Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Sheffield Dermatology Research

The University Of Sheffield.

Skin bioMARkers for atopic eczema Therapy evaluation study 2 (SMART2)

Study Title	Validation of a novel composite of skin biomarkers as a primary outcome measure for evaluating the safety of treatments for atopic dermatitis study 2: a randomised controlled trial (phase 2) comparing the effects of crisaborole 2% ointment to betamethasone valerate 0.025% cream on skin structure and function in participants with atopic dermatitis.
Short Title	Skin bioMARkers for atopic eczema Therapy evaluation study 2
Acronym	SMART2
Date and Version	30 Sep 2022, Version 2.0
IRAS#	1005095
ISRCTN#	xxx
EudraCT#	2022-000560-21
Chief Investigator	Prof Michael J. Cork, Consultant Dermatologist & Head of Sheffield Dermatology Research
Co-Investigators	Dr Simon Danby, Research Fellow (Trial Manager and non-medical Co-PI) Dr Sura Muneer Sahib, Specialist Registrar (medical Co-I) Prof Stephen Matcher, Professor of Biophotonics (non-medical Co-I), Miss Rosie Taylor, Principal Consultant (statistician Co-I), Dr Rob Byers, Research Associate (non-medical Co-I)
Study Site	The Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF Tel: 0114 2268515 Mobile: 07834392439 (direct number for study team)
Governance Sponsor	Sheffield Teaching Hospitals (STH) NHS Trust
Sponsor Reference	STH20466
Research Ethics Committee (REC)	xxx
REC Reference	xxx
Funder	Pfizer, Inc.
Funder Reference	70111305

DOCUMENT DETAILS

This protocol has regard for the HRA guidance

AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date Issued	Author(s) of changes	Details of changes made
0	1.0	19Jan2022	SGD	Original protocol
1	1.1	15Feb22	SGD	Inclusion of target lesion severity scoring, a global severity assessment at visit 8 and other minor changes following Pfizer Scientific Committee review.
2	1.2	25Feb22	SGD	Reduced maximum participant age from 65 to 64 in line with current definition of an elderly participant. Provided additional information on the marketing experience with crisaborole and use on delicate skin sites A series on minor clarifications following Sponsor review of the protocol. Provided additional detail on the planned analysis of biopsy tissue. Provided criteria to trigger participant retraining. Provided additional description of the source data in section 11.5
3	1.3	02Mar22	SGD	Minor clarifications made to sections 2.7 and 2.8.3
4	1.4	04Mar22	SGD	Update to exploratory objectives, including removal of the objective to compare HPLC derived NMF levels between treatment groups to focus on validation of FTIR determined carboxylate levels.

				Further clarification of the source data pertaining to biopsy analysis.
5	2.0	30Sep22	SGD	Male or female condom used with a spermicide product is not considered a highly effective method of contraception. This has therefore been removed from the list of highly effective contraceptive methods.

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

For and on behalf of the Trial Sponsor:

Signature: …… Date: …..…/…..…/…..…

Name (please print): ……

Position: ……

Chief Investigator:

Signature: …… Date: …..…/…..…/…..…

Name (please print): ……

Position: ……

KEY TRIAL CONTACTS

Role	Team Member
CHIEF INVESTIGATOR: (Medical Expert)	[redacted]
SUB-INVESTIGATORS:	[redacted]

	[redacted]
TRIAL MANAGER	[redacted]
RESEARCH COORDINATOR (SPONSOR): (Sheffield Teaching Hospitals Trust)	[redacted]
FUNDER REPRESENTATIVES	[redacted]
STUDY ADMINISTRATIOR	[redacted]

SYNOPSIS

Title	Validation of a novel composite of skin biomarkers as a primary outcome measure for evaluating the safety of treatments for atopic dermatitis study 2: a randomised controlled trial (phase 2) comparing the effects of crisaborole 2% ointment to betamethasone valerate 0.025% cream on skin structure and function in participants with atopic dermatitis.	
Acronym	SMART2	
Clinical Phase	Phase 2	
	Objectives:	**Outcome measures:**
Primary	1. To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy (on the volar forearm) in patients with AD.	The difference in the change in epidermal thickness (day 29 – day 1) on the volar forearm, measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream.
Secondary	2. To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy (on the antecubital fossa) in patients with AD.	The difference in the change in epidermal thickness (day 29 – day 1) on the antecubital fossa, measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream.
	3. To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 2 weeks is a cause of skin atrophy on the cheeks in patients with AD.	The difference in the change in epidermal thickness (day 15 – day 1) on the cheeks, measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream.
	4. To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate	The difference in the change in epidermal thickness measured by structural OCT and angiographic OCT (superficial plexus depth in µm) during and after 28 days

	(0.025%) cream at different anatomical sites	treatment on the volar forearms, antecubital fossae and cheeks.
	5. To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream	The difference in the change in visual redness/erythema score and objective redness (erythema index from skin images and redness from the Mexameter) during and after treatment.
	6. To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on skin barrier function	The difference in the change in Trans-Epidermal Water Loss (TEWL) during and after treatment.
		The difference in the change in skin barrier integrity/STS (day 29 – day 1) on the volar forearms ($TEWL_{ts20}$) and antecubital fossae ($TEWL_{ts10}$).
		The difference in the change in visual skin dryness on the volar forearms, antecubital fossae and cheeks during and after treatment.
Trial Design	An observer-blind randomised within-subject (bilateral) controlled clinical trial	
Trial Participants	Participants 18-64 years old with AD not currently undergoing, or requiring, active drug treatment.	
Planned Sample Size	A single cohort of 40 participants will be recruited with a target of 33 for completion (allowing for a 18% drop out rate)	
Key eligibility criteria	1. Volunteers with AD, defined according to the UK working party diagnostic criteria, not currently undergoing, or requiring, active drug treatment at baseline (visit 1) 2. Male or female aged 18-64 years old at baseline (Visit 1) 3. Volunteer understands the purpose, modalities and potential risk of the trial 4. Participants able to read and understand English 5. Participants willing to sign the informed consent Exclusion criteria are detailed in section 6.3.	

Investigational medicinal product(s)	• Crisaborole (2%) ointment • Betamethasone valerate (0.025%) cream
Formulation, Dose, Route of Administration	Twice-daily self-administered application of 1 finger-tip unit (FTU) of either crisaborole (2%) ointment or betamethasone valerate (0.025%) cream to the designated treatment areas on the arms (volar forearm and including the corresponding antecubital fossa) of the respective side of the body for 28 days. Additionally, ¼ FTU of the respective treatment to be applied to the cheek on the same side of the body twice-daily 14 days.
Treatment duration	28 days on the volar forearm and antecubital fossa, 14 days on the cheeks
Follow-up duration	28 days on the volar forearm and antecubital fossa, 42 days on the cheeks
Planned Trial Period	The duration participants will participate in this study is approximately 10 weeks, including the 1-week run-in period (no topical product use on measurement sites). The study is expected to last 12 months in total.

CONTENTS

i. List of abbreviations

AD	Atopic dermatitis
AE	Adverse Event
AR	Adverse Reaction
ATR	Attenuated total reflectance
BSA	Body surface area
CA	Competent Authority
CF	Cubital/antecubital fossa
CH	Cheek
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EASI	Eczema Area and Severity Index
EC	European Commission
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMEA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
EudraVIGILANCE	European database for Pharmacovigilance
FA	Forearm (volar face)
FTIR	Fourier Transform Infrared
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography

IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File (This forms part of the TMF)
ISRCTN	International Standard Randomised Controlled Trials Number
LCF	Left cubital/antecubital fossa
LCH	Left cheek
LFA	Left (volar) forearm
MA	Marketing Authorisation
MHRA	Medicines and Healthcare products Regulatory Agency
MS	Member State
NHS R&D	National Health Service Research & Development
NIMP	Non-Investigational Medicinal Product
NMF	Natural moisturizing factor
OCT	Optical Coherence Tomography
PI	Principal Investigator
PIC	Participant Identification Centre
PIL	Participant information leaflet
PIS	Participant Information Sheet
PS-OCT	Polarization sensitive OCT
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
RCF	Right cubital/antecubital fossa
RCH	Right cheek
RCT	Randomised Control Trial
REC	Research Ethics Committee
RFA	Right (volar) forearm
SAE	Serious Adverse Event

SAR	Serious Adverse Reaction
SDV	Source Data Verification
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SRSD	Single reference safety document
SSI	Site Specific Information
STS	Skin tape-stripping
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCI	Topical calcineurin inhibitor
TCS	Topical corticosteroid
TEWL	Trans-Epidermal Water Loss
TMF	Trial Master File
TMG	Trial Management Group
TS	Tape-strip
TSC	Trial Steering Committee
USPI	United States prescribing information
US	Ultrasound

ii. Funding and support in kind

Funder(s) *(Names and contact details of ALL organisations providing funding and/or support in kind for this trial)*
Pfizer Inc. **John Schenkel** Research Collaboration Project Manager Global Medical Grants, Pfizer Worldwide Research, Development and Medical Pfizer Inc. Office: +1-212-733-1097 Email: John.Schenkel@pfizer.com

iii. Role of the trial sponsor and funder

The sponsor, Sheffield Teaching Hospitals Trust, assumes overall responsibility for the initiation and management of the trial.

The investigative team, in the employ of the University of Sheffield, assumes responsibility for trial design, conduct, data analysis and interpretation, manuscript writing, and dissemination of results.

The funder, Pfizer, assumes the role of collaborator, by reviewing the study design, supplying the study medication, supporting the study set-up and helping ensure the study is conducted safely by communicating any pertinent safety updates relating to crisaborole (2%) ointment to the PI.

iv. Roles and responsibilities of trial management committees/groups and individuals

A Trial Management Group (TMG) will monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself.

The group will comprise the Chief Investigator, co-investigators, trial manager, study co-ordinator and statistician.

The group will convene at regular intervals during the course of the study. Further to the overall role of the group as a whole the following individuals will undertake specific responsibilities:

- The study coordinator will be responsible for providing recruitment metrics
- The statistician will be responsible for providing blinded safety data
- The trial manager will be responsible for updates on the completeness, integrity and security of research data and updates on safety reporting.

Due to the low level of assessed risk, the single centre nature of the trial and the straight-forward design a Data Monitoring Committee and Trial Steering Committee are not considered to be required. The TMG will therefore assume the roles of both the DMC and TSC.

v. Protocol contributors

Role	Team Member
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vi. Key words

Atopic Dermatitis; Corticosteroid; PDE4 Inhibitor; Skin Barrier; Epidermal Atrophy; Topical

vii. Trial flow chart

1. LAY SUMMARY

The first-choice drug treatment for mild-moderate eczema is currently a topical corticosteroid. By topical, we mean the treatment is intended for application directly to the skin. Whilst topical corticosteroids are effective at treating eczema, they have been found to cause unwanted skin changes, such as skin thinning, if used inappropriately over long periods of time. Exactly how much unwarranted thinning is caused by different treatment routines is unclear, so we want to use some new non-invasive ways to measure skin thinning to better understand the problem. One of these ways is to take a 3D image of the skin using a technique called OCT, which is similar to ultrasound. Because the methods are so sensitive, the signs of skin thinning can be seen before the skin becomes visibly damaged.

Crisaborole ointment is a new non-steroidal drug treatment for eczema that appears to be as effective as some topical corticosteroids and is not expected to cause abnormal skin thinning. Betamethasone valerate cream is one of the most commonly prescribed topical corticosteroids for eczema in the UK and is available in a number of preparations with different potencies or strengths. Therefore, the aim of the SMART program of studies is to conduct trials in eczema patients involving treatment of separate areas of their skin with either crisaborole ointment or betamethasone valerate cream of different potencies. The effects of the treatments will be assessed using the non-invasive skin imaging techniques.

Both types of treatment have already been tested in clinical trials for clinical efficacy, and so efficacy will not be assessed again here. This study will confirm whether or not crisaborole ointment causes the same unwarranted skin thinning caused by the moderately potent betamethasone valerate 0.025% cream in a direct comparison.

The first SMART study (described in a separate protocol) is currently underway and investigates the effects of crisaborole ointment compared to betamethasone valerate 0.1% cream, which is a more potent (stronger) preparation. Having a better understanding of the unwanted effects of these treatments will be informative for prescribers/doctors and patients. The findings will also help identify important ways of measuring skin thinning for use in future clinical trials.

2. BACKGROUND

2.1 Socioeconomic impact of Atopic Dermatitis/Eczema (AD)

AD is a chronic, relapsing, inflammatory disease of the skin. Its prevalence continues to increase throughout the world, affecting 15–30% of children and 2–10% of adults[1]. In the UK AD accounts for the highest number of consultations with a General Practitioner (GP) for a skin complaint[2].

The primary event in the development of AD is breakdown of the 'skin barrier', formed by the intact stratum corneum (Figure 2.1).[3] A dysfunctional skin barrier permits the penetration of irritants and allergens, which subsequently trigger immune system hyper reactivity. AD is the first step along the atopic march leading to the development of food allergy, asthma, and allergic rhinitis.[4] Together these are the most common chronic diseases of childhood and a major financial burden to health services: in the UK alone the direct costs to the National Health Service (NHS) are estimated at over £1billion per annum[5].

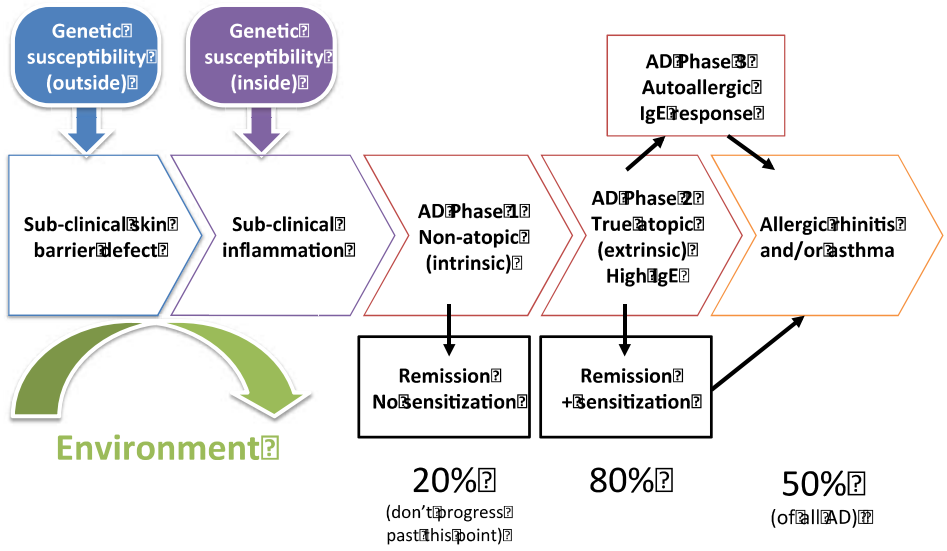

Fig 2.1: The development and progression of AD

2.2 The limitations of current treatment strategies

The mainstay of treatment for flares of AD[6] is the use of topical corticosteroids (TCS). It is well established that intensive treatment (once or twice daily) with TCS is effective at supressing clinically visible skin inflammation (Figure 2.2). However, the overuse, or inappropriate use, of TCS causes atrophy of the skin tissue, characterised by whole skin thinning and damage to the skin barrier (sub-clinical adverse effects). The atrophic changes associated with TCS use are progressive, starting with thinning of the epidermal and dermal layers and leading to telangiesctasia, hematomas, and eventually skin lacerations/fissures, loss of skin barrier function and delayed healing (clinical adverse effects). This process, whereby the skin is eroded away until it loses functional capacity is sometimes referred to as dermatoporosis. Even a defective/sub-optimal skin barrier cannot provide proper protection from

harmful exogenous agents such as irritants and allergens[7], and predisposes the skin to further flares[3]. The adverse effects of chronic TCS use therefore appear to contribute to the chronicity of AD.

In recognition of these adverse effects of TCS on the skin, current guidance[6] suggests limiting intensive TCS treatment to 'short-courses', for which there is no clear definition. This creates a clinical dilemma: on one hand TCS treatment should be limited to avoid adverse effects, but on the other hand TCS treatment should be continued long enough to fully suppress inflammation (including sub-clinical inflammation) and maintain remission. In clinical practice, the main problem with TCS is its underuse due to inappropriate fear of side effects, yet clear guidance based on evidence for exactly how long TCS should be used and in what way is lacking. Recent evidence[8] suggests that the period of remission (flare free period) can be prolonged, by gaining control of clinical and sub-clinical inflammation early on (Figure 2.3). However, the relationship between the duration of treatment of sub-clinical inflammation (induction of remission), the development of local/skin adverse effects, and the long-term control of AD (the frequency of flares) has not been fully established.

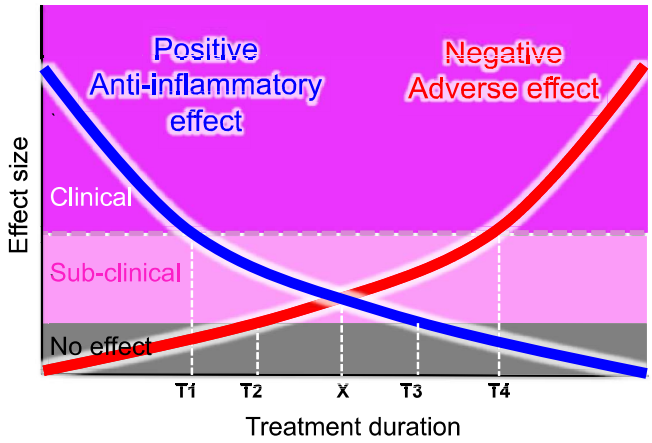

Fig 2.2: Positive and negative effects of TCS treatment on the skin (blue and red lines, respectively). Initially TCS exert positive effects by effectively reducing clinical/visible inflammation (T1). Clinically visible adverse effects (skin thinning) arise with prolonged, or inappropriate, use of TCS (T4). Sub-clinical (non-visible) inflammation persists in the skin following resolution of clinical inflammation, and is suppressed upon continued intensive treatment (T3). It is not known when sub-clinical adverse effects arise (T2) in AD patients undergoing treatment, or what the optimum trade-off is (X).

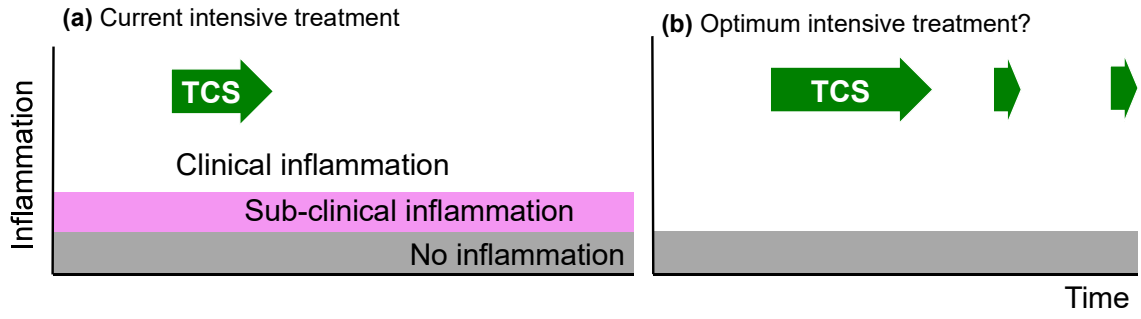

Fig 2.3: The effect of sub-clinical inflammation on the control of AD. The period of remission (flare-free) can be prolonged, by prolonged use of TCS to control clinical and sub-clinical inflammation early on.

Topical anti-inflammatory treatments without local adverse effects, or treatment approaches that minimise these effects, have the potential to change the current treatment paradigm for mild-moderate AD. Topical calcineurin inhibitors (TCI), with comparable efficacy to mild and moderately potent TCS, do not appear to cause local adverse effects on the skin barrier or epidermal atrophy. The use of TCI is limited, however, due to the burning/stinging sensation they induce upon application in AD patients and the warning relating to an unconfirmed cancer risk. In both cases there is a need to further establish the local adverse effects of different treatment approaches to provide better guidance on their optimum safe use.

2.3 Preliminary data on the local adverse effects of TCS on the skin

We have previously investigated the comparative effects of the TCS betamethasone valerate (0.1%) cream and equivalent potency TCI tacrolimus (0.1%) ointment on the skin barrier and epidermal thickness (see attached publications). In short betamethasone valerate (0.1%) cream, but not tacrolimus (0.1%) ointment: decreases skin barrier function (increased Trans-epidermal water loss, TEWL) (Fig 2.4); decreases skin barrier integrity and inter-corneocyte cohesion (Fig 2.5); suppresses natural moisturising factor (NMF) constituent (2-pyrrolidone-5-carboxylic acid and urocanic acid) levels (Fig 2.6); and induced epidermal atrophy measured using structural optical coherence tomography (OCT) (Fig 2.7).

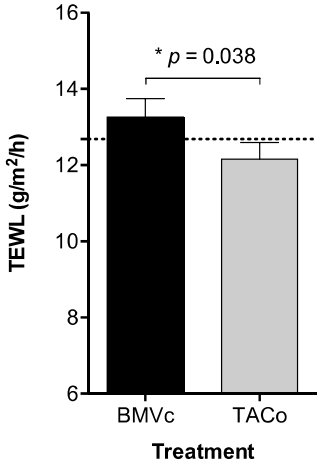

Fig 2.4: The effect of betamethasone valerate (0.1%) cream (BMVc) and Tacrolimus (0.1%) ointment (TACo) on skin barrier function in patients with quiescent AD. TEWL was significantly different post-treatment, accounting for baseline measurements (n = 25, one-way ANCOVA, $p = 0.038$). The dashed line indicates mean TEWL before treatment.

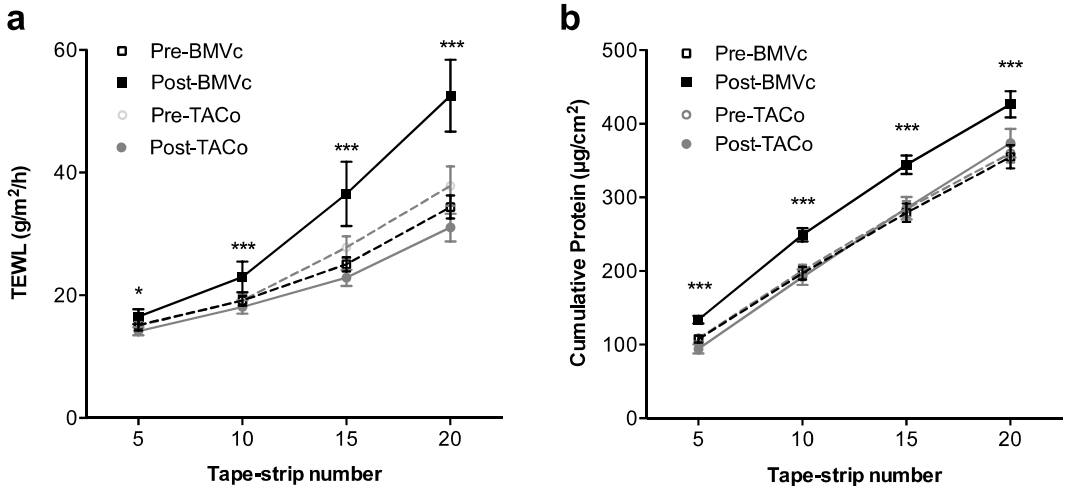

Fig 2.5: The effect of betamethasone valerate (0.1%) cream (BMVc) and Tacrolimus (0.1%) ointment (TACo) on skin barrier integrity and cohesion. (a) TEWL measured in conjunction with skin tape-stripping (STS) was significantly higher following treatment with BMVc compared with TACo (n = 25, $p = 0.0024$). (b) The cumulative amount of protein removed by the tape-strips (TS) was significantly higher following treatment with BMVc compared with TACo (n = 25, $p < 0.0001$). Asterisks indicate the results of a Tukey post-test comparing BMVc and TACo treated sites (*$p<0.05$, ***$p<0.001$).

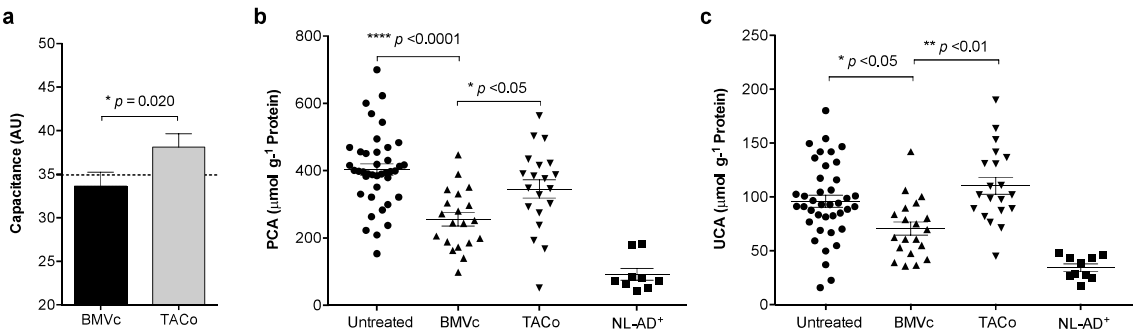

Fig 2.6: The effect of betamethasone valerate (0.1%) cream (BMVc) and Tacrolimus (0.1%) ointment (TACo) on the water holding capacity of the SC. (a) SC hydration measured using the capacitance method. A one-way ANCOVA revealed a significant difference between the two treatments (n = 25, $p = 0.020$). The dashed line indicates mean capacitance before treatment. (b) The level of NMF components 2-pyrrolidone-5-carboxylic acid (PCA) and (c) urocanic acid (UCA) detectable in the SC, as measured *ex vivo* by HPLC (n = 25). BMVc significantly lowered PCA (****p <0.0001) and UCA (*p <0.05) levels compared to untreated skin. For clinical relevance both PCA and UCA were quantified in *FLG* mutation carriers ($n = 6$) with active AD at non-lesional sites (NL-AD). $^{+}$All groups were significantly different from NL-AD (*p <0.05).

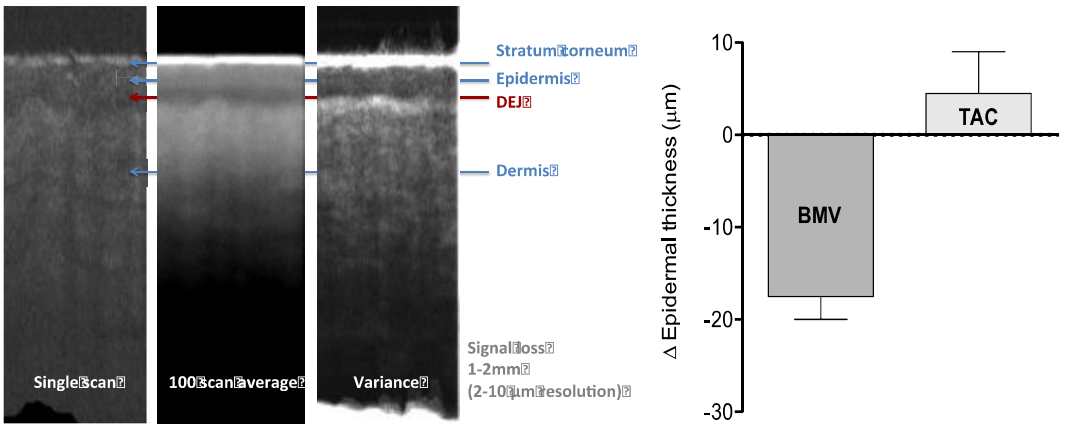

Fig 2.7: (Left) Representative raw B scan of epidermis (highlighted) determined by structural OCT. **(Right)** Change in epidermal thickness following a 4-week, twice-daily treatment regimen using betamethasone valerate (0.1%) cream (BMV) and Tacrolimus (0.1%) ointment (TAC).

The suppression of NMF is important for 2 reasons: (1) NMF plays an important role in skin barrier homeostasis (pH regulation) and moisturization and (2) NMF is a downstream product of Filaggrin catabolism and so its reduction suggests a decrease in filaggrin levels/ *FLG* gene (encoding filaggrin) expression. Reduced filaggrin levels/*FLG* gene expression is a key cause of skin barrier dysfunction, and patients exhibiting a deficit of functional filaggrin are predisposed to more severe, persistent, AD, depending on the extent of the deficit. What is not clear from the literature is whether filaggrin function itself is important, or its role in providing the material for NMF or a combination of both (the latter being most likely) due to the pivotal functions of both filaggrin and NMF.

Together the data clearly evidences the negative local adverse effects of TCS, which can worsen AD after the ant-inflammatory effects have waned following TCS discontinuation, and helps establish a set of benchmarks upon which to compare alternative topical ant-inflammatory treatments.

2.4 Preliminary data on new novel biomarkers of TCS-induced skin damage

Whilst structural OCT analysis of epidermal thickness provides a useful measure of TCS-induced skin atrophy (Fig 2.7), its application in AD patients is limited due to a loss of definition in the boundary between the epidermis and dermis in inflamed skin. Through additional image analysis using software developed in-house structural OCT images acquired using the VivoSight clinical OCT system can reveal detailed angiographs of the skin. We have shown that vascular changes can be associated with structural skin changes, including epidermal thickness, and that the vascular architecture of the skin changes appreciably in patients with AD (even in the absence of clinical inflammation). Importantly the ability to visualise and analyse the vascular architecture of the skin is not affected by disease severity, making it a more robust analytical approach compared to structural measurements. Figure 2.8 illustrates the analysis of the OCT images. In addition, angiographic OCT may offer additional early biomarkers relating to the later clinical adverse effects of TCS (telangiectasia and hematomas for example). We therefore propose that angiographic OCT measurements will offer more robust and informative biomarkers of TCS-induced adverse effects.

A variant of OCT, referred to polarisation sensitive (PS)-OCT can be used to measure skin birefringence, which relates to the density and directionality of collagen fibres. The undue inhibition of collagen synthesis is an adverse effect associated with epidermal atrophy that occurs following prolonged, or inappropriate, use of TCS. We propose using a custom-built PS-OCT to measure birefringence before and after treatment to explore whether this modality can provide a new novel biomarker for the adverse effects of TCSs.

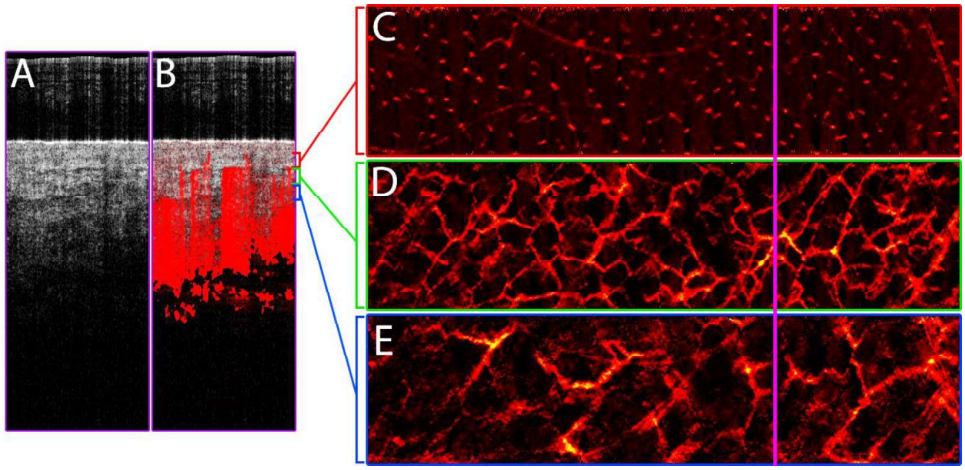

Fig 2.8: Angiographic analysis of OCT images

We have also used Fourier-Transform InfraRed (FTIR) spectroscopy to quantify the molecular structure of the skin, including the levels of carboxylate groups (which relate to NMF levels and filaggrin gene expression), the arrangement of lamellar lipids (which also relates to skin permeability barrier function, TEWL), and the water content of the skin among other structural parameters (Figures 2.9 and 2.10). Treatment with TCS has been shown to inhibit epidermal differentiation and negatively affect lipid metabolism in the skin. These changes underpin the negative effects of TCS on skin barrier function. We have already demonstrated that TCS-treatment leads to the suppression of NMF levels in stratum corneum samples using a HPLC-based technique. The application of FTIR to the assessment of TCS-induced skin changes could therefore introduce a new panel of early biomarkers for skin barrier structural defects.

Angiographic OCT, PS-OCT, TEWL and FTIR skin structure measurements all have the potential to provide early biomarkers relating to the adverse effects of TCS which require further development and validation in order to define an optimum limited panel for future use as safety biomarkers in future clinical trials.

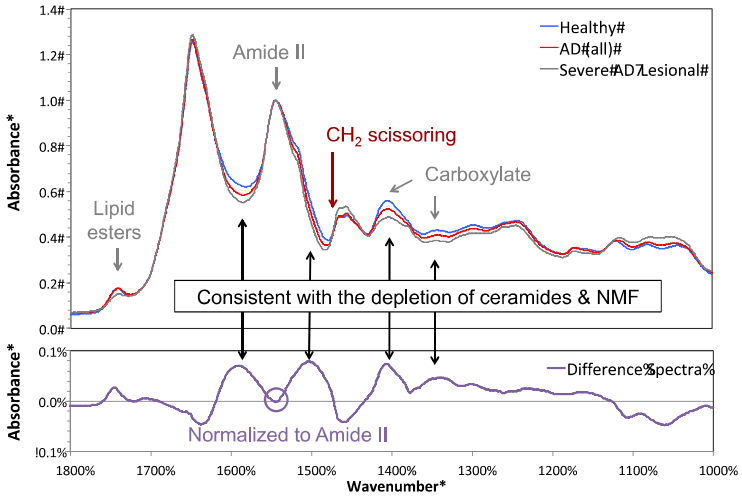

Fig 2.9: Top: Mean ATR-FTIR absorbance spectra collected at the antecubital fossa for healthy participants (blue line), all AD patients (red line), and severe AD patients with clinical signs at the test sites (grey line). Bottom: Difference spectra (Healthy – all AD).

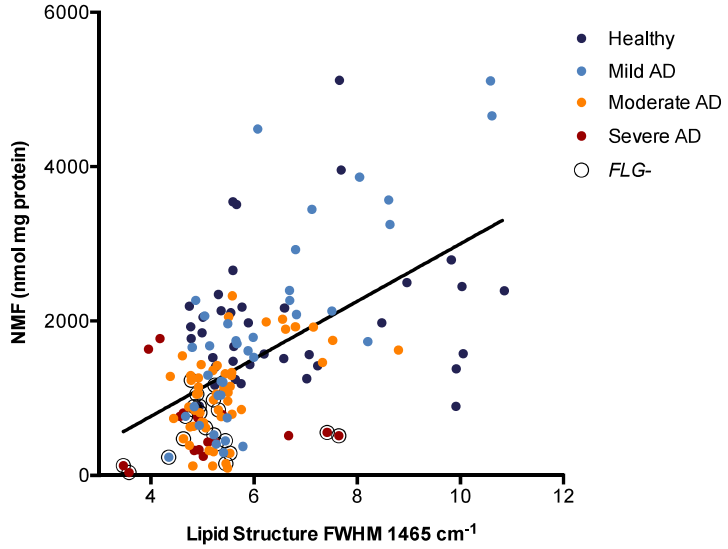

Fig 2.10: Correlation between stratum corneum NMF levels and lipid structure. Lipid structure was determined according to a previously published protocol. FWHM, full width at half maximum – a spectral feature associated with lipid membrane lateral chain packing. A highly ordered orthorhombic state (toward FWHM of 12) is associated with optimum permeability barrier function (TEWL).

2.5 Key questions to be answered

In summary the overuse, or inappropriate use, of ***TCS causes local adverse effects that exacerbate the underlying skin barrier defect in patients with AD*** leading to poor control of the condition. In acknowledgement of this fact, coupled with the lack of first-line alternative treatment options (beyond TCI's, currently reserved as a second-line treatment with poor uptake), a key question of healthcare

practitioners and patients alike is "***how long can we use TCS before they induce clinically relevant skin barrier damage and skin thinning***"[9].

With the introduction of new treatment options the pressing question will become: "does the new treatment cause local adverse effects, and therefore does it alter the current treatment paradigm".

A barrier to addressing these questions is the availability of validated non-invasive early biomarkers for the assessment of TCS-induced adverse effects. ***We aim to tackle this barrier by evaluating two new non-invasive technologies for assessing skin properties to identify and validate a panel of safety biomarkers.***

2.6 Selection of the study TCS

There are a number of TCS preparations available, and to conduct a robust controlled comparison a single comparator is required to represent TCS's as a class. A breakdown of the 10 most commonly prescribed TCS preparations (excluding preparations with additional active ingredients, such as anti-fungal and antibiotic agents) by the National Health Service in England for 2016 is provided in the table below. In terms of risk, mild TCS's such as hydrocortisone cream 1%, exhibit the least risk of local adverse effects, and so were eliminated from our selection. Betamethasone valerate cream is therefore the most widely prescribed TCS in the UK and appropriate for our study population, making it our chosen representative TCS for this study in both potent (0.1%) and moderately potent (0.025%) cream forms. The first SMART study makes use of the potent preparation and this second study makes use of the moderately potent preparation.

Table 2.1: TCS ranked by prescriptions

Rank	Name (active compound)	Potency (2007 NICE guidelines)	Number of Items issued
1	Hydrocortisone cream 1%	Mild	1,504,569
2	Betamethasone Valerate cream 0.1%	Potent	1,053,999
3	Betamethasone Valerate ointment 0.1%	Potent	619,214
4	Clobetasone butyrate 0.05% cream	Moderately potent	608,586
5	Clobetasone butyrate 0.05% ointment	Moderately potent	521,258
6	Hydrocortisone ointment 1%	Mild	471,768
7	Mometasone Furoate ointment 0.1%	Potent	436,618
8	Clobetasone Proprionate oint. 0.05%	Very potent	389,292
9	Clobetasone Proprionate 0.05% cream	Very potent	352,078
10	Mometasone Furoate cream 0.1%	Potent	344,302

2.7 Crisaborole

Crisaborole 2% ointment has been approved and marketed for twice daily use in the topical treatment of mild to moderate AD in patients 2 years of age and older in Australia (Staquis), Canada (Eucrisa), Columbia, China (Staquis), Hong Kong (Staquis), Israel (Staquis), Macau, South Africa, Taiwan (Staquis) and the United States (US, Eucrisa). In the US, Canada and Taiwan the indication has been expanded to the topical treatment of mild to moderate AD in adult and paediatric patients 3 months of age and older. This same expanded indication has been approved in Argentina, Lebanon, Oman, Qatar and the UAE under the brand name Staquis. To date, no significant information has arisen regarding the marketed use of this product.

Crisaborole is a low molecular weight nonsteroidal benzoxaborole phosphodiesterase 4 (PDE-4) inhibitor. The route of administration is topical in a 2% ointment preparation. PDE-4 inhibition results in increased intracellular cyclic adenosine monophosphate (cAMP) levels. The specific mechanism(s) by which crisaborole exerts its therapeutic action is not well defined. Crisaborole reduces the production of several inflammatory cytokines implicated in the pathophysiology of AD and other inflammatory skin diseases, including TNF-α, IFN-γ, IL-2, IL-5, IL-6, IL-10, IL-12, and IL-23. Crisaborole applied to human skin *ex vivo* or on AD lesions of patients reduces expression of key drivers of AD including T-cell derived cytokines interleukin (IL)-13, IL-31, and interferon gamma (IFN-γ) as well as innate markers of inflammation such as matrix-metalloproteinase (MMP)-12. The clinical efficacy and safety of crisaborole have been demonstrated in 2 Phase 3 randomised controlled trials and 1 Phase 3 open label extension study.[10,11] Crisaborole 2% ointment, applied twice per day to affected skin, recently demonstrated clinical efficacy in the treatment of mild-moderate AD.

The two Phase 3, multicenter, randomised, double-blind, vehicle-controlled studies evaluated the efficacy and safety of crisaborole in patients $\geq$ 2 years of age with mild to moderate AD affecting at least 5% body surface area (BSA).[11] A statistically significantly higher percentage of patients in the crisaborole group met criteria for success as measured by the Investigator's Static Global Assessment (ISGA) scale (score of 0 or 1 and $\geq$ 2-point improvement from baseline) compared with those given vehicle at Day 29 (study AD-301: p=0.038 and study AD-302: p<0.001). Crisaborole treatment resulted in a statistically significantly higher percentage of patients with AD rated as clear or almost clear (score of 0 or 1 on ISGA) at Day 29 than vehicle treatment (study AD-301: p=0.005 and study AD-302: p<0.001). A pre-defined supportive efficacy endpoint of Time to Improvement in Pruritus was defined as a pruritus score of None [0] or Mild [1] with at least a 1-grade improvement from baseline (on a scale of 0 [none], 1 [mild], 2 [moderate], 3 [severe]). The crisaborole group achieved Improvement in Pruritus earlier than vehicle (pooled data, 1.37 days vs 1.70 days, p=0.001).

2.8 Assessment and management of risk

2.8.1 Potential ethical issues arising from this study

In order to determine a set of safety biomarkers it is necessary to characterise an unsafe treatment scenario, which raises important ethical questions about asking participants/patients to undertake a

treatment that may cause them harm. It is important to highlight that this study focuses on 'early' 'transient' signs of 'skin atrophy', and so there is no intention to induce clinical (visible) adverse effects. We have already established that 4 weeks of treatment with betamethasone valerate 0.1% induces sub-clinical skin barrier disruption and epidermal skin thinning without inducing clinical adverse effects. In this study the less potent TCS, betamethasone valerate 0.025%, will be used following the same regimen. Published literature further demonstrates that epidermal skin thinning induced during short courses of TCS treatment is transient, with skin returning to pre-treatment thickness within a matter of weeks following cessation of treatment.

The treatment regimen proposed for the antecubital fossa and volar forearms (4 weeks twice daily) conforms to the current marketing licence for Betnovate RD cream. However, the duration of treatment proposed for the cheeks (2 weeks twice daily) exceeds the guidance in the label and SmPC, which suggests treatment is restricted to a maximum of 5 days. A justification for this is provided below, including steps to mitigate any risks. It is important to recognise that the treatment regimens proposed are in line with current clinical practice (including secondary care), making a strong ethical argument to identify any potential harm using this product may have under the proposed conditions.

In short, we do not expect to compromise the safety of participants as a result of their taking part. Nevertheless, we will monitor and report any adverse events, and cease treatment in any participant showing clinical signs of clinical skin atrophy, adding a level of safety above that of standard care.

The study is not expected to raise any other significant ethical issues above and beyond those associated with conducting clinical research.

2.8.2 Justification for the proposed treatment regimen

The treatment period has been determined based on clinical guidelines, practice and evidence from previous clinical trials of TCS use. Current UK guidance recommend that "As a rough guide, steroid use should be limited to a few days to a week for acute eczema and up to 4–6 weeks to gain initial remission for chronic eczema".[12] Published trials investigating TCS use for the treatment of AD have focused on once to twice daily regimens of between 2-4 weeks, although there are examples of longer treatment regimens up to 8 weeks duration. A recent systematic review of these studies found no evidence of skin thinning when TCS were used intermittently 'as required' to treat flares, but noted that the majority of data was from short-term studies.[13] A review of TCS-induced atrophy found evidence of reversible skin thinning in response to short-term TCS treatment, related to TCS potency, dose and duration in weeks.[14] Moderately potent TCS were associated with 3 to 19% (whole) skin thinning depending on dose (3 time daily to 3 times weekly) and duration (4 weeks to 8 weeks). The very potent TCS clobetasol propionate was associated with between 6 and 22% (whole) skin thinning (8-59% thinning of the epidermis) depending on dose and duration. The effects of TCS treatment on skin thickness appear to reverse within about 2 weeks following discontinuation.[14] Moreover, another systematic review of steroid addiction highlights that the clinical consequences of this thinning develop following years of misuse.[15] In a large study examining the long-term effects of TCS use, just one episode of skin thinning was reported in 1213 children using mild/moderate TCS 'as required' with a 5-year follow-up.[13]

Given that the current guidance recommends up to 6 weeks continuous use, and that epidermal thinning is observed following just 4 weeks of use, we propose a 4-week treatment duration on the antecubital fossa and volar forearm with evaluations at 1 and 2 weeks to assess the speed of onset of epidermal thinning. A follow-up 4 weeks after cessation of treatment is included to confirm whether the thinning fully reverses.

Most investigations of TCS induced atrophy have focused on the volar forearm and in some cases the abdomen and back. As such we propose additionally monitoring the effects of treatment at other anatomical sites, including the antecubital fossa and cheek. The cheek is subject to additional restrictions within the current guidance however. The product information leaflet states that the "use on children or on the face should be limited to 5 days". This is in line with guidance stating that "On the face mild TCS should be used. For severe flares use moderate potency TCS for 3-5 days only".[16] This appears to be based on the fact that drug uptake is greater and the observation that the risk of TCS addiction is greater with excessive use on the face over long periods of time. Despite the guidance and the warning in the product label, use of TCS on the face for longer periods is not uncommon in practice to control inflammation and there is a need to explore the effects of longer treatment durations.

In order to better understand the effects of TCS treatment on the structure of the skin of the face we propose a 2-week treatment regimen for the cheek, with an interim assessment after the first week. **To mitigate any risks of prolonged treatment we will stop treatment after 1 week if epidermal thinning reaches ≥30%.** Whilst the cheek exhibits a thinner stratum corneum, the thickness of the epidermis appears to be similar to the volar forearm and less than the antecubital fossa.[17] A reduction in the thickness of the epidermis by 30% can be expected after 4 weeks of treatment at other anatomical locations with a potent TCS and is reversible.[14] Thinning of up to -59% has been observed with clinically acceptable regimens on the volar forearms with clobetasol propionate. Given the transient nature of epidermal thinning of this scale the 30% limit should mitigate the risk of excessive epidermal thinning due to the increased absorption of drug at the cheek. **As a precaution we will review epidermal thickness data collected after the 5th and 10th participants and adjust the treatment duration down if excessive levels of epidermal thinning are observed.** This will ensure the study is conducted safely whilst also providing data on whether the 5-day limit on application in the label is appropriate for this anatomical location.

2.8.3 Potential risks associated with the IMP crisaborole

Crisaborole has been well tolerated across completed clinical studies (28 to date, involving 2558 participates from 3 months to adults >18 years of age). No clinically important systemic safety signals have been identified. Most adverse events (AEs) have been mild, and most considered unrelated or unlikely to be related to study drug. The most common drug-related AEs have been application site reactions. Studies in pregnant or lactating populations have not been conducted, and so this group will be excluded from participation and steps taken to reduce the risk of pregnancy whilst using the study medication.

A number of studies have investigated the skin tolerability of crisaborole 2% ointment to date, including 3 phase 2 studies and 3 phase 3 studies. A phase 1 study (AN2728-PSR-107) specifically investigated the local tolerability of crisaborole 2% ointment at delicate/sensitive skin areas. Thirty-

two healthy participants applied crisaborole 2% ointment twice daily to 13 anatomic areas including the face and genitals for 21 days. No marked differences in tolerability (burning/stinging, erythema, pruritus) were found between participants who received crisaborole 2% ointment or vehicle control. In subsequent phase 2 and 3 therapeutic clinical trials in AD patients only the scalp has been excluded as a treatment site (relating to acceptability of the ointment vehicle at this area with dense hair), with the decision to treat particular anatomical areas based on clinical need (presence of clinical inflammation). The marketing authorisation for crisaborole 2% ointment in the US does not restrict treatment to particular anatomical areas and establishes no maximum duration of treatment or maximum body surface area for application. The majority of safety and efficacy data pertaining to the therapeutic use of crisaborole 2% ointment for AD is derived from treatment regimens of 28-days duration (BID).

2.8.4 Potential risks and burdens associated with the skin procedures

This study involves a series of non-invasive procedures to test the biophysical properties of the skin. These procedures have all been used safely in previous clinical studies, and so the risks of harm are minimal. The OCT and PS-OCT equipment comprises a class 1M laser and Class 3 laser attenuated down to class 1 AEL respectively, making them safe for the proposed testing under normal operating conditions. A regular maintenance plan and risk assessment has been developed to mitigate the risks of participants becoming exposed to the unattenuated Class 3 laser. The skin sites for assessment are located on the volar side of the forearm, the elbow crease (antecubital fossa) and face (cheek), and so participants will only be required to roll up their sleeves, and where appropriate remove face masks*, for the tests to be performed.

The following steps will be taken to avoid any risk of physical and/or psychological harm to participants:

- Participants will be notified in advance of the anatomical locations we are interested in and encouraged to wear appropriate clothing.
- Experienced members of staff who have received appropriate training will conduct all of the test procedures. This includes laser safety training in order to operate the OCT and PS-OCT devices.
- The equipment regularly undergoes maintenance, performance verification and biomedical engineering testing for accuracy and safety. This includes assessment of laser safety by the Sponsor biomedical engineering department.
- 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.
- *The Sheffield Dermatology Research team has a detailed COVID-19 working policy and risk assessment; the policy includes safety precautions for close contact working (<2-meter social distancing) and collection of measurements from participants without face coverings – in each case close contact PPE is required for staff members (apron, mask and gloves). Visors to be worn in addition to close contact PPE when performing procedures with a risk of generating aerosols, such as when taking buccal swab samples.

2.8.5 Potential risks associated with the sample collection

Three types of samples will be collected in a non/minimally-invasive way: (1) superficial stratum corneum (skin) samples by STS, (2) saliva samples by buccal swabbing, and (3) skin biopsy collection. STS 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. STS will not be performed on broken skin. The procedure is now routinely used in clinical trials in participants of all ages from birth upwards. The stratum corneum regenerates fully every 2 weeks and so the 28-day follow-up is sufficient to ensure STS sites are fully healed.

The collection of skin biopsy samples may cause both physical and psychological harm to participants; however, the risk of any physical harm is minimal due to the procedure being routine in dermatology clinics and being performed by an experienced clinician. Ten adult participants, out of 40 recruited, are required to provide 2 biopsies each. The procedure will be treated as optional (although it will be compulsory for the final 10 recruits if 10 participants do not voluntarily opt to provide biopsies), on a first come first served basis, and require specific consent. Additional remuneration of £100 per biopsy will be provided in recognition of the burden of providing the samples. In our experience, with this rate of remuneration, we see good uptake, with all adult participants in our recent SPOT study consenting to provide biopsies. Obtaining the biopsies is necessary to validate the non-invasive techniques under investigation against histological analysis (current gold standard). It should be noted that by providing a robust validation of the non-invasive techniques under investigation here the need to collect biopsies in future clinical studies could be avoided.

2.8.6 Potential burden of taking part

Due to the number of site visits and the frequency of compliance checks there is a significant burden on participants' time. This burden will be lessened by providing taxi transport to and from the test centre. Participants will also be remunerated for the burden they endure, which may involve time taken out of work for example (with remuneration being commensurate with the level of involvement for those not completing the full study).

2.8.7 Data management issues arising from this study

The study involves the collection of a number of instrumental skin measurements (readings taking from skin diagnostic devices), skin images and spectra (as raw data files). Data management activities will be undertaken by The Sheffield Clinical Trials Research Unit (CTRU), an experienced provider that has undergone MHRA inspection. A bespoke database based on the Prospect system, which is fully compliant with current clinical trial regulations, will be developed by the CTRU for this study.

To preserve the integrity of skin images and spectra, the raw data files will be directly transferred via intranet connection to the secure study server managed by the University of Sheffield. All files will be date stamped and collection will be tracked in the associated eCRF.

Access to the eCRF and study server will be restricted to only the system administrators and the direct study team as per delegation in the study delegation log.

2.8.8 Retention of samples at the end of the study

The TS samples are not considered relevant material under the Human Tissue Act as only enucleated non-viable corneocytes are collected.

The saliva samples collected will be processed during the course of this study to isolate genomic DNA. Specific consent will be obtained from participants to store this genomic DNA beyond the end of this study.

The skin biopsy samples will be retained by the SDR group (University of Sheffield) for use in future research at the end of this study (subject to additional REC approval). Sample will only be collected where specific consent for this is freely provided.

2.8.9 Risk of breach of confidentiality

We will take steps to preserve the confidentiality of participants. The participants in this study will be advised that their study data will be held securely, identified only by a unique study number, and that all personal information will be kept strictly confidential. Identifying personal details will only be kept on the study registration form and the Participant Screening, Enrolment & Completion Log. These documents provide the only link between personal identifiable information and the study participant number, and will be kept separately from study data, and stored securely in the study office. The information in the study registration form is also stored separately in the SDR volunteer database where specific consent is provided in the consent form for a period limited to no more than 5 years.

3. RATIONALE

The first-line drug treatment for mild-moderate AD is currently a TCS. TCS are clinically efficacious, however their prolonged, or inappropriate use, can lead to local adverse effects. These effects include epidermal atrophy, suppression of skin barrier homeostasis (i.e. decreased expression of filaggrin and its downstream metabolites), and reduced permeability barrier function. For the potent TCS betamethasone valerate (0.1%) cream these effects appear within 4 weeks of treatment when applied to clinically clear skin. These adverse effects limit the clinical utility of TCS, and underpin the clinical need for alternative treatment approaches and alternative topical anti-inflammatory treatments for AD. A barrier to assessing the safety (local adverse effects) of new treatments and treatment approaches is the ability to non-invasively monitor early skin (subclinical) changes associated with the local clinical adverse effects of TCS.

Our preliminary research demonstrates that OCT and FTIR spectroscopy can be used in a clinical trial to non-invasively monitor the sub-clinical changes at the volar forearm associated with atrophy and skin barrier damage respectively. To validate the outcomes derived using these technologies the first SMART study compares the effects of 4 weeks twice daily topical therapy with the potent topical corticosteroid betamethasone valerate 0.1% cream, which is known to induce sub-clinical epidermal atrophy, to that of a new topical anti-inflammatory treatment crisaborole 2% ointment. In two phase-3 clinical studies, twice daily treatment with crisaborole (2%) ointment for 4 weeks was found to be an efficacious and well-tolerated treatment for mild-moderate AD when compared to no treatment. As a non-steroidal therapy, crisaborole is not expected to induce epidermal atrophy and skin barrier damage making it an ideal comparator. In this study we aim to compare crisaborole to a less potent TCS to ascertain the local adverse effects of these treatments at a broader range of anatomical and further validate the biomarkers for these sub-clinical changes.

3.1 Study Aim

- To directly compare the effects of crisaborole (2%) ointment to the moderately potent TCS betamethasone valerate (0.025%) cream on the properties of the skin at different anatomical locations (volar forearm, antecubital fossa and cheek) using an established model for quantifying the local adverse effects of TCS.
    - 4 weeks twice daily applications to the antecubital fossa and volar forearm
    - 2 weeks twice daily applications to the cheek
- By achieving this aim, we will provide the data needed to validate a panel of non-invasive biomarkers of epidermal atrophy/local adverse effects of TCS treatment with future potential for the development and testing of topical treatments for inflammatory skin conditions.
- The study will confirm biomarker data gathered using OCT and FTIR spectroscopy, specifically in the context of TCS-induced atrophy, through histological analysis of the skin tissue for vascular, collagen, lipid and protein (filaggrin) changes.

3.2 Hypothesis

The associated hypotheses are:

- Treatment with a moderately potent TCS induces atrophic changes (reduced epidermal thickness determined by structural/angiographic OCT), and skin barrier disruption (reduced NMF levels and altered stratum corneum lipid structure) not observed with comparable crisaborole (2%) ointment treatment.
- Epidermal atrophy and skin barrier disruption is more pronounced and develops sooner at delicate skin sites, defined as sites of predilection to dermatitis and having a thinner epidermis and lower skin barrier function.

4. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

The objectives of this study, and the associated outcome measures and timepoints, are detailed in Table 4.1.

Table 4.1: Objectives and outcome measures

Objectives	Outcome Measures	Timepoint(s) of evaluation
Primary		
1. To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy (on the volar forearm) in patients with AD.	The difference in the change in epidermal thickness (day 29 – day 1) on the volar forearm, measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream.	Structural OCT images of epidermal thickness taken on day 1 and day 29
Secondary		
2. To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy (on the antecubital fossa) in patients with AD.	The difference in the change in epidermal thickness (day 29 – day 1) on the antecubital fossa, measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream.	Structural OCT images of epidermal thickness taken on day 1 and day 29
3. To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 2 weeks is a cause of skin atrophy on the cheeks in patients with AD.	The difference in the change in epidermal thickness (day 15 – day 1) on the cheeks, measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream.	Structural OCT images of epidermal thickness taken on day 1 and day 15
4. To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream at different anatomical sites	The difference in the change in epidermal thickness measured by structural OCT and angiographic OCT (superficial plexus depth in µm) during and after 28 days treatment on the volar forearms, antecubital fossae and cheeks.	Structural OCT images of epidermal thickness taken on day 1, 15, 29 and day 57 from the volar forearm and antecubital fossa and on day 1, 15, and day 57 from the cheeks. *This outcome will also be assessed at Day 8 as part of the exploratory analysis.

5. To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream	The difference in the change in visual redness/erythema score and objective redness (erythema index from skin images and redness from the Mexameter) during and after treatment.	Visual redness/erythema score and objective redness determined on day 1, 15, 29 and day 57 from the volar forearm and antecubital fossa and on day 1, 15, and day 57 from the cheeks. *This outcome will also be assessed at Day 8 as part of the exploratory analysis.
6. To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on skin barrier function	The difference in the change in TEWL during and after treatment.	TEWL measurements on day 1, 15, 29 and day 57 from the volar forearm and antecubital fossa and on day 1, 15, and day 57 from the cheeks. *This outcome will also be assessed at Day 8 as part of the exploratory analysis.
	The difference in the change in skin barrier integrity/STS (day 29 – day 1) on the volar forearms ($TEWL_{ts20}$) and antecubital fossae ($TEWL_{ts10}$).	TEWL measurements during and after STS on day 1 (FA1) and day 29 (FA2 and CF)
	The difference in the change in visual skin dryness on the volar forearms, antecubital fossae and cheeks during and after treatment.	Visual skin dryness scored on day 1, 15, 29 and day 57 from the volar forearm and antecubital fossa and on day 1, 15, and day 57 from the cheeks. *This outcome will also be assessed at Day 8 as part of the exploratory analysis.
Exploratory*:		
7. To visually confirm that structural OCT measurement of epidermal thickness provides an accurate indication of epidermal atrophy in response to TCS treatment.	Visualise epidermal thickness of skin tissue sections by histology	Skin biopsies collected from a subset of AD participants at day 29
8. To determine the effect of crisaborole (2%) ointment, compared to betamethasone	The difference in the change in blood vessel diameter (μm) and blood vessel density (segments/mm^2) derived from	OCT images taken on the volar forearms and antecubital fossae on day 1, 8, 15, 29 and day 57 and

valerate (0.025%) cream, on epidermal vascular structure	baseline measured by angiographic OCT.	at the cheeks on day 1, 8, 15 and day 57.
a. To visually confirm that angiographic OCT-derived biomarkers provide an accurate indication of vascular changes associated with epidermal atrophy in response to TCS treatment.	Visualise epidermal tissue vascular structure by histological analysis	Skin biopsies collected from a subset of AD participants at day 29
9. To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on collagen matrix structure (fibrosis)	The difference in the change in collagen matrix index (an index derived from birefringence images of collagen density and arrangement) from baseline measured by PS-OCT.	PS-OCT images taken on the volar forearms on day 1, 29 and day 57.
a. To visually confirm that PS-OCT-derived biomarkers provide an accurate indication of collagen matrix changes (fibrosis) associated with epidermal atrophy in response to TCS treatment.	Visualise collagen structure by second harmonic generation imaging of frozen tissue sections	Skin biopsies collected from a subset of AD participants at day 29
	Visualise dermal collagen staining by skin tissue sections	Skin biopsies collected from a subset of AD participants at day 29
10. To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on stratum corneum NMF levels (key skin metabolites linked to skin homeostasis, skin microbiome, and skin moisturization.	The difference in the change in carboxylate levels (indirect measure of NMF levels, filaggrin phenotype) in the stratum corneum from baseline measured by FTIR spectroscopy.	FTIR spectrum of the skin surface taken at the volar forearms and antecubital fossae on day 1, 15, 29 and day 57 and at the cheeks on day 1, 15 and day 57.
a. To confirm that FTIR carboxylate levels enable the accurate quantification of NMF changes associated with epidermal atrophy in response to TCS treatment.	Describe the relationship between HPLC derived NMF levels and FTIR carboxylate levels	FTIR spectra and superficial stratum corneum samples collected at days 1 and 29

b. To visually confirm that FTIR carboxylate levels provide an accurate indication of changes in filaggrin expression (source of NMF) associated with epidermal atrophy in response to TCS treatment.	Visualise skin tissue filaggrin staining by immunohistochemistry	Skin biopsies collected from a subset of AD participants at day 29
11. To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on stratum corneum lipid structure (a key determinant of permeability barrier function and general skin health that is adversely affected by TCS treatment).	The difference in the change in stratum corneum lipid structure (day 29 - day 1) measured by FTIR spectroscopy in conjunction with STS on the volar forearm.	FTIR spectra taken through the stratum corneum from the volar forearm (during STS) on day 1 (FA1) and day 29 (FA2)
a. To confirm that FTIR lipid structure provides an accurate indication of lipid changes associated with epidermal atrophy in response to TCS treatment.	Visualise skin tissue total lipid properties by histology	Skin biopsies collected from a subset of AD participants at day 29
12. To investigate the number of participants with *FLG* loss-of-function mutations and explore if there is any evidence of a relationship to treatment effects	Number of *FLG* loss-of-function mutation carriers Descriptive tabulations of TEWL, epidermal thickness, and carboxylate levels by mutation status, if sufficient participants with mutation are detected.	Saliva sample at visit 1 for *FLG* genotyping As described above

*Exploratory analysis also includes the outcomes identified as exploratory and listed under the secondary outcomes (marked with an asterisks)

5. TRIAL DESIGN

An observer-blind randomised within-subject (bilateral) controlled clinical trial in 33 AD patients is proposed (recruitment target of 40 allowing for an 18% loss-to-follow rate), wherein each participant will undergo treatment with crisaborole (2%) ointment on one side and betamethasone valerate (0.025%) cream on the other (twice daily application in each case and randomised site allocation) for 4 weeks (28 days) on the volar forearms and antecubital fossae and 2 weeks (14 days) on the cheeks. At the start of the study the skin of the measurement sites (except the antecubital fossa measurement sites) will be clear of the signs of AD so that the investigation focuses on local adverse effects on the skin as opposed to anti-inflammatory effects (focus on local adverse effects and not clinical efficacy). The condition of the skin will be assessed before, during and after treatment. An overview of the design is provided below (Figure 5.1).

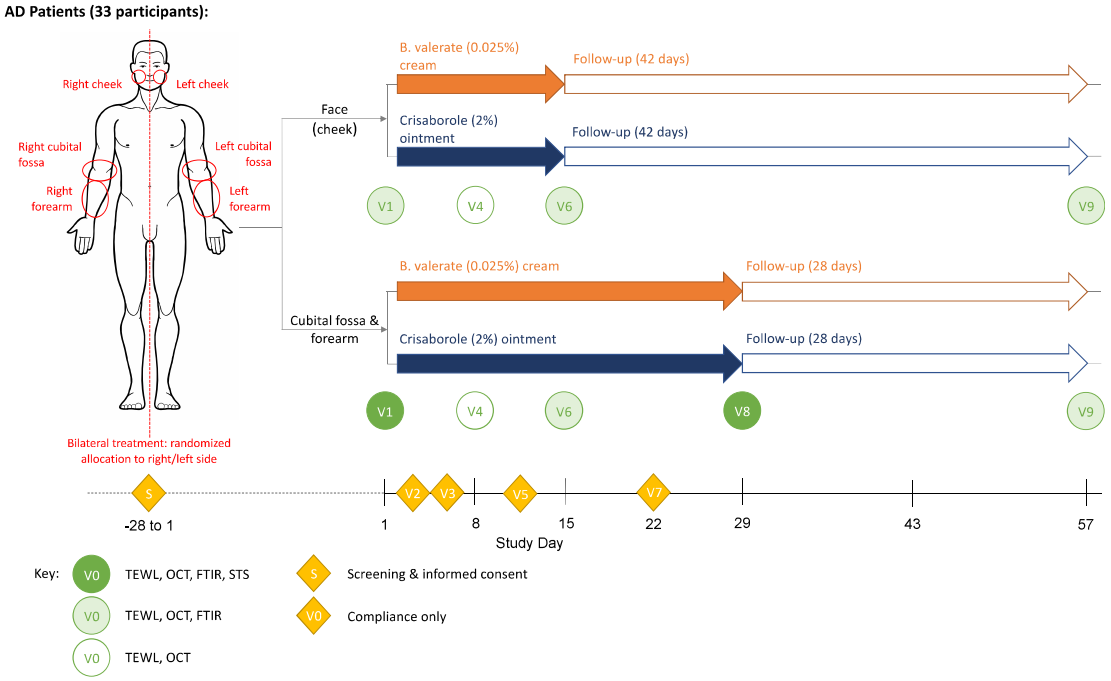

Figure 5.1: Study overview

STS will be performed, to assess skin barrier integrity before and after treatment at each site (to 20 TS on the volar forearm, and 10 on the antecubital fossa).

A post-treatment washout period of ≥28 days post-treatment is included to establish whether skin changes re-adjust to baseline and to obtain post-treatment safety information.

5.1 Trial Duration

The duration participants will participate in this study is approximately 10 weeks, including the 1-week run-in period (no topical product use on measurement sites).

The study is expected to last 12 months in total, with recruitment taking 9 months at a rate of ≥4.5 recruits per calendar month.

5.2 Trial setting

This single-centre clinical trial will take place at:

Sheffield Teaching Hospitals NHS Foundation Trust

The Royal Hallamshire Hospital,

Sheffield, S10 2JF

6. PARTICIPANT ELIGIBILITY CRITERIA

6.1 Study Population

A cohort of 40 adult participants (18-64) with AD not currently undergoing, or requiring, active drug treatment at baseline (visit 1) will be recruited to meet the target of 33 for completion (based on a sample size calculation and allowing for a +18% drop out rate).

6.2 Inclusion criteria

1. Volunteers with AD, defined according to the UK working party diagnostic criteria, not currently undergoing, or requiring, active drug treatment at baseline (visit 1)
2. Male or female aged 18-64 years old at baseline (Visit 1)
3. Volunteer understands the purpose, modalities and potential risk of the trial
4. Participants able to read and understand English
5. Participants willing to sign the informed consent

6.3 Exclusion criteria

1. Participants with a known allergy/hypersensitivity to any of the excipients of the trial preparations.
2. Participants with acne, suntan, birth marks, multiple nevi, tattoos, blemishes or dense body hair that obstruct the test areas.
3. Investigator assessment of eczema severity at the measurement (anatomical) sites on the volar forearm and cheek is almost clear or greater (score ≥1) based on the Investigators static global assessment (ISGA) scale at screening and baseline. At the start of the study the skin of the volar forearm and cheek measurement sites will therefore be clear (0) of the signs of eczema.
4. Investigator assessment of eczema severity at the measurement (anatomical) sites on the antecubital fossae is moderate or severe (score ≥3) based on the Investigators static global assessment (ISGA) scale at screening and baseline. At the start of the study the skin of the antecubital fossa measurement sites will therefore be clear (0) of the signs of eczema, almost clear (1) or mild (2).
5. Participants with a condition that in the opinion of the investigator contradicts participation in the study.
6. Pregnant female participants; breastfeeding female participants; and female participants of childbearing potential who are unwilling or unable to use a highly effective method of contraception as outlined in this protocol for the duration of the study and for at least 28 days after the last dose of investigational product.
7. Use of any topical product on the test areas within 7 days prior to Baseline/Day 1, including cosmetic moisturizers and sunscreen. *Participants using any topical products on the test areas within 7 days at the screening visit will be eligible if they are willing and able to wash-out these products for 7 days in total and for the duration of the trial. Such participants will be potentially eligible at screening and will be confirmed as eligible if adequate washout is confirmed at visit 1. Use of moisturizers and/or sunscreen is permitted during the study to*

manage dry skin and sun exposure in areas surrounding but not on or overlapping the test areas.

8. Participants who have used a tanning bed within 28 days of baseline (visit 1). *Participants who have used a sunbed within 28 days at the screening visit will be eligible if they are willing and able to wash-out for 28 days in total and for the duration of the trial. Such participants will be potentially eligible at screening and will be confirmed as eligible if adequate washout is confirmed at visit 1.*
9. Participants who have used any medication that could interfere with the trial aim prior to the start of the study (baseline/visit 1). *Participants using such medication at the screening visit will be eligible if they are willing and able to wash-out these treatments for the applicable washout period as defined by in section 8.8 'Prior and Concomitant Medication' and for the duration of the trial. Such participants will be potentially eligible at screening and will be confirmed as eligible if adequate washout is confirmed at visit 1.*
10. Participants currently participating in another interventional clinical trial.
11. Volunteer is incapable of giving fully informed consent.
12. Participants judged by the PI to be inappropriate for the trial.

6.4 Instructions to participants and lifestyle changes

Participants will be asked not to apply any topical leave-on products to the skin sites of interest for 1 week prior to attending Visit 1/baseline and throughout the study.

The use of their normal wash products is permitted throughout the trial. Participants should plan washes such that the investigational products are applied after (rather than before) washing. Participants should not swim, bathe or wash the treatment areas for at least 4 hours after application of investigational products.

When applying investigational product, the participants will generally not be required to wear gloves. However, they must be instructed to wash their hands with mild soap and water before and after each application.

Participants must not apply the test products in the morning before study visits.

The following life-style restrictions apply:

- Tanning bed use 28 Days Prior to Baseline/Day 1 and throughout the study.
- Use of moisturisers, leave-on cosmetics or sunscreen *on the test areas*, within 7 days prior to Baseline/Day 1 and throughout the study. Use of moisturizers and/or sunscreen is permitted during the study to manage dry skin and sun exposure in areas surrounding but not on or overlapping the test areas.

Where these exceptions are met the PI will determine whether the participant should be withdrawn. In all cases a protocol deviation will be logged.

6.5 Contraception

All fertile female participants who are of childbearing potential as applicable to the study who are, in the opinion of the investigator, sexually active and at risk for pregnancy with their partner(s) must agree to use a highly effective method of contraception consistently and correctly for the screening period, the duration of the active treatment period and for at least 28 days after the last dose of investigational product. The investigator or his or her designee, in consultation with the participant, will confirm that the participant has selected an appropriate method of contraception for the individual participant from the permitted list of contraception methods (see below) and will confirm that the participant has been instructed in its consistent and correct use. At time points indicated in the Schedule of Events, the investigator or his/her designee will inform the participant of the need to use highly effective contraception consistently and correctly and document the conversation and the participant's affirmation in the participant's notes (participant needs to affirm their consistent and correct use of at least 1 of the selected methods of contraception). In addition, the investigator or his/her designee will instruct the participant to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected in the participant.

Highly effective methods of contraception are those that, alone or in combination, result in a failure rate of less than 1% per year when used consistently and correctly (ie, perfect use) and include the following:

1. Established use of hormonal methods of contraception associated with inhibition of ovulation (eg, oral, inserted, injected, implanted, transdermal) provided the participant plans to remain on the same treatment throughout the entire study and has been using that hormonal contraceptive for an adequate period of time to ensure effectiveness.
2. Correctly placed copper-containing intrauterine device (IUD).
3. Male sterilization with absence of sperm in the post vasectomy ejaculate.
4. Bilateral tubal ligation/bilateral salpingectomy or bilateral tubal occlusive procedure (provided that occlusion has been confirmed in accordance with the device's label).

NOTE: Sexual abstinence, defined as completely and persistently refraining from all heterosexual intercourse (including during the entire period of risk associated with the study treatments) may obviate the need for contraception ONLY if this is the preferred and usual lifestyle of the participant.

6.6 Screening failure

All individuals who sign the consent form and either (1) withdraw their participation before the first assessment, (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/visit will be considered a "screening failure."

7. STUDY PROCEDURES

7.1 Overview of procedures

An overview of the study procedures is provided in the Table below, with further details provided in the sub-sections below.

Table 7.1: Schedule of events

Study Procedures: [a]		**Consent & screening**	**Baseline**	**Treatment Phase**						**EoT**	**Follow-up**
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
Study Week:		-3 to 0	0	0	0	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 +3	5 ±3	8 ±2	11 ±3	15 ±3	22 ±4	29 ± 3	57 + 7[b]
Duration (minutes)		20 - 60 min	3 h	20 min	20 min	2 h	20 min	2.5 h	20 min	4 h	2.5 h
Screening, consent and enrolment in a clinical area designated for unblinded assessments											
1	Informed Consent (Informed Consent sheet)	X									
2	Collect demographic information	X	(X)								
3	Capture medical history	X	(X)								
4	Capture concomitant medication	X	(X)								
5	Capture participant height and weight	X	(X)								
6	Carry out EASI and ISGA assessment of eczema severity [d]	X	(X)							X	
7	Confirm suitability against the eligibility criteria [c]	X	(X)								
8	Re-confirm consent verbally		X	X	X	X	X	X	X	X	X
9	Capture of new AE and concomitant medication		X	X	X	X	X	X	X	X	X
10	Conduct a urine pregnancy test (female participants only) [e]	X	X							X	
11	Check that an appropriate method of contraception is in use [e]	X	X			X		X		X	

	Study Procedures: [a]	**Consent & screening**	**Baseline**	**Treatment Phase**						**EoT**	**Follow-up**
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
Study Week:		-3 to 0	0	0	0	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 +3	5 ±3	8 ±2	11 ±3	15 ±3	22 ±4	29 ± 3	57 + 7[b]
Duration (minutes)		20 - 60 min	3 h	20 min	20 min	2 h	20 min	2.5 h	20 min	4 h	2.5 h
12	Access and update patient notes. If the participant has no STH notes already, a new set of notes should be requested.	X	X	X	X	X	X	X	X	X	X
13	Completion of Screening, Enrolment & Completion Log as appropriate	X	X								X
14	Issue of a Randomisation number		X								
15	Check treatment compliance with dosing conditions			X	X	X	X	X	X	X	
Skin assessments and sample collection at the skin barrier suite											
16	Acclimatise measurement sites for 20 minutes		X			X		X		X	X
17	Identify and demarcate the treatment and measurement sites		X			X		X		X	X
18	Indicate the required treatment areas to the participant and document the area of each in cm^2.		X								
19	Visually score eczema severity, skin dryness and redness/erythema at each measurement site [g]		CH, CF, FA1, FA2			CH, CF, FA2		CH, CF, FA2		CF, FA2	CH, CF, FA2
20	Capture the 2D skin images [g]		CH, CF, FA1, FA2			CH, CF, FA2		CH, CF, FA2		CF, FA2	CH, CF, FA2
21	Perform Mexameter measurements at each measurement site (skin redness, 4 repeats per site) [g]		CH, CF, FA2			CH, CF, FA2		CH, CF, FA2		CF, FA2	CH, CF, FA2
22	Take OCT (structural & angiographic) images/scans with the Vivosight at each measurement site (in triplicate) [g]		CH, CF, FA2			CH, CF, FA2		CH, CF, FA2		CF, FA2	CH, CF, FA2
23	Safety measure: Assessment of skin thinning on the cheek		CH			CH					
24	Take PS-OCT images/scans with the PS-OCT machine at each measurement site (in triplicate) [g]		FA2							FA2	FA2
25	Measure TEWL at each measurement site (in triplicate) [g]		CH, CF, FA1, FA2			CH, CF, FA2		CH, CF, FA2		CF, FA2	CH, CF, FA2

	Study Procedures: [a]	**Consent & screening**	**Baseline**	**Treatment Phase**						**EoT**	**Follow-up**
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
Study Week:		-3 to 0	0	0	0	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 +3	5 ±3	8 ±2	11 ±3	15 ±3	22 ±4	29 ± 3	57 + 7[b]
Duration (minutes)		20 - 60 min	3 h	20 min	20 min	2 h	20 min	2.5 h	20 min	4 h	2.5 h
26	Collect FTIR spectra at each measurement site (duplicates) [g]		CH, CF, FA1, FA2					CH, CF, FA2		CF, FA2	CH, CF, FA2
27	Perform STS in conjunction with TEWL and FTIR on the FA (different sub-site for baseline and follow-up visits) [g]		FA1 [j]							FA2 [j]	
28	Perform STS (to 10 TS) in conjunction with TEWL on the CF – FTIR measurements at the end of STS [g]									CF	
29	Collect TS samples [g]		FA1							FA2	
30	Collect buccal swab sample for *FLG* genotyping		X								
Collection of skin biopsies at the dermatology department											
31	Collect skin biopsies from FA									X [i]	
32	Check condition of skin biopsy sites										X
Treatment and compliance in a clinical area designated for unblinded assessments											
33	Issue IMP's (weighed) and provide demonstration of dosing by fingertip unit		X								
34	Issue treatment diary, complete the relevant sections, and provide training on its completion		X								
35	Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage. [h]			X	X	X	X	X	X	X	
36	Copy diary for records and check all entries for missing applications and AE (skin reactions reported at the time of application).			X	X	X	X	X	X	X	
37	Supervised product application (after measurements).		X	X	X	X	X	X	X		
38	Provide re-training/guidance to improve on application technique and/or dosing 'as required'		(X)	(X)	(X)	(X)	(X)	(X)	(X)		

Study Procedures: [a]		**Consent & screening**	**Baseline**	**Treatment Phase**						**EoT**	**Follow-up**
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
Study Week:		-3 to 0	0	0	0	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 +3	5 ±3	8 ±2	11 ±3	15 ±3	22 ±4	29 ± 3	57 + 7[b]
Duration (minutes)		20 - 60 min	3 h	20 min	20 min	2 h	20 min	2.5 h	20 min	4 h	2.5 h
39	Retrieve diary and IMP's									X	
40	Arrange taxi transfer for next session		X	X	X	X	X	X	X	X	
41	Remind participants not to apply products in the morning before study visits. Reminders should be sent out the day before the appointment is due.[f]			X	X	X	X	X	X		
42	Complete claim form for remuneration										X
43	Study completion										X

a, A description of the procedures carried out at every visit is provided in the subsequent sub-sections.

***b*, must be ≥28 days following end of treatment (Visit 8).**

c, the Admission Form provides a step-by-step guide to the screening and admission process. A medically qualified study Investigator must make the final assessment of eligibility. Where potential participants are found to require a washout period at the consent visit in order to become eligible, a follow-up admission form must be completed and signed off by a medically qualified study investigator prior to enrolment.

d, the EASI assessment should be repeated whenever the admission process is conducted (i.e. at screening and after a given washout period)

e, female participants of childbearing potential only

f, reminders are required to prevent overdosing where supervised on-site applications are to be conducted and to ensure product residues do not interfere with skin assessments.

g, the anatomical locations of each test are indicated using the following code: CH, cheek; CF, antecubital fossa; FA1, forearm (volar) site 1; FA2, forearm (volar) site 2. Both the right and left sites are tested at each instance.

h, the weight should be entered into the treatment diary, which includes the expected amounts participants should have used at each visit so that compliance with the dosing can be discussed with the participant.

i, should be collected at a location adjacent to FA2 (not on FA2, biophysical measurements to be repeated at FA2 on V9); can be undertaken at a separate visit (see guidance below)

j, STS damages the stratum corneum and so STS sites must not be re-tested during the remainder of the study. Follow-up assessment (at end of treatment) using the STS procedure must therefore take place at an adjacent site.

7.2 Recruitment

Recruitment will be conducted in several ways:

1. General advertisement using posters. The poster contains telephone and email contact details for the study team and the SDR research page address. 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. Sheffield Teaching Hospitals premises, including the Royal Hallamshire Hospital
    d. Online, on the University of Sheffield website and departmental pages/portals, the SDR website, SHU website (student portal), Facebook, Twitter
    e. Local circulars, magazines and newspapers. For example, Tito, Grapevine, Active8, LookLocal, Westside, Sixer, and Mercury
    f. Local shops, libraries, community centres and practices with the permission of the owner/manager in each case.
2. Email lists. An email, containing study details from the PIS and the poster 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.
3. Social media including posts on institutional pages and via advertising containing study details from the PIS and the poster
    a. Twitter including @Shef_Derm Twitter pages
    b. Facebook and Instagram
    c. University and group website

The study office will handle all expressions of interest. Participants responding to our adverts/letters by phone/email will be asked to complete a volunteer registration form (this may be done by phone or online). *The registration form will make it clear that the next step will be a phone call from a researcher on the study to conduct the pre-screening.*

A copy of the full PIS will be sent by post/email to each volunteer completing the registration form.

7.2.1 Pre-screening

A member of the team will contact interested volunteers to conduct a pre-screening session by phone. If the participant does not answer, a voicemail to a personal mobile number may be left once but this will not detail any study or clinical information, it will be limited to a request to call back on a given number. Four documented attempts will be made at most.

The call will be conducted according to a prescribed format (see the Pre-Screening Form), which runs through the basic inclusion/exclusion criteria – this will help avoid wasting the time of volunteers who do not meet the eligibility criteria.

All study appointments may be arranged upon successful pre-screening, 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 measurement sites prior to visit 1/baseline.

7.3 Informed consent and eligibility

7.3.1 Informed consent

At the beginning of the consent study visit (at least 24 hours after the PIS has been issued), A medically qualified member of the study team, trained in informed consent taking, will conduct the informed consent process and complete the screening according to the inclusion and exclusion criteria. All participants will be provided with a consent form to sign before any study related procedures are undertaken, of which they will be provided a copy to keep.

Once informed consent has been obtained participants will be assessed for eligibility. This will involve capturing a medical history and other background information pertinent to the study.

Participants should ideally be enrolled within 29 days of obtaining informed consent. Where this is not possible the informed consent process should be repeated. Informed consent will not be retaken where the participant is returning after a defined/scheduled washout period of up to 16 weeks (12 weeks + 28 days). Volunteers who reapply to take part after this time or where a washout period was not scheduled (and documented in the admission form), will be required to re-consent.

7.3.2 Eligibility assessment

Eligibility to take part may only be determined by a medically qualified investigator.

There are 3 possible outcomes to the eligibility assessment:

1. **the participant is found not to be eligible –** exclude the participant from the study and document this outcome;
2. **the participant meets all of the criteria and is eligible to take part –** proceed straight to Visit 1. Visit 1 may take place at any point up to 28 days after the assessment of eligibility. If more than 28 days passes the screening and consent visit should be repeated.
3. **the participant may become eligible after an appropriate wash-out period**. Some participants, who are currently using treatments for eczema, will need to undergo a 'wash out' period, where the use of these treatments is stopped, prior to the first study visit. The duration of the wash-out depends on the type of treatment, and varies from 7 days for emollients to 12 weeks for intravenous biologic therapies (see section 8). The required wash-out period should be documented and the admission process repeated at the start of visit 1. This will ensure that the participant is in fact eligible to take part before proceeding onto any study procedures, and that valid background information is captured.

7.4 Study visits

At the start of every visit consent will be reconfirmed and enquiries will be made about potential AE and concomitant medication (AE and Concomitant Medication forms will be completed as required).

New entries should be made in the participants hospital notes for each visit, and an account of the visit recorded.

A description of the visits is provided below. All visits should be conducted in the indicated order. The measurement sites to be tested at each visit are detailed in the schedule of events (Table 7.1)

7.4.1 Screening & Consent Visit (approx. 30-60 min)

- A member of the study team should provide an overview of the study and answer any questions. Informed consent should then be taken.
- Participants should be admitted onto the study by following the admission process:
    - Collect basic demographic information about the participant
        - Recording the participants date of birth
        - Recording the participant's sex
        - Recording the participants ethnicity
    - Medical history should be collected through discussion with the participant, and from the patient notes where they are available (not all participants will be registered at Sheffield Teaching Hospitals).
    - Details of concomitant medication to be captured and recorded in the Concomitant Medication Log.
    - Measure the participants height and weight
    - Gather information on the participants skin condition
        - Assessing and recording the participants Fitzpatrick skin type
        - Assessing and recording the participants history of eczema according to the UK Work Party Diagnostic Criteria
    - A physical examination of the participants skin should be performed by a suitably trained researcher to grade the severity of eczema overall. The researcher will need to see the signs of eczema to assess its severity.
        - Grade and record the severity of eczema using the EASI scoring system
        - Grade and record the severity of eczema according to the Investigators static global assessment
        - Record the time since the participant's last flare
        - Record how many times the participant's condition has relapsed/flared up in the last 12 months
    - Screen against the study criteria.
- Female participants should be asked to take a urine pregnancy test
- For female participants only, the undertaking of a reliable form of contraception for the duration of the study should be discussed, and a method agreed.
- Access and update patient medical notes with study details. If the participant has no notes already, a new set of notes should be prepared.
- Determine if the participant is eligible to take part.

7.4.2 Visit 1, Day 1 (approx. 2.5-3 hours)

- *If a wash-out period was required*, the admission process should be repeated:
    - Update basic demographic/background information about the participant if necessary

  - Update medical history
  - Update concomitant medication
  - Measure the participants height and weight
  - Update skin condition information
  - Perform a physical examination of the participants skin to grade the severity of eczema overall.
  - Re-assess eligibility against the inclusion and exclusion criteria
- Capture AE and new concomitant medications
- Female participants should be asked to take a urine pregnancy test
- Check that an appropriate method of contraception is in use
- **Check that a medically qualified investigator has assessed the participant as eligible within the last 28-days and that a valid signed consent form is on record before proceeding to the next step.**
- Issue a randomisation number and order the prescription

Transfer to the skin barrier lab for skin assessments by the blinded investigators

- Participants need to acclimatize the skin on their volar forearms, antecubital fossa and cheeks to the room conditions for 20 minutes.
- The treatment areas and measurement sites should be identified and marked out using a marker pen (as discreetly as possible). The marker is easily removed by washing the skin at the end of the study visit. Hair interferes with the measurements, so where present we may need to trim it.
- **Indicate the required treatment areas to the participant and document the area of each in cm^2.**
- Once acclimatised the condition of the skin should be assessed:
  - Visually score eczema severity, skin dryness and redness/erythema at each measurement site
  - Capture 2D skin images of each measurement site
  - Perform Mexameter measurements of objective skin redness at each measurement site
  - Take OCT (structural & angiographic) images/scans with the Vivosight at each measurement site
  - **Safety measure:** Document epidermal thinning observed at the cheek sites (RCH and LCH); this is required in V4 to determine the percentage of epidermal thinning.
  - **Take PS-OCT images/scans with the PS-OCT machine at each measurement site**
  - Measure TEWL at each measurement site
  - **Collect FTIR spectra at each measurement site**
  - **Perform STS in conjunction with TEWL and FTIR assessments on FA1 (the second volar forearm site)**
  - **Collect TS samples from FA1**
- **A mouth swab sample should be collected**

Transfer back to the clinical area designated for unblind assessments

- Issue IMP's (weighed) and provide demonstration of dosing by fingertip unit
- Issue treatment diary, complete the relevant sections, and provide training on its completion
- Ask the participant to perform a supervised product application (after measurements).
- