EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 February 2022
EMA/152430/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Kapruvia

International non-proprietary name: difelikefalin

Procedure No. EMEA/H/C/005612/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

AcOH	Acetic acid
ACN	Acetonitrile
ADME	Absorption, distribution, metabolism, and excretion
ADR	Adverse drug reaction
AE	Adverse event
ANCOVA	Analysis of covariance
AUC	Area under the curve
BBB	Blood brain barrier
Cara	Cara therapeutics, inc.
CI	Confidence interval
CKD-aP	Chronic kidney disease-associated pruritus
C_{max}	Maximum plasma concentration
CMQ	Custom meddra query
CNS	Central nervous system
CPP	Critical process parameter
CQA	Critical quality attribute
CR845	Difelikefalin
CSR	Clinical study report
CYP	Cytochrome P450
DB	Double-blind
ECG	Electrocardiogram
EEA	European economic area
EMA	European medicines agency
ESRD	End-stage renal disease
EU	European union
FDA	United States Food and Drug Administration
GC	Gas chromatography
HD	Haemodialysis
HPLC	High performance liquid chromatography
ICH	International Council on Harmonisation
ICP-MS	Inductively coupled plasma mass spectrometry
ISE	Integrated Summary of Effectiveness
ISS	Integrated Summary of Safety
ITT	Intent-to-treat
IV	Intravenous or intravenously
KF	Karl-fisher
KOR	Kappa opioid receptor
LC	Liquid chromatography
LS	Least squares

MACE	Major cardiovascular event
MedDRA	Medical Dictionary for Regulatory Activities
MAA	Marketing authorisation application
MOR	Mu opioid receptor
MS	Mass spectometry
NMR	Nuclear magnetic resonance
OLE	Open-label Extension
Pd	Palladium
PGIC	Patient global impression of change
PIP	Paediatric investigation plan
PK	Pharmacokinetic or pharmacokinetics
PY	Patient-years
QoL	Quality of life
QTc	QT interval corrected for heart rate
SAP	Statistical analysis plan
SCE	Summary of Clinical Efficacy
SmPC	Summary of Product Characteristics
SM-1	Starting material 1
SMQ	Standardised meddra query
$t_{½}$	Apparent plasma elimination half-life
TEAE	Treatment-emergent adverse event
TFA	Trifluoroacetic acid
UK	United Kingdom (of Great Britain and Northern Ireland)
US	United States (of America)
USRDS	United States renal data system
USP/NF	United States pharmacopoeia/national formulary
UV	Ultraviolet
WI-NRS	Worst itching-numerical rating scale
XPRD	X-ray (powder) diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Vifor Fresenius Medical Care Renal Pharma France submitted on 8 March 2021 an application for marketing authorisation to the European Medicines Agency (EMA) for Kapruvia, through the centralised procedure under Article 3 (2)(a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 March 2020. The applicant applied for the following indication:

Treatment of moderate-to-severe pruritus associated with chronic kidney disease in adult patients on haemodialyis.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision (P/172/2020) on the agreement of a paediatric investigation plan (PIP). At the time of submission of the application, the PIP (P/172/2020) was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.4.2. New active Substance status

The applicant requested the active substance difelikefalin contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.5. Scientific advice

The applicant did not seek Scientific advice from the CHMP.

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Thalia Marie Estrup Blicher Co-Rapporteur: František Dráfi

The application was received by the EMA on	8 March 2021
The procedure started on	25 March 2021
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	11 June 2021
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	14 June 2021
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	18 June 2021
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 July 2021
The applicant submitted the responses to the CHMP consolidated List of Questions on	15 October 2021
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	22 November 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	02 December 2021
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	16 December 2021
The applicant submitted the responses to the CHMP List of Outstanding Issues on	24 January 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	09 February 2022
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to KAPRUVIA on	24 February 2022
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product	24 February 2022

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The therapeutic indication originally applied for by the applicant was:

Treatment of pruritus associated with chronic kidney disease in adult patients on haemodialyis, including patients where other treatment options have failed.

Chronic pruritus is defined as itch lasting 6 weeks or more. Stemming from a wide variety of causes ranging from primary dermatologic to secondary effects of underlying medical conditions, chronic pruritus can be a debilitating condition that significantly decreases quality of life and affects mood, sleep, personal relationships, and self-esteem. Chronic kidney disease-associated pruritus (CKD-aP), or uremic pruritus, is a frequently underdiagnosed but severely distressing condition that occurs in greater than 60% of patients undergoing dialysis. With between 20 and 40% of patients reporting intense, generalized systemic itching in the moderate-to-severe range, CKD-aP has been associated with depression, worsened sleep quality, increased risk of infection, decreased quality of life, and an increased risk of death.

2.1.2. Epidemiology

Of the over ~460,000 patients undergoing HD in the United States (US) (2019, USRDS), >60% have some degree of pruritus, with 20-40% suffering from moderate-to-severe CKD-aP. Comparable proportions are reported worldwide and in the major countries of the European Union (EU). However, CKD-aP is often under-recognized and under-treated by physicians and clinical staff.

2.1.3. Biologic features

While the pathogenesis of CKD-aP is still incompletely understood, four major hypotheses have arisen: 1) uremic toxin (such as Vitamin A, aluminium, calcium, phosphorus, and magnesium) deposition in the subcutaneous tissue, 2) peripheral neuropathy secondary to dysautonomia, 3) immune system dysregulation and 4) opioid imbalance. The last, endogenous opioid imbalance has been the focus of many new therapies for this condition. Specifically, it has been proposed that chronic pruritus, in general, may result from an imbalance in receptor activity between the mu- and kappa- opioid receptors. Mu-opioid receptor (MOR) activation has been shown to be pruritus-inducing, whereas kappa-opioid receptor (KOR) activation has been shown to be largely antipruritic. MOR upregulation and KOR downregulation has been reported in chronic itch of many etiologies, including uremic pruritus and cholestatic itch. In addition, this seems to explain why pruritus is a common side effect of both prescribed opioids for pain management (such as morphine, oxycodone, oxycontin, and hydromorphone) and commonly abused opioids (such as heroin), as they tend to exert larger activating effects on the MOR, inducing a MOR/KOR imbalance. This dichotomy also suggests that reversing the MOR/KOR imbalance, through decreasing MOR activation and/or increasing KOR activation, may be an effective treatment target for minimizing pruritus.

2.1.4. Clinical presentation, diagnosis

CKD-aP is 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, including sleep disturbance, insomnia, chronic fatigue, shame, social isolation, and an increased incidence of depression. Scratching often leads to an increased risk of infections (e.g., cellulitis, sepsis, bacteraemia, 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. Patients with CKD undergoing HD have a shortened life expectancy and lower quality of life compared with the general population. Their quality of life (QoL) and life expectancy may be further reduced when they suffer from CKD-aP.

2.1.5. Management

The prevalence of CKD-aP remains high despite improved efficiency of dialysis techniques, improved skin hydration with emollients, and use of phosphate binders and calcimimetics. There is currently no medicinal treatment approved specifically for CKD-aP in the EU. Several medications have been used largely as off-label treatments for CKD-aP (e.g., antihistamines, corticosteroids, gabapentin, and pregabalin). However, these off-label therapies are not always well tolerated by patients, and the evidence of their antipruritic efficacy is limited and lacking support from randomized, well-controlled studies. Taken together, the severe impact and consequences of CKD-aP and the lack of safe and effective treatment options underscore a significant unmet medical need for patients undergoing HD.

2.2. About the product

According to the applicant, difelikefalin is a selective and full agonist at kappa opioid receptors, with no identified off-target activity. As such, difelikefalin has no binding or functional activity at mu opioid receptors the main target of opioid analgesics. The selective activity of difelikefalin at KORs 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) minimizes passive diffusion or active transport across membranes, thus having limited permeability into the central nervous system (CNS).

The pathophysiology of 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 was 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.

The proposed indication for difelikefalin is the treatment of pruritus associated with chronic kidney disease in adult patients on HD, including patients where other treatment options have failed.

Difelikefalin is formulated as a parenteral solution for intravenous (IV) injection (0.05 mg/mL) and is intended to be administered as an IV bolus injection (0.5 mcg/kg dry body weight per dose) 3 times per week, following each HD session.

2.3. Quality aspects

2.3.1. Introduction

The finished product is presented as a solution for injection containing 50 microgram/ml difelikefalin as active substance. The product contains difelikefalin acetate.

Other ingredients are: acetic acid, sodium acetate trihydrate, sodium chloride and water for injections.

The product is available in in a single use 2 mL glass vial (type I), with a bromobutyl rubber stopper, an aluminium seal and a blue flip-off plastic cap, as described in section 6.5 of the SmPC.

2.3.2. Active substance

General information

The chemical name of difelikefalin is N-[(2R)-1-{[(2R)-1-{[(2R)-6-Amino-1-(4-amino-4-carboxy-1-piperidinyl)-1-oxo-2-hexanyl]amino}-4-methyl-1-oxo-2-pentanyl]amino}-1-oxo-3-phenyl-2-propanyl]-D-phenylalaninamide corresponding to the molecular formula $C_{36}H_{53}N_7O_6$. Difelikefalin acetate has a relative molecular mass of 679 and the following structure:

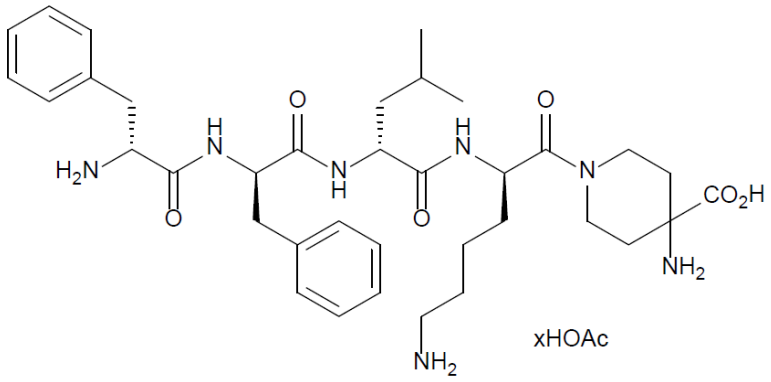


Figure 1: active substance structure

The chemical structure of difelikefalin was elucidated by ^{1}H and ^{13}C NMR spectra, molecular weight and amino acid sequence was confirmed by fragmentation and sequence analysis by MS/MS.

The active substance is an amorphous solid oligopeptide with approximately 1 to 2 mols of acetic acid per mol of peptide.

Difelikefalin is a lyophilised white to off-white powder; it is very hygroscopic and freely soluble in water. The measured pH is between 6 and 8.

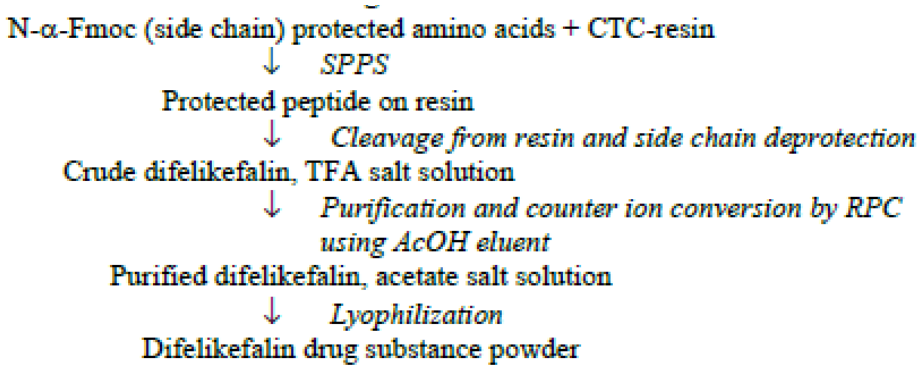
Difelikefalin exhibits stereoisomerism due to the presence of several chiral centres. 15 potential isomers (diastereomers and the enantiomer) can be formed by epimerisation during peptide synthesis or by carry-over of the chiral impurity from the starting materials, these are controlled at the level of starting materials, process parameters and active substance specification.

Specific optical rotation (Ph. Eur.) of the active substance has been investigated. The chirality of the individual amino acids has been confirmed by GC-MS on the reference standard, confirming the relative and absolute stereochemistry of difelikefalin and demonstrating that the amino acids Phe, Leu and Lys had the D-configuration. Chirality is controlled at the level of the starting materials and stereoisomeric impurities are controlled in the specifications of the active substance.

Polymorphism has been assessed by XRPD. No crystalline or polymorphic forms are known for the lyophilised amorphous difelikefalin.

Manufacture, characterisation and process controls

Difelikefalin is synthesised in four main stages: synthesis of the peptide on the resin, cleavage from the resin, purification and lyophilisation of the peptide as highlighted in Scheme 1. The manufacturing process uses well-defined starting materials with acceptable specifications; with the exception of SM-1, all the starting materials are well established commercially available staring materials, commonly used in solid phase peptide manufacture. SM-1 is a custom synthesised small molecule with acceptable specifications and, as such, it is accepted as starting material, it is supplied by two suppliers. All starting materials are single amino acid derivatives in D-configuration except (4-carboxy-4-amino)-piperidinyl residue (which has no chiral centre).



Scheme 1: active substance manufacturing process

The manufacturing method uses the well-established Fmoc solid phase peptide synthesis (SPPS) which is based on sequential assembly of protected amino acids, see Scheme 2. The growing peptide chain is anchored to a solid polymeric support, which allows for efficient removal of excess starting materials and reagents between each reaction step. After incorporation of the last D-phenylalanyl residue, the assembly of the resin-bound peptide is complete. The combination of side chain protection and the linker selection enables concomitant deprotection and cleavage from the solid support at acidic conditions (with TFA/water). Purification and concomitant acetate conversion of the difelikefalin crude in solution are performed by reverse-phase HPLC, see Scheme 3. Ion exchange of the crude peptide solution is achieved by applying isocratic aqueous ammonium acetate buffer, then purification is performed with a linear gradient of acetonitrile in water containing acetic acid buffer. Fractions that meet the main pool criteria are pooled together and concentrated, the solution is filtered through a 0.2 µm filter prior to lyophilisation. Isolation of the active substance is performed by lyophilisation. The bulk active substance is jar milled.

During the procedure, the manufacturing description has been updated with details on the amounts of reagent solution and solvents used in the process. Pooling of fractions is critical for the final purity of the peptide and details regarding fraction sizes, selection and pooling have been included in the description of the purification process. Reprocessing is not proposed as part of the application.

Critical steps have been identified and are controlled by adequate in-process controls during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. All the 15 potential diastereomers were investigated, of which only two of the were detected; however, four diastereomers are included as specified impurities in the active substance specification since they may potentially be present due to the presence of the chiral impurity up to 0.2 % in the starting materials. The remaining potential isomers are adequately controlled by starting material specifications and process parameters and can, if present, be detected by the analytical method used for testing the purity of the active substance. Insertion and deletion impurities have been investigated and are adequately controlled by the proposed specification.

Process related impurities have been investigated. Two t-butyl derivatives, obtained by the reaction of an amino group on the peptide with the tert-butyl cation formed during Boc cleavage, are controlled by IPC and in the active substance specification (as unspecified impurities).

The reagent trifluoroacetic acid (TFA) is used in the cleavage and deprotection process step to remove the peptide protecting groups. The reagents and solvents used in purification are ammonium acetate, ammonium hydroxide, acetic acid (AcOH), acetonitrile (ACN) and water. TFA, AcOH and ACN are controlled by the active substance specifications. A risk assessment has successfully demonstrated that benzene is not a potential impurity in acetonitrile, hence the absence of its control is justified.

In summary, potential and actual impurities were well-discussed with regards to their origin and characterised.

The manufacturing process has been developed in parallel with the clinical development program, using a combination of conventional univariate studies and elements of QbD such as risk assessment. Critical quality attributes (CQAs), critical material attributes (CMAs) and critical process parameter (CPPs) were identified and the impact of the CMAs and PPs on the CQAs was investigated, focusing on minimising critical impurities that are difficult to resolve and remove during the chromatography purification process.

Each step of manufacturing process for difelikefalin active substance was optimised separately. As a result of the optimisation exercise, the proposed commercial manufacturing process is robust and under a state of control to ensure the identity, assay, purity and quality of difelikefalin active substance. Confirmation of the successful optimisation was demonstrated through the manufacture of 6 consecutive commercial scale Process Performance Qualification (PPQ) batches. The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The active substance is packaged in a high-density polyethylene (HDPE) bottle with a polypropylene (PP) screw closure which complies with the EC directive 2002/72/EC and EC 10/2011 as amended. The plastic container is placed in a heat-sealed aluminium foil pouch. The secondary packaging component provides additional protection from moisture.

Specification

The active substance specification shown in Table 2 includes tests for: appearance, pH of solution (Ph. Eur.), clarity of solution (Ph. Eur.), colour of solution (Ph. Eur.), identification (molecular mass, Ph. Eur.; amino acid sequence MS/MS sequencing; retention time of HPLC-peak, LC-UV; structural identity ^{1}H-NMR), specific optical rotation (Ph. Eur.), assay (LC-UV, mass-balance), peptide relate impurities (LC-UV), residual TFA content (LC-UV), residual solvent acetonitrile (HS-GC), elemental impurities Pd (ICP-MS, Ph. Eur.), water content (KF – Ph. Eur.), acetic acid content (LC-UV), microbial examination (Ph. Eur.), bacterial endotoxin (Ph. Eur.).

The specification contains tests expected for a peptide active substance. The tests clarity (Ph. Eur. 2.2.1) and colour (Ph. Eur. 2.2.2) of the solution, which are able to detect impurities at levels not detected by the impurity methods, have been included. Four diastereomers are included as specified impurities since they may potentially be present due to the presence of the chiral impurity in the starting materials.

The pH acceptance criteria have been tightened during the assessment in line with batch data. During characterisation of the active substance the chiral purity of each amino acid has been demonstrated by chiral GC; therefore, it is acceptable that a test for enantiomeric purity is not included in the specification; optical rotation is included as specification test to demonstrate the identity of the chiral difelikefalin active substance and to demonstrate the absence of the racemate.

Specification limits are in line with ICH Q3A and have been satisfactorily justified from a reaction mechanistic point of view.

Limits for residual solvents (TF, ACN and AcOH) and catalysts (Pd) have been included in the specification.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data on six commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from 6 batches of active substance from the proposed manufacturers stored in the intended commercial package for up to 48 months under long term (-20°C) and intermediate (5°C) conditions and for up to 24 months under accelerated conditions (25°C / 60% RH) according to the ICH guidelines were provided. Batches were manufactured using SM-1 from both suppliers.

All results comply with the proposed specifications and no special trends are observed. An unidentified impurity was detected at -20°C with at a content of 0.09 % after 48 months storage. An increase in unspecified impurities is observed under accelerated conditions.

Photostability testing following the ICH guideline Q1B was performed on one batch. The active substance is not light sensitive.

Results on stress conditions (i.e. exposure to heat, aqueous solutions of neutral, acidic and basic pH and oxidation) have been provided. The results of the stress stability show that difelikefalin was degraded by exposure to dry heat (80°C for 7 days) and by exposure to oxidative stress (3 % H_2O_2 at 25°C for 15 hours). None of the degradants were identified during long-term stability studies;

however, two acetylated forms, which were identified after dry heat exposure, were also observed after 12 months storage at accelerated conditions (25°C /60% RH).

Additionally, the impact of repeated cycling from long-term storage conditions (-20°C) to ambient temperature (25°C) conditions (5 cycles) has been studied. There was no change in any of the parameters tested for the 5 cycles.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 48 months when stored at -20°C in the proposed container.

2.3.3. Finished medicinal product

Description of the product and pharmaceutical development

The finished product is solution for injection, containing 50 μg of the active substance difelikefalin.

Other ingredients are acetic acid, sodium acetate trihydrate, sodium chloride and water for injection.

The pharmaceutical development of the finished product focused on the choice of buffer, investigation on the solution density and the effect of concentration and fill volume on formulation stability.

The choice of excipients is justified, and their functions has been explained and it is satisfactory. All excipients are well-known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

Only fill volume and concentration of the active substance have been changed during the development of the finished product. Minor optimisation of the manufacture of the active substance has been conducted during the development of the finished product but no major changes to the synthesis of the active substance have been implemented. The formulation used during clinical studies is the same as that intended for marketing, hence no bioequivalence study has been conducted and this is acceptable. The development of the manufacturing process has been described in detail. To address a major objection raised during the procedure, the sterilisation method now includes an additional terminal sterilisation step at compendial conditions (≥121°C, ≥ 15 min) performed on the aseptically filled product.

The finished product as a ready to use single dose IV solution is drawn from the vial by the use of a sterile, single-use syringe and needle. No dilution or reconstitution prior to administration is needed. A compatibility study was conducted to evaluate the stability of the dosing solution in polypropylene syringes. Samples were tested for appearance, assay, impurities and pH. Testing was conducted with the validated HPLC method for assay and impurities or by compendial methods for appearance (and pH value. Results met the release specifications demonstrating compatibility with the polypropylene syringes.

The primary packaging is clear glass vials single use 2 mL glass vial (type I), with a bromobutyl rubber stopper, an aluminium seal and a blue flip-off plastic cap. The glass vial is a 2 R hydrolytic type I, the material complies with Ph. Eur. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of five main stages: compounding, biorburden reduction, sterile filtration, aseptic filling, terminal sterilisation. The process is considered to be a standard manufacturing process.

Initially, critical steps in the finished product manufacturing process have been identified and they included sterile filtration and aseptic filling, which was the originally chosen sterilisation process for the finished product. No additional critical steps were proposed for the introduction of the additional terminal sterilisation step since it is performed at compendial standards. The manufacturing process has been successfully validated through enhanced monitoring, sampling, and testing of three consecutive commercial scale batches of the aseptically manufactured finished product intermediate. Since the product is further terminally sterilised at compendial conditions, validation of the terminal sterilisation is not required to be submitted in the MAA and it will be completed prior to marketing. Nevertheless, data for two commercial batches manufactured with terminal sterilisation have been included in the dossier. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of pharmaceutical form/ manufacturing process and consist of measuring of pH, pre/post-filtration integrity test (bioburden reduction and sterility) and fill weight test.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (in house), pH (Ph. Eur.), osmolality (Ph. Eur.), particulate contamination (Ph. Eur.), visible particulates in injections (Ph. Eur.), container closure integrity (USP), test for extractable volume of parenteral preparations (Ph. Eur.), identification of difelikefalin (UV-VIS and HPLC), determination of difelikefalin content (free base, RP-HPLC), related substances (RP-HPLC), sterility (Ph. Eur.), bacterial endotoxins (LAL – Ph. Eur.).

Overall, the finished product specification has been adequately set in accordance with ICH Q6A and Ph. Eur. During the procedure, the limit for the assay at the end of shelf life has been brought in line with the release limit. Following the implementation of the terminal sterilisation method, a specified impurity has been added to the specification.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on three batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for identification, assay and impurities testing has been presented.

Batch analysis results are provided for one production scale batch manufactured by terminal sterilisation; supportive data on three production scale batches, which underwent aseptic filling, have been provided, confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data from one full scale batch of the terminally sterilised finished product stored for up to three months under long term conditions (25°C / 60% RH) and for up to three months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines were provided. Additional supportive stability data on three full scale process validation batches of the aseptically filled finished product intermediate stored for up to 36 months under long term conditions (25°C / 60% RH) and for up to 6 months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines were provided. Satisfactory stability data on a number of pilot batches of the finished product prior to and after terminal sterilisation has also been provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Stability data generated with the terminally sterilised pilot batches show a decrease of the assay value after the terminal sterilisation. However, the effect of the sterilisation is compensated with a respective manufacturing overage in order to meet the assay specifications. Exposure to terminal sterilisation results in a maximum single impurity/degradation product, which has been identified and specified as related substance . Apart from this difference, the batches that are terminally sterilised behave during stability like the batches that are manufactured aseptically. Hence, the data provided for the aseptically sterilised batches is accepted as supportive. Three full scale commercial batches of the terminally sterilised finished product have been put on stability

The analytical procedures used are stability indicating. No trends or significant changes in any stability parameters have been observed. However, it should be noted that there is an inherent variability of the assay results of approximately 2% observed between different time points which is typical of oligopeptides. An excursion outside this variability was observed at t = 9 months and it was linked to the change in reference standard.

In addition, two batches were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. No significant changes have been observed, all attributes evaluated have met the specifications: the finished product is not light sensitive.

A thermal degradation study to identify degradation products resulting from heat stress was performed on a simulated finished product solution containing difelikefalin active substance at a higher concentration of 1 mg/mL, exposing it to 121 °C, but for a longer time period of 4 h in order to generate a sufficient amount of degradation products.

Based on available stability data, the proposed shelf-life of 2 years, without any special storage conditions, as stated in the SmPC (section 6.3) are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.3.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The applicant has revised the sterilisation method from aseptic filling to compendial terminal sterilisation, thus resolving the Major Objection raised. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.3.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.3.6. Recommendations for future quality development

Not applicable.

2.4. Non-clinical aspects

2.4.1. Introduction

Difelikefalin, a small synthetic peptide and full agonist at kappa opioid receptors (KORs), is being developed for the treatment of chronic kidney disease-associated pruritus (CKD-aP) in adult patients undergoing haemodialysis, including patients where other treatment options have failed. Chronic kidney disease-associated pruritus is a common, distressing, and underrecognized condition that affects more than 60% of patients undergoing haemodialysis (HD), with 20 to 40% of patients reporting moderate-to-severe pruritus. It is characterized by a generalized, persistent, and intractable itch that affects their ability to sleep and results in chronic fatigue, feelings of shame, increased social isolation, and an increased incidence of depression. The pathophysiology of CKD-aP is not fully understood but is thought to be multifactorial, including systemic inflammation and 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 which is the basis for the development of difelikefalin for the proposed indication.

2.4.2. Pharmacology

2.4.2.1. Primary pharmacodynamic studies

Five *in vitro* pharmacology studies were conducted to determine the binding characteristics, functional activity and anti-inflammatory effects of difelikefalin. Binding affinity assays showed that difelikefalin binds to human recombinant KOR expressed in human embryonic kidney cells (HEK-293) with an inhibitory constant (Ki) and half-maximal inhibitory concentration (IC_{50}) of 0.32 and 0.799 nM, respectively. Difelikefalin also exhibited significant affinity for the rat recombinant KOR expressed in Chinese hamster ovary cells resulting in 93% inhibition of control specific binding at 10 µM. In a

binding kinetic study conducted in CHO cells transfected with human recombinant KORs, the binding half-life ($t_{½}$) of difelikefalin was estimated at 92 minutes with an equilibrium dissociation constant (K_d) of 0.14 nM. Together, these three *in vitro* studies demonstrated that difelikefalin is a potent and full agonist at human and rat KORs with a slow receptor off-rate that might contribute to a long duration of activity *in vivo*.

The potency and efficacy of difelikefalin were assessed in an *in vitro* functional cyclic adenosine monophosphate (cAMP) production assay using thymoma cells expressing mouse KORs and a reporter gene assay in HEK-293 cells transiently transfected with cDNA for human KORs. The concentration of difelikefalin necessary to produce 50% of the maximal response (EC_{50}) in the assays was 0.048 nM at the mouse KORs and 0.16 nM at the human KORs, with 96 and 99% efficacy, respectively. Thus, difelikefalin is a potent full agonist at the human and mouse KORs. Difelikefalin also displayed anti-inflammatory activity. *In vitro*, difelikefalin at 2.0 to 50 nM reduced pro-inflammatory cytokine production (e.g. IL-1, TNF-α, granulocyte colony stimulating factor) by human monocyte-derived macrophages stimulated with lipopolysaccharide and IFN-γ.

The *in vivo* pharmacological activity of difelikefalin was evaluated in several mouse and rat studies. Also, a high degree of overall homology for KORs, MORs, DORs and orphanin FQ (or nociception) receptors exists across vertebrate species. The key models used to evaluate the antipruritic and anti-inflammatory activity of difelikefalin are well-characterized and commonly used in drug evaluation. Hence, the choice of animal models is considered suitable.

In vivo, i.v. difelikefalin displayed potent, dose-dependent, long-duration antipruritic effects in two well-characterized mouse models of chemically-induced itch using the kappa antagonist, 5' guanidinonaltrindole (5' GNTI), and the mast cell secretagogue, compound 48/80. Difelikefalin dose necessary to reduce scratching activity in the neck region of male mice near the pruritogen injection site by 50% (ED_{50}) was 0.05 and 0.08 mg/kg i.v. for 5' GNTI and compound 48/80 induced itch behaviour, respectively. A time-course study, which evaluated the anti-scratching activity of difelikefalin pre-treatment against compound 48/80-induced itch at an estimated submaximal ED80 dose (0.3 mg/kg i.v.), demonstrated a statistically significant mean percent inhibition of scratching of approximately 70% or more compared with the control value through 12 hours post dose. At the doses evaluated, the effect of difelikefalin was similar to the effect of nalfurafine, a centrally acting KOR agonist and partial MOR agonist approved for the treatment of CKD-aP in HD patient. The two models evaluating the antipruritic effect of difelikefalin were relevant as they are sensitive to the effect of other kappa agonists, such as nalfurafine, a centrally acting KOR agonist and partial MOR agonist.

The anti-inflammatory activity of difelikefalin shown *in vitro* was also characterized in two *in vivo* studies. Inhibition of LPS-evoked pro-inflammatory cytokine release was demonstrated *in vivo* in a mouse model of sepsis (endotoxemia). Difelikefalin dose dependently reduced the release of TNF-α compared with the mean vehicle control value when administered by s.c. injection prior to LPS challenge, with a minimum effective dose (MED) of 3 mg/kg and an efficacy similar to the positive control, prednisolone (3 mg/kg). Difelikefalin, administered by s.c. injection at 10 mg/kg prior to LPS challenge, decreased a panel of cytokines in a qualitatively similar pattern to prednisolone, significantly reducing TNF-α, macrophage inflammatory protein-1β, IL-1β, IL-2, and IL-12 production by 19 to 47% compared with the vehicle control. Pre-treatment with difelikefalin prior to intraplantar injection of carrageenan also resulted in a dose-related anti-inflammatory effect in rats as indicated by a significant reduction in hind paw swelling. The MED for difelikefalin was 0.3 mg/kg i.v., with an effect in the inflammatory pain model similar to the non-steroidal anti-inflammatory drug, ibuprofen (100 mg/kg s.c.).

