Clinical Trial Protocol

Trial title:	A parallel group (2-arm), randomised, double-blind, 12-week trial to explore the efficacy and safety of MC2-25 cream and MC2-25 vehicle in women diagnosed with vulvar lichen sclerosus (VLS)
Trial short name	Orchid 1
Trial short title	Efficacy and safety of MC2-25 cream & vehicle in women with vulvar lichen sclerosus (VLS)
Indication:	Vulvar lichen sclerosus (VLS)
Trial design:	Parallel group (2-arm), randomised, double-blind
Trial no.:	MC2-25-C3
Investigational medicinal product:	MC2-25 cream MC2-25 vehicle
Development phase:	Phase 2
Trial registry numbers:	EU-CT number: 2023-503516-32-00
Document Status, Version, and Date:	Final, v4.0, 31-Jul-2024

Name of the sponsor:	MC2 Therapeutics Ltd 1A Guilford Business Park Guilford GU2 8XG United Kingdom	EU representative: MC2 Therapeutics A/S Agern Allé 24-26 2970 Hørshom Denmark

This trial will be conducted in compliance with the protocol, with regulation (EU) No 536/2014, and with the principles of Good Clinical Practice (GCP).

Clinical trial protocol approval

Investigational Medicinal Product: MC2-25 cream

Protocol number: MC2-25-C3

Protocol title: A parallel group (2-arm), randomised, double-blind, 12-week trial to explore the efficacy and safety of MC2-25 cream and MC2-25 vehicle in women diagnosed with vulvar lichen sclerosus (VLS).

The following persons have approved this clinical trial protocol:

Maj Dinesen, Head of Clinical Development, MC2 Therapeutics

Gudula Kirtschig, International Coordinating Investigator, Consultant Dermatologist with expertise in lichen sclerosus

Signature pages are separate documents adjoined to this document.

Table of contents

List of tables

List of figures

List of abbreviations and definitions

Abbreviation	Definition
AE	Adverse Event
Ala-Gln	Alanyl-Glutamine
CFR	Code of Federal Regulation
CGA	Clinician's Global Assessment
CGIC	Clinician's Global Impression of Change
CKD	Chronic Kidney Disease
CKD-aP	Chronic Kidney Disease-associated Pruritus
ClinRO	Clinician-Reported Outcome
COA	Clinical Outcome Assessment
CRF	Case Report Form
CRO	Contract Research Organisation
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
EoT	End of Treatment
EU	European Union
FAS	Full Analysis Set
FTU	Fingertip Unit
FU	Follow-Up
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
hCG	Human Chorionic Gonadotropin
HRQoL	Health-Related Quality of Life
IB	Investigator's Brochure
ICH	International Council for Harmonisation
ICIQ-UI-SF	International Consultation on Incontinence Questionnaire Urinary Incontinence Short Form
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
LS	Lichen Sclerosus
MedDRA	Medical Dictionary for Regulatory Activities
MFAS	Modified Full Analysis Set
MMRM	Mixed Model of Repeated Measures
PGIC	Patient's Global Impression of Change
PPS	Per Protocol Set
PRO	Patient Reported Outcome
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SoA	Schedule of Assessments
SmPC	Summary of Product Characteristics
SS	Screened Set

Abbreviation	Definition
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TOMS	Technical Operational Measures
UV-A	Ultraviolet A
UV-B	Ultraviolet B
UTI	Urinary Tract Infection
VAS	Visual Analogue Scale
VASSI	Vulvar lichen sclerosus Area and Sign Severity Index
VLS	Vulvar Lichen Sclerosus (Note: in the context of this trial 'Vulvar' region includes the clitoris and periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal skin)
VQLI	Vulvar disease Quality of Life Index
w/w	Weight by weight
WI	Worst Itch
WI-NRS	Worst Itch – Numeric Rating Score
WPP	Worst Penetrative sex related Pain
WPP-NRS	Worst Penetrative sex related Pain – Numeric Rating Score
WP	Worst Pain
WP-NRS	Worst Pain – Numeric Rating Score

1 Protocol summary

1.1 Synopsis

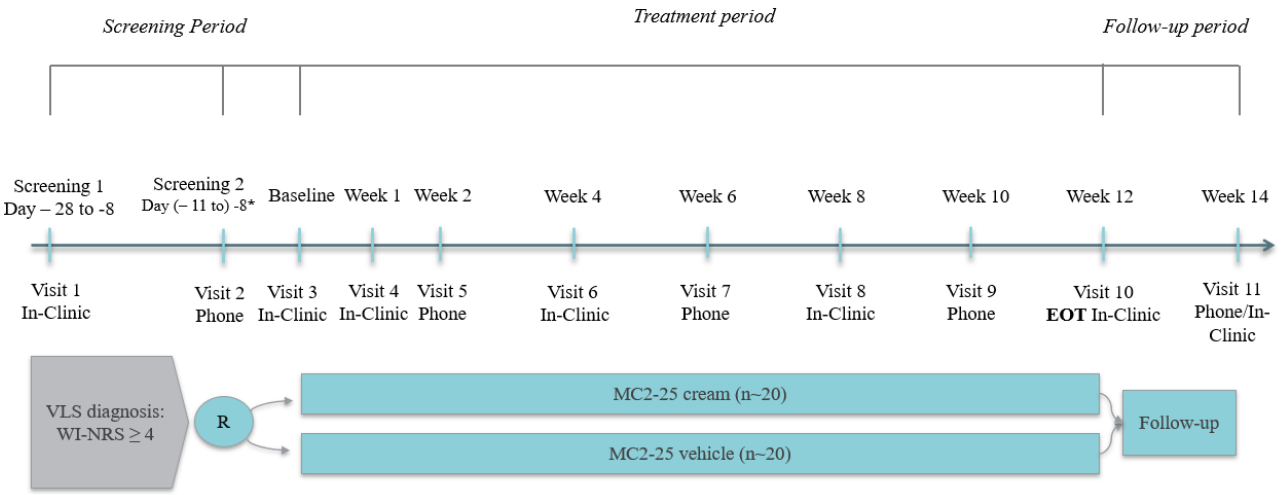
Title	A parallel group (2-arm), randomised, double-blind, 12-week trial to explore the efficacy and safety of MC2-25 cream and MC2-25 vehicle in women diagnosed with vulvar lichen sclerosus (VLS).
Protocol Number (EU trial number)	MC2-25-C3 (EU-CT 2023-503516-32-00)
Rationale	VLS is a serious condition with major impact on the quality of life of affected women. There is evidence that urea-derived isocyanate could be an important triggering factor in the development of signs and symptoms in patients with VLS. There is a high unmet need for an authorised, efficient, and safe treatment of VLS. Topically applied cream with Ala-Gln as an effective isocyanate scavenger in form of MC2-25 cream may prove to be an effective and safe treatment of VLS.
Phase	Phase 2
Objectives	Primary objective: To explore the efficacy of MC2-25 cream compared to MC2-25 vehicle in vulvar lichen sclerosus (VLS). Secondary objectives: • To explore the safety of MC2-25 cream compared to MC2-25 vehicle in VLS. • To explore the burden of VLS on women's lives.
Design	Multicentre, phase 2, randomised, double-blind, 2-arm, parallel-group, vehicle-controlled trial in VLS. The trial consists of 3 consecutive periods adding up to a maximum trial duration for each patient of 18 weeks: Screening (up to 4 weeks), Treatment (12 weeks) and Follow-Up (2 weeks).
Planned Sample Size:	33 patients will be randomised in a 1:1 ratio to MC2-25 cream or MC2-25 vehicle.
Trial population	Main inclusion criteria: • Women, of any race or ethnicity, who are ≥18 years of age at the time of screening. • Clinical diagnosis of VLS (i.e., biopsy for histological confirmation not required except if needed to rule out malignancy or differential diagnosis of e.g., vulvar lichen planus) made by either a dermatologist or a gynaecologist familiar with VLS.

	• Presence of <u>at least one of the following signs of VLS</u>: a. Hyperkeratosis (i.e., patches/plaques of bright white skin with a 'powdery' texture) and/or b. Sclerosis (i.e., areas of yellowish/ivory white skin with a smooth/waxy/firm texture. Sclerosis is often seen at the tips of the labia minora, or on periclitoral or perineal skin) in <u>at least one of the following vulvar areas:</u> Clitoris and/or periclitoral skin (C); Right interlabial sulcus and labium minus (R); Left interlabial sulcus and labium minus (L); Posterior Fourchette and/or perineum (P) (see protocol section 8.5.1.2). • First symptoms of VLS (e.g., itching and/or pain) noticed by the patient at least 6 months before baseline. • At least moderate itch defined as average WI-NRS ≥4 at the Baseline visit (average WI-NRS is calculated as the average of all available and at least four WI-NRS scores which are to be reported once daily by the patient in the diary for 7 days prior to the Baseline visit (7 days in total)). <u>Main exclusion criteria:</u> • Any (other than VLS) ongoing localised or systemic disease involving the vulvar region – e.g., lichen planus, psoriasis, eczema, ulcerative colitis or known active infection (bacterial, viral or fungal). • Ongoing or prior diagnosis of any genitoanal malignancy or pre-malignancy (e.g., differentiated intraepithelial neoplasia or squamous cell carcinoma). • Any kind of ongoing cancer (or anti-cancer treatment within 3 months or 5 half-lives (whichever is longest) prior to the Baseline visit). Note: non-metastasising basal cell carcinoma which does not involve the genitoanal area is acceptable. • Use of emollients (including but not limited to creams, ointments, oils, or soaps with emollient properties) on the vulvar region within 3 days prior to the Baseline visit. Note: Only water and, if necessary, a soap bar without emollient properties, may be used to wash the vulvar region (intimate hygiene) when bathing or showering from within 3 days prior to the Baseline visit. • Use of any topical treatment on the vulvar region, including but not limited to calcineurin inhibitors, corticosteroids or anti-infectives within 14 days prior to the Baseline visit. • Use of any light therapy on the vulvar region, including but not limited to UV-B, UV-A, and laser, within 28 days prior to the Baseline visit.
Main endpoints	
<u>Primary endpoint:</u>	Mean change in weekly mean Worst Itch Numeric Rating Score (WI-NRS) recorded in the patient'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 patient's diary for 7 days prior to the in-clinic visits).

Secondary endpoints:	• Mean change in weekly mean Worst Pain Numeric Rating Score (WP-NRS) recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. (Weekly mean WP-NRS is calculated as the average of WP-NRS values recorded in the patient's diary for 7 days prior to the in-clinic visits.) • Percentage of patients obtaining a ≥4-point improvement in weekly mean WI-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a ≥4-point improvement in weekly mean WP-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a ≥3-point improvement in weekly mean WI-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a ≥3-point improvement in weekly mean WP-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Change in Skindex-29 domains from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.
Other endpoints:	• Frequencies of treatment emergent adverse events (AEs), SAEs, adverse drug reactions, AEs leading to treatment discontinuation or trial withdrawal, Other important AEs and deaths during the trial for MC2-25 cream compared to MC2-25 vehicle. • Mean changes in Vital signs from Baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.
Trial sites	6 sites will be opened in Denmark.
Investigational Medicinal Product	Trial participants will be randomized (1:1) to receive either: • MC2-25 cream (with active product ingredient) or • MC2-25 vehicle (without active product ingredient) The IMP must be applied after each toilet visit during the 12-week treatment period.
Participant duration (including follow-up)	For each participant, there will be three consecutive trial periods, adding up to a maximum trial duration of 18 weeks: • Screening (up to 4 weeks) • Treatment (12 weeks) and • Follow-up (2 weeks).
Statistical Methods	The primary endpoint, mean change in weekly mean Worst Itch – Numeric Rating Score (WI-NRS) from baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle, will be analysed by a mixed model of repeated measures (MMRM) adjusting for visit, menopausal status at baseline, urinary incontinence status (ICIQ-UI-SF score) at baseline and weekly mean WI-NRS

	at baseline. At Week 12, the estimated treatment difference will be reported together with the associated standard error, 95% confidence intervals and p-values based on F-statistics.
Benefit-risk	There are only few known potential risks associated with the investigational medicinal product or the trial, i.e., application site reaction, use during pregnancy or lack of effect due to use of MC2-25 vehicle. Use of a vehicle control is considered justified as there are no approved treatments for VLS in Europe, off label systemic treatments for VLS may be continued during the trial and the IMP is an emollient and emollients are normally recommended for VLS. In view of the major impact of VLS on patient's quality of life, the potential benefits of MC2-25 cream on VLS, and the benign safety profile of MC2-25 cream, the benefit-risk ratio for the current trial is considered positive.

