CLINICAL STUDY PROTOCOL

A phase 2 randomised controlled trial of a NOVel moisturiser for Atopic dermatitis: effect on the skin barrier in adults with a predisposition to a skin barrier defect

Short Title:	A **NOV**el moisturiser for **A**topic dermatitis: effect on the skin barrier
NCT number:	NCT03901144
Date:	2019-08-15

CONFIDENTIAL

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Short Title:	A NOVel moisturiser for Atopic dermatitis: effect on the skin barrier		
Acronym:	NOVA – Skin Barrier	IRAS number:	251853
Study code:	Novum ACO-CT-2018-01	Study development phase:	Phase 2
Amendment:	Novum ACO-CT-2018-01-V3(Amendment #2)	Indication:	Skin barrier strengthening
EudraCT number:	2018-002945-12	Investigational product:	Miniderm Novum
Version:	3.0	Date:	2019-08-15

Principal Investigator: Professor Michael J Cork and Dr Simon Danby (Co-PI)

Sponsor Signatory: Anneleen Spooren, PhD

1 SYNOPSIS

Name of the Sponsor/Company: ACO Hud Nordic AB		**Study Code:** Novum ACO-CT-2018-01
Name of Investigational Product: Miniderm Novum		**EudraCT No.:** 2018-002945-12
Development Phase of the Study: Phase 2		**Trial under an IND:** Yes
TITLE OF THE STUDY: A phase 2 randomised controlled trial of a NOVel moisturiser for Atopic dermatitis: effect on the skin barrier in adults with a predisposition to a skin barrier defect		
	OBJECTIVES:	**OUTCOME MEASURES:**
Primary	To determine whether applying a new moisturizing cream (the test cream) for 4 weeks is superior in terms of skin barrier strengthening, when compared with (1) no treatment and (2) two reference creams in adults with a predisposition to a skin barrier defect.	1. Trans Epidermal Water Loss (TEWL) after induction of skin irritation 2. Redness after induction of skin irritation
Secondary	To determine whether there is a difference between the new moisturising cream (the test cream) and (1) no treatment and (2) the two reference creams in: 1. Resting skin barrier function 2. Skin moisturisation 3. Tolerability 4. Cream consumption 5. Safety	1. TEWL after 4 weeks treatment 2. Skin moisturisation: a. Capacitance measurements b. Skin surface dryness (3D skin images) c. NMF levels 3. Tolerability properties: a. Participant tolerability scores by VAS b. Investigator visual scores for redness c. Objective erythema from 2D colour skin images 4. Cream consumption (g) 5. Number of adverse events
Tertiary	To investigate the number of participants with FLG loss-of-function mutations and explore if there is any evidence of a	1. Number of FLG loss-of-function mutation carriers 2. Descriptive tabulations of TEWL by mutation status, if sufficient participants with mutation

	relationship to treatment effects	are detected.

TRIAL CONFIGURATION:
A randomised, controlled, bilateral single centre trial.

SETTING:

Participants will be recruited from the general population by open advertisement and all trial related activities will be performed in the Skin Barrier Research Facility, operated by Sheffield Dermatology Research, based in the Royal Hallamshire Hospital, Sheffield.

OVERALL STUDY DESIGN:

Consenting volunteers with atopic dermatitis will be screened against the inclusion/exclusion criteria. Figure 1 and Table 1 illustrate the overall study design and the study flow-chart. Enrolled participants will be randomised to treat the lower or upper parts of the lower volar forearms (i.e. 4 treatment areas) with the new active emulsion (Miniderm Novum), the reference cream Miniderm 20% cream or the reference cream Diprobase cream. One area is left untreated as control. One Finger Tip Unit (FTU) of the creams will be applied twice daily for 28 (± 3) days. On day 1 and 29 (± 3) the transepidermal water loss (TEWL) and skin capacitance is measured on their forearms to evaluate the effect on skin barrier function and skin hydration. Furthermore, on day 31 (± 3), after challenge with 1 % sodium lauryl sulphate (SLS) on day 29 (± 3), the susceptibility to irritation caused by SLS will be evaluated visually and by measuring TEWL on their forearms. Study participants will attend visits at the start of randomised therapy and on day 5 (± 2), 15 (± 3), 29 (± 3) and 31 (± 3). During the study period the participants will also grade and evaluate the tolerability of the different creams.

INVESTIGATIONAL PRODUCTS (dose, route, administration)	**Test product**	• Miniderm Novum cream (investigational product), 1 FTU twice daily to the previously randomised volar forearm for 28 (± 3) days
	Comparator	• Miniderm 20% cream (reference product), 1 FTU twice daily to the the previously randomised volar forearm for 28 (± 3) days
		• Diprobase cream (reference product), 1 FTU twice daily to the the previously randomised volar forearm for 28 (± 3) days

DURATION OF TRIAL:
31 (± 3) days - the primary end point of the trial will be measured on day 31.

NUMBER OF PARTICIPANTS and statistical evaluation:

50 participants will be recruited into the study. This sample size is sufficient to provide more than 90% power to detect a difference (test treatment vs no treatment) of 3.5 in change in TEWL for the primary endpoint, if the data is suitable for a parametric analysis. If the observed data is not suitable for a parametric analysis then the sample size would provide greater than 80% power in a non parametric analysis.

INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria:

The participants have to meet all of the following criteria to be eligible to enter the study:

- Willing and able to provide informed consent
- Male or female and aged 18 years or above

- Volunteers able to read and understand English
- A personal history of atopic dermatitis

Exclusion criteria:

Participants meeting any of the following criteria will not be permitted to enter the study:

- Eczema on the volar forearms requiring anti-inflammatory treatment
- Possible allergy to ingredients in the study medications.
- Any serious current medical condition which, in the opinion of the Investigator, may interfere with the evaluation of the results or may be contraindicated by the use of the test medications
- Use of any concomitant medication that may interfere with the study related activities or assessment of efficacy, as judged by the Investigator
- Use of any topical product, including cosmetic leave-on products on the volar forearms, within 1 week prior to, and throughout the study
- Female participant who, according to the participant, is pregnant or breast-feeding, or plans to become pregnant during the course of the study
- Any participant-related factor suggesting potential poor compliance with study procedures (e.g. psychiatric disorders, history of alcohol or substance abuse), as judged by the Investigator
- Enrolment in any interventional study or use of an investigational drug within 3 months prior to the screening visit
- Volunteers judged by the PI to be inappropriate for the trial.

STATISTICAL METHODS:

The primary analysis will compare the test treatment to no treatment and to the two reference creams in terms of change in TEWL and redness between days 31 and 29 (after and before the induction of skin irritation).

These analyses will be carried out on the FAS and PPS.

Full details of the analyses to be carried out will be detailed in the SAP and will take account of both the distribution of the observed data and the matched nature of the data (4 treatment areas per subject). Where possible and appropriate the baseline value will be included as a covariate. The secondary analyses will be carried out on the FAS and will follow the same analysis plan as the primary analysis where baseline data is available (TEWL (baseline to day 19); skin moisturisation as measured by capacitance and skin surface dryness; tolerability properties as measured by investigator visual scores for redness and objective erythema from 2D colour skin images).

Skin moisturisation as measured by NMF levels and patient tolerability scores will not be recorded prior to treatment and so will be summarised by timepoint and the treatments compared using an appropriate analysis, taking account of the distribution of the data and the ‘paired’ nature of the observations.

STUDY PERIOD:

First participant first visit is expected to be in Febuary 2019.

Last participant last visit is expected to be in April 2019.

Figure 1 Trial flow chart

Table 1 Schedule of events

	Screening[a]	Visit 1 Baseline[a,b]	Visit 2 5 days[b] (+/- 2 days)	Visit 3 15 days[b] (+/- 3 days)	Visit 4 29 days[b] (+/- 3 days)	30 days[c] (+/-3 days)	Visit 5 31 days[b] (+/-3 days)
Screening & Enrolment:							
Informed consent	X						
Confirm eligibility	X						
Demographic data	X						
Medical history	X						
Screening (& admission)	X						
Randomisation		X			X		
Reconfirm ascent		X	X	X	X		X
Concomitant medication	X	X	X	X	X		X
Adverse events	X	X	X	X	X		X
Skin assessments:							
Acclimatize test sites		X			X		X
TEWL measurement		X			X		X[d]
Capacitance measurement		X			X		
Image skin (2D – colour)		X		X	X		X[d]
Objective redness measurement					X		X[d]
Visual grading of redness					X		X[d]
Image skin (3D - roughness)		X			X		
Saliva sample collection		X[e]					
NMF sample collection					X		
SLS application					X		
SLS removal						X	
Measurement of SLS-exposed skin (TEWL and redness[d])							X[d]
Treatment:							
Dispensation of investigative products and participant diary		X					
Instructions to participants		X					
Supervised product application		X	X	X			
Review of participant diary and weigh treatments		X	X	X	X		
Collection of participant diary					X		
Collection of investigational products					X		

[a] The screening and baseline visit may be combined providing sufficient time is given to allow parents to properly consider the study.

[b] Face-to-face meeting

[c] Participants removes SLS-patch after 24 hours and rinses with lukewarm water.

[d] Measurement of skin irritation only

[e] Saliva sample collection can be made at any visit

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3 LIST OF ABBREVIATIONS

AD	Atopic Dermatitis
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CRF	Case Report Form
CRO	Contract Research Organisation
FAS	Full Analysis Set
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
NMF	Natural Moisturising Factor
PI	Principal Investigator
PIS	Patient Information Sheet
PPS	Per Protocol Set
QoL	Quality of Life
PPS	Per-Protocol Set
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SLS	Sodium Lauryl Sulfate
SOP	Standard Operating Procedure
TEWL	Transepidermal Water Loss

4 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

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5 INTRODUCTION

5.1 Disease Background

Atopic dermatitis (AD) is among the most common chronic types of inflammatory skin disease. AD is a relapsing and remitting disease characterised by exacerbations or relapses over years. The symptoms, e.g. pruritus, erythema, scaling, vesiculation, weeping, crusting, and lichenification have considerable impact on the patient's quality of life (QoL) (1). The disease predominantly affects children (2).

AD is a multifactorial disease that is influenced by inheritance as well as the environment. The patients have a genetically impaired stratum corneum barrier (3). The skin barrier integrity can be evaluated by measuring the transepidermal water loss (TEWL), which is increased in both dry skin and clinically normal skin in AD patients (4, 5). A major predisposing factor for the disease is mutations in the filaggrin gene (6-8).

Increased TEWL has been suggested to induce pathological effects by over-stimulating cytokines in the skin. The immunological response may result in flare-ups; red, scaly, itching, and inflamed patches (9, 10). Elevation of TEWL may therefore be regarded as a risk factor for developing flare-ups/eczema. Consequently the improvement of the defect barrier function is likely to prevent persistent dermatitis by mitigating the cytokine cascade (10).

5.2 Standard Treatment

Moisturising creams are the most prescribed products in dermatology and the market is still increasing. The use of moisturisers in AD is almost instinctive and is emphasised by healthcare personnel, also when the eczema is cleared (11). Moisturiser therapy is suggested to prolong the clinical improvement after discontinuation with anti-inflammatory treatment (12) and to enhance degree of clearance of the eczema in AD (13, 14) i.e. moisturizers were found to be steroid-sparing (15).