The peripheral action of difelikefalin was investigated in a chemically induced pancreatitis and spinal nerve ligation model. The antinociceptive activity of difelikefalin (1 mg/kg, i.p.) was significantly

inhibited in male rats following i.p. administration of naloxone methiodide or the selective KOR antagonist nor-BNI in chemically induced pancreatitis. Inhibition of the difelikefalin effect by nor-BNI demonstrates that the mechanism of action is mediated through KOR. The inhibitory effect of the peripherally restricted mu and kappa antagonist, naloxone methiodide, supports that difelikefalin is acting via the activation of peripherally located opioid receptors. The anti-allodynic effect of difelikefalin (1 mg/kg; i.v.) in a spinal nerve ligation (SNL) model was reversed by intraplantar administration of nor-BNI into the affected paw of male rats, further indicating that difelikefalin acts primarily by activating KORs located on primary afferent sensory neurons outside of the central nervous system.

The lack of tolerance to the antinoceptive effect of difelikefalin was supported in two *in vivo* studies. Sustained anti-allodynic activity was observed in SNL male rats following repeat daily *i.v.* administration of difelikefalin (1 mg/kg/day), with a statistically significant increase in paw withdrawal threshold (PWT) compared with daily baseline values observed across all days. There was a slight downward trend in efficacy by approximately 28% to 33% from Day 1 beginning Day 5 through Day 7. To ascertain whether the diminution in the difelikefalin response could be related to technical issues associated with repeat *i.v.* injections, the 7-day repeat-dose administration of difelikefalin was repeated using the p.o. route of administration instead of *i.v.* and no decrease in efficacy was observed on Day 7 compared with Day 1. Overall, the data support that there is no tolerance to the anti-allodynic effect of difelikefalin in the rat SNL model.

Overall, these *in vitro* and *in vivo* studies were relevant in relation to the disease to be treated and the proposed indication. Proof of concept and mode of action of difelikefalin were demonstrated and this is endorsed.

2.4.2.2. Secondary pharmacodynamic studies

The *in vitro* binding of difelikefalin at 10 µM was assessed across five studies to characterize the potential secondary pharmacodynamics activity in a comprehensive panel of ion channels, enzymes, transporters, and receptors including the human MOR, DOR, and ORL1. The studies demonstrated that difelikefalin is unlikely to exhibit biologically relevant activity at any target tested, except the intended KOR target. Furthermore, difelikefalin up to 1 M exhibited no activity at the at MORs and DORs in a functional assay. The data indicate that difelikefalin is highly selective for the rodent and human KORs and is unlikely to exhibit biologically relevant secondary pharmacodynamics activity at any of the targets tested.

2.4.2.3. Safety pharmacology programme

The potential effects of difelikefalin on the CNS were evaluated in both, mice and rats. Difelikefalin i.v. administered to mice at doses up to 0.1 mg/kg was not associated with any effect on locomotor activity. A statistically significant, dose-dependent decrease in total locomotor activity through 1 hour after dose administration compared with the vehicle control was observed in mice at ≥0.3 mg/kg.

A functional observational battery (FOB) GLP study in rats was conducted 2 and 24 hours after administration of i.v. difelikefalin at 1 to 25 mg/kg. An effect on all functional domain groups evaluated in the FOB was noted across all dose groups, with an effect designated as adverse based on magnitude, duration, and/or dose response relationship. The adverse effects included decreased activity and arousal in the open field, decreased rearing and posture abnormalities (CNS parameters); increased urination and miosis (autonomic nervous system effects); delayed tail flick response (sensorimotor effects); gait impairments or abnormalities, surface righting, and air righting (neuromuscular effects); and decreased body temperature. Difelikefalin-related effects had resolved or were resolving within 24 hours of dosing, and animals appeared normal by 48 hours post dose. The

increased urination and decreased behavioural and motor effects are consistent with a pharmacological response to the activation of KORs, occurring at large multiples (>300-fold) of the human exposure at the clinical dose to treat CKD-aP in HD patients. The lowest observed adverse effect level (LOAEL) was considered to be 1 mg/kg, which is approximately 386-fold higher than the anticipated C_{max} in CKD-aP patients undergoing HD administered the proposed clinical dose of 0.5 µg/kg.

In addition, five other CNS safety pharmacology studies in mice and rats evaluating the safety potential of difelikefalin were presented and the results are outlined in the table below. Their results were in line with the two aforementioned pivotal CNS studies.

Other CNS safety pharmacology studies of difelikefalin

Study No	Organ systems evaluated	Species	Doses i.v.	Noteworthy Findings	GLP
CR845-SP033	CNS/Rotarod	Mouse	0.7 mg/kg	Difelikefalin significantly decreased time spent on rotarod with maximal decrease at 20 minutes and return to control level at 180 minutes post dose.	No
SBL069-130	CNS/Rotarod	Mouse	0.1, 0.3, 1, 3, 10 mg/kg	Administration of ≤0.3 mg/kg had no effect on the latency to fall off the rotarod. ≥1 mg/kg difelikefalin induced motor discoordination in mice for at least 90 minutes post dose. The mean ED_{50} was 0.63 mg/kg and 0.74 mg/kg at 30 and 60 minutes post dose, respectively. NOEL = 0.3 mg/kg	Yes
SBL069-131	CNS/Sleep	Mouse	0.03, 0.1, 0.3, 1, 3 mg/kg 40 mg/kg IP: pentobarbital	Difelikefalin doses ≥0.1 mg/kg induced a dose-dependent increase in pentobarbital-induced loss of righting reflex (sleeping time) in mice compared with the control. NOEL = 0.03 mg/kg	Yes
CR845-SP034	CNS/Rotarod	Rat	0, 0.3, 1, 3, 10, 30 mg/kg	≤1 mg/kg = no effects. >50% of rats administrated ≥3 mg/kg difelikefalin exhibited motor discoordination and reduced activity for up to 60 minutes after dosing. 30 mg/kg was also associated with abnormal posture up to 90 minutes post dose. NOEL = 1 mg/kg	No
9718	CNS/Sleep wake cycle	Rat	0, 0.1, 0.3, 1mg/kg	Spontaneous brain waves were normal across all groups. Increased autonomic movement was observed in 1, 2, and 6 of 6 rats at 0.1, 0.3, and 1 mg/kg, respectively. Difelikefalin at ≥0.1 mg/kg was associated with changes in the sleep-wake cycles, most notably at ≥1 mg/kg. Also, at ≥0.3 mg/kg, the brain waves never reached deep sleep although rats exhibited a supine position. LOEL = 0.1 mg/kg	No

CNS=central nervous system; GLP=Good Laboratory Practices; LOEL=lowest observed effect level; MAP=mean arterial pressure; NOEL=no observed effect level.

The safety pharmacology assessment of difelikefalin was carried out in accordance with the ICH S7A and ICH S7B guidelines and presented in a comprehensive manner. *In vitro* and *in vivo* safety

pharmacology studies evaluating the effects of difelikefalin on the central nervous, respiratory and cardiovascular systems were conducted in compliance with GLP.

2.4.2.4. Pharmacodynamic drug interactions

A pharmacodynamic drug-drug interaction study was conducted to investigate the potential additive or synergistic effect on locomotor activity when difelikefalin is co-administered with the antihistamine, ketotifen. The study was undertaken because HD patients with CKD-aP often take antihistamines, and antihistamine can induce drowsiness and decrease motor function. Difelikefalin at 0.3 and 0.6 mg/kg administered by i.v. injection or ketotifen at 30 mg/kg administered orally to rats resulted in a statistically significant decrease in mean locomotor activity compared with the vehicle at the three-time intervals evaluated: 0 to 10, 10 to 20, and 20 to 30 minutes. The i.v. administration of 0.3 mg/kg difelikefalin 30 minutes after administration of ketotifen exhibited neither synergistic nor additive effects when compared with ketotifen alone.

At a dose of 0.6 mg/kg, difelikefalin appeared to significantly potentiate the 30 mg/kg ketotifen-related decrease in locomotor activity. It is stated that these effects occur at high exposure multiples to the MRHD. However, in the pooled data of two well-controlled Phase 3 studies, subjects who used an antihistamine prior anti-itch medication (most common drugs used were antihistamines diphenhydramine and hydroxyzine) showed a higher risk of somnolence indicating that there is a drug-drug interaction. This is adequately reflected in the SmPC.

2.4.3. Pharmacokinetics

The PK of difelikefalin was evaluated in relevant animal species including mice, rats, rabbits and monkeys.

Absorption: Difelikefalin PK following a single intravenous dose demonstrated peak plasma concentrations at the first timepoint post dose that declined with time in a biexponential pattern characterised by a relatively fast distribution phase followed by a slower elimination phase. Exposure measured by area under the concentration vs time curve (AUC) generally increased in a roughly dose-proportional manner. Elimination half-life was relatively short and tended to be slightly longer in the rat compared to the monkey. The clearance was moderate in the rat and low in monkey. Several single- and repeat-dose toxicokinetic analyses of difelikefalin were performed on samples collected from male and female rats and monkeys following i.v. bolus administration in toxicology studies. In the general toxicology studies, rats received daily difelikefalin doses at 0.25 to 25 mg/kg/day for up to 26 weeks by i.v. injection and monkeys were administered daily i.v. doses of 0.25 to 4 mg/kg/day for 4 weeks or 0.06 to 1.0 mg/kg/day for up to 39 weeks. Repeat-dose toxicokinetics were also determined in pregnant rats at 0.25 to 25 mg/kg/day and in pregnant rabbits at 0.025 to 0.1 mg/kg, where difelikefalin was administered through the period of organogenesis.
In both, rat and monkey, i.v. exposure to difelikefalin increased in a dose-proportional to slightly greater than dose-proportional manner. Accumulation was generally not observed in the rat, but when noted, it was only minor (<2-fold) and occurred only at the high dose.
There was no consistent pattern of gender-related effects on any toxicokinetic parameters in either species.
The kinetics of and exposure to difelikefalin in pregnant rats from gestation day (GD) 7 through GD 17 was similar to that reported in nonpregnant animals. Qualitatively similar kinetics to the pregnant rat was observed in the pregnant rabbit administered difelikefalin from GD 7 through GD 19 characterised by a dose-proportional increase in exposure and plasma concentration that exhibited a biexponential decline following maximum concentration.

In general, toxicokinetic data for difelikefalin in the intravenous repeat-dose studies demonstrated that exposure for both C_{max} and AUC was roughly dose-proportional to slightly greater than dose-proportional, independent of sex and with a short half-life.

Repeat-dose toxicokinetic studies by the s.c. route were conducted to support carcinogenicity testing in mouse and rat, as well as chronic administration in the rat. Mice were administered difelikefalin by s.c. injection at doses of 3 to 33 mg/kg/day in a dose-range finding study and 3 to 30 mg/kg/day in a 26-week transgenic carcinogenicity study. Rats were administered difelikefalin by s.c. injection at doses of 10 and 50 mg/kg/day in a 10-day preliminary study, 1 to 25 mg/kg/day in a 26-week study, and 0.25 to 1.0 mg/kg/day for up to 183 days in a 2-year carcinogenicity study toxicokinetic cohort. Absorption in both mice and rats was relatively quick (t_{max} = 0.25 to 0.5 hours and 0.25 to 1 hour, respectively). Exposure to difelikefalin increased with increasing dose resulting in a wide range of exposures for both species. In the mouse, there was minimal to no accumulation with repeat dosing and no gender-related differences. In the rat, marked accumulation and/or increase in difelikefalin plasma concentration with repeat dosing was observed. Gender-related differences (i.e., ≥2-fold difference) were dependent on dose in the rat, with no differences following repeat dose administration at 5 and 25 mg/kg/day but with females exhibiting notably greater exposure than males following repeat dose administration at doses of 0.25 to 1 mg/kg/day.

Distribution: Protein binding of difelikefalin was low across all species (<50% in mice, rats, monkeys, and humans) as assessed via equilibrium dialysis and/or ultracentrifugation of radiolabelled difelikefalin *in vitro* and by ultracentrifugation in plasma samples from monkeys following a single i.v. dose. Results of the studies demonstrated that *in vitro* protein binding ranged from 15.8 to 36.8% at 1 µM and 17.7 to 49.1% at 10 µM difelikefalin in plasma from mice, rats, dogs, monkeys and humans. The *ex vivo* plasma protein binding in monkeys was approximately 17.3 to 17.8% at 0.5 and 2 hours, respectively, following i.v. dose administration of 1 mg/kg of [^{14}C]-difelikefalin. Assessment of difelikefalin general tissue distribution was conducted in rats and monkeys using whole tissue dissection and quantitative whole-body autoradiography (QWBA) or qualitative whole-body autoradiography (ARG) methods. Four comprehensive tissue distribution studies were conducted in rats following a single i.v. administration of 1 mg/kg [^{14}C]-difelikefalin in male albino rats and male pigmented rats, as well as male and female SD rats, and following 7 days of difelikefalin i.v. administration. In monkeys, qualitative head and whole-body ARG was conducted following a single i.v. dose of 1 mg/kg [^{14}C]-difelikefalin. Additional studies were conducted to further characterise the potential distribution to whole brain and other nervous tissues in rats and distribution to the cerebrospinal fluid (CSF) in rats and monkeys. Overall, the distribution studies in both species and across all studies demonstrated a similar kinetic profile following an i.v. injection of radiolabelled difelikefalin in both the rat and the monkey, the highest levels of radioactivity were observed in difelikefalin excretory organs including the kidney (urine primary excretory route) and to a lesser extent the liver (faeces minor excretory route). In all other tissues the tissue:blood ratios were generally <1 at all timepoints.

Distribution of radioactivity to CNS tissues protected by the blood-brain barrier (BBB) was negligible, even at exposures based on C_{max} and AUC_{last} that are ≥400-fold and ≥40-fold higher, respectively, than the exposure in subjects with CKD-aP undergoing HD at the clinical dose of 0.5 mcg/kg IV every 2 to 3 days.

In general, the studies demonstrated a similar distribution profile in albino and pigmented rats, no gender-related differences were noted, and comparable tissue radioactivity concentrations after a single dose and daily doses for 7 days suggest that repeat dosing does not impact distribution. The QWBA results were considered qualitatively similar to the tissue dissection studies in rats. Transplacental transfer of difelikefalin was demonstrated in pregnant rats with foetal:maternal plasma ratios ranging from 0.025 to 0.027.

Metabolism: The metabolism of difelikefalin was negligible in vitro and in rats and monkeys with no major metabolites identified consistent with data from a human ADME study in healthy and HD subjects using [^{14}C]-difelikefalin.

Excretion: The excretion of difelikefalin was evaluated in rats and monkeys administered a single i.v. dose of 1 or 3 mg/kg [^{14}C]-difelikefalin. The main route of excretion for difelikefalin was in the urine with a mean cumulative excretion of the radioactive dose through 168 hours post dose of 57.1 to 74 and 70.1% in the rat and monkey, respectively. In monkeys, the urinary excretion was underestimated compared to the aforementioned studies. Through 168 hours post dose, the percent of the radioactive difelikefalin dose excreted in the faeces ranged from 13 to 37.2% in the rat and was 12.7% in the monkey. In bile duct cannulated animals, 65, 13, and 2.6% of radioactivity was excreted in urine, bile, and faeces, respectively, through 48 hours after dosing.
Faecal excretion was reported to be 13 to 37, 12.7 and 11.3% of the total radioactive dose in rats, monkeys, and humans with normal renal function, respectively. As expected, the amount of difelikefalin excreted in the urine was notably lower (11.2%) in HD subjects. The total percent of the radioactive dose recovered from the dialysate was 19.5%, whereas faecal excretion represented 58.8% of the radioactive dose. The percentage of the radioactive dose excreted in the faeces of HD subjects is greater than that observed in either the rat or monkey. Based on the doses administered in the rat and monkey chronic toxicology studies that were not associated with any hepatic or biliary lesions (i.e., the high dose of 25 and 1 mg/kg/day, respectively), the total dose excreted via the bile into the faeces compared with HD subjects (0.5 µg/kg every 2 to 3 days) would result in a bile concentration exposure multiple of greater than several thousand-fold and approximately 32.2-fold in the rat and monkey, respectively. The data in the rat and monkey, therefore, support that the increase in faecal excretion of difelikefalin in CKD patients on HD would represent negligible to no safety risk, which is supported. The potential milk transfer of difelikefalin in relation to plasma exposure was investigated in lactating rats on lactation day 14 in addition to plasma levels of the nursing pups. The data indicate that difelikefalin is transferred to the milk when lactating rats were administered difelikefalin with milk:plasma ratio of 0.044, 0.051, and 0.042 at 0.6, 2.5, and 10 mg/kg/day, but detectable levels of difelikefalin were not measured in the nursing pups.

Pharmacokinetic drug interactions: A battery of *in vitro* assays evaluating the potential effect of difelikefalin on CYP induction and inhibition, UGT inhibition, transporter test systems (substrate or inhibitor) as well as low protein binding (*in vitro* and *in vivo*) was undertaken to determine the potential for drug interactions.
Overall, the study results indicated that permeability was similar in the presence and absence of the efflux-transporter P-glycoprotein (P-gp)-inhibitor, verapamil, and difelikefalin did not result in appreciable inhibition of a P-gp substrate (digoxin) efflux, suggesting that difelikefalin is not a substrate nor inhibitor of P-gp.
In transporter test systems, difelikefalin at concentrations up to 10, 30 or 300 µM (approximately 7 to 200 µg/mL) was not a substrate or inhibitor of the bile salt export pump (BSEP), multidrug resistance associated protein 2 (MRP2), breast cancer resistance protein (BCRP), organic anion transporter 1 (OAT1), OAT3, organic cation transporter 1 (OCT1), OCT2, OCT3, organic anion transporting polypeptide 1B1 (OATP1B1), OATP1A2, OATP1B3, multidrug and extrusion (MATE) transporter 1 (MATE1), MATE2K, L-type amino acid transporter 1 (LAT1), and peptide transporter 1 (PEPT1) or PEPT2. Furthermore, difelikefalin is not a substrate of the apical sodium-dependent bile acid transporter (ASBT), OAT2, OATP2B1, organic cation/carnitine transporter 1 (OCTN1), OCTN2, and organic solute transporter α/β (OSTαβ).
The totality of the in vitro drug interaction studies showed that difelikefalin was not a substrate (<2-fold difference in uptake) or an inhibitor (<22% inhibition) of any major human drug transporter. The

effect of other drugs on difelikefalin pharmacokinetics have not been directly evaluated, but the low degree of plasma protein binding and lack of interaction of difelikefalin with common drug transporters and metabolic enzymes in vitro suggest a low likelihood of drug interactions that could impact the safety or efficacy of difelikefalin or concomitantly administered drugs based on pharmacokinetic drug interactions. Therefore, the potential risk of interaction between difelikefalin and other drugs is considered minimal, and this is endorsed by the CHMP.

Other pharmacokinetic studies
No other pharmacokinetic studies were conducted.

2.4.4. Toxicology

Mice, rats, rabbits and monkeys were considered pharmacological relevant species for toxicity studies due to findings from the conducted pharmacology and toxicology studies in addition to a high degree of KORs homology across vertebrate species. Due to the extensiveness for the submitted data, only selected studies considered essential for the sought indication were assessed. The test article used in the pivotal nonclinical studies are according to the applicant comparable to the drug substance used in the clinical trials and in the drug substance to-be-marketed. Additionally, no new excipients are used and the excipients in the formulation (i.e., compendial sodium chloride, sodium acetate, and acetic acid in water) are at concentrations at or below currently approved products.

2.4.4.1. Single dose toxicity

Single-dose toxicity studies were conducted in mice, rats and monkeys with i.v. administration. The rat and monkey study were made with i.v. infusion over a 5-minute interval. The mouse study was conducted to GLP, as it is part of a dose-range finding for the micronucleus test, whereas the remaining rat and monkey study are non-GLP. This is acceptable. The single-dose toxicity studies are generally conducted as a part of a dose range finding studies for either the micronucleus test or repeat-dose toxicity studies. This is supported, as stand-alone single-dose studies are not encouraged from a 3R perspective in accordance with ICH M3(R2).

Primary difelikefalin-related observations were lethargy in all the species even at the lower doses (mouse and rats ≥50 mg/kg/day, monkeys ≥ 0.5 mg/kg/day) and reduced food consumption, leading to a dose-dependent effect on body weight gain primarily in male rats (and female rats at 75 mg/kg/day). Clinical signs generally increased in number and severity with increasing doses in all species.

Mortality was seen for all mice receiving 200 mg/kg/day i.v. and veterinary intervention was needed for a monkey in the 16 mg/kg/day group (ataxia, loss of body temperature, emesis resulting in Naloxone treatment). MTDs of 100 mg/kg/day and 8 mg/kg/day in mice and monkey is therefore supported, however, it should be noted that adverse effects of varying severity were still observed at these doses. An MTD in rats were not established but could be set to 100 mg/kg/day, using criteria as lack of mortality and highly severe illness. However, observations of reduced food consumption and body weight (BW)/BW gain led to a reduction of the high dose for the repeat-dose study in rats to 25 mg/kg/day, which is supported.

2.4.4.2. Repeat dose toxicity

The choice of repeat-dose studies as pivotal are generally supported according to ICH M3(R2) guideline. Of the non-pivotal studies, the dose range finding study in rats and in monkeys are

considered relevant in support of the doses used for the repeat-dose studies. The SC repeat-dose studies in mice and rats supports the conducted CARC studies.

Rats: A maximum difelikefalin doses of 25 mg/kg/day for i.v. administration in repeat-dose toxicity studies in rats was determined based on data from the dose range finding study. The dose was generally well tolerated, however, transient lethargy, lower BW gain and reduced food consumption was detected. Decreased activity were detected in all dose groups during the first week of dosing in the 28-day rat study. Generally, male rats appear to be more prone to adverse effect even though no gender-related difference was observed in exposure. In male rats, a reduction in BW and reduced food consumption were noted already at 5 mg/kg/day, compared to female animals primarily showing reduced BW gain. In the 13- and 26-week rat studies similar findings of transient decrease in activity, food consumption and BW were noted during the first weeks of IV administration difelikefalin, especially in male animals. However, the effect on reduced BW and food consumption appeared to normalize over longer periods of administration.

Testicular and epididymal changes were noted in the 4-week study with reduced testicular weight at 25 mg/kg/day and germinal epithelium degeneration at doses ≥5mg/kg/day. Changes were most pronounced in the 25 mg/kg/day group but mostly resolved after recovery. The effect observed in the lower dose groups appeared to mitigate due to changes in housing from wire-bottomed to solid-bottomed cages in the subsequent 13- and 26-week studies. However, atrophy and/or degeneration in seminiferous tubular and cell debris in epididymis were still seen at doses of ≥25 mg/kg/day in the two longer-term studies. In the 4-week and 26-week study, the effect was bilateral and even though it mostly resolved after recovery, a difelikefalin-related effect on the spermatogenesis cannot be excluded. The changes in the 13-week study were, however, only observed unilaterally and a link to a systemic effect of difelikefalin is therefore more uncertain. A reduction of ovary weight of 33% was seen in female rats in the 26-week study, however, no correlating histopathological findings were noted.

In the kidneys, non-reversable tubular basophilia were seen primarily in male rats at 25 mg/kg/day in both the 13- and 26-week study, as an example of rodent-specific chronic progressive nephropathy (CPN). Red pigment granules in pars convolute were noted in female rats in both the 13- and 26-week study from doses of 2.5 mg/kg/day in addition to hyaline droplet in males in the 26-week study. However, since the changes was reversible and not associated with histopathological lesions in the surrounding tissue, the Applicant did not consider them to be toxicologically important. This is supported. Additionally, in the 13-week i.v. rat study urinary output was transiently decreased from doses of ≥2.5 mg/kg/day, however, this could not be reproduced in the 26-week study, where a tendency toward increased urinary output were seen, if any. An effect on organ weight (i.e., decreased liver, heart, kidney, spleen and prostatic weight) were noted in males in the 4-week study, which could be related to the decreased BW. In female rats, a decrease in thyroid, thymus and heart weight were seen (significant at 25 mg/kg/day in the 4-week study), however, no corelating gross or histopathological findings were noted to explain the finding.

The lowest NOAEL were set to 2.5 mg/kg/day in male rats based on testicular/epididymal changes in the 26-week study, whereas a NOAEL of 25 mg/kg/day were general for female rats across all three studies. The NOAEL of 2.5 mg/kg/day ensured a clinical exposure margin of approximately 211-fold, which is considered acceptable. All pivotal studies were conducted to GLP, as required according to guideline.

Monkeys: An MTD of 4 mg/kg/day was estimated in a non-GLP i.v. dose range finding study in monkeys. However, it should be noted that rather severe clinical signs were still observed at this dose (i.e., lethargy, decreased activity, decrease faeces, reduced food consumption and BW in addition to sporadic occurrence of emesis, ataxia and intermittent tremors). Four pivotal GLP compliant repeat-

dose monkey studies were conducted with IV administration of difelikefalin, a 4-week, a 13-week and two 39-week studies. The two 39-week studies differed only by i.v. administration given as slow bolus or infusion respectively, however, major findings were to a large degree comparable between the studies. Mortality was noted in one female receiving 1 mg/kg/day in the 39-week study. Common for all four monkey studies, was the initiation of transient clinical signs of decreased activity, lethargy, decreased responsiveness, hunched position, sporadic vomiting and reduced food consumption with corresponding decreased BW primarily from dose of 0.25 mg/kg/day. The clinical signs exhibited a dose-dependent increased in incidence, duration and severity, but they were all transient and normalized over 1-3 weeks. This indicated that IV administration of difelikefalin may require some time of habituation and may be associated with discomfort during the first weeks of administration. However, as the findings were transient, they did not affect the determination of the NOAELs.

A dose-dependent decrease in heart rate from dose of ≥0.25 mg/kg/day were noted primarily in female monkeys in the 4-week study. However, since no effect were seen on ECG or at histopathology and no corresponding findings were noted in the 13- and 39-week study at similar dose levels, the issue will not be further pursued.

An effect on ovarian and uterus weight were seen the 4-week monkey study at dose ≥ 0.25 mg/kg/day but not in any of the longer-term studies conducted in monkeys. Similar finding was noted in the 26-week rat study, however, as no corresponding histopathological changes were noted, the relationship to difelikefalin is unclear but a potential link is expected to be further enlightened in the reproductive studies. Conversely to the rat studies, no testicular changes were observed in the monkeys.

A significant decrease in urine volume were seen at dose of 0.06 mg/kg/day in the 13-week and in one of the 39-week monkey studies. It is acknowledged that the urine output normalized during the study. Similar findings were seen in the 13-week rat study and the applicant has provided a discussion, where dehydration due to decreased water consumption was highlighted as the main cause in rats.

Pigment depositions corresponding to hemosiderin in lamina propria of the duodenum and in macrophages in the liver and spleen were sporadically occurring across all groups including the control group in the 4-week and the two 39-week studies. The findings were therefore considered to be a monkey-specific background finding, which was supported by literature. Additionally, presence of lipofuscin pigment in pars recta/pars convoluta of the renal proximal tubules, appeared to show a duration and dose-dependent pattern with occurrence at studies from 13-weeks of duration at doses from 0.25 mg/kg/day. However, the lipofuscin findings were considered non-adverse, as they were not associated with corresponding histopathological lesions, no effect was noted on kidney function and literature was presented in supported of the findings as background lesions.

The lowest NOAELs in the conducted monkey studies were set to 0.25 mg/kg/day in the 4-week and in the 39-weeks studies. In the two remaining studies NOAEL≥1 mg/kg/day. Especially, in the 39-week study determination of NOAEL≥0.25 mg/kg/day was due to a potential difelikefalin-related death at 1 mg/kg/day and clinical observations pronounced enough to require a single day dosing holiday. The NOAELs of 0.25 mg/kg/day ensured a clinical exposure margin of at least 50-fold in the 4-week study and much higher in the 39-week study, which is considered acceptable. All pivotal studies were conducted to GLP, as required according to guideline.

Subcutaneous studies in mice and rats to support CARC: Studies with s.c. administration was conducted in mice and rats. For the current indication of i.v. difelikefalin treatment, the studies are mostly assessed with regards to dose support for the conducted CARC studies and the lack of GLP-compliance for three out of four studies are therefore acceptable. Transient findings of decreased activity, impaired general condition and reduced food consumption with effect on BW were noted even at lower doses (≥1 mg/kg/day in rats and ≥3 mg/kg/day in mice) during the first weeks of administration in both mice and rats and corresponded largely with findings from the i.v. rat studies.

As previously addressed, this confirmed that both s.c. and i.v. administration of difelikefalin may be associated with discomfort during the first weeks of treatment and require some time of habituation. However, as the findings were transient it did not affect the setting of the NOAEL of 25 mg/kg/day in rats and an MTD in mice of 33 mg/kg/day, which is supported. Findings of CPN (i.e. evidenced by tubular basophilia) were also noted at long-term s.c. administration in rats with increasing severity and/or incidence compared with controls at doses of 25 mg/kg/day. CPN is an adverse effect in rats possibly exacerbated by the difelikefalin treatment, however, CPN is considered rat-specific and therefore not relevant to humans.

In conclusion, the s.c. repeat-dose studies supported a maximum dose of 30 mg/kg/day in mice and 1 mg/kg/day in rats for long-term treatment in the conducted CARC studies.

Interspecies comparison: Safety margins was addressed in an extensive interspecies comparison table, comparing exposure (AUC) at NOAEL levels for the majority of non-clinical studies with human clinical exposure. As human patients are dosed at 3 days interval after HD, only human $AUC_{0h\text{-}72h}$ values were obtained. In order to compare animal $AUC_{0h\text{-}24h}$ with human $AUC_{0h\text{-}72h}$ for calculation of safety margins the following equation AUC_{last} x 3/human $AUC_{0h\text{-}72h}$ was applied. This is accepted.

The lowest safety margins based on exposure, observed in the repeat-dose studies in rats and monkeys were 211-fold and 50-fold respectively, which is considered acceptable. Safety margins for the conducted carcinogenicity and reproductive studies are also considered acceptable with the majority of studies being well above the clinical exposure, giving rise to many-fold safety margins. The lowest observed safety margin is in the embryofoetal developmental study in rabbits (5 to 30-fold), which is still considered acceptable.

2.4.4.3. Genotoxicity

The genotoxicity of difelikefalin was studied in accordance with guideline (ICH S2(R1), 2011) and did not show mutagenic or clastogenic potential in Ames test, *in vitro* chromosome aberration test in human PBL cells and *in vivo* bone marrow micronucleus assay in mice. Ames test was conducted with the required bacterial strains with and without metabolic activation (S9). The use of concentration dose up to 5000 mcg/mL in the PBL cell study is accepted according to the old guideline (ICH S2A, 1995), under which the study was conducted. In conclusion all tests were GLP-compliant and considered adequately performed. No evidence for a relevant genotoxic potential of difelikefalin was detected.

2.4.4.4. Carcinogenicity

Two GLP compliant carcinogenicity studies were conducted in accordance with ICH guideline S1B, a 26-weeks transgenic mouse study and a 2-year study in SD rats. Both studies were conducted with s.c. administration, even though the intended clinical route of administration is IV. This was, however, sufficiently addressed by the applicant and considered acceptable, mainly based on the argument that TK assessments indicated that adequate exposure could be achieved by s.c. injection. In the 2-year rat study. The only significant neoplastic finding was an increase of pheochromocytomas in adrenal gland at mid dose in males only. This effect was not dose dependent and did not meet the significance level recommended for common tumours in rats ($P \leq 0.0204$ vs $p \leq 0.01$ for common tumours). Irrelevance of this single dose effect of adrenal pheochromocytoma in males is therefore acceptable. No other significant neoplastic findings were observed. In the medium-term transgenic bioassay in Tg.ras H2 mice no treatment related effects on neoplastic or pre-neoplastic lesion were observed. The positive control exhibited the expected tumorigenic response showing validity of the study.

A higher number of clinical adverse effect were noted in the transgenic mouse study compared to the rat study. However, this is expectable as the maximum dose of 30 mg/kg/day in the mice was close to

the MTD determined in the s.c. repeat-dose studies, whereas the maximum dose in the rat CARC study was significantly lower than the NOAEL of 25 mg/kg/day estimated based on the s.c. repeat-dose studies in rats. As argued by the applicant, doses in the transgenic mouse were based on toxicity endpoints, whereas doses in the rat were based on PK endpoints (i.e., with an exposure multiple of ≥ 25-fold over the exposure at the clinical dose), which is considered acceptable. The clinical signs noted in the transgenic mice were to a large degree comparable with previous observations, as were the transient clinical signs in the rats. Renal lesions of infarcts and/or tubular degeneration was observed to a larger degree in difelikefalin treated mice than in control animals, which potentially could be concerning as the intended difelikefalin patient group already is kidney compromised. However, it is agreed with the applicant that a clear relationship between the renal lesions and difelikefalin-treatment cannot be established in the mice, as the lesions generally were unilateral (not bilateral, as excepted due to systemic effect), without clear dose-response relationship and not associated with evidence of vascular events.

In conclusion, difelikefalin was not carcinogenic in either male or female transgenic mice or SD rats compared with the concurrent control animals.

2.4.4.5. Reproductive and developmental toxicity

Reproduction and development were assessed in a fertility and early embryonic development study in rats, two embryofoetal development study in rats and rabbits, respectively, and a pre- and postnatal development study in rats. The conducted studies covered all stages of the reproductive process from premating to conception through weaning to sexual maturity of the F1 generation. All pivotal studies were conducted in accordance with GLP regulations and the current ICH S5 guideline.

Fertility and early embryonic development: Findings of altered testicular weigh and testicular/epididymal degeneration was noted in the repeat-dose studies in rats. However, in the fertility and early embryonic development study, male fertility was evaluated based on mating performance, sperm assessments (morphology, motility, concentration), reproductive organ weight parameters (epididymides, prostate, seminal vesicles, testes), and histopathology of the testes/epididymides and no changes were noted in any of the parameters. Normal progression of the spermatogenic cycle and the expected cell associations and cell proportions in the various stages of spermatogenesis were present. Similar, for female animals, no effect was noted on fertility, pregnancy indices, or ovarian or uterine parameters. Persistent diestrus were noted, reaching statistical significance at doses ≥ 2.5 mg/kg/day and resulting in a decreased number of estrous cycles per 14 days and an increased in the mean number of days to mating. However, since the days to mating were within historical control and the fertility and pregnancy parameters where comparable across all groups, the relevance of the finding was considered to be of little toxicological concern. Nevertheless, the persistent diestrus was still taken into consideration when determining the NOAEL of 0.25 mg/kg/day for female mating, which is supported.

Clinical signs were transient and corresponded to findings from the repeat-dose studies in rats. As for the repeat-dose studies, a higher incidence and severity of clinical signs were noted in male rats and often at lower doses. The effects of difelikefalin were most prevalent during the first two weeks of the dose period, and in most cases, the clinical signs and effects on body weight gain had resolved before the initiation of mating.

NOAEL for male and female fertility and EED was determined to 25 mg/kg/day, whereas the NOAEL for female mating was reduced to 0.25 mg/kg/day based on a potential effect on diestrus. Clinical exposure margin for fertility were several 1000 folds, whereas it was 15-fold for female mating.

Embryo-foetal development: In accordance with the ICH S5(R3) guideline, EFD studies was conducted in a rodent (rat) and a non-rodent species (rabbit) and difelikefalin was administered by IV injection from implantation to closure of the hard palate (GD7-17 in rats and GD7-19 in rabbits).

In rats, doses of 0.25, 2.5 and 25 mg/kg/day was selected for the pivotal rat EFD study based on preliminary dose-range finding study, which is supported. No evidence of teratogenicity or difelikefalin-related effect on embryofoetal development or maternal reproductive parameters (i.e. effects on ovarian or uterine parameters) were seen at any dose. The observed increased incidence of skeletal variations (i.e. wavy ribs and incomplete ossified ribs) were not considered adverse and did therefore not affect the EFD NOAEL of 25 mg/kg/day, which is in line with the ICH S5(R3) guideline for this type of changes and provides a safety margin to clinical exposure levels of 2133-fold.Clinical signs, corresponding with finding from the repeat-dose studies, were seen in the mother animals at doses of ≥ 0.25 mg/kg/day and calling them transient is slightly misleading, as they occurred almost throughout the dosing period due to the short duration of the study. Thus, no maternal toxicity NOAEL was determined. NOAEL of the maternal reproductive function were determined to 25 mg/kg/day.

In rabbit, doses of 0.025, 0.05, and 0.1 was selected for the pivotal EFD study based on a combined tolerability (1st phase) and dose-range study in pregnant rabbits (2nd phase). The choice of a maximum dose of 0.1 mg/kg/day for the pivotal EFD study is supported, as maternal toxicity and foetal loss was detected at doses of 0.5 mg/kg/day. Nevertheless, in the 0.1 mg/kg/day group in the pivotal EFD study two animals died, respectively, prematurely at GD16 or aborted and was euthanized at GD23, most likely due to difelikefalin-related reduction in food consumption and severe BW loss.

No evidence of teratogenicity or difelikefalin-related effect on embryofoetal development (i.e., survival, foetal body weights, gender distribution, external/soft tissue/skeletal abnormalities or ossification sites) was detected in the rabbit offspring. However, as for the rats, most findings were related to maternal toxicity initiating already at the lowest dose of 0.025 mg/kg/day with decreased BW/BW gain and food consumption, thin body condition and dehydration. The magnitude and persistence of the effect on BW and food consumption prevented the establishment of a NOAEL for maternal toxicity.

Fewer pregnancies were observed at 0.1 mg/kg/day and a test article-related effect is suspected. However, it was speculated that the implantation disruption at 0.1 mg/kg/day could be secondary to the notable maternal toxicity observed at the high dose and not a direct foetal effect. An EFD NOAEL of 0.1 mg/kg/day is therefore supported due to the lack of findings in the offspring and provides a safety margin to clinical exposure of 30-fold. Due to the reduction in pregnancies at 0.1 mg/kg/day but an absence of effect on ovarian or uterine parameters, the NOAEL for maternal reproductive function was determined to be 0.05 mg/kg/day.

The pre- and postnatal developmental study was conducted in accordance with ICH S5(R3) guideline and covered an exposure period with dose of 0.6, 2.5, and 10 mg/kg/day from pregnancy in F0 animals (GD7) through lactation (LD20) and the potential subsequent effect on F1 weaning, mating, implantation and gestation with evaluation of F1 foetuses (F2 animals). Hence, covering the full pre- and postnatal developmental period.

As for the other studies, the most significant finding was clinical signs of transient maternal toxicity with a dose-dependent pattern from doses of ≥0.6 mg/kg/day and a persistent effect on BW/BW gain from dose of ≥2.5 mg/kg/day. NOAEL for F0 maternal toxicity was therefore determined to 0.6 mg/kg/day. A potential effect on increased duration of gestation and number of stillborn pups at ≥2.5 mg/kg/day were dismissed, as it is within the historical control of the facility, did not correspond with EFD findings and were mostly related to a high number of dead pups in a single litter. This explanation is considered acceptable.

No difelikefalin-related effect was seen in F1 animals including reproductive endpoints of the generation and no effect was noted in the F2 fetuses. The NOAEL for pre- and postnatal development were therefore determined to 10 mg/kg/day, which is supported. Data indicative of placental transfer of difelikefalin were noted in addition to evidence that difelikefalin is excreted into milk. Findings of excretion to milk is sufficiently addressed in the SmPC section 4.6.

2.4.4.6. Toxicokinetic data

Toxicokinetics of difelikefalin was investigated in the pharmacokinetic studies, see section 2.4.3.

2.4.4.7. Local Tolerance

Perivascular irritancy of difelikefalin was considered to be slight but reversible, based on data from a SC local tolerance study in rabbits and evaluation of the local tolerance parameters from the conducted toxicity studies. Acute and chronic inflammation with fibroses and vessel necrosis/degeneration were seen in the 28-day i.v. monkey study, however, similar findings were not seen in the longer-term i.v. repeat-dose studies. Stand-alone local tolerance studies are generally not endorsed according to the ICH M3 guideline and the necessity of the rabbit study for the current indication is unclear.

2.4.4.8. Other toxicity studies

Immunotoxicity: Findings in the 4-week i.v. monkey study of thymic and splenic lymphoid depletion suggested a potential immune toxic effect even though similar findings were not detected in longer-term monkey studies. However, based on the assessment of immune system parameters from the 13-week rat and monkey studies, no difelikefalin-related immunotoxicity was expected at clinically relevant doses.

Abuse potential and dependence: Abuse liability studies to study drug discrimination, self-administration and withdrawal were conducted in accordance with ICH M3 guideline, as difelikefalin exhibits activity through the opioid receptor family even though only peripherally. The lack of GLP compliance and the use of rodents for the studies was considered acceptable according to guideline and the selected doses was based on prior PK/toxicology studies in rats in addition to dose range-finding phases in the drug discrimination and self-administration studies.

The conducted studies showed no evidence to support self-administration of difelikefalin with levels similar to saline control, difelikefalin did not induce a place preference and no signs of the withdrawal symptoms, as seen for the positive control, was noted for difelikefalin. The drug discrimination study demonstrated only a weak partial generalization (35% at 0.125 mg/kg) for difelikefalin indicating that difelikefalin did not share discriminative cues with (-)-pentazocine to the same extent as observed for butorphanol. Overall, it was concluded that difelikefalin was not likely to present a risk of physical dependence or a meaningful risk of an abuse potential at clinically relevant exposure, which is supported based on the submitted studies. The results correlated with difelikefalin being a selective KOR agonist with poor CNS penetration. Additionally, the results are also in line with the outcomes of the analysis of AEs from Phase 1 and Phase 3 clinical studies and a dedicated human abuse potential study.

Impurities: No stand-alone toxicity studies have been conducted to qualify impurities and the impurities are therefore more thoroughly addressed in other parts of the report. Most of the impurities do not exceed the qualification limit of ICH Q3A(R2) and do not exceed the threshold of toxicological concern (TTC) of 1.5 mcg/day as set in ICH M7(R1), which is considered acceptable.

2.4.5. Ecotoxicity/environmental risk assessment

An environmental risk assessment (ERA) covering phase I was submitted evaluating the potential environmental risks of difelikefalin (as the acetate) for IV treatment of pruritus associated with chronic kidney disease (CKD-aP) in adult patients undergoing haemodialysis (HD). The log D_{ow} of difelikefalin (as the acetate) were determined experimentally as a function of pH to -2.11 at pH 5.0, -2.12 at pH 7.0 and -1.74 at pH 9.0. The following method was used to determine the log D_{ow} experimentally: Difelikefalin was mixed with octanol and buffered aqueous solution to form a mixture with a top organic solvent layer and a bottom aqueous buffer layer. Upon tumbling at room temperature for 24 hours, an equilibrium of the distribution of difelikefalin between the two layers was reached. The two layers were then separated, and the partition coefficient was determined by means of LC-MS. The calculation of Fpen on a worst-case scenario is supported and it is agreed that a phase II assessment is not triggered based on the $PEC_{SURFACEWATER}$ calculation ($PEC_{SURFACEWATER}$ difelikefalin = 0.00045 µg/L), as the value is below the action limit of 0.01 µg/L. Furthermore, the Applicant has provided an evaluation of repeat-dose and reproductive toxicity studies showing that no evidence of endocrine disruption was seen for difelikefalin, which is supported.

Summary of main study results

Substance (INN/Invented Name): Difelikefalin (as the acetate salt)			
CAS-number (if available): 1024829-44-4			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log Kow	Not according to OECD guideline Nos 123 or 107	pH = 5 -> Log D = -2.11 pH = 7 -> Log D = -2.12 pH = 9 -> Log D = -1.74	Potential PBT: No
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{SURFACEWATER}$, refined with prevalence and treatment regime	0.00045	µg/L	> 0.01 threshold: No
Other concerns (e.g., chemical class)	-	-	None

Difelikefalin (as the acetate) $PEC_{SURFACEWATER}$ value is below the action limit of 0.01 µg/L and is not a PBT substance as log K_{OW} does not exceed 4.5. Therefore, difelikefalin is not expected to pose a risk to the environment.

2.4.6. Discussion on non-clinical aspects

The binding properties, functional activity and anti-inflammatory effects of difelikefalin were adequately characterized in pharmacodynamic *in vitro* and *in vivo* studies. A series of *in vitro* binding assays demonstrated that difelikefalin is a potent and full agonist at human, rat and mouse KORs with a slow receptor off-rate that might contribute to a long duration of activity *in vivo*.

In vitro difelikefalin also displayed anti-inflammatory activity by reducing pro-inflammatory cytokine production (e.g. IL-1, TNF-α, granulocyte colony stimulating factor) by human monocyte-derived macrophages stimulated with lipopolysaccharide and IFN-γ. The anti-inflammatory activity of difelikefalin is relevant to the antipruritic effect because CKD-aP in HD patients comprise an inflammatory component.

The *in vivo* pharmacological activity of difelikefalin was evaluated in several mouse and rat studies. Species in which the drug was pharmacologically active. In addition, a high degree of overall homology for KORs, MORs, DORs and orphanin FQ (or nociception) receptors exists across vertebrate species. *In vivo*, i.v. difelikefalin (0.01 to 0.3 mg/kg) displayed potent, dose-dependent antipruritic effects in chemically induced mouse itch models when mice were dosed 15 minutes prior to administration of the

pruritogens 5'-GNTI (KOR antagonist) and compound 48/80 (mast cell activator). The effective dose of compound at 50% of the ED_{50} value in the 5'GNTI- and compound 48/80-models was 0.05 and 0.08 mg/kg i.v., respectively. Thus, the *in vivo* pharmacology studies also support that difelikefalin effect is mediated by peripheral KOR and suggest a lack of tolerance development.

In vitro studies characterised potential secondary pharmacodynamic activity/binding and the data indicated that difelikefalin is unlikely to exhibit biologically relevant off-target activity. In addition, difelikefalin displayed no activity at the mu opioid receptors and delta opioid receptors in a functional assay.

The safety pharmacology assessment of difelikefalin was carried out in accordance with the ICH S7A and ICH S7B guideline. In general, the effects on the CNS, including decreased locomotor function and abnormal posture and/or gait, occurred at doses that represented large exposure multiples of the maximum observed plasma concentration (C_{max}) in the CKD-aP subjects undergoing HD at the proposed clinical dose of 0.5 µg/kg every 2 to 3 days. However, more relevant exposure multiples based on HED and AUCs were only 5-fold in a Pentobarbital Sleeping Duration study in mice, 13-fold at the LOAEL (lowest dose, 1 mg/kg) in the FOB in rats based on AUCs, which were bridged from a rat repeat-dose toxicity study at 1 mg/kg. Given that effects were already seen at the lowest dose in the FOB and no NOAEL could be determined, safety margins would even be lower at a no effect dose. Studies in rats of Sleep-Wake Cycle resulted in no safety margins based on AUC. These findings are in line with findings in all repeat-dose toxicity studies across species where CNS effects were reported already at the lowest doses. In addition, reported adverse drug reactions (ADRs) in placebo controlled and uncontrolled Phase 3 clinical trials were somnolence (1.1%), paraesthesia (including hypoesthesia, paraesthesia oral and hypoesthesia oral) (1.1%), dizziness (0.9%), headache (0.6%), nausea (0.7%), vomiting (0.7%), mental status changes (including confusional state) (0.3%) and diarrhoea (0.2%). It appears that even at clinically relevant doses, difelikefalin has CNS effects albeit only to a small extent with mild or moderate severity in patients. The data also indicated that difelikefalin represented a negligible risk for any notable adverse effects on the cardiovascular, respiratory and gastrointestinal systems. The SmPC and the RMP have been updated with appropriate warnings and measures to monitor these by the healthcare professionals and minimise the impact on patients.

A pharmacodynamic drug-drug interaction study was conducted to investigate the potential additive or synergistic effect on locomotor activity when difelikefalin was co-administered with the antihistamine, ketotifen. At a dose of 0.6 mg/kg, difelikefalin appeared to significantly potentiate the 30 mg/kg ketotifen-related decrease in locomotor activity. It is stated that these effects occur at high exposure multiples to the MRHD. However, in the pooled data of two well-controlled Phase 3 studies, subjects who used an antihistamine prior anti-itch medication (most common drugs used were antihistamines diphenhydramine and hydroxyzine) showed a higher risk of somnolence indicating that there is a drug-drug interaction. This is adequately reflected in the SmPC.

Toxicokinetics from pivotal toxicology studies were carried out in accordance with GLP consistent with the guideline S3A. In general, data in the intravenous repeat-dose studies in rats and monkeys demonstrated that exposure for both C_{max} and AUC was roughly dose-proportional to slightly greater than dose-proportional, independent of sex and with a short half-life. Accumulation was generally not observed in rats, but moderate accumulation was observed in male and female monkeys. Following an i.v. injection, difelikefalin was relatively quickly distributed to tissues; highly distributed to the kidney, especially the renal cortex; and minimally distributed to the central nervous system in brain regions protected by the blood brain barrier. It was concluded that there were no gender-related differences in distribution of difelikefalin in rats and monkeys. Overall, the metabolism of difelikefalin was negligible *in vitro* and in rats and monkeys with no major metabolites identified, which is consistent with data from a human ADME study in healthy and HD subjects using [^{14}C]-difelikefalin. The main route of

excretion for difelikefalin was *via* the urine (57 to 74%) with lesser amounts found in the bile (13%) and faeces (13 to 37%) in rats and monkeys.

In the i.v. repeat-dose studies in rats, the lowest NOAEL were determined to be 2.5 mg/kg/day in the 26-week study due to degenerative changes in testis/epididymis of in male rats. A general NOAEL of 25 mg/kg/day existed for female rats across all three studies. However, this still ensured an acceptable safety margin of 221-fold to clinical exposure for the lowest NOAEL. In monkeys, the lowest NOAELs were set to 0.25 mg/kg/day based on the 4-week and 39-weeks study and this ensured an acceptable clinical exposure margin of at least 50-fold for the 4-week study and much higher for the 39-week study. Transient signs of decrease activity, lethargy, decreased responsiveness and reduced food consumption with corresponding effect on BW gain were noted across species during the first weeks of difelikefalin administration at all doses, especially in male animals. However, as the findings were transient, they did not affect the setting of the NOAELs but indicated that a habituation period may exist for the treatment. Reassuringly, repeat-dose studies conducted with s.c. administration in mice and rats supported the dose selection for CARC studies and similar findings as for i.v. administration was noted. No evidence for a relevant genotoxic potential of difelikefalin was detected and no evidence for tumorigenic potential of difelikefalin was identified.

No effect was seen on fertility, early embryonic development, teratogenicity in rats or rabbits, embryofetal development in rats or pre- and postnatal development. Maternal toxicity consistent with the transient findings from the conducted repeat-dose studies were detected at most doses in all studies. Local tolerance studies in rabbits showed a slight but reversible perivascular irritancy to difelikefalin. Furthermore, it was concluded that difelikefalin was not likely to present a risk of physical dependence or a meaningful risk of an abuse potential at clinically relevant exposure, which is endorsed by the CHMP. No concern regarding immunotoxicity, phototoxicity or impurities were identified.

Difelikefalin PEC $_{surfacewater}$ value is below the action limit of 0.01 μg/L. and is not a PBT substance as log K_{ow} does not exceed 4.5. Therefore, difelikefalin is not expected to pose a risk to the environment.

2.4.7. Conclusion on the non-clinical aspects

Overall, *in vitro* and *in vivo* primary pharmacodynamic studies of difelikefalin demonstrated proof of concept and mode of action. Both the role of peripheral KORs in mitigating itch and inflammation, but also the effect of difelikefalin at these receptors was shown. Secondary pharmacodynamic studies indicated that difelikefalin displayed no off-target activity. From the safety pharmacology studies, no safety concern was identified. In general, the effects on the CNS occurred at doses that represent large exposure multiples of the C_{max} in HD subjects with CKD-aP at the proposed maximum dose of 0.5 μg/kg every 2 to 3 days. No pharmacodynamic drug interactions were expected. The pharmacodynamic and safety profile of difelikefalin was characterised in mice, rats, or monkeys, species in which the drug was pharmacologically active. In addition, there was a high degree of overall homology for KORs, MORs, DORs and orphanin FQ receptors across vertebrate species.

The toxicokinetic data by intravenous route in rats and monkeys demonstrated that exposure for both C_{max} and AUC was roughly dose-proportional to slightly greater than dose-proportional, independent of sex and with a short half-life. Accumulation was generally not observed in rats, but moderate accumulation was observed in male and female monkeys. Protein binding was low across all species. Distribution of difelikefalin to the CNS protected by the BBB was negligible in rats and monkeys with no increase in distribution over time. Metabolism of difelikefalin was negligible in animals with no major metabolites identified, consistent with data from a human ADME study in healthy and HD subjects

using [^{14}C]-difelikefalin; the major route of elimination in animals was *via* urinary excretion. A battery of *in vitro* assays also suggested that clinically relevant drug interactions with difelikefalin was unlikely.

Overall, the toxicology programme revealed that difelikefalin was well tolerated in relevant animal species and no major concerns was identified and the agreed SmPC adequately reflects the non-clinical profile of this medicinal product. Difelikefalin is not expected to pose a risk to the environment.

2.5. Clinical aspects

2.5.1. Introduction

GCP aspects

The clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Tabular overview of clinical studies

Study	Phase	Country	Abbreviated Study Description	Study Design	Dosing Regimen	Study Population	First Subject/ Patient, First Visit	Duration	Number of Subjects
Section 5.3.3.1 - Healthy subject PK and initial tolerability Study reports and related information									
CR845-CLIN1004	1	US	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	01-JUL-2010	24 hours	28 CR845 14 placebo
Section 5.3.3.2 - Patient PK and initial tolerability Study reports and related information									
CR845-CLIN1003	1	US	Safety/PK in patients with ESRD on haemodialysis	Single-center, randomized, DB, PC ascending single-dose	1, 3, and 6 mcg/kg (15-minute IV infusion)	Patients with ESRD on haemodialysis	15-OCT-2009	Single dose	18 CR845 6 placebo
CR845-CLIN1005	1	US	Safety/PK in healthy volunteers and patients with 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	09-AUG-2013	Single dose	24 renally-compromised 12 healthy volunteers
PR-13A9-P1-B	1	Japan	Safety/PK in haemodialysis 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)	Haemodialysis patients	27-AUG-2015	1 week	14 CR845 5 placebo
CR845-100302	1	US	PK and Metabolism of [^{14}C]-CR845 in healthy volunteers and haemodialysis patients	Single-center, open-label, single-dose	230 mcg CR845 solution containing 100 microcuries [^{14}C]-CR845, IV bolus (the total dose of CR845 ranged from 1.7 to 3.0 mcg/kg)	Healthy volunteers and patients with ESRD on haemodialysis	28-Dec-2018	Single dose	12 CR845

Study	Phase	Country	Abbreviated Study Description	Study Design	Dosing Regimen	Study Population	First Subject/ Patient, First Visit	Duration	Number of Subjects
Section 5.3.4.1 - Healthy subject PD and PK/PD Study reports and related information									
CR845-CLIN1001	1	UK	Safety/PK/PD (biomarkers) in healthy volunteers	Single-center, randomized, DB, PC, ascending single-dose	2 to 40 mcg/kg (15-minute IV infusion)	Healthy volunteers	07-APR-2008	Single dose	Part 1: 31 CR845 16 placebo Part 2: 6 CR845 1 placebo
CR845-CLIN1006	1	US	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	29-JUL-2014	Single dose	44
CR845-CLIN1009	1	Canada	Respiratory effects of CR845 IV	Single-center, randomized, DB, PC, 3-way crossover	1 and 5 mcg/kg single IV bolus dose	Healthy volunteers	06-FEB-2017	Single dose	15
PR-13A9-P1-A	1	Japan	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	27-OCT-2013	Step 1: single dose Step 2: 21 hours	Step 1: 36 CR845 12 placebo Step 2: 30 CR845 10 placebo
CR845-100201	1	US	Effect difelikefalin (CR845) on the QTc interval	Single-center, randomized, DB, PC, positive-controlled, four-way crossover study	0.5 mcg/kg IV as a bolus injection (therapeutic dose) and 3 mcg/kg IV as a bolus injection (supratherapeutic dose)	Healthy volunteers	20-JUN-2019	Single dose	58

Study	Phase	Country	Abbreviated Study Description	Study Design	Dosing Regimen	Study Population	First Subject/ Patient, First Visit	Duration	Number of Subjects
Section 5.3.4.2 - Patient PD and PK/PD Study reports and related information									
CR845-100303	1	US	Relative physical withdrawal potential in patients on haemodialysis	Open-label treatment phase (3 weeks) with CR845 DB, PC randomized withdrawal phase (2 weeks)	0.5 mcg/kg IV bolus 3 times a week after each dialysis session	Haemodialysis patients	31-DEC-2019	3 weeks	Open label: 35 CR845 Double blind: 16 CR845 14 placebo
Section 5.3.5.1 - Study reports and related information of controlled clinical studies pertinent to the claimed indication									
CR845-CLIN2005	2	US	Safety, PK, and efficacy in haemodialysis 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 haemodialysis Part B: haemodialysis patients with moderate-to-severe CKD-aP	21-JUL-2014 (2005A) 21-NOV-2014 (2005B)	2 weeks	Part A: 19 CR845 5 placebo Part B: 33 CR845 32 placebo
CR845-CLIN2101	2	US	Efficacy and safety in haemodialysis 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	Haemodialysis patients with moderate-to-severe CKD-aP	13-JUL-2016	8 weeks	129 CR845 45 placebo
PR-13A9-P2-A	2	Japan	Efficacy and safety in haemodialysis patients with pruritus	Multicenter, randomized, DB, PC, parallel-group	0.25, 0.5, 1.0, and 1.5 mcg/kg IV bolus 3 times a week for 2 weeks	Haemodialysis patients with moderate-to-severe CKD-aP	03-AUG-2016	2 weeks	84 CR845 21 placebo
CR845-CLIN3102 DB	3	US	Safety and efficacy in haemodialysis patients with pruritus	Multicenter, randomized, DB, PC with an open-label extension	0.5 mcg/kg IV bolus 3 times a week for 12 weeks	Haemodialysis patients with moderate-to-severe CKD-aP	06-FEB-2018	12 weeks	189 CR845 188 placebo
CR845-CLIN3103 DB	3	Global	Safety and efficacy in haemodialysis patients with pruritus	Multicenter, randomized, DB, PC with an open-label extension	0.5 mcg/kg IV bolus 3 times a week for 12 weeks	Haemodialysis patients with moderate-to-severe CKD-aP	20-JUL-2018	12 weeks	235 CR845 236 placebo

2.5.2. Clinical pharmacology

2.5.2.1. Pharmacokinetics

Bioanalytical methods utilising LC-MS/MS analysis were validated to quantify the concentration of difelikefalin in K2EDTA plasma and in urine. Sample analysis were conducted at multiple analytical sites. The percentage of below limit of quantification (BLQ) samples in the oral studies were rather high and most BLQ samples occurred in the elimination phase. The PK parameters for difelikefalin were estimated using non-compartmental methods with WinNonlin. Pop PK analyses were conducted via nonlinear mixed effects modelling in NONMEM. A population PK data set was developed from the pooled data across difelikefalin studies with available PK information including oral and IV administration. The full analysis dataset was comprised of 811 subjects, contributing a total of 22,332 observations. Of these, 3,488 samples were BLQ (15.6%). The BLQ samples were mainly from the oral studies (83%). Population and individual model parameters were estimated using stochastic approximation expectation maximization followed by Monte Carlo importance sampling. The final model parameters were re-estimated using Bayesian methods run across four chains, each with up to 5000 burn-in iterations followed by 10,000 post-burn-in iterations. Parameter estimates were summarized across all four chains. A three-compartment population PK model with a first order rate of elimination could describe the exposure of difelikefalin i.v. data. Residual random effects were described by a proportional error model. Fixed effects of body weight were included on all CL and V terms using the typical values of 0.75 and 1, respectively. A full covariate model was constructed and included effects of age, AST and BILI on CL, and of MDRD on CL and V.

The typical patient was 47 years old, weighed 78 kg, had an MDRD value of 83.502, a BILI value of 8.552 and an AST value of 19.

Absorption/distribution/metabolism/elimination: The applicant conducted a mass balance study to investigate the ADME characteristics of difelikefalin in healthy and HD subjects. Difelikefalin was eliminated primarily in the urine in healthy volunteers (80.5%) and primarily in faeces and dialysate, 58.8 % and 19.5 % respectively, in HD subjects. As expected, the Cmax was similar, but AUC increased for HD subjects compared with healthy subjects. Metabolism appears to be minimal as the most prevalent metabolite (MP1) accounted for 0.48% and 0.10% of total plasma exposure in healthy volunteers and HD subjects respectively.

Plasma protein binding was evaluated in two clinical studies: in subjects with normal renal function and subjects with mild, moderate and severe impairment of renal function, and in subjects on haemodialysis. Plasma protein binding of difelikefalin ranges from 24 to 32 % and remains unaffected by renal impairment. Mean volume of distribution at steady state ranged from 145 to 189 mL/kg in healthy subjects and from 214 to 301 mL/kg in HD patients.

The primary route of elimination for difelikefalin in the target population, HD patients, is faecal, accounting on average for about 59 % of the dose; about 19 % were recovered in dialysate; and about 11 % were found in urine. In healthy subjects, the primary route of elimination for difelikefalin is renal, accounting for about 81 % of the dose being excreted in urine as compared to 11 % via faecal excretion. In HD patients mean total clearance decreased and half-lives increased about 10-fold with ranges of 5.3 to 7.5 mL/h/kg and 23 to 31 hours, respectively, compared to healthy subjects (54 to 71 mL/h/kg and mean half-lives from 2 to 3 hours). Difelikefalin is not metabolized in neither HD nor subjects with normal kidney function.