1.2 Schematic of trial design



* *Site to call patient on day (-11 to) -8 to remind about daily WI-NRS and WP-NRS assessment in diary (day-7 to day -1),*

1.3 Schedule of assessments (SoA)

Table 1: Schedule of assessments

Trial Period	**Screening**		**Treatment**								**FU**
Visit Number[1] (Visit name)	**1 (Screening1)**	**2 (Screening 2)**	**3 (Baseline)**	**4**	**5**	**6**	**7**	**8**	**9**	**10 (EoT[2])**	**11 (FU[3])**
Trial Week			**0**	**1**	**3**	**4**	**7**	**8**	**11**	**12**	**14**
Days from Baseline *Visit Window*	-28 to -8 *NA*	(-11 to) -8	0 *NA*	7 *±1*	18 *±2*	28 *±4*	46 *±2*	56 *±4*	74 *±2*	84 *±4*	98 *±4*
Visit type[4]	In-Clinic	Phone	In-Clinic	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	In-Clinic/ Phone
Informed Consent[5]	X										
Demographics	X										
Eligibility Assessment	X		X								
Medical History	X										
Menopausal status and Menstrual cycle status			X							X	
Prior & concomitant therapies[6]	X		X	X	X	X	X	X	X	X	X
VLS History	X										
VLS Patient Journey[7]	X										
ICIQ-UI-SF			X							X	
WI-NRS and WP-NRS – in-clinic assessment[7,8]	X		X	X		X		X		X	

Trial Period	**Screening**		**Treatment**								**FU**
Visit Number[1] (Visit name)	**1 (Screening1)**	**2 (Screening 2)**	**3 (Baseline)**	**4**	**5**	**6**	**7**	**8**	**9**	**10 (EoT[2])**	**11 (FU[3])**
Trial Week			**0**	**1**	**3**	**4**	**7**	**8**	**11**	**12**	**14**
Days from Baseline *Visit Window*	-28 to -8 *NA*	(-11 to) -8	0 *NA*	7 *±1*	18 *±2*	28 *±4*	46 *±2*	56 *±4*	74 *±2*	84 *±4*	98 *±4*
Visit type[4]	In-Clinic	Phone	In-Clinic	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	In-Clinic/ Phone
WI-NRS and WP-NRS Diary (Handout)[9]	X		X	X		X		X			
WI-NRS and WP-NRS diary completion reminder[4]		X	X		X		X		X		
WI-NRS and WP-NRS Diary (return & review) [10]			X	X		X		X		X	
WPP-NRS – in-clinic assessment[8]	X		X			X		X		X	
Patient's Global Impression of Change (PGIC)[7]						X		X		X	
Patient's rating of importance of improvement[7]						X		X		X	
VQLI[7]			X			X		X		X	
Skindex-29[7]			X			X		X		X	
Imaging of VLS[11]	X		X	X		X		X		X	
VASSI	X		X	X		X		X		X	
CGA	X		X	X		X		X		X	

Trial Period	**Screening**		**Treatment**								**FU**
Visit Number[1] (Visit name)	**1 (Screening1)**	**2 (Screening 2)**	**3 (Baseline)**	**4**	**5**	**6**	**7**	**8**	**9**	**10 (EoT[2])**	**11 (FU[3])**
Trial Week			**0**	**1**	**3**	**4**	**7**	**8**	**11**	**12**	**14**
Days from Baseline *Visit Window*	-28 to -8 *NA*	(-11 to) -8	0 *NA*	7 *±1*	18 *±2*	28 *±4*	46 *±2*	56 *±4*	74 *±2*	84 *±4*	98 *±4*
Visit type[4]	In-Clinic	Phone	In-Clinic	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	Phone	In-Clinic	In-Clinic/ Phone
CGIC				X		X		X		X	
Physical examination[12]	X		X							X	(X)
Vital signs[13]			X							X	(X)
Urine pregnancy test[14]	X		X	X		X		X		X	(X)
Randomisation			X								
Application Diary (Handout)			X	X		X		X			
Application Diary (Return and review)[10]				X		X		X		X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X
IMP dispensation			X			X		X			
IMP return, compliance and accountability						X		X		X	
IMP application[15]			X								

1. An unscheduled visit can be conducted to perform any of the post-baseline assessments or procedures included in the schedule of assessments (SoA) if deemed necessary by the Investigator.
2. In the case of trial withdrawal or treatment discontinuation, all assessments of the Week 12 (End of Treatment [EoT]) visit must be performed to the extent possible. The end of treatment (EoT) form must be completed when treatment is permanently discontinued, and the end of trial form must be completed at end of the trial or in case of trial withdrawal.

3. (X) indicates assessments to be performed if judged necessary by the investigator. This visit could be an in-clinic visit or a phone visit depending on the follow up assessments considered needed by the investigator.
4. The patients should be reminded about when to fill in the WI-NRS and WP-NRS diary. In case of phone visits, the phone call should be made as close as possible to the first WI-NRS and WP-NRS diary completion day. **Note: if Screening 1 visit is performed on day -8, then Screening 2 visit will be omitted.**
5. Informed consent must be signed by both patient and investigator before any trial-related procedures are performed.
6. "Therapies" encompassing medications and non-pharmacological treatments.
7. At all in-clinic visits, all patient-reported outcome (PRO) assessments should be completed before all other clinical assessments or procedures.
8. The WI-NRS and WP-NRS must also be reported by the patient at in-clinic visits.
9. WI-NRS and WP-NRS diary assessments must be performed at home once daily (i.e., every day around the same timepoint, e.g., morning, afternoon, or evening) for a total of 7 days before each in-clinic visit (i.e., from day -7 to day -1 before the next in-clinic visit).
10. When returned the Diaries should be checked for completeness and any irregularities while the patient is still in the clinic.
11. Imaging is mandatory, and must be done at Screening 1, Baseline, Week 1, Week 4, Week 8 and Week 12 (or early termination).
12. Physical exam: A complete physical examination is required at Screening 1 and Week 12 (EoT or Early Termination) visits. An abbreviated physical examination is required at the Baseline visit and at the FU visit (if physical examination is judged necessary by the investigator).
13. Vital signs include blood pressure, heart rate, and body temperature measurement. Blood pressure and pulse rate must be taken with the patient in the sitting position with approximately 5 minutes rest prior to measurement.
14. Highly sensitive urine pregnancy tests are conducted for women of childbearing potential.
15. Before the first IMP application, the site staff shall provide thorough instruction to the patient regarding the area to apply the cream to, the amount of cream to be applied and the required frequency of application.

2 Introduction

2.1 Purpose of trial

As explained in the following sections, VLS is a serious condition with major impact on the quality of life of affected women. There is a high unmet need for an authorised, efficient, and safe treatment of VLS. The aetiology of VLS is largely unknown, but one hypothesis is that exposure to isocyanate (a degradation product from urea in the urine) may be a triggering factor via a process known as carbamylation. Based on currently available in-vitro data the active drug substance (Alanyl-Glutamine) in MC2-25 cream appears to be an efficient isocyanate scavenger and MC2-25 cream may thereby counteract the effects of carbamylation and potentially alleviate VLS. The purpose of this trial is to explore the efficacy and safety of MC2-25 cream in women diagnosed with vulvar lichen sclerosus (VLS).

2.2 Background

2.2.1 Vulvar Lichen Sclerosus (VLS)

The true prevalence of VLS is unknown, and probably underestimated as it is often either asymptomatic or under-recognised (Goldstein et al., 2005; Walkden et al., 1997). Prevalences found in a few very small studies in highly selected patient groups are frequently cited and range from 3% in nursing home residents to 1.7% in gynaecology practice and down to 0.1% in dermatology departments (Goldstein et al., 2005; Leibovitz et al., 2000; Wallace, 1971). There are only a few studies investigating the incidence or prevalence of VLS in larger and broader populations, but they all conclude that the incidence and or prevalence increase with age (Bleeker et al., 2016; Halonen et al., 2020; Melnick et al., 2020).

VLS is an inflammatory, potentially scarring dermatosis, that has a predilection for the genital skin (Smith & Haefner, 2004). The characteristic sites involved are the interlabial sulci, labia minora, clitoral hood, clitoris with possible extension to the perineal body and perianal area giving rise to the characteristic ‘figure of eight’ shape. In adult women with VLS, itch is the main symptom, but pain may be a consequence of erosions or fissures. Rarely VLS may be asymptomatic and is an incidental finding on examination. In the women with itch, the itch may be difficult to withhold and can create embarrassment. The itch may be worse at night and may be sufficiently severe to disturb sleep. Dyspareunia occurs in the presence of erosions, fissures or introital narrowing. VLS may cause resorption of the labia minora, sealing of the clitoral hood, covering of the clitoris and narrowing of the vaginal introitus. The vagina and cervix are generally not involved (in contrast to lichen planus), unless there is a significant vaginal prolapse when the mucosa may become keratinised (Bhargava & Lewis, 2013; Corazza et al., 2021; Zendell & Edwards, 2013). Perianal lesions occur in women in about 30% of cases. There may be extension to the buttocks and genitocrural folds (Lewis et al., 2018). Squamous cell carcinoma in women has been associated with VLS. The risk of developing malignancy is approximately 3.5–5% (Micheletti et al., 2016).

VLS affects otherwise healthy individuals with a major impact on quality of life. A survey among 1.492 patients with LS (1.421 adult women) showed that for 95% of the patients their quality of life was impacted "sometimes, frequently, or always" (for 93% of the patients this was also the case for impact on their sexual life). Overall, the impact of VLS was rated as moderate or severe on overall quality of life for approximately 85% of the patients. According to the same survey, one in ten women with VLS have considered suicide because of VLS (GlobalSkin, 2018).

The aetiology of VLS is not well defined but available evidence indicates the role of two main pathomechanisms, i.e., immunological dysreactivity and chronic inflammation as well as abnormal collagen synthesis, which act on a susceptible genetic background and triggering factors (Corazza et al., 2021; Tran et al., 2019). One such triggering factor could be irritation from exposure to urine e.g., due to incontinence. There is very little data on the role of urinary incontinence in VLS in women, but recent data found that the prevalence of urinary incontinence was 63% in LS women compared to 34% in non-LS women (Kirby et al., 2021). This may indicate an association between VLS in women and urinary incontinence.

Urea, which is a degradation product excreted in urine, is via a slow equilibrium a source of formation of isocyanate. Isocyanate is a highly reactive electrophile that can quickly react with nucleophilic groups which in the body often is in the form of amino groups on e.g., proteins, peptides or free amino acids. The reaction between isocyanate and an amino group is irreversible and often referred to as "carbamylation". The impact of carbamylation on protein properties is believed to be an important contributor to the development of a number of chronic diseases such as chronic kidney disease, cataract, atherosclerosis and rheumatoid arthritis (Jaisson et al., 2018). See IB Section 2.1 for further details. It is conceivable that carbamylation in the skin affects afferent small nerve fibres resulting in the sensation of itch. In addition, epidermal haemorrhagic bleedings, which are a typical finding in VLS, may be a result of mechanical ruptures of small blood vessels, where supporting connective tissues (collagen and elastin) have lost their elastic properties due to carbamylation (Adamolekun, 2011; Kimani et al., 2014). It is hypothesised that prolonged skin exposure to urea from urine may lead to formation of isocyanate and in turn increased protein carbamylation triggering skin changes characteristic for VLS in women genetically predisposed to develop LS.

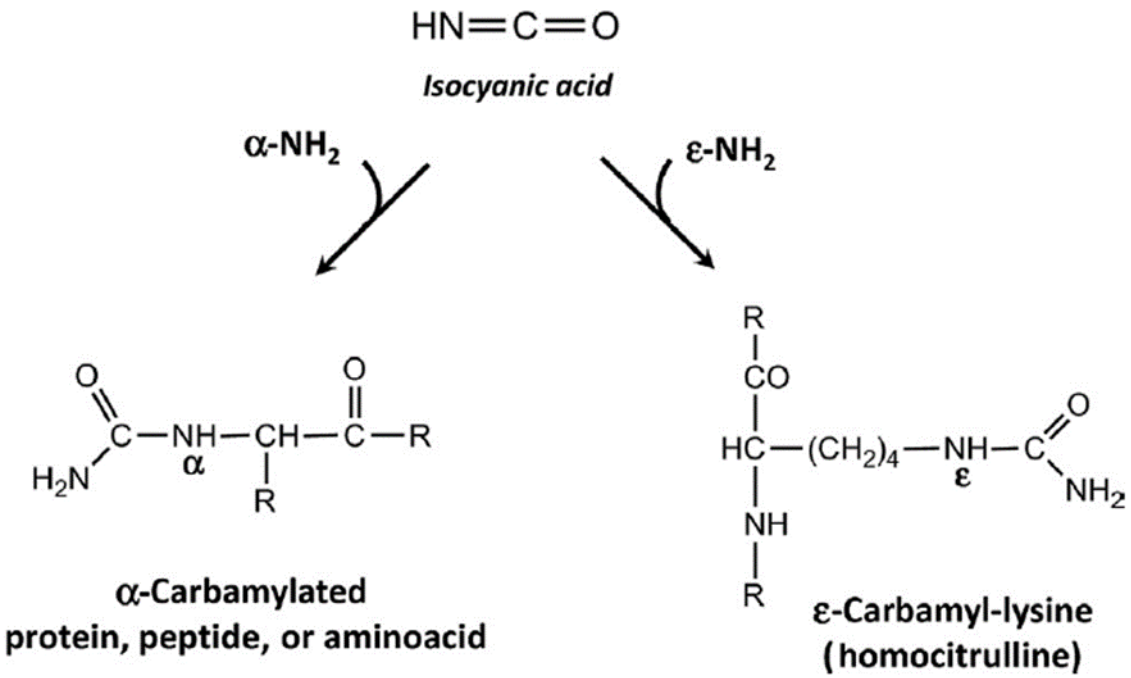
There are no treatments specifically approved for VLS in Europe or the UK. However, British and European guidelines recommend 3-month regimens with high potency topical corticosteroids. For many patients, long-term treatment, lasting for years or even life long, is often necessary, even if there are few signs and/or symptoms of LS (Kirtschig et al., 2015; Lewis et al., 2018). In women there is growing evidence that VLS under good control has a reduced risk of scarring and malignancy. However, the risk is not eliminated (Cooper et al., 2004; Lee et al., 2015). According to a survey including around 1400 women with VLS, only 21% of patients feel they are managing their VLS condition "Well" or "Very Well" and only half achieved remission for an average duration of 7 months (GlobalSkin, 2018). As such there is an unmet need for an approved safe and effective treatment for VLS.

