Clinical Trial Protocol

Trial Title:	**A Parallel-group (2-Arm), Randomized, Double-blind, 12-week Trial to Evaluate the Efficacy and Safety of MC2-25 Cream and MC2-25 Vehicle in Subjects with Chronic Kidney Disease-associated Pruritus (CKD-aP)**
Investigational product:	**MC2-25 cream**
Active Comparator:	**Not applicable**
Protocol No:	**MC2-25-C1**
Short title:	**ITCHINESS**
EUDRACT:	**2021-006971-40**
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Sponsor:	**MC2 Therapeutics Ltd** **1A Guilford Business Park** **Guilford GU2 8XG** **United Kingdom** **EU representative:** **MC2 Therapeutics A/S** **c/o Agern Alle 24-26** **2970 Hørsholm** **Denmark**

CLINICAL TRIAL PROTOCOL APPROVAL

Product: MC2-25 cream

Protocol number: MC2-25-C1

Protocol title: A Parallel-group (2-Arm), Randomized, Double-blind, 12-week Trial to Evaluate the Efficacy and Safety of MC2-25 Cream and MC2-25 Vehicle in Subjects with Chronic Kidney Disease-associated Pruritus (CKD-aP)

Short title: The MC2-25 cream in subjects wITh CHronic KIdNEy disease-aSsociated pruritus (ITCHINESS) trial

The following persons have approved this clinical trial protocol, as reflected in separate documents adjoined to this document:

Maj Dinesen, Director of Clinical Development, MC2 Therapeutics

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SIGNATURE PAGE FOR INTERNATIONAL COORDINATING INVESTIGATOR

Product: MC2-25 cream

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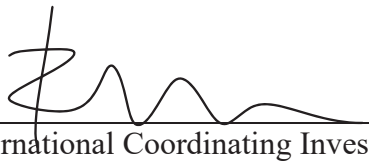
The signature of the International Coordinating Investigator below constitutes his/her approval of this protocol and provides the necessary assurances that this trial will be conducted according to all stipulations, clinical and administrative, as detailed in the protocol. The trial will not be initiated without the approval of an appropriate Institutional Review Board or Ethics Review Committee and the approval from the appropriate health authority.

Kieran McCafferty

International Coordinating Investigator's printed name



International Coordinating Investigator's signature

30/Nov/2022

Date

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AD	Atopic dermatitis
ADR	Adverse drug reaction
AE	Adverse event
BSA	Body surface area
CGA	Clinician's Global Assessment
CTA	Clinician's Targeted Assessment
CKD	Chronic kidney disease
CKD-aP	Chronic kidney disease-associated pruritus
ClinRO	Clinician-reported outcome
COVID-19	Coronavirus disease 2019
CRO	Contract research organization
DOPPS	Dialysis Outcomes and Practice Patterns Study
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EoT	End of treatment
EQ-5D-5L	EuroQoL 5 Dimensions, 5 levels
ESRD	End-stage renal disease
EU	European Union
FAS	Full analysis set
FTU	Fingertip unit
GCP	Good Clinical Practice
HD	Haemodialysis
HDF	Haemodiafiltration
HRQoL	Health-related quality of life
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
IRB	Institutional Review Board
IV	Intravenous(ly)
IWR	Interactive web response
LOCF	Last observation carried forward
MFAS	Modified full analysis set
NMF	Natural moisturizing factor
PPS	Per-protocol set

Abbreviation	Definition
PRO	Patient-reported outcome
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety analysis set
SD	Skin Dryness
SD-NRS	Skin Dryness Numeric Rating Score
SGIC	Subject's Global Impression of Change
SI	Sleeploss due to Itch
SI-NRS	Sleeploss due to Itch Numeric Rating Score
SoA	Schedule of assessments
SmPC	Summary of Product Characteristics
SS	Screened set
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
TOM	Technical-operational measures
USPI	United States prescribing information
UV-B	Ultraviolet B
VAS	Visual analogue scale
WI	Worst Itch
WI-NRS	Worst Itch Numeric Rating Score
w/w	Weight by weight

1.0 SYNOPSIS

Trial Title:	A Parallel-group (2-Arm), Randomized, Double-blind, 12-week Trial to Evaluate the Efficacy and Safety of MC2-25 Cream and MC2-25 Vehicle in Subjects with Chronic Kidney Disease-associated Pruritus (CKD-aP)
Protocol Number:	MC2-25-C1
Sponsor:	MC2 Therapeutics Ltd
Development Phase:	2
Trial Objectives:	The primary objective is to explore the clinical efficacy of MC2-25 cream compared to MC2-25 vehicle in adults with chronic kidney disease-associated pruritus (CKD-aP). The secondary objectives are to explore the safety of MC2-25 cream compared to MC2-25 vehicle in adults with CKD-aP and to explore the subclinical effects of MC2-25 cream in adults with CKD-aP.
Trial Design:	This is a multicentre, phase 2, randomized, double-blind, 2-arm, parallel-group and vehicle-controlled trial in subjects with CKD-aP. Eligible subjects will be randomised in a 2:1 ratio to MC2-25 cream or MC2-25 vehicle, respectively. Subjects will apply the assigned investigational medicinal product (IMP) twice daily for 12 weeks. Subjects will be seen at the trial sites at Screening, Baseline, Week 1, Week 4, Week 8, and Week 12 (end of treatment, EoT). Subjects who have ongoing serious adverse events (SAEs) or related adverse events (AEs) at Week 12 will have a follow-up visit at Week 14 or (in case of early treatment discontinuation) 14 days after the EoT visit, whichever comes first. Additionally, phone contacts are planned at Week 2, Week 6, and Week 10.
Planned Sample Size:	Approximately 108 subjects will be randomised in the trial in a 2:1 ratio of MC2-25 cream to MC2-25 vehicle, including an assumption of 10% dropouts.
Trial Population:	Males or non-pregnant females at least 18 years of age who have chronic (>3 months) kidney disease (CKD) stages G3-G5 (estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2) and at least moderate CKD-aP, defined as WI-NRS ≥4 (i.e., the average of all and at least 4 non-missing scores reported by the subject in the diary for 7 days prior to and including the Baseline day, 8 days in total). Both subjects on dialysis (haemodialysis or haemodiafiltration) and not on dialysis will be enrolled.
Investigational Medicinal Product:	MC2-25 cream for topical application (MC2-25 cream)
Vehicle:	MC2-25 vehicle for topical application (MC2-25 vehicle)
Primary Endpoint:	• Mean change in weekly mean Worst Itch Numeric Rating Score (WI-NRS) recorded in the subject's diary from Baseline to Week 12 for

	MC2-25 cream compared to MC2-25 vehicle. (Weekly mean WI-NRS is calculated as the average of WI-NRS values recorded in the subject's diary 7 days prior to and including the visits.)
Secondary Endpoints:	• Percentage of subjects obtaining a ≥4-point improvement in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects obtaining a ≥3-point improvement in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects obtaining a complete response in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. (Complete response is defined as scores equal to 0 or 1 in ≥80% of the non-missing WI-NRS values recorded in the subject's diary 7 days prior to and including the visits.)
Other Endpoints:	Additional endpoints are included in the Clinical Trial Protocol.
Safety Endpoints:	• Frequencies of treatment-emergent adverse events (TEAEs), SAEs, adverse drug reactions (ADRs), AEs leading to treatment discontinuation or trial withdrawal, and deaths during the trial for MC2-25 cream compared to MC2-25 vehicle. • Changes in mean safety assessments: vital signs (heart rate, systolic blood pressure, diastolic blood pressure, temperature), and blood samples (biochemistry, haematology) from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Frequency of clinically significant abnormal physical examinations and ECGs from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects who missed 1 or more dialysis visits during the Double-blind Treatment Period.
Statistical Methods:	Efficacy analysis: The primary endpoint of the trial, mean change in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle, will be analysed by a mixed model of repeated measures with Baseline weekly mean WI-NRS as covariate and treatment, CKD stage stratum at baseline, systemic CKD-aP treatment status at baseline and visit as fixed factors with a two-sided $\alpha = 0.05$. Treatment groups are compared for the superiority of MC2-25 cream over MC2-25 vehicle after 12 weeks of treatment. Due to the Phase II type of the trial, no alpha adjustment will be carried out for secondary endpoints. Safety Analysis: Safety data will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) for continuous

	variables and frequency distributions (counts and percentages) for categorical variables.
Trial Sites:	Approximately 26 sites will be opened in United Kingdom (UK) and (EU).
Planned Dates of Trial:	FPFV: H1 2022 LPLV: H2 2023

2.0 INTRODUCTION

2.1 Background

2.1.1 Chronic kidney disease-associated pruritus

The global prevalence of Chronic Kidney Disease (CKD) is around 9·1% and the prevalence of CKD increases with age (Collaboration, Chronic Kidney Disease, 2020; Hill 2016). Patients with chronic kidney disease (CKD) are afflicted by severe pruritus, referred to as CKD-associated pruritus (CKD-aP) or uremic pruritus. Pruritus has most thoroughly been investigated in patients with end-stage renal disease (ESRD) on haemodialysis. The pruritus often presents with fluctuating intensity, often worse during nighttime than daytime, resulting in sleep disturbances. Most patients report itching over large, discontinuous, non-dermatomal regions of skin, often with bilateral symmetry (Mathur 2010).

The significant impact of itch on ESRD patients' quality of life is generally accepted. According to the Dialysis Outcomes and Practice Patterns Study (DOPPS), which included 23,264 haemodialysis patients from 21 countries, the proportion of CKD patients on haemodialysis who are at least moderately bothered by self-reported pruritus is 37%, and 7% are extremely bothered. The trial also found that pruritus was associated with higher risk for hospitalizations and death, higher rates of withdrawal from dialysis and missing scheduled dialysis treatments, and lower rates of employment. Additionally, severe pruritus was strongly associated with self-reported depression, self-reported restless sleep, and progressively poorer self-reported mental and physical Health Related-Quality of Life (Sukul 2021). In a previous DOPPS trial the increased mortality in patients with severe itch was no longer significant after adjusting for sleep quality measures (Pisoni 2006).

Recent studies indicate that moderate to severe/extreme patient-reported pruritus is common regardless of whether or not patients are on haemodialysis and that more severe pruritus is associated with negative effects on several patient-reported outcomes (PROs). A major study with more than 5,600 CKD-aP patients from the United States, Brazil, and France found that the prevalence of moderate to extreme pruritus was 24% and there was only weak correlation between the degree of kidney failure and the intensity of pruritus. The same trial also indicated older age was associated with higher prevalence of moderate-to-extreme pruritus (Sukul 2019).

The pathogenic basis of pruritus in patients with renal failure is unknown. Numerous suggestions for the aetiology of the pruritus (e.g., hyperparathyroidism, allergic sensitization, neuropathy, various abnormal laboratory values) have been evaluated without positive results (Martin 2020). One hypothesis is that the symptoms typical of chronic kidney failure can be explained by excessive protein carbamylation, referring to the binding of isocyanic acid on free amino acids and proteins. Urea that is elevated in CKD is via a slow equilibrium a source of formation of isocyanic acid (Jaisson 2018, Kraus 1998).

It is known that the metabolism of many amino acids and proteins is altered in advanced renal failure to the extent that uremic patients acquire symptoms of malnutrition (Kopple 1978). One of the key reasons for this may be the extensive carbamylation of serum essential amino acids that is seen in uremic patients. The level of carbamylation of such amino acids can be extensive with up to 10 times molar excess of carbamylated amino acids compared to non-carbamylated amino acids (Kraus 1998). The carbamylated amino group cannot participate in the protein synthesis, which requires an un-derivatized amino acid, and it has been demonstrated that carbamylated amino acids inhibit protein synthesis (Kraus 2001).

The concentration of urea is higher in sweat than in other body compartments (Baker 2020). Furthermore, the evaporation of water from the skin will lead to concentration of urea on the surface of the skin. The high level of urea on the skin surface creates a concentration gradient of urea through the uppermost layers of the skin resulting in extensive carbamylation of amino acids and proteins in the epidermis. Free amino acids are readily carbamylated, which means that the levels of un-derivatized amino acids are reduced in the stratum corneum skin in patients with CKD. Since skin amino acids are important humectants, this may be the reason that uremic skin is most often dry, as carbamylated amino acids presumably are not good humectants. Isocyanate is known to be neurotoxic in animals (Kimani 2014) and in humans (Adamolekun 2011). In patients with CKD, intra-epidermal fibres often demonstrate nerve sprouting (Johansson 1989), probably because of nerve damage. Although not proven, carbamylation in the skin may affect afferent small nerve fibres resulting in the sensation of itch. Scratch marks in uremic patients are often lined with epidermal haemorrhagic bleedings – a result of mechanical ruptures of small blood vessels where supporting connective tissues (collagen and elastin) have lost their elastic properties due to carbamylation (Gorisse 2016, Jaisson 2006).

A number of treatment strategies exist for CKD-aP (e.g., optimization of dialysis parameters, topical emollients and analgesics, antihistamines, GABA analogues (gabapentin and pregabalin), kappa opioid agonists (nalfurafine, diefelikefalin), ultraviolet light B (UV-B) phototherapy) but apart from GABA analogues and kappa opioid receptor agonists the evidence supporting their efficacy is limited (Hercz 2020, Rayner 2017, Shirazian 2017). Furthermore, except for nalfurafine (Remitech®), which is only marketed in Japan (Nalfurafine Report 2008), and difelikefalin (Korsuva®), which at the present time is only approved in the US and carries a risk of gastrointestinal and nervous system side effects (Korsuva USPI 2021), there are currently no approved and marketed drugs for treatment of CKD-aP. Therefore, there is a high unmet need for a safe and efficient treatment option.

2.1.2 MC-25 cream

MC2-25 cream consists of the dipeptide L-Alanyl-L-Glutamine (Ala-Gln) as the active drug substance in a cream base. Ala-Gln is intended to act as a scavenger for isocyanate, thereby preventing protein carbamylation. A series of in vitro assays demonstrated that Ala-Gln was

among the most efficient amino acid and peptide compounds in terms of preventing protein carbamylation, that Ala-Gln achieved ~65% inhibition of protein carbamylation compared with histidine, for example, that only achieved ~39% inhibition, and that Ala-Gln showed a dose-dependent inhibition of protein carbamylation reaching 89% inhibition at a 3% Ala-Gln strength. Further details are provided in the Investigator's Brochure (IB) Section 4.2.1, Primary pharmacology.