Dose proportionality and time dependency: There was a proportionate increase in PK exposure between the 8.0 mcg/kg and 24 mcg/kg difelikefalin dosages in female subjects when administered the day following elective laparoscopic-assisted hysterectomy. Dose proportionality was also established

across the dose range studied (0.5 to 2.5 mcg/kg) in haemodialysis patients on both day 1 and at steady state. PK analysis from two phase 2 studies in the target population showed steady-state concentrations were generally observed 44 hours after the first dose. Accumulation of difelikefalin with respect to AUC values was low; mean relative accumulation (RA) values varied between 1.08 and 1.33 for the 3 dose levels. The mean RA for Week 8 Cmax was also low and varied between 0.873 and 1.28 across all doses.

Minimal accumulation was observed (mean accumulation ratios: 1.0 to 1.2) in the target population. After an initial distribution phase, plasma concentrations of difelikefalin in HD subjects decrease slowly until mostly cleared during HD. Data on accumulation is reported from all 3 studies with repeated administration in HD subjects. Although AUC accumulation is mild, there is a great variability of 50 to 70% in this parameter among patients. While the usual dosing regimen includes 3 doses per week, the applicant enables to use one extra dose if 4th haemodialysis during a week is performed. Based on the dossier review, it however seems that PK data for such a situation are not available.

Variability: The intraindividual variability data shown from the Pop-PK model were low with 18.2%. The interindividual variability in healthy volunteer's single dose studies was low, with most coefficient of variation values <20% for all parameters across all doses. interindividual variability data are shown from the Pop Pk model and mostly explained by few covariates. The available data on interindividual variability suggest that it is usually high (above 30%) in HD subjects at 0.5 mcg/kg. For example, in the study 2101 the CV% was at week 8 around 40% for AUC, 76% for T_{half} and 76% for Cmax. However, the number of subjects in two PK evaluations was low (7 and 9 subjects) which could increase the variability itself.

Pharmacokinetics in target population

In study 1003 the mean CL values of difelikefalin in HD subjects (8 to 16 mL/min) were significantly less than in normal subjects resulting in a t½ that was ~10-fold longer (17.9 to 24.3 hours) with plasma exposures that were ~3- to 6-fold higher than those established at comparable doses in normal subjects. Dialysis clearance was similar across the 3 and 6 mcg/kg dose groups and was substantially higher than plasma clearance (128 and 143 mL/min, respectively). The target population for difelikefalin i.v. formulation is subjects with CKD undergoing HD. The PK properties of difelikefalin were assessed in subjects with normal renal function as well as those with CKD who require HD. There were 9 clinical studies where the PK of difelikefalin was evaluated in subjects with normal renal function, seven studies were conducted in the HD patient population and one study evaluated the PK of difelikefalin in subjects with normal renal function as well as mild, moderate and severe renal impairment. Difelikefalin was substantially, but incompletely cleared from plasma by a 4-hour dialysis session. On average, a 4-hour dialysis session resulted in a 79% (CV=4.15%) reduction in plasma difelikefalin concentration. Mean t½ following oral and IV dosing was 26.6 and 22.9 hours, respectively. The mean (SD) $C_{extracted\%}$ was 79% (3.26). Individual reduction values ranged from 70.3 to 82.1%. The mean (90% CI) absolute bioavailability of difelikefalin was 0.085 (0.044, 0.160).

Special populations

PK in special populations has been investigated in patients with severe renal impairment and Japanese patients. The Pop-PK model was used to describe PK in special populations further. No other special population studies were conducted. Hepatic function has little impact on the PK of difelikefalin since it is essentially not metabolized. There are no standalone studies evaluating i.v. difelikefalin in hepatically impaired patients. However, the population PK model indicated that difelikefalin exposure in subjects with mild and moderate hepatic impairment was similar to those with normal hepatic function. Therefore, no dose adjustments are recommended for HD subjects with mild or moderate hepatic impairment. Because insufficient PK data are available for HD subjects with severe hepatic impairment,

PK and any dose adjustments in this patient population could not be assessed or predicted. The SmPC is adequately updated.

The posology for difelikefalin is weight-based and any impact of weight therefore already considered. It is planned to market a 1 mL vial in EU. With the weight-based dosing, subjects above 104 kg dry body weight will need two vials. Since weight has not been included as a covariate in the PopPK model, the impact on weight on exposure is unknown. The PK development programme encompasses weights from 40 to 129 kg in i.v. studies.

Pharmacokinetic interaction studies

Results of *in vitro* studies indicate that difelikefalin is metabolically stable and is not a significant inhibitor or substrate for clinically relevant enzymes and transporters. No drug-drug interaction studies have been performed. Because difelikefalins major clearance pathway is excretion of the unchanged parent compound into urine dialysate, and/or faeces and difelikefalin is not a substrate/inhibitor/enhancer of any CYP enzymes or transporter proteins, it should not significantly affect the clearance and/or metabolism of any co -administered drug and the clearance and/or metabolism should not be impacted by the co-administration of other drugs.

Pharmacokinetics using human biomaterials

Not applicable.

2.5.2.2. Pharmacodynamics

Mechanism of action

Difelikefalin is a selective KOR agonist with no activity at mu and delta-opioid receptors, and no off-target activity detected at a comprehensive panel of >120 additional receptors, ion channels, or transporters, as demonstrated by binding and/or functional assays. Difelikefalin has been shown to have minimal to no penetration into the CNS in non-clinical studies.

Primary and Secondary pharmacology

A phase 2 proof of concept study 2005 showed statistically significant reduction in itch intensity as measured by the change from baseline to the average of Week 2 worst itching scores (P=.02) and improvement in itch-related quality of life (QoL) (based on the Skindex-10 scale) (P=.03) with difelikefalin treatment in HD subjects with moderate-to-severe pruritus which supports the claimed mode of action.

Difelikefalin had aquaretic effects in healthy male subjects, with a statistically significant increase in the volume of urine excreted over the 12 hours after dosing for all doses of the substance in comparison to placebo, with the exception of the lowest dose of 0.002 mg/kg of difelikefalin. The increase in urine output did not appear to be associated with a decrease in vasopressin levels, increased urine osmolality or increased electrolyte excretion. Thus, this change is of no concern in the target population with little to no kidney function.

In a phase 1 study in healthy subjects difelikefalin caused a rapid and marked increase in serum prolactin concentrations. Prolactin levels returned to normal within 12 to 24 hours and were of no clinical concern. No TEAEs potentially indicative of the effects of hyperprolactinemia were reported. In phase 2 study PR-13A9-P2-A in subjects undergoing HD and experiencing CKD-aP, a dose-dependent drop in levels of thyroid-stimulating hormone was observed on the fourth dialysis day of the administration period in the dose groups 1 and 1.5 µg/kg, however this decrease had resolved at the end of the observation period. No changes were seen in the 0.5 µg/kg group. Only few thyroid related

AEs were reported and there is no concern regarding the impact of difelikefalin on the hypothalamic-pituitary-thyroid axis.

A 4-way crossover study assessing the abuse potential of difelikefalin in healthy, recreational polydrug users with lifetime and recent hallucinogenic use, showed that difelikefalin produced some "drug liking" compared to placebo, however significantly lower Drug Liking VAS Emax, with no evidence of a dose-relation when compared to pentazocine (a C-IV KOR agonist/MOR partial agonist).

A randomised, double-blind, placebo-controlled study in subjects undergoing haemodialyis was conducted to assess the potential of physical withdrawal from difelikefalin. Patients 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. Data of the randomised, double-blind, placebo and positive-controlled, four-way crossover study to assess the effects of CR845 at therapeutic and supratherapeutic doses on cardiac repolarization (QTc) following single IV dose administration relative to placebo in healthy adult male and female subjects showed no clinically significant effect of CR845 on heart rate, PR interval, or QRS duration. The largest placebo corrected change from baseline for heart rate was 8.0 bpm, occurring in the 3.0 mcg/kg treatment group 4 hours following dosing.

Dose-response relationship: Dose-response relationship was investigated a randomised, double-blind, placebo-controlled study where 3 dose levels of difelikefalin (1.5, 1.0, and 0.5 mcg/kg) were evaluated relative to placebo in HD subjects with moderate-to-severe pruritus. The systemic exposure of difelikefalin increased with dose, however dose proportionality over the 0.5 to 1.5 mcg/kg dose range could not be concluded. Both, the Cmax and AUC for 0.5 and 1.0 mcg/kg doses were proportional, but both parameters for the 1.5 mcg/kg dose were lower than expected at the highest dose. The reason for this behavior and difference from historical data is unknown.

2.5.3. Discussion on clinical pharmacology

The pharmacokinetic properties of difelikefalin were studied in a multitude of studies described in the dossier. Overall, the studies are considered appropriately designed and conducted, especially PK data from the target population was collected which is endorsed. The study populations contain different ages, sex, weight, race and are considered in whole representative for the population to be treated as well as adequate for exploring the pharmacokinetic properties of difelikefalin. Difelikefalin penetration into the central nervous system has been studied in the non-clinical setting and is assumed to be very limited and of no clinical concern. However, CNS-related adverse events like mental status changes, dizziness and somnolence are observed and the relevant information has been included in the SmPC for the healthcare professional treating the patients.

Mild to moderate hepatic impairment seemed not to have any impact on the PK of difelikefalin. It is therefore acceptable that the SmPC does not provide dose recommendations for the mild to moderate hepatically impaired. It is acknowledged that difelikefalin is not metabolized, but there is no clinical data on the pharmacokinetics of difelikefalin in HD subjects with severe hepatic impairment and this is reflected in the SmPC including relevant recommendation for prescribers. A fixed effect of weight was used in the Pop PK model. The posology for difelikefalin is weight-based and any impact of weight therefore already considered.

The PK development programme encompasses weights from 40 to 129 kg in IV studies. The applicant provides dosing recommendations also for patients whose weight is above 129 kg. Weight has been included as a covariate in the POP PK model through allometrically scaled model parameters. This approach might be appropriate for dose determination in obese patients, especially considering physical and PK characteristics of difelikefalin. Dose-capping was implemented by the applicant at 200

kg in order to prevent excessive exposure in obese patients. The applicant showed with simulation that this approach leads to exposures comparable with non-obese patients. The pop PK showed no effect of subject race or gender on difelikefalin exposure. Furthermore, the pharmacokinetics of difelikefalin is not altered in the elderly. The investigated patients ranged from 25 to 80 years of age and thus, the population is considered appropriate to conclude that no dose adjustment is necessary for the elderly. There are no data available in paediatric subjects. The date for completion of the PIP is March 2026. The lack of information on the paediatric population is reflected in the SmPC.

The applicant discussed the potential for abuse comprehensively. The results from the abuse liability study in humans showed that increasing the dose of difelikefalin to 15 µg/kg did not increase the small drug liking signal, whereas drugs of abuse usually show strong dose-response functions. Along with not producing physical dependence and the fact that difelikefalin is administered by hospital staff after dialysis only, the conclusion that it does not hold abuse potential is accepted.

For patients with intact blood-brain-barrier the potential for pharmacodynamic interactions with other medicinal products or substances is considered to be very low. However, concerns have been raised regarding the observed CNS symptoms in the target population. Relevant wording has been added in section 4.4. of the SmPC. Although AUC accumulation is mild, there is a great variability of 50 to 70% in this parameter among patients. A statement on variability has been added in Section 5.2 of the SmPC: "The available data on interindividual variability in haemodialysis subjects receiving 0.5 microgram/kg difelikefalin suggest that variability of AUC can exceed 30%."

The selection of a difelikefalin dose of 0.5 mcg/kg for the Phase 3 studies seems meaningful as the efficacy seen across dose groups was similar and dose-response trends observed in the safety results achieved the most favourable benefit-risk profile. Doses greater than 0.5 mcg/kg did not appear to provide any additional treatment benefit and the selection of the minimally effective dose is endorsed.

2.5.4. Conclusions on clinical pharmacology

The clinical pharmacology testing concerning PK, PD effects of difelikefalin is sufficient and results are adequately reflected in the product information and in the agreed RMP. The CHMP did not considers any measures necessary to address the issues related to pharmacology in the post-marketing phase.

2.5.5. Clinical efficacy

Difelikefalin full clinical development programme, consisted of 18 completed clinical studies. The efficacy evaluation focuses on results of the 2 pivotal Phase 3 clinical studies, CR845-CLIN3102 and CR845-CLIN3103, which evaluated the efficacy of difelikefalin *versus* placebo in double-blind treatment periods of 12 weeks duration (see table below). Study CR845-CLIN3102 was conducted in the US while CR845-CLIN3103 was a global study with sites in the US, Europe, and Asia-Pacific.

Summary of Completed Difelikefalin Clinical Efficacy and Safety Studies in CKD-aP

Study Number (Objective)	Study Sites/ Location(s)	Study Start/End Total Enrollment/ Enrollment Goal	Design	Study Drug Regimen	Subjects Entered/ Completed (by Study Arm)	Duration	Gender Median Age (Range)	Diagnosis Inclusion Criteria	Primary Objectives/ Endpoints
CR845-CLIN2005 (Safety, PK, and efficacy)	**Part A:** 1 site, US	**Part A:** Jul 2014 – Sept 2014 24/24	Randomized, Double Blind	**Part A:** IV bolus 3x/week 0.5 mcg/kg 1 mcg/kg 2.5 mcg/kg Placebo	**Part A:** 7/7 7/7 5/5 5/5	2 weeks	**Part A:** 17/7 47 (37-66)	**Part A:** Patients undergoing HD	Change from baseline to the average of Week 2 worst itching
	Part B: 19 sites, US	**Part B:** Dec 2014 – Jul 2015 65/60		**Part B:** IV bolus 3x/week 1 mcg/kg Placebo	**Part B:** 33/32 32/30		**Part B:** 33/32 60 (26-88)	**Part B:** HD patients with moderate-to-severe CKD-aP	
CR845-CLIN2101 (Efficacy and safety)	33 sites, US	Jul 2016 - March 2017 226/160	Randomized, Double Blind	IV bolus 3x/week 0.5 mcg/kg 1.0 mcg/kg 1.5 mcg/kg Placebo	44/39 41/35 44/33 45/42	8 weeks	105/69 58.5 (26-84)	HD patients with moderate-to-severe CKD-aP	Change from baseline to the weekly mean of the daily 24h WI-NRS score
PR-13A9-P2-A (Efficacy and safety)	45 sites, Japan	Aug 2016 - Oct 2017 121/100	Randomized, Double Blind Placebo	IV bolus 3x/week 0.25 mcg/kg 0.5 mcg/kg 1.0 mcg/kg 1.5 mcg/kg Placebo	21/19 21/16 20/19 23/14 21/21	2 weeks	71/34	HD patients with moderate-to-severe CKD-aP	VAS variation
CR845-CLIN3102 DB (Safety and efficacy)	56 sites, US	Feb 2018 – Apr 2019 503/350	Randomized, Double Blind	IV bolus 3x/week 0.5 mcg/kg Placebo	189/162 188/170	12 weeks	230/147 58 (22-88)	HD patients with moderate-to-severe CKD-aP	Proportion of subjects achieving a >3-point improvement from baseline
CR845-CLIN3103 DB (Safety and efficacy)	75 sites, North America, Europe, Asia-Pacific	Jul 2018 – Feb 2020 620/350	Randomized, Double Blind	IV bolus 3x/week 0.5 mcg/kg Placebo	235/206 236/223	12 weeks	274/197 60 (23-88)	HD patients with moderate-to-severe CKD-aP	Proportion of subjects achieving a >3-point improvement from baseline
CR845-CLIN3105 (Safety and efficacy)	43 sites, Eastern Europe, US	Apr 2019 - Mar 2020 286/400	Open-label	IV bolus 3x/week 0.5 mcg/kg	222/197	12 weeks	121/101 59 (22-85)	HD patients with moderate-to-severe CKD-aP	Evaluation of safety and efficacy
CR845-CLIN3102 OLE (Safety and efficacy)	54 sites, US	May 2018 - Mar 2020 378/not defined	Open-label	IV bolus 3x/week 0.5 mcg/kg	151[1]/94 162[2]/95	Up to 52 weeks	189/124 58 (22-88)	HD patients with moderate-to-severe CKD-aP	Maintenance of the effect of difelikefalin on itch
CR845-CLIN3103 OLE (Safety and efficacy)	71 sites, North America, Europe, Asia-Pacific	Nov 2018 - Mar 2020 473/not defined	Open-label	IV bolus 3x/week 0.5 mcg/kg	189[1]/2 210[2]/3	Up to 52 weeks	234/165 60 (23-87)	HD patients with moderate-to-severe CKD-aP	Maintenance of the effect of difelikefalin on itch

[1] Number of subjects randomized to CR845 group during DB phase/received CR845 during OLE phase.
[2] Number of subjects randomized to placebo group during DB phase/received CR845 during OLE phase.
CKD-aP = chronic kidney disease-associated pruritus; DB = Double blind; HD = haemodialysis; IV = intravenous or intravenously; OLE = Open-label Extension; PK = Pharmacokinetics; US = United States; VAS = visual analogue scale; WI-NRS = Worst Itching-Numerical Rating Scale.

2.5.5.1. Dose response studies

In the supportive dose-ranging study CR845-CLIN2101, which examined the efficacy of 3 doses of difelikefalin (0.5, 1.0, and 1.5 mcg/kg), IV treatment with difelikefalin after each HD session (3 times/week) over an 8-week period resulted in a significant reduction in itch intensity in HD subjects with moderate-to-severe pruritus, which was further supported by significant improvement in multiple itch-related QoL measurements (e.g., sleep, mood, and social functioning) compared with placebo. This study included a post-hoc evaluation of the percentage of subjects achieving a ≥3 or ≥4-point improvement in the WI-NRS. The percentage of subjects with ≥3-point improvement in the weekly mean WI-NRS score by Week 8 was significantly higher for the 0.5 mcg/kg (62.4%, P=.003) dose group compared with placebo (29.5%). The percentage of subjects with ≥4-point improvement in the weekly mean WI-NRS score by Week 8 was significantly higher for the 0.5 mcg/kg (48%, P=.019)

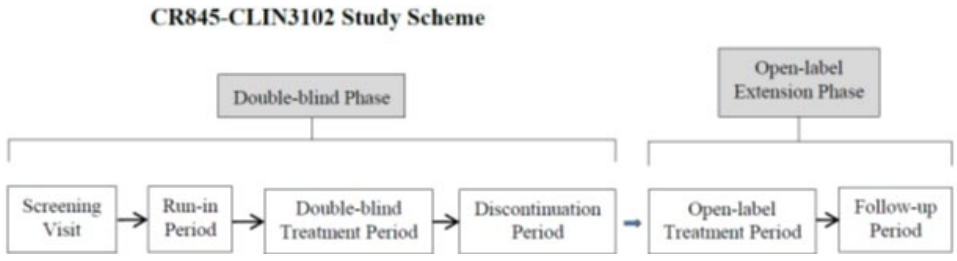
difelikefalin group. No apparent dose-response in antipruritic efficacy was observed among the 3 doses (0.5, 1.0, and 1.5 mcg/kg) studied in CR845-CLIN2101. Based on the similar efficacy across dose groups and dose-response trends observed in the safety results, a difelikefalin dose of 0.5 mcg/kg appeared to achieve the most favourable benefit-risk profile and was thus selected as the dose to be further evaluated in the phase 3 studies.

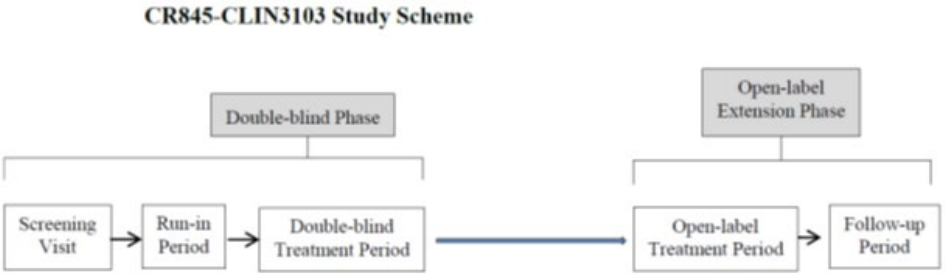
2.5.5.2. Main studies

Title of study: CR845-CLIN3102 and CR845-CLIN3103: A Multicentre, Double-Blind, Randomised, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Intravenous CR845 in Haemodialysis Patients with Moderate-to-Severe Pruritus, with a 52-Week Open-Label Extension

Methods

Studies CR845-CLIN3102 and CR845-CLIN3103 were Phase 3, multicentre, randomised, 12-week double-blind, placebo-controlled studies to evaluate the safety and efficacy of difelikefalin at a dose of 0.5 mcg/kg. Difelikefalin was administered after each HD session 3 times a week in subjects with moderate-to-severe pruritus (due to end stage renal disease) who were undergoing HD. Both studies included a double-blind phase and an OLE phase (see figure below). During the double-blind phase in both studies, an interim analysis was conducted in order to adjust for final sample size.





The eligible subjects at the end of the 7-day Run-in Period were randomized in a 1:1 ratio to receive either difelikefalin 0.5 mcg/kg or placebo in the double-blind treatment period. Subjects were stratified according to their use or non-use of concomitant medications to treat pruritus during the week prior to randomization (Run-in Period), as well as the presence or absence of specific medical conditions (e.g., falls/fractures due to falls, gait disturbance, and mental status change).

Interim Analysis: During the double-blind phase an interim analysis was planned to be conducted when approximately 50% of the planned sample size was randomised. The interim analysis was performed by an independent data monitoring committee with a prespecified charter. The IDMC was to

recommend extending, or maintain, final sample size dependent on the conditional power obtained from an unblinded interim analysis and a set of predefined ranges of the conditional power. This together with masked descriptive and comparative results, i.e., actual name of treatment group masked, was provided to the Applicant.

Open-label Extension Phase: Subjects who received at least 30 doses of study drug (either placebo or active) during the 12-week Double-blind Treatment Period and continued to meet other eligibility criteria had the option to receive open-label difelikefalin for an additional period of up to 52 weeks. Each subject received difelikefalin at a dose of 0.5 mcg/kg after each HD session, 3 times per week, regardless of whether he/she had been previously administered placebo or difelikefalin. Whenever an extra dialysis was performed, the study drug was administered after each additional dialysis session up to a maximum of 4 times per week.

Follow-up Period: For all subjects in both studies, a follow-up visit of 7 to 10 days was planned after the End-of-Treatment/Early Termination Visit.

Study Participants

Inclusion criteria: The eligible subjects were male or female, 18 years of age or older, with end-stage renal disease (ESRD) and had been undergoing HD 3 times per week for at least 3 months prior to the start of screening, had a prescription dry body weight between 40.0 and 135.0 kg inclusive, had at least 2 single-pool measurements ≥1.2 for [dialyzer clearance of urea × dialysis time] / volume of distribution of urea (or Kt/V), or at least 2 urea reduction ratio measurements ≥65%, or 1 single-pool Kt/V measurement ≥1.2 and 1 urea reduction ratio measurement ≥65% on different dialysis days during the 3-month period prior to screening, had completed at least 4 WI-NRS worksheets prior to randomization, had a mean baseline WI-NRS score >4 (CLIBN3102) or ≥5 (CLIN3103), defined as the average of all non-missing pre-randomization scores for the seven daily assessments before randomisation.

Exclusion Criteria: A subject was excluded from the Double-blind Phase of the study if he/she was scheduled to receive a kidney transplant during the study; had known history of allergic reaction to opiates, such as hives; had a concomitant disease or a history of any medical condition such as known or suspected history of alcohol, narcotic, or other drug abuse, or substance dependence within 12 months prior to screening, significant systolic or diastolic heart failure, severe mental illness or cognitive impairment, any other relevant acute or chronic medical or neuropsychiatric condition within 3 months prior to screening; had received new or changed treatment for itch, including antihistamines, corticosteroids, opioids, gabapentin, or pregabalin within 14 days prior to screening; had received another investigational drug within 30 days prior to the start of screening or was planning to participate in another clinical study while enrolled in this study; in the opinion of the investigator, had pruritus attributed to a cause other than ESRD or its complications; had localized itch restricted to the palms of the hands; had pruritus only during the haemodialysis session; was receiving ongoing ultraviolet B treatment and anticipated receiving such treatment during the study; had participated in a previous clinical study with difelikefalin.

- **Treatments**

Subjects received difelikefalin at a dose of 0.5 mcg/kg or placebo after each haemodialysis session, generally 3 times per week for up to 64 weeks (12 weeks as double-blind treatment and 52 weeks as open-label treatment). A maximum of 4 doses per week was allowed.

- **Objectives**

Primary: To evaluate the efficacy of IV difelikefalin 0.5 mcg/kg in reducing the intensity of itch.

Secondary: To evaluate the efficacy of IV difelikefalin 0.5 mcg/kg in improving the itch-related quality of life and to evaluate the safety of difelikefalin 0.5 mcg/kg.

For all comparative analyses covered by the control of family wise type 1 error, the hypotheses tested was formulated as superiority.

- **Outcomes/endpoints**

Primary efficacy endpoint: The primary efficacy endpoint in the two phase 3 studies was the event of achieving at least 3-point reduction (i.e., improvement) from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 12 of the Double-blind Treatment Period. At most 3 daily assessments were required to construct the average, if not it was set to missing. Subjects were in the screening period instructed/trained on the use of the PRO tools. For the WI-NRS, it was documented how it was constructed and shown fit-for -purpose prior to the phase 3 studies. Daily assessments in WI-NRS were instructed to be performed at a similar time at day, whether at home, or during dialysis days, at site.

Secondary endpoints: Key secondary endpoints are listed in table below:

Gate-keeping and Hierarchical Testing Procedures – Phase 3 Studies

CR845-CLIN3102	CR845-CLIN3103
≥3-point improvement from baseline in WI-NRS at Week 12 (primary)	≥3-point improvement from baseline in WI-NRS at Week 12 (primary)
Change from baseline in total 5-D Itch Scale score at Week 12	≥4-point improvement from baseline in WI-NRS at Week 12
Change from baseline in total Skindex-10 Scale score at Week 12	≥3-point improvement from baseline in WI-NRS at Week 8
≥4-point improvement from baseline in WI-NRS at Week 12	≥3-point improvement from baseline in WI-NRS at Week 4
	≥4-point improvement from baseline in WI-NRS at Week 8
	≥4-point improvement from baseline in WI-NRS at Week 4
	Change from baseline in total Skindex-10 Scale score at Week 12
	Change from baseline in total 5-D Itch Scale score at Week 12

WI-NRS = Worst Itching-Numerical Rating Scale.

- **Randomisation and Blinding (masking)**

Subjects were randomized in a 1:1 ratio, using a central IWRS. Randomization was stratified according to their use or non-use of concomitant medications to treat their itch during the week prior to randomization (run-in period) as well as the presence or absence of specific medical conditions (History of fall or fracture (related to fall), confusional state or mental status change or altered mental status or disorientation, Gait disturbance or movement disorder) for a total of 4 strata. During the Double-blind Treatment Period, subjects, investigators, study staff, and the sponsor were blinded to study drug assignment.

Both studies included a planned single IA for sample size re-estimation, which was agreed as appropriate by the FDA, to be conducted after approximately 50% of the first 350 randomized subjects either completed the 12-week Double-blind Treatment Period or discontinued study drug prematurely. The IA was implemented by an Independent Data Monitoring Committee (IDMC) who could decide

either to keep the original sample size of 350 or to increase it to up to 500 subjects based on the conditional power of the primary and key secondary endpoints of the protocols, and the respective IDMC charters. The study team was blinded to IA results until after the database lock.

- **Statistical methods**

Sample size: The planned sample size of 350 subjects (175 per treatment group) in each trial was chosen to provide up to 96% power to detect a treatment difference between placebo and difelikefalin for the proportion of subjects with a ≥3-point improvement from baseline in WI-NRS scores at Week 12 using a 2-sided continuity corrected Chi-square test, assuming a placebo response of 30% and a difelikefalin response ranging from 46% to 50% (based on estimates of response consistent with results from Phase 2 study CR845-CLIN2101). This sample size also provided adequate power (≥80%) to detect a treatment difference with respect to the proportion of subjects with a ≥4-point improvement from baseline. According to IA for sample size re-estimation, for CR845-CLIN3102, the sample size was not increased (378 subjects were included in the intent-to-treat [ITT] population). For CR845-CLIN3103, the planned sample size was increased to 430 as per recommendation from the IDMC (473 subjects were included in the ITT population).

Analyses: To counter the bias associated with the interim analysis and to maintain control of the family wise error rate on the statistical inference at the end of the study, test statistics and estimates on treatment effects and their difference were adjusted according to Hung, Lawrence respectively Cui, Hung, Wang.

Missing values of weekly averages of WI-NRS was imputed using a multiple imputation approach within each treatment group: intermittent missing values by MCMC and otherwise by monotone regression using baseline scores, stratification factors and non-missing scores during treatment. The applied MI process was completely independently among subjects contributing the interim results and those following the interim analysis. The family wise error rate was controlled by applying a hierarchical test strategy for the primary and key secondary endpoints as they are listed in the table above. Treatment groups were compared by means of logistic regression, modelling the probability being a responder (i.e. having a reduction of at least 3 in average weekly WI-NRS scores), with effects representing treatment, baseline score and stratification factors.

Sensitivity analyses: A series of sensitivity analyses was performed using the same modelling, but for different approaches on handling missing values:

- Early discontinuations and/or termination of drug use regarded as being non-responders
- Multiple imputation, assuming not missing at random: intermittently missing by MCMC, and otherwise either by sampling from baseline distribution, in case discontinuation was done due to presence of an AE, or for other reasons by the observed WI-NRS profile from non-missing scores in the same time period within the corresponding treatment group.
- Tipping point analysis: a variant to the MI used for the primary analysis, in which non-intermittent missing values in the active treatment groups are imputed progressively in a series of analyses with different shift parameters in order to investigate any departures from the missing at random assumption.
- No adjustment for interim: similar to primary analysis by without adjustment of estimates and test statistic.
- Per protocol population analysis

Analyses on key secondary binary endpoints: Missing values for of weekly average WI-NRS scores for secondary endpoints will imputed in an identical manner as was applied to the primary endpoint. Missing values of 5-D Itch and Skindex-10 will be imputed for each domain within a questionnaire in a manner identical to the weekly average WI-NRS scores. The 5-D Itch and Skindex-10 total scores at week 12 was compared between treatment groups by means of an ANCOVA model, with effects

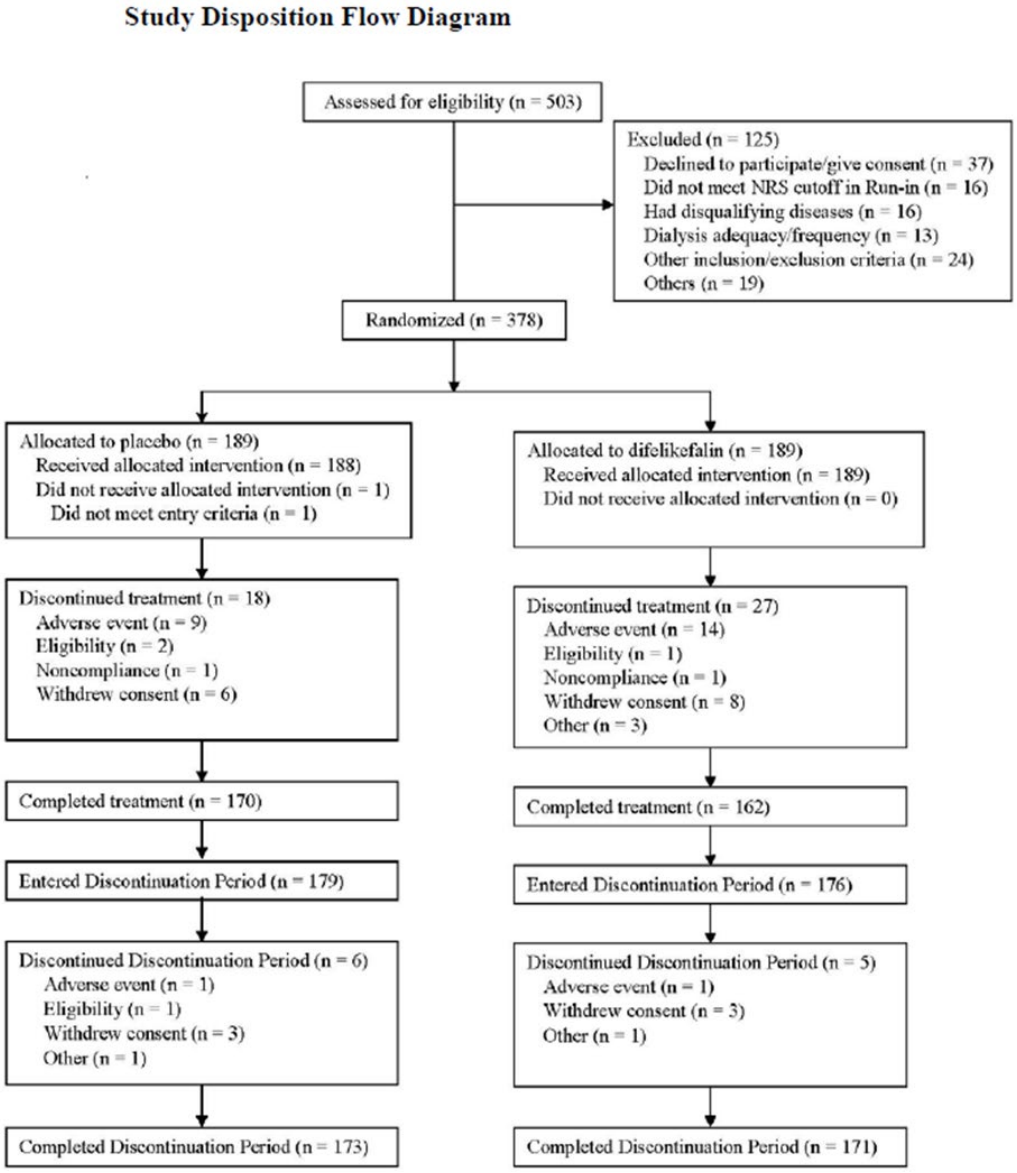
corresponding to treatment, baseline score, region and stratification. For sensitivity purpose a Mixed Model Repeated Measurement model were used without any imputation. Key secondary endpoints of same nature as the primary, i.e., derived from weekly average of WI-NRS at some time point and using a threshold for the responder definition, was analysed by the same model and method of imputation as was used for the primary analysis.

Subgroups: Pooled analyses for the primary endpoint and the key secondary endpoint of ≥4-point improvement in WI-NRS was conducted for the ITT population on the following subgroups: age categories (<65 years, ≥65 years), gender (male, female), race, geographic region, stratification factors (anti-itch medication use during the Run-in Period, presence of specific medical conditions).

Results

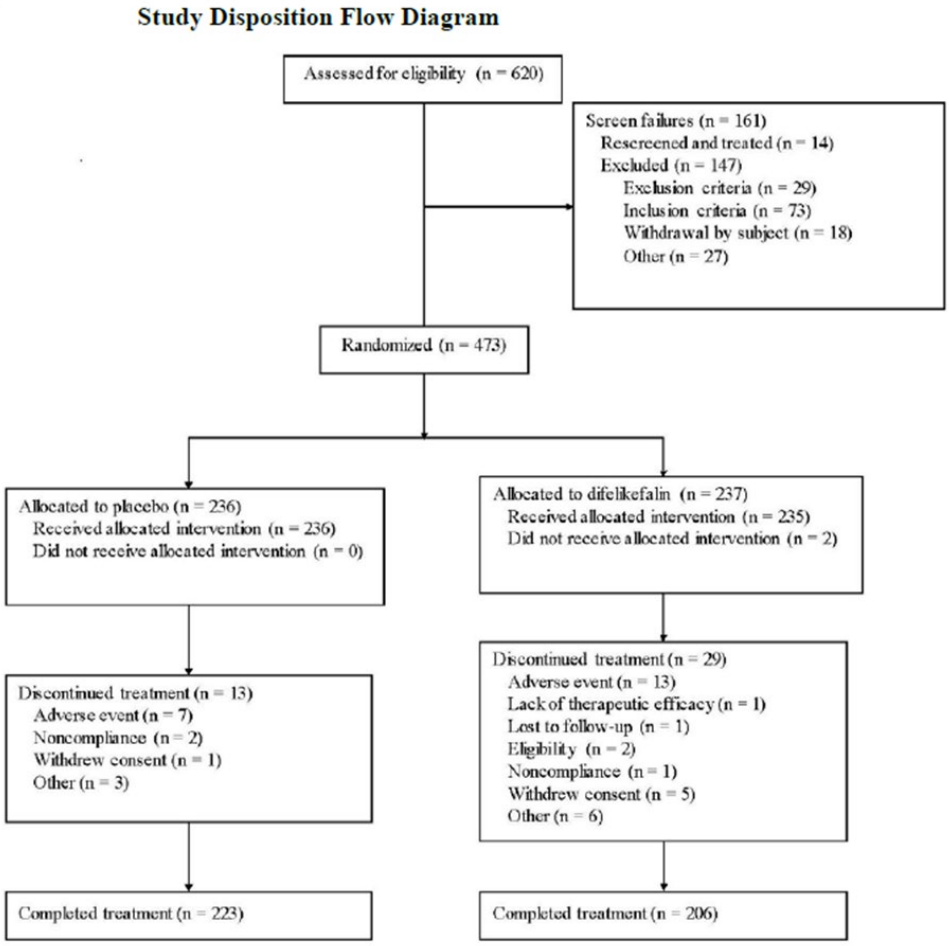
- **Participant flow**

Study CR845-CLIN3102



In CR845-CLIN3102, a total of 503 subjects were screened in the US, of whom 125 failed screening, 378 were randomised (intent-to-treat [ITT] Population), and 377 received at least 1 treatment with difelikefalin (189 subjects) or placebo (188 subjects) during the Double-blind Treatment Period.

Study CR845-CLIN3103



In CR845-CLIN3103, a total of 620 subjects were screened in the US and 10 other countries (Canada, Germany, UK, Hungary, Poland, Czech Republic, Australia, New Zealand, Taiwan, and South Korea), of whom 161 failed screening, 473 were randomised (ITT Population), and 471 received at least 1 treatment with difelikefalin (235 subjects) or placebo (236 subjects) during the Double-blind Treatment Period.

- **Recruitment**

CR845-CLIN3102: Date first subject enrolled: 06FEB2018; Date last subject completed: 14APR2019

CR845-CLIN3103: Date first subject enrolled: 20JUL2018; Date last subject completed: 28FEB2020

- **Conduct of the study**

For CR845-CLIN3102, prior to the interim analysis, minor changes to the SAP were made regarding data handling for subjects who reported WI-NRS scores after discontinuing study drug; all changes were finalized prior to the interim analysis, and no additional changes were made to the primary analysis between Versions 1.1 and 2.0 of the SAP. For CR845-CLIN3103, all changes were finalized prior to the interim analysis, and no changes were made to the primary alpha-controlled analysis between Versions 1.0 and 1.1 of the SAP.

- **Baseline data**

CR845-CLIN3102: Sixty-one percent (61.0%) was male, and the median age was 58.0 years (range 22 to 88 years). The majority of subjects (214 subjects [56.8%]) were in the age group ≥45 to <65

years; 30 subjects (8.0%) were age 75 years or older. 10.6% of subjects aged ≥75 years were enrolled in the placebo group versus 5.3% in the difelikefalin group. The predominant races were white (48.8%) and black or African American (41.6%), and the predominant ethnicity was non-Hispanic/non-Latino (64.5%). The median prescription dry body weight was 84.00 kg (range 42.0 to 135.0 kg). The median duration of CKD-aP for all subjects was 2.50 years (range 0.1 to 26.5 years), and the median WI-NRS score at baseline was 7.14 (range 4.1 to 10.0). At the baseline, 39.8% of subjects were using anti-itch medications (a stratification factor), and 14.1% reported at least 1 of the specific medical conditions used for stratification (History of fall or fracture (related to fall), confusional state or mental status change or altered mental status or disorientation, or gait disturbance or movement disorder). The median time intervals since the diagnoses of CKD and end-stage renal disease (ESRD) for all subjects were 5.45 years (range 0.3 to 42.9 years) and 3.92 years (range 0.3 to 28.7 years), respectively. The median time interval of undergoing chronic HD was 3.32 years (range 0 to 26.5 years). The most common (≥20% of all subjects) aetiologies of CKD were hypertension (71.1%) and diabetes (53.3%), although multiple aetiologies could be reported.

CR845-CLIN3103: Males represented 58.2% of population (274 out of 471 total subjects), and the median age of all subjects was 60.0 years (range 23 to 87 years). Slightly over half of subjects (243 subjects [51.6%]) were in the age group ≥45 to <65 years; 68 subjects (14.4%) were age 75 years or older. The predominant races were white (70.3%) and black or African American (19.3%), the predominant ethnicity was non-Hispanic/non-Latino (69.9%), and the predominant regions were the US (59.0%) and Eastern Europe (24.2%). The median prescription dry body weight was 78.00 kg (range 42.0 to 135.0 kg). The median duration of CKD-aP for all subjects was 2.28 years (range 0.0 to 58.4 years), and the median baseline WI-NRS score was 7.13 (range 4.5 to 10.0). At baseline, 36.5% of subjects were using anti-itch medications (a stratification factor), and 16.6% reported at least 1 of the specific medical conditions used for stratification. The median time intervals since the diagnosis of CKD and ESRD for all subjects were 7.53 years (range 0.3 to 48.3 years) and 4.03 years (range 0.3 to 30.2 years), respectively. The median time interval of undergoing chronic HD was 3.86 years (range 0.3 to 30.2 years). The most common (≥20% of all subjects) aetiologies of CKD were hypertension (49.9%) and diabetes (48.8%), although multiple aetiologies could be reported.

- **Numbers analysed**

CR845-CLIN3102: Intent-to-treat (ITT) Population: 378 subjects

Per Protocol Population: 332 subjects

Double-blind Safety Population: 377 subjects

Double-blind Discontinuation Safety Population: 355 subjects

Double-blind Discontinuation Population: 300 subjects

CR845-CLIN3103: Intent-to-treat (ITT) Population: 473 subjects

Per Protocol Population: 418 subjects

Double-blind Safety Population: 471 subjects

- **Outcomes and estimation**

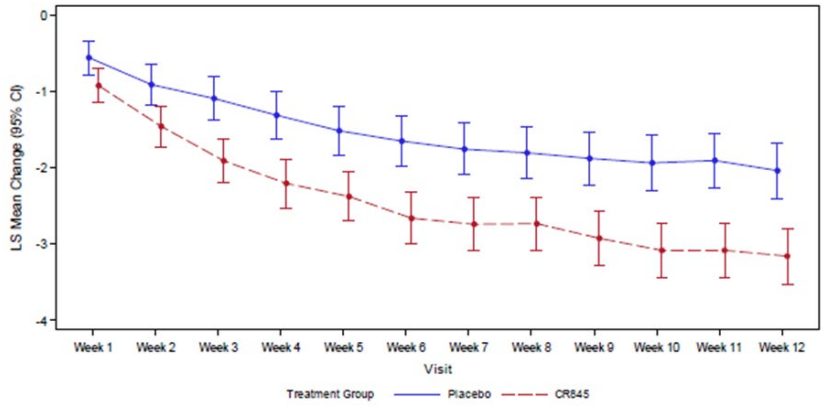
CR845-CLIN3102 - Primary endpoint

In Study CR845-CLIN3102, 51.0% of difelikefalin subjects versus 27.6% placebo subjects met the primary efficacy endpoint by achieving a ≥3-point improvement (reduction) in WI-NRS at Week 12 (odds ratio = 2.72; P <.001).

CR845-CLIN3102 – Key secondary endpoints

At the end of Week 12, change from baseline in the total 5-D Itch Scale score for difelikefalin and placebo were (LS mean) -5.0 and -3.7, respectively (P <.001) (Table 20) and change from baseline in the total Skindex-10 Scale score for difelikefalin and placebo were (LS mean) -17.2 and -12.0, respectively (P <.001) (Table 17). The proportion of subjects achieving a ≥4-point improvement in WI-NRS from baseline at Week 12, were consistent with the primary endpoint; 38.9% (LS mean) of subjects treated with difelikefalin and 18.0% subjects treated with placebo achieved a ≥4-point improvement in WI-NRS from baseline (odds ratio = 2.89; P <.001).

Mean Change From Baseline in Worst Itching Intensity Numerical Rating Scale Score by Study Week (Primary Efficacy Imputation) (Population: ITT)



CI = confidence interval; ITT = Intent-to-treat; LS = least squares
Note: LS means, standard errors, and CIs were based on a mixed model repeated measures analysis with effects for treatment, visit, treatment-by-visit interaction, baseline score, use of anti-itch medication during the week prior to randomization, and the presence of specific medical conditions. The model was fit using an unstructured covariance structure.
Missing values were imputed using multiple imputation under missing-at-random missing data assumption.

CR845-CLIN3103 - Primary endpoint

In Study CR845-CLIN3103, the percentage of difelikefalin and placebo subjects achieving a ≥3-point improvement (reduction) in WI-NRS at Week 12 were 54.0% and 42.2% (odds ratio = 1.61; P = .020).

The proportion of ITT subjects achieving a ≥3-point improvement in the mean 24-hour WI-NRS scores from baseline at Week 12 by use of anti-itch medications at baseline (stratification variable). The subjects using anti-itch medications at baseline had a greater treatment difference (odds ratio = 2.15; 95% CI, 1.09 to 4.25) favouring difelikefalin than subjects not using anti-itch medications at baseline (odds ratio = 1.36; 95% CI, 0.84 to 2.20).

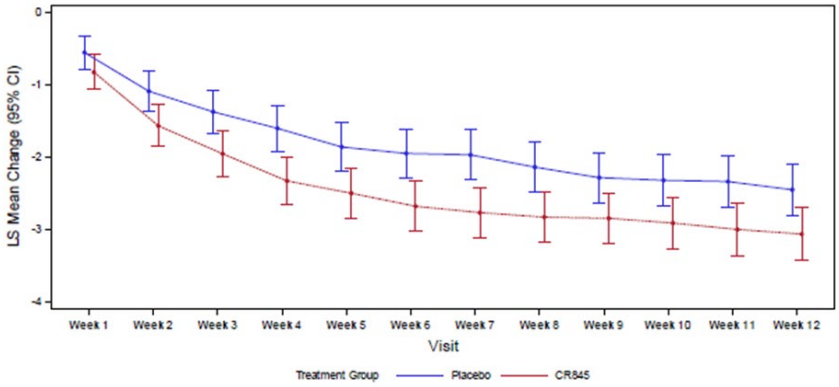
CR845-CLIN3103 – Key secondary endpoints

For the first key secondary endpoint, a greater proportion of subjects who received difelikefalin than placebo achieved a ≥4-point improvement in WI-NRS from baseline at Week 12 (41.2% versus 28.4%; odds ratio = 1.77; P = .010).

A greater percentage of difelikefalin subjects than placebo subjects achieved a ≥3-point improvement (reduction) in WI-NRS at Week 8 (49.0% versus 36.2%; P = .010) and Week 4 (38.3% versus 23.8%; P = .002). For a ≥4-point improvement in WI-NRS, there were 36.1% difelikefalin subjects versus 23.7% placebo subjects at Week 8 (P = .010) and 26.1% versus 16.7% at Week 4 (P = .036). With

respect to the time course of WI-NRS improvement, treatment group differences in favour of difelikefalin were observed as early as Week 2 for a ≥3-point reduction and as early as Week 3 for a ≥4-point reduction, which were maintained throughout the remainder of the Double-blind Treatment Period (Figure below).

Mean Change From Baseline in Worst Itching Intensity Numerical Rating Scale Score by Study Week (Primary Efficacy Imputation) (Population: ITT)



CI = confidence interval; ITT = Intent-to-treat; LS = least squares
Note: LS means, standard errors, and CIs were based on a mixed model repeated measures analysis with effects for treatment, visit, treatment-by-visit interaction, baseline score, region, use of anti-itch medication during the week prior to randomization, and the presence of specific medical conditions. The model was fit using an unstructured covariance structure.
Missing values were imputed using multiple imputation under missing-at-random missing data assumption.
Source: Figure 14.2.3

The final 2 secondary endpoints in the hierarchy were change in the total Skindex-10 Scale score and the total 5-D Itch Scale score from baseline to the end of Week 12. At week 12, the treatment group difference for LS mean reduction in total Skindex-10 Scale score was -1.8 (P = .171). Subsequently, the reported LS mean change reduction in the total 5-D Itch Scale score (ANCOVA) at the end of Week 12 could not be tested (nominal P value was 0.002).

- **Ancillary analyses**

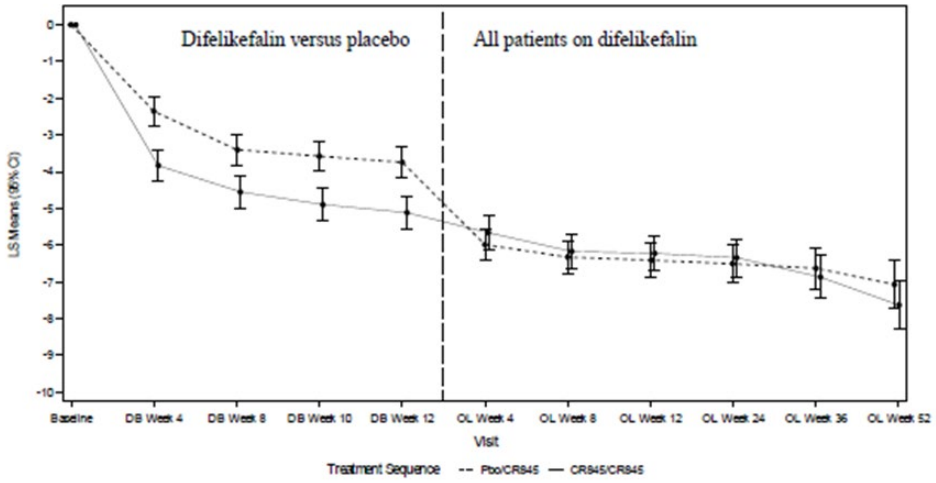
The subgroup analyses were conducted using the pooled data from CR845-CLIN3102 and CR845-CLIN3103 only with respect to ≥3- and ≥4-point changes in the WI-NRS at each timepoint and specifically at Week 12. Post-hoc analyses have been performed to analyse efficacy according to baseline itch severity. Given the small number of subjects with a very severe baseline score and the fact that difelikefalin is proposed for the treatment of moderate to severe CKD-aP, the following 2 categories were considered regarding the baseline itch intensity: WI-NRS ≥4 to <7: moderate itch; and WI-NRS ≥7: severe itch. Difelikefalin was shown to be effective for both CKD-aP subjects with moderate baseline itch and CKD-aP subjects with severe baseline itch.

The Applicant has performed post-hoc analyses in subjects with ≥3-point improvement and with ≥4-point improvement in the WI-NRS by region (EU versus non-EU) in the pooled ITT population. Regarding the ≥3-point improvement at Week 12, the treatment effect in the EU was at least as large (OR (CI) of improvement with difelikefalin versus placebo 2.56 (1.26-5.19)) as in the other regions (OR (CI) 1.81 (1.32-2.48)) and it was statistically significant in both the EU (p=0.009) and the other regions (p<0.001). Regarding the ≥4-point improvement at Week 12, a similar relation was observed; i.e., the treatment effect in the EU was at least as large as in the other regions (EU: OR (CI) 2.47 (1.17-5.21), p=0.018; non-EU OR (CI) 1.99 (1.41-2.82), p<0.001)).

Long-term effectiveness data were explored in the OLE of CR845-CLIN3102 for up to 52 weeks. The OLE of CR845-CLIN3103 was stopped early by the sponsor and only 5 patients completed 52 weeks of

treatment. For CR845-CLIN3102- OLE, after 52 weeks of Open-label treatment, the LS mean (standard error [SE]) change from baseline in total 5-D Itch Scale score was -6.9 (0.39) and -7.8 (0.39) for placebo/difelikefalin (n = 94) and difelikefalin/difelikefalin subjects (n = 90), respectively. Pooled OLE population reached 712 patients, see below.

Mean Change From Double-blind Baseline in Total 5-D Itch Score by Double-blind and Open-label Visits (Population: Pooled Open-label Safety from CLIN3102 and CLIN3103)



Solid line = data for subjects treated with difelikefalin during the double blind (DB) treatment period (through Week 12) and who remained on treatment during the open label (OL) extension.
Dashed line = data for subjects treated with placebo during the DB treatment period and then switched to difelikefalin for the OL extension.
Y axis shows least square (LS) means and 95% confidence intervals (CI).
X axis shows study week for DB treatment period and OL extension.
Note: Least square means and CIs were based on a mixed model repeated measures analysis with effects for treatment sequence, visit, treatment-by-visit interaction, baseline score, region/study combined variable and randomization stratification variables. The model was fit using an unstructured covariance structure.
Note: Baseline was the last assessment prior to the start of Double-blind treatment.
Program: OL_EF_FBA.sas, Generated: 08JAN21:14:17

To further support clinical relevance of the treatment response in patients with very severe baseline pruritus, additional post-hoc analyses were performed based on the classification of the response using relative thresholds of 30% and 50%, as see below.

Subjects with ≥30% Improvement from Baseline at Weeks 11 and 12 with Respect to the WI-NRS Score, by Baseline WI-NRS Severity

	Baseline WI-NRS ≥9		Overall	
	Placebo (N=63)	Difelikefalin (N=56)	Placebo (N=425)	Difelikefalin (N=426)
DB Week 11				
Observed ≥30% WI-NRS improvement - n (%)[1]				
Yes	18 (32.7)	25 (51.0)	174 (45.0)	221 (61.6)
No	37 (67.3)	24 (49.0)	213 (55.0)	138 (38.4)
Missing	8	7	38	67
LS means estimate of percent with improvement[2]				
Percent (95% CI)	33.0 (20.8, 48.1)	52.0 (35.7, 68.0)	46.1 (40.2, 52.1)	62.3 (56.3, 68.0)
Odds ratio (95% CI)		2.20 (1.02, 4.78)		1.94 (1.46, 2.57)
P-value		0.046		<0.001
DB Week 12				
Observed ≥30% WI-NRS improvement - n (%)[1]				
Yes	18 (33.3)	26 (55.3)	179 (48.1)	220 (63.2)
No	36 (66.7)	21 (44.7)	193 (51.9)	128 (36.8)
Missing	9	9	53	78
LS means estimate of percent with improvement[2]				
Percent (95% CI)	35.6 (22.9, 50.7)	53.5 (37.1, 69.2)	50.6 (44.5, 56.6)	65.0 (59.1, 70.6)
Odds ratio (95% CI)		2.08 (0.96, 4.48)		1.82 (1.37, 2.40)
P-value		0.062		<0.001

Subjects with ≥50% Improvement from Baseline at Weeks 11 and 12 with Respect to the WI-NRS Score, by Baseline WI-NRS Severity

	Baseline WI-NRS ≥9		Overall	
	Placebo (N=63)	Difelikefalin (N=56)	Placebo (N=425)	Difelikefalin (N=426)
DB Week 11				
Observed ≥50% WI-NRS improvement - n (%)[1]				
Yes	11 (20.0)	19 (38.8)	104 (26.9)	164 (45.7)
No	44 (80.0)	30 (61.2)	283 (73.1)	195 (54.3)
Missing	8	7	38	67
LS means estimate of percent with improvement[2]				
Percent (95% CI)	19.5 (10.2, 33.8)	41.5 (25.9, 59.0)	27.8 (22.8, 33.4)	46.5 (40.4, 52.7)
Odds ratio (95% CI)		2.93 (1.21, 7.09)		2.26 (1.67, 3.06)
P-value		0.017		<0.001
DB Week 12				
Observed ≥50% WI-NRS improvement - n (%)[1]				
Yes	12 (22.2)	19 (40.4)	107 (28.8)	164 (47.1)
No	42 (77.8)	28 (59.6)	265 (71.2)	184 (52.9)
Missing	9	9	53	78
LS means estimate of percent with improvement[2]				
Percent (95% CI)	22.9 (12.7, 37.9)	40.5 (25.2, 57.8)	29.4 (24.2, 35.1)	47.0 (41.0, 53.1)
Odds ratio (95% CI)		2.28 (0.98, 5.33)		2.13 (1.59, 2.86)
P-value		0.056		<0.001

- **Summary of main efficacy results**

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Summary of Efficacy for trial *CR845-CLIN3102 (KALM-1)*

Title: A Multicenter, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Intravenous CR845 in Haemodialysis Patients with Moderate-to-Severe Pruritus, with a 52 Week Open-Label Extension			
Study identifier	CR845-CLIN3102, KALM-1, NCT03422653		
Design	Multicenter, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of difelikefalin at a dose of 0.5 mcg/kg administered after each haemodialysis session 3 times a week in subjects with moderate-to-severe pruritus who were undergoing haemodialysis. Stratification by use of anti-itch medication at baseline (Yes/No) and history of specific medical conditions (Yes/No). The study included a Double-blind Phase and Open-label Extension Phase.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 weeks 7 days 2-week Discontinuation Period, up to 52 weeks Open-label Extension Phase
Hypothesis	Superiority		
Treatments groups	Difelikefalin		Difelikefalin 0.5 mcg/kg as an IV bolus after each haemodialysis session (generally 3 times per week). 12 weeks, 189 subjects
	Placebo		Matching placebo as an IV bolus after each haemodialysis session (generally 3 times per week). 12 weeks, 189 subjects
	Primary endpoint	≥3-point improvement WI-NRS	Proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS (Worst Itching Intensity Numerical Rating Scale) at Week 12
	Key Secondary endpoint	5-D Itch	Change from baseline at the end of Week 12, as assessed by the 5-D Itch Scale
	Key Secondary endpoint	Skindex-10	Change from baseline at the end of Week 12, as assessed by the total Skindex-10 Scale score
	Key Secondary endpoint	≥4-point improvement WI-NRS	Proportion of subjects achieving a ≥4-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 12
Database lock	21 May 2019		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent-to-treat (ITT) Population: 378 subjects; Week 12		
Descriptive statistics and estimate variability	Treatment group	Difelikefalin	Placebo
	Number of subjects	189	189
	≥3-point improvement WI-NRS (LS mean)	51.0%	27.6%
	95% confidence interval	42.9%, 58.9%	20.2%, 36.6%

	5-D Itch (LS mean)	-5.0	-3.7
	95% confidence interval	-5.7, -4.4	-4.4, -3.1
	Skindex-10 (LS mean)	-17.2	-12.0
	95% confidence interval	-19.6, -14.7	14.5, -9.6
	≥4-point improvement WI-NRS (LS mean)	38.9%	18.0%
	95% confidence interval	29.8%, 48.7%	12.1%, 26.0%
Effect estimates per comparison	≥3-point improvement WI-NRS	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	2.72
		95% confidence interval	1.72, 4.30
		P-value (Cui, Hung, Wang)	<0.001
	5-D Itch	Comparison groups	Difelikefalin vs. Placebo
		Difference in LS means	-1.3
		95% confidence interval	-2.0, -0.5
		P-value (ANCOVA)	<0.001
	Skindex-10	Comparison groups	Difelikefalin vs. Placebo
		Difference in LS means	-5.1
		95% confidence interval	-8.0, -2.3
		P-value (ANCOVA)	<0.001
	≥4-point improvement WI-NRS	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	2.89
		95% confidence interval	1.75, 4.76
		P-value (Cui, Hung, Wang)	<0.001
		P-value (Cochran-Mantel-Haenszel)	<0.001
Notes	None.		

Summary of Efficacy for trial *CR845-CLIN3103 (KALM-2)*

Title: A Multicenter, Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Intravenous CR845 in Haemodialysis Patients with Moderate-to-Severe Pruritus, with a 52 Week Open-Label Extension			
Study identifier	CR845-CLIN3103, KALM-2, 2018-001930-17, NCT03636269		
Design	Multicenter, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of difelikefalin at a dose of 0.5 mcg/kg administered after each haemodialysis session 3 times a week in subjects with moderate-to-severe pruritus who were undergoing haemodialysis. Stratification by use of anti-itch medication at baseline (Yes/No) and history of specific medical conditions (Yes/No). The study included a Double-blind Phase and Open-label Extension Phase.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 weeks 7 days up to 52 weeks Open-label Extension Phase
Hypothesis	Superiority		
Treatments groups	Difelikefalin		Difelikefalin 0.5 mcg/kg as an IV bolus after each haemodialysis session (generally 3 times per week). 12 weeks, 237 subjects
	Placebo		Matching placebo as an IV bolus after each haemodialysis session (generally 3 times per week). 12 weeks, 236 subjects
Endpoints and definitions	Primary endpoint	≥3-point improvement WI-NRS Week 12	Proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS (Worst Itching Intensity Numerical Rating Scale) at Week 12
	Key Secondary endpoint	≥4-point improvement WI-NRS Week 12	Proportion of subjects achieving a ≥4-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 12
	Key Secondary endpoint	≥3-point improvement WI-NRS Week 8	Proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS (Worst Itching Intensity Numerical Rating Scale) at Week 8
	Key Secondary endpoint	≥3-point improvement WI-NRS Week 4	Proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS (Worst Itching Intensity Numerical Rating Scale) at Week 4
	Key Secondary endpoint	≥4-point improvement WI-NRS Week 8	Proportion of subjects achieving a ≥4-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 8
	Key Secondary endpoint	≥4-point improvement WI-NRS Week 4	Proportion of subjects achieving a ≥4-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 4
	Key Secondary endpoint	Skindex-10	Change from baseline at the end of Week 12, as assessed by the total Skindex-10 Scale score
	Key Secondary endpoint	5-D Itch	Change from baseline at the end of Week 12, as assessed by the 5-D Itch Scale
Database lock	31 March 2020		

Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent-to-treat (ITT) Population: 473 subjects Weeks 4, 8, 12		
Descriptive statistics and estimate variability	Treatment group	Difelikefalin	Placebo
	Number of subjects	237	236
	≥3-point improvement WI-NRS Week 12 (LS mean)	54.0%	42.2%
	95% confidence interval	43.9%, 63.9%	32.5%, 52.5%
	≥4-point improvement WI-NRS Week 12 (LS mean)	41.2%	28.4%
	95% confidence interval	33.0%, 50.0%	21.3%, 36.7%
	≥3-point improvement WI-NRS Week 8 (LS mean)	49.0%	36.2%
	95% confidence interval	38.3%, 59.9%	27.3%, 46.2%
	≥3-point improvement WI-NRS Week 4 (LS mean)	38.3%	23.8%
	95% confidence interval	28.5%, 49.1%	16.6%, 32.8%
	≥4-point improvement WI-NRS Week 8 (LS mean)	36.1%	23.7%
	95% confidence interval	28.0%, 45.1%	17.2%, 31.8%
	≥4-point improvement WI-NRS Week 4 (LS mean)	26.1%	16.7%
	95% confidence interval	18.8%, 34.9%	11.4%, 23.9%
	Skindex-10 (LS mean)	-16.6	-14.8
	95% confidence interval	-19.3, -14.0	-17.4, -12.2
	5-D Itch (LS mean)	-4.9	-3.8
	95% confidence interval	-5.6, -4.2	-4.5, -3.1
		Comparison groups	Difelikefalin vs. Placebo

Effect estimates per comparison	≥3-point improvement WI-NRS Week 12	Odds ratio (Lawrence, Hung)	1.61
		95% confidence interval	1.08, 2.41
		P-value (Cui, Hung, Wang)	0.020
	≥4-point improvement WI-NRS Week 12	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	1.77
		95% confidence interval	1.14, 2.74
		P-value (Cui, Hung, Wang)	0.010
	≥3-point improvement WI-NRS Week 8	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	1.69
		95% confidence interval	1.13, 2.53
		P-value (Cui, Hung, Wang)	0.010
	≥3-point improvement WI-NRS Week 4	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	1.99
		95% confidence interval	1.29, 3.06
		P-value (Cui, Hung, Wang)	0.002
	≥4-point improvement WI-NRS Week 8	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	1.82
		95% confidence interval	1.16, 2.86
		P-value (Cui, Hung, Wang)	0.010
	≥4-point improvement WI-NRS Week 4	Comparison groups	Difelikefalin vs. Placebo
		Odds ratio (Lawrence, Hung)	1.76
		95% confidence interval	1.04, 2.98
		P-value (Cui, Hung, Wang)	0.036
	Skindex-10	Comparison groups	Difelikefalin vs. Placebo
		Difference in LS means	-1.8
		95% confidence interval	-4.3, 0.8
		P-value (ANCOVA)	0.171
	5-D Itch	Comparison groups	Difelikefalin vs. Placebo
		Difference in LS means	-1.1
		95% confidence interval	-1.7, -0.4
		P-value (ANCOVA)	0.002
Notes	None		

2.5.5.3. Analysis performed across trials (pooled analyses and meta-analysis)

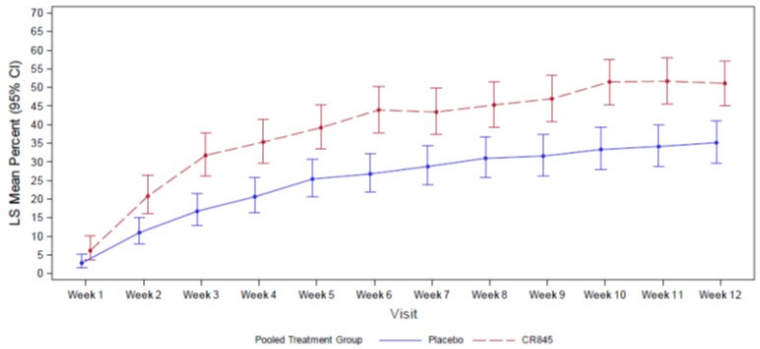
The placebo estimates varied between studies by more than 10%. The proportion of subjects on placebo who also achieved a ≥3-point reduction from baseline in the WI-NRS at Week 12 was higher in the global pivotal study CR845-CLIN3103 (42.2%) than in the US only study CR845-CLIN3102 (27.6%). A meta-analysis comparing active treatments versus placebo in chronic dermatological

conditions with pruritus, showed that placebo treatment significantly reduced itch compared to baseline, with an overall reduction in itch severity of 24% (Van Laarhoven, et al 2015). Thus, itch can be reduced by placebo in a variety of chronic pruritic conditions by as much as 1.3 points out of 10 (95% CI 1.02-1.61) (Van Laarhoven et al, 2015). Placebo response rates in US subjects were also higher in CR845-CLIN3103 (37.3%) than in CR845-CLIN3102 (27.6%). CR845-CLIN3103 was a larger study (n = 473) and included more sites (75) than CR845-CLIN3102 (n = 378; 56 sites). The number of study sites may have influenced the placebo response. There is literature evidence to suggest that more study sites may inflate placebo responses in randomized controlled studies (Meske et al, 2019).

Proportion of Subjects Achieving ≥3- and ≥4-point Improvement in WI-NRS by Study Week

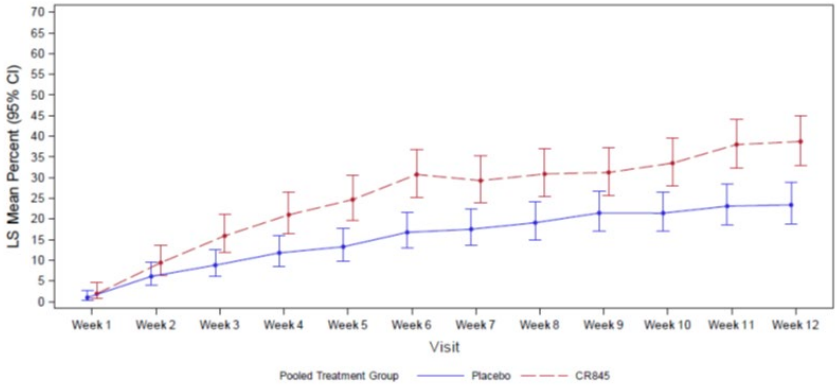
The analyses of ≥3- and ≥4-point changes in the WI-NRS at each time point were also conducted using the pooled data from CR845-CLIN3102 and CR845-CLIN3103 (see figures below).

Percentage of Subjects with ≥3-point Improvement with Respect to the WI-NRS Score by Week – MI with MAR Assumption (Population: ITT - Pooled Dataset)



Note: Estimated percent and confidence interval used logistic regression model with terms for treatment group, baseline score, use of anti-itch medication during the week prior to randomization, and the presence of specific medical conditions. Missing values were imputed using MI under MAR missing data assumption for interim subjects and post-interim subjects separately. Region was also included in the model for the 3103 DB analysis, while a region/study combined variable was also included in the model for the pooled analysis.
CI = confidence interval; ITT = intent-to-treat; LS = least squares; MAR = missing at random; MI = multiple imputation; WI-NRS = Worst Itching-Numerical Rating Scale.
Source: Integrated Summary of Efficacy, Figure 3.1.4.1

Percent of Subjects with ≥4-point Improvement with Respect to the WI-NRS Score by Week (Population: ITT - Pooled Dataset)



2.5.5.4. Supportive study

Not applicable.

2.5.6. Discussion on clinical efficacy

The initially proposed indication for difelikefalin has been requested by the CHMP to be amended on CHMP's request as follows "*the treatment of moderate-to-severe pruritus associated with chronic kidney disease in adult patients on haemodialysis"* in order to reflect the population truly treated in the clinical trial. The recommended dose of difelikefalin is 0.5 micrograms/kg dry body weight (i.e., the target post-dialysis weight) by intravenous bolus injection three times per weekThe evidence of the efficacy of difelikefalin is based on data from the double-blind parts of two phase 3 clinical trials (Studies CR845-CLIN3102 and CR845-CLIN3103). Maintenance of the effect is based on open label extensions of these studies. However, OLE periods are not sufficient for continued evaluation of efficacy beyond 12 weeks due to very early termination of one of the studies and also no reporting on the scale used for primary endpoint in the double-blind phases. Dose finding was performed in the dose-ranging study CR845-CLIN2101 which examined the efficacy of 3 doses of IV difelikefalin (0.5, 1.0, and 1.5 mcg/kg) treatment after each HD session (3 times/week) over an 8-week period. The 0.5 mcg/kg dose was selected for the Phase 3 studies given the similarities in efficacy results across doses and a more favourable safety profile over the higher doses.

Design and conduct of clinical studies

The design of the pivotal trials is 12-week double-blind randomized placebo-controlled parallel group confirmatory trial. Overall, the design for double-blind phase could be considered acceptable for this treatment/indication. The provision of potentially un-masked comparative results from the interim analyses were questioned, no impact on study integrity was observed.

The studies consisted of screening (7-28 days), Run-in (7 days), double-blind treatment (12 weeks) periods in the double-blind trial phase, and open-label treatment (up to 52 weeks) with safety follow up (7-10 days) periods in the open-label extension phase. In CR845-CLIN3102, the double-blind treatment period was followed by a 2-week discontinuation period designed to assess potential signs/symptoms of drug withdrawal, during which subjects did not receive any study drug. The study was overseen by and independent data monitoring committee (IDMC).

Eligible subjects were randomized in a 1:1 ratio to receive either difelikefalin 0.5 mcg/kg or placebo as an IV bolus after the end of each HD session, generally 3 times per week (maximum 4 doses per week), in the Double-blind Treatment Period. Subjects were stratified according to their use or non-use of concomitant medications to treat pruritus during the week prior to randomization (Run-in Period), as well as the presence or absence of specific medical conditions (e.g., falls/fractures due to falls, gait disturbance, and mental status change).

The inclusion and exclusion criteria can generally be considered suitable to define a relevant patient population. Eligible subjects were males or females aged 18 years or older undergoing in-center haemodialysis 3 times a week for end-stage renal disease (ESRD). Prior to randomization, subjects had to have completed at least 4 WI-NRS worksheets during the Run-in Period and have a mean baseline WI-NRS score >4 for CR845-CLIN3102 and ≥5 for CR845-CLIN3103, defined as the average of all non-missing pre-randomization scores, to ensure moderate-to-severe pruritus at baseline. They were not eligible if they had pruritus attributed to a cause other than ESRD or its complications, in the opinion of the investigator. The subjects were excluded from the Double-blind Phase of the study if they were scheduled to receive a kidney transplant during the study; had known history of allergic reaction to opiates, such as hives; had a concomitant disease or a history of any medical condition such as known

or suspected history of alcohol, narcotic, or other drug abuse, or substance dependence within 12 months prior to screening, significant systolic or diastolic heart failure, severe mental illness or cognitive impairment (e.g., dementia), any other relevant acute or chronic medical or neuropsychiatric condition within 3 months prior to screening (e.g., diagnosis of encephalopathy, coma, delirium); had localized itch restricted to the palms of the hands; had pruritus only during the haemodialysis session (by subject report). Both treatment naïve and previously treated patients were enrolled, however, patients were excluded if they had received new or changed treatment for itch, including antihistamines, corticosteroids (oral, IV, or topical), opioids, gabapentin, or pregabalin within 14 days prior to screening; had received another investigational drug within 30 days prior to the start of screening or were planning to participate in another clinical study while enrolled in this study; were receiving ongoing ultraviolet B treatment and anticipated receiving such treatment during the study. Upon query the Applicant added warning in section 4.4. of SmPC on treatment of patients with clinically important disruptions to the BBB (e.g., primary brain malignancies, CNS metastases or other inflammatory conditions, active multiple sclerosis, advanced Alzheimer's disease) due to a potentially higher risk for difelikefalin entry into the CNS. Kapruvia should be prescribed with caution in such patients taking into account their individual benefit-risk balance with observation for potential CNS effects.

The primary efficacy endpoint was the proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 12 of the Double-blind Treatment Period. The WI-NRS scale is considered relevant, and the Applicant performed the validation of the scale in this indication. The cut off of 3 points was determined as minimal clinically significant change by the Applicant. FDA noted that "a success (or responder) defined as a ≥4-point improvement from baseline on an 11-point NRS has been proposed for other indications", "the definition of a clinically meaningful change in the WI-NRS should be based on an absolute change rather than a percentage change from the baseline value", and "recommended that a 4-point improvement be used as a threshold for the primary efficacy endpoint instead of a ≥3-point improvement from baseline". The choice of ≥3-point improvement instead of ≥4-point improvement from baseline as minimal clinically meaningful change on WI-NRS is not queried further as analyses with both revealed significant results. Different sensitivity analyses were conducted for the primary analysis using interim or post-interim analysis subjects, counting early discontinuations as non-responders, using multiple imputation and tipping point analysis. Additional post-hoc tables of ≥3-point and ≥4-point improvements of the WI-NRS have been generated based on the daily scores, rather than on the weekly scores, which have also supported the primary analyses.

The list of key secondary endpoints and their hierarchical testing differ between the two pivotal studies. For CR845-CLIN3102, secondary endpoints were change from baseline in total 5-D Itch Scale score at week 12, change from baseline in total Skindex-10 Scale score at week 12, and ≥4-point improvement from baseline in WI-NRS at week 12. For CR845-CLIN3103, there were 7 secondary endpoints in hierarchy which included 3 or 4 points or more improvements from baseline to weeks 12, 8 or 4, together with total Skindex-10 Scale score and total 5-D Itch Scale score. In general, the scales and analyses used for key secondary/secondary/ exploratory endpoints are relevant to the indication. According to testing hierarchy in SAP, the primary endpoint was to be tested first at $\alpha = 0.05$ in the overall ITT population. If the primary endpoint was statistically significant in the ITT population, each secondary endpoint was tested with a hierarchical order differing between trials. Thus, there was strict control on the family wise error rate for all endpoints covered by the pre-specified test strategy. The sample size of 350 subjects (175 per treatment group) in each trial was chosen to provide up to 96% power to detect a treatment difference between placebo and difelikefalin for the proportion of subjects with a ≥3-point improvement from baseline in WI-NRS scores at Week 12 using a 2-sided continuity corrected Chi-square test, assuming a placebo response of 30% and a difelikefalin response ranging from 46% to 50% (based on estimates of response consistent with results from Phase 2 study CR845-

CLIN2101). This sample size also provided adequate power (≥80%) to detect a treatment difference with respect to the proportion of subjects with a ≥4-point improvement from baseline.

Efficacy data and additional analyses

Study CR845-CLIN3102 randomized subjects at 56 sites and was conducted exclusively in the US, whereas study CR845-CLIN3103 randomized subjects at 75 sites and was conducted in the US, Europe, and Asia-Pacific. Despite planned target population size of 350 subjects in each trial, the pre-planned interim analysis for sample size re-estimation resulted in an advice from Independent Data Monitoring Committee to increase sample size for CR845-CLIN3103. A total of 503 subjects were screened in the US, of whom 125 failed screening, 378 were randomised (intent-to-treat [ITT] Population), and 377 received at least 1 treatment with difelikefalin (189 subjects) or placebo (188 subjects) during the Double-blind Treatment Period. A total of 332 subjects (88.1% of the 377 subjects randomized and exposed to study drug) completed the Double-blind Treatment Period. Forty-five subjects (11.9%) discontinued early, 14.3% in difelikefalin group and 9.6% in the placebo group. The most common reasons for early discontinuation were AE (6.1%) and subject withdrawal of consent (3.7%).

In CR845-CLIN3103, a total of 620 subjects were screened (in US, Canada, Germany, UK, Hungary, Poland, Czech Republic, Australia, New Zealand, Taiwan, and South Korea), of whom 161 failed screening, 473 were randomised (ITT Population), and 471 received at least 1 treatment with difelikefalin (235 subjects) or placebo (236 subjects). A total of 429 subjects (91.1% of the 471 subjects randomized and exposed to study drug) completed the Double-blind Treatment Period. Forty-two subjects (8.9%) discontinued early, 12.3% in the difelikefalin group versus 5.5% in the placebo group. The most common reason for early discontinuation was AE (4.2%). Overall, study population reflects the intended indication. The study arms were in general comparable with respect to median values for demographic characteristics and the baseline characteristics. Slight differences between placebo and active groups are not expected to have significant impact on the primary endpoint and key secondary endpoints. Across both Phase 3 studies, males represented 59.6% of the ITT population, and the median age was 60.0 years for difelikefalin subjects and 59.0 for placebo subjects (range 22 to 90 years). The majority of subjects were <75 years old (88.4%). 106/377 and 172/471 patients were at or above the age of 65. The predominant races were white (60.8%) and black or African American (29.3%), and the predominant regions were the US (77.2%) and Eastern Europe (13.4%). EU patient data comes from CR845-CLIN3103 and 63 patients who were recruited in Europe were exposed to difelikefalin treatment. Around 37% of the population treated with difelikefalin were using another anti-itch medication and the study populations were stratified for this factor. This supports the inclusion of all *de-novo* or previously treated CKDaP patients in the indication. Treatment compliance seems to be different in terms of range between two pivotal studies, however mean and median values are comparable between the studies. Study CR845-CLIN3102 is considered a positive study to show the efficacy of difelikefalin in the treated population, in line with the estimations in the study design.

In Study CR845-CLIN3102, 51.0% of difelikefalin subjects versus 27.6% placebo subjects met the primary efficacy endpoint by achieving a ⩾3-point improvement (reduction) in WI-NRS at Week 12 (odds ratio = 2.72; P <.001). Absolute baseline scores were 7.2 (SD 1.61) for placebo and 7.1 (SD 1.44) for difelikefalin groups. The change at 12 weeks was -2 for placebo and -3.2 for active arms with a treatment difference of -1.1. The sensitivity analyses of primary analysis reached statistical significance (<0.05) and were supportive. Per protocol analysis results removed some concerns around the protocol deviations. Primary analysis results for interim and post-interim populations or for subjects using anti-itch medication or not did not differ significantly. Secondary endpoints were met in study CR845-CLIN3102. At the end of Week 12, change from baseline in the total 5-D Itch Scale score for difelikefalin and placebo were (LS mean) -5.0 and -3.7, respectively (P <.001) and change from baseline in the total Skindex-10 Scale score for difelikefalin and placebo were (LS mean) -17.2 and -

12.0, respectively (P <.001). The proportion of subjects achieving a ⩾4-point improvement in WI-NRS from baseline at Week 12, were consistent with the primary endpoint which was reassuring; 38.9% (LS mean) of subjects treated with difelikefalin and 18.0% subjects treated with placebo achieved a ⩾ 4-point improvement in WI-NRS from baseline (odds ratio = 2.89; P <.001)

Primary endpoint was met in study CR845-CLIN3103, however, there are some concerns and limited support from sensitivity analyses of the primary analysis, analyses with interim/post-interim populations and secondary endpoints with scales other than WI-NRS. In Study CR845-CLIN3103, the percentage of difelikefalin and placebo subjects achieving a ⩾3-point improvement (reduction) in WI-NRS at Week 12 were 54.0% and 42.2% (odds ratio = 1.61; P = .020). Absolute baseline scores were 7.1 (SD 1.36) for placebo and 7.3 (SD 1.36) for difelikefalin groups. The change at 12 weeks was -2,5 for placebo and -3.1 for active arms with a treatment difference of -0.6. Primary analysis results for interim and post-interim populations did differ substantially with insignificant treatment differences (OR 1.88 (p=0.62) and 1.42 (p=0.152)). Sensitivity analyses with patients who discontinued early being treated as non-responders resulted in a non-significant difference between active and placebo arms. This was concerning given the high discontinuation rate in active arms compared to placebo especially in this study. However, reasons for discontinuations were not related to safety or efficacy issues, thus they did not raise concerns regarding safety or lack of efficacy. Other two sensitivity analyses were supportive. The subjects using anti-itch medications at baseline had a greater treatment difference (odds ratio = 2.15; 95% CI, 1.09 to 4.25) in favour of difelikefalin than subjects not using anti-itch medications at baseline (odds ratio = 1.36; 95% CI, 0.84 to 2.20).

Secondary endpoints differed from study CR845-CLIN3102 in the extent and hierarchical order. The significant results seen with the proportion of subjects achieving a ⩾4-point improvement in WI-NRS from baseline at Week 12 and also with ⩾3-point improvement at week 8 and week 4 are supportive of the results for the primary analysis. The total 5-D Itch Scale score and the total Skindex-10 Scale score were first two secondary endpoints for study CR845-CLIN3102, but this was not the case with study CR845-CLIN3103 and Skindex-10 Scale gave insignificant results and stopped the testing at that level.

The subgroup analyses were conducted using the individual or pooled data from CR845-CLIN3102 and CR845-CLIN3103 only with respect to ⩾3- and ⩾4-point changes in the WI-NRS at each timepoint and specifically at Week 12. A subgroup analysis of efficacy according to the baseline itch severity was presented: categorised to two groups - WI-NRS ⩾4 to <7: moderate itch; and WI-NRS ⩾7: severe itch. Based on these post-hoc analyses from week 12, it can be concluded that the primary and secondary endpoints have been achieved regardless of the baseline itch severity. The small observed differences on primary endpoint (and also achievement of ⩾4-point improvement from baseline in WI-NRS at Week 12) between treatment arms in patients above 65 years (n=279) were not considered significant. The regional analysis of primary endpoint and key secondary endpoint addressing proportion of subjects achieving a ⩾4-point improvement from baseline in WI-NRS at Week 12 in EU region was consistent with overall results. No paediatric patients have been treated with difelikefalin. The effects of hepatic impairment on the pharmacokinetics of difelikefalin has not been formally studied. Long-term effectiveness data were explored in the OLE of CR845-CLIN3102 for up to 52 weeks. After 52 weeks of Open-label treatment, the LS mean (standard error [SE]) change from baseline in total 5-D Itch Scale score was -6.9 (0.39) and -7.8 (0.39) for placebo/difelikefalin (n = 94) and difelikefalin/difelikefalin subjects (n = 90), respectively.

Pooled analyses were performed, however, were not prespecified. Some concerns are summarized here. In general, for the primary endpoint, there is a significantly higher amount of missing data in difelikefalin group in both pivotal studies despite similar number of patients per arm (24 and 29 patients in placebo groups versus 32 and 53 patients in difelikefalin groups, Table 12). There is minimal difference in primary endpoint for difelikefalin arms in between two pivotal trials (50.9%

versus 53.4%). The placebo estimates varied between studies by more than 10%. The difference between placebo arms of the pivotal studies is 14.3% (28.3% vs 42.6%) which is significant (larger than the effect size observed for primary endpoint in CR845-CLIN3103 (10.8%) and similar to the effect size in pooled dataset (15.9%)). A meta-analysis comparing active treatments versus placebo in chronic dermatological conditions with pruritus, showed that placebo treatment significantly reduced itch compared to baseline, with an overall reduction in itch severity of 24% (Van Laarhoven, et al 2015). Thus, itch can be reduced by placebo in a variety of chronic pruritic conditions by as much as 1.3 points out of 10 (95% CI 1.02-1.61) (Van Laarhoven et al, 2015). In the light of variability in placebo response, the Applicant discussed the clinical relevance of the 15.9% increase in responders for primary endpoint and 3 points difference as a cut off for primary endpoint. The Applicant has provided cumulative distribution function of the unimputed weekly WI-NRS change from baseline at Week 12 by baseline WI-NRS score and Patient Global Impression of Change (PGIC) categories in pooled data of both clinical studies CR845-CLIN3102 and CR845-3103. In a group of very severe baseline pruritus (WI-NRS ⩾9) for PGIC "minimally or much improved" the median WI-NRS change was -3.17.

A *post-hoc* analysis was performed documenting WI-NRS score improvement according to the location of measurement (dialysis unit vs home), showing no substantial difference between the measurements conducted at dialysis unit and at home in terms of WI-NRS score improvement. Further *post-hoc* analyses were conducted by the applicant following the list of questions. The ⩾3 points improvement in WI-NRS by baseline pruritus severity was demonstrated. This analysis has shown that although not statistically significant (p=0.065), the proportion of patients in the very severe subgroup (WI-NRS ⩾9) achieving ⩾3 points improvement was comparable to the overall population, 53 % vs 51 % respectively. Additionally, when the ⩾4 points improvement in WI-NRS by baseline pruritus severity was analyzed, the highest proportion of subjects achieving this endpoint was in the very severe subgroup (WI-NRS ⩾9) and this was statistically significant. Similarly, the highest proportion of subjects achieving ⩾5 points improvement was observed in this subgroup as well and this was statistically significant. It was also this subgroup where the highest mean reduction in WI-NRS points was observed from baseline. Furthermore, the applicant conducted post-hoc analyses based on the classification of the response using relative thresholds of 30% and 50%. These analyses have shown that the response is similar in the overall population and very severe subgroup, although not statistically significant in the latter.

2.5.7. Conclusions on the clinical efficacy

All phase 3 confirmatory studies for difelikefalin met their primary endpoints. The individual or pooled data from CR845-CLIN3102 and CR845-CLIN3103 with respect to ≥3- and ≥4-point changes in the WI-NRS have shown a modest but clinically relevant treatment effect for difelikefalin for patients with moderate-to-severe pruritus associated with chronic kidney disease in adult patients on haemodialysis. The OLE studies are regarded as supportive.

2.5.8. Clinical safety

2.5.8.1. Patient exposure

In the primary safety pool, almost 80% (79.7%, n=338) of difelikefalin treated patients had at least three months of treatment and 85.1% (n=361) of placebo patients. In the difelikefalin exposure safety pool, 412 HD patients were exposed to difelikefalin for at least 12 months, which is sufficient according to the ICH E1 guidance on population exposure. However, only three months exposure was placebo-

controlled. In the Primary safety pool, a total of 13.2% and 7.3% discontinued treatment early for difelikefalin and placebo, respectively during the 12 weeks study. Adverse events were the primary reason for early treatment discontinuation, and more so for the difelikefalin treated patients (6.4%) compared to placebo treated patients (3.8%). Only 1 patient was lost to follow-up. During the long-term treatment, in the difelikefalin exposure safety pool, 26.4% of patients discontinued treatment early (excluding the stopping by sponsor). Adverse events were one of the primary reasons for early treatment discontinuation in the difelikefalin exposure safety pool (9.3%) but also the other category (6.8%). The adverse event category has been pooled and consist of AEs, pregnancy, safety of subject may be compromised, and sponsor requested subject to discontinue due to safety concerns. Almost 1/3 of the population in the difelikefalin exposure safety pool did not complete the treatment because the studies CLIN3101, CLIN3102, and CLIN3103 was stopped early by sponsor due to filing to the FDA for Breakthrough therapy Designation. The stopping of the trials was unrelated to safety for these patients.

2.5.8.2. Adverse events

In the primary safety pool, slightly more difelikefalin treated patients than placebo treated patients reported TEAEs, i.e., any TEAE, non-fatal serious TEAEs, TEAEs leading to discontinuation of study treatment and TEAEs of special interest (fall, gait disturbance, mental status changes, dizziness, somnolence, seizure, syncope, unusual feeling/sensation, mood altered, tachycardia, and palpitations). Around 2/3 of patients reported at least one TEAE. 71.2% and 65.3% for difelikefalin and placebo, respectively. Although, slightly more patients treated with difelikefalin reported more adverse events, the placebo treated patients also had a high reporting of adverse events. This is in line with the rather comorbid patient population of patients treated with haemodialysis reporting hypertension (97.4% and 94.8% of pooled difelikefalin and pooled placebo subjects, respectively), anaemia of CKD (78.8% and 73.8%), diabetes (62.5% and 55.7%), hyperphosphataemia (58.0% and 56.6%), hyperparathyroidism secondary (51.9% and 54.0%), hyperlipidaemia (45.5% and 37.5%), gastroesophageal reflux disease (39.4% and 42.7%), hypotension (38.9% and 40.6%), iron deficiency anaemia (36.1% and 35.4%), nausea (35.6% and 40.1%). Non-fatal serious TEAEs were reported by 25.2% and 22.6% for difelikefalin and placebo, respectively. TEAEs of special interest were reported more in the difelikefalin group (17.7%) compared with placebo (13.0%). Severity and relatedness of TEAEs were almost similar between difelikefalin and placebo groups.Five (n=5; 1.2%) placebo treated patients died during the in the two phase 3 pivotal placebo-controlled studies compared with three (n=3; 0.7%) in the difelikefalin group. Overall, any TEAE, TEAEs leading to death, and nonfatal serious TEAEs was reported more in the difelikefalin exposure safety pool than in the primary safety pool, due to the longer observation time. Incidence rates are calculated for the events in the difelikefalin exposure safety pool due to different observation times in the studies and to be able to compare to historical data.

The most common (≥15% of the subjects) system organ classes of TEAEs were gastrointestinal disorders (25.9% for difelikefalin and 19.1% for placebo), infections and infestations (21.7%, and 21.5%, respectively), nervous system disorders (20.3% and 14.2%), and general disorders and administration site conditions (15.3% and 12.0%). The most common TEAEs reported by ≥ 2% of difelikefalin patients and with ≥1 percentage point higher than placebo was diarrhoea, dizziness, nausea, hyperkalemia, headache, somnolence and back pain. 10% or less of patients in the 12 weeks DB placebo-controlled study period reported these preferred terms. Most of these events were mild to moderate in severity. The incidences of diarrhoea, dizziness, and nausea were higher in the pooled difelikefalin group than in the pooled placebo group. Diarrhoea (9.0% for difelikefalin and 5.7% for placebo), dizziness (6.8% and 3.8%, respectively), and nausea (6.6% and 4.5%). Constipation was also reported by 2.6% and 1.7% for difelikefalin and placebo, respectively. The incidences of SAEs were low: diarrhoea – 0.7% for difelikefalin and 0.7% for placebo; dizziness – 0.2% and 0%,

respectively; somnolence – 0.2% and 0%; hyperkalaemia – 1.9% and 1.9%; There were no reports of serious TEAEs with nausea, headache, or back pain. During long term treatment in the difelikefalin exposure safety pool, diarrhoea and nausea were reported by up to 17% of difelikefalin treated patients. Vomiting by up to 13%. Fall was reported by up to 15% and dizziness by up to 13%. Constipation was reported by 4.4%. Most (>60%) were mild to moderate in severity. Somnolence was reported by less than 5% of patients. Hence, the most commonly reported adverse events in the safety pools presented here are GI-events diarrhoea and nausea; nervous system disorders dizziness, headache and somnolence, see table below.

Incidence of Treatment-emergent Adverse Events Occurring in ≥2% of Difelikefalin Subjects and With ≥1 Percentage Point Higher Incidence Than Placebo by Preferred Term - Primary Safety Pool (Population: Safety)

	CLIN3102 DB		CLIN3103 DB		Pooled	
Preferred Term	Placebo (N = 188) n (%)	CR845 0.5 mcg/kg (N = 189) n (%)	Placebo (N = 236) n (%)	CR845 0.5 mcg/kg (N = 235) n (%)	Placebo (N = 424) n (%)	CR845 0.5 mcg/kg (N = 424) n (%)
Subjects with any event	130 (69.1%)	142 (75.1%)	147 (62.3%)	160 (68.1%)	277 (65.3%)	302 (71.2%)
Diarrhoea	11 (5.9%)	19 (10.1%)	13 (5.5%)	19 (8.1%)	24 (5.7%)	38 (9.0%)
Dizziness	3 (1.6%)	13 (6.9%)	13 (5.5%)	16 (6.8%)	16 (3.8%)	29 (6.8%)
Nausea	9 (4.8%)	8 (4.2%)	10 (4.2%)	20 (8.5%)	19 (4.5%)	28 (6.6%)
Hyperkalaemia	9 (4.8%)	11 (5.8%)	6 (2.5%)	9 (3.8%)	15 (3.5%)	20 (4.7%)
Headache	4 (2.1%)	9 (4.8%)	7 (3.0%)	10 (4.3%)	11 (2.6%)	19 (4.5%)
Somnolence	5 (2.7%)	6 (3.2%)	5 (2.1%)	12 (5.1%)	10 (2.4%)	18 (4.2%)
Back pain	1 (0.5%)	7 (3.7%)	3 (1.3%)	4 (1.7%)	4 (0.9%)	11 (2.6%)

Note: Adverse events are coded using MedDRA version 22.0. The table is sorted by descending subject incidence in the Pooled CR845 0.5 mcg/kg column for preferred term. A subject is counted only once for each preferred term if he/she had multiple events of the same preferred term.
DB = Double-blind Phase; MedDRA = Medical Dictionary for Regulatory Activities.

More than 50% of reported TEAEs were of mild to moderate severity. Less than 14% reported a severe TEAEs in both the difelikefalin and placebo. In line with the longer exposure in the difelikefalin exposure safety pool, the nature of the severe TEAEs is different from the Primary safety pool. More infections (sepsis and pneumonia) rated as severe were seen over time along with cardiovascular events.

The phase 2 dose-finding study (CR845-CLIN2101 Part A) in HD patients with CKD-aP receiving 0.5, 1 or 1.5 mcg/kg for 8 weeks, did not show any dose effect on overall TEAEs. Treatment-emergent AEs with a dose-dependent increase were mental status change, somnolence, paresthesia, fatigue, and hypertension. In the other two phase 2 studies (CR845-CLIN2005 Part A, and PR-13A9-P2-A) evaluating different difelikefalin doses (treatment duration 1-2 weeks) in HD patients with CKD-aP, a higher incidence for TEAEs were seen for the 1.5 mcg/kg dose, although the differences were small. Preferred terms of TEAEs that appeared to show dose-related increases in incidence were vertigo, constipation, increased blood prolactin level, and decreased free thyroxine in PR-13A9-P2-A. Treatment discontinuations were higher in the 1.5 mcg/kg group in studies CR845-CLIN2101 Part A and PR-13A9-P2-A. Based on the dose-response trends observed with difelikefalin in CR845-CLIN2101 and the similar efficacy seen across dose groups, the 0.5 mcg/kg dose was selected for further development in the Phase 3 studies in subjects undergoing HD.

In the primary safety pool, the median time to onset was shorter (<10 days) for difelikefalin treated patients compared to placebo for TEAEs of special interest (dizziness, falls, mood changes, palpitations, seizures, somnolence, syncope and tachycardia), as well as for the selected TEAEs diarrhoea, hyperkalaemia and hypotension. Nausea and vomiting occurred earlier in the placebo group. See table below for the time to onset for the preferred terms listed in SmPC section 4.8 (except headache). Placebo and difelikefalin (DB) is from the Primary safety pool and difelikefalin (OL) is from the difelikefalin exposure safety pool:

Pts I 4.8	placebo	Difelikefalin (DB)	Difelikefalin (OL)
Median time to onset (days)			
Dizziness	37	22	63
Somnolence	29	17	19
Diarrhoea	41	22.5	57
Nausea	29	39.5	90
Mental status changes	16	18.5	124

The median event duration for any category of TEAE of special interest in the pooled difelikefalin group of the Primary Safety Pool ranged from 0.80 day for syncope to 20.50 days for somnolence, with the upper limit of the range being consistent with that in the pooled difelikefalin group in the difelikefalin Exposure Safety Pool. Palpitations and gait disturbance had a longer median duration in placebo treated patients.

Pts I 4.8	placebo	Difelikefalin (DB)	Difelikefalin (OL)
Median time to onset (days)			
Dizziness	1	1	2
Somnolence	29.5	20.5	22
Diarrhoea	3	3	5
Nausea	4	2	3
Mental status changes	4	4	4.5

The incidence and prevalence were evaluated for patients with continuous exposure for 6 and 12 months. Both the incidence and prevalence diminished over time for dizziness, somnolence, diarrhoea, nausea and vomiting. Falls were reported at a constant rate throughout the studies.

Overall, the median duration of the adverse drug reactions listed in SmPC section 4.8 is a few days except for somnolence, with a median duration of around 3 weeks (range 1-86 days in primary safety pool and 0.2-312 days in the difelikefalin exposure safety pool). However, the median duration of somnolence in the placebo group was even longer 29.5 days (range 1-84 days). In addition, incidence and prevalence of somnolence seemed to diminish over time. Somnolence was reported by 4.2% of difelikefalin patients compared to 2.4% of placebo treated patients. Although, median duration for somnolence was the same for both placebo and difelikefalin, the TTO was shorter for difelikefalin.

Adverse events of special interest: TEAEs of special interest included fall, gait disturbance, mental status changes, dizziness, somnolence, seizure, syncope, unusual feeling/sensation, mood altered, tachycardia, and palpitations. Data from the primary safety pool showed that dizziness and somnolence were reported more in the difelikefalin treated patients compared to placebo. These two preferred terms have been included in section 4.8 of the SmPC. Also falls were reported by around 5% of patients, although equally in the two treatment arms. Gait disturbance and mental status changes

were also reported more in the difelikefalin treated group, although small numbers (<2%). Mental status changes are included in the SmPC in section 4.8. In the long-term safety evaluation, 11.2% of difelikefalin patients reported falls. Incidence rates for falls were similar in the Primary safety pool and the difelikefalin exposure safety pool (IR difelikefalin 265.2 and 255.1 events/1000 PY; IR for placebo was 207.8 events/1000PY). In addition, the Applicant has referenced literature that shows even higher fall rates among patients treated with HD than found in these studies. In conclusion, the AESIs have been evaluated further by looking at custom MedDRA queries (CMQs), since the standardised MedDRA queries (SMQs) for the areas of interest were found too broad. The fall and potentially drug-related injury CMQ was comparable in difelikefalin and placebo treated patients (7.8% for difelikefalin and 6.4% for placebo), as well were the incidence rates. Hence, there is no indication that the occurrence of falls is related to study treatment and acceptable not to include in the SmPC section 4.8.

Based on adverse events seen in the pivotal phase 3 double-blind studies, SMQs for acute central respiratory depression, cardiac arrythmias, cardiac failure and vestibular disorders have been evaluated. In the SMQs, there is an imbalance for serious TEAEs for atrial fibrillation in the Primary Safety Pool with an incidence rate (IR) of 30.6 events per 1000 PY in the difelikefalin treated patients compared with an incidence rate of 9.9 events/1000 PY in placebo patients. In the difelikefalin exposure safety pool, the IR for serious TEAEs of atrial fibrillation is 13.6 events/1000 PY and the literature suggests an IR of 12.5 events/1000 PY (Abbot, 2003). Hence, the IR for serious adverse events for AF was higher in the Primary safety pool. However, the overall number of patients is small: 3 difelikefalin (n=424) and 1 placebo (n=424) patients reported an SAE of atrial fibrillation (PT) in the placebo-controlled phase, and 11 in the difelikefalin exposure safety pool (n=1306). The IR is higher in difelikefalin patients (30.9 events/1000 PY) compared to placebo (9.9 Events/1000 PY), the difelikefalin exposure safety pool (13.6 events/1000 PY) and the literature (12.5 events/1000 PY for hospitalisation for AF), but as mentioned, this higher IR in the placebo study is based on 3 vs 1 patient.

IR for cardiac failure (SMQ) SAEs were also higher in difelikefalin treated patients: 49.5 events/1000 PY for placebo, 102 and 72.7 for difelikefalin in the DB and OL phase, respectively. USRDS data from 2013 suggest a rate of 140 events/1000 PY for hospitalisation for heart failure in HD patients. Hence, although there is a higher incidence rate of serious adverse events of heart failure (cardiac failure SMQ) in the difelikefalin patients vs placebo patients (49.5 vs 102 events/1000 PY), this rate is lower than the IR seen in the background population (based on USRDS, 2013).

Treatment emergent adverse events considered related to study drug: In the primary safety pool, any event of TEAEs were considered related to study drug for 6.4% placebo patients and 8.0% difelikefalin patients. In the difelikefalin exposure safety pool 6.6% patients reported TEAEs that was judged to be related to study drug. In the Primary safety pool, 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. Same picture was seen in the difelikefalin exposure safety pool: 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 paraesthesia (0.5%). Some patients also reported related constipation, 2 patients in both the placebo and difelikefalin group, and 5 patients in the pooled difelikefalin group.

Adverse drug reactions: Somnolence, dizziness, headache, nausea, diarrhoea, mental status changes (including confusional state), vomiting and paraesthesia (including paraesthesia, hypoesthesia, paraesthesia oral and hypoesthesia oral) were included in section 4.8 as adverse drug reactions.

2.5.8.3. Serious adverse event/deaths/other significant events

Serious adverse events

During the two pivotal double-blind studies, 107 subjects (25.2%) in the pooled difelikefalin group reported at least 1 nonfatal serious TEAE (SAE), compared to 96 subjects (22.6%) in the pooled placebo group. In total, 200 and 188 SAEs were reported and the incidence rates (IR) for SAEs were 2040 and 1860 events/1000 PY, for difelikefalin and placebo, respectively. Hence, more difelikefalin treated patients reported a serious adverse event compared with placebo during the 12 weeks DB period. The IR of any SAE in the difelikefalin exposure safety pool was 1824.3 events /1000 PY, same IR as placebo group.

In the Primary safety pool, the most common (≥4% of subjects) system organ classes of SAEs in patients 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%). As also seen in the standardised MedDRA queries (SMQs) discussed earlier, events classified as cardiac disorders were reported more frequently in the pooled difelikefalin group than in the pooled placebo group (incidence rate of 244.8 and 128.6 events/1000 PY, respectively).

The most common (≥1% of subjects) preferred terms of SAEs were chest pain (1.9% for difelikefalin and 0.9% for placebo), hyperkalaemia (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 the SAEs 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). The picture of serious adverse events reported, corresponds well to the patient population in question, and there are imbalances that both disfavours difelikefalin and placebo. Apart from an imbalance in cardiac disorders, which are discussed later, the profile for SAEs reported are as expected.

Incidence of Nonfatal Serious Treatment-emergent Adverse Events by System Organ Class and Preferred Term for Preferred Terms With ≥10 Subjects in the Pooled Difelikefalin Treatment Group - Difelikefalin Exposure Safety Pool (Population: Safety)

System Organ Class Preferred Term	CLIN3101 CR845 0.5 mcg/kg (N = 288) n (%)	CLIN3102 DB+OLE CR845 0.5 mcg/kg (N = 351) n (%)	CLIN3103 DB+OLE CR845 0.5 mcg/kg (N = 445) n (%)	CLIN3105 CR845 0.5 mcg/kg (N = 222) n (%)	Pooled CR845 0.5 mcg/kg (N = 1306) n (%)
Subjects with any event	145 (50.3%)	192 (54.7%)	162 (36.4%)	43 (19.4%)	542 (41.5%)
Blood and lymphatic system disorders	5 (1.7%)	5 (1.4%)	5 (1.1%)	2 (0.9%)	17 (1.3%)
Anaemia	3 (1.0%)	3 (0.9%)	5 (1.1%)	2 (0.9%)	13 (1.0%)
Cardiac disorders	40 (13.9%)	40 (11.4%)	21 (4.7%)	6 (2.7%)	107 (8.2%)
Acute myocardial infarction	13 (4.5%)	5 (1.4%)	7 (1.6%)	1 (0.5%)	26 (2.0%)
Cardiac failure congestive	5 (1.7%)	11 (3.1%)	2 (0.4%)	0	18 (1.4%)
Angina pectoris	8 (2.8%)	7 (2.0%)	1 (0.2%)	1 (0.5%)	17 (1.3%)
Atrial fibrillation	4 (1.4%)	5 (1.4%)	2 (0.4%)	0	11 (0.8%)
Gastrointestinal disorders	24 (8.3%)	39 (11.1%)	26 (5.8%)	5 (2.3%)	94 (7.2%)
Gastrointestinal haemorrhage	6 (2.1%)	8 (2.3%)	5 (1.1%)	2 (0.9%)	21 (1.6%)
Abdominal pain	4 (1.4%)	9 (2.6%)	4 (0.9%)	0	17 (1.3%)
Diarrhoea	1 (0.3%)	5 (1.4%)	4 (0.9%)	0	10 (0.8%)
General disorders and administration site conditions	18 (6.3%)	25 (7.1%)	22 (4.9%)	2 (0.9%)	67 (5.1%)
Asthenia	4 (1.4%)	10 (2.8%)	4 (0.9%)	1 (0.5%)	19 (1.5%)
Chest pain	1 (0.3%)	3 (0.9%)	15 (3.4%)	0	19 (1.5%)
Non-cardiac chest pain	8 (2.8%)	7 (2.0%)	0	1 (0.5%)	16 (1.2%)
Infections and infestations	55 (19.1%)	71 (20.2%)	70 (15.7%)	17 (7.7%)	213 (16.3%)
Pneumonia	12 (4.2%)	25 (7.1%)	23 (5.2%)	2 (0.9%)	62 (4.7%)
Sepsis	9 (3.1%)	10 (2.8%)	14 (3.1%)	3 (1.4%)	36 (2.8%)
Cellulitis	6 (2.1%)	5 (1.4%)	3 (0.7%)	0	14 (1.1%)
Osteomyelitis	5 (1.7%)	4 (1.1%)	3 (0.7%)	2 (0.9%)	14 (1.1%)
Septic shock	3 (1.0%)	5 (1.4%)	4 (0.9%)	0	12 (0.9%)
Influenza	1 (0.3%)	3 (0.9%)	4 (0.9%)	2 (0.9%)	10 (0.8%)
Injury, poisoning and procedural complications	25 (8.7%)	33 (9.4%)	26 (5.8%)	8 (3.6%)	92 (7.0%)
Arteriovenous fistula thrombosis	5 (1.7%)	2 (0.6%)	5 (1.1%)	3 (1.4%)	15 (1.1%)
Fall	3 (1.0%)	6 (1.7%)	3 (0.7%)	2 (0.9%)	14 (1.1%)
Arteriovenous fistula site complication	1 (0.3%)	3 (0.9%)	7 (1.6%)	0	11 (0.8%)
Vascular access malfunction	3 (1.0%)	7 (2.0%)	1 (0.2%)	0	11 (0.8%)

Incidence of Nonfatal Serious Treatment-emergent Adverse Events by System Organ Class and Preferred Term for Preferred Terms With ≥10 Subjects in the Pooled Difelikefalin Treatment Group - Difelikefalin Exposure Safety Pool (Population: Safety) (Cont'd)

System Organ Class Preferred Term	CLIN3101 CR845 0.5 mcg/kg (N = 288) n (%)	CLIN3102 DB+OLE CR845 0.5 mcg/kg (N = 351) n (%)	CLIN3103 DB+OLE CR845 0.5 mcg/kg (N = 445) n (%)	CLIN3105 CR845 0.5 mcg/kg (N = 222) n (%)	Pooled CR845 0.5 mcg/kg (N = 1306) n (%)
Metabolism and nutrition disorders	35 (12.2%)	36 (10.3%)	21 (4.7%)	6 (2.7%)	98 (7.5%)
Fluid overload	17 (5.9%)	18 (5.1%)	6 (1.3%)	2 (0.9%)	43 (3.3%)
Hyperkalaemia	15 (5.2%)	14 (4.0%)	8 (1.8%)	5 (2.3%)	42 (3.2%)
Nervous system disorders	23 (8.0%)	40 (11.4%)	21 (4.7%)	6 (2.7%)	90 (6.9%)
Syncope	7 (2.4%)	8 (2.3%)	4 (0.9%)	3 (1.4%)	22 (1.7%)
Metabolic encephalopathy	1 (0.3%)	11 (3.1%)	2 (0.4%)	0	14 (1.1%)
Psychiatric disorders	12 (4.2%)	15 (4.3%)	9 (2.0%)	1 (0.5%)	37 (2.8%)
Mental status changes	8 (2.8%)	10 (2.8%)	7 (1.6%)	1 (0.5%)	26 (2.0%)
Respiratory, thoracic and mediastinal disorders	23 (8.0%)	43 (12.3%)	32 (7.2%)	7 (3.2%)	105 (8.0%)
Respiratory failure	8 (2.8%)	11 (3.1%)	9 (2.0%)	2 (0.9%)	30 (2.3%)
Dyspnoea	4 (1.4%)	12 (3.4%)	9 (2.0%)	0	25 (1.9%)
Chronic obstructive pulmonary disease	4 (1.4%)	6 (1.7%)	7 (1.6%)	1 (0.5%)	18 (1.4%)
Pulmonary oedema	6 (2.1%)	5 (1.4%)	0	1 (0.5%)	12 (0.9%)
Acute respiratory failure	3 (1.0%)	6 (1.7%)	0	1 (0.5%)	10 (0.8%)
Pleural effusion	2 (0.7%)	4 (1.1%)	4 (0.9%)	0	10 (0.8%)
Vascular disorders	26 (9.0%)	26 (7.4%)	24 (5.4%)	4 (1.8%)	80 (6.1%)
Hypotension	9 (3.1%)	9 (2.6%)	5 (1.1%)	1 (0.5%)	24 (1.8%)
Hypertensive urgency	2 (0.7%)	6 (1.7%)	2 (0.4%)	2 (0.9%)	12 (0.9%)

Notes: Adverse events are coded using MedDRA version 22.0. The table is sorted alphabetically by system organ class and descending subject incidence in the Pooled CR845 0.5 mcg/kg column for preferred term. A subject is counted only once for each preferred term if he/she had multiple events of the same preferred term.
DB = Double-blind Phase; MedDRA = Medical Dictionary for Regulatory Activities; OLE = Open-label Extension Phase.

Looking at the long-term safety in the difelikefalin exposure safety pool, 41.5% reported at least one SAE, which is, as expected, higher than the around 25% in the placebo-controlled studies of 12 weeks duration. As stated above the overall IR was comparable to placebo in the 12 weeks placebo-controlled studies: the IR of any SAE in the difelikefalin exposure safety pool was 1824.3 events /1000 PY, and in the placebo group it was 1860 events/1000 PY. The most common (≥8% of subjects) system organ classes of SAEs in the pooled difelikefalin group of the Difelikefalin Exposure Safety Pool aligned with those in the pooled difelikefalin group of the Primary Safety Pool.

Major adverse cardiovascular events (MACE): Primary safety pool: Patients experiencing a MACE event (MACE event includes non-fatal stroke and MI, CV-death, heart failure and revascularisation) was 16 patients (3.8%, IR 193.8 events/1000 PY) vs 10 patients (2.4%, IR 128.6/1000 PY), in the difelikefalin and placebo group, respectively. The MACE events were driven by cardiac heart failure and myocardial infarction, and less by cerebrovascular events. In the difelikefalin exposure safety pool, 119 (9.1%; IR 199,7 events/1000 PY) patients experienced at least one MACE event (in total 162 MACE events). The IR of 199.7 events/1000 PY is in line with the IR seen for MACE in the primary safety pool (IR 193.8 events/1000 PY), despite the shorter observation time in the primary safety pool. The MACE events were driven by myocardial infarction and heart failure. Overall, a higher incidence of MACE was seen in the difelikefalin treated patients compared with placebo.

Looking at the SMQ for cardiac failure in the Primary safety pool, 20 difelikefalin patients (IR 234.6 events/1000 PY) vs 14 placebo patients (IR 158.3 events/1000 PY) reported event(s) of cardiac failure SMQ. For SAEs in SMQ for cardiac failure in the Primary safety pool, the incidence was 9 (2.1%, IR of

102/1000 PY) and 4 (0.9%, IR of 49.5/1000 PY) for difelikefalin and placebo, respectively. USRDS data from 2013 suggest a rate of 140 events/1000 PY for hospitalisation for heart failure in HD patients. Hence, although there is a higher incidence rate of SAEs of heart failure (cardiac failure SMQ) in the difelikefalin patients vs placebo patients, this rate is lower than the IR seen in the background population based on USRDS, 2013.

Within the SMQ of cardiac failure, patients are reporting TEAEs within the different PTs. The imbalance between difelikefalin and placebo pertains to a few more difelikefalin patients reporting events of cardiac failure and cardiac failure acute (N=4). 3 of these 4 events are serious with one being fatal. The events were reported by patients with known comorbidities including previous heart failure. All events occurred concurrently with other significant medical events.

The applicant has evaluated the cardiac failure SMQ further by looking at the reporting of cardiac failure SMQ for patients who was treated with placebo in the DB phase and then crossed over to difelikefalin in the open label phase of the two pivotal studies. The incidence of TEAEs in the Cardiac Failure SMQ remained the same for placebo subjects who crossed over to difelikefalin treatment. 3.0% during the Double-blind Phase and 3.0% during the first 3 months of the Open-label Extension Phase. Similarly, there were no events of cardiac failure or acute cardiac failure in the placebo group during the double-blind treatment Phase and once subjects crossed over to difelikefalin for the first 3 months, there were no events of cardiac or acute cardiac failure. The rates for the other common preferred terms showed no notable increase over those in the Primary Safety Pool. 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; Table 12), in which study the patients had a longer period on dialysis, which may have contributed to increased adverse cardiac outcomes. Pharmacologically, KOR agonists as aquaretic agents promoting diuresis in patients with normal functioning nephrons and potentially could be cardiac protective. In conclusion, small imbalances are consistently seen for adverse events related to cardiac safety. The information on cardiac safety was reflected in the SmPC and in the adopted RMP.

Adverse events leading to death: In total (all studies pool), 59 patients treated with difelikefalin died while on treatment with difelikefalin (3.1%; n=1879; IR 69.0 events/1000 PY) compared with 6 patients on placebo (0.8%; n=714; IR 50.8 events/1000 PY). All deaths occurred in patients treated with HD. Note that placebo patients were observed for a maximum of 12 weeks, whereas difelikefalin treated patients had a much longer observation time (up to 15 months), why it is difficult to compare the incidence rates for placebo and difelikefalin for the long-term effects such as fatal events. Since there are no placebo control longer than 12 weeks, a literature reference to incidence rates for e.g. deaths and cardiovascular events is needed in order to be able to evaluate if difelikefalin has any impact on these kinds of events. The Applicant has used references from different sources, e.g. the United States Renal Data System (USRDS), which is a database on patients treated with haemodialysis in the US. Overall, cardiac events and infections were the primary reasons for the fatal events, in line with what is known as the primary causes if deaths in patients on HD. For the 6 placebo treated patients, 1 patient died from cardiac arrest and 3 from infections. Of the 59 patients, who died on difelikefalin treatment, 28 patients died from cardiac disorders and 13 patients from infections. All fatal events in the studies were also evaluated as not related to study treatment by the investigator.

Nevertheless, for deaths within cardiac disorders SOC, the IR was 3 times higher for difelikefalin than placebo (33.1 vs 8.7 events/1000 PY in HD patients, all studies pool).

Hence, there is an imbalance in deaths due to cardiac disorders disfavouring difelikefalin, with incidence rates for deaths due to cardiac disorders being trice the IR seen in placebo patients. However, short observation times in the placebo studies (up to 12 weeks), are for the occurrence of deaths a rather short observation time, and therefore the IRs are difficult to compare to the longer

exposure time for difelikefalin treated patients. To have a reference of IRs for this patient population of HD patients, the USRDS (United States Renal Data System) has statistics on e.g. CV events in HD patients.

The incidence rate of fatal TEAEs in the overall difelikefalin group, 69.0 events/1000 PY, is consistent with or lower than death rates reported in the literature for patients undergoing HD, eg, 167 events/1000 PY (unadjusted IR of death in US patients undergoing HD; USRDS, 2019) or 132 events/1000 PY (all-cause mortality of European HD patients, Stirnadel-Farrant, 2019).

In the All studies pool, the most common preferred term leading to death was cardiac arrest (11 subjects [0.8%, IR of 13.6/1000 PY]) and cardio-respiratory arrest (3 subjects [0.2%, IR of 3.7/1000 PY]). The USDRS reports cardiac arrest as the most common reason for death in subjects undergoing HD, with an incidence of 53.5/1000 PY (USRDS, 2019). Hence, the IR for cardiac arrest are comparable with those data reported in the literature.

In addition, the Study CLIN3101 had the highest number of cardiac-related deaths (12 [4.2%]) compared to the other studies (CLIN3102 DB +OLE: 7 [2.0%] and CLIN3103 DB+OLE 7 [1.6%]); there were no cardiac-related deaths in Study CLIN3105, (12 weeks study). Of note, patients in CLIN3101 had a slightly longer dialysis vintage, with a mean duration of 6.21 years compared to subjects in the other Phase 3 studies, which ranged from a mean of 4.59 years in CLIN3102 DB+OLE to 5.42 years in CLIN3105.

In conclusion, more deaths occurred in the difelikefalin group compared with placebo, mostly due to cardiovascular events and infections. Due to shorter observation times in the placebo-controlled studies, the IR on deaths cannot easily be compared between difelikefalin and placebo. When comparing to historical data from large databases on dialysis patients, e.g. the USRDS, the IR of CV deaths are in line with what you would expect in this patient population.

2.5.8.4. Laboratory findings

In the studies, haemoglobin was defined as high if exceeding a value of 14 g/dL. It is recommended in guidelines, e.g. from KDIGO that haemoglobin levels should be kept lower than in healthy subjects, due to increased risk of thromboembolic events. In the primary placebo pool, more difelikefalin patients had a haemoglobin value above 14 g/dL than placebo 7 (1.7%) vs 3 (0.8%) patients, but no one experienced a thromboembolic event in the DB phase. In the difelikefalin exposure safety pool 39 patients (3.1%) had a haemoglobin value above 14 g/dL, and 3 patients experienced a serious event of thrombosis. Potassium was also evaluated, the critical high value being >7 mmol/L. In the primary safety pool, this critical value was observed in 11 (2.8%) and 4 (1.0%) patients for difelikefalin and placebo, respectively. No one reported any TEAE within cardiac disorders in the DB phase. In the Difelikefalin Exposure Safety Pool, 54 subjects (4.4%) experienced a treatment-emergent potassium value >7 mmol/L. Two subjects 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

It is expected that co-morbidities, such as lower Gi bleeding, pneumonia and multi-organ failure, are the likely reasons for the observed liver abnormalities, including the fact that the patient was treated with difelikefalin for almost one year, with normal liver parameters. Hence, difelikefalin does not seem to impact liver parameters to a clinically relevant extent.

Blood pressure and heartbeat were measured throughout the studies. As expected, these parameters fluctuated over the time course of the studies, in both the placebo and difelikefalin groups. No safety concerns aroused from the vital signs picture. A thorough QT/QTc study did not show any effects from

difelikefalin on heart rate, PR and QRS interval duration, cardiac repolarization, or other ECG parameters.

2.5.8.5. Safety in special populations

The Applicant has presented a number of subgroup analyses in the Primary safety pool evaluating intrinsic and extrinsic factors on the incidence of TEAEs (65 and ≥65 years, sex, race, renal and hepatic impairment and other pre-existing specific medical conditions; geography, use of prior ant-itch medication, concomitant use of opioids and CNS-depressant medication and use of other drugs [drug-drug interactions]).

Overall, these data do not suggest that a dose adjustment is necessary based on intrinsic/extrinsic factors subgroup analyses of TEAEs and serious TEAEs evaluated. A few differences in the reporting of TEAEs was noted, e.g. more headache in female patients compared to male patients treated with difelikefalin and more somnolence in patients ≥65 years of age, patients with prior use of anti-itch medication and concomitant use of opioids.

Renal and hepatic impairment: Difelikefalin is intended for use in the dialysis population. Oral difelikefalin was evaluated in patients with CKD stages 3-5, and no unexpected safety signals were observed. Likewise, was oral difelikefalin well tolerated in patients with mild, moderate and severe hepatic impairment.

Around 2/3 of patients in the Primary safety pool were aged <65 years. Patients ≥65 years of age reported a few more TEAEs than patients <65 years of age. Somnolence was reported more by the ≥ 65-year-old patients, patients treated with prior use of anti-itch medication and patients with concomitant use of opioids.

Use in Pregnancy, Lactation and Fertility: For difelikefalin no or very limited amount of data on pregnant patients are available. Animal studies do not indicate direct or indirect harmful effects with respect to reproductive toxicity.It is unknown whether difelikefalin is excreted in human breast milk. Animal studies have shown excretion of difelikefalin in breast milk.

Overdose: Single dose of difelikefalin up to 12 times and multiple doses of difelikefalin up to 5 times the clinical dose of 0.5 mcg/kg were administered in clinical studies in subjects undergoing HD. A dose-dependent increase in adverse events including dizziness, somnolence, mental status changes, paraesthesia, fatigue, hypertension, and vomiting, was observed in study CR845-CLIN1003. Difelikefalin is cleared by dialysis, however the use of dialysis as a treatment for difelikefalin overdose has not been studied.