2.2.2 MC2-25 cream

The drug product MC2-25 cream consists of a cream base and the active dipeptide drug substance Alanyl-Glutamine (Ala-Gln). Ala-Gln is intended to scavenge isocyanate, a toxic degradation product from urea which is present in high concentrations in urine, and which may be an important triggering factor in VLS via carbamylation of proteins as described above.

The carbamylation reaction is illustrated in Figure 1. Both free amino acids and proteins can be carbamylated. All free amino acids have an α-amino group, but in proteins the α-amino groups of amino acids are incorporated in the peptide bonds that link the amino acids and they are therefore not available for carbamylation. However, the essential amino acid lysine – whether free or incorporated in proteins – also has a positively charged ε-amino group side chain. The rate of the reaction between isocyanate and amino groups increases with decreasing pKa values, and this means that at pH 7 or below, the α-amino groups react about 100 times faster than the lysine ε-amino groups (Gillery & Jaisson, 2013; Stark, 1965). The pH in the vulvar region (interior aspect of labia majora and perineum) is around 5.4-5.6 (Runeman, 2005).

Figure 1: The reaction of isocyanic acid with protein amino groups (Jaisson et al., 2018)



Free amino acids compete for isocyanate binding and thus act as natural scavengers for carbamylation (Kalim et al., 2014). However, long-term use of amino acids as isocyanate scavengers may pose problems due to formation of carbamylated amino acids, which may interfere with protein synthesis (Kraus & Kraus, 2001). As such, using peptides rather than amino acids as isocyanate scavengers may be a better alternative and in fact di- and oligopeptides have been demonstrated to be more efficient isocyanate scavengers than amino acids (Berg et al., 2013; Stark, 1965).

Considering these discoveries, MC2 undertook an in vitro screening assay of protein carbamylation with a range of different compounds including amino acids and peptides. Ala-Gln was demonstrated to be among the most efficient compounds in terms of preventing protein carbamylation through isocyanate scavenging, showing a ~65% inhibition of protein carbamylation, compared with e.g., Histidine which showed ~39% inhibition (see IB, Section 4.2.1.2). Furthermore, in another in vitro study in reconstructed human epidermis, Ala-Gln inhibited protein carbamylation in a dose-dependent manner, resulting in ~89% inhibition at the highest concentration (3%) (see IB Section 4.2.1.3).

These data support the hypothesis that MC2-25 cream may be an efficient isocyanate scavenger and may thereby counteract the effects of carbamylation and potentially alleviate VLS. MC2-25 cream is being tested for the first time in VLS in this trial. It is currently being tested for the first time in humans in an ongoing clinical trial in Chronic Kidney Disease associated Pruritus (CKD-aP) patients (MC2-25-C1, EUCT 2022-500044-38-01, NCT05482698).

2.3 Risk/benefit assessment

2.3.1 Known potential risks

A summary of risks that may be associated with MC2-25 cream or specific trial procedures/circumstances and mitigation strategies to address these are provided in Table 2.

Table 2: Summary of risks and mitigation strategies

Risk	MC2-25 cream
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). In addition, in an ongoing randomised, double-blind, vehicle controlled clinical trial in chronic kidney disease associated pruritus (CKD-aP) where MC2-25 cream (3%), or MC2-25 vehicle is applied twice daily to unlimited areas of CKD-aP skin for 12 weeks only 3 adverse events within the System Organ Class 'Skin and subcutaneous tissue disorders' (Preferred terms: pruritus, pruritus and alopecia) have been reported among 36 patients who completed 8–12 weeks treatment as of medio May 2023. All events were considered mild (Data on file). Consequently, the risk of application site reactions is considered low. However, in this trial MC2-25 cream will be applied topically in VLS for the first time. Therefore, at the present time application site reactions cannot be ruled out.
	Risk Mitigation: Adverse events will be evaluated more frequently during the first 2 weeks of the treatment phase (weekly visits) compared to the subsequent weeks of the treatment phase (bi-weekly visits). In addition, a patient 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.

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 with resulting systemic safety margins even more favourable than those presented in Section 4.2. 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, patients can only be randomised 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.
Risk	**Trial procedures/circumstances**
Use of MC2-25 vehicle	Risk Description: Half of the patients will be treated with MC2-25 vehicle during the 12-week treatment period. This might result in deterioration of VLS.
	Risk Mitigation: No marketed products are approved for treatment of VLS. Therefore, patients will not miss out on approved treatment by participation in this trial. However, as described in Section 2.2.1, VLS has a significant impact on quality of life and therefore off-label treatments are not uncommon. Current UK and European guidelines are based on potent to ultrapotent topical corticosteroids. To limit the risk of VLS deterioration after entering the trial, all patients will be allowed to continue existing stable systemic treatments with a known or potential effect on VLS during the trial. Half of the patients will be randomised to receive the MC2-25 vehicle. There is no available data on the effect of MC2-25 vehicle in VLS patients, Consequently, it is currently not possible to establish an expected vehicle effect size, However, MC2-25 vehicle has emollient properties and as such is not strictly considered a placebo treatment. Patients 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 or lack of improvement. Withdrawal from the trial or discontinuation of trial treatment will not affect or prejudice the patient's further care or treatment.

2.3.2 Known potential benefits

There are currently no data on the clinical effect of MC2-25 cream in VLS. However, as described in Section 2.2.2 there are data to support the hypothesis that MC2-25 cream may be an efficient isocyanate scavenger and may potentially alleviate VLS.

2.3.3 Assessment of potential risks and benefits

As described above, VLS is a serious disease and patients with VLS often suffer from 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 itch and pain associated with VLS.

This is the first clinical trial in VLS with MC2-25 cream, so no clinical data are available. MC2 is also developing MC2-25 cream for topical treatment of chronic kidney disease associated Pruritus (CKD-aP). The first clinical trial (Phase 2) with MC2-25 cream in patients with CKD-aP is

currently ongoing (MC2-25-C1, EUCT 2022-500044-38-01, NCT05482698). So far, only 3 adverse events within the System Organ Class 'Skin and subcutaneous tissue disorders' (Preferred terms: pruritus, pruritus and alopecia) have been reported among 36 patients who completed 8–12 weeks treatment as of medio May 2023. All events were considered mild (Data on file). As described in Section 4.2 below, the systemic safety margin for MC2-25 cream in this trial compared to the maximum recommended dose for IV infusion of Dipeptiven® ranges from 200 to 20.000 if 100% respectively 1% of Ala-Gln in the MC2-25-cream is absorbed to the systemic circulation. In view of these favourable systemic safety margins no safety laboratory samples are included in the trial. Furthermore, Ala-Gln is rapidly split into alanine and glutamine after reaching the systemic circulation. 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 after IV infusion of Ala-Gln in the form of Dipeptiven® (see IB Section 5.2). So, any Ala-Gln which has not scavenged isocyanate in the skin after topical application of MC2-25 cream, may reach systemic circulation, but will rapidly be metabolised 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. Furthermore, any metabolite formed as a consequence of the isocyanate scavenging role of Ala-Gln in the skin, are presumably already present in VLS patients, given that Ala-Gln and its degradation products appears to be present endogenously in human skin, see 'IB Section 1.3'.

As also described in Section 4.2, MC2-25 cream was well tolerated when applied to the skin on a twice-daily basis for 2 weeks in minipigs and for 8–12 weeks in CKD-aP patients. Furthermore, Ala-Gln was well tolerated when applied in high doses to the peritoneal surface on a daily basis for 8 weeks in patients with chronic renal failure. Therefore, the risk of local adverse effects (local application site reactions) is considered low in this trial. All excipients used in MC2-25 cream are known from topical use in other drug products.

VLS prevalence increases with age as described in Section 2.2.1. Consequently, a considerable amount of elderly (>65 years of age) patients is expected to be enrolled. Elderly patients are considered a vulnerable population due to potential comorbidities and polypharmacy but not due to their VLS. In view of the favourable systemic and local safety profiles described above, there are no specific eligibility criteria for elderly patients. Likewise, there are no specific safety surveillance measures during the trial for elderly patients. Exclusion criterion 6, Section 5.2 'Any chronic or acute medical condition that, in the opinion of the investigator, may pose a risk to the safety of the patient or may interfere with the assessment of efficacy in this trial' will ensure that elderly patients are not enrolled in the trial if they have significant comorbidities.

In view of the major impact of VLS on patient's quality of life, the potential benefits of MC2-25 cream on VLS, and the benign safety profile of MC2-25 cream, the benefit-risk ratio for the current trial is considered positive. In view of the benign nature of the potential risks a Data Safety Monitoring Board is not considered necessary.

3 Objectives and endpoints

Primary objective:	**Primary endpoint:**
To explore the efficacy of MC2-25 cream compared to MC2-25 vehicle in vulvar lichen sclerosus (VLS).	Mean change in weekly mean Worst Itch Numeric Rating Score (WI-NRS) recorded in the patient'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 patient's diary for 7 days prior to the in-clinic visits). **Secondary endpoints:** • Mean change in weekly mean Worst Pain Numeric Rating Score (WP-NRS) recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. (Weekly mean WP-NRS is calculated as the average of WP-NRS values recorded in the patient's diary for 7 days prior to the in-clinic visits.) • Percentage of patients obtaining a ≥4-point improvement in weekly mean WI-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a ≥4-point improvement in weekly mean WP-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a ≥3-point improvement in weekly mean WI-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a ≥3-point improvement in weekly mean WP-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Change in Skindex-29 domains from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle. **Other endpoints:** • Percentage of patients obtaining a complete response (score ≤1) in weekly mean WI-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Percentage of patients obtaining a complete response (score ≤1) in weekly mean WP-NRS recorded in the patient's diary from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle • Mean change in Worst Itch Numeric Rating Score (WI-NRS) recorded at in-clinic visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle. • Mean change in Worst Pain Numeric Rating Score (WP-NRS) recorded at in-clinic visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.

- Mean change in Worst Penetrative sex related Pain Numeric Rating Score (WPP-NRS) recorded at in-clinic visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Percentage of patients obtaining a ≥3-point improvement in WI-NRS recorded at in-clinic visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Percentage of patients obtaining a ≥3-point improvement in WP-NRS recorded at in-clinic visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Percentage of patients obtaining a ≥3-point improvement in WPP-NRS recorded at in-clinic visits from Baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Percentage of patients who reported 'Very much better', 'Much better' or 'A little better' in Patient's Global Impression of Change (PGIC) for Worst Itch (WI), Worst Pain (WP) or Worst Penetrative sex related Pain (WPP) from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Percentage of patients who reported an Important improvement in Patient's Global Impression of Change (PGIC) for Worst Itch (WI), Worst Pain (WP) or Worst Penetrative sex related Pain (WPP) from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Mean change in VQLI total score from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.
- Mean change in Vulvar lichen sclerosus Area and Sign Severity Index (VASSI) from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle
- Mean change in VASSI Area scores from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle
- Mean change in VASSI Sign scores from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle
- Mean change in Clinician's Global Assessment (CGA) of VLS from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle
- Percentage of patients with a ≥2-point improvement in Clinician's Global Assessment (CGA) of VLS from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle
- Percentage of patients with an improvement in the Clinician's Global Impression of Change (CGIC) assessment (i.e., 'Very much better', 'Much better' or 'A little better') from baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.

Secondary objectives:	**Other endpoints:**
• To explore the safety of MC2-25 cream compared to MC2-25 vehicle in VLS.	• Frequencies of treatment emergent adverse events (AEs), SAEs, adverse drug reactions, AEs leading to treatment discontinuation or trial withdrawal, Other important AEs and deaths during the trial for MC2-25 cream compared to MC2-25 vehicle. • Mean changes in Vital signs from Baseline to week 12 for MC2-25 cream compared to MC2-25 vehicle.
• To explore the burden of VLS on women's lives.	• Burden of VLS on women's lives as reported on the "VLS patient journey sheet".

4 Trial design

4.1 Description of trial design

This is a multicentre, phase 2, randomised, double-blind, 2-arm, parallel-group and vehicle-controlled trial in VLS. The trial consists of 3 consecutive periods adding up to a maximum trial duration for each patient of 18 weeks:

- Screening period (up to 4 weeks)
- Treatment period (12 weeks) and
- Follow-up (FU) period: (2 weeks).

33 patients will be randomised in a 1:1 ratio to MC2-25 cream or MC2-25 vehicle. During the treatment period, patients are treated for 12 weeks with MC2-25 cream or MC2-25 vehicle. In-clinic visits are planned at Screening 1, Baseline, Week 1, Week 4, Week 8 and Week 12 (End of Treatment). Phone visits are planned at Screening (Visit) 2, Week 2, Week 6, and Week 10. The week 14 visit may be either a phone visit on an in-clinic visit.

A schematic overview of the trial is provided in Section 1.2 and a schedule of assessments and visits is provided in Section 1.3.

4.2 Rationale for trial design

4.2.1 Design

This is a randomised, double-blind, and vehicle-controlled trial. By blinding, randomisation and inclusion of a group receiving MC2-25 vehicle (without the active ingredient), the trial design controls for potential influence on the results other than that arising from the pharmacological action of MC-25 cream (containing the active ingredient alanyl-glutamine [Ala-Gln])).