A pilot clinical test (n=2) indicated that application of a urea cream to healthy human skin (to mimic urea and isocyanate levels in CKD-aP skin) leads to a decrease in amino acids and an increase in carbamylated proteins in the skin. This was supported by a subsequent pilot clinical trial in CKD-aP patients (n=3) and an age-matched control group (n=3) that indicated that the total level of natural moisturizing factors (NMFs) (amino acids are an important source of NMFs) is reduced in CKD-aP compared to healthy human skin (see IB Section 4.2, Nonclinical pharmacology for further details).

An in vitro assay indicated that homogenized healthy human skin contains significant levels of both Ala-Gln and its metabolites. It is likely that in CKD-aP skin endogenous levels of Ala-Gln and its metabolites are considerably lower due to carbamylation. In a skin penetration study on whole fresh human abdominal skin samples, only ~ 4% of the applied Ala-Gln dose was recovered in the skin and <1% of the applied dose reached the receptor fluid (a surrogate for systemic exposure) (see IB Section 4.3.1, Absorption and pharmacokinetics for further details). Due to itching and dryness, CKD-aP skin may theoretically resemble atopic dermatitis (AD) skin with resulting higher skin permeation than across healthy skin. An in vitro study (n=35) indicated that permeation of theophylline was 2 to 2.5-fold higher across AD epidermis compared to healthy control skin (Yoshiike 1993). In man the plasma half-life of Ala-Gln is <5 min (varies between 2.4 and 3.8 min; 4.2 min in terminal renal insufficiency) (Dipeptiven SmPC 2015). There are no available data on the distribution, excretion, and drug-drug interaction properties of Ala-Gln. However, based on the expected low systemic availability of Ala-Gln after application of MC2-25 cream (<1%) and the short half-life in plasma (<5 min), the contribution of topically applied MC2-25 cream to the endogenous amino acid homeostasis or to drug-drug interactions is expected to be negligible (see IB Section 4.3, Pharmacokinetics and product metabolism in animals for further details).

MC2-25 cream was found to be safe and well tolerated when applied topically, twice daily for 2 weeks in minipigs. Systemic toxicity studies have not been performed with MC2-25 cream. However, the systemic toxicity of Ala-Gln was explored (up to 13 weeks in rats and dogs) as part of the development of Dipeptiven®, which is a product for intravenous (IV) infusion with Ala-Gln as the active ingredient. As described in the following section, safety margins can be inferred from the maximum clinical dose and the benign safety profile provided in the Dipeptiven® SmPC 2015.

Dipeptiven®, a product containing Ala-Gln that was approved in 1995 in the EU for parenteral or enteral administration in association with hypercatabolic conditions, has a benign safety profile. According to the European SmPC, Dipeptiven® can be administered to adult patients up to maximum daily doses equivalent to 0.5 g per kg body weight for 3 weeks (Dipeptiven® SmPC 2015). In addition, no adverse safety signals were observed in a phase 2 randomized controlled trial evaluating biomarkers of peritoneal health that included 50 patients who were randomized to Ala-Gln (8 mM) or placebo added to their peritoneal dialysis fluid for 8 weeks (Vychytil 2018). Therefore, as systemic administration of Ala-Gln in humans appears safe, dermally applied Ala-Gln is also considered to be safe in humans although the safety of MC2-25 cream (dermally applied Ala-Gln) in humans has not been investigated yet.

2.2 Rationale of the trial

The trial uses a randomized, double-blind, vehicle-controlled design to minimize the potential for bias. By using blinding and randomization and including a group that receives vehicle, the trial design controls for potential influences on the results other than those arising from the pharmacologic action of MC-25 cream (Ala-Gln).

Based on reported plasma and skin urea concentrations, and literature regarding urea chemistry and biology, it can be estimated that the amount of isocyanate formed and present in the skin of dialysis patients can be as high as ~10 nmol/cm^2 due to elevated levels of plasma urea (see IB Section 2, Introduction for further details). As a rough estimate, to effectively scavenge this amount of isocyanate at any time, the amount of nucleophile (Ala-Gln) added via a cream would have to be at least in the same range. One fingertip unit (FTU) of MC2-25 cream weighs approximately 500 mg (data on file). This is in line with findings for other topical formulations and it is generally accepted that 1 FTU can cover approximately two handprints (Finlay 1989, Finlay 2012), which correspond to 2% of the body surface area (BSA) or an average of 358 cm^2 in adult men and women (Sacco 2010, Thomas 2007). The amount of scavenger needed in one gram of cream can then be calculated by multiplying 10 nmol Ala-Gln/cm^2 skin by 716 cm^2 skin/g cream, which adds up to 7.2 x 10^{-6} mol Ala-Gln/g cream. With a molecular weight of 217.22 g/mol this corresponds to a cream strength of 0.0016 g Ala-Gln/g cream, i.e., a ~ 0.16% (w/w) formulation. However, several factors may affect the estimation, including extent of absorption/penetration of Ala-Gln, reaction efficiency between Ala-Gln and isocyanate, stability of the Ala-Gln in the skin, and the amount of "natural" isocyanate scavengers in the skin (e.g., amino acids/peptides).

Based on an in vitro human skin penetration study with Ala-Gln cream, if ~4% of the total applied Ala-Gln dose is recovered (see IB Section 4.3.1.1, Skin penetration study using whole human skin for additional information), this would represent the total amount of Ala-Gln available for reaction with isocyanate during penetration through the skin layers over time. Taking this into account, the required amount of Ala-Gln to be added would then correspond to a

~3.91% (w/w) formulation instead of 0.16% (w/w) to account for retention of most of the product on the skin surface. Further, factoring in the apparent scavenging efficiency of Ala-Gln, which on the basis of the in vitro protein carbamylation assay using BSA as a model protein can be assumed to be ~65% (see IB Section 4.2.1.1, In vitro protein carbamylation inhibition assay), and also considering other factors such as potential degradation of Ala-Gln in the skin, then a dose strength of ~6.0% (w/w) would seem reasonable to ensure optimal scavenging effect towards isocyanate. However, currently available data only support physical-chemical stability of Ala-Gln in the MC2-25 cream up to 3% (w/w).

The twice-daily dosing regimen has been chosen to compensate for the fact that the Ala-Gln strength in the MC2-25 cream may be at the lower end of the hypothetical efficiency range as explained above and because it is thought to be the highest feasible frequency for achieving both good treatment compliance and effect.

There is no available data on the effect of MC2-25 vehicle in CKD-aP patients, and there are no approved medicinal products for topical treatment of CKD-aP. Consequently, it is currently not possible to establish an expected vehicle effect size. In a clinical trial comparing IV administration of Korsuva® (i.e., the only approved treatment for CKD-aP in Europe; tradename Kapruvia®) and placebo for CKD-aP the reduction in weekly mean Worst Itch Numeric Rating Score (WI-NRS) from baseline to week 8 was approximately 2 in the placebo group (Spencer 2018). The MC2-25-C1 trial is using a cream vehicle (and therefore has emollient properties) as control as opposed to a pure placebo control. Therefore, a reduction in weekly mean WI-NRS of at least 2 is expected in the MC2-25 vehicle arm in the MC2-25-C1 trial.

The 12-week treatment duration was selected based on the assumption that if no differentiation to the MC2-25 vehicle is observed within that timeframe, then the treatment may be less clinically relevant.

The primary endpoint assessment Worst Itch Numeric Rating Score (WI-NRS) was selected based on clinical and regulatory precedent with Korsuva® for CKD-aP and Dupixent® for another extremely itchy disease, namely AD.

2.3 Benefit-risk assessment

As described above, CKD is a serious disease and patients with CKD often have dry and itchy skin with a major impact on their quality of life. While the benefits of MC2-25 cream are not yet known, an effective isocyanate scavenger Ala-Gln may reduce the adverse effects resulting from carbamylation of skin proteins and amino acids and thereby alleviate pruritus and dry skin in CKD-aP patients.

A summary of risks that may be associated with the investigational medicinal product (IMP) MC2-25 cream or the trial procedures and mitigation strategies to address these is provided in

Table 1. More detailed information about the risks associated with the IMP may be found in the IB.

The MC2-25 cream is not authorised in EU or in other countries outside the EU. This is the first clinical trial with MC2-25 cream and MC2-25 vehicle so no clinical data are available. As noted above, the active ingredient Ala-Gln is included in the product Dipeptiven®, which has been approved for IV human use at doses up to 0.5 g per kg body weight per day. According to the Dipeptiven® SmPC (2015) there are no known undesirable effects when administered correctly. In the present trial a maximum of 50 g MC2-25 cream 3% (w/w) will be used per day, i.e., 0.5 g cream per 2% body surface area (see fingertip unit rule described above) and twice daily application. This corresponds to ~0.02 g Ala-Gln/kg/day (1.5 g Ala-Gln per day in a 70-kg adult) and a resulting safety margin of 25 compared to IV administration assuming that all topically applied Ala-Gln is absorbed into the systemic circulation. However, less than 1% of the total applied Ala-Gln dose was recovered in the receptor fluid (a surrogate for absorption into the systemic circulation) in an in vitro whole human skin penetration study with Ala-Gln cream (see IB Section 4.3.1.1, Skin penetration study using whole human skin). This corresponds to 0.0002 g Ala-Gln/kg/day and a resulting systemic safety margin of 2500. Assuming that the skin defect in CKD-aP is similar to AD skin (worst case scenario), and consequently the skin permeation is 2.5-fold higher than in healthy skin (Yoshiike 1993), then the resulting systemic safety margin would be 10 or 1000 if either all or less than 1% of topically applied Ala-Gln was absorbed, respectively. In view of the benign safety profile of Dipeptiven® and the high safety margin, the risk of treatment-related systemic adverse effects is considered to be negligible in this trial.

Ala-Gln is rapidly split into alanine and glutamine after infusion. In man, half-lives of between 2.4 and 3.8 min (4.2 min in terminal renal insufficiency) and a plasma clearance of between 1.6 and 2.7 l/min were determined (see IB Section 4.3, Pharmacokinetics and product metabolism in animals). So, even if topical application of MC2-25 cream leads to systemic exposure to Ala-Gln, then it will rapidly be metabolized to the two respective amino acids and become part of the body's amino acid homeostasis. In view of the rapid elimination of any systemically available Ala-Gln, the risk of systemic drug-drug interactions is considered negligible in this trial.

MC2-25 cream was safe and well tolerated when topically applied on a twice-daily basis for 2 weeks in minipigs. All excipients used in MC2-25 cream are known from topical use in other drug products (references on internal file). Therefore, the risk of local adverse effects (local application site reactions) is considered to be low in this trial.

A significant portion of CKD-ap patients in the trial are expected to be mid-aged to elderly and may have chronic comorbidities such as diabetes and hypertension. In view of the favourable safety profile, there are no eligibility criteria specific to the elderly population in this trial but

exclusion criterion 10 ‘Has a concurrent or recent (within 12 months prior to screening) medical condition that, in the opinion of the investigator, could pose undue risk to the subject…..’ would ensure that any patients (including elderly) who are vulnerable due to comorbidities are not enrolled in the trial. Due to the general frailness of CKD-aP patients rather than any known or suspected side effects of the trial treatment, regular general safety monitoring in the form of physical examinations, vital signs measurements (systolic and diastolic blood pressure, pulse, and body temperature), safety blood samples (haematology and biochemistry), and ECGs are included in this trial. Patients will be enrolled through specialist nephrology sites experienced in the care for CKD patients including those who are elderly.

In view of the major impact of CKD-aP on patient’s quality of life, the potential benefits of MC2-25 cream on CKD-aP, and the benign safety profile of MC2-25 cream, the benefit-risk ratio for the current trial is considered to be positive.

Table 1: Summary of Risks and Mitigation Strategies

Investigational Medicinal Product (MC2-25 cream)	
Risk Title: Application site reaction	**Risk Description:** No application site reactions were observed after topical (dermal) application of MC2-25 cream and MC2-25 vehicle to minipigs (twice daily for 2 weeks). Consequently, the risk of application site reactions is considered low. However, in this trial MC2-25 cream and MC2-25 vehicle will be applied topically in humans for the first time. Therefore, at the present time application site reactions in humans cannot be ruled out.
	Risk Mitigation: The first IMP application will be performed in the clinic. Adverse events will be evaluated more frequently during the first 2 weeks of the trial treatment phase (weekly) compared to the subsequent weeks of the trial treatment phase (bi-weekly). In addition, a subject may withdraw from the trial or discontinue trial treatment at any time at his/her own request or at the discretion of the investigator due to unacceptable adverse events.
Risk Title: Use during Pregnancy	**Risk Description:** The toxicological profile of the active product ingredient Ala-Gln has been evaluated as part of the approval of Dipeptiven® with no indications of teratogenic or other embryotoxic and peri-postnatal injuries observed up to an intravenous dosage of 1.6 g N(2)-L-alanyl-L-glutamine/kg body weight per day. Reproductive toxicity studies have not been conducted with MC2-25 cream.

	Risk Mitigation: According to the SmPC Dipeptiven® should not be administered during pregnancy due to lack of experience. Therefore, female subjects can only be randomized in the trial if they are of either non-childbearing potential or of childbearing potential and have a negative pregnancy test at the Baseline visit and use a highly effective method of contraception throughout the trial (see inclusion criteria for details). Due to the short half-life in plasma (<5 min), pregnancy testing is only mandatory while subjects receive double-blind treatment.
Trial Procedures	
Risk Title: Blood sampling	**Risk Description:** Blood sampling is invasive and there is always a slight risk of bruising, infections, pain, etc.
	Risk Mitigation: All blood sampling will be performed by trained staff and the volume of blood to be drawn at each occasion is small (approximately 10 mL).
Risk Title: Tape stripping	**Risk Description:** Repeated tape stripping may lead to transient, minor redness and irritation at the (~4 cm^2) tape stripping site.
	Risk Mitigation: If unacceptable redness and or irritation develops during the procedure, the procedure must be stopped.
Risk Title: Skin biopsies	**Risk Description:** As is the case with all invasive procedures, punch skin biopsies carry a small risk of bleeding and infection. In addition, a small scar may develop (Nischal 2008).
	Risk Mitigation: Skin biopsies will only be performed under sterile conditions by trained staff. Skin biopsies are optional and additional informed consent will be obtained from subjects who agree to have skin biopsies performed. Subjects will be informed about the risk of bleeding, infection, and scarring. In addition, subjects who will have skin biopsies performed must not have any diagnosed bleeding disorders or known hypersensitivity to the local anaesthetic used.
Other	
Risk Title: Use of MC2-25 vehicle	**Risk Description:** One third of the subjects will be treated with MC2-25 vehicle during the 12-week treatment period. This might result in deterioration of CKD-aP.

	Risk Mitigation: Except for Remitch® in Japan and Korsuva® in US, no marketed products are approved for treatment of CKD-aP. Therefore, subjects will not miss out on approved treatment by participation in this trial. However, as described in Section 2.1.1, CKD-aP has a significant impact on quality of life and therefore off-label treatments are not uncommon. A recent Cochrane review concluded that, of all treatments for uremic pruritus, gabapentinoids (gabapentin and pregabalin) were the most studied and show the greatest reduction in itch scores (Hercz 2020). To limit the risk of deterioration of CKD-aP after entering the trial, all subjects are allowed to continue existing systemic treatment (including gabapentin and pregabalin) for CKD-aP during the trial, as described in more detail in exclusion criterion 13. Only one third of subjects will be randomized to receive the MC2-25 vehicle, which has emollient properties and as such is not strictly considered a placebo treatment. Subjects are free to withdraw from the trial or discontinue trial treatment at any time at their own request or at the discretion of the investigator in case of unacceptable adverse events. Withdrawal from the trial or discontinuation of trial treatment will not affect or prejudice the subject's further care or treatment.
Risk Title: COVID-19	**Risk Description:** In view of the negligible risk of systemic adverse effects and/or drug-drug interactions, the risk of experiencing an aggravation of COVID-19 or experiencing interactions with COVID-19 vaccination is considered negligible in this trial. Consequently, subjects who have not been diagnosed with COVID-19 and do not have symptoms suggestive of COVID-19 can safely be enrolled in the trial despite the ongoing pandemic and can follow COVID-19 vaccination programs. Subjects who have been diagnosed with or have symptoms suggestive of COVID-19 carry a significant risk of transmitting the disease to other trial participants and trial staff.
	Risk Mitigation: All subjects will be asked to inform the site immediately (via phone – NOT in person!) if they are diagnosed with or have symptoms suggestive of COVID-19 and they will not be randomized (see exclusion criterion 10.d) and will not be allowed to attend trial visits until local COVID-19 regulations allow it. Subjects who are diagnosed with COVID-19 after randomization may subsequently be asked to complete trial visits (to the extent possible) via phone until applicable negative test results can be provided (if necessary, additional trial supplies may be shipped to the subject) or be withdrawn from the trial at the investigator's discretion (see Section 5.4, Withdrawal from trial and discontinuation of treatment). COVID-19 diagnosis and/or COVID-19 vaccinations should be reported in the eCRF (as AEs or Concomitant therapies, respectively) to enable evaluation of any impacts on the safety of trial subjects or the integrity of trial data.

3.0 TRIAL OBJECTIVES AND PURPOSE

The primary objective is to explore the clinical efficacy of MC2-25 cream compared to MC2-25 vehicle in adults with chronic kidney disease-associated pruritus (CKD-aP).

The secondary objectives are to explore the safety of MC2-25 cream compared to MC2-25 vehicle in adults with CKD-aP and other objectives are to explore the subclinical effects of MC2-25 cream in adults with CKD-aP.

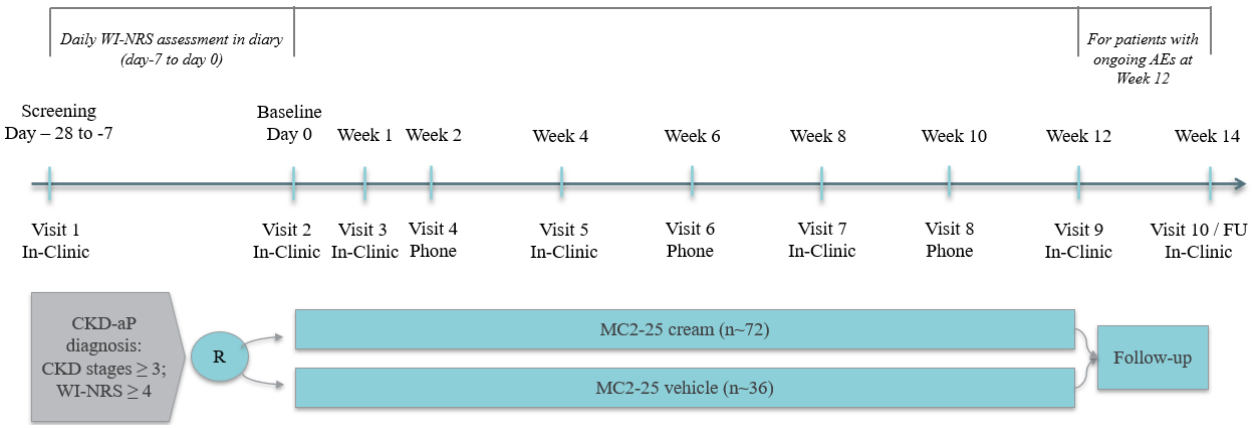
4.0 TRIAL DESIGN

4.1 Overall trial design

This is a multicentre, phase 2, randomized, double-blind, 2-arm, parallel-group and vehicle-controlled trial in subjects with CKD-aP. Approximately 108 eligible subjects will be randomised in a 2:1 ratio to MC-25 cream or MC2-25 vehicle, respectively. Subjects will apply the assigned IMP twice daily for 12 weeks.

The overall trial duration for each subject is maximum 18 weeks. Subjects will be seen at the trial sites at Screening, Baseline, Week 1, Week 4, Week 8, and Week 12 (end of treatment, EoT). Subjects who have ongoing serious adverse events (SAEs) or related adverse events (AEs) at Week 12 will have a follow-up visit at Week 14 or (in case of early treatment discontinuation) 14 days after the EoT visit, whichever comes first. Additionally, phone contacts are planned at Week 2, Week 6, and Week 10.

Figure 1: MC2-25-C1 Clinical Trial Design



4.2 Trial endpoints

Primary Objective: To explore the clinical efficacy of MC2-25 cream compared to MC2-25 vehicle in adults with CKD-aP	**Primary Endpoint:** • Mean change in weekly mean Worst Itch Numeric Rating Score (WI-NRS) recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. (Weekly mean WI-NRS is calculated as the average of WI-NRS values recorded in the subject's diary 7 days prior to and including the visits.) **Secondary Endpoints:** • Percentage of subjects obtaining a ≥4-point improvement in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects obtaining a ≥3-point improvement in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects obtaining a complete response in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. (Complete response is defined as scores equal to 0 or 1 in ≥80% of the non-missing WI-NRS values recorded in the subject's diary 7 days prior to and including the visits.) **Other Endpoints:** • Mean change in WI-NRS recorded during on-site visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Mean change in Sleeploss due to Itch Numeric Rating Score (SI-NRS) from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Mean change in Skin Dryness Numeric Rating Score (SD-NRS) from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.

	• Percentage of subjects who reported "Much better" or "A little better" in Subject's Global Impression of Change (SGIC) for Worst Itch (WI), Sleeploss due to Itch (SI), or Skin Dryness (SD) from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects who reported an important improvement in SGIC for WI, SI, or SD at Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Change in treatment area size from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Change in 5D-Itch from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Change in Skindex-10 from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Change in EQ-5D-5L from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects obtaining a ≥2-step improvement in Clinician's Global Assessment (CGA) of skin appearance from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects obtaining a ≥2-step improvement in one or more individual signs or in the Clinician's Targeted Assessment (CTA) of skin appearance from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.
Secondary Objective: To explore the safety of MC2-25 cream compared to MC2-25 vehicle in adults with CKD-aP	**Other Endpoints:** • Frequencies of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), adverse drug reactions (ADRs), AEs leading to treatment discontinuation or trial withdrawal, and deaths during the trial for MC2-25 cream compared to MC2-25 vehicle. • Changes in mean safety assessments: vital signs (heart rate, systolic blood pressure, diastolic blood pressure, temperature) and blood samples (biochemistry,

	haematology) from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Frequency of clinically significant abnormal physical examinations and ECGs from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of subjects who missed 1 or more dialysis visits during the Double-blind Treatment Period.
Other Objective: To explore the subclinical effects of MC2-25 cream compared to MC2-25 vehicle in adults with CKD-aP	**Other Endpoints:** • Changes in biomarkers in skin tape stripping samples from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Changes in biomarkers in skin punch biopsy samples from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.

5.0 SELECTION OF TRIAL POPULATION

5.1 Subject population

The trial population is males or non-pregnant females at least 18 years of age who have chronic kidney disease (CKD) stages G3-G5 (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2) and at least moderate CKD-aP (as defined in inclusion criterion 6). Both subjects on dialysis haemodialysis or haemodiafiltration and not on dialysis will be enrolled (=randomized).

Subjects who fail screening because they do not meet minimum WI-NRS criteria cannot be re-screened. Subjects who fail screening for other reasons may be re-screened once. Reason(s) for screen failure will be recorded in the eCRF. Subjects fulfilling all inclusion and none of the exclusion criteria can be randomized in the trial. Pre-randomization approval of deviations to inclusion or exclusion criteria is not permitted. If deviations to inclusion or exclusion criteria are identified post-randomization, subjects shall continue in the trial as planned unless discontinuation of IMP treatment is warranted for safety reasons.

5.2 Inclusion criteria

Subjects must meet all of the following criteria to be eligible for participation in the trial:

1. Adult males or non-pregnant females of any race or ethnicity who are ≥ 18 years of age at the time of screening
2. Able to understand the trial and willing to comply with trial requirements
3. Has provided written informed consent
4. Chronic (>3 months) kidney disease (CKD) stages G3-G5 (i.e., estimated glomerular filtration rate [eGFR] by CKD-EPI creatinine 2021 equation <60 mL/min/1.73 m^2)
5. Specifically for CKD subjects on haemodialysis (HD) or haemodiafiltration (HDF):
    a. Subjects must be established on HD or HDF 3 times per week continuously for at least 3 months prior to the start of screening (Note: at least the 2 last weeks of the 3-month period must have been in-centre dialysis) and must not have plans to change from HD to HDF or vice versa during the trial.
    b. Subjects who require an occasional additional HD or HDF treatment to manage fluid overload may be enrolled as long as it is anticipated that no more than 4 such treatments will be required in any given month.
6. At least moderate CKD-aP defined as WI-NRS ≥4 (i.e., the average of all and at least 4 non-missing scores reported by the subject in the diary for 7 days prior to and including the Baseline day, 8 days in total)
7. Female subjects must be of either:
    - Non-childbearing potential, i.e., postmenopausal* or confirmed sterile (e.g., hysterectomy, bilateral salpingectomy or bilateral oophorectomy).
      (*Note: a postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient) or,
    - Childbearing potential with a negative highly sensitive urine pregnancy test at the Baseline visit or (in the case of anuria) a negative serum pregnancy test at the Baseline visit that is no more than 3 days old.
8. Female subjects of childbearing potential must agree to use a highly effective method of contraception (i.e., a method with a failure rate of less than 1% per year when used consistently and correctly) while receiving double-blind treatment. Highly effective contraception is defined as follows:

- combined (estrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner (provided that is the sole sexual partner of the subject and that the vasectomised partner has received medical assessment of the surgical success)
- sexual abstinence if in line with the preferred and usual lifestyle of the subject and defined as refraining from heterosexual intercourse during the entire period of the trial. Periodic methods of abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) are not accepted methods of contraception.

5.3 Exclusion criteria

Subjects who fulfil any of the following criteria will be ineligible to participate in the trial:

1. In the opinion of the investigator, the subject is unlikely to comply with the clinical trial protocol.
2. Has a functioning kidney transplant or is scheduled to receive a kidney transplant during the trial (Note: subject can be on waiting list for kidney transplant)
3. Subjects who receive peritoneal dialysis
4. In the opinion of the investigator has pruritus attributed to a cause other than CKD or its complications, including but not limited to dermatological disease (e.g., atopic dermatitis, psoriasis) or liver disease (cholestatic pruritus)
5. Has localized itch restricted to the palms of the hands
6. Only has pruritus during haemodialysis sessions
7. Has concurrent skin conditions (including but not limited to pruritic dermatoses, active skin infections, ulcerations) that may limit or prevent application of MC2-25 cream or MC2-25 vehicle or that may interfere with evaluation of the effects of MC2-25 cream or MC2-25 vehicle on the skin at the Screening or Baseline visits
8. Subjects who will have skin biopsies performed must not have any known hypersensitivity to the local anaesthetic or diagnosed bleeding disorders. Note: subjects with suspected uremic platelet dysfunction, without other bleeding diatheses, can be enrolled if the investigator agrees.

9. Known history of allergic reaction to any ingredients in MC2-25 cream or MC2-25 vehicle (see Section 6.1)
10. Has a concurrent or recent (within 12 months prior to screening) medical condition that, in the opinion of the investigator, could pose undue risk to the subject, impede completion of the trial procedures, or would compromise the validity of the trial measurements, including, but not limited to:
    a. known or suspected abuser of alcohol, drugs, or narcotic substances
    b. severe physical, mental, or cognitive disorder other than CKD
    c. malignancy
    d. failure to comply with local COVID-19 regulations on vaccination or testing (due to risk of transmitting the disease to other trial participants or trial staff).
11. Has an uncontrolled Human Immunodeficiency Virus (HIV) or a known active generalized infection (bacterial, viral, or fungal) that, in the opinion of the investigator, may interfere with the assessment of safety or efficacy in this trial
12. Is pregnant, breast feeding, or planning a pregnancy
13. Start of a new or change to existing systemic treatment for CKD-aP, including but not limited to antihistamines, corticosteroids, opioids, GABA analogues, or kappa opioid receptor agonists within 21 days prior to the Baseline visit
14. Use of emollients on CKD-aP areas within 10 days prior to the Baseline visit
15. Use of any topical treatment on CKD-aP areas, including but not limited to antihistamines, or corticosteroids within 21 days prior to the Baseline visit (Note: standard topical treatments used on or around a haemodialysis access site are allowed, e.g., local anaesthetics, sterilants)
16. Use of any light therapy for CKD-aP, including but not limited to UV-B within 35 days prior to the Baseline visit
17. Start of a new or change of existing non-biologic systemic immunosuppressive treatment, including but not limited to corticosteroids, cyclosporin, and tacrolimus within 21 days prior to the Baseline visit
18. Start of a new or change of existing biologic systemic treatment, including but not limited to etanercept, adalimumab, alefacept, infliximab, and ustekinumab within 3 months or 5 half-lives (whichever is longer) prior to the Baseline visit
19. Subjects who consent to having skin biopsies performed who are using anticoagulation treatment and are judged by the investigator to have an unacceptable risk of excessive bleeding in association with the skin biopsy

20. Subjects not currently on dialysis but who are likely to initiate routine dialysis during participation in the trial
21. Received another investigational drug within 30 days or 5 half-lives (whichever is longer) prior to screening or is planning to participate in another clinical trial while enrolled in this trial
22. Previously randomized in this trial

5.4 Withdrawal from trial and discontinuation of treatment

In accordance with legal requirements and International Council for Harmonisation (ICH) – Good Clinical Practice (GCP) guidelines, every subject has the right to refuse further participation in this trial (withdrawal from trial) or refuse further treatment (discontinuation of treatment) at any time and without providing reasons. A subject's refusal should be effectuated immediately upon his/her request.

In addition, a subject may be withdrawn from the trial or be discontinued from treatment at any time at the discretion of the investigator.

Reasons for withdrawal from trial or discontinuation of treatment include (but are not necessarily limited to) progressive disease, AE, pregnancy, death, protocol violation, and lost to follow-up. If the IMP application is discontinued regardless of the reason for discontinuation, the subject should be encouraged to continue in the trial.

The reasons for trial withdrawal or treatment discontinuation are to be fully documented on the electronic case report form (eCRF) and an EoT visit should be completed as soon as possible to the extent possible. Subjects who withdraw from trial before randomization will be considered screen failures. In case of treatment discontinuation, the subject should be encouraged to attend remaining trial visits in line with the schedule of assessments (SoA). If a subject is withdrawn from the trial due to an AE or treatment is discontinued due to an AE, the AE must be followed up as outlined in Section 7.12.2.

If subjects are diagnosed with or have symptoms suggestive of COVID-19 they may not be allowed to attend trial visits until local regulations allow it. Subjects who are diagnosed with COVID-19 after randomization may subsequently be asked to complete trial visits (to the extent possible) via phone until applicable negative test results can be provided (if necessary, additional trial supplies may be shipped to the subject) or be withdrawn from the trial at the discretion of the investigator.

5.5 Replacement policy

Subjects who discontinue treatment after the administration of the first dose of trial medication will not be replaced. See Section 7.13 for replacement of subjects missing assessments due to COVID-19.

6.0 TRIAL TREATMENTS

6.1 Investigational medicinal product

MC2-25 cream 30 mg/g is a white oil-in-water, topical emulsion containing L-Alanyl-L-Glutamine in the aqueous phase at concentrations of 3% (w/w). In addition to the active ingredient, the formulation contains Triglycerides medium chain, Paraffin liquid, Glycerol, Carbomer interpolymer type A, Phenoxyethanol, Macrogol lauryl ether (4), Citric acid, Sodium Benzoate, Polysorbate (20), Sodium hydroxide, and Purified water.

6.2 Vehicle product

MC2-25vehicle is identical to the IMP except for the active ingredient.

6.3 Dosing regimen

MC2-25 cream or MC2-25 vehicle is to be applied twice daily, in the morning and evening, to areas with CKD-aP. The treatment must be applied in a thin even layer to affected areas and rubbed in gently to ensure that the treatment areas are saturated. Treatment of all areas with CKD-aP must be continued until the Week 12 (or EoT) visit. This is also applicable for areas becoming itch-free during trial participation and new areas of CKD-aP identified after the Baseline visit.

The daily dose should not exceed 50 g. Detailed application instructions will be provided in the subject instructions.

6.4 Dose modification

Not applicable.

6.5 Packaging, labelling and storage

MC2-25 cream and MC2-25 vehicle are identical in appearance and packaging.

Medication labels for the IMPs will comply with the legal requirements of the country where the trial is performed and be printed in the local language.

The IMPs will be supplied by MC2's designated vendor and will be stored securely at the site under the control of the investigator. The temperature will be monitored and documented.

The IMP will be supplied to the clinical site(s) as tubes containing 60 g of product. The IMP is to be stored at a temperature of 2° to 8°C at the site, and below 25°C after dispensing to the subject. At all times, the IMP tube should be protected from direct sunlight exposure.

6.6 Assignment to treatment

6.6.1 Randomisation

Randomisation will be performed using a validated system that automates the random assignment of treatment groups to randomisation numbers. Treatment assignment will be via a central interactive web response (IWR) system in accordance with a pre-planned computer-generated randomisation schedule in a 2:1 ratio.

Randomisation will be stratified according to the following CKD stage strata:

A. ≥40% of subjects must be on dialysis (HD or HDF) and must be CKD stages 4-5

B. ≥20% of subjects must Not be on dialysis and must be CKD stages 4-5

C. ≥20% of subjects must Not be on dialysis and must be CKD stage 3

Randomisation data will be kept strictly confidential, accessible only to authorised persons, until the time of unblinding.

A subject who fulfils the trial eligibility requirements will be randomly assigned to treatment.

6.6.2 Blinding

This is a double-blind trial. The sponsor, sponsor representatives, and all subjects, monitors, and trial personnel will be blinded throughout the trial. Instructions for emergency unblinding are provided in Section 13.1.

6.7 Prior, concomitant, and prohibited therapies

Medications and therapies are hereafter referred to as therapies. All therapies (i.e., prescription drugs [including COVID-19 vaccination programs, see Table 1], dialysis treatment regimen, over-the-counter [OTC] drugs and vitamins, herbal and dietary supplements) taken within 30 days prior to screening will be recorded as prior therapies at the Screening visit.

Thereafter, all therapies used during the course of the trial will be recorded as concomitant therapies. Information regarding the total daily dose, route of administration, start and discontinuation dates, and indication are to be recorded on the subject's eCRF.

6.7.1 Prohibited therapies prior to baseline

A number of therapies are prohibited during the period leading up to baseline as outlined in Table 2.

Table 2: Prohibited Therapies Prior to Baseline

Prohibited Therapy	Exclusion Criterion	Applicable Period Prior to Baseline (Day 0)
Start of a new or a change to existing systemic treatment for CKD-aP, including but not limited to antihistamines, corticosteroids, opioids, GABA analogues, or kappa opioid receptor agonists (Section 5.3).	13	21 days
Use of emollients on CKD-aP areas within 10 days prior to the Baseline visit	14	10 days
Use of any topical treatment on CKD-aP areas, including but not limited to antihistamines, or corticosteroids. Note: standard topical treatments used on or around a haemodialysis access site are allowed, e.g., local anaesthetics, sterilants.	15	21 days
Use of any light therapy for CKD-aP, including but not limited to UV-B	16	35 days
Start of new or change of existing non-biologic systemic immunosuppressive treatment, including but not limited to corticosteroids, cyclosporin, and tacrolimus.	17	5 weeks
Start of new or change of existing biologic systemic treatment, including but not limited to etanercept, adalimumab, alefacept, infliximab, and ustekinumab	18	3 months or 5 half-lives (whichever is longer)

Therapies not listed are allowed.

6.7.2 Prohibited therapies from baseline to the end of the treatment period

Use of any therapy that would exclude the subject from participation in the trial (as specified in Section 5.3) is also prohibited during the treatment period, which includes therapies in the following categories:

- Start of new or change of existing systemic treatment for CKD-aP, including but not limited to antihistamines, corticosteroids, opioids, GABA analogues, or kappa opioid receptor agonists (but stable systemic CKD-aP treatment can be continued).
- Any topical treatment on CKD-aP areas, including but not limited to emollients, antihistamines, and corticosteroids

- Therapeutic anticoagulation treatment for subjects who have skin biopsies performed (heparin administered in association with dialysis is allowed but in such cases skin biopsies must not be performed on a dialysis day)
- Any light therapy for CKD-aP, including but not limited to UV-B
- Start of new or change of existing non-biologic systemic immunosuppressive treatment, including but not limited to corticosteroids, cyclosporin and tacrolimus
- Start of new or change of existing biologic systemic treatment, including but not limited to etanercept, adalimumab, alefacept, infliximab, and ustekinumab

If a prohibited therapy is used during the trial, treatment with the IMP shall not automatically be discontinued. After consultation with the sponsor's or designee's Medical Monitor, the investigator shall use his/her judgement to decide if (in the following prioritized order) 1) The prohibited therapy can safely be stopped; 2) the prohibited therapy cannot safely be stopped but the IMP treatment can safely continue alongside the prohibited therapy; or 3) the prohibited therapy cannot safely be stopped and the IMP treatment cannot safely continue alongside the prohibited therapy. The event must be reported as a protocol deviation.

6.8 Therapies not listed are allowed. Treatment compliance and drug accountability

Records of trial product used, and dosages administered will be kept during the trial. The trial monitor will note product accountability during site visits and at the completion of the trial. Subjects will be asked to return all used and unused tubes in the outer box at each visit to ensure drug accountability. All returned tubes that had been dispensed to a subject and have broken seals will be weighed to determine the amount of the IMP.

At all on-treatment visits, the subject will be asked if he/she has used the trial medication as prescribed and to indicate the CKD-aP treatment area size. Any degree and nature of noncompliance will be specified. In addition, subjects will be asked to complete a dosing diary during the treatment period as a measure of treatment compliance. Subjects who are consistently noncompliant will be counselled.

7.0 VISIT SCHEDULE AND ASSESSMENTS

7.1 Visit schedule

The schedule of assessments (SoA) is provided in Table 3.

The informed consent process must be completed before any other assessment or procedure. The following order of assessments must always be adhered to:

- Patient Reported Outcomes should be assessed before all other clinical assessments or procedures.
- Skin tape stripping and skin biopsies of CKD-aP target areas must be taken after imaging and CTA of CKD-aP target areas.

On-site visit days should preferably be on the same weekday throughout the trial for subjects on dialysis. On-site visits should be aligned with dialysis visits to the extent possible to avoid unnecessary travelling for subjects on dialysis.

Table 3: Visit Schedule

Trial Period	**Screening Period**	**Treatment Period**								**FU period[1]**
Visit Number[2]	**1 (Screening)**	**2 (Baseline)**	**3**	**4**	**5**	**6**	**7**	**8**	**9 (EoT)[3]**	**10 (FU)**
Trial Week		**0**	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Days from Baseline (Visit 2) ***Visit Window***	**-28 to -1** ***NA***	**0** ***NA***	**7** ***±1***	**14** ***±2***	**28** ***±4***	**42** ***±2***	**56** ***±4***	**70** ***±2***	**84** ***±4***	 ***±2***
In-clinic or Phone Visits[4]	In-Clinic	In-Clinic	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	In-Clinic
Informed Consent[5]	X									
Eligibility Assessment	X	X								
Demographics	X									
CKD History	X									
Medical History	X									
CKD-aP Treatment History	X									
Worst Itch NRS (WI-NRS) – at-home (diary) assessments[6]		X	X		X		X		X	
Worst Itch NRS (WI-NRS) – On-site assessment[7, 8]	X	X	X		X		X		X	
Sleeploss due to Itch NRS (SI-NRS)[8]	X	X	X		X		X		X	
Skin Dryness NRS (SD-NRS)[8]	X	X	X		X		X		X	
Subject's Global Impression of Change (SGIC)[8]			X		X		X		X	
Subject´s rating of importance of improvement[8]			X		X		X		X	
Subject's indication of CKD-aP treatment area size[8, 9]		X	X		X		X		X	
5D-Itch[8]		X			X		X		X	
Skindex-10[8]		X			X		X		X	
EQ-5D-5L[8]		X			X		X		X	
CKD-aP target area identification		X								
CGA of Skin Appearance	X	X	X		X		X		X	
CTA of Skin Appearance		X	X		X		X		X	
Physical examination[10]	X	X							X	(X)
Vital signs[11]	X	X			X		X		X	(X)

Trial Period	**Screening Period**	**Treatment Period**								**FU period[1]**
Visit Number[2]	**1 (Screening)**	**2 (Baseline)**	**3**	**4**	**5**	**6**	**7**	**8**	**9 (EoT)[3]**	**10 (FU)**
Trial Week		**0**	**1**	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Days from Baseline (Visit 2) ***Visit Window***	**-28 to -1** ***NA***	**0** ***NA***	**7** ***±1***	**14** ***±2***	**28** ***±4***	**42** ***±2***	**56** ***±4***	**70** ***±2***	**84** ***±4***	 ***±2***
Single 12-lead ECG		X			X				X	(X)
Pregnancy Testing[12]	X	X			X				X	(X)
Laboratory Assessments[13]	X	X			X				X	(X)
Imaging of CKD-aP[14]		X			X				X	
Randomisation		X								
Diary (Handout)	X	X	X		X		X			
Biopsy[15]		X							X	
Tape stripping[16]		X							X	
Prior & concomitant therapies[17]	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X
Investigational medicinal product (IMP) dispensation		X			X		X			
IMP return, compliance and accountability					X		X		X	
IMP application[18]		X								
Diary (Review)		X	X		X		X		X	

1. Follow-up (FU) visit is only applicable if there are ongoing serious adverse events (SAEs) or related adverse events (AEs) at Week 12. (X) indicates assessments to be performed if judged necessary by the investigator.
2. An unscheduled visit can be conducted to perform any of the postbaseline assessments or procedures included in the SoA if deemed necessary by the Investigator.
3. In the case of trial withdrawal or treatment discontinuation, all assessments of the Week 12 (End of Treatment [EoT]) visit have to be performed. The end of treatment form must be completed when treatment is discontinued and the end of trial form must be completed at end of the trial or in case of trial withdrawal.
4. Phone calls: To remind subjects about diary documentation (in particular once-daily WI-NRS assessments that must be performed for 8 days leading up to each on-site visit [i.e., from day -7 to day 0 where day 0 = next on-site visit day]) and to ask for AEs. Phone visits may be replaced by in-clinic visits where relevant, e.g., in subjects going to the dialysis unit for haemodialysis.
5. Informed consent must be signed by both subject and investigator before any trial-related procedures are performed.

6. At-home (diary) assessments must be performed for 8 days leading up to each visit, i.e., from day -7 to day 0 where day 0 = next on-site visit day.
7. The WI-NRS must also be reported by the subject at on-site visits
8. At all on-site visits, all patient-reported outcome (PRO) assessments should be completed before all other clinical assessments or procedures.
9. The trial staff must estimate the % body surface area treated based on the subject's indication of CKD-aP treatment area size on the CKD-aP treatment area diagram.
10. Physical exam: A complete physical examination is required at Screening and Week 12 (EoT or Early Termination) visits. An abbreviated physical examination is required at the Baseline visit (and if judged necessary by the investigator) at the FU visit if the FU visit is applicable.
11. Vital signs include blood pressure, heart rate, and body temperature measurement. For subjects on haemodialysis vital signs are to be assessed prior to dialysis. Blood pressure and pulse rate will be taken with the subject in the sitting position with approximately 5 minutes rest prior to measurement.
12. Serum pregnancy tests are conducted for women of childbearing potential. At Baseline a urine pregnancy test must also be conducted except for dialysis subjects with anuria.
13. For subjects on dialysis, the blood samples must be collected before dialysis.
14. Only at selected sites and only for subjects who have provided additional consent. CKD-aP target area selected at Baseline. Images of CKD-aP target area must be taken before tape stripping and skin biopsies.
15. Only at selected sites and only for subjects who have provided additional consent. Biopsy: 4 mm skin punch biopsy (optional) is taken from the CKD-aP target area.
16. Tape stripping is conducted in all subjects from the CKD-aP target area.
17. "Therapies" refers to medications and therapies.
18. The first IMP application shall preferably be performed in the clinic under surveillance, and assistance if necessary, of trial staff.

7.2 Demographics and medical history

The following demographic and medical history must be collected in the eCRF:

- Year of birth
- Sex
- Race
- Subject-reported ethnicity
- CKD stage and history, including age of diagnosis, severity, and in subjects on haemodialysis (HD or HDF), information on haemodialysis
- All other current and past medical/surgical conditions within the previous 12 months

7.3 Prior and concomitant therapies

Prior or concomitant therapies as defined in Section 6.7 must be recorded in the eCRF at all trial visits. Information regarding the total daily dose, route of administration, start and discontinuation dates, and indication are to be recorded on the subject's eCRF.

7.4 Physical examination

A complete physical examination (i.e., including all organ systems as well as the skin) is required at Screening and Week 12 or (EoT or Early termination). The height and weight can be self-reported. An abbreviated physical examination (i.e., including the skin as a minimum; other organ systems may be included depending on present signs, symptoms or investigation outcomes) is conducted at Baseline. For subjects with SAEs or ongoing related AEs at Week 12, an abbreviated physical examination is also conducted at Week 14 (Follow-up) if judged necessary by the investigator.

Any abnormalities must be assessed as "clinically significant" or "not clinically significant" by the investigator. Abnormalities classified as "clinically significant" must be recorded in the eCRF as AEs except those observed at Screening visit 1, which will be recorded as medical history.

7.5 Vital signs

Vital signs must be recorded in the eCRF. Vital signs (systolic and diastolic blood pressure, heart rate, and body temperature) will be measured at Screening, Baseline, Week 4, Week 8, and Week 12 (EoT or Early Termination) visits. For subjects with ongoing SAEs or related AEs at Week 12, vital signs are also measured at Week 14 (Follow-up) if judged necessary by the investigator. In haemodialysis subjects, vital signs are to be recorded prior to dialysis. Blood pressure and heart rate will be taken with the subject in the sitting position with approximately 5 minutes rest prior to measurement. Body temperature (oral or ear) will also be measured.

Any abnormalities must be assessed as “clinically significant” or “not clinically significant” by the investigator. Abnormalities classified as “clinically significant” must be recorded in the eCRF as AEs except those observed at Screening visit 1, which will be recorded as medical history.

7.6 Electrocardiogram (ECG)

A single 12-lead ECG must be recorded using local equipment at Baseline, Week 4, and Week 12 or Early Termination. For subjects with ongoing SAEs or related AEs at Week 12, a single 12-lead ECG will also be performed at Week 14 (Follow-up) if judged necessary by the investigator. In haemodialysis subjects, ECGs are to be performed preferably prior to dialysis. Interpretation of ECGs will be based on machine read-out and local physician evaluation (cardiologist overread will not be performed).

Any abnormalities must be assessed as “clinically significant” or “not clinically significant” by the investigator. Abnormalities classified as “clinically significant” must be recorded in the eCRF as AEs except those observed at Screening visit 1, which will be recorded as medical history.

7.7 Pregnancy test

Women of childbearing potential will undergo a serum pregnancy test at Screening, Baseline, Week 4, and Week 12 or Early Termination. At Baseline a highly sensitive urine pregnancy test (i.e. with sensitivity down to at least 25 mIU/ml for human chorionic gonadotrophin (hCG)) must also be conducted except in dialysis subjects with anuria. At the investigator’s discretion, additional testing for pregnancy may be performed at the FU visit if this visit is applicable.

All pregnancies must be recorded in the eCRF and should be immediately reported to the sponsor or designee and followed through to resolution (i.e., delivery, miscarriage, or abortion). The report should be submitted within the same timelines as an SAE (within 24 hours of knowledge), although a pregnancy per se is not considered an SAE.

7.8 Laboratory assessments

Clinical laboratory specimens will be analysed by a central licensed and accredited laboratory facility according to the laboratory’s standard operating procedures.

Any abnormalities must be assessed as “clinically significant” or “not clinically significant” by the investigator. Abnormalities classified as “clinically significant” must be recorded in the eCRF as AEs except those observed at Screening visit 1, which will be recorded as medical history.

7.8.1 Haematology and biochemistry

For all subjects, the following tests will be performed at Screening, Baseline, Week 4, and Week 12 or Early Termination. For subjects with ongoing SAEs or related AEs at Week 12, laboratory tests will also be performed at Week 14 (Follow-up) if judged necessary by the investigator. In patients on dialysis, the blood samples must be collected before dialysis:

- Haematology: haemoglobin, haematocrit, red blood cell (RBC) count, mean corpuscular volume (MCV), and white blood cells (WBC) count (basophils, eosinophils, neutrophils, lymphocytes, and monocytes), including differential count and platelet count
- Serum biochemistry: urea, creatinine, albumin, sodium, potassium, chloride, calcium, phosphate, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)

7.9 Patient-reported outcome (PRO) assessments

At all in-clinic visits, all PRO assessments should be completed before other clinical assessments and procedures.

7.9.1 Worst itch numeric rating score (WI-NRS)

The subject's WI-NRS must be recorded by the subject in a diary once daily (at the same time of the day throughout the trial, i.e., morning, afternoon, or evening for all recordings) on day -7 to day 0 (where day 0 = next on-site visit day) leading up to each visit (8 days in total) at Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using an 11-point numeric rating scale ranging from 0 to 10:

Please score the intensity of the Worst Itch you have experienced in the past 24 hours: 0 (no itch), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (very severe/extreme itch).

In addition, the subject's WI-NRS must be evaluated at on-site visits at Screening, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using the 11-point verbal rating scale above.

7.9.2 Sleeploss due to itch numeric rating score (SI-NRS)

The subject's SI-NRS must be evaluated at Screening, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using an 11-point numeric rating scale ranging from 0 to 10:

Please score how much Sleeploss due to Itch you have experienced in the past 24 hours: 0 (no sleeploss due to itch), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (very severe/extreme sleeploss due to itch).

7.9.3 Skin dryness numeric rating score (SD-NRS)

The subject's SD-NRS must be evaluated at Screening, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using an 11-point numeric rating scale ranging from 0 to 10:

Please score the intensity of the Worst Skin Dryness you have experienced in the past 24 hours in the areas treated with trial medication: 0 (no skin dryness) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (very severe/extreme skin dryness).

7.9.4 Subject's global impression of change (SGIC)

SGIC in Worst Itch (WI), Sleeploss due to Itch (SI), and Worst Skin Dryness (SD) must be evaluated at Week 1, Week 4, Week 8, and Week 12 or Early Termination using a 7-item verbal rating scale:

Please choose the response below that best describes the overall change in your (Worst Itch*) since you started taking the trial medication:

- Very much better
- Much better
- A little better
- No change
- A little worse
- Much worse
- Very much worse

*SGIC must be repeated with SI and SD.

7.9.5 Subject's rating of importance of improvement

Subjects reporting an improvement in SGIC ("Very much better," "Much better," or "A little better") must rate the importance of improvements in WI, SI, and SD at Week 1, Week 4, Week 8, and Week 12 or Early Termination using a 2-item Importance Questionnaire:

Question 1:You have reported an improvement in your (Worst Itch*) since you started taking the trial medication. Was the improvement important to you? (Yes/No). If yes, please proceed to question 2.

Question 2: In which way(s) was the improvement in your (Worst Itch*) important to you? (free text)

*Subject's rating of importance of improvements must be repeated with SI and SD.

7.9.6 Health-related quality of life (HRQoL)

HRQoL must be evaluated by the subject at Baseline, Week 4, Week 8, and Week 12 or Early Termination using the Skindex-10, 5D-Itch and EQ-5D-5L questionnaires.

7.9.6.1 Skindex-10

The Skindex-10 consists of 10 questions (beginning with "During the past week, how often have you been bothered by….") exploring the impact of itch on 3 domains (disease, mood/emotional distress and social functioning) with a 1 week recall period.

Each question must be scored on a 7-point scale ranging from 0 (never bothered) to 6 (always bothered).

The domain scores are the sums of the following: disease domain (questions 1 to 3), mood/emotional distress domain (questions 4 to 6), and social functioning domain (questions 7 to 10).

The total score is the sum of the numeric value of each answered question. The total score ranges from 0 to 60.

7.9.6.2 5D-Itch

The 5D-Itch consists of 5 domains numbered 1-5. Domains 1-3 are single item (question) domains exploring the Duration (Duration domain), Degree (Degree domain) and Direction (Direction domain) of Itch. Domain 4 (Disability domain) includes four items (questions) assessing the impact of itching on daily activities: sleep, leisure/social activities, housework/errands and work/school). Domain 5 (Distribution domain) assesses the number of affected body parts out of 16 possible options. For each of domains 1-3 the domain score is equal to the value indicated below the response choice (range 1–5). For domain 4 the score is achieved by taking the highest score on any of the four items (range 1-5). For domain 5 the score is obtained by tallying the number of affected body parts and the sum is sorted into five scoring bins: sum of 0–2 = score of 1, sum of 3–5 = score of 2, sum of 6–10 = score of 3, sum of 11-13 = score of 4, and sum of 14-16 = score of 5 (range 1-5).

The total score is achieved by summing together the 5 domain scores and can therefore range between 5 (no pruritus) and 25 (most severe pruritus).

7.9.6.3 EQ-5D-5L

The EuroQoL 5 Dimensions, 5 levels (EQ-5D-5L) is a generic, multidimensional, health-related, quality-of-life instrument and comprises a Visual Analogue Scale (VAS) and a short descriptive system questionnaire.

The VAS records the subject's overall current health on a vertical VAS where the endpoints are labelled "(100) The best health you can imagine" and "(0) The worst health you can imagine." The VAS provides a quantitative measure of the subject's perception of his/her overall health.

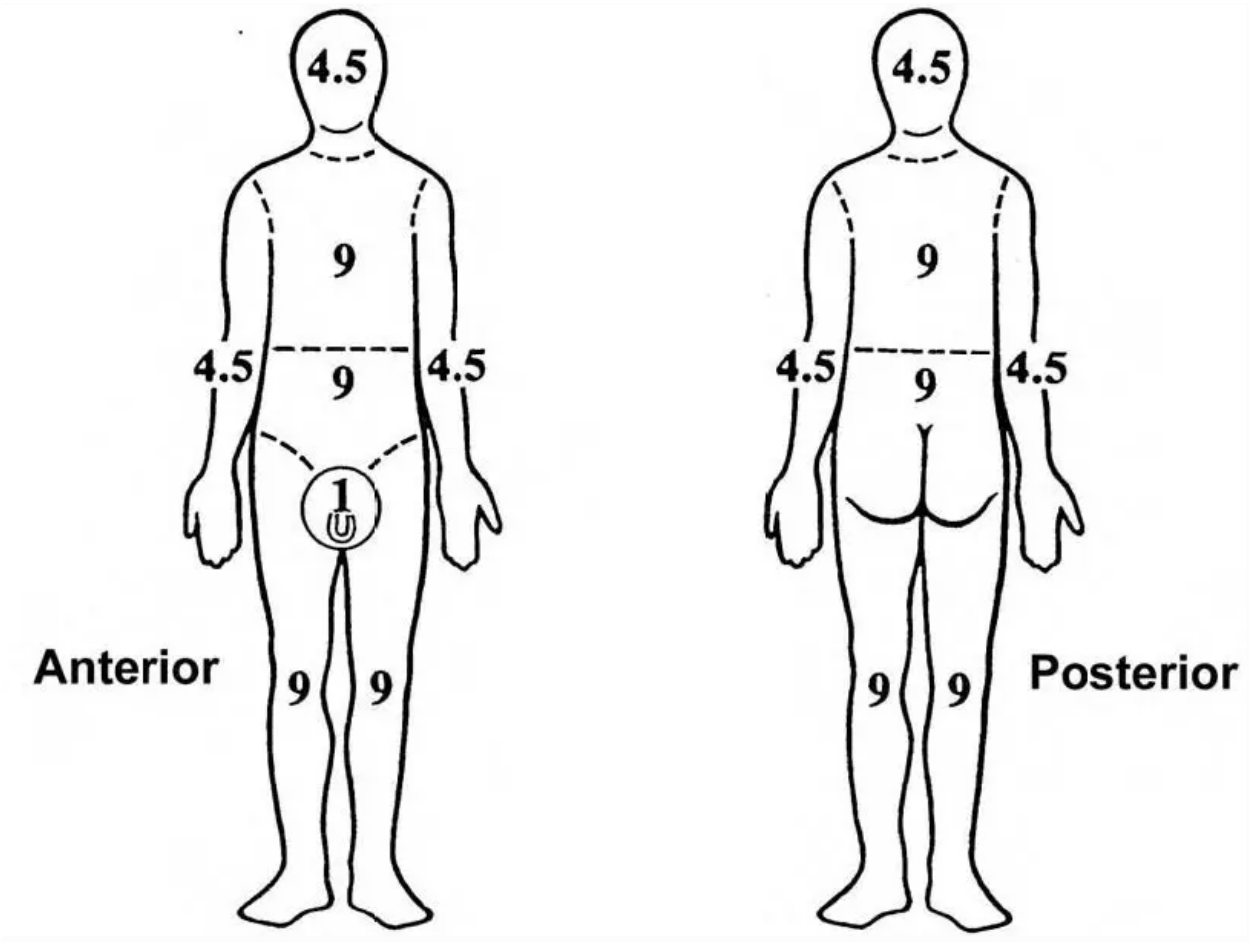
The descriptive system questionnaire allows subjects to rate their health in 5 domains: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression using a 5-level scale

indicating: 1. no problem, 2. slight problem, 3. moderate problem, 4. severe problem, or 5. unable to/extreme problems. The perceived problem levels for each domain are combined into a 5-digit health state that is converted to an index value, which reflects how good or bad a health state is according to the preferences of the general population with higher scores indicating better quality of life.

7.9.7 Subject's indication of CKD-aP treatment area size

The subject must indicate the size of the CKD-aP treatment area (by greying out all areas treated) at Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using the CKD-aP treatment area diagram below, if necessary, with assistance from trial staff. Subsequently, trial staff must estimate the size of the treatment area (in terms of % body surface area treated) based on the CKD-aP treatment area diagram:

CKD-aP treatment area diagram – according to subject (example)



7.10 Clinician-reported outcome (ClinRO) assessments

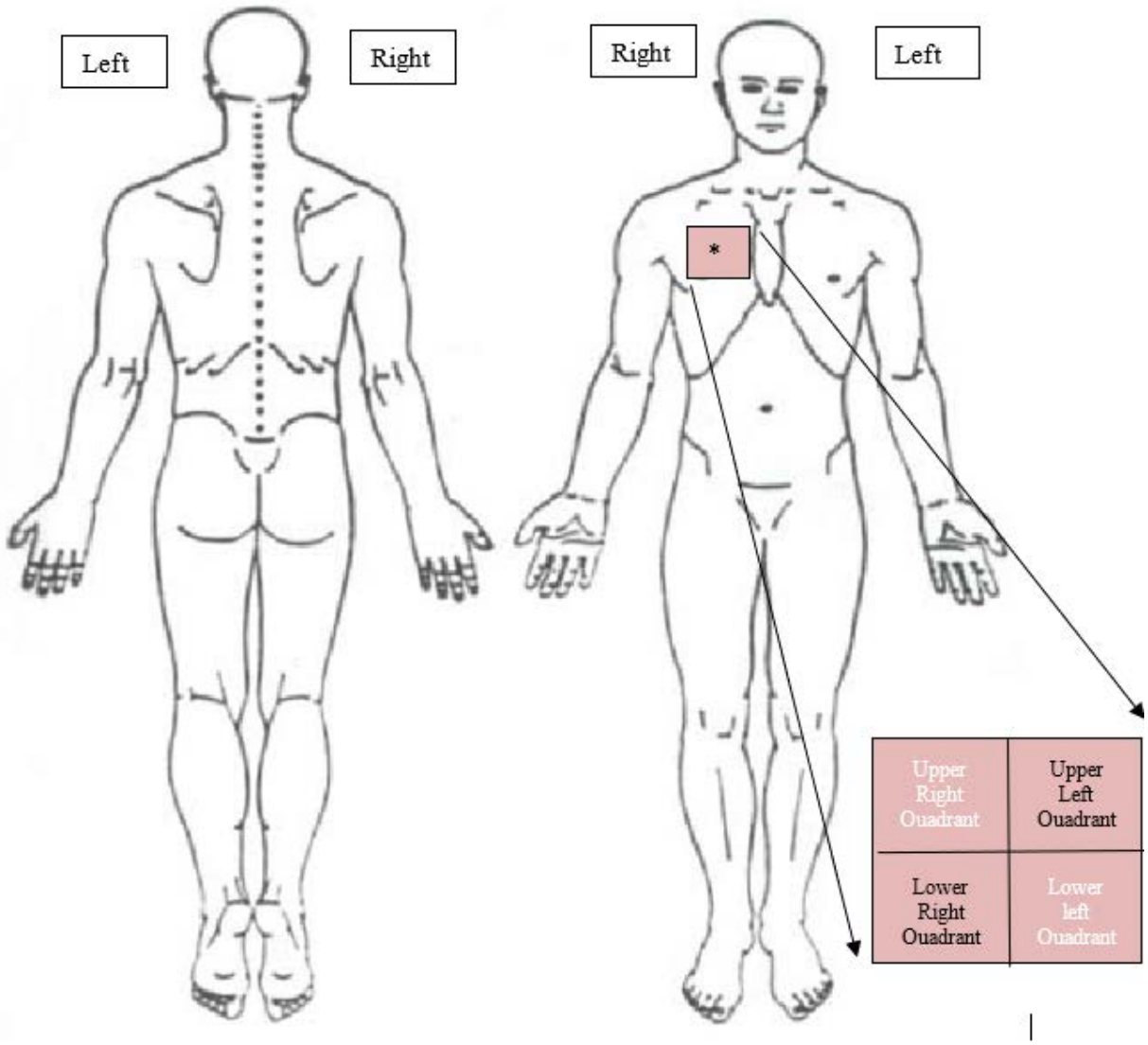
7.10.1 Identification of CKD-aP target area

At the Baseline visit a CKD-aP target area that fulfils the following criteria must be identified by trained clinical staff:

- The area must be among the areas with the worst CKD-aP according to the subject
- The area must measure minimum 10 cm x 10 cm in size (roughly the palm of a hand)
- The area must be easy for the subject to reach, to apply the IMP on and must be treated with the IMP throughout the double-blind treatment phase of the trial
- The area must not be in close proximity of dialysis access sites in haemodialysis subjects

When the CKD-aP target area has been identified it must be marked with a square on the "CKD-aP diagram." An example of a CKD-aP target area marked with an asterisk * is shown on the CKD-aP diagram below.

CKD-aP target area diagram (*example of CKD-aP target area)



7.10.2 Clinician's global assessment (CGA) of skin appearance

CGA of skin appearance on the entire body surface area must be evaluated by trained clinical staff (preferably the same staff member throughout the trial for an individual subject) and recorded in the eCRF at Screening, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using a 5-point verbal rating scale (from clear to severe) as follows:

Please choose the description below that best describes the severity of skin dryness and skin signs of itch on the entire body surface area:

CGA score	Skin Dryness Description	Skin signs of Itch Description
Clear	No skin dryness	No active skin signs of itch* (chronic skin signs of itch may be present)
Almost clear	Barely perceptible skin dryness	Barely recognizable active skin signs of itch*
Mild	Easily perceptible skin dryness	Easily recognizable active skin signs of itch*
Moderate	Moderate [between Mild and Severe] skin dryness	Moderate [between Mild and Severe] active skin signs of itch*
Severe	Marked skin dryness	Marked active skin signs of itch*

*Active skin signs of itch include but are not necessarily limited too excoriation, crusting, superficial bleeding or intradermal bleeding (ecchymoses) or prurigo nodules and must be distinguished from chronic skin signs of itch, e.g., skin discoloration, thickening or scarring.

The overall CGA intensity is defined based on the intensity of the individual signs skin dryness and skin signs of itch. In case of difference in intensity of skin dryness and skin signs of itch then the worst intensity shall prevail in the overall CGA score (e.g., in case of mild skin dryness and severe skin signs of itch, then the overall CGA must be scored as severe).

7.10.3 Clinician's targeted assessment (CTA) of skin appearance

CTA of skin appearance in the CKD-aP target area (see Section 7.10.1 for description of CKD-aP target area) must be evaluated by trained clinical staff (preferably the same staff member throughout the trial for an individual subject) and recorded in the eCRF at Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination. CTA includes assessments of skin dryness and skin signs of itch and overall CTA in the CKD-aP target area using a 5-point verbal rating scale (from clear to severe) as follows:

Please choose the description below that best describes the skin dryness and skin signs of itch on the CKD-aP target area:

CTA score	Skin Dryness Description	Skin Signs of Itch Description
Clear	No skin dryness	No active skin signs of itch* (chronic skin signs of itch may be present)
Almost clear	Barely perceptible skin dryness	Barely recognizable active skin signs of itch*
Mild	Easily perceptible skin dryness	Easily recognizable active skin signs of itch*
Moderate	Moderate (between Mild and Severe) skin dryness	Moderate (between Mild and Severe) active skin signs of itch*
Severe	Marked skin dryness	Marked active skin signs of itch*

*Active skin signs of itch include but are not necessarily limited to excoriation, crusting, superficial bleeding or intradermal bleeding (ecchymoses), or prurigo nodules and must be distinguished from chronic skin signs of itch, e.g., skin discoloration, thickening, or scarring.

The overall CTA intensity is defined based on the intensity of the individual skin dryness and skin signs of itch assessments. In case of difference in intensity of skin dryness and skin signs of itch then the worst intensity shall prevail in the CTA score (e.g., in case of mild skin dryness and severe skin signs of itch, then the overall CTA must be scored as severe).

7.10.4 Imaging of CKD-aP

Imaging of the CKD-aP target area (see Section 7.10.1 for description of CKD-aP target area) will be obtained by the trained clinical staff at Baseline, Week 4, and Week 12 or Early Termination in a subset of subjects who provide additional consent. The same area must be used for imaging at the subsequent timepoints indicated in the SoA. Images must always be taken before skin tape stripping and skin biopsies.

The imaging procedure will be described in a separate manual. Steps will be taken to ensure that the identities of the subjects are protected. Additional informed consent will be obtained with regard to collection, transmission and use of images.

7.11 Biomarker assessments of CKD-aP

The subclinical effects of MC2-25 on CKD-aP will be evaluated by analyses of relevant biomarkers in skin biopsies and skin tape strips after completion of the trial.

Skin tape stripping provides information on biomarker changes in the most superficial layer of the skin, i.e., the stratum corneum, which is the uppermost part of the epidermis. In contrast, a skin biopsy can provide information on biomarker changes through all layers of the skin, i.e., the epidermis and the dermis. As this trial is the first in humans with the MC2-25 cream it is

important to understand not only whether the treatment has effects on biomarkers in the skin but also to understand to which skin depth such effects extend.

7.11.1 Skin biopsies

Skin biopsies will be obtained (at selected trial sites) by a physician at Baseline and Week 12 or Early Termination (after the Week 4 visit) in a subset of subjects who provide additional consent to have skin biopsies performed.

Skin biopsies will be taken from the upper right quadrant at the Baseline visit and from the upper left quadrant at the Week 12 (EoT or Early Termination) visit following the directions described in Section 7.10.1. Skin biopsies should be performed at a site without scratch marks and at least 2 cm apart.

Skin punch biopsies must be performed by trained staff under local anaesthesia using a disposable 4-mm puncher. The puncher is pushed into the skin by rotary movement until a “give away” feel is perceived. Suture is generally not needed. A cotton bandage is sufficient (Nischal 2008).

The skin biopsy specimens will be stored for relevant analyses of biomarkers of CKD-aP. The samples will be destructed within 1 year after trial completion.

7.11.2 Skin tape strips

Skin tape strips will be obtained by a physician at Baseline and Week 12 or Early Termination in all subjects.

Tape strips will be taken from the lower right quadrant at the Baseline visit and from the lower left quadrant at the Week 12 (EoT or Early Termination) visit following the directions described in Section 7.10.1.

Tape stripping is a minimally invasive technique to sequentially remove stratum corneum by repeated application of appropriate adhesive tape. Areas with, e.g., eczema and scratch marks should be avoided. Round adhesive tape discs are attached to the skin for 10 seconds using a disc pressure applicator. The tapes are gently removed with a tweezer and stored in closed plastic vials at room temperature at the site and during shipment. At the central laboratory the samples must be stored between -20°C and -30°C until analysis. The first strip is discarded, and the following are stored together in groups as follows: strips 2-4, strips 5-7, and strips 8-10 (Kezic 2011).

The skin tape strips will be stored for relevant analyses of biomarkers of CKD-aP. The samples will be destructed within 1 year after trial completion.

7.12 Adverse events

7.12.1 Adverse events assessments

The investigator or designee is responsible for obtaining, assessing, and documenting all AEs during the trial.

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered an investigational or non-investigational medicinal product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs will be documented in the eCRF, including a description of each AE, AE relationship to IMP administration, start and stop dates, seriousness, severity, action taken and outcome.

Any AE that meets the serious criteria must be reported as an SAE to PharmaLex (PLX) within 24 hours of first awareness.

Contact information:

Company: PharmaLex A/S, Agern Allé 24, DK-2970 Hørsholm, Denmark

E-mail: PV-nordic@pharmalex.com

Throughout the trial, the occurrence of AEs should be monitored by nondirective questioning of the subject at each visit. Information on AEs can also be obtained from signs and symptoms detected during examinations, observations made by the trial site personnel, or spontaneous reports from subjects. Pre-existing conditions that worsen during the trial should also be recorded as AEs. This will also include worsening of the disease as judged by the investigator.

AEs requiring therapy must be treated in accordance with recognised standards of medical care to protect the health and well-being of the subject. Treatment due to an AE will be recorded in the subject's records and on the appropriate eCRF.

7.12.2 Reporting of adverse events

Adverse event information will be collected from individual trial subjects from signature of the informed consent form until the last trial visit.

If the event is reported as an SAE, the timelines for SAE reporting apply (see Section 7.12.10). If the event is reported as non-serious AE, it should be reported on the appropriate eCRF page.

All SAEs and related non-serious AEs must be followed by the investigator until they are resolved or until judged by the investigator to be stable/needing no further follow-up. All

relevant follow-up information will be reported in the eCRF (and to PharmaLex if it qualifies as an SAE, see Section 7.12.1).

7.12.3 Severity of adverse events

The investigator is to classify the severity (intensity) of an AE according to the following definitions:

- Mild – The subject was aware of the signs and symptoms, but the signs and symptoms were easily tolerated and does not interfere with daily activity.
- Moderate – The signs and symptoms were sufficient to restrict, but did not prevent, usual daily activity for the subject. The subject is still able to function.
- Severe – The subject was unable to perform usual daily activities.

The maximum severity recorded during the course of an AE (mild, moderate, or severe) will be considered the final severity.

7.12.4 Relationship of adverse events to trial treatment

The investigator is responsible for assessing the relationship of an AE to the IMP treatment using good clinical judgment and the following definitions:

- Not related – The AE is clearly explained by another cause not related to the IMP administration; the temporal relationship of the AE to IMP administration makes a causal relationship clearly improbable or concomitant medication, therapeutics interventions, or underlying condition provide a sufficient explanation for the observed AE.
- Unlikely related – The AE is most likely related to aetiology other than the IMP administration; the temporal relationship of the AE to IMP administration makes a causal relationship unlikely, and other concomitant medication, therapeutic interventions or underlying disease provide plausible explanations for the observed AE.
- Possibly related – The AE and administration of IMP are temporally related, but the AE can be explained equally well by causes other than the IMP administration. A causal relationship is conceivable and cannot be dismissed.
- Probably related – The AE and use of IMP are temporally related, and the AE is more likely explained by IMP administration than by other causes. Good reason and sufficient documentation to assume a causal relationship.

The last relationship recorded during the course of an AE will be considered the final relationship as the investigator is assumed to have had the most information to perform the relationship assessment at that time.

7.12.5 Outcome of adverse event

The outcome of an AE will be classified as:

- Recovered/resolved
- Recovered/resolved with sequelae (note: in case of sequelae, the sequelae must be reported in eCRF)
- Recovering/resolving
- Not recovered/not resolved
- Fatal
- Unknown

7.12.6 Unexpected serious adverse events

All SAEs will be assessed for expectedness by the sponsor or designee. An SAE is considered "unexpected" if its nature or severity is not included in the "Reference safety information" section in the MC2-25 cream IB.

7.12.7 Trial medication overdose

An overdose of the IMP is a dose that is higher than the highest dose under clinical investigation.

In the event of a clinically significant overdose, as judged by the investigator, the event should be reported as an AE and supportive treatment or discontinuation of the IMP may be applied as judged by the investigator. In view of the >40-fold systemic safety margin (see Section 2.3), the administered dose may be several fold higher than the highest dose under clinical investigation without necessarily qualifying as a clinically significant overdose.

7.12.8 Pregnancy

Any pregnancy occurring from date of the informed consent signature until trial completion -- must be recorded in the eCRF and reported to PharmaLex as soon as it becomes known to the investigator and not later than within 24 hours of his/her knowledge of the occurrence of the pregnancy (see Section 7.12.10).

The investigator must actively follow-up, document, and report to PharmaLex the progress of the pregnancy until outcome is reached.

7.12.9 Serious adverse event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening

- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other serious or important medical event

Any medical important events that may not result in death, be life-threatening, or require hospitalisation may be considered an SAE when, based on appropriate medical judgment, they may jeopardise the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in the above definitions.

The death of a subject enrolled in a trial is per se not an event, but an outcome. Any event resulting in a fatal outcome must be fully documented and reported, regardless of the causality relationship to the IMP.

Any pre-planned hospitalisations that are known at the time of signing the informed consent form will not be recorded as SAEs, however they will be recorded as AEs only.

Any SAE, whether or not deemed IMP-related or expected, must be reported immediately to PharmaLex as soon as it becomes known to the investigator and not later than within 24 hours of his/her knowledge of the occurrence of an SAE (see Section 7.12.10). The investigator will document such events in the best possible detail on the SAE Report Form.

A suspected unexpected serious adverse reaction (SUSAR) is an SAE that is both unexpected (not listed in the Reference Safety Information (RSI) section of the IB, and for which there is evidence to suggest a causal relationship between the drug and the SAE. The Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be informed of SUSARs according to local requirements. All investigators participating in the trial will also be notified of SUSARs.

Investigator instructions for reporting SAEs are provided in Section 7.12.10.

SAEs occurring after a subject has completed the trial and deemed IMP-related by the investigator should be reported to the sponsor if the investigator becomes aware of them.

7.12.10 Reporting of serious adverse events and pregnancies

7.12.10.1 Contact person(s) and number(s)

SAEs and pregnancies must be reported immediately (i.e., not later than 24 hours after first knowledge). The SAE or pregnancy report should be e-mailed or faxed to PharmaLex using the following e-mail or fax-number:

Email: PV-nordic@pharmalex.com
Fax number: +45 74 44 19 37

7.12.10.2 Reporting procedures

Serious adverse events

For each SAE, the investigator will complete a Serious Adverse Event Report Form and assess the relationship of each SAE to trial treatment. The completed form(s) should be sent to PharmaLex within 24 hours of first knowledge of the SAE.

Follow-up reports regarding the status of the SAE and the subject's subsequent course should be submitted until the SAE has resolved, is stabilised or needing no further follow-up (in the case of persistent impairment) or the subject dies. The form and acknowledgment of receipt will be retained. Contacts for reporting SAEs, pregnancies and other safety concerns are provided to each site.

7.13 COVID-19 Considerations

COVID-19 control measures in different countries may impact the ability to adhere to some of the trial procedures described in this protocol. Due to challenges that include, but are not limited to, subject preferences, site closures, travel restrictions, and quarantines, some modifications to trial conduct during the COVID-19 pandemic may be necessary to ensure trial continuity. The following are allowable, as necessary, modifications to trial conduct during the COVID-19 pandemic.

- Prior to a trial visit at the site, the subject may be contacted and may be asked to be screened for potential exposure or infection to COVID-19 per local requirements. If the subject is suspected or known to be infected with COVID-19, the on-site visit may either be re-scheduled or a virtual visit may be performed instead, as applicable.
- If a subject cannot attend the regularly scheduled trial visits in person due to COVID-19 necessitating a limit on in-person contact, the investigator may perform safety and efficacy assessments by phone or video to the extent possible. The investigator may use technology platforms that are considered suitable by the site.
- Source documentation and the eCRF should note that the visit was performed virtually (not face-to-face). If certain trial procedures or assessments cannot be completed per the SoA, the reason for the missed assessment must be noted in the source documentation (e.g., COVID-19) and captured in the protocol deviations documentation.

A detailed assessment of COVID-19-related risk and mitigation measures will be documented in the appropriate trial plans, e.g., trial risk management plan.

Subjects who missed significant WI-NRS assessments due to COVID-19 may be replaced if the total number exceeds 10% of subjects enrolled.

8.0 STATISTICAL AND ANALYTICAL PLANS AND DETERMINATION OF SAMPLE SIZE

8.1 General considerations for data analysis

The methodology presented below represents a brief overview of the statistical methods that will be fully detailed in the statistical analysis plan (SAP). The SAP will be finalised before the database is locked. Any changes to the methods described in the final SAP will be described and justified in the clinical trial report. All statistical analyses will be performed using SAS statistical software (Version 9.4 or higher).

Statistical significance will be tested at the two-sided 5% level unless otherwise specified. Superiority is concluded if a p-value of statistical hypothesis testing is below the level and the estimate of treatment difference is in favourable direction for MC2-25 cream.

Categorical variables will be described by summary tables of frequencies (counts and percentages) per treatment group, continuous parameters by displaying arithmetic mean, median, minimum and maximum values, standard deviation, and, where applicable, 1st and 3rd quartiles.

All collected and derived data will be presented in individual subject data listings.

8.2 Sample size and power considerations

Approximately 108 subjects will be randomized in the trial in 2:1 ratio of MC2-25 cream to MC2-25 vehicle, including an assumption of 10% dropouts. For a two-sided test with a 5% level of significance (expected d=1.5 and s=2.46, d/s=0.61) this sample size will provide adequate power (≥80%) to compare mean change in WI-NRS from Baseline to Week 12 between MC2-25 cream and MC2-25 vehicle with 72 and 36 subjects (Spencer 2018).

8.3 Analysis populations

The analysis populations are defined as follows:

Screened set (SS): all subjects who sign the informed consent form.

Safety analysis set (SAS): all subjects who are randomized and dispensed the trial medication at Randomisation/Day 0, excluding subjects to whom trial medication is not dispensed or who return all of the trial medication unused. Subjects will be analysed according to the actual treatment.

Full analysis set (FAS): all subjects who are randomized. Subjects will be analysed according to the randomized treatment.

Modified full analysis set (MFAS): all subjects who are randomized and returned for at least one post-baseline scheduled visit with data on WI-NRS. Subjects will be analysed according to the randomized treatment.

Per-protocol set (PPS): a subset of the MFAS subjects who completed the trial with WI-NRS data for Week 12 and are deemed to have no important protocol deviations that could interfere with the objectives of this trial. Important deviations of eligibility criteria and other deviations from the protocol will be assessed. Important deviations from the protocol may lead to exclusion of a subject or data points from the PPS, which could have interfered with the administration of the treatment or the evaluation of treatment effect. All such decisions will be identified and documented before the final trial database is unblinded. Important protocol deviations may include, but are not limited to, medication non-compliance, missing consecutive application days, and intake of prohibited concomitant therapies, which may have an impact on the primary endpoint.

The SAS will be used for all safety analyses. FAS, MFAS, and PPS will be used for efficacy analyses.

8.4 Background and demographic characteristics

For the FAS (and MFAS if it differs from the FAS) and the SAS, summary tables will describe the trial population by treatment group and overall, with regard to demographic and other baseline characteristics. Demographics include age, sex, race, and ethnicity (Section 7.2). Baseline characteristics include medical history, CKD stage and duration, whether subjects are on dialysis or not and time since initiation of dialysis, weekly mean WI-NRS, time since start of CKD-aP, and selected laboratory parameters.

8.5 Trial medication/exposure

Descriptive statistics will be used to summarise trial medication exposure for the SAS. Measures of IMP exposure will include the total duration of treatment, the total weight of trial medication used, the average weight of trial medication used and the total number of applications.

8.6 Prior and concomitant therapies

For the SAS, prior and concomitant therapies (as defined in Section 6.7) will be summarized by treatment. Separate summaries will be given for prior therapies, for prior therapies ongoing at baseline, and for concomitant therapies.

8.7 Analysis of safety

On the SAS, safety will be analysed by means of incidences of AEs, abnormalities in laboratory values, vital signs, physical examination and ECG findings. In addition, laboratory values will also be presented as described for continuous variables in Section 8.1.

8.7.1 Adverse events

Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) terminology. Treatment-emergent adverse events (TEAEs), related TEAEs, non-serious TEAEs, serious TEAEs, related serious TEAEs, TEAEs leading to treatment discontinuation and Deaths

will be summarised by the overall incidence of at least one event, incidence by body system, and incidence by body system and preferred term. Each subject will contribute only once (e.g., the first occurrence) to each of the rates, regardless of the number of occurrences (events) the subject experiences. The number of occurrences will also be displayed.

Treatment-emergent AEs will be summarised by severity (mild, moderate, or severe), and by relationship to trial product (not related, unlikely, possibly, or probably). In summaries of severity and relationship, subjects who report more than one event that are mapped to the same preferred term will be counted only once under the strongest severity and relationship, accordingly. An AE is treatment-emergent if its date of onset is on or after the day of start of trial medication. An AE is related if relationship to trial product is not assessed as unrelated.

8.8 Analysis of efficacy

On the FAS, all efficacy endpoints will be analysed, the primary endpoint also on the MFAS and the PPS. All endpoints, including PROs and HRQoL questionnaires, will be summarized as observed. For statistical analyses of primary and secondary endpoints, missing values will be imputed by different approaches described below. Further details will be given in the SAP.

8.8.1 Primary endpoint

The primary endpoint, mean change in weekly mean WI-NRS recorded in the subject's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle, will be analysed by a mixed model of repeated measures with Baseline weekly mean WI-NRS as covariate and treatment, CKD stage stratum at baseline, systemic CKP-aP treatment status at baseline and visit as fixed factors with a two-sided α=0.05. The weekly mean WI-NRS is calculated as the average of minimum 4 WI-NRS values recorded once daily in the subject's diary from 7 days prior to and including the visit days (i.e., must be recorded for 8 days total).

On the FAS, the primary analysis and one sensitivity analysis will follow a treatment policy strategy irrespective of any potential intercurrent events. On primary analysis, missing values will be replaced following a multiple imputation procedure adequate for the endpoint assuming missingness appearing at random and based on 20 imputations. On the FAS, sensitivity analyses will include imputation by last observation carried forward (LOCF; treatment policy strategy) and multiple imputation of missing data and all data after start of rescue medication (hypothetical strategy).

Applying the same statistical model, further estimands include multiple imputation on the MFAS and as observed on the PPS, both following a treatment policy strategy. If deaths occur, further estimands may be defined following a composite endpoint strategy.

Supportive subgroup analyses will be conducted by dialysis status and CKD stages and by country on the FAS.

8.8.2 Secondary endpoints

Due to the phase 2 type of the trial, no alpha adjustment will be carried out for secondary endpoints.

For the FAS, secondary endpoints will be analysed as derived from the WI-NRS data by multiple imputation. A general estimating equations model applies with baseline weekly mean WI-NRS as covariate and treatment, dialysis status at baseline, systemic CKD-aP treatment status at baseline and visit as fixed factors with a two-sided α=0.05. Derived secondary endpoints are the rates of subjects with 4-point improvement, with 3-point improvement and with complete response in weekly mean WI-NRS, respectively. Complete response is given if scores equal 0 or 1 in ≥80% of the non-missing WI-NRS values recorded in the subject's diary 7 days prior to and including the post-baseline visit.

9.0 CHANGES IN THE PLANNED TRIAL

9.1 Protocol amendments

Except for administrative changes, any changes or additions to this clinical trial protocol require a written protocol amendment that must be approved by the IRB/IEC, and if applicable the regulatory authorities, before implementation.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or MC2 in the interests of preserving the safety of all subjects included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons, MC2 or designee should be notified, and the IRB/IEC should be informed according to their reporting requirements.

9.2 Completion, Termination or suspension of the trial

The trial will be considered completed when the last subject has completed the last visit. Trial completion must be distinguished from premature termination of the trial and temporary suspension of the trial.

The sponsor (MC2 Therapeutics) reserves the right to prematurely terminate or temporarily suspend trial sites or the trial at any time for any reason. The sponsor may terminate one or several sites/investigators at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early termination of a trial site or the trial by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator

- Discontinuation of further IMP development

If the trial is prematurely terminated or suspended, the sponsor (or designee) shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) (CRO) used in the trial of the reason(s) for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the subjects and should assure appropriate subject therapy and/or follow-up.

10.0 DATA HANDLING AND RECORD KEEPING

10.1 Recording of data

Data handling and record keeping will be performed in compliance with the requirements of Articles 33 and 34 in conjunction with Article 28 (3) sentence 3 **(EU) 2016/679** with its related local laws.

All parties involved in this clinical trial will be obliged by law, as well as the established Standard Contractual Clauses (SCCs) to processing of any personalized data within the framework of this clinical trial in compliance with the EU GDPR and all related laws and regulations. The parties involved undertake to instruct and supervise their employees in regard to the requirements and compliance with EU GDPR with all related laws and regulations.
At the sites, a very limited group of dedicated study team members have access to the subject source data: site staff, selected staff members of the CRO, authority representatives and the sponsor's auditors. Any subject personal and medical source data will be pseudonymized before leaving the sites. All subject personal and medical data are encrypted while at transfer, and appropriately protected at rest.

The technical and organizational measures put in place by all parties result from the requirements of Articles 32 and 25 of the EU GDPR and form the minimal standard that all involved parties will comply with. For this purpose, the respective technical-operational measures (TOMs) of the parties involved are attached to their respective SCCs. The TOMs will have to address all aspects of data security, i.e.:

(a) the pseudonymisation and encryption of personal data

(b) the ability to ensure the ongoing confidentiality, integrity, availability and resilience of processing systems and services

(c) the ability to restore the availability and access to personal data in a timely manner in the event of a physical or technical incident

(d) a process for regularly testing, assessing and evaluating the effectiveness of technical and organisational measures for ensuring the security of the processing

If one of the parties involved deviates or observes a deviation from these data security regulations, a notification to the sponsor's data protection officer will be made in accordance with the requirements of Articles 33 and 34 in conjunction with Article 28 (3) sentence 3 (EU) 2016/679. The latter shall carry out an assessment of the situation in accordance with those articles and shall report to the competent supervisory authority and, if and when appropriate, to the subjects concerned, within the time limit set out therein. The measures to be taken depend on the seriousness of the deviation and the situation and shall be coordinated with the competent authority. Typically measures include at least an overall assessment of the magnitude of the data breach, the risk for involved data subjects (one or several), an impact assessment, as well as measures for correction of the actual occurrence and future prevention.

10.1.1 Source documents

Source data are all the information in original records and copies of original records of clinical findings, observations, or other activities in the trial that are necessary for the reconstruction and evaluation of the trial.

Trial data collection procedures must ensure that each data element can be traced with a high level of confidence from its originator or recorder to its representation in the trial database and then to its place in the analysis and report of trial results. Once recorded, the trial data must be protected from unauthorised modification or deletion, and all authorised modifications and deletions must be securely linked in the permanent record with their author, time of change, and reason for change (i.e., the audit trail must be maintained). Applicable data protection regulations must be followed to protect the privacy of trial subjects.

The investigator will permit trial-related monitoring, audit(s), IRB/IEC review(s) and regulatory inspection(s), with direct access to all the required source records.

The Principal Investigator will certify the trial data to be accurate and complete and will release the trial data for transmittal to MC2 or designee.

Source records need to be preserved for the minimum period of time permitted by local regulations (see Section 10.2). For each subject enrolled, the investigator will indicate in the source record(s) that the subject participated in the trial.

10.1.2 Case report forms

The primary data collection tool for the trial is an eCRF designed specifically for the trial. For each subject enrolled in the trial, an eCRF will be completed by the trial coordinator and signed by the investigator or his/her designate. Data recorded on source documents and required to be captured in the database will be entered in the eCRFs using a validated a 21 CFR Part 11-compliant electronic data capture (EDC) system.

The investigator will be responsible for ensuring the accuracy of all data entered in the eCRFs. All eCRFs are to be completed in a timely manner.

Errors occurring in the eCRFs will be queried. Queries raised by data reviewers must be addressed by site personnel.

On request, the investigator will provide MC2 with additional data relating to the trial, or copies of relevant source records, duly anonymized (i.e., subject's name is redacted).

10.2 Retention of documents

The investigator shall take responsibility for maintaining adequate and accurate source documents of all observations and data generated during this trial, including any data clarification forms received from MC2 or designee. Such documentation is subject to inspection by the sponsor or its agents, the European Medicines Agency (EMA), and/or other regulatory agencies. The investigator is responsible for retention of essential documents including the Investigator Trial File for 25 years in accordance with the Clinical Trials Regulation (Regulation (EU) No 536/2014).

11.0 QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Direct Access to source documents

As specified in the investigator's agreement, the investigator agrees to allow trial-related monitoring, audit(s), IRB/IEC review(s) and regulatory inspection(s), with direct access to all the required source records, and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any issues.

11.2 Monitoring procedures

The Clinical Trial Monitor will contact and/or visit the investigator site periodically to verify the adherence to the protocol, the maintenance of trial-related source records, and the completeness and accuracy of all eCRF entries compared to source data. The investigator will cooperate with the trial monitor to ensure that any discrepancies that may be identified are resolved.

11.3 Audit and inspection

The investigator will make all the trial-related source data and records available to a quality assurance auditor mandated by the sponsor, or to domestic or foreign regulatory inspectors, after reasonable notice. The main purposes of an audit or inspection are to confirm that the rights and well-being of the subjects have been adequately protected, and that all data relevant for the evaluation of the IP have been processed and reported in compliance with GCP/ICH and applicable regulatory requirements.

The investigator is to notify the MC2 or designee immediately of any inspection by regulatory authorities or IRBs.

12.0 ETHICS

12.1 Ethical conduct of the trial

This trial must be carried out in compliance with the protocol and the applicable laws and regulatory requirements of the appropriate regulatory agency. The trial must be conducted in accordance with the ethical principles originating from the Declaration of Helsinki and amendments, Regulation [EU] No 536/2014 and applicable ICH-GCP guidelines.

12.2 Institutional review board (IRB) or independent ethics committee (IEC)

This protocol, the proposed informed consent form, and other information for subjects must be reviewed and approved by an IRB or IEC, before the start of the trial, in compliance with local regulations. This committee must also approve any amendments to the protocol, other than administrative ones, before initiation of the amendment procedures.

12.3 Subject information and consent

Before participation in the trial, each subject or guardian is required to provide written consent to participate in the trial. No trial-specific procedures will be performed before a subject's informed consent is obtained.

12.4 Disclosure and confidentiality

12.4.1 Confidentiality of trial documentation

By signing the protocol, the investigator agrees to keep all information provided by the sponsor in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC. Trial documents provided by the trial sponsor (i.e., protocols, Investigators' Brochures, eCRFs and other material) will be stored appropriately to ensure their confidentiality. The information provided by the sponsor to the investigator may not be disclosed to others without direct written authorisation from the sponsor, except to the extent necessary to obtain informed consent from subjects who wish to participate in the trial.

12.4.2 Privacy of individual health information

The investigator will undertake to protect the privacy of all individually identifiable health information and personalized information as specifically agreed to by each individual subject through the written informed consent as per the applicable data protection rules in each of the countries, where the study is conducted. The Informed Consent document will include a request of the subject's consent to release the collected data for research purposes in such a way that the individual's identity remains masked for the specified purposes, the specified duration, by the specified parties and at the specified locations. While all data records will be identified by the corresponding subject number, the identity of the subject will be held in confidential source documents at the trial site. All trial personnel with access to this information are legally bound

not to disclose such information. The sponsor and all involved parties will comply with the respective data protection rules, which are applicable individually.

12.5 Subject involvement in the design of the clinical trial

Facilitated by Kidney Research UK (KRUK), a panel including nephrologists, dermatologists, and 8 patients with CKD-aP were given the opportunity to review and provide comments on the protocol before finalization.

13.0 EMERGENCY PROCEDURES

13.1 Emergency unblinding

The IWR system will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a subject's intervention assignment is warranted. Subject safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's intervention assignment unless this could delay emergency treatment of the subject. If a subject's intervention assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and case report form, as applicable.

14.0 INSURANCE

MC2 has taken out appropriate insurance policies covering the subjects in the clinical trial in accordance with applicable laws and regulations.

15.0 PUBLICATION POLICY

The clinical trial will be registered and results will be posted on www.clinical trial.gov and EudraCT in accordance with applicable local regulations.

All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this trial must be submitted to MC2 for review, as specified in the Clinical Trial Agreement between the institution, investigator and MC2 or its designee.

16.0 REFERENCES

Adamolekun B. Neurological disorders associated with cassava diet: a review of putative etiological mechanisms. Metab Brain Dis. 2011;26(1):79-85.

Baker LB, Wolfe AS. Physiological mechanisms determining eccrine sweat composition. Eur J Appl Physiol. 2020;120(4):719-752.

Collaboration, Chronic Kidney Disease. 2020. 'Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017', Lancet, 395: 709-33.

Dipeptiven® Summary of Product Characteristics (SmPC) 2015.

Finlay AY. Defining the fingertip unit. BMJ. 2012;344:e3061

Finlay AY, Edwards PH, Harding KG. “Fingertip unit” in dermatology. Lancet. 1989;2(8655):155.

Gorisse L, Pietrement C, Vuiblet V, et al. Protein carbamylation is a hallmark of aging. Proc Natl Acad Sci U S A. 2016;113(5):1191-6.

Hercz D, Jiang SH, Webster AC. Interventions for itch in people with advanced chronic kidney disease. Cochrane Database Syst Rev. 2020;12(12):CD011393.

Hill, Nathan R., Samuel T. Fatoba, Jason L. Oke, Jennifer A. Hirst, Christopher A. O'Callaghan, Daniel S. Lasserson, and F. D. Richard Hobbs. 2016. 'Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis', PLoS ONE, 11: e0158765-e65.

Jaisson S, Pietrement C, Gillery P. Protein carbamylation: chemistry, pathophysiological involvement, and biomarkers. Adv Clin Chem. 2018;84:1-38.

Jaisson S, Lorimier S, Ricard-Blum S, et al. Impact of carbamylation on type I collagen conformational structure and its ability to activate human polymorphonuclear neutrophils. Chem Biol. 2006;13(2):149-59.

Johansson O, Hilliges M, Ståhle-Bäckdahl M. Intraepidermal neuron-specific enolase (NSE)-immunoreactive nerve fibres: evidence for sprouting in uremic patients on maintenance hemodialysis. Neurosci Lett. 1989;99(3):281-6.

Kezic S, O’Regan GM, Yau N, Sandilands A, Chen H, Campbell LE, et al. Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. Allergy. 2011;66(7):934-40.

Kimani S, Moterroso V, Morales P. Cross-species and tissue variations in cyanide detoxification rates in rodents and non-human primates on protein-restricted diet. Food Chem Toxicol. 2014;66:203-9.

Kopple JD. Abnormal amino acid and protein metabolism in uremia. Kidney Int. 1978;14(4):340-8.

Korsuva™ United States Package Insert (USPI). Stamford, CT: Cara Therapeutics, Inc; 2021.

Kraus LM, Jones MR, Kraus AP. Essential carbamoyl-amino acids formed in vivo in patients with end-stage renal disease managed by continuous ambulatory peritoneal dialysis: isolation, identification, and quantitation. J Lab Clin Med. 1998;131(5):425-31.

Kraus LM, Kraus AP Jr. Carbamoylation of amino acids and proteins in uremia. Kidney Int Suppl. 2001;78:S102-7.

Martin CE, Clotet-Freixas S, Farragher JF, et al. Have we just scratched the surface? A narrative review of uremic pruritus in 2020. Can J Kidney Health Dis. 2020;7:2054358120954024.

Mathur VS, Lindberg J, Germain M, et al. A longitudinal study of uremic pruritus in hemodialysis patients. Clin J Am Soc Nephrol. 2010;5(8):1410-9.

(Nalfurafine Report) Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare (Japan). Report on the Deliberation Results. 06 November 2008.

Nischal U, Nischal Kc, Khopkar U. Techniques of skin biopsy and practical considerations. J Cutan Aesthet Surg. 2008;1(2):107-11.

Pisoni RL, Wikström B, Elder SJ, et al. Pruritus in haemodialysis patients: international results from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Nephrol Dial Transplant. 2006;21(12):3495-505.

Rayner HC, Larkina M, Wang M, et al. International comparisons of prevalence, awareness, and treatment of pruritus in people on hemodialysis. Clin J Am Soc Nephrol. 2017;12(12):2000-7.

Sacco JJ, Botten J, Macbeth F. The average body surface area of adult cancer patients in the UK: a multicentre retrospective study. PLoS One. 2010;5(1):e8933.

Shirazian S, Aina O, Park Y, et al. Chronic kidney disease-associated pruritus: impact on quality of life and current management challenges. Int J Nephrol Renovasc Dis. 2017;10:11-26.

Spencer. 2018. Clinically meaningful itch reduction by CR845. Poster

Sukul N, Karaboyas A, Csomor PA, et al. Self-reported pruritus and clinical, dialysis-related, and patient-reported outcomes in hemodialysis patients. Kidney Med. 2021;3(1):42-53 e1.

Sukul N, Speyer E, Tu C, et al. Pruritus and patient reported outcomes in non-dialysis CKD. Clin J Am Soc Nephrol. 2019;14(5):673-81.

Thomas CL, Finlay AY. The “handprint” approximates to 1% of the total body surface area whereas the “palm minus the fingers” does not. Br J Dermatol. 2007;157(5):1080-1.

Vychytil A, Herzog R, Probst P. A randomized controlled trial of alanyl-glutamine supplementation in peritoneal dialysis fluid to assess impact on biomarkers of peritoneal health. Kidney Int. 2018;94(6):1227-37.

Yoshiike T, Aikawa Y, Sindhvananda J, et al. Skin barrier defect in atopic dermatitis: increased permeability of the stratum corneum using dimethyl sulfoxide and theophylline. J Dermatol Sci. 1993;5(2):92-6.

17.0 APPENDICES

17.1 Appendix 1
Contact list of MC2 and protocol authors

Contact details for MC2 representatives are provided to the trial sites on a list outside of the protocol, which is included in the clinical trial application. The name and address of each third-party vendor (e.g. clinical laboratory) used in this trial will be maintained in the investigator´s and sponsor´s files

Sponsor

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United Kingdom

Protocol Authors

Maj Dinesen, Director Clinical Development MC2 Therapeutics

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Carsten Makus, Advisor Clinical Operations, SRE GmbH

Frank Freischläger, Senior Advisor Biostatistics, Estimondo

17.2 Appendix 2
Vendor overview

Vendor	Role
SRE GmbH	CRO
Pharmalex	Pharmacovigilance Provider

CLINICAL TRIAL PROTOCOL APPROVAL FORM

Product: MC2-25 cream

Protocol number: MC2-25-C1

Protocol title: A Parallel-group (2-Arm), Randomized, Double-blind, 12 week Trial to Evaluate the Efficacy and Safety of MC2 25 Cream and MC2-25 Vehicle in Subjects with Chronic Kidney Disease-associated Pruritus (CKD aP)

Version: 3.0_EU

Date: 30-Nov-2022

The following person has approved this clinical trial protocol:

Maj Dinesen
Head of Clinical Development
MC2 Therapeutics

Maj Dinesen

Digitally signed by Maj Dinesen
DN: cn=Maj Dinesen, o=MC2 Therapeutics, ou=Director Clinical Development, email=mdi@mc2therapeutics.com, c=DK
Date: 2022.11.30 13:21:02 +01'00'

Date and signature

CLINICAL TRIAL PROTOCOL APPROVAL FORM

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Version: 3.0_EU

Date: 30 Nov 2022

The following person has approved this clinical trial protocol:

Frank Freischläger Senior Advisor
Biostatistics Estimondo

Frank Freischläger

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Date: 2022.11.30 13:38:13 +01'00'

Date and signature

CLINICAL TRIAL PROTOCOL APPROVAL FORM

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Version: 3.0_EU

Date: 30 Nov 2022

The following person has approved this clinical trial protocol:

Irene Sandholdt
Senior Advisor Clinical Operations
MC2 Therapeutics

Irene Sandholdt

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