Drug Abuse: The clinical data include a systematic review of AE preferred terms suggestive of the potential for abuse in all clinical studies in subjects who received at least 1 dose of difelikefalin and a dedicated human abuse-potential study in healthy recreational polydrug users with opioid and hallucinogenic drug experience in which difelikefalin doses 10- and 30-fold the planned clinical dose were evaluated relative to pentazocine, a mixed partial mu opioid receptor (MOR) agonist/antagonist with KOR agonist activity, and placebo (Study CR845-CLIN1006). The human abuse potential study in recreational polydrug users with opioid and hallucinogen experience demonstrated that difelikefalin, at doses of 5 and 15 mcg/kg IV (resulting in peak exposures that were 10.5- and 28.6-fold higher that at the proposed human IV dose of 0.5 mcg/kg in subjects with CKD-aP undergoing HD) had an abuse potential profile that was significantly lower than with pentazocine. There were no dysphoric or euphoric effects reported that would be consistent with CNS-penetrant kappa agonists or mu opioids, respectively.

In all clinical studies of difelikefalin, there were no AE reports related to misuse, abuse, diversion, or dependence with IV difelikefalin at dose up to 80-fold the clinical dose for the proposed indication. Paraesthesia, hypoaesthesia, dizziness, somnolence and sedation (not confirmed by sedation scale) were the most commonly and consistently potentially abuse-related TEAEs reported following single and repeated administration of IV difelikefalin in healthy subjects (pooled population of 376 subjects) at doses resulting in peak and area-under-the-curve exposures up to 60-fold and 25-fold those at the proposed human IV dose of 0.5 mcg/kg in subjects with CKD-aP undergoing HD, respectively. Dizziness and somnolence were also the most common and dose-related abuse-related TEAE in subjects with CKD-aP undergoing HD, followed by events of paraesthesia and hypoaesthesia occurring at much lower incidence compared to healthy subjects.

Confusional state was reported at a higher incidence in subjects with CKD-aP undergoing HD, 1.4% among difelikefalin-treated subjects compared with 0.5% in placebo-treated subjects but did not appear to be dose-related.

Withdrawal and rebound: Clinical assessments of physical dependence of difelikefalin were conducted in two double-blind clinical studies, including assessment of a 2-week Discontinuation Period in a phase 3 study of subjects with CKD-aP undergoing HD (CR845-CLIN3102) and a dedicated physical dependence study in which subjects undergoing HD were randomized in a double-blind fashion to withdrawal of difelikefalin (CR845-100303). The results of the clinical assessments of physical dependence in 2 double-blind clinical studies indicated that abrupt discontinuation of difelikefalin was not associated with physical withdrawal signs or symptoms. The overall incidence of Discontinuation-emergent Adverse Events was similar between placebo and difelikefalin treatment groups in double-blind studies utilizing the IV formulation in CKD-aP subjects undergoing dialysis, as well as studies utilizing the oral formulation in subjects with chronic pain or CKD.

2.5.8.6. Immunological events

Difelikefalin is a small molecule, and an immunological assessment is not relevant.

2.5.8.7. Safety related to drug-drug interactions and other interactions

No clinical drug-drug interaction studies are performed. The major pathway for clearance of difelikefalin is excretion of the unchanged parent compound into urine dialysate and/or faeces. Nonclinical data suggest that difelikefalin exhibits negligible potential for PK drug-drug interactions based on the cytochrome P450 metabolic profile. Similarly, the absence of difelikefalin interactions with multiple transporter proteins suggests that difelikefalin will have no interactions with other drugs that are substrates for these clinically relevant transporters. In the primary safety pool, somnolence and dizziness was reported more by patients with prior use of anti-itch medication and patients with concomitant use of opioids.

2.5.8.8. Discontinuation due to adverse events

Overall, less than 10% of patients discontinued study drug due to adverse events during the 12 weeks of treatment in the DB, placebo-controlled primary safety pool and the up to 52 weeks treatment in the difelikefalin exposure safety pool. More difelikefalin treated patients discontinued study drug compared to placebo-treated patients. In the primary safety pool, dizziness, nausea, headache, anxiety, insomnia and mental status changes were reported as reasons for discontinuing the study treatment, although in less than 1% of patients in both difelikefalin and placebo patients. In the difelikefalin exposure safety pool, difelikefalin patients reported reasons for discontinuing difelikefalin dizziness and nausea along

with cardiovascular events and deaths, somnolence and respiratory failure. All events in less than 1% of patients. Hence, study treatment was discontinued due to adverse events that are related to the study treatment as well as for reasons related to the study population, i.e. cardiovascular events, which occurs frequently in these patients treated with haemodialysis.

2.5.8.9. Post marketing experience

Difelikefalin has not been marketed in the EU yet.

2.5.9. Discussion on clinical safety

Safety of difelikefalin was evaluated in 18 completed clinical studies and 1879 patients received at least 1 dose of difelikefalin, of whom 1592 were undergoing Haemodialysis (HD), the target population for difelikefalin in this MAA. Safety data were pooled in 5 relevant study groupings for the safety assessment. The two pivotal studies were placebo-controlled (primary safety pool), but only for 12 weeks, hence for the more long-term adverse events e.g. deaths, the safety profile was to be evaluated against historical data.

Four clinical studies comprised the evaluation of long-term safety (difelikefalin exposure safety pool), and sufficient number of patients (n=412) were exposed to difelikefalin in at least 12 months, in accordance with the ICH E1 guidance. However, the early treatment discontinuation rates were rather high in three of the studies (24-34%); in addition, several categories have been pooled, so the early discontinuation reasons were not entirely clear, but this was not further pursued by the CHMP.

Adverse events of special interest related to the pharmacology of difelikefalin as a kappa opioid receptor agonist was evaluated along with the abuse potential, although there seems to be a limited CNS penetration. In addition, due to the occurrence of cardiac events, cardiac safety was also evaluated separately. Overall, the safety data seem robust, and the applicant has addressed the safety issues thoroughly.

Adverse events: The safety profile observed is to be seen in the light of the rather co-morbid patient population of dialysis patients where >80% were above 45 years, the median number of years since diagnosis of ESRD was 3.99 years (range 0.3 to 30.2 years), with a median of 6.64 years (range 0.3 to 48.3 years) since diagnosis of CKD, and a median of 3.67 years (range 0.0 to 30.2 years) on chronic HD. The most common (≥20%) of subjects' aetiologies of CKD were concomitant diabetes and hypertension (26.5%); hypertension only (25.2) and diabetes only (20.5%). Around 65.3% placebo patients and 71.2% difelikefalin patients reported at least one TEAE during the 12 weeks double-blind phase. Diarrhoea, nausea, paraesthesia, dizziness, hyperkalemia, headache, and somnolence were the most commonly reported events. Hyperkalaemia has been reflected in section 4.4. of the SmPC, monitoring of patients is advised. Most were mild to moderate in severity and of short duration (few days) except for somnolence that had a median duration of 3 weeks.

Somnolence and paraesthesia (including paraesthesia, hypoesthesia, paraesthesia oral and hypoesthesia oral) were evaluated as a common adverse drug reaction. Dizziness, headache, nausea, diarrhoea, vomitting and mental status changes as uncommon ADRs listed in the SmPC section 4.8. Overall, the median duration of these adverse drug reactions is a few days except for somnolence that has a median duration of around 3 weeks (range 1-86 days in primary safety pool and 0.2-312 days in the difelikefalin exposure safety pool). The median duration of somnolence in the placebo was even longer 29.5 days (range 1-84 days). In addition, incidence and prevalence of somnolence seemed to diminish over time. Somnolence was reported by 4.2% of difelikefalin patients compared to 2.4% of placebo treated patients, and more by the ≥ 65-year-old patients, patients treated with prior use of

anti-itch medication and patients with concomitant use of opioids. Although, median duration for somnolence was the same for both placebo and difelikefalin, the time to onset was shorter for difelikefalin. The observed CNS related adverse events indicate some distribution to CNS. However, dizziness and somnolence were commonly present in the medical history of the CKD-aP subjects and it is generally accepted that the CNS TEAES seen in the studies are prevalent in the population treated.

Serious adverse events: During the 12 weeks of double-blind treatment, 107 subjects (25.2%) in the pooled difelikefalin group reported at least 1 non-fatal serious adverse event (SAE), compared to 96 subjects (22.6%) in the pooled placebo group. In total, 200 and 188 SAEs were reported and the incidence rates (IR) for SAEs were 2040 and 1860 events/1000 PY, for difelikefalin and placebo, respectively. In the difelikefalin exposure safety pool, 41.5% reported at least one SAE. More difelikefalin treated patients reported a serious adverse event compared with placebo during the 12 weeks DB period. The IR of any SAE in the difelikefalin exposure safety pool was 1824.3 events /1000 PY, same IR as placebo group. Although it is difficult to compare the IR due to different observation times in the two safety pools, it does not seem that the incidence of SAEs increases over time.

Cardiac safety: Cardiac safety was evaluated with cardiac disorders system organ class and preferred terms (SOC and PT), cardiac failure and cardiac arrhythmias SMQs and an evaluation of MACE events. The most frequently reported serious adverse events were related to cardiac disorders and infections, in line with the expected co-morbidities in the target population of HD patients. Small imbalances are consistently seen for adverse events related to cardiac safety, but no association of the cardiac events with the timing or dosage of difelikefalin treatment was seen. A causal relationship between difelikefalin and the cardiac events cannot be conclusively established – neither excluded. Mechanistically and due to nonclinical and clinical data of KOR agonists, the selective activation of KORs is not expected to adversely alter cardiac function in CKD-aP subjects. The applicant has committed to provide additional safety data from the planned Phase 3 trials of oral difelikefalin in other indications, avoiding the high background incidence of cardiac events confounding factors of the HD-population, post-approval (see RMP). This is agreed by the CHMP.

The underlying medical history, particularly the change of treatment (i.e. discontinuation or missing treatment) of atrial fibrillation in HD patients is considered as potential risk, which is included as a warning in the section 4.4 of the SmPC. Cardiac failure imbalances in SAEs in the cardiac failure SMQ (4 placebo and 9 difelikefalin patients) were observed, although total number of patients were few. IR for cardiac failure SMQ SAEs were higher in difelikefalin treated patients: (IR 49.5 events/1000 PY for placebo (n=4), and IR 102 events/1000 PY (n=9) and IR 72.7 events/1000 PY (n=59) for difelikefalin in the DB and OL phase, respectively. USRDS data from 2013 suggest a rate of 140 events/1000 PY for hospitalisation for heart failure in HD patients. Hence, although there is a higher incidence rate of SAEs of heart failure (cardiac failure SMQ) in the difelikefalin patients vs placebo patients, this rate is lower than the IR seen in the background population based on USRDS, 2013. The applicant has evaluated the cardiac failure SMQ further by looking at the placebo patients in the DB phase that crossed over to difelikefalin in the open label phase of the two pivotal studies. The incidence in the cardiac failure SMQ remained the same for placebo subjects who crossed over to difelikefalin treatment. Around 3.0% during the Double-blind Phase and 3.0% during the first 3 months of the Open-label Extension Phase.

MACE: Patients experiencing a MACE event were 16 patients (3.8%, IR 193.8 events/1000 PY) vs 10 patients (2.4%, IR 128.6/1000 PY), in the difelikefalin and placebo group, respectively. In the difelikefalin exposure safety pool, 119 (9.1%; IR 199,7 events/1000 PY) patients experienced at least one MACE event (in total 162 MACE events). The IR of 199.7 events/1000 PY is in line with the IR seen for MACE in the Primary safety pool (IR 193.8 events/1000 PY), despite the shorter observation time in the Primary safety pool. The MACE events were driven by cardiac heart failure and myocardial infarction, and less by cerebrovascular events. The imbalance was driven by 13 patients in CLIN3101 (OL study) who have had longer time on dialysis, and hence more prone to adverse cardiac events.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.5.10. Conclusions on the clinical safety

The clinical safety profile of difelikefalin was evaluated in 1879 subjects receiving at least one dose of difelikefalin, of whom 1306 haemodialysis patients with CKD associated pruritus received 0.5 µg/kg difelikefalin. By the entirety of available data, the safety profile of difelikefalin is considered favourable, although imbalances are seen for adverse events related to cardiac safety. This is adequately reflected in section 4.4 of the SmPC and the adopted RMP. Treatment emergent adverse events related to study drug and listed in SmPC section 4.8 are somnolence (1.1%), paraesthesia (including hypoesthesia, paraesthesia oral and hypoesthesia oral) (1.1%), dizziness (0.9%), headache (0.6%), nausea (0.7%), vomiting (0.7%), diarrhoea (0.2%) and mental status changes (0.3%) and is considered satisfactory by the CHMP.

2.6. Risk Management Plan

Summary Table of Pharmacovigilance Activities and Risk Minimisation Activities by Safety Concern

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Cardiac failure and arrhythmias including atrial fibrillation in haemodialysis patients with a medical history of atrial fibrillation	Routine risk minimisation measures: • SmPC Section 4.4 • PIL Section 2 • Legal status: Prescription only medicine. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Targeted Questionnaire for Cardiac failure and arrythmias including atrial fibrillation in haemodialysis patients with a medical history of atrial fibrillation Additional pharmacovigilance activities: None
Use in pregnant and lactating women	Routine risk minimisation measures: • SmPC Section 4.6. • PIL Section 2 • Legal status: Prescription only medicine. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Specific adverse reaction follow-up questionnaire for use in pregnant women (Report on Exposure to Medicines During Pregnancy) Additional pharmacovigilance activities: None
Use in patients with impaired blood brain barrier	Routine risk minimisation measures: • SmPC Section 4.4. • PIL Section 2 • Legal status: Prescription only medicine. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Use in patients with severe hepatic impairment	Routine risk minimisation measures: • SmPC Section 4.2. • PIL Section 3 • Legal status: Prescription only medicine. Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None

Notes: PIL=Patient Information Leaflet; SmPC=Summary of Product Characteristics.

2.6.1. Conclusion

The CHMP considers that the risk management plan version 1.4 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 23 August 2021. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.8. Product information

2.8.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

2.8.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Kapruvia (difelikefalin) is included in the additional monitoring list as it contains a new active substance which was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Chronic kidney disease-associated pruritus (CKD-aP), or uremic pruritus, is characterised by a generalised and intractable itch, a frequently underdiagnosed but severely distressing condition that occurs in greater than 60% of patients undergoing dialysis. The aim of new treatments is to improve symptoms, whilst minimising the toxicity.

3.1.2. Available therapies and unmet medical need

The prevalence of CKD-aP remains high, despite improved efficiency of dialysis techniques, improved skin hydration with emollients, and use of phosphate binders and calcimimetics. There is currently no medicinal treatment approved specifically for CKD-aP in the EU though several medications have been used largely as off-label treatments for CKD-aP (e.g., antihistamines, corticosteroids, gabapentin, and pregabalin). The antipruritic efficacy of these therapies is limited and currently lacking support from randomised, well-controlled studies.

3.1.3. Main clinical studies

The main evidence of efficacy submitted is two phase III multicenter, randomized, double-blind studies (CR845-CLIN3102 and CR845-CLIN3103) comparing IV difelikefalin 0.5 mcg/kg (n=189 and 236) vs. placebo (n=189 and 237) in newly diagnosed or previously treated adult patients with end stage renal disease, undergoing haemodialysis and experiencing moderate-to-severe pruritus. The two studies were comparable in design, but subjects were included if they had WI-NRS scores of >4 for study CR845-CLIN3102 conducted in the US and ≥5 for study CR845-CLIN3103 conducted in US and 10 other countries. Patients received difelikefalin or placebo after each haemodialysis session, generally 3 times per week for up to 12 weeks (some continued up to 52 weeks as open-label treatment). Study CR845-CLIN3102 continued as planned with all subjects rolling over to open-label treatment for up to 52 additional weeks.

3.2. Favourable effects

Dose finding was performed in the dose-ranging study CR845-CLIN2101 which examined the efficacy of 3 doses of IV difelikefalin (0.5, 1.0, and 1.5 mcg/kg) treatment after each HD session (3 times/week) over an 8-week period. The 0.5 mcg/kg dose was selected given the similarities in efficacy results across doses and a more favourable safety profile than the higher doses.

In both pivotal studies the primary efficacy endpoint was met. In Study CR845-CLIN3102, 51.0% of difelikefalin subjects versus 27.6% placebo subjects achieved a ≥3-point improvement (reduction) in WI-NRS at Week 12 (odds ratio = 2.72; P <.001). In Study CR845-CLIN3103, the percentages were 54.0% for difelikefalin and 42.2% for placebo (odds ratio = 1.61; P = .020). The change at 12 weeks for absolute scores showed a treatment difference of -1.1 and -0.6 for each study respectively.

Secondary endpoints were met in study CR845-CLIN3102. At the end of Week 12, change from baseline in the total 5-D Itch Scale score for difelikefalin and placebo were (LS mean) -5.0 and -3.7,

respectively (P <.001) and change from baseline in the total Skindex-10 Scale score for difelikefalin and placebo were (LS mean) -17.2 and -12.0, respectively (P <.001). The proportion of subjects achieving a ≥4-point improvement in WI-NRS from baseline at Week 12, were consistent with the primary endpoint which was reassuring; 38.9% (LS mean) of subjects treated with difelikefalin and 18.0% subjects treated with placebo achieved a ≥4-point improvement in WI-NRS from baseline (odds ratio = 2.89; P <.001)

Secondary endpoints for CR845-CLIN3103 differed from study CR845-CLIN3102 in the extend and hierarchical order. Only the secondary endpoints related to WI-NRS had given significant results (proportion of subjects achieving a ≥4-point improvement in WI-NRS from baseline at Week 12 and ≥3-point improvement at week 8 and week 4).

Around 37% of the population treated with difelikefalin were using another anti-itch medication and the study populations were stratified for this factor. This supports the inclusion of all de-novo or previously treated CKDaP patients in the indication.

Long-term effectiveness data were explored in the OLE of CR845-CLIN3102 for up to 52 weeks. After 52 weeks of Open-label treatment, the LS mean (standard error [SE]) change from baseline in total 5-D Itch Scale score was -6.9 (0.39) and -7.8 (0.39) for placebo/difelikefalin (n = 94) and difelikefalin/difelikefalin subjects (n = 90), respectively.

3.3. Uncertainties and limitations about favourable effects

All scales used for primary and key secondary endpoints are informant-based scales and do not include input from treating physician.The primary efficacy endpoint was the proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 12 of the Double-blind Treatment Period. The WI-NRS scale is considered relevant, and the Applicant performed the validation of the scale in this indication. The cut off of 3 points was determined as minimal clinically significant change by the Applicant, this is questionable, but significant results with 4 points cut off for overall population are reassuring.

The pivotal studies were designed assuming a placebo response of 30% and a difelikefalin response ranging from 46% to 50% for the primary endpoint. The actual placebo response varied between studies by more than 10%. The proportion of subjects on placebo who also achieved a ≥3-point reduction from baseline in the WI-NRS at Week 12 was higher in the global pivotal study CR845-CLIN3103 (42.2%) than in the US only study CR845-CLIN3102 (27.6%). Placebo response rates in US subjects were also higher in CR845-CLIN3103 (37.3%) than in CR845-CLIN3102 (27.6%), but this does not impact the overall efficacy.

Key secondary endpoints using scales other than WI-NRS have failed to show significant differences in favour of the difelikefalin therapy for CR845-CLIN3103 (total 5-D Itch Scale score and the total Skindex-10 Scale score). Some sensitivity analyses for primary analysis also failed to reach significance in this study. Data for EU patients is coming from this study only. Regarding the ≥3-point improvement at Week 12, the treatment effect in the EU was at least as large (OR (CI) of improvement with difelikefalin versus placebo 2.56 (1.26-5.19)) as in the other regions (OR (CI) 1.81 (1.32 2.48)) and it was statistically significant in both the EU (p=0.009) and the other regions (p<0.001). Regarding the ≥4-point improvement at Week 12, a similar relation was observed, i.e., the treatment effect in the EU was at least as large as in the other regions (EU: OR (CI) 2.47 (1.17-5.21), p=0.018; non-EU OR (CI) 1.99 (1.41-2.82), p<0.001)).

Maintenance of the effect is based on open label extensions of these studies. However, OLE periods are only supportive for continued evaluation of efficacy beyond 12 weeks and post-authorisation phase will monitor the efficacy beyond (see RMP).

There were additional questions regarding the clinical relevance in a very severe population (WIN-NRS ≥9), however, the applicant has provided sufficient data in a form of post-hoc analyses supporting efficacy in this subgroup.

3.4. Unfavourable effects

The overall safety profile of difelikefalin in HD patients with CKD-aP appears favourable, although a number of unfavourable effects and uncertainties were identified. Long-term safety was evaluated in open-label studies of up to 12 months treatment, hence no placebo beyond 12 weeks to compare with.

Common (>2% in difelikefalin and >1%-point higher than placebo) AEs were observed in both difelikefalin and placebo treated patients. Nausea, diarrhoea, and headache were considered adverse drug reactions, i.e. related to difelikefalin treatment. These adverse drug reactions were mostly mild to moderate in severity and resolved within few days, and also diminished over time. Less than 1% of patients discontinued treatment with difelikefalin due to these events. Due to the pharmacological effect of kappa opioid receptor agonism, a number of adverse events of special interest were evaluated. This evaluation showed that dizziness, somnolence and mental status changes were more frequently reported by difelikefalin treated patients. These events are considered related to difelikefalin and could be caused by central effects of difelikefalin. Median duration of these events was confined to a few days, except for somnolence with a median duration of 3 weeks. Most were mild to moderate in severity. For mental status changes, 2% were reported as serious.

The target population of haemodialysis patients has an inherent increased risk of cardiovascular events and deaths, which are the most frequent cause of morbidity and mortality in patients treated with haemodialysis. Overall, small, but consistent imbalances disfavoured difelikefalin for cardiac disorders SOC, MACE, and serious adverse events of cardiac arrhythmias SMQ and cardiac failure SMQ compared to placebo.

3.5. Uncertainties and limitations about unfavourable effects

Sufficient number of patients have been exposed to assess long-term safety. The uncertainty is related to the lack of placebo control beyond 12 weeks, and that the rates of adverse events of difelikefalin is to be evaluated against literature data from large databases, i.e. not in a controlled setting of the clinical trial. For example, for MACE events, a small imbalance was seen compared to placebo (3.8% vs 2.4%), but the observation time was only 12 weeks, which is short in relation to a possible impact on MACE events. Hence, the number of events observed in the long-term setting are comparable to literature data. The databases that collect information and health data on dialysis patients are large and hold comprehensive information about mortality rates, causes of death, comorbidities etc. which are comprehensive enough for comparison. Small numerical imbalances disfavoured difelikefalin for cardiac disorders, and a causal relationship could not be excluded nor confirmed. Cardiac failure and cardiac arrythmias have been included as important potential risks in the RMP, along with medical history of atrial fibrillation. Difelikefalin is not supposed to give any effects from the CNS, due to allegedly low distribution over the blood brain barrier. However, Somnolence, dizziness and mental status change were reported more in difelikefalin treated patients than placebo patients and were also considered related to difelikefalin. Indeed, somnolence was reported more by patients >65 years of age 7% vs 2.8% for < 65 years of age as well as for patients with concomitant use of opioids and prior

use of anti-itch treatment. Both somnolence and dizziness were commonly reported in the medical history and included in SmPC section 4.8.

3.6. Effects Table

Effects Table for [Kapruvia, CKDaP] (data cut-off: 21 May 2019 for CR845-CLIN3102 and 31 March 2020 for CR845-CLIN3103).

Effect	Short Description	Unit	Treatment Difelikefalin 0.5 µg/kg	Control Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
≥3-point improvement WI-NRS (Primary endpoint)	Proportion of subjects achieving a ≥3-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS (Worst Itching Intensity Numerical Rating Scale) at Week 12	LS mean (95% CI)	51.0% (42.9%, 58.9%)	27.6% (20.2%, 36.6%)	3 points cut off queried. Absolute difference: -1.1 in favour of difelikefalin.	CR845-CLIN3102
		Odds ratio (Lawrence, Hung) (95% CI)	2.72 (1.72, 4.30)		P-value <0.001 (Cui, Hung, Wang)	
		LS mean (95% CI)	54.0% (43.9%, 63.9%)	42.2% (32.5%, 52.5%)	High placebo response. Absolute difference: -0.6 in favour of difelikefalin.	CR845-CLIN3103
		Odds ratio (Lawrence, Hung) (95% CI)	1.61 (1.08, 2.41)		P-value =0.010 (Cui, Hung, Wang)	
5-D Itch	Change from baseline at the end of Week 12, as assessed by the 5-D Itch Scale	LS mean (95% CI)	-5.0 (-5.7, -4.4)	-3.7 (-4.4, -3.1)		CR845-CLIN3102
		Difference in LS means (95% CI)	-1.3 (-2.0, -0.5)		P-value <0.001 (ANCOVA)	
		LS mean (95% CI)	-4.9 (-5.6, -4.2)	-3.8 (-4.5, -3.1)	P value not tested due to testing hierarchy	CR845-CLIN3103
		Difference in LS means (95% CI)	-1.1 (-1.7, -0.4)			
Skindex-10	Change from baseline at the end of	LS mean (95% CI)	-17.2 (-19.6, -14.7)	-12.0 (14.5, -9.6)		CR845-CLIN3102

Effect	Short Description	Unit	Treatment Difelikefalin 0.5 µg/kg	Control Placebo	Uncertainties/ Strength of evidence	References
	Week 12, as assessed by the total Skindex-10 Scale score	Difference in LS means (95% CI)	-5.1 (-8.0, -2.3)		P-value <0.001 (ANCOVA)	
		LS mean (95% CI)	-16.6 (-19.3, -14.0)	-14.8 (-17.4, -12.2)	No significant change	CR845-CLIN3103
		Difference in LS means (95% CI)	-1.8 (-4.3, 0.8)		P= 0.171	
≥4-point improvement WI-NRS	Proportion of subjects achieving a ≥4-point improvement from baseline with respect to the weekly mean of the daily 24-hour WI-NRS at Week 12	LS mean (95% CI)	38.9% (29.8%, 48.7%)	18.0% (12.1%, 26.0%)		CR845-CLIN3102
		Odds ratio (Lawrence, Hung) (95% CI)	2.89 (1.75, 4.76)		P-value <0.001 (Cui, Hung, Wang) (Cochran-Mantel-Haenszel)	
		LS mean (95% CI)	41.2% (33.0%, 50.0%)	28.4% (21.3%, 36.7%)		CR845-CLIN3103
		Odds ratio (Lawrence, Hung) (95% CI)	1.76 (1.04, 2.98)		P-value =0.036 (Cui, Hung, Wang)	
Unfavourable Effects						
AEs	proportion	%	71.2	65.3		Primary safety pool
SAEs	proportion	%	25.2	22.6		Primary safety pool
Cardiac disorders SOC AEs	proportion	%	7.8	6.4		Primary safety pool
Cardiac disorders SOC SAEs	Proportion	%	4.5	1.9		Primary safety pool
Gastrointestinal disorders SOC AEs	proportion	%	25.9	19.1		Primary safety pool
Infections and infestations SOC AEs	Proportion	%	21.7	21.5		Primary safety pool
Nervous system disorders SOC AEs	Proportion	%	20.3	14.2		Primary safety pool

Effect	Short Description	Unit	Treatment Difelikefalin 0.5 µg/kg	Control Placebo	Uncertainties/ Strength of evidence	References
MACE events	Proportion	%	3.8	2.4	Small imbalance disfavouring difelikefalin	Primary safety pool
Dizziness PT AEs	Proportion	%	6.8	3.8	Uncertainty of central effect of difelikefalin	Primary safety pool
Somnolence PT AEs	Proportion	%	4.2	2.4	Uncertainty of central effect of difelikefalin	Primary safety pool
Diarrhoea PT AEs	Proportion	%	9.0	5.7		Primary safety pool
Nausea PT AEs	Proportion	%	6.6	4.5		Primary safety pool

Abbreviations: AEs: adverse events; SAEs: serious adverse events

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Pruritus is burdensome for a large proportion of patients in haemodialysis. There are no approved or truly effective treatments and the treatments used in clinical practise tend to have adverse events including CNS effects. There is a clear unmet medical need. Both in vitro and in vivo primary pharmacodynamic studies of difelikefalin has demonstrated proof of concept and mode of action, both the role of peripheral KORs in mitigating itch and inflammation, but also the effect of difelikefalin at relevant receptors. Difelikefalin is proposed to act mainly peripherally, on the imbalance of mu-opioid versus KOR activity, which has been shown to arise in uraemia and cause pruritus.

The main clinical evidence come from two randomised placebo-controlled clinical trials conducted in a population relevant to the target population, i.e. both treatment naïve and previously treated patients.

In the study CR845-CLIN3103 conducted outside of the US and with a slightly more affected study population, the primary endpoint was met. In both studies, the placebo response was considerable, especially in the non-US study where more than 40 % in the placebo group were responders. A high placebo response is well-known in pruritus studies.

Treatment with difelikefalin is symptomatic and would be expected to be continued long-term in patients if a clinical effect is experienced. Though the studies were limited to 12 weeks, OLE studies and pooled analyses bring some evidence for maintenance of effect beyond 12 months though the efficacy measure in OLE is the 5-D Itch Scale and not WI-NRS.

The overall safety profile of difelikefalin appears favourable, but comparative safety data are only available for 12 weeks treatment. Distribution to CNS is expected to be minimal in subjects with intact blood-brain barrier, but the identification of CNS related adverse events, somnolence, dizziness and mental status change, questions if this also apply to the HD population. "Use in patients with impaired blood-brain barrier" has been identified as missing information in the RMP.

Given the pharmacology of difelikefalin it is reassuring that there were no signs for potential abuse. Cardiovascular events are together with infections the most common causes of mortality and morbidity in haemodialysis patients, and there are small but consistent imbalances in MACE, cardiac failure and cardiac arrythmia. A thorough QTc study did not show any influence of difelikefalin on the cardiac repolarisation. There is no mechanistic likely explanation for an increased risk of cardiovascular events. Important potential risks for cardiac failure and cardiac arrythmias have been included in the RMP. The underlying medical history, particularly the change of treatment (i.e. discontinuation or missing treatment) of CV conditions in HD patients has been identified as a potential risk and it has been proposed to be included as a warning in the section 4.4 of the SmPC and in the adopted RMP.

3.7.2. Balance of benefits and risks

In the conducted nonclinical programme and in the clinical trials, a clear benefit of Kapruvia was demonstrated with respect to easing the burden of pruritus in patients with CKD. Primary and most secondary objectives of the trials were met. The safety is manageable and overall favourable although limited to 12 weeks data, which will be further expanded pot-authorisation. The applicant has satisfactorily addressed the concerns of the CHMP and PRAC and the benefit-risk balance is positive.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable.

3.8. Conclusions

The overall benefit-risk balance of Kapruvia is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus decision that the benefit-risk balance of Kapruvia is favourable in the following indication(s):

Kapruvia is indicated for the treatment of moderate-to-severe pruritus associated with chronic kidney disease in adult patients on haemodialysis (see section 5.1).

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and

any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency.
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that difelikefalin is to be qualified as a new active substance in itself as it is a constituent of a medicinal product previously authorised within the European Union.