A reduced risk of AD relapse may be expected by strengthening of an impaired barrier function, since an abnormal stratum corneum has been suggested to be the primary exacerbant of inflammatory skin diseases. However, differences among moisturisers have been noted and three different patterns are noted in skin barrier disorders:

1. The elevated TEWL level may remain unchanged (i.e. no influence of the barrier) (16, 17)
2. TEWL could increase further (i.e. weakening of the barrier) (18, 19)
3. TEWL could decrease towards normal values (i.e. improvement of the barrier) (20)

Therefore, the benefit of a general use of moisturisers has been questioned (21) and finding the most suitable product for an individual is currently a matter of trial and error. Furthermore, when applying creams on impaired skin, the patients may experience local side effects like stinging and burning sensations (22, 23). These side effects may negatively affect compliance to treatment. Hence, the influence on moisturisers is far from elucidated and more clinical studies are considered indispensable.

The influence on moisturisers is far from elucidated and more clinical studies are considered necessary to investigate the influence of the skin barrier function.

The investigational product is an oil-in-water emulsion with two active ingredients, urea (carbamide) at 2 % and glycerol (glycerine) at 20 %. In addition, it consists of a mixture of oils and waxes that have a documented beneficial emollient effect on the skin including the hydrogenated canola oil.

Several studies have shown that urea-containing creams relieve clinical signs of dryness (24, 25). Previous studies have shown that application of an emulsion with 5% urea improves skin barrier function to water, (i.e. reduces TEWL) (26-29) on atopic skin as well as normal skin and reduces skin susceptibility to surfactant-induced irritation (20, 29).

These findings suggest a reduced risk for the recurrence of AD by daily treatment with a moisturiser. This was later confirmed in two clinical trials:

- Treatment with 5% urea emulsion in patients with clear/almost clear AD resulted in significantly longer time to relapse vs untreated control group (30)
- Treatment with 5% urea emulsion in patients with clear/almost clear resulted in significantly lower risk of relapse and significantly longer time to relapse vs reference cream with no humectants (31)

Moreover, the 5% urea emulsion was well-tolerated in patients with atopic dermatitis. All patients in this relapse prevention study was above 18 years old.

Several studies on glycerol-containing creams suggest that the creams increase skin hydration (32, 33) and relieve clinical signs of dryness (24). Furthermore, a recent systematic Cochrane meta-analysis concluded that there is moderate to high quality evidence that glycerol-containing moisturizers are more effective than ’vehicle’ or placebo (34).

5.3 Miniderm Novum

The investigational product is an oil-in-water emulsion with two active ingredients, urea (carbamide) at 2 % and glycerol (glycerine) at 20 %, giving the product water binding properties.

5.4 Study Rationale

The product is intended for people with dry skin symptoms, such as dryness (xerosis), itching (pruritus), and flaking in people with dry skin of different origin, e.g.: atopic dermatitis, contact dermatitis and psoriasis. The target population is infants and children but may also be a treatment option for adults, when relevant.

As most people with dry skin of different origin have an impaired skin barrier function, it is important to investigate the possible influence on the skin barrier after long-term (several weeks’) treatment.

This study is the first step in a sequence of studies investigating the effect of the newly developed moisturising emulsion Miniderm Novum. The primary objective of this study is to investigate the skin barrier strengthening effect and the tolerability of Miniderm Novum in adults, before continuing to investigate the potential of Miniderm Novum in children. Clinical trials

following the trial presented in this protocol will be performed on children (relapse prevention study) and high-risk infants (primary prevention study).

The comparator creams are Minderm 20% cream and Diprobase cream. Miniderm 20% cream contains commonly used cream ingredients and the humectant glycerol. It has been shown to neither improve nor impair the skin barrier function in patients with AD (35). The reference product Diprobase cream contains commonly used cream ingredients (cetomacrogol, cetostearyl alcohol, liquid paraffin, white soft paraffin), but no humectant. It was used in the pilot study by Simpson et al (36) showing that emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention, and is one of the creams in the ongoing NIHR Health Technology Assessment (HTA) Barrier Enhancement Eczema Prevention (BEEP) study (https://www.nottingham.ac.uk/research/groups/cebd/projects/1eczema/beep-maintrial.aspx).

It is of utmost importance to evaluate different moisturisers head-to-head in order to facilitate an evidence-based choice of moisturiser.

5.5 Potential Risks and Benefits

The overall risks for the participants participating in the study are considered to be negligible. The potential benefits come from the development of an emollient with skin barrier strengthening effect but reduced risk of stinging sensation that might impact compliance.

5.5.1 The potential risk associated with the intervention

Adverse effects commonly reported after treatment with moisturising products are local sensations of burning and heat. It is well known that topical preparations may cause transient disagreeable sensations immediately after application.

The study involves the repeated use of a topical skin care product that is not currently marketed. The product has been formulated using only ingredients already regarded as safe for topical use in humans. The formulation of the test product has also been reviewed independently for safety and is considered safe for human testing. The actives, urea and glycerol, have a long history of use in emollients and the reported use concentrations in cosmetics are up to 10% and 79%, respectively. The concentration of urea and glycerol in Miniderm Novum is 2% and 20%, respectively. All ingredients, except two, have a long history of safe use and are used in other licensed medicinal emollients marketed by ACO Hud Nordic on the Nordic market, i.e. Canoderm, Miniderm, Hyderm and Fenuril. The two new ingredients are panthenol and triacetin. Panthenol is added for its soothing function and it is commonly used in emollients targeted for children, i.e. Bepanthen. Triacetin is added to stabilize the urea and it is only present at 1%. Both ingredients have been evaluated in the *Investigator's Brochure* and are regarded as safe to use. Whilst the risk is therefore minimal there is always a chance that someone may experience an adverse reaction to a topically applied product. The risk of this occurring will be minimized by excluding individuals who have previously reacted to products containing any of the ingredients in the test products (or similar complete formulations). The first product application will be made by the research team on site so that they can monitor any adverse skin changes. A Dermatologist is on-call throughout the study to treat any serious adverse reactions.

The study team includes medically qualified clinical researchers, who will be available throughout the trial. Due to the low risk of the intervention and the mechanistic design of the study involving volunteers from the general population (participants are not selected based upon their use of NHS services) the PI may delegate activities, including informed consent and

medical history taking (facilitated using the medical history form), to non-medically qualified and otherwise appropriately trained researchers. Because not all participants will be undergoing treatment for their eczema, and because the intervention is not herein being used therapeutically, GP's will not ordinarily be contacted about the study unless the PI determines they need to be made aware of an adverse event. For this reason, GP contact details will be collected upon enrollment.

Risk category	Justification
Type A: No higher risk than the risk of standard medical care	The intervention comprises active substances (urea at 2 % and glycerol at 20 %) part of other medicinal products licenced in the EU. Reduction in risk from Type B is justified based upon the extensive clinical and cosmetic use of the product ingredients and there is no reason to suspect a different safety profile in this study population

5.5.2 The potential for physical and/or psychological harm to participants

This study involves a series of harmless procedures to test the properties of the skin. All types of procedures to test the skin are non-invasive and have been used safely in previous clinical studies, and so the risks of harm are minimal.

Patch testing is performed using the standard skin irritant sodium lauryl sulfate (SLS). This is done to assess the sensitivity of the skin, and a mild irritant reaction is expected to evolve that will pass in a few days. The level of reaction, ranging from barely perceptible to prominent redness, varies between individuals. The test is routine in dermatology clinics. SLS is a common ingredient in cosmetic and household wash products such as shampoo and toothpaste. The patched site may become dry and require the use of a moisturizer.

The risk of psychological distress is also low. Our questions are limited to the collection of basic demographic information, information about the participants skin history and details of any medical conditions and current treatments for safety monitoring purposes. We have limited the test sites to the forearms and clearly specify the test sites in the participant information. If at any point a participant becomes uncomfortable or distressed (however unlikely) we will cease procedures immediately and will only resume the study with the participant's assent once they are comfortable/relaxed (which may be at a later date if possible within the bounds of the study). We will not enroll/continue to include participants who remain visibly uncomfortable or distressed.

5.5.3 The steps taken to avoid physical and/or psychological harm to participants

The following steps will be taken to protect participants from physical and/or psychological harm:

- Informed consent will be performed, and we will make it clear to all participants that participation is entirely voluntary and that they are free to withdraw at any time.

- Participants will be notified in advance of the anatomical locations we are interested in (forearms only) and encouraged to wear appropriate clothing.
- Experienced members of staff who have received appropriate training will conduct all of the test procedures.
- The equipment regularly undergoes biomedical engineering testing for safety.
- Individual procedures will only be performed with participant assent (in addition to consent), and where participants feel discomfort we will cease the procedures and offer a break – we would then only continue with assent.

6 STUDY OBJECTIVES

6.1 Primary Objective

The primary objective is to determine whether applying a new moisturizing cream (the test cream) for 4 weeks is superior in terms of skin barrier strengthening, when compared with (1) no treatment and (2) two reference creams in adults with a predisposition to a skin barrier defect.

- TEWL after induction of skin irritation
- Redness after induction of skin irritation

The hypotheses for the study are provided below in order of importance:

a) Skin irritation, indicated by elevated TEWL and redness, will be reduced following treatment with the test cream compared to the untreated control.

b) Skin irritation, indicated by elevated TEWL and redness, will be reduced following treatment with the test cream compared to the reference creams, Miniderm 20% cream and Diprobase cream.

6.2 Secondary Objectives

Secondary objectives: To determine whether there is a difference between the new moisturising cream (the test cream) and (1) no treatment and (2) the two reference creams in:

- Resting skin barrier function
- Skin moisturisation
- Tolerability
- Cream consumption
- Safety

7 STUDY DESIGN

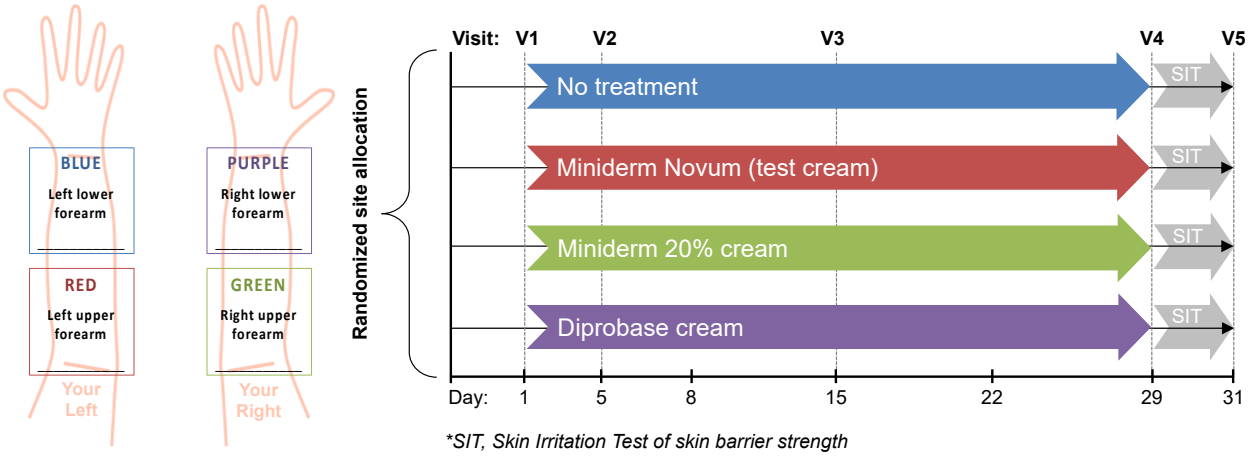
7.1 Study Design and Plan-Description

A prospective phase 2 within-subject multilateral randomised controlled trial to determine the superiority of the test treatment at strengthening the skin barrier compared to no treatment and two reference treatments in adults with a predisposition to a skin barrier defect.

Participants will treat their lower volar forearms for 28 days with three different creams (Miniderm Novum (test cream), Miniderm 20% cream or Diprobase cream) and leave one area untreated as a control. Each forearm will have two different treatment areas and treatment allocation will be randomized (right arm upper, right arm lower, left arm upper, left arm lower). One Finger Tip Unit (FTU) of each cream will be applied twice daily on the designated study area. As such there will be four treatment conditions (with each participant undertaking all 4 treatment conditions):

1. No treatment (control),
2. Miniderm Novum (test cream),
3. Miniderm 20% cream or
4. Diprobase cream

Study overview:



**SIT, Skin Irritation Test of skin barrier strength*

The participants will record details of cream application frequency, as well as participant tolerability in a diary. At each study visit, concomitant medication and any AEs are noted in the Case Report Form (CRF). The study medication will be collected and weighed in order to estimate cream consumption.

Participants will attend 5 clinic visits during the study:

- Visit 1 (screening) will be performed at baseline
- Visit 2 will be performed after 5 days of treatment

- Visit 3 will be performed after 15 days of treatment
- Visit 4 will be performed after 29 days of treatment
- Visit 5 will be performed 1 day after SLS-patch removal (Day 31 after start of treatment)

The study design is visualised in **Figure 2**.

Figure 2 Overall Study Design

7.2 Trial Setting

This single-centre trial will be conducted The Sheffield Dermatology Research Clinical Research Facility.

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The Royal Hallamshire Hospital, B-Road,

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Email: s.danby@sheffield.ac.uk, dermatologyresearch@sheffield.ac.uk

7.3 Recruitment Period

The expected recruitment period is 3 months. This is calculated to be sufficient to include 50 participants with a mean accrual rate of 3.3 participants per week.

7.4 Study Period

First participant first visit is expected to be in February 2019.

Last participant last visit is expected to be in April 2019.

7.5 End of Study

The end of study is defined as the date of the last participant's last visit.

8 INVESTIGATIONAL PLAN

8.1.1 Schedule of events

The schedule of events for the study is shown in **Error! Reference source not found.**.

Table 2 Schedule of events

	Screening[a]	Visit 1 Baseline[a,b]	Visit 2 5 days[b] (+/- 2 days)	Visit 3 15 days[b] (+/- 3 days)	Visit 4 29 days[b] (+/- 3 days)	30 days[c] (+/-3 days)	Visit 5 31 days[b] (+/-3 days)
Screening & Enrolment:							
Informed consent	X						
Confirm eligibility	X						
Demographic data	X						
Medical history	X						
Screening (& admission)	X						
Randomisation		X			X		
Reconfirm ascent		X	X	X	X		X
Concomitant medication	X	X	X	X	X		X
Adverse events	X	X	X	X	X		X
Skin assessments:							
Acclimatize test sites		X			X		X
TEWL measurement		X			X		X[d]
Capacitance measurement		X			X		
Image skin (2D – colour)		X		X	X		X[d]
Objective redness measurement					X		X[d]
Visual grading of redness					X		X[d]
Image skin (3D - roughness)		X			X		
Saliva sample collection		X					
NMF sample collection					X		
SLS application					X		
SLS removal						X	
Measurement of SLS-exposed skin (TEWL and redness[d])							X[d]
Treatment:							
Dispensation of investigative products and participant diary		X					
Instructions to participants		X					
Supervised product application		X	X	X	X		
Review of participant diary and weigh treatments		X	X	X	X		
Collection of participant diary					X		
Collection of investigational products					X		

[a] The screening and baseline visit may be combined providing sufficient time is given to allow parents to properly consider the study.

[b] Face-to-face meeting

[c] Participants removes SLS-patch after 24 hours and rinses with lukewarm water.

[d] Measurement of skin irritation only

8.1.2 Recruitment

Recruitment will be conducted in several ways:

1. General advertisement using posters (see attached *PosterAdvert* and *AltMediaAdvert*). The poster contains telephone and email contact details for the study team. Posters (see attached) will be displayed at:
    a. The University of Sheffield premises, including the Medical School
    b. Sheffield Hallam University (SHU) premises, with permission
    c. Online, on the University of Sheffield website, SHU website (student portal), Facebook, Twitter, including @Shef_Derm Twitter pages
2. Email lists. An email, containing the information in the **Advert* (and a copy of the *Participant Information sheet, PIS*), will be distributed to:
    a. an email list of people who have either expressed an interest in or have taken part in our research projects previously (where consent has been provided to do this)
    b. staff and students at the University of Sheffield, and SHU using the appropriate moderated email lists at each institution.

The study office will handle all expressions of interest. Participants responding to our adverts/letters by phone/email will be asked to complete a registration form/expression of interest form (this may be done by phone/email/post).

8.1.3 Informed Consent

People responding to study advertisements will be provided with a *Participant Information Sheet (PIS)* and a study *Registration Form*.

A member of the study team will then contact interested volunteers to arrange a screening visit at the test centre. While it is preferable to conduct the screening visit in person, it can be conducted by telephone at the participants request.

A screening number will be assigned using the *SDR Subject Screening Log* at the point of first contact to record approaches.

At the start of the screening visit (at least 24 hours after provision of the PIS), a study researcher (as delegated by the PI), trained in informed consent taking, will conduct the informed consent process. All participants will be provided with a *Consent Form* to sign, of which they will be provided a copy to keep.

Following Informed Consent, we will complete the study *Registration Form* (if not already complete), the *Background Information Form* (includes medical history), together with the *NESS Body Map form*, and determine whether the participant meets the inclusion/exclusion criteria detailed on the *Admission Form.*

Upon successful screening, all study appointments will be arranged such that participants have undergone a 1-week wash-out period for any topical products (excludes wash products) they may use on the skin of their test sites.

A screening number will be assigned using the *Screening, Enrolment and Completion Log* at the point of first contact to record approaches.

The Enrolment Log will be completed and all enrolled participants assigned a unique study ID.

Note for remote screening sessions: Where the screening is conducted by telephone the participants will be notified of the inclusion and exclusion criteria and asked to confirm whether they think they meet the criteria or not without further discussion or collection of the participants medical history. This will help minimise the number of volunteers who are invited to the test site who are then found to be unsuitable upon completion of screening, whilst also protecting the participants privacy. Under these circumstances Consent, Screening, and completion of the *Background Information Form* will need to be undertaken at the start of Visit 1.

8.1.4 Study Visits

The study assessments described in the sections below are presented in detail in Section 11.2 (efficacy assessments), Section 11.3 (demographic data and baseline characteristics) and Section 11.4 (safety assessments). Recording and reporting of AEs are described in detail in Section 12.

The timing of all study events is shown in **Error! Reference source not found.** in Section 8.1.1.

8.1.4.1 Visit 1 (Screening and baseline)

Before any study specific procedures are performed, the participants will sign the informed consent form. After written informed consent has been obtained, the following assessments and activities will be performed:

- History of AD will be diagnosed according to UK working party criteria (37) (see also Section 9.2)
    - *According to the UK working party diagnostic criteria (37), eczema is defined as exhibiting an itchy skin condition in the past 12 months plus 3 or more of:*
        - *History of involvement of the skin creases*
        - *Personal history of asthma or hay fever*
        - *History of generally dry skin in past year*
        - *Visible flexural dermatitis*
        - *Onset below age 2*
- Demographic data and baseline characteristics will be recorded (sex, date of birth, race)
- Medical history will be recorded
- Prior (see section 8.3 for details) and concomitant medication will be recorded
- Inclusion and exclusion criteria will be evaluated
- Eligible participants will be given a participant number

- Participant randomization
- AE recording will start
- The test sites will be acclimatized to the room conditions for ≥20 minutes
- Baseline measurements will be performed
    - Measurement of Trans Epidermal Water Loss (TEWL)
    - Measurement of skin capacitance
    - 3D skin images captured (roughness evaluation)
    - 2D skin images captured (colour evaluation)
- Collect a saliva sample for genotyping
- Study creams (weighed) will be dispensed
- Diary will be handed out (for recording cream application and tolerability)
- The researcher will provide instructions to the participant
- The participant will be asked to perform the first treatment applications under supervision
- The first patient-orientated skin tolerability assessment will be recorded in the diary buy the participant

The participants will start applying the creams on the same day as Visit 1 is performed.

Before leaving the clinic, the participants will be informed about the restriction criteria and they will be instructed to return to the clinic 29 days (±3 days) later for measurement of TEWL and skin capacitance. The participant will also be instructed how to fill out the diary and to bring the investigational products to the last visit.

8.1.4.2 Visit 2 (Review of participant diary and weigh treatments, Day 5 (±3 days))

At Visit 2, the following assessments and activities will be performed:

- Review of participant diary
- Weigh test and reference products
- AEs recorded
- Concomitant medications will be recorded
- Remind the participant to complete the second patient-orientated skin tolerability assessment in the diary

8.1.4.3 Visit 3 (Review of participant diary and weigh treatments, skin image, Day 15 (±3 days))

At Visit 3, the following assessments and activities will be performed:

- Review of participant diary

- Weigh test and reference products
- Image analysis of the skin
- AEs recorded
- Concomitant medications will be recorded
- 2D skin images captured (colour evaluation)

8.1.4.4 Visit 4 (Evaluation of TEWL change, start of SLS exposure, Day 29 (±3 days))

At Visit 4, the following assessments and activities will be performed:

- Diary will be collected and reviewed
- Ensure the participant has completed the third patient-orientated skin tolerability assessment in the diary
- Collect and weigh test and reference products
- Concomitant medications will be recorded
- AEs recorded
- Restriction criteria will be evaluated
- The test sites will be acclimatized to the room conditions for ≥20 minutes
- Skin measurements will be performed
    - Measurement of Trans Epidermal Water Loss (TEWL)
    - Measurement of skin capacitance
    - 3D skin images captured (roughness evaluation)
    - 2D skin images captured (colour evaluation)
    - Objective skin redness will be measured using the Mexameter
    - Visual scoring of redness
- Collect NMF samples
- SLS application
- The researcher will provide instructions to the participant

8.1.4.5 Visit 5 (Evaluation of TEWL change, Day 31 (±3 days))

At Day 30, the SLS-patch will be removed by the participant at home and the skin will be left to recover for 24 h.

At Visit 5, the following assessments and activities will be performed:

- Concomitant medications will be recorded
- Restriction criteria will be evaluated

- The test sites will be acclimatized to the room conditions for ≥20 minutes
- Skin measurements will be performed
    - Measurement of Trans Epidermal Water Loss (TEWL)
    - 2D skin images captured (colour evaluation)
    - Objective skin redness will be measured using the Mexameter
    - Visual scoring of redness
- AEs recorded

8.2 Collection of human samples

The following types of human tissue/biological material will be collected as part of this study:

1. Stratum corneum samples for quantification of Natural Moisturising Factor (NMF) levels
2. Saliva Samples to obtain genomic DNA for the determination of *FLG* gene status.

The samples will be stored in linked-anonymised form, meaning that they will be labelled with a participant ID and participant initials for identification (and not personal identifiable information, PII). The link between the participant ID and PII will be kept in the site file and only be accessible to the direct study team.

The samples will be stored at the test site under the custodianship of the PI. All samples, except the extracted DNA, will be destroyed within 1 year following the last participants last visit. Extracted genomic DNA will be kept under the custodianship of the PI for use in future research project, unless specific consent for this is not obtained (separately specified on the ICF).

8.2.1 Stratum corneum samples

Superficial skin samples are collected by tape stripping. Tape stripping is a painless procedure that removes the dead cells (denucleated corneocytes) from the surface of the skin that will eventually be lost/shed as a result of normal desquamation. It can cause some discomfort and redness, but any redness usually dissipates in a matter of hours/days. No dressings are required. The only skin preparation required before tape stripping is the removal of visible contaminants by dry wipe if necessary.

The samples collected by tape-stripping described herein do not meet the definition of relevant material under the Human Tissue Act because they contain no viable human cells (corneocytes are non-viable terminally differentiated/dead cells).

8.2.2 Saliva Samples

A saliva sample will be collected from the inside of the participants cheek during visit 1 in order to obtain a sample of genomic DNA. It can be easily collected by the participant themselves. Extracted genomic DNA will be genotyped for the 5 most common *FLG* gene mutations. The filaggrin gene (*FLG*) encodes proteins that are key to the normal barrier function of the skin. Null mutations in *FLG* are associated with an increased risk of eczema. Understanding how these mutations affect the responses to topical treatment is therefore of interest.

9 SELECTION OF STUDY POPULATION

9.1 Number of Participants

It is estimated that 50 evaluable participants will be needed (see Section 13.2 for sample size calculation).

9.2 Inclusion Criteria

The participants have to meet all of the following criteria to be eligible to enter the study:

1) Willing and able to provide informed consent
2) Male or female and aged 18 years or above
3) Volunteers able to read and understand English
4) A personal history of atopic dermatitis

The diagnosis of AD history will be set according to UK working party 1994 (37).

9.3 Exclusion Criteria

Participants meeting any of the following criteria will not be permitted to enter the study:

1) Eczema on the volar forearms requiring anti-inflammatory treatment
2) Possible allergy to ingredients in the study medications.
3) Any serious current medical condition which, in the opinion of the Investigator, may interfere with the evaluation of the results or may be contraindicated by the use of the test medications
4) Use of any concomitant medication that may interfere with the study related activities or assessment of efficacy, as judged by the Investigator
5) Use of any topical product, including cosmetic leave-on products on the volar forearms, within 1 week prior to, and throughout the study
6) Female participant who, according to the participant, is pregnant or breast-feeding, or plans to become pregnant during the course of the study
7) Any participant-related factor suggesting potential poor compliance with study procedures (e.g. psychiatric disorders, history of alcohol or substance abuse), as judged by the Investigator
8) Enrolment in any interventional study or use of an investigational drug within 3 months prior to the screening visit. Cosmetic studies on emollient use are allowed and the wash out period is 7 days, as with any emollient used
9) Volunteers judged by the PI to be inappropriate for the trial.

9.4 Restrictions

- Medications Prohibited >28 Days Prior to Baseline/Day 1 and throughout the study
    - Use of systemic (oral, parenteral) corticosteroids prohibited less than 3 months prior to baseline/day 1 and throughout the study
- Medications Prohibited 28 Days Prior to Baseline/Day 1 and throughout the study
    - Use of TCS, or TCI, anywhere on the body.
    - Inhaled glucocorticoids of low to moderate doses for treatment of asthma are allowed
    - Use of systemic immunosuppressive agents, including but not limited to, methotrexate, ciclosporin, azathioprine, hydroxychloroquine, and mycophenolate mofetil.
    - Tanning bed use, or light therapy Ultraviolet (UV), Ultraviolet B (UV-B), psoralen–UV-A [PUVA]).
    - Use of topical retinoids.
- Medications Prohibited 14 Days Prior to Baseline/Day 1 and throughout the study
    - Use of systemic antibiotics.
    - Use of topical antihistamines anywhere on the body.
- Medications Prohibited 7 Days Prior to Baseline/Day 1 and throughout the study
    - Use of topical antibacterial medications or products, including antibacterial soaps, bleach baths, topical sodium hypochlorite-based products anywhere on the body.
    - Systemic sedating antihistamines (eg, hydroxyzine or diphenhydramine or other sedating antihistamines).
    - Use of systemic non-sedating antihistamines in a nonstable (eg, escalating or decreasing, or as needed) regimen. Subjects on a stable non-sedating systemic antihistamine regimen with ≤7 days of consistent use prior to Visit 1 are permitted to continue but must not alter or stop their regimen during the study.
    - Use of bland (non-medicated) emollients, moisturisers or sunscreen on the test areas, within 7 days prior to Visit 1. Use of bland (non-medicated) emollient(s) is permitted during the study to manage dry skin in areas surrounding but not on or overlapping the test areas.
- Medications prohibited from Baseline/Day 1 and throughout the study
    - Use of vasoactive drugs in a non-stable (eg, escalating or decreasing, or as needed) regimen including: metaraminol, adrenaline/epinephrine, noradrenaline/norepinephrine, phenylephrine, dobutamine, dopamine, dopexamine, oxymetazoline.
    - Peroral anti-fungal medication
    - Topical anti-fungal creams on the test areas

9.5 Screening failure

All individuals who sign the consent form and either (1) withdraw their participation before the first assessment/procedure, (2) fail to meet all of the eligibility criteria and/or (3) for technical or logistical reasons do not participate in the first assessment session will be considered a "screening failure."

9.6 Removal of Participants from Treatment or Assessment

Participants are free to discontinue their participation in the study at any time. Withdrawal from the study will not affect or prejudice the participant's further care or treatment. Participants may be withdrawn from study treatment and assessments at any time, if deemed necessary by the Investigator.

Potential reasons for withdrawal of participants from this study are:

- If a participant decides to withdraw from the study (including if the participant withdraws informed consent)
- Intercurrent illness
- If the participant uses any prohibited concomitant medication or treatments listed in Section 9.4, at the discretion of the Investigator
- If the participant experiences an unacceptable AE
- If the participant is lost to follow-up

The reason and date the participant is withdrawn from the study will be documented in the CRF (e.g. lost to follow-up, consent withdrawn, incorrect enrolment, AEs, etc.). If a participant is withdrawn from further treatment with the investigational creams, the Investigator should complete the study termination page of the CRF. All AEs should be followed up in accordance with Section 12.2.

If a participant is withdrawn from the study, all data collected until the time of withdrawal will be used in the analyses.

9.7 Premature Termination of the Study

The Investigator or the Sponsor may terminate this study prematurely for any reasonable cause. The Independent Ethics Committee(s) (IECs) and Competent Authority(ies) (CAs) should be informed promptly.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the participants enrolled in the study, or potential study participants

If the CA obtains information that raises doubts about the safety or scientific validity of the clinical study, the CA can suspend or prohibit the study. Before the CA reaches its decision, it shall, except where there is imminent risk, ask the Sponsor and/or the Investigator for their opinion, to be delivered within one week (Directive 2001/20/EC, Article 12, Section 1).

If the study is prematurely terminated or suspended for any reason, the Investigator/institution should promptly inform the study participants and should assure appropriate therapy and follow-up for the participants.

9.8 Remuneration

All participants enrolled onto the study will be compensated for their time. A payment in Amazon Vouchers will be made as follows:

- £100 for completing the study

This is based upon a rate of £20 per visit to our test site.

Participants who fail to complete the study, for whatever reason, may claim a proportion of the remuneration commensurate with the extent of their participation.

Travel to and from the test site by taxi will be additionally arranged at our expense (not taken out of the remuneration).

10 TREATMENT OF PARTICIPANTS

10.1 Investigational Products

10.1.1 Treatment Regimens

The participants will apply 3 different creams on their lower volar forearms and the treatment area will be randomized. One randomized area will be left untreated. One FTU of the creams will be applied twice daily for 28 (± 3 days).

10.1.2 Identity of Investigational Products

The following creams will be used:

- Test cream: Miniderm Novum, moisturising cream containing 2% urea and 20% glycerol (no marketing authorization, Investigator´s brochure provided with the protocol)
- Reference cream: Miniderm 20% cream, moisturising cream containing 20% glycerol (marketing authorization in Sweden, Norway and Finland, SmPC provided with the protocol)
- Reference cream: Diprobase cream (no pharmacologically active component), paraffin based cream (marketing authorization, SmPC provided with the protocol)

10.1.3 Packaging and Labelling of Investigational Products

The test cream will be produced by Pharmavize NV Kleimoer 4, 9030 Mariakerke, Belgium.

Miniderm 20% cream will be produced by Medgenix Benelux, Vilegveld 21, 8560 Wevelgem, Belgium.

Miniderm Novum and Miniderm 20% cream will be filled in identical plastic tubes of 100 mL at Pharmavize.

Diprobase cream will be used in its original tube of 50 g and labelled as the other products.

The products will be labelled with:

- Name, address and telephone number of the sponsor
- pharmaceutical dosage form, route of administration, quantity of dosage units and the identifier of the product
- batch number
- trial reference code
- trial subject identification number
- name of Investigator

- name of Sponsor
- “directions for use, please see patient diary”
- “For clinical trial use only”
- Storage conditions
- Expiry date in month/year format
- “keep out of reach of children”
- Randomized application site (upper left arm, lower left arm, upper right arm or lower right arm)

10.1.4 Storage and Handling of Investigational Products

All creams in the study should be stored in a locked cupboard below 25°C and out of reach of children. Temperature logs throughout the study will be kept. Only personnel authorised by the principal investigator should dispense the study medication, and the accountability is the responsibility of investigator. The investigator/study nurse/pharmacist must complete and return the study medication supply form verifying the receipt of study medication to the study monitor.

A study medication inventory (dispensing records) for all medication dispensed must be maintained at all times and always kept current.

For application of the creams, please refer to Section 10.4.

10.2 Method of Assigning Participants to Treatment Groups

Randomisation (allocation of the treatments to the test sites) will be performed according to a randomisation list with balanced allocation to the 4 different test areas (to avoid site-dependent effects). Participants eligible for the study will be provided with a participant ID number at Visit 1.

10.3 Selection of Doses in the Study

The doses of the creams will be 1 FTU of cream per treatment area.

10.4 Selection and Timing of Dose for Each Participant

The creams should be applied morning and evening according to the randomisation for 28 days (± 3 days).

10.5 Blinding

The Investigator should keep the randomisation key and the numbering of the products in a secure, limited-access location to prevent inadvertent breaking of the blind. In an emergency requiring information about the study treatment, the appropriate envelope may be opened and the date, time and reason should be recorded and signed by the Investigator or his/her delegate. If the code should be broken without prior announcement to the Sponsor, the person who breaks the code should inform the Sponsor immediately.

Data will be collected and entered into the database on an ongoing basis. A clean file declaration will take place when all data have been entered and cleaned, and the database will then be locked before starting the analyses.

All study personnel at the clinic, as well as the CRO and Sponsor staff will remain blinded to the individual treatments during the study. Miniderm Novum and Miniderm will be packed in identical tubes and therefore blinded to both participants and study personnel. Diprobase cream will be provided in its original tube and thus not blinded. A product code will be indicated on the label to enable identification of the different treatments. The products are labelled upon arrival to the clinical research facility and therefore none of the study personnel can discriminate Miniderm Novum from Miniderm.

10.6 Prior and Concomitant Therapy

The concomitant medication and therapies listed in Section 9.4 should be avoided during the study.

Medication, which is considered necessary for the participants safety and well-being, may be given at the discretion of the Investigator. However, concomitant medication administered during the study may lead to withdrawal of the participant from the study (see Section 9.6).

10.7 Treatment Compliance

Compliance will be noted in the participant diaries. Use of creams will be recorded daily by the participants.

The test and reference creams will also be weighed before dispensing of the creams to the participants, during the study at day 5 and 15, and after return of the creams. Cream consumption (test or reference creams) based on weight will be calculated in order to analyse exposure to treatment. Patients that apply treatment on less than 75% of the days in the study period are considered to be violating the protocol and will be excluded from the per protocol population.

10.8 Drug Accountability

All study medications supplied for this study should be stored in a secure place. The Investigator or designated person at the study site will keep a record of the inventory and dispensing of all study medications. The Investigator is responsible for the product accountability.

11 STUDY ASSESSMENTS

11.1 Primary and Secondary Endpoints

	Objectives	Outcome measures
Primary	To determine whether applying a new moisturizing cream (the test cream) for 4 weeks is superior in terms of skin barrier strengthening, when compared with (1) no treatment and (2) two reference creams in adults with a predisposition to a skin barrier defect.	1. Trans Epidermal Water Loss (TEWL) after induction of skin irritation (TEWL on day 31 – TEWL on day 29) 2. Redness after induction of skin irritation (redness on day 31 – TEWL on day 29)
Secondary	To determine whether there is a difference between the new moisturising cream (the test cream) and (1) no treatment and (2) the two reference creams in: 1. Resting skin barrier function 2. Skin moisturisation 3. Tolerability 4. Cream consumption 5. Safety	1. TEWL after 4 weeks treatment (day 29-baseline) 2. Skin moisturisation: a. Capacitance measurements (day 29-baseline) b. Skin surface dryness (3D skin images, day 29-baseline) c. NMF levels on day 29 3. Tolerability properties: a. Participant tolerability scores by VAS on day 1, day 5 and day 29 b. Investigator visual scores for redness (day 15 – baseline and day 29 – baseline) c. Objective erythema from 2D colour skin images (day 15 – baseline and day 29 – baseline) 4. Cream consumption after 28 days of treatment (g) 5. Number of adverse events
Tertiary	To investigate the number of participants with FLG loss-of-function mutations and investigate and relationshhip with treatment effects	1. Number of FLG loss-of-function mutation carriers 2. Descriptive tabulations of TEWL by mutation status, if sufficient participants with mutation are detected

11.2 Efficacy Variables and Assessments

Unless otherwise indicated all procedures will be conducted by the investigative team, comprising experienced skin/dermatology researchers delegated duties by the PI, at the Sheffield Dermatology Research CRF. All assessments will be conducted at each of the 4 treatment sites in each participant unless otherwise indicated. None of the procedures listed would be routinely performed as part of standard clinical care.

11.2.1 Skin irritation testing

This test is a previously published method for determining skin sensitivity. It is based upon the skin's response to the standard irritant sodium lauryl sulphate (SLS). Exposure to 1% SLS for 24 hr under occlusion is followed by evaluation of redness and TEWL readings of the transient irritation. The procedure is summarised below:

1. Demarcate the test sites; upper volar forearms – both sides, 2 patches each (refer to "Test Sites").
2. Score visible redness for the test sites using the scales provided
3. Capture skin site images as described above (for determination of erythema index)
4. Measure objective skin redness (mexameter) and TEWL (as described above) at each patch site
5. Apply Finn Chambers (12mm aluminium chambers on Scanpor tape) containing a filter insert and 50µL 1% SLS to designated test sites. Record the time of patch application.
6. Cover all test sites with a waterproof adhesive membrane (Patch Protect).
7. Instruct the participants to:
    a. Wash as normal – the dressings are water proof
    b. Contact us if the patches come away before the 24hr period is complete
    c. Remove the patches themselves in 24hrs (day 30)
    d. Wash the skin of their forearms with water after removing the patches, and then avoid washing the sites until after the next visit
    e. Not to apply any products to the patch test sites
8. 24 ± 2 hours after patch removal (48 ± 2 hours after patch application), remark the test sites where necessary
9. Locate/demarcate the test sites
10. Repeat the visual scoring, image capture and objective assessments of redness, and TEWL.

The procedure is conducted over 49 hours, and involves 60 minutes to conduct the baseline assessments, 40 minutes to apply the patches, 10 minutes to remove them 24 hours later and a further 60 minutes to evaluate the results.

Quantification of skin irritation will support the two primary efficacy outcomes (further details on these variables are provided below):

1. Trans Epidermal Water Loss (TEWL) after induction of skin irritation (TEWL on day 31 – TEWL on day 29)
2. Redness after induction of skin irritation (redness on day 31 – TEWL on day 29). Redness is measured using the following techniques (described in more detail below):

a. Visual scoring by the investigator
b. Measurement of skin redness objectively using the Mexameter
c. Determination of the erythema index from 2D skin images

11.2.2 Trans Epidermal Water Loss

Skin barrier function will be evaluated by measuring the Trans Epidermal Water Loss (TEWL) of the untreated area and the areas treated with creams, before and after treatment (secondary outcome). TEWL will also be measured before and after induction of skin irritation by application of SLS (see "induction of skin irritation") as a means of quantifying skin irritation (primary outcome). TEWL measurements, recorded in $g/m^2/h$, will be performed using an AquaFlux AF200 condensing chamber probe (Biox Systems Ltd., London, UK).

Three repeat TEWL measurements, taking ~2 minutes each, will be collected at each site. Measurements will be repeated if an anomaly occurs during measurement (i.e. deviation from a typical bell shaped TEWL curve), which can occur if a participant becomes uncomfortable, or if the reading appears abnormally low or high. In either event the measurement will not be taken until at least 4 minutes has passed from the collection of the first measurement to permit restoration of normal TEWL. If TEWL was retaken due to a measurement anomaly the first reading will be discarded. If the reading was repeated for any other reason both measurements will be recorded the average of all readings will be used for subsequent analysis. After use in each participant the probe will be decontaminated with a 70% alcohol wipe.

All assessments will be performed in a room maintained at 21 ± 2 ° C and 38–50% relative humidity according to published guidelines (38). All test sites will be acclimatized to room conditions for 20-30 min before assessment.

11.2.3 2D Skin Imaging of erythema

A single close-up (50x magnification) image of each skin site will be captured using the c-cube camera with glass lens (Pixience, France). Each image takes approximately 5 minutes to capture. A 16x12mm area of the test site skin is imaged therefore there are no data confidentiality concerns. Captured 2D images are analysed to determine the skin erythema index (degree of redness, arbitrary numerical value) for the region of interest. This quantitative metric supports the primary outcome and the secondary tolerability outcome. The images are stored by the c-cube clinical database software within a protocol specific folder locally and on the server within the study specific folder.

11.2.4 Visual scoring of skin redness

Visual redness will be scored by an experienced grader using the scale below to assess both tolerability (secondary outcome) and skin irritation following patch/irritation testing (primary outcome). The assessment takes no more than 2 minutes.

Table 9.3: Visual assessment scale for erythema

Score	Description
0	No reaction.

0.5 / +	Slight, patchy erythema – barely perceptible
1	Slight uniform erythema – mild erythema
2	Moderate, uniform erythema – Moderate erythema
3	Strong erythema – Marked erythema

11.2.5 Objective skin redness

Skin redness will be measured 3 times within each patch test site using a C&K Mexameter probe to quantify SLS-induced skin irritation (primary outcome measure). All 3 measurements (arbitrary numerical scale) will be recorded. A fourth measurement should be collected if the variation is deemed to be too great (at the discretion of the Investigator), under which circumstance all 4 measurements will be recorded (no measurement is discarded). After use in each participant the probe will be decontaminated with a sanitation/detergent wipe. It takes approximately 5 minutes per test site to perform this procedure (20 minutes in total)

11.2.6 Skin capacitance

Capacitance, an indirect measure of skin water content, will be measured using a Corneometer CM825 (CK electronic GmbH, Cologne, Germany). Three repeats will be taken from each test site. Between every treatment site the probe head should be cleaned and dried to prevent cross-over contamination. All 3 measurements will be recorded. A fourth measurement should be collected if the variation is deemed to be too great (at the discretion of the Investigator), under which circumstance all 4 measurements will be recorded (no measurement is discarded). Capacitance units are reported using an arbitrary scale from 0 to 130, where values below 30 indicate very dry skin and values above 45 indicate sufficiently moisturised skin. It takes approximately 5 minutes per test site to perform this procedure (20 minutes in total)

11.2.7 3D Skin Imaging of surface dryness (roughness)

A close-up (50x magnification) image of each skin site will be captured using the c-cube camera (Pixience, France) without glass lens. Each image takes approximately 6 minutes to capture. A 16x12mm area of the test site skin is imaged therefore there are no data confidentiality concerns. Captured images are analysed for topographical changes (skin surface roughness in this case as a parameter associated with surface dryness). The images are stored by the c-cube clinical database software within a protocol specific folder locally and on the server within the study specific folder. An arbitrary quantitative metric for surface roughness is derived from the images with the c-cube software.

11.2.8 Natural Moisturizing Factors (NMF)

Samples for analysis of stratum corneum NMF levels (including urocanic acid and pyrrolidine carboxylic acid) will be collected by tape stripping

Tape-stripping involves the repeated application and removal of D-Squame cutaneous stripping discs (CuDerm cooperation, Dallas, USA) to/from the skin in a standardised manner.[42,46] A specially designed plunger is used to exert a standardised pressure (225g/cm^2) to each disc. Approximately 3 corneocyte layers (incomplete layers/uneven coverage) are removed per disc, depending on the participant and the treatment conditions. The amount of stratum corneum/corneocytes collected, and the associated depth through the stratum corneum sampled, is determined by measuring the amount of corneotypes removed by each disc using the SquameScan Device.

In this case the application and removal of 3 consecutive D-squame discs to a single site constitutes a single sample. Two samples will be collected from each sampling site (each of the 4 treatment areas), comprising tape-strips/discs 1-3 and 4-6, representing different depths through the stratum corneum. Samples will be stored at -20ºC before extraction and HPLC analysis. A quantitative value for the NMF components urocanic acid and pyrrolidine carboxylic acid will be obtained. It takes approximately 10 minutes to collect all samples from all 4 treatment sites.

11.2.9 Filaggrin mutations

A single saliva sample will be collected from each enrolled participant at Visit 1 to obtain genomic DNA for determination of participant *FLG* gene status. It takes no more than 5 minutes to collect the sample, which is done by the participant themselves using a passive spit or facilitated swab technique. *FLG* loss-of-function mutations are a risk factor for AD and predispose to more severe disease. Samples will undergo DNA extraction and genotyping at the University of Sheffield for the 5 common European loss-of-function FLG mutations that have been reported to confer increased AD risk.

11.2.10 Cream Consumption

Cream consumption will be based on the weight of the reference and test creams before (Visit 1), during (Visit 2 and 3) and after use (Visit 4). The study team will weigh the treatments at each visit.

11.2.11 Tolerability

The participants will be asked to score the degree of smarting/burning sensation (a sharp, local, superficial effect which can be experienced during contact with for example acidic solutions) using a VAS scale, at the start of treatment, after 5 days and again after 4 weeks of treatment. These participant assessments will be recorded in the treatment diary. The assessments can be made at the test site or at the participants home.

11.3 Demographic and Other Baseline Characteristics

The following information will be collected by the investigative team, comprising experienced skin/dermatology researchers delegated duties by the PI, at the Sheffield Dermatology Research CRF.

11.3.1 Demographic and Baseline Data

The following demographic and baseline data will be collected at Visit 1 by the study team as part of a guided 'background information' questionnaire taking approximately 30 minutes to complete:

- Sex
- Date of birth
- Race/ethnicity
- Time since AD diagnosis (at Visit 1)
- Investigator global assessment (IGA) of AD severity
- Time since last flare/episode of AD (according to the participant)
- Number of relapses during last 12 months (at Visit 1)

11.3.2 Medical History

Medical history according to the participant will be recorded at the screening visit. Changes in medical history from the screening visit will be recorded at Visit 1. Medical history will be captured as part of the background information questionnaire (above).

Relevant medical history includes, but is not limited to, asthma and allergy, other skin diseases and all chronic conditions.

11.3.3 Prior and Concomitant Medication and Therapy

Medications and therapies will be recorded on the concomitant medication form.

At Visit 1, all prior medications (oral and topical) used within 3 weeks prior to screening, as well as any topical products or ultraviolet light therapy used on the eczematous areas within 3 weeks prior to screening, will be recorded. This will take an average of 15 minutes depending upon the number of medications and involve completion of the Concomitant Medication Form.

During the treatment phase, participants will be asked to fill in any concomitant medication or therapy in the diaries. At Visits 4 and 5, information about concomitant medication and therapies used will be entered into the concomitant medication form. This is anticipated to take an average of 5 minutes per participant per visit.

11.4 Safety Assessments

The safety assessments are limited to recording of AEs.

AEs will be recorded from Visit 1 to the completion of Visit 5. During the treatment phase, participants will record AEs in their diaries. Information about AEs will then be entered into the CRF at Visits 4 and 5. An average of 10 minutes has been allocated for AE reporting per participant per visit.

For further information about definitions and reporting of AEs and Serious Adverse Events (SAEs), see Section 12 below.

11.5 Appropriateness of Measurements

Instrumental assessment of the skin condition will be used to evaluate the skin barrier function as well as skin hydration.

Two instruments to be used, the AquaFlux and the Corneometer, measure the water evaporation through the stratum corneum (TEWL) and the surface capacitance, respectively. TEWL is elevated when the skin barrier function is disturbed (39). Therefore, in atopic participants with active dermatitis, both TEWL and percutaneous absorption of hydrocortisone are elevated (40). Also the unaffected skin of atopic participants show increased levels of TEWL (4, 5, 41). Measurements of TEWL are considered valuable to demonstrate treatment effects in damaged skin. Furthermore, measurements of TEWL are often used to detect irritant effects from topical treatment. Induction of skin irritation using the model irritant sodium lauryl sulphate (SLS) is a well established method (42). This method is utilized in this protocol to evaluate if the creams provide protection from irritation, which is an indication of a skin barrier strengthening effect. A protective effect is shown if the TEWL of the area treated with cream is lower after induction of irritation, compared with the untreated area. TEWL measurement is a highly sensitive and precise measure of SLS irritant effects on skin with dose-effect relationship easily demonstrated in the concentration ranges normally used in provocative testing (42).

Data from skin surface capacitance measurements with a Corneometer is though less easy to interpret. Corneometer measurements is claimed to reflect the hydration status of the skin. However, the reading can be influence by other agents than water, for instance by body hair and cream residues (43, 44). The active ingredients in the tested creams, such as urea, can also induce changes in keratin dipole orientation by virtue of its protein denaturant properties and thereby affect the electrical properties of the skin. Therefore, data from Corneometer measurements need careful interpretation.

TEWL measurements will be performed using an AquaFlux AF200 condensing chamber probe (Biox Systems Ltd, London, U.K.). Skin surface capacitance will be measured using a Corneometer CM825 (CK electronic GmbH, Cologne, Germany). All assessments will be performed in a room maintained at 21 ± 2 °C and 38–50% relative humidity according to published guidelines.

Invisible skin reactions to topically applied drugs and cosmetics are a common problem among subjects who consider their skin as sensitive. The reactions are often purely sensory phenomena without clinical expression, making dermatologist or instrumental evaluation

impossible. One test method for evaluation of smarting / stinging potential is the grading by the subjects themselves (45). The participants are asked to mark in their diary on a 10 cm visual analogue scale (VAS) the degree of subjective discomfort following the use of the test creams. 0 cm represents no discomfort at all and 10 cm the worst possible state. It is considered more appropriate that the grading is performed at the participants' home rather than during the clinical visit since the participant will probably give a more accurate rating in conjunction to the application of the creams. The degree of discomfort will be graded and evaluated in the beginning, after 5 days and at the end of the study.

The criteria used to describe response are widely used and considered to be reliable.

12 ADVERSE EVENTS

12.1 Definitions

12.1.1 Adverse Event

Any untoward medical occurrence in a participant or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Comment: An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

If eczema appears on a study area or the untreated control area, the participant should note the date in the diary and immediately contact the Investigator in order to have the eczema investigated. The participant should continue to treat the eczema with the randomised cream until he/she has seen the Investigator. The Investigator judges if the eczema should be documented as an AE and if the participant will continue in the study. The participant will then be instructed to continue his/her treatment with the randomised cream or discontinue the study.

12.1.2 Adverse Reaction

All untoward and unintended responses to an investigational medicinal product related to any dose administered.

Comment: All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

12.1.3 Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unauthorised investigational product or Summary of Product Characteristics for an authorised product).

Comment: When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

12.1.4 Serious Adverse Event

Any untoward medicinal occurrence or effect that at any dose:

- Results in death
- Is life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Comments: Life-threatening in the definition of a serious adverse event (SAE) or serious adverse reaction refers to an event in which the participant was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it was more severe.

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

12.1.5 Serious Adverse Drug Reaction

A Serious adverse Drug Reaction (SADR) is an adverse drug reaction which results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, causes a congenital anomaly/birth defect in offspring.

12.1.6 Unexpected Serious Adverse Reaction

An unexpected serious adverse Drug Reaction is an adverse which complies with the definition of a serious adverse reaction and, at the same time, that of an unexpected adverse reaction.

12.1.7 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is an adverse reaction that is both unexpected (not consistent with the applicable product information) and meets the definition of a Serious Adverse Event in a causal relationship with the investigational medicinal product.

12.2 Reporting of Adverse Events

All study participants will be carefully monitored for the occurrence of AEs during the study period from Visit 1 to the completion of Visit 3. During the treatment phase, the participants will record all AEs in their diaries. At each visit, the researcher will then transfer AEs recorded in the diaries to the CRF. The Investigator will also collect AEs using a non-leading question such as “have you experienced any new health problems or worsening of existing conditions”, as well as report any AEs directly observed.

Clearly related signs, symptoms and abnormal diagnostic procedure results should be grouped together and reported as a single diagnosis or syndrome whenever possible.

All AEs including but not limited to events reported by the participant, or reported in answer to an open question by the Investigator or member of this team, which fall into any of the above definitions must be recorded as an AE in the CRF and should include the following information:

- Brief description of the event (diagnosis)
- Start date (and time, if relevant)
- Stop date (and time, if relevant) (or resolution)
- Severity

- Action taken regarding study drug
- Opinion on causality
- Seriousness
- Outcome

Severity

Severity describes the intensity of an event, and will be assessed as:

Mild

The AE does not interfere in a significant manner with the participant's normal functioning level. It may be an annoyance.

Moderate

The AE produces some impairment of function but not hazardous to health. It is uncomfortable and/or an embarrassment.

Severe

The AE produces significant impairment of functioning or incapacitation and/or it is a hazard to the participant.

If an AE changes in severity, the worst severity should be reported.

Causality

Causality will be assessed as:

Probable

A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.

Possible

A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Unlikely

A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Follow-up of Participants after Adverse Events

Any AE that is ongoing when the participant is withdrawn from the study should be followed up until the AE is resolved or the Investigator decides that the AE is stable and needs no further

follow-up. The date when the Investigator considers one of these outcomes to have occurred for the last ongoing AE for a participant will be considered the last visit for this participant, and the outcome should be recorded in the CRF.

12.3 Reporting of Serious Adverse Events

The Investigator is responsible for ensuring that all SAEs are reported to the Sponsor immediately, but in any event no later than 24 hours of any site staff becoming aware of the event. Initial reports should be followed as soon as possible by detailed written reports. The initial and follow-up reports should identify participants by unique code numbers assigned in the study rather than by the participants' names, personal identification numbers, and/or addresses. The following information is **mandatory** for the initial report:

- Participant study ID
- Study treatment (blinded, if applicable)
- Start date (time, if relevant) of the study treatment
- Brief description of the event (diagnosis)
- Start date (time, if relevant) of the event
- Seriousness criteria
- Causality assessment

All Serious or Unexpected Adverse Events must be reported by e-mail within 24 hours to the Sponsor, CHCIGlobalSafety@perrigo.com. Where appropriate, hospitalisation or autopsy reports should be made available.

The sponsor shall ensure that all relevant information about suspected serious unexpected adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the competent authorities in all the Member States concerned, and to the Ethics Committee, and in any case no later than seven days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight days.

All other suspected serious unexpected adverse reactions shall be reported to the competent authorities concerned and to the Ethics Committee concerned as soon as possible but within a maximum of 15 days of first knowledge by the sponsor. All Serious Adverse Events will be followed up until resolution (i.e. asymptomatic, stabilisation or death).

12.4 Pregnancy

Female participants will be instructed to notify the Investigator immediately if they become pregnant during the study, but they will not be withdrawn from study treatment. The participants will also be instructed to report pregnancies discovered after the last visit, if they believe that conception occurred during their participation in the study.

A pregnancy as such is not an AE, unless there is a possibility that the investigational product has interfered with the efficiency of any contraceptive measures. However, the Investigator

should report pregnancies according to the procedures and timelines described for reporting of SAEs (Section 12.3). Pregnancies do not need to be followed up.

12.5 Responsibilities

The following section details the responsibilities for reporting and reviewing AE's by each party.

Principal Investigator (PI):

Checking for AEs and ARs when participants attend for treatment / follow-up.

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
2. Using medical judgement in assigning seriousness, causality and whether the event/reaction was anticipated using the Reference Safety Information approved for the trial.
3. Ensuring that all SAEs are recorded and reported to the sponsor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available. Ensuring that SAEs are chased with Sponsor if a record of receipt is not received within 2 working days of initial reporting.
4. Ensuring that AEs and ARs are recorded and reported to the sponsor in line with the requirements of the protocol.

Sponsor: (NB where relevant these can be delegated to the CRO)

1. Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol onto a database.
2. Assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs and SARs.
3. Expedited reporting of SUSARs to the Competent Authority (MHRA in UK) and REC within required timelines.
4. The unblinding of a participant for the purpose of expedited SUSAR reporting. In the case of SUSAR, the appropriate envelope may be opened and the date, time and reason should be recorded and signed by the Investigator or his/her delegate. If the code should be broken without prior announcement to the Sponsor, the person who breaks the code should inform the Sponsor immediately.
5. Checking for (annually) and notifying PIs of updates to the Reference Safety Information for the trial.
6. Preparing and final sign off of the Development Safety Update Report (DSUR) as required and ensuring timely submission to the MHRA and REC.

Trial Steering Committee (TSC):

Due to the mechanistic nature of the study, the short duration and the low risk posed by the intervention there will be no TSC for this study.

Data Monitoring Committee (DMC):

Due to the short duration of the study and the low risk posed by the intervention there will be no DMC for this study.

13 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

13.1 Statistical and Analytical Plans

Before the unblinding of the study data, a separate statistical analysis plan (SAP), which will provide the technical details of the statistical analysis outlined below, will be prepared and approved.

13.1.1 Data Sets to be Analysed

The participant population sets are defined as follows:

Full analysis set (FAS): All participants who are diagnosed with a history of AD and who are randomised for the treatment.

Per-protocol set (PPS): All participants who are deemed to have no major protocol violations that could interfere with the objectives of this study. This is a sub-population of the FAS.

Note: Important violations of eligibility criteria and other deviations from the protocol will be assessed in cooperation with the Sponsor. Important deviations from the protocol may lead to exclusion of a participant from the PPS. All deviations will be discussed and agreed prior to the unblinding of the data.

Safety set: All randomised participants who receive at least 1 dose of test or reference cream.

Although the primary analysis will be performed on both the FAS and the PPS, the FAS will be considered the primary analysis population. Secondary analyses will be performed on the FAS only.

Safety summaries will be performed on the safety set.

13.1.2 Statistical Issues

Handling of Drop-outs or Missing Data

Missing data will not be replaced. The drop out rate will be calculated and analysed. Further details will be presented in the SAP.

Blind Review

Prior to code breaking, a blind review of the data will be performed. The objective of the review is to identify problems and to make decisions regarding data analytical issues under blind conditions.

13.1.3 Summary Statistics

Data will be summarised using appropriate descriptive statistics such as mean and standard deviation, minimum and maximum. If appropriate, median and quartiles may be presented in place of the mean and standard deviation. Further details will be given in the statistical analysis plan.

Demographic and patient history data collected at screening and baseline visits will be summarised.

13.1.4 Primary Efficacy Analysis

The primary analysis will compare the test treatment to no treatment and to the two reference creams in terms of change in TEWL and redness between days 31 and 29.

The statistical analysis technique used must take account of the design of the study, such that each participant has four 'matched' treatment areas. In addition, the choice of analysis must consider the distribution of the data, as it is likely that both TEWL and redness score will be skewed. Therefore, both transformations combined with parametric tests and non-parametric tests which take account of the 'matched' nature of the data will be considered. Where possible and appropriate the baseline values will be included as a covariate. Further details will be outlined in the SAP.

These analyses will be carried out on the FAS and PPS.

13.1.5 Secondary Efficacy Analyses

The secondary analyses will compare the test treatment to no treatment and to the two reference treatments in terms of change in TEWL and skin moisturisation as measured by capacitance and skin surface dryness from baseline to day 29.

The analyses of these outcomes will follow the same plan as the primary analyses.

Skin moisturisation as measured by NMF levels will not be recorded at baseline and therefore the analysis will compare the treatments in terms of the data observed at day 29 using a suitable analysis, taking account of the distribution of the data and the 'matched' nature of the observations.

Patient tolerability scores will not be recorded prior to treatment and so will be summarised by timepoint (day 1, day 5 and day 29) and the treatments compared using a suitable analysis, taking account of the distribution of the data and the 'paired' nature of the observations.

Tolerability properties as measured by investigator visual scores for redness and objective erythema from 2D colour skin images: The change from baseline to day 15 and from baseline to day 31 will be compared between treatments using a suitable analysis, in a similar way to the primary analyses.

The secondary analyses will be carried out on the FAS.

13.1.6 Other Analyses

The total consumption of the creams (test or reference cream), based on cream weight, will be calculated and tabulated descriptively. If appropriate consideration will be given to including the cream consumption as a covariate in the model or in an exploratory analysis of the primary endpoints.

The number of FLG loss-of-function mutation carriers will be summarised. If sufficient participants with the mutation are detected then descriptive tabulations of TEWL by mutation status will be presented.

13.1.7 Demographic and Other Baseline Characteristics

Participant disposition, demographic and other baseline data will be presented using summary statistics. Both the FAS will be used for this presentation.

13.1.8 Exposure to Treatment and Treatment Compliance

For exposure to treatment, the total consumption of the test and reference creams will be calculated. The FAS set will be used for this presentation.

For treatment compliance, data from the participant diaries will be used.

13.1.9 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities and tabulated by system organ class and by preferred term.

The total number of participants with at least 1 AE and the total number of AEs will be presented.

The number of participants and the number of AEs will be tabulated by system organ class and by preferred term. AEs will also be tabulated versus worst severity and worst relationship to treatment. Finally, for those AEs which are specifically recorded as related to one of the test sites the AEs will be summarised by test site. A listing, by participant number, will also be produced which lists the AEs with worst severity and worst relationship to treatment.

13.2 Determination of Sample Size

The sample size for this study is based on the primary end point, change in TEWL after induction of skin irritation (change between day 19 and day 21), with the primary comparison being between the test treatment and no treatment and the secondary comparison being between the test treatment and the two reference treatments.

In a similar study the standard deviation of the difference in change in TEWL after induction of skin irritation was seen to be approximately 8 $g/m^2/h$ when comparing the no treatment group to an active treatment. (that is: standard deviation of {[Change after skin irritation in no treatment area]-[Change after skin irritation in active treatment area]}).

When comparing the difference between the test treatment and one of the two reference treatments the standard deviation was approximately 3.

If the assumptions of a paired t-test are found to be valid, for a sample of 40 participants this analysis would have >90% power to detect a difference (in change) of 3.5 g/mÇ.h between test treatment and no treatment. The same sample size would also have >90% power to detect a difference (in change) of 2 g/mÇ.h between test treatment and active treatment. If a parametric analysis is indicated then an analysis which includes the baseline as a covariate will be considered which, if there is variation at baseline may increase the power of the analysis further.

If the assumptions of a paired t-test are not met (and a suitable transformation cannot be found) then a sign test (or a wilcoxon matched pairs test) may be used. A power calculation can be carried out for the sign test (46) although this too makes some assumptions. This calculation shows that a sample size of N=40 would have >80% power to detect a difference such that the probability of X>0 (where X is the difference in change, as above) is approximately 0.7. This probability is not unrealistic given the data seen previously, and, if the clinically relevant difference detailed above is seen, the probability of X>0 may in fact be increased leading to higher power. To account for dropouts, 50 subjects will be recruited.

13.3 Procedures for Reporting any Deviation(s) from the Original Statistical Analysis plan

Any deviation(s) from the original statistical analysis plan (as described in the study protocol or in the SAP) will be described and justified in a revised SAP and in the final report, as appropriate.

14 INVESTIGATOR/SPONSOR RESPONSIBILITIES

14.1 Ethics

14.1.1 Independent Ethics Committee (IEC)

This protocol and any amendments will be submitted to a properly constituted IEC, in accordance with the International Conference on Harmonisation (ICH) guidelines, the applicable European Directives and local legal requirements, for approval of the study. Approval must be obtained in writing before the first participant can be recruited.

14.1.2 Ethical Conduct of the Study

The study will be conducted in compliance with the protocol, regulatory requirements, Good Clinical Practice (GCP) and the ethical principles of the latest revision of the Declaration of Helsinki as adopted by the World Medical Association.

14.1.3 Participant Information and Consent

All participants will receive written and verbal information regarding the study. This information will emphasise that participation in the study is voluntary and that the participant may withdraw from the study at any time and for any reason. All participants will be given the opportunity to ask questions about the study and will be given sufficient time to decide whether to participate in the study.

Before any study-related procedures are performed at the first study visit, the informed consent form will be signed and personally dated by the participant (or their legally acceptable representative and/or witness, as applicable) and by the researcher who conducted the informed consent discussion.

The consent includes information that data will be recorded, collected, and processed in accordance with the Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC, the data will not identify any persons taking part in the study.

A copy of the participant information including the signed consent form will be provided to the participant.

The study team includes medically qualified clinical researchers, who will be available throughout the trial. Due to the low risk of the intervention and the mechanistic design of the study involving volunteers from the general population (participants are not selected based upon their use of NHS services) the PI may delegate activities, including informed consent and medical history taking (facilitated using the medical history form), to non-medically qualified and otherwise appropriately trained researchers.

14.2 Participant Records and Source Data

All data will be collected during the conduct of the study. No patient notes will be accessed. The following documents will capture source data:

- Medical History Form: Medical history according to the participant
- Participant Diaries: Treatment compliance, adverse events and participant reported tolerability. The information in the diary will be transferred to an electronic CRF at each study visit.
- Adverse Events Form.
- Concomitant Medication Form: recent (Visit 1) and concomitant medication (Visits 2-5) according to the participant

All other study information will be directly recorded into an electronic CRF. Completed electronic CRF's will be printed, dated and signed (by the researcher) upon completion to verify them. Verified printed records of the eCRF's will be kept in the site file.

The following data may be recorded directly in the eCRF(s), which will then be considered as source data:

- Race
- Sex
- Time since AD diagnosis (at Visit 1)
- Number of relapses during last 12 months (at Visit 1)
- Cream consumption (weight of creams)
- TEWL measurements
- Capacitance measurements (skin hydration)
- Objective skin redness measurements

Skin images will be captured directly in the Pixience clinical software database, stored by participant ID, visit number and anatomical site/test site. Colour and dryness measures determined from the images within the software will be directly reported in an annotated database.

The Investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the CRFs. Data reported in the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. Signed sections of CRFs will be monitored and collected on a regular basis.

14.3 Access to Source Data and Documentation

Access to study information will be managed in the following ways:

- **Personal identifiable information (PII):** Collected in order to conduct the study, this data will be accessible only to the direct study team at the site. PII collected on the

registration form will be kept for up to 5 years in order to contact participants about future studies, pending specific informed consent is obtained for this, otherwise contact details will be destroyed at the end of the study. PII will be kept in paper form. Where participants consent to being contacted about future studies, their contact details will also be stored in the electronic volunteer register. The volunteer register is located on a secure server with access control in a separate location to the source data.

- **Source data:** All source data will be in a pseudonymised fashion using a participant ID and participant initials to identify records. Patient ID's will be generated upon enrolment using the enrolment log. The enrolment log will be kept in paper form in the study site file. Source data will be retained by the site under the custodianship of the Principle Investigator. The Investigator will guarantee access to source documents for the monitor and auditors as well as for inspection by appropriate regulatory agencies, and the IEC, if required. Source data in paper form will be stored in a locked cupboard in the study rooms, and electronic source data will be stored on a secure severed, with access control, managed by the University of Sheffield.
- **Study data (collated and verified by site)**: The collated and verified study data will have any PII removed and will be shared with the study Statistician and the Sponsor.

14.4 Monitoring

The monitor will visit the study site on a regular basis to ensure that the study is conducted and documented in accordance with this protocol, ICH GCP guidelines, regulatory requirements and any study specific documents such as CRF completion guidelines.

Monitoring visits will be conducted to confirm that e.g.:

- The investigational team is adhering to the study protocol
- Informed consent has been obtained from all participants
- AEs have been reported as required
- Data are being accurately recorded in the CRFs
- The creams are being stored correctly and drug accountability is being performed on an ongoing basis
- Facilities are, and remain, acceptable throughout the study
- The Investigator and the site are receiving sufficient information and support throughout the study

Moreover, during monitoring visits the data recorded in the CRFs, source documents and other study-related records will be compared against each other in order to ensure accurate data that reflect the actual existence of the participant in the study i.e. source data verification.

14.5 Data Management

Data management and handling will be conducted according to the study specific Data Management Plan in accordance with ICH guidelines and Investigator's standard operating procedures (SOPs).

Data entry, validation, and data queries will be handled by the Investigator Data Management Team. The data will be subjected to validation according to Investigator SOPs in order to ensure accuracy in the collected CRF data.

Before database closure a reconciliation will be performed between the SAEs entered in the safety database TrackWise and the study SAE forms after database closure.

Any deviations, i.e. discrepancies and additions from the process defined in the Data Management Plan, will be described in a study specific Data Management Report.

14.6 Quality Assurance and Audit

Audits or inspections, including source data verification, may be performed by representatives of the CRO, the Sponsor, a CA and/or an IEC.

14.7 Record Retention

The Investigator/institution should maintain essential documents (as defined in ICH E6 GCP, Section 8) as required by the applicable regulatory requirement(s). The Investigator/institution should take measures to prevent accidental or premature destruction of the documents.

The Investigator/institution shall retain all Clinical Trial records for a period of 25 years after Trial Completion.

It is the responsibility of the Sponsor to inform the Investigator/institution in writing as to when the documents no longer need to be retained.

14.8 Protocol Deviations

Deviations from the study protocol will be documented in a Protocol Deviation Log.

The classification of participants into protocol violators will be made during a meeting before database lock. The classification will be mutually agreed between the Sponsor, the Investigators, the statistician and the CRO before breaking the randomisation codes.

14.9 Insurance

The Sponsor must provide insurance or must indemnify (legal and financial coverage) the Investigator/the institution against claims arising from the study, except for claims that arise from malpractice, negligence or non-compliance with the protocol.

14.10 Report and Publication

After completion of the study, a clinical study report will be prepared according to the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3) by Sponsor in close collaboration with the Investigator.

All publications and presentations must be based upon the clinical study report.

All information supplied by the Sponsor in connection with this study will remain the sole property of the Sponsor and is to be considered confidential information. No confidential

information will be disclosed to others without obtaining prior written consent from the Sponsor and will not be used except in the performance of this study.

If the Investigator wishes to publish results from this clinical study, written permission to publish must be obtained from the Sponsor in advance. As some of the information regarding the investigational products and development activities at the Sponsor may be of a strictly confidential nature, the Sponsor must first review any publication manuscript prior to their submission to journals, meetings or conferences.

The Sponsor may choose to publish or present data from this study. If an Investigator is offered authorship, he/she will be asked to critically review the article for important intellectual content and approve the version to be published. The Sponsor has the right to use the results for registration and internal presentation and for promotion of the Sponsor’s commercial interests.

15 REFERENCE LIST

1. Halvarsson K, Loden M. Increasing quality of life by improving the quality of skin in patients with atopic dermatitis. Int J Cosmet Sci. 2007;29(2):69-83.
2. Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. Allergy. 2014;69(1):3-16.
3. Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. J Allergy Clin Immunol. 2006;118(1):3-21; quiz 2-3.
4. Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. Acta Derm Venereol. 1985;65(2):102-5.
5. Loden M, Olsson H, Axell T, Linde YW. Friction, capacitance and transepidermal water loss (TEWL) in dry atopic and normal skin. Br J Dermatol. 1992;126(2):137-41.
6. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006;38(4):441-6.
7. Morar N, Cookson WO, Harper JI, Moffatt MF. Filaggrin mutations in children with severe atopic dermatitis. J Invest Dermatol. 2007;127(7):1667-72.
8. Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol. 2006;118(1):214-9.
9. Loden M. The skin barrier and use of moisturizers in atopic dermatitis. Clin Dermatol. 2003;21(2):145-57.
10. Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses. American journal of contact dermatitis : official journal of the American Contact Dermatitis Society. 1999;10(3):119-26.
11. Holden C, English J, Hoare C, Jordan A, Kownacki S, Turnbull R, et al. Advised best practice for the use of emollients in eczema and other dry skin conditions. J Dermatolog Treat. 2002;13(3):103-6.
12. Szczepanowska J, Reich A, Szepietowski JC. Emollients improve treatment results with topical corticosteroids in childhood atopic dermatitis: a randomized comparative study. Pediatr Allergy Immunol. 2008;19(7):614-8.
13. Giordano-Labadie F, Cambazard F, Guillet G, Combemale P, Mengeaud V. Evaluation of a new moisturizer (Exomega milk) in children with atopic dermatitis. J Dermatolog Treat. 2006;17(2):78-81.
14. Draelos ZD. The effect of ceramide-containing skin care products on eczema resolution duration. Cutis. 2008;81(1):87-91.
15. Lucky AW, Leach AD, Laskarzewski P, Wenck H. Use of an emollient as a steroid-sparing agent in the treatment of mild to moderate atopic dermatitis in children. Pediatr Dermatol. 1997;14(4):321-4.
16. Halkier-Sorensen L, Thestrup-Pedersen K. The efficacy of a moisturizer (Locobase) among cleaners and kitchen assistants during everyday exposure to water and detergents. Contact Dermatitis. 1993;29(5):266-71.
17. Vilaplana J, Coll J, Trullas C, Azon A, Pelejero C. Clinical and non-invasive evaluation of 12% ammonium lactate emulsion for the treatment of dry skin in atopic and non-atopic subjects. Acta Derm Venereol. 1992;72(1):28-33.

18. Kolbe L, Kligman AM, Schreiner V, Stoudemayer T. Corticosteroid-induced atrophy and barrier impairment measured by non-invasive methods in human skin. Skin Res Technol. 2001;7(2):73-7.
19. Ganemo A, Virtanen M, Vahlquist A. Improved topical treatment of lamellar ichthyosis: a double-blind study of four different cream formulations. Br J Dermatol. 1999;141(6):1027-32.
20. Loden M, Andersson AC, Lindberg M. Improvement in skin barrier function in patients with atopic dermatitis after treatment with a moisturizing cream (Canoderm). Br J Dermatol. 1999;140(2):264-7.
21. Tsang M, Guy RH. Effect of Aqueous Cream BP on human stratum corneum in vivo. Br J Dermatol. 2010;163(5):954-8.
22. Lonne-Rahm S, Berg M, Marin P, Nordlind K. Atopic dermatitis, stinging, and effects of chronic stress: a pathocausal study. J Am Acad Dermatol. 2004;51(6):899-905.
23. An S, Lee E, Kim S, Nam G, Lee H, Moon S, et al. Comparison and correlation between stinging responses to lactic acid and bioengineering parameters. Contact Dermatitis. 2007;57(3):158-62.
24. Loden M, Andersson AC, Anderson C, Bergbrant IM, Frodin T, Ohman H, et al. A double-blind study comparing the effect of glycerin and urea on dry, eczematous skin in atopic patients. Acta Derm Venereol. 2002;82(1):45-7.
25. Serup J. A double-blind comparison of two creams containing urea as the active ingredient. Assessment of efficacy and side-effects by non-invasive techniques and a clinical scoring scheme. Acta Derm Venereol Suppl (Stockh). 1992;177:34-43.
26. Andersson AC, Lindberg M, Lodén M. The effect of two urea-containing creams on dry, eczematous skin in atopic patients. I. Expert, patient and instrumental evaluation. Journal of Dermatological Treatment. 1999;10(3):165-9.
27. Lodén M, Bárány E, Mandahl P, Wessman C. The Influence of Urea Treatment on Skin Susceptibility to Surfactant-Induced Irritation: A Placebo-Controlled and Randomized Study. Exog Dermatol. 2004;3(1):1-6.
28. Loden M. Barrier recovery and influence of irritant stimuli in skin treated with a moisturizing cream. Contact Dermatitis. 1997;36(5):256-60.
29. Buraczewska I, Berne B, Lindberg M, Torma H, Loden M. Changes in skin barrier function following long-term treatment with moisturizers, a randomized controlled trial. Br J Dermatol. 2007;156(3):492-8.
30. Wiren K, Nohlgard C, Nyberg F, Holm L, Svensson M, Johannesson A, et al. Treatment with a barrier-strengthening moisturizing cream delays relapse of atopic dermatitis: a prospective and randomized controlled clinical trial. J Eur Acad Dermatol Venereol. 2009;23(11):1267-72.
31. Akerstrom U, Reitamo S, Langeland T, Berg M, Rustad L, Korhonen L, et al. Comparison of Moisturizing Creams for the Prevention of Atopic Dermatitis Relapse: A Randomized Double-blind Controlled Multicentre Clinical Trial. Acta Derm Venereol. 2015;95(5):587-92.
32. Loden M, Wessman W. The influence of a cream containing 20% glycerin and its vehicle on skin barrier properties. Int J Cosmet Sci. 2001;23(2):115-9.
33. Blichmann CW, Serup J, Winther A. Effects of single application of a moisturizer: evaporation of emulsion water, skin surface temperature, electrical conductance, electrical capacitance, and skin surface (emulsion) lipids. Acta Derm Venereol. 1989;69(4):327-30.
34. van Zuuren EJ, Fedorowicz Z, Christensen R, Lavrijsen A, Arents BWM. Emollients and moisturisers for eczema. The Cochrane database of systematic reviews. 2017;2:CD012119.
35. Loden M, Andersson AC, Andersson C, Frodin T, Oman H, Lindberg M. Instrumental and dermatologist evaluation of the effect of glycerine and urea on dry skin in atopic dermatitis. Skin Res Technol. 2001;7(4):209-13.

36. Simpson EL, Chalmers JR, Hanifin JM, Thomas KS, Cork MJ, McLean WH, et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. J Allergy Clin Immunol. 2014;134(4):818-23.
37. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. Br J Dermatol. 1994;131(3):406-16.
38. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis. 1990;22(3):164-78.
39. Loden M. Biophysical methods of providing objective documentation of the effects of moisturizing creams. Skin Res Technol. 1995;1(3):101-8.
40. Aalto-Korte K. Improvement of skin barrier function during treatment of atopic dermatitis. J Am Acad Dermatol. 1995;33(6):969-72.
41. Watanabe M, Tagami H, Horii I, Takahashi M, Kligman AM. Functional analyses of the superficial stratum corneum in atopic xerosis. Arch Dermatol. 1991;127(11):1689-92.
42. Tupker RA, Willis C, Berardesca E, Lee CH, Fartasch M, Agner T, et al. Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. Contact Dermatitis. 1997;37(2):53-69.
43. Loden M, Hagforsen E, Lindberg M. The presence of body hair influences the measurement of skin hydration with the Corneometer. Acta Derm Venereol. 1995;75(6):449-50.
44. Loden M, Lindberg M. The influence of a single application of different moisturizers on the skin capacitance. Acta Derm Venereol. 1991;71(1):79-82.
45. Sahlin A, Edlund F, Loden M. A double-blind and controlled study on the influence of the vehicle on the skin susceptibility to stinging from lactic acid. Int J Cosmet Sci. 2007;29(5):385-90.
46. Noether GE. Sample Size Determination for Some Common Nonparametric Tests. Journal of the American Statistical Association. 1987;82(398):645-7.

16 APPENDICES

Appendix A: Supplementary files

- Poster advert
- Alternative media advert
- Participant Information Sheet
- Informed Consent Form
- Registration Form
- SDR Subject Screening Log
- Background Information Form (includes medical history)
- Admission Form
- SDR Screening, Enrolment and Completion Log
- SDR Concomitant medication Form
- SDR Adverse Events Form
- Serious Adverse Event and Pregnancy Report Form
- Participant Diary
- Skin Assessment Visit Case Record Forms
- Compliance Monitoring Case Record Form
- Substantial amendment no 1 table

Appendix B: Risk Assessment