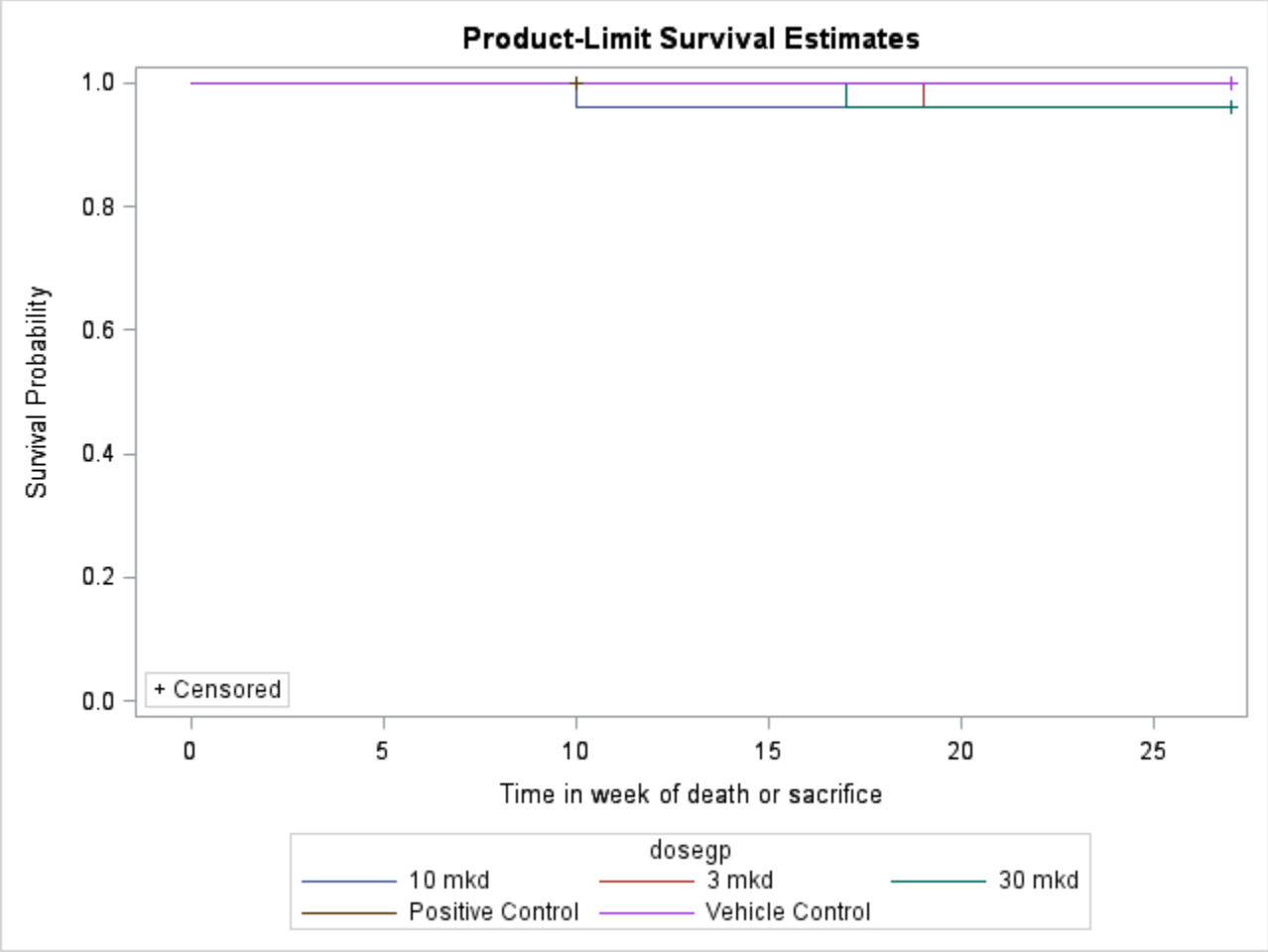


Figure 4: Kaplan-Meier Survival Functions for Female Mice



6. References

- Kaplan EL and Meier P (1958) Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc., 53, 457-481.
- Mantel N (1966) Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemotherapy Reports, 50, 163-170.
- Peto R (1974) Guidelines on the analysis of tumour rates and death rates in experimental animals. British J. Cancer, 29, 101-105.
- Lin KK (2000) Carcinogenicity Studies of Pharmaceuticals. In: Encyclopedia of Biopharmaceutical Statistics, ed. Shein-Chung Chow, Marcel Dekker, New York.
- Peto R et al. (1980) Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: Long term and Short term Screen Assays for Carcinogens: A Critical Appraisal. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Supplement 2, pp.311-426. WHO International Agency for Research on Cancer, Lyon.
- SAS Institute (2002) SAS OnlineDoc® Version Nine. SAS Institute Inc., Cary, NC, USA.
- Peto, R., M.C. Pike, N.E. Day, R.G. Gray, P.N. Lee, S. Parish, J. Peto, Richards, and J.Wahrendorf, “Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments”, Long term and short term screening assays for carcinogens: A critical appraisal, International agency for research against cancer monographs, *Annex to supplement, World Health Organization, Geneva,* 311-426, 1980.
- Bailer AJ, Portier CJ (1988). “Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples.” *Biometrics*, 44, 417-431.
- Bieler, G. S. and Williams, R. L. (1993). “Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity”. *Biometrics* 49, 793-801.
- Tarone RE, “Test for trend in life table analysis”, *Biometrika* 1975, 62: 679-82
- Lin K.K. and Rahman M.A.,” Overall false positive rates in tests for linear trend in tumor incidence in animal carcinogenicity studies of new drugs”, *Journal of Biopharmaceutical Statistics,* 8(1), 1-15, 1998.

- Rahman, A.M., and K.K. Lin (2008), "A Comparison of False Positive Rates of Peto and Poly-3 methods for Long-Term Carcinogenicity Data Analysis Using Multiple Comparison Adjustment Method Suggested by Lin and Rahman", Journal of Biopharmaceutical Statistics, 18:5, 849-858.
- Haseman, J, “A re-examination of false-positive rates for carcinogenesis studies”, *Fundamental and Applied Toxicology*, 3: 334-339, 1983.
- Guidance for Industry. Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Statues of Pharmaceuticals (Draft Guidance). U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.
- Lin, KK, and MA Rahman (2019), Comparisons of False Negative Rates from a Trend Test Alone and from a Trend Test Jointly with a Control-High Groups Pairwise Test in the Determination of the Carcinogenicity of New Drugs, Journal of Biopharmaceutical
  Statistics, 29(1):128-142.
- Lin, K.K., M.A. Rahman (2018), “Chapter 8: Expanded Statistical Decision Rules for Interpretations of Results of Rodent Carcinogenicity Studies of Pharmaceuticals”, IN Biopharmaceutical Applied Statistics Symposium, Volume 3: Pharmaceutical Applications, Editors: Peace, Karl E., Chen, Ding-Geng, Menon, Sandeep (Eds.), recently published by Springer in August 2018, Pages 151-183.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

ZHUANG MIAO
05/19/2021 01:59:23 PM

KARL K LIN
05/20/2021 08:47:37 AM
Concur with review.