4.2.2 Comparator

No marketed products are approved by regulatory authorities for treatment of VLS. Therefore, patients will not miss out on approved treatment by participation in this trial. MC2-25 vehicle has emollient properties. So, patients randomised to MC2-25 vehicle treatment will not receive active treatment, but they will receive emollient treatment during the treatment period. Available guidelines recommend the use of emollients and ultrapotent topical corticosteroids (Kirtschig et al., 2015; Lewis et al., 2018).

4.2.3 Duration of trial

The 12-week double-blind treatment duration is based on the assumption that if no differentiation to the MC2-25 vehicle is observed within this timeframe, then the treatment may be of less clinical relevance as current standard of care is a 12 weeks treatment with potent to ultrapotent topical

corticosteroids according to EU and UK guidelines (Kirtschig et al., 2015; Lewis et al., 2018). In addition, the 12-week treatment duration is supported by 13-weeks repeat dose toxicity studies in rats and dogs with Ala-Gln administered intravenously in form of Dipeptiven® (Fresenius, 2015). The double-blind treatment period is preceded by a screening period of up to 28 days duration allowing for wash out of other treatments and emollients before baseline as/if applicable and a 14-day follow-up period, to ensure safety follow-up.

4.2.4 Trial population

Traditional teaching is that there is a bimodal onset of VLS in prepubertal children and in post-menopausal women. However, recent studies suggest that VLS prevalence is extremely low in childhood and increases steadily with age (Bleeker et al., 2016; Halonen et al., 2020; Melnick et al., 2020). Consequently, the trial allows enrolment of adult women with no upper age limit. Children will not be enrolled as this is the first clinical trial with MC2-25 cream in VLS. As outlined in Section 2.2.1, chronic exposure to urine and from occlusion and moisture in the genitoanal area may be triggering factors for Lichen sclerosus. There is very little data on the role of urinary incontinence in VLS in women, but a recent cross-sectional analysis found that the prevalence of urinary incontinence was 63% in LS women compared to 34% in non-LS women (Kirby et al., 2021). Consequently, women will be allowed to enter the trial regardless of their urinary incontinence status, but they will be screened for urinary incontinence when entering the trial so urinary incontinence status can be taken into account during analysis of trial data. According to a recent survey including around 1400 women with VLS, itch is the predominant symptom reported by >90% of the survey respondents and relief of itching was rated the most important efficacy parameter to be addressed in a future treatment (GlobalSkin, 2018). In a recent cross-sectional study including around 500 pre-menopausal women with VLS the most prevalent symptom was dyspareunia. However, pruritus prompted patients to seek medical attention at a frequency similar to dyspareunia (Krapf et al., 2022). Consequently, patients will be required to have a minimum Worst Itch Numeric Rating Score (WI-NRS) ≥ 4 (on a scale ranging from 0 [no itch] to 10 [worst imaginable itch) to ensure that trial patients suffer from itch and that it is at least of moderate intensity to ensure that there is room for improvement.

4.2.5 Dose strength

Urea is in equilibrium with isocyanate which is a reactant in a process known as carbamylation. Carbamylation is believed to be an important contributor to the development of a number of chronic diseases including CKD. It is conceivable that carbamylation may also contribute to itch and dry skin associated with CKD, as well as the symptoms of itch and pain in VLS, as outlined in Section 2.2.1. The concentration of urea in urine ranges from 9 to 23 g/L (Thomas, 2019). With a molar weight of 60.06 g/mole for urea, this corresponds to a urinary urea concentration ranging from 0.15 to 0.38 mole/L. For the following calculation, an average urea concentration of 0.3 mole/L in urine is assumed.

As mentioned above a recent cross-sectional analysis indicated that urinary incontinence is more frequent in women with VLS than in women without LS (Kirby et al., 2021). A previous trial

showed that the average pad weight gain in a one-hour pad test for incontinence was 16.5 g in 18 women with proven genuine stress incontinence (Wall et al., 1990), which is classified as the lower end of moderate incontinence (Ferreira & Bo, 2015). Assuming a urine density of ~1 (i.e. 16.5 g ~16.5 mL urine), this amount of urine contains ~4950 µmole of urea. The concentration of isocyanate in urine is not easy to calculate. The concentration of isocyanate in serum is very low due to high concentration of scavenging activity of amino and sulfhydryl groups of amino acids, peptides, and proteins (Nilsson et al., 1996). However, in the ultrafiltrate from the nephron, isocyanate will start accumulating. Kinetic data on the rate of formation of isocyanate depends on factors like temperature, pH of urine, and concentration of urea, but data suggest that equilibrium is reached fairly quickly (Dirnhuber & Schutz, 1948). Based on an accepted equilibrium ratio of urea to isocyanate of 99:1 (Jaisson et al., 2018; Nicolas et al., 2018), a urine amount containing 4950 µmole of urea (the hourly urine leakage in patients with moderate incontinence) will contain up to 49,5 µmole isocyanate. It is well known that urea is quickly absorbed into the skin (Gabard & Chatelain, 2003), thus the genital skin of LS patients with urinary incontinence is expected to have constant high levels of urea and isocyanate.

To reduce the concentration of free isocyanate in the skin, the molar amount of scavenger (Ala-Gln) added via a cream should be higher than the molar concentration of isocyanate. At each treatment application one fingertip unit (FTU) (approximately 500 mg) should be used. It is generally accepted that one FTU covers 2% of the body surface area corresponding to appr. 358 cm^2 (Sacco et al., 2010; Thomas & Finlay, 2007) which should be sufficient to cover the vulvar region which in the context of this trial includes the clitoris and periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal skin. There is 15 mg (0.015 gram) of Ala-Gln in 500 mg (1 FTU) of MC2-25 3% cream corresponding to 69 µmole dipeptide.

Based on these calculations, administration of one FTU of MC2-25 cream in the vulvar region will provide a slight excess of scavenger (~69 µmole dipeptide scavenger vs 49,5 µmole isocyanate). Considering that the above calculations are based on average hourly urine leakage in patients with moderate urinary incontinence, the application of MC2-05 has to be repeated (see next section).

4.2.6 Dosing-regimen

A FTU corresponding to 0.5 g of MC2-25 cream, or MC2-25 vehicle is to be applied on the vulvar region (i.e., the clitoris and periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal skin) after each toilet visit (during day and night) starting on the day of the Baseline visit. This dosing regimen has been chosen to ensure replenishment of Ala-Gln on the vulvar region (as the cream will likely be removed due to wiping after urination) and to counteract any increase in urea concentration e.g., due to insufficient cleaning/wiping of urine residues after toilet visits. This will also counteract any increase in urea due to urinary leakage between toilet visits. Limited information is available on the normal range of urination frequencies in women. However, a recent survey investigating urination frequencies in 2534 healthy women reported reference ranges for urination frequencies between 2-10 times/day and 0-4 times/night (Wyman et al., 2022). Consequently, the maximum number of applications will be 12 per day. Assuming up to

12 toilet visits per day leads to a maximum total daily dose of 6.0 g of MC2-25 cream (3%) corresponding to 0.18 g Ala-Gln per day or 17 mg MC2-25 cream per cm^2 skin per day (assuming that 6.0 g cream per day will be distributed on a skin area corresponding to two handprints or 358 cm^2).

Systemic safety margin for Ala-Gln: Ala-Gln in the form of Dipeptiven® can safely be administered intravenously up to a maximum daily dose of 0.5 g Ala-Gln/kg/day. Under the assumption that an average adult woman weighs around 70 kg this corresponds to a maximum IV dose of 35 g Ala-Gln per day. If all Ala-Gln applied per day as MC2-25 cream was to penetrate into the systemic circulation this would correspond to a systemic safety margin of approximately 200. However, less than 1% of the topically 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 trial with Ala-Gln cream (see IB Section 4.3.1.2) which would correspond to a systemic safety margin of approximately 20,000. Whether whole human skin penetration data can be extrapolated to VLS or not can be debated as VLS skin could be argued both to be a deficient or an efficient skin barrier.

Local tolerability of MC2-25 cream (3%) and Ala-Gln: In a local tolerability study, MC2-25 cream was well tolerated when applied topically on a twice-daily basis for 2 weeks in minipigs which is in accordance with regulatory requirements for 'stand-alone' local tolerance testing (EMA/CHMP/SWP/2145/2000 Rev. 1, Corr. 1). In this study the animals were treated with a total daily dose of 20 mg/cm^2 of MC2-25 cream (i.e.10 mg/cm^2 of MC2-25 cream per application, twice daily) under semi-occlusion to avoid that the cream was rubbed off. The total daily duration of local exposure to MC2-25 cream was 20 hours (divided in a 6-hour and a 14-hour exposure period), as the high dose volume results in residual cream on the skin. Thus, on average animals were exposed to 1 mg/cm^2/hour over a 20-hour period.

In the MC2-25-C3 trial patients are instructed to apply ~1.4 mg/cm^2 of MC2-25 cream per application (i.e. 1 FTU ~0.5 g cream applied to an area corresponding to two handprints, or ~358 cm^2), after each toilet visit both day and night, and up to 12 times daily, resulting in a total daily dose of 16.8 mg/cm^2 of MC2-25 cream. Thus, over a 24-hour period patients will on average be exposed to ~0.7 mg/cm^2/hour (1.4 mg/cm^2 x 12 applications per day : 24 hours per day), if they use the maximum number of 12 daily applications.

The maximum total daily dose of cream per area, the amount of cream per area per application and the average hourly local exposure per area are therefore lower in the proposed clinical trial than in the minipig study.

In an ongoing randomised, double-blind, vehicle controlled clinical trial in chronic kidney disease associated pruritus (CKD-aP) where MC2-25 cream (3%), or MC2-25 vehicle is applied twice daily to unlimited areas of CKD-aP skin for 12 weeks only 3 adverse events within the System Organ Class 'Skin and subcutaneous tissue disorders' (Preferred terms: pruritus, pruritus and alopecia) have been reported among 36 patients who completed 8–12 weeks treatment as of medio May 2023. All events were considered mild (Data on file) (Data on file).

In view of the favourable systemic safety margin for Ala-Gln as well as the lack of local tolerability issues both after administration of MC2-25 cream on the skin in minipigs and CKD-aP patients, the proposed dosing regimen is considered safe.

4.2.7 Endpoints

As described in the 'Trial population' section above, itch is an important symptom in VLS patients. In addition, according to a recent publication from the Core Outcomes for Research in Lichen sclerosus (CORALS) initiative, it is recommended that symptoms followed by lichen sclerosus specific quality of life and clinical signs of lichen sclerosus are evaluated in all clinical trials in lichen sclerosus (Simpson, 2022). Consequently, the primary endpoint the symptom itch which is evaluated by use of the clinical outcome assessment (COA), patient reported Worst Itch Numeric Rating Score (WI-NRS) with a 24-hour recall period. This COA was selected based on clinical relevance and regulatory acceptability precedent with two other extremely itchy diseases (namely Korsuva® for Chronic Kidney Disease associated Pruritus (CKD-aP) and Dupixent® for Atopic Dermatitis).

Furthermore, in line with the above-mentioned CORALS initiative, the trial includes a number of other endpoints evaluating symptoms, lichen sclerosus specific quality of life and clinical signs reported by clinicians as follows. The trial includes endpoints evaluating patient reported outcomes such as Worst Pain- and Worst Penetrative sex related Pain Numeric Rating Scores (WP-NRS and WPP-NRS). To enable determination of minimum important difference (MID) in these patient-reported numeric rating scores, the trial also includes endpoints evaluating Patient's Global Impression of Change (PGIC) (on a verbal rating scale) for Worst Itch, Worst Pain and Worst Penetrative sex related Pain in accordance with recent Patient-Focused Drug Development (PFDD) Guidances[1]. Furthermore, patients will be asked to complete the VLS patient journey sheet at the Screening 1 visit. The VLS patient journey sheet seeks to understand what the most burdensome issues for VLS patients are. Also, patients will be asked to complete quality of life questionnaires (Skindex-29 and VQLI) throughout the trial to evaluate any effects of treatment on quality of life. The trial also includes a number of endpoints evaluating clinician reported outcomes to explore correlations with patient reported endpoints.

4.3 Start of trial and end of trial definition

The start of trial is defined as the date of the first visit of the first patient. The end of trial is defined as the date of the last visit of the last patient.

[1] https://www.fda.gov/drugs/development-approval-process-drugs/fda-patient-focused-drug-development-guidance-series-enhancing-incorporation-patients-voice-medical - accessed 10 October 2022

5 Trial population

5.1 Inclusion criteria

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

1. Women, of any race or ethnicity, who are ≥18 years of age at the time of Screening 1.
2. Able to understand the trial and willing to comply with trial requirements.
3. Has provided written informed consent.
4. Clinical diagnosis of VLS (i.e., biopsy for histological confirmation not required except if needed to rule out malignancy or differential diagnosis of e.g., vulvar lichen planus) made by either a dermatologist or a gynaecologist familiar with VLS.
5. Presence of at least one of the following signs of VLS: a. Hyperkeratosis (i.e., patches/plaques of bright white skin with a 'powdery' texture) and/or b. Sclerosis (i.e., areas of yellowish/ivory white skin with a smooth/waxy/firm texture. Sclerosis is often seen at the tips of the labia minora, or on periclitoral or perineal skin) in at least one of the following vulvar areas: Clitoris and/or periclitoral skin (C); Right interlabial sulcus and labium minus (R); Left interlabial sulcus and labium minus (L); Posterior Fourchette and/or perineum (P) (see protocol section 8.5.1.2).
6. First symptoms of VLS (e.g., itching and/or pain) noticed by the patient at least 6 months before baseline.
7. At least four WI-NRS scores available in the diary for calculation of the average WI-NRS at the baseline visit.
8. At least moderate itch defined as average WI-NRS ≥4 at the Baseline visit (average WI-NRS is calculated as the average of all available and at least four WI-NRS scores which are to be reported once daily by the patient in the diary for 7 days prior to the Baseline visit (7 days in total)).
9. Women must be of either:
    a) Non-childbearing potential, i.e., post-menopausal* 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,
    b) Childbearing potential with a negative highly sensitive* urine pregnancy test at the Baseline visit (*Note: a highly sensitive urine pregnancy test must have a sensitivity down to at least 25 mIU/ml for human chorionic gonadotropin (hCG)).

10. Women 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:

    a) combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal

    b) progestogen-only hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable

    c) intrauterine device (IUD)

    d) intrauterine hormone-releasing system (IUS)

    e) bilateral tubal occlusion

    f) vasectomised partner (provided that is the sole sexual partner of the patient and that the vasectomised partner has received medical assessment of the surgical success.)

    g) sexual abstinence if in line with the preferred and usual lifestyle of the patient 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).

5.2 Exclusion criteria

Participants who meet any of the following criteria are ineligible for participation in the trial:

Conditions/Diseases

1. Pregnant, breast feeding, or planning to become pregnant during the trial.
2. Any (other than VLS) ongoing localised or systemic disease involving the vulvar region – e.g., lichen planus, psoriasis, eczema, ulcerative colitis or known active infection (bacterial, viral or fungal).
3. Ongoing symptomatic Urinary Tract Infection (UTI). Note: symptomatic is defined as recent start of pain (e.g., burning or stinging) with urination along with increased urination frequency.
4. Ongoing or prior diagnosis of any genitoanal malignancy or pre-malignancy (e.g., differentiated intraepithelial neoplasia or squamous cell carcinoma).
5. Any kind of ongoing cancer (or anti-cancer treatment within 3 months or 5 half-lives (whichever is longest) prior to the Baseline visit). Note: non-metastasising basal cell carcinoma which does not involve the genitoanal area is acceptable.
6. Any chronic or acute systemic medical condition that, in the opinion of the investigator, may pose a risk to the safety of the patient or may interfere with the assessment of efficacy in this trial.

7. Known history of allergic reaction to any ingredients in MC2-25 cream or MC2-25 vehicle (see Section 6.1.1.1).

Treatments

8. Start of a new or change of existing non-biologic systemic treatment, including but not limited to corticosteroids, cyclosporin, methotrexate and tacrolimus, within 21 days prior to the Baseline visit.
9. 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 longest) prior to the Baseline visit.
10. Start of a new or change of existing systemic or intravaginal treatment with estrogen containing products, within 21 days prior to the Baseline visit
11. Start of new or change of menstrual care routines within 21 days prior to the Baseline visit.
12. Use of emollients (including but not limited to creams, ointments, oils, or soaps with emollient properties) on the vulvar region within 3 days prior to the Baseline visit. Note: Only water and, if necessary, a soap bar without emollient properties, may be used to wash the vulvar region (intimate hygiene) when bathing or showering from within 3 days prior to the Baseline visit.
13. Use of any topical treatment on the vulvar region, including but not limited to calcineurin inhibitors, corticosteroids or anti-infectives within 14 days prior to the Baseline visit.
14. Use of any light therapy on the vulvar region, including but not limited to UV-B, UV-A, and laser, within 28 days prior to the Baseline visit.
15. Received a non-marketed or blinded drug within 28 days or 5 half-lives (whichever is longer) prior to the Baseline visit.

Compliance/Administrative

16. If in the opinion of the investigator, the patient is unlikely to comply with the clinical trial protocol. Note: Patient must be able to apply the IMP without need for assistance from e.g., spouse or caregiver.
17. If previously randomised in this trial.

5.3 Inclusion of vulnerable populations

A considerable number of elderly (>65 years of age) patients are expected to be enrolled in this trial. This is considered acceptable in view of the favourable safety profile of the trial and that no specific safety surveillance measures have been implemented for elderly patients specifically, see Section 2.3.3 for details.

5.4 Lifestyle considerations

Not applicable

5.5 Screen failures and re-screening

Patients who fail screening because they do not meet the minimum WI-NRS criterion at baseline cannot be re-screened. Patients who fail screening for other reasons may be re-screened once. Reasons for screen failure must be recorded in the eCRF.

5.6 Strategies for recruitment and retention

Patients may be identified from pre-existing databases, recruited in an outpatient setting or hospital environment. Recruitment plans will be site specific with a common goal to create awareness/support for the trial among all site-affiliated staff working with this patient population.

Due to the rarity of the disease, the identification of all potential patients for enrolment is critical. Advertising may be introduced to create awareness of the trial, e.g., at the enrolling centre as well as at any affiliates, referral clinics, or via patient organisations.

To prevent patients from dropping out early, frequent visits are planned in the beginning of the trial and phone-visits will be conducted throughout the trial, and patients may also receive appointment reminders.

A patient travel stipend sufficient to meet patients' out-of-pocket travel costs will be available.

6 Trial intervention

6.1 Trial interventions administration

6.1.1 Trial intervention description

6.1.1.1 Investigational medicinal product (IMP)

MC2-25 cream

MC2-25 cream is a white oil-in-water, topical emulsion containing the active ingredient, Ala-Gln in the aqueous phase at a concentration of 30 mg/g. The formulation components are the following: L-alanyl-L-glutamine, triglycerides medium chain, paraffin liquid, glycerol, carbomer interpolymer type A, phenoxyethanol, macrogol lauryl ether (4), citric acid, sodium benzoate, polysorbate (20), sodium hydroxide, purified water.

MC2-25 cream is not authorised in any markets.

MC2-25 vehicle

MC2-25 vehicle is identical to MC2-25 cream but without the active ingredient.

6.1.2 Dosing and administration

MC2-25 cream, or MC2-25 vehicle must be applied after each toilet visit following urination (during day and night) starting on the day of the Baseline visit. The MC2-25 cream, or MC2-25 vehicle should be applied by the patient in a thin saturating layer to the vulvar region (i.e., vulvar region includes clitoris and periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal skin). Approximately one fingertip-unit (FTU), roughly corresponding to 0.5 g cream is to be used after each toilet visit (during night and day). The vulvar region may be gently cleaned with water and dabbed dry with toilet paper or a clean cloth before each application of the trial treatment. Assuming, a maximum of 12 toilet visits are needed over a period of 24 hours, the maximum daily dose is expected to be around 6.0 g. A treatment application manual will be available for patients.

Reference is made to Section 4.2 for the rationale behind the dosing-regimen.

6.2 Preparation/ handling/ storage/ accountability

6.2.1 Acquisition and accountability

The IMP will be supplied by MC2's designated vendor.

The investigator is responsible for ensuring that all IMP received at the site is inventoried and accounted for throughout the trial. The IMP dispensed to the patient and returned to the site must be documented on the drug accountability form and in the eCRF. All IMP will be stored and disposed of according to the sponsor's instructions.

IMP must be handled in strict accordance with the protocol and the label and must be stored at the trial site in a limited-access area. Used and unused IMP tubes returned by the patient must be available for verification by the trial monitor during on-site monitoring visits.

IMP should be dispensed under the supervision of the investigator or a qualified member of the site staff, or by a hospital/clinic pharmacist. IMP will be supplied only to randomised patients. IMP cannot be relabelled or reassigned for use by other patients. The investigator agrees neither to dispense the IMP from, nor store it at, any site other than the trial sites agreed upon with the sponsor.

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. Patients will be asked to return all used and unused tubes in the accompanying box at each visit to ensure drug accountability. All returned tubes (used and unused) will be weighed to determine the amount of IMP used.

6.2.2 Formulation, appearance, packaging, and labelling

Sponsor will provide Solural Pharma with the MC2-25 cream and MC2-25 vehicle. The tubes are identical in appearance and packaging.

MC2-25 cream and MC2-25 vehicle will be labelled in accordance with applicable regulatory requirements and Good Manufacturing Practice Guidelines and delivered to the clinics in boxes.

IMP for one patient consists of the following: One box containing 3 boxes with 8 tubes in each adding up to a total of 24 tubes per patient. Labels for the IMP will comply with the legal requirements of the country where the trial is performed and will be printed in the local language(s).

6.2.3 Product storage and stability

The IMP must 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 according to the label. At all times, the IMP tube should be protected from direct sunlight exposure.

6.2.4 Preparation and destruction

No preparation of the IMP is needed.

Destruction: IMP must only be destructed after sponsor's approval. Unused IMP (i.e., both dispensed/returned as well as not dispensed IMP) should preferably be destructed locally at the site (to be documented on the IMP destruction form). If the IMP is not destructed at site but returned for destruction, to sponsor's designated vendor, this must also be documented on the IMP return form.

6.3 Measures to minimise bias: randomisation and blinding

Randomization will be performed in a 1:1 ratio. Randomisation data will be kept strictly confidential, accessible only to authorised personnel, until the time of un-blinding.

A patient who fulfils the trial eligibility requirements will be randomly assigned to treatment with IMP.

An external statistician who is independent from the study team will prepare the randomization list using appropriate software tool ensuring equal allocation of patients into the two treatment arms. The external statistician will also prepare the emergency unblinding envelopes. The randomization list will be shared with Solural Pharma (who are responsible for labelling) and with PharmaLex (who are responsible for handling of SAEs) and will be kept strictly confidential, filed securely, and will be accessible only to authorized persons from Solural Pharma and PharmaLex until unblinding. This is a double-blind trial. The sponsor, sponsor representatives, and all patients, monitors, and site staff will be blinded throughout the trial. Instructions for emergency unblinding are provided in Section 10.2.1. The staff at the study treatment manufacturing site and the external statistician responsible for preparing the randomization list are not blinded.

Each patient will be assigned a unique randomization number in ascending order determining which study treatment the patient will receive. These randomization numbers will be based on a supply list accompanying the IMP provided to the sites and the same randomization number (named as kit no. will also be listed to the applicable trial medication. At each dispense, this kit no. will be documented in the eCRF linking the trial medication to correct patient. No patient ID, other than the kit no. will be written on the trial medication.

6.4 Treatment compliance

At all on-treatment visits, the patient will be asked if he/she has used the trial medication as prescribed. Any degree and nature of noncompliance will be specified. In addition, patients will be asked to complete an Application Diary during the treatment period as a measure of treatment compliance. Patients who are consistently noncompliant will be counselled.

6.5 Prohibited therapies

6.5.1 Prohibited therapies prior to baseline

Several therapies are prohibited during the period leading up to baseline as outlined in Table 3. Therapies not listed in the table are allowed.

Table 3: Prohibited therapies prior to baseline

Prohibited Therapy	Exclusion Criterion	Applicable Wash-Out Period Prior to Baseline (Day 0)
Use of anti-cancer treatment	5	3 months or 5 half-lives (whichever is longest)
Start of a new or change of existing non-biologic systemic treatment, including but not limited to corticosteroids, cyclosporin and tacrolimus.	8	21 days
Start of a new or change of existing biologic systemic treatment, including but not limited to etanercept, adalimumab, alefacept, infliximab, and ustekinumab	9	3 months or 5 half-lives (whichever is longest)

Start of a new or change of existing systemic or intravaginal treatment with estrogen containing products	10	21 days
Start of new or change of menstrual care routines	11	21 days
Use of emollients (including but not limited to creams, ointments, oils, or soaps with emollient properties) on the vulvar region Note: Only water and, if necessary, a soap bar without emollient properties, may be used to wash the vulvar region (intimate hygiene) when bathing or showering.	12	3 days
Use of any topical treatment on the vulvar region, including but not limited to calcineurin inhibitors, corticosteroids or anti-infectives.	13	14 days
Use of any light therapy on the vulvar region, including but not limited to UV-B, UV-A and laser.	14	28 days
Received a non-marketed or blinded drug.	15	28 days or 5 half-lives, whichever is longer

6.5.2 Prohibited therapies after Baseline

Use of any therapies that would exclude the participant from participation in the trial (as specified in Section 5.2 Exclusion Criteria) is also prohibited during the treatment and follow-up periods, see Table 3. Therapies not listed in Table 3 are allowed.

6.5.3 Rescue therapies

If prohibited therapies are used as rescue therapies during the screening period, the patient is not eligible for the trial. If prohibited therapies are used as rescue therapies during the treatment period, they must be recorded as concomitant therapies and reported as protocol deviations.

7 Trial intervention discontinuation and participant discontinuation / withdrawal

7.1 Participant discontinuation / discontinuation of trial intervention

In accordance with legal requirements and International Council for Harmonisation (ICH) – Good Clinical Practice (GCP) guidelines, every patient 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 patient's refusal should be effectuated immediately upon her request.

A patient will be withdrawn from the trial for any of the following reasons:

- Lost to follow-up
- Withdrawal of consent
- Death
- Trial terminated by sponsor

In addition, a patient 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) lack of treatment effect, unacceptable AE(s), pregnancy, and protocol violations.

In the case of trial withdrawal or treatment discontinuation, all assessments of the Week 12 (End of Treatment [EoT]) visit must be performed to the extent possible. The reasons for trial withdrawal or treatment discontinuation must be fully documented in the eCRF and the end of treatment form must be completed when treatment is permanently discontinued, and the end of trial form must be completed at end of the trial or in case of trial withdrawal.

Patients who withdraw from the trial before randomisation will be considered screen failures. After randomisation, in case of treatment discontinuation, the patient should be encouraged to attend remaining trial visits in line with the schedule of assessments (SoA). If a patient 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 8.8.4.1.

7.2 Lost to follow-up

If a patient is lost to follow-up, every reasonable effort must be made by the site staff to contact the patient and determine the reason for discontinuation/withdrawal. The measures taken to follow-up must be documented.

7.3 End-of-treatment visit

Unless a patient withdraws consent for trial participation, or is lost to follow-up, an End-of-Treatment Visit should be performed as soon as possible (no later than 30 days) after the treatment

has been discontinued. Every effort should be made to conduct the End-of-Treatment Visit before the patient starts subsequent therapy. If a patient is unable to return to the site for the End-of-Treatment Visit, then the patient should be contacted (e.g., by phone) to collect information on AEs and concomitant therapies and other EOT visit assessments (in particular PROs) to the extent possible that occurred within 30 days after the last treatment. Additional information on reporting of AEs can be found in Section 8.8.4.1.

Patients who permanently discontinue treatment at some point after the administration of the first dose of trial medication will not be replaced.

8 Trial assessments and procedures

8.1 Visit schedule

The schedule of assessments (SoA) is provided in Table 1.

The following order of assessments must always be adhered to:

- At Visit 1, the informed consent process must be completed before Patient Reported Outcomes or any other clinical assessment or procedure.
- At all visits, Patient Reported Outcomes should be assessed before all other clinical assessments or procedures.

8.2 Screening

Up-front waivers for inclusion or exclusion criteria are not allowed. Any deviation from inclusion or exclusion criteria identified post-randomisation, will be reported as a protocol deviation, patients shall continue in the trial as planned unless discontinuation of IMP treatment is warranted for safety reasons.

8.3 Demographics and medical history

The following demographic and medical history must be collected:

- Age
- Height (can be self-reported)
- Weight (can be self-reported)
- Race
- Ethnic origin
- Complete VLS history (year of first VLS symptoms, year of VLS diagnosis, diagnosis made by gynaecologist, dermatologist or other [specify], diagnosis confirmed by biopsy, any family members with VLS, currently seeing a VLS treating physician regularly, current VLS treating physician is gynaecologist, dermatologist or other [specify])
- All other current and past medical/surgical conditions within the previous 12 months.

8.4 Prior and concomitant therapies

All medications and treatments (i.e., prescription drugs [including COVID-19 vaccination programs, see Table 3], over the counter [OTC] drugs and vitamins, herbal and dietary supplements, and lubricants used with sex), hereafter referred to as therapies, taken within 30 days prior to screening will be recorded as prior therapies at the Screening 1 visit.

All therapies used after the screening 1 visit will be recorded as concomitant therapies.

Information about the total daily dose, route of administration, start and discontinuation dates, and indication are to be recorded in the eCRF.

8.5 Efficacy assessments

8.5.1 Clinician reported outcomes (ClinRO) assessments

8.5.1.1 Imaging of VLS

Imaging of the vulvar region (i.e., clitoris and periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal skin), must be obtained by the investigator (preferably the same investigator at all visits for a specific patient) at Screening 1, Baseline, Week 1, Week 4, Week 8 and Week 12 (or Early Termination) for all patients. Imaging of VLS should preferably be performed BEFORE the scoring of the VASSI.

Detailed description of the imaging procedure will be provided in a separate imaging manual. Steps will be taken to ensure that the images are pseudonymised.

8.5.1.2 Vulvar lichen sclerosus Area and Sign Severity Index (VASSI)

The Vulvar lichen sclerosus Area and Sign Severity Index (VASSI) evaluates the severity of 3 non-permanent signs of VLS in 5 different vulvar areas. It must be performed by the investigator (preferably the same investigator at all visits for a specific patient) at Screening 1, Baseline, Week 1, Week 4, Week 8 and Week 12 (or Early Termination) for all patients. It should preferably be performed AFTER Imaging of VLS and BEFORE scoring of the CGA.

Areas: The 5 different vulvar areas are Clitoris and periclitoral skin (**C**), Right interlabial sulcus and labium minus (**R**), Left interlabial sulcus and labium minus (**L**), Posterior fourchette and perineum (**P**), Anus and perianal skin (**A**)), see Figure 2.

Signs: The 3 non-permanent signs of VLS are **Fissures/erosions** (longitudinal/patchy ruptures of the skin surface), **Ecchymoses** (bleedings within the skin) and **Hyperkeratoses** (patches/plaques of bright white skin with a ‘powdery’ texture). IMPORTANT: Hyperkeratoses must be distinguished from Sclerosis (areas of yellowish/ivory white skin with a smooth/waxy/firm texture) and Pallor (areas of pale whitish skin that differ from hyperkeratosis in that they are not “powdery”). Sclerosis and pallor are permanent signs of VLS.

Severity: The severity must be scored on a 4-point scale from 0-3 (i.e., 0 (no lesion), 1 (length of lesion ≤5 mm), 2 (length of lesion > 5mm and ≤ 10 mm) or 3 (length of lesion > 10 mm)) for each non-permanent sign in each vulvar area (i.e., the light grey cells in Table 4). Note: The measure included in the photocard used for imaging of VLS must be used as reference when assigning severity scores. In case a non-permanent sign extends from one vulvar area into another, then it must be scored in both areas according to the length of the lesion in the particular area. In contrast, if a non-permanent sign involves the border between two areas, it should only be scored for the area that is most involved/affected to avoid scoring the same lesion twice.

A scoring manual will be available.

For endpoint analyses, the VASSI Area scores, VASSI Sign scores and Total VASSI score (i.e., the white cells in Table 4) will be calculated as follows:

1. A VASSI Area score (**VASSI$_{C, -R, -L, -P, -A}$**) is calculated by summing up the 3 severity scores for Fissures/erosions, Ecchymoses and Hyperkeratoses in the particular vulvar area [range 0-9].
2. A VASSI Sign score (**VASSI$_{F, -E, -H}$**) is calculated by summing up the severity scores for a particular sign across all 5 vulvar areas [range 0-15]
3. The Total VASSI (**VASSI**) score is calculated by summing the 5 VASSI Area scores or VASSI Sign scores [range 0-45]

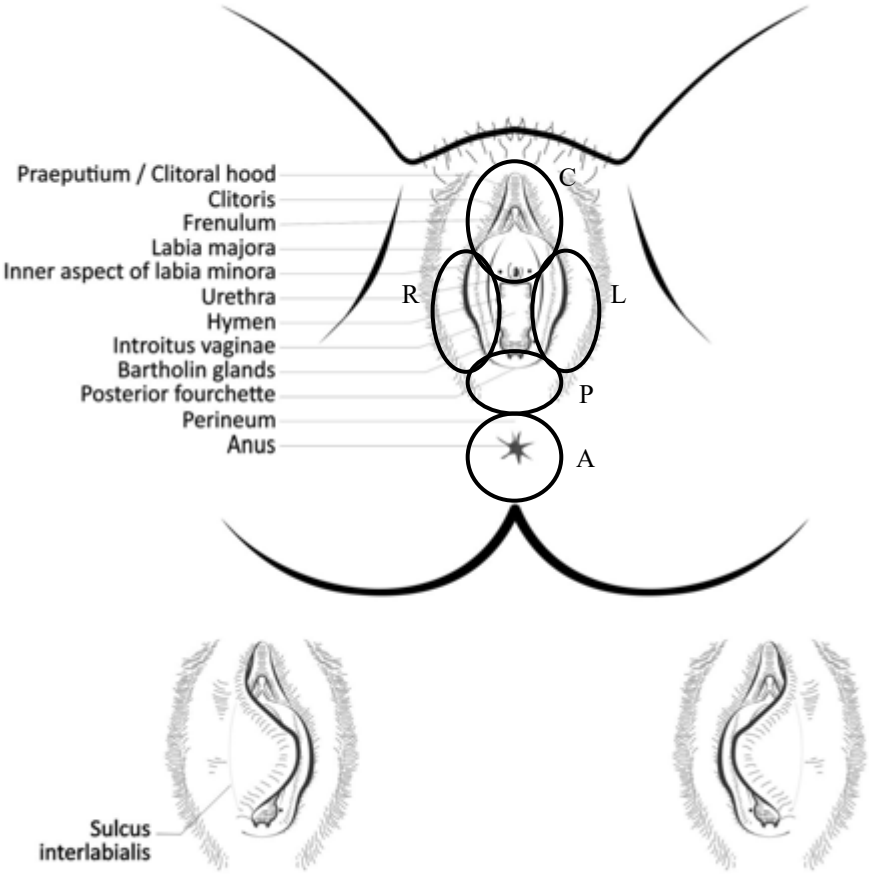
Table 4: Vulvar lichen sclerosus Area and Sign Severity Index (VASSI)

Vulvar areas / **Signs**	Clitoris and periclitoral skin (C)	Right interlabial sulcus and labium minus (R)	Left interlabial sulcus and labium minus (L)	Posterior fourchette and perineum (P)	Anus and perianal skin (A)	**VASSI Sign scores**
Fissures / erosions (F)						VASSI $_F$ =
Ecchymoses (E)						VASSI $_E$ =
Hyperkeratoses (H)*						VASSI $_H$ =
VASSI Area scores	VASSI $_C$ =	VASSI $_R$ =	VASSI $_L$ =	VASSI $_P$ =	VASSI $_A$ =	**Total VASSI score =**

Light grey cells must be filled in by the investigator with severity scores. The severity must be scored on a 4-point scale (i.e., 0 (no lesion), 1 (length of lesion ≤5 mm), 2 (length of lesion > 5mm and ≤ 10 mm) or 3 (length of lesion > 10 mm) for each non-permanent sign in each vulvar area; White cells will be calculated.

*IMPORTANT: Hyperkeratoses (patches/plaques of bright white skin with a 'powdery' texture) is a non-permanent sign of VLS and must be distinguished from Sclerosis (areas of yellowish/ivory white skin with a smooth/waxy/firm texture) and Pallor (areas of pale whitish skin that differ from hyperkeratosis in that they are not "powdery"). Sclerosis and pallor are permanent signs of VLS.

Figure 2: Vulvar areas



8.5.1.3 Clinician's Global Assessment (CGA) of VLS

The Clinician's Global Assessment (CGA) of VLS evaluates the global (overall) severity of VLS in the entire vulvar region (i.e., including the clitoris, periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal areas). It should preferably be performed AFTER the scoring of the VASSI and BEFORE scoring of the CGIC.

The CGA must be evaluated by the investigator (preferably the same investigator at all visits for a specific patient) on a 5-point scale ranging from Clear to Severe at Screening 1, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using Table 5 below :

Table 5: Clinician's Global Assessment (CGA) of VLS

CGA score	Description

Clear	No visible Non-permanent signs* of VLS (Fissures/Erosions, Ecchymoses or Hyperkeratoses) in the vulvar region. Note: Permanent signs[#] of VLS may be present
Almost clear	Barely visible Non-permanent signs* of VLS (Fissures/Erosions, Ecchymoses or Hyperkeratoses) in the vulvar region.
Mild	Slightly visible Non-permanent signs* of VLS (Fissures/Erosions, Ecchymoses or Hyperkeratoses) in the vulvar region.
Moderate	Easily visible Non-permanent signs* of VLS (Fissures/Erosions, Ecchymoses or Hyperkeratoses) in the vulvar region.
Severe	Markedly visible Non-permanent signs* of VLS (Fissures/Erosions, Ecchymoses or Hyperkeratoses) in the vulvar region.

*Non-permanent signs of VLS include fissures/erosions (longitudinal/ respectively patchy ruptures of the skin surface), ecchymoses (bleedings inside the skin) and hyperkeratoses (patches/plaques of bright white skin with a 'powdery' texture). Hyperkeratoses must be distinguished from Sclerosis (areas of yellowish/ivory white skin with a smooth/waxy/firm texture) and pallor (areas of pale whitish skin that differ from hyperkeratosis in that they are not "powdery")

[#] Permanent signs of VLS include e.g., atrophy, sclerosis, pallor, scarring or fusion.

A scoring manual will be available.

8.5.1.4 Clinician's Global Impression of Change (CGIC) of VLS

Clinician's Global Impression of Change (CGIC) in non-permanent signs of VLS including fissures/erosions (longitudinal/respectively patchy ruptures of the skin surface), ecchymoses (bleedings inside the skin) and hyperkeratoses (patches/plaques of bright white skin with a 'powdery' texture) must be assessed by the investigator (preferably the same investigator at all visits for a specific patient) at Week 1, Week 4, Week 8, and Week 12 or Early Termination visits using the below 7-item verbal rating scale. It should preferably be performed AFTER the scoring of the CGA:

Please choose the response below that best describes the overall change in the patient's non-permanent signs of vulvar lichen sclerosus since the trial medication was started at baseline, based on the baseline image of VLS (see Section 8.5.1.1 'Imaging of VLS'):

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

8.5.2 Patient reported outcomes (PRO) assessments

8.5.2.1 Worst Itch Numeric Rating Score (WI-NRS) of VLS

The patient's WI-NRS score must be recorded by the patient in a diary once daily (i.e., every day around the same timepoint, e.g. morning, afternoon or evening) for a total of 7 days (i.e. starting 7 days before the next in-clinic visit and ending on the day before the next in-clinic visit) leading up to each in-clinic visit day 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 associated with your lichen sclerosus, that you have experienced in the past 24 hours: 0 (no itch), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (worst imaginable itch).

In addition, the patient's WI-NRS score must be reported to the clinical staff at in-clinic visits at Screening 1, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using the 11-point numeric rating scale above.

8.5.2.2 Worst Pain Numeric Rating Score (WP-NRS) of VLS

The patient's WP-NRS score must be recorded by the patient in a diary once daily (i.e., every day around the same timepoint, e.g. morning, afternoon or evening) for a total of 7 days (i.e. starting 7 days before the next in-clinic visit and ending on the day before the next in-clinic visit) leading up to each in-clinic visit day 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 Pain* associated with your lichen sclerosus, that you have experienced in the past 24 hours: 0 (no pain), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (worst imaginable pain).

*Pain includes sensations such as Burning, Stinging, Soreness and Irritation.

In addition, the patient's WP-NRS score must be reported to the clinical staff at in-clinic visits at Screening 1, Baseline, Week 1, Week 4, Week 8, and Week 12 or Early Termination using the 11-point numeric rating scale above.

8.5.2.3 Worst Penetrative sex related Pain Numeric Rating Score (WPP-NRS) of VLS

At Screening 1, Baseline, Week 4, Week 8, and Week 12 or Early Termination visit all patients must indicate whether they are sexually active or not by answering Question 1 below. Patients who answer No to Question 1, must subsequently answer only Question 2. Patients who answer Yes to Question 1 must subsequently answer Questions 2, 3, and 4, and finally in Question 5 report their Worst Penetrative sex related Pain on an 11-point numeric rating scale ranging from 0 to 10 as also described below:

1. Have you had penetrative sex during the past 4 weeks? (Yes/No)
   If No, please answer Question 2 only. If Yes, please answer Questions 2, 3, 4 and 5.
2. Have you abstained from penetrative sex during the past 4 weeks due to your vulvar lichen sclerosus? (Yes/No)

3. How many times have you had penetrative sex during the past 4 weeks? (X times)
4. Have you used lubricants during penetrative sex during the past 4 weeks? (Yes/No)
5. Please score the intensity of the Worst Penetrative sex related Pain* you have experienced due to your lichen sclerosus in the past 4 weeks: 0 (no penetrative sex related pain), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (worst imaginable penetrative sex related pain).

*Pain includes sensations such as Burning, Stinging, Soreness and Irritation.

8.5.2.4 Patient's Global Impression of Change (PGIC) of VLS

PGIC in Worst Itch (WI), Worst Pain (WP) and Worst Penetrative sex related Pain (WPP) must be reported to the clinical staff at Week 4, Week 8, and Week 12 or Early Termination visits using a 7-item verbal rating scale:

Please choose the response below that best describes the overall change in your [Worst Itch*] associated with your lichen sclerosus since you started applying the trial medication:

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

*Repeat with Worst Pain and Worst Penetrative sex related Pain.

8.5.2.5 Patient's rating of importance of improvements of VLS

Patients reporting an improvement in PGIC (i.e., 'Very much better', 'Much better' or 'A little better') must report the Importance of the improvement to the clinical staff at Week 4, Week 8, and Week 12 or Early Termination visits using a 2-item Importance Questionnaire:

Question 1: You have reported an improvement in your [Worst Itch*] associated with your lichen sclerosus since you started applying 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*] associated with your lichen sclerosus important to you? (*Free text*).

*Repeat with Worst Pain and Worst Penetrative sex related Pain.

8.5.2.6 International Consultation on Incontinence Questionnaire Urinary Incontinence Short Form (ICIQ-UI-SF)

Incontinence status must be evaluated by the patient at Baseline and Week 12 or Early termination visits using the ICIQ-UI-SF questionnaire.

The ICIQ-UI-SF is a validated questionnaire with a recall period of 4 weeks comprising a total of 4 questions about when, how often and how much urine is leaked and how much urine leaking interferes with everyday life. The ICIQ-UI-SF score ranges from a maximum score of 21 indicating maximum burden) and a minimum score of 0 (indicating no burden). A score ≥ 1 indicates urinary incontinence (Avery et al., 2004).

8.5.2.7 Menopausal status and Menstrual cycle status

Menopausal status (Pre-menopausal or Menopausal) in accordance with inclusion criterion 9, Section 5.2 must be recorded for all women at Baseline and Week 12 (or Early Termination) visits.

Menstrual cycle status (i.e., whether the patient is menstruating or not, reason in case of no menstruation, number of days since the first day of the latest menstruation period, average duration of menstruation period in days, regularity of menstrual cycle) must be evaluated by all pre-menopausal women at Baseline, and Week 12 (or Early Termination) visits.

8.5.2.8 Skindex-29

Skindex-29 is a skin specific multidimensional quality-of-life instrument. It must be evaluated by the patient at Baseline, Week 4, Week 8, and Week 12 or Early Termination visits using the Skindex-29 Quality of Life Index questionnaire. It inquires how often (1. Never, 2. Rarely, 3. Sometimes, 4. Often, 5. All the time) during the previous 4 weeks the patient experienced the effect described in each item. Seven items address the Symptoms domain, ten items the Emotional domain, and twelve items the Functioning domain. All responses are transformed to a linear scale of 100, varying from 0 (no effect) to 100 (effect experienced all the time). Skindex-29 scores are reported as three scale scores, corresponding to the three domains; a scale score is the average of a patient's responses to items in a given domain (Chren et al., 1997).

8.5.2.9 VQLI

The Vulvar Quality of Life Index (VQLI) questionnaire is a vulvar specific quality-of-life instrument which was validated in cohorts of women including 40-50% Lichen Sclerosus patients. It must be evaluated by the patient at Baseline, Week 4, Week 8, and Week 12 or Early Termination visits using the Vulvar Quality of Life Index (VQLI) questionnaire. In the context of this trial, the vulvar region includes clitoris and periclitoral skin, interlabial sulci, labia minora, posterior fourchette and perineum, anal and perianal skin.

The questionnaire comprises a total of 15 questions with a recall period of 1 month under the following domains: Symptoms (Questions 1–2); Feelings and Emotions (Questions 3–5); Activities of daily living (Questions 6–10); Relationships (Question 11); Sexual function (Questions 12–13);

Future health concerns (Question 14); and Treatment (Question 15). Each of the questions are graded zero to three on a Likert scale, with 0 representing no symptom or complaint ('Not at all' or 'Not applicable'), 1 ('A little'), 2 ('A lot') and 3 ('Very much'), resulting in a maximum score of 45/45 and a minimum score of 0/45 (Saunderson et al., 2020).

8.5.2.10 VLS patient journey sheet

The Burden of living with VLS before entering the trial must be described by the patient by filling in the 'VLS patient journey sheet' at the Screening 1 visit. The patient journey sheet is a questionnaire which collects information about:

- which issues the VLS patient has experienced in relation to VLS
- which issues experienced in relation to VLS the patient has found most burdensome.

8.6 Safety and other assessments

8.6.1 Physical examination

A complete (i.e., including all organ systems as well as the vulvar region) physical examination is required at Screening 1 and Week 12 (EoT or Early Termination) visits.

An abbreviated physical examination (including the vulvar region as minimum; other organ systems may be included depending on present signs, symptoms, or investigation outcomes) is required at the Baseline visit (and if judged necessary by the investigator) at the FU visit.

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.

8.6.2 Vital signs

Vital signs (Systolic Blood Pressure, Diastolic Blood Pressure, Heart rate and Temperature) will be recorded by trained clinical staff at Baseline and Week 12 (or Early termination) visits. Vital signs are also recorded 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.

8.6.3 Urine pregnancy test

A highly sensitive urine pregnancy test (i.e., with a sensitivity down to at least 25 mIU/ml for human chorionic gonadotrophin (hCG) will be collected from women of childbearing potential (see inclusion criterion 9, Section 5.1) by trained clinical staff at all in-clinic visits and must be recorded in the eCRF. Urine pregnancy test samples will be destroyed after the results of the test have been recorded.

If there is suspicion of pregnancy at any time during the trial, an additional urine sample will be obtained and analysed. Should a participant become pregnant during the trial, treatment must be discontinued, and the patient must be withdrawn from the trial.

All pregnancies (occurring before or after start of IMP administration) should be immediately reported to MC2 or designee and followed through to resolution (i.e., delivery, miscarriage, or elective 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.

8.7 Laboratory assessments

8.7.1 Analysis of laboratory samples

There will be no scheduled sampling for clinical laboratory analyses in this trial.

If there is a suspicion of a medical condition (considered related or not to the IMP), relevant laboratory samples may be taken and analysed at the discretion of the investigator. No samples will be stored for future analysis.

8.8 Adverse events and serious adverse events

8.8.1 Definition of adverse events (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation participant 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, symptom, or disease temporally associated with the use of a medicinal product, whether considered related to the medicinal product or not. Such unfavourable signs, symptoms or diseases may be discovered via any trial assessment (e.g., laboratory findings, PRO questionnaires, physical examination).

8.8.2 Definition of serious adverse events (SAE)

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 important medical 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 participant or the participant may require medical or surgical intervention to prevent one of the outcomes listed in the above definitions.

The death of a participant 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 or procedures that are known at the time of signing the informed consent form will not be recorded as SAEs but will be recorded as AEs only.

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 IB, Section 6.2.12.

8.8.3 Classification of an adverse event

8.8.3.1 Severity of adverse events

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

- Mild – The patient 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 patient. The patient is still able to function.
- Severe – The patient 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.

8.8.3.2 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, therapeutic 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.

8.8.3.3 Outcome of adverse events

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 as a comment in the eCRF)
- Recovering/resolving
- Not recovered/not resolved
- Fatal
- Unknown

8.8.4 Time period and frequency for event assessment and follow-up

8.8.4.1 Adverse event reporting

The investigator or designee (as per site delegation log) is responsible for obtaining, assessing, and documenting all AEs during the trial.

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

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

If the event is reported as an SAE, the timelines for SAE reporting apply (see Section 8.8.4.2). If the event is reported as non-serious AE, it should be reported on the appropriate eCRF page.

Throughout the trial, the occurrence of AEs should be monitored by nondirective questioning of the patient at each visit. Information on AEs can also be obtained from signs and symptoms detected during examinations, observations made by the site staff, or spontaneous reports from patients. 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 patient. Treatment due to an AE will be recorded in the patient's records and on the appropriate eCRF.

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. Unrelated non-serious AEs must be followed by the investigator until the patient's last trial visit. All relevant follow-up information will be reported in the eCRF (and to PharmaLex if it qualifies as an SAE, see Section 8.8.9.1).

8.8.4.2 Serious adverse event reporting

Any SAE, whether by the investigator considered IMP-related or expected or not, 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 8.8.9.1). For each SAE, the investigator will complete a Serious Adverse Event Report Form and assess the relationship of each SAE to trial treatment.

Follow-up report forms regarding the status of the SAE and the participant's subsequent course should be submitted to PharmaLex immediately and not later than 24 hours of follow up information awareness and until the SAE has resolved, is stabilised or needing no further follow-up (in the case of persistent impairment) or the patient dies.

The forms and acknowledgment of receipt will be retained by both the site and PharmaLex. For contact details for reporting SAEs, pregnancies and other safety concerns, refer to Section 8.8.9.1. 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 6.2.12 of the IB, and for which there is evidence to suggest a causal relationship between the IMP and the SAE. The National Competent Authorities (NCA), Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be informed of SUSARs and other safety issues according to local requirements, via Eudravigilance. All investigators participating in the trial will also be notified of SUSARs and other safety issues as applicable.

Investigator instructions for reporting SAEs and contact information are provided in Section 8.8.9.1.

SAEs occurring after a patient has completed the trial and deemed IMP-related by the investigator should be reported to the sponsor within 24 hours from the investigator's awareness.

8.8.5 Reporting events to participants

Not applicable

8.8.6 Events of special interest

Not Applicable

8.8.7 Reporting of pregnancy

Any pregnancy occurring from the date of the informed consent signature until trial completion must be recorded in the eCRF and reported to PharmaLex as soon as possible, but no later than 24 hours from the investigator's awareness of the pregnancy (see Section 8.8.9.1).

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

8.8.8 Trial medication overdose and trial medication errors

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 instigated as judged by the investigator. In view of the high systemic safety margin (see Section 4.2), the administered dose may be several folds higher than the highest dose under clinical investigation without necessarily qualifying as a clinically significant overdose.

A trial medication error is when the trial medication is used outside the dosing regimen specified in the protocol. In the event of a clinically significant trial medication error, as judged by the investigator, the event should be reported as an AE and supportive treatment or discontinuation of the IMP may be instigated as judged by the investigator.

8.8.9 Reporting of serious adverse events and pregnancies

8.8.9.1 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

9 Statistical considerations

9.1 Statistical hypotheses

On the primary and all secondary endpoints, treatment groups will be compared for superiority of MC2-25 cream over MC2-25 vehicle. The null hypothesis is of no difference between the two treatment groups. For further details see Section 9.4.1.

9.2 Sample size determination

Sixty two (62) patients will be screened. 33 patients will be randomised in a 1:1 ratio of MC2-25 cream to MC2-25 vehicle. For a two-sided test with a 5% level of significance and an effect size of 1 standard deviation, this sample size will provide adequate power (≥78%) to compare mean change in mean weekly WI-NRS from Baseline to Week 12 between MC2-25 cream and MC2-25 vehicle with 16 to 17 patients per treatment group.

9.3 Populations for analyses

The analysis populations are defined as follows:

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

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

Full analysis set (FAS): all patients who are randomised. Patients will be analysed according to the randomised treatment.

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

Per-protocol set (PPS): a subset of the MFAS patients 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 patient 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.

9.4 Statistical analyses

9.4.1 General approach

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% significance level unless otherwise specified. Superiority is concluded if a p-value of statistical hypothesis testing is below the significance level and the estimate of treatment difference is in favourable direction for MC2-25 cream. Estimates of treatment effects and differences at timepoints other than Week 12 will be reported exploratively.

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, interquartile ranges.

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

9.4.2 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 summarised 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.

9.4.2.1 Analysis of the primary efficacy endpoint

The primary endpoint, mean change in weekly mean WI-NRS from baseline to Week 12 for MC2-25 cream compared to MC2-25 vehicle, will be analysed by a mixed model of repeated measures (MMRM) including all post-baseline assessments. The model will include the fixed effects of treatment, visit, treatment-by-visit interaction, menopausal status at baseline, ICIQ-UI-SF score at baseline, and weekly mean WI-NRS at baseline. The mean change in WI-NRS at Week 12, as well as the treatment difference will be estimated using LS-means and reported together with the associated standard error, 95% confidence intervals and p-values based on F-statistics.

On the FAS, for the primary analysis assuming missingness at random, missing data will be imputed 20 times and summarised following Rubin’s rule. Following a treatment policy approach, all data will be considered.

Applying the same statistical model, in sensitivity analyses MMRM will be applied to observed cases on the FAS, MFAS and PPS, respectively, without imputation. In a while-on-treatment approach, in another sensitivity analysis on the FAS, only data up to the week of treatment

discontinuation will be considered. As detailed in the SAP, further sensitivity analyses may consider assumption of missingness not at random and other potential intercurrent events.

9.4.2.2 Analysis of the secondary endpoints

Secondary endpoints include mean changes in weekly mean WP-NRS and in Skindex-29 domain scores as well as percentages of participants obtaining a ≥3-point or a ≥4-point improvement in in weekly mean WI-NRS and weekly mean WP-NRS, respectively.

Mean changes in weekly mean WP-NRS and in Skindex-29 domain scores on the FAS will be analysed as described in Section 9.4.2.1 for primary and sensitivity analyses on mean changes in weekly mean WI-NRS. On the FAS, percentages of participants obtaining a ≥3-point or a ≥4-point improvement in weekly mean WI-NRS and weekly mean WP-NRS will be analysed by means of logistic regression adjusting for respective NRS scores at baseline. If the endpoint is missing, no improvement is assumed.

9.4.3 Safety analyses

9.4.3.1 Safety analyses – General

On the SAS, safety will be analysed by means of incidences of AEs, changes in vital signs and physical examination.

9.4.3.2 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 patient will contribute only once (e.g., the first occurrence) to each of the rates, regardless of the number of occurrences (events) the patient 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, patients who report more than one event that are mapped to the same preferred term will be counted only once under the strongest severity and last 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.

9.4.4 Baseline descriptive statistics

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, regarding demographic and other baseline characteristics. Demographics include age, sex, race, and ethnicity (see Section 8.2). Baseline characteristics include medical history, and relevant history/details of VLS related information

(VLS history, VLS patient journey, VLS treatment history, and NRS and other scores at screening and/or baseline).

9.4.5 Planned interim analyses

The current trial is a phase 2 trial and is powered to detect statistical significance on the primary endpoint and to inform further clinical development. The expected recruitment period is relatively short, and the double-blind treatment period is only 12 weeks. Thus, no interim analysis is planned.

9.4.6 Sub-group analyses

In descriptive summary tables, results on the primary endpoints will be displayed per site and per country. If deemed appropriate, sub-group analyses may be conducted as detailed in the SAP.

9.4.7 Tabulation of individual participant data

9.4.7.1 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.

9.4.7.2 Prior and concomitant therapies

For the SAS, prior and concomitant therapies (as defined in Section 6.5) will be summarised by treatment. Separate summaries will be given for prior therapies, for prior therapies ongoing at baseline, and for concomitant therapies.

9.4.7.3 Further tabulation of individual participant data

As collected and derived, all individual participant data will be listed in individual participant data listings.

9.4.8 Exploratory analyses

Exploratory endpoints include complete responders, WI-NRS, WP-NRS and WPP-NRS recorded at in-clinic visits, PGIC, Importance of improvements in PGIC, VQLI total score, VASSI, CGA, CGIC and burden of living with VLS.

Exploratory endpoints will be described in summary tables as observed in the FAS. If deemed appropriate, statistical hypothesis testing may be conducted on exploratory endpoints to compare treatment groups as detailed in the SAP.

10 Supporting documentation and operational considerations

10.1 Regulatory, ethical, and trial oversight considerations

10.1.1 Ethical considerations and conduct of the trial

There are no marketed products approved for the treatment of VLS. Therefore, patients will not miss out on approved treatment by participation in this trial. VLS has a significant impact on quality of life and therefore off-label treatments are not uncommon. Current UK and European guidelines are based on potent to ultrapotent topical corticosteroids. To limit the risk of VLS deterioration during the trial, the patients are allowed to continue existing stable systemic treatments with a known or potential effect on VLS. Furthermore, patients are free to withdraw from the trial or discontinue treatment in case of unacceptable adverse events or lack of improvement.

The active ingredient included in the MC2-25 cream is approved for use in an already marketed product (Dipeptiven®), which is given intravenously in considerably higher concentration than used in this trial. There are no known undesirable effects for this marketed product when used as prescribed. 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). Furthermore, the excipients used in MC2-25 cream are known from topical use in other approved drug products. Therefore, the risk of local side effects on the skin is considered low in this trial.

There is no blood drawing or other procedures considered to introduce risks involved in the trial.

Women of childbearing potential not using adequate contraception are excluded from the trial to avoid potential risks related to pregnancy or breastfeeding.

The use of MC2-25 cream during the trial may improve VLS in individual participants. However, the effectiveness of the MC2-25 cream has not yet been proven and half of the participants will receive the vehicle cream. This might result in deterioration of VLS. It should be noted however, that MC2-25 vehicle has emollient properties and as such is not strictly considered a placebo.

In view of the major impact of VLS on quality of life, the benign risk profile of MC2-25 cream, and MC2-25 vehicle are considered outweighed by the potential benefits of MC2-25 cream. The results of the trial may, if they are favourable, help bring a new treatment for VLS on the market in the future. In conclusion, based on the above considerations conduct of the trial is considered ethical.

This trial must be carried out in compliance with the protocol, with regulation (EU) No 536/2014, and with 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 and applicable ICH-GCP guidelines.

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

This protocol, the proposed informed consent form, and other information for patients 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.

10.1.3 Informed consent process

Before participation in the trial, each patient is required to provide written consent to participate in the trial. Each patient will receive oral and written information of the trial. The patient should be given every opportunity to ask for clarification of any point she does not understand and, if necessary, ask for more information. At the end of the interview, the patient should be given all the time she needs to consider the trial. No trial-specific procedures will be performed before a patient's informed consent is obtained.

10.1.4 Trial completion, discontinuation, and closure

The trial will be considered completed when the last patient 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 patients 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 organisation(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 patients and should assure appropriate patient therapy and/or follow-up.

10.1.5 Confidentiality and privacy

10.1.5.1 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 patients who wish to participate in the trial.

10.1.5.2 Privacy of individual health information

The investigator, site staff, sponsor and any third parties working for sponsor and involved in the clinical trial will undertake to protect the privacy of all individually identifiable health information and personalised information ("Personal Data") as per the applicable data protection rules in each of the countries, where the trial is being conducted. All Personal Data will be processed in accordance with the appropriate legal basis as described in the Informed Consent document. While all data records will be identified by the corresponding patient number, the identity of the patient will be held in confidential source documents at the trial site. Any Personal Data will be pseudonymised before leaving the sites and will be encrypted while at transfer, and appropriately protected at rest. All site staff, monitors, auditors and health authorities with access to this information are legally or contractually bound not to disclose such information. The sponsor and all involved parties will comply with the respective data protection rules.

Data handling and record keeping will be performed in compliance with the applicable data protection rules.

All parties involved in this clinical trial will be obliged by law, as well as by contractual arrangements to process any Personal Data in compliance with the EU GDPR and all other related laws and regulations. The parties involved undertake to instruct and supervise their employees regarding the requirements and compliance with EU GDPR and with all related laws and regulations.

The Sponsor will put in place and make sure that anyone involved in the clinical trial puts in place appropriate technical and organisational measures such as:

(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 in the clinical trial deviates or observes a deviation from the agreed security measures, a notification will be promptly sent to the sponsor's data protection responsible, and the sponsor will assess whether a personal data security breach has occurred and act accordingly.

10.1.5.3 Patient involvement in the design of the clinical trial

Two VLS patients (facilitated by the Danish lichen sclerosus association) have reviewed and provided comments to the protocol before finalisation.

10.1.6 Future use of stored specimens and data

There is no scheduled sampling for clinical laboratory analyses in this trial. No samples or specimens will be stored for future analysis.

10.1.7 Safety oversight

In view of the benign nature of the potential risks (see Section 2.3.3) a Data Safety Monitoring Board is not implemented in the trial.

10.1.8 Quality assurance and quality control

10.1.8.1 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.

10.1.8.2 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. 100% SDV is selected for this trial. The investigator will cooperate with the trial monitor to ensure that any discrepancies that may be identified are resolved.

10.1.8.3 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 patients 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.

10.1.9 Data handling and record keeping

10.1.9.1 Recording of data

Data handling and record keeping will be performed in compliance with any applicable law including applicable data protection rules. Personal data will be handled in accordance with chapter V of the of the General data Protection Rules (GDPR).

10.1.9.2 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 patients.

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 permitted by local regulations (see Section 10.1.9.4). For each patient screened, the investigator will indicate in the source record(s) that the patient participated in the trial.

10.1.9.3 Case report forms

The primary data collection tool for the trial is an eCRF designed specifically for the trial. For each patient screened in the trial, an eCRF will be completed by the site staff and signed by the investigator or his/her designate. Any 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 staff.

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

Any transfer of personal data to third countries will be in accordance with Chapter V of the General Data Protection Regulation (GDPR)

10.1.9.4 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).

10.1.10 Protocol deviations

Protocol deviations will be reported. Important deviations related to study inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be reported in the CSR. as per the Clinical Study Reporting Guideline (CPMP/ICH/137/95).

10.1.11 Publication and data sharing policy

The clinical trial will be registered, and results will be posted at least on https://euclinicaltrials.eu and 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.

10.1.12 Conflict of interest policy

The investigators should provide a curriculum vitae or equivalent documentation of suitability to be responsible for the trial, including valid GCP training, documentation of current licensure (as applicable), and should sign a financial disclosure on conflict of interests.

10.2 Additional considerations

10.2.1 Emergency unblinding

Patient safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator may contact the sponsor prior to unblinding a patient's intervention assignment unless this could delay emergency treatment of the patient. Emergency unblinding envelopes will be available at all clinical trial sites. If a patient'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. Emergency unblinding envelopes will also be available at the external pharmacovigilance provider (Pharmalex) responsible for handling of SAEs and reporting of SUSARs.

10.3 Insurance

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

10.4 Protocol amendments

All substantial changes 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 patients 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.

Signature page for principal investigator

Investigational Medicinal Product: MC2-25 cream

Protocol number: MC2-25-C3

Protocol title: A parallel group (2-arm), randomised, double-blind, 12-week trial to explore the efficacy and safety of MC2-25 cream and MC2-25 vehicle in women diagnosed with vulvar lichen sclerosus (VLS)

The signature of the principal 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 Institutional Review Board and/or Independent Ethics Committee.

Principal Investigator's printed name

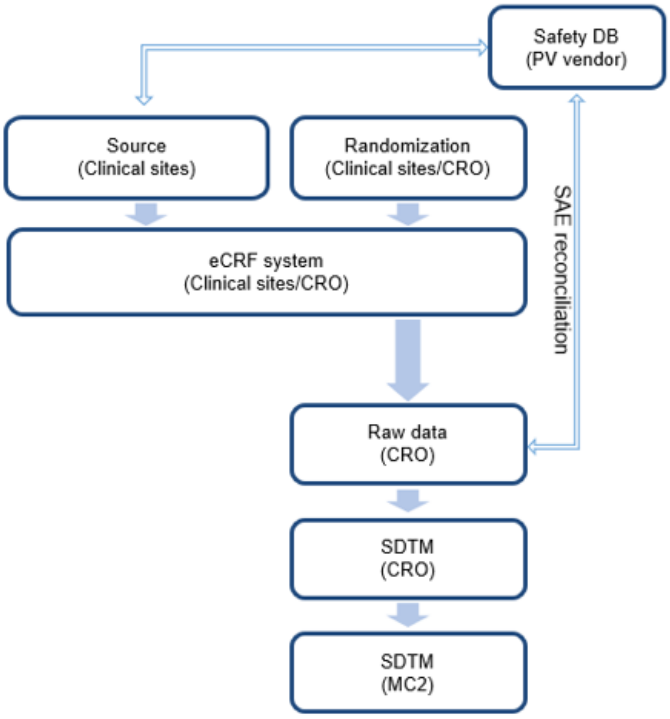
Investigational Site Address

Principal Investigator's signature

Date (dd-mmm-yyyy)

Appendices

Appendix 1 Overall data flow of the clinical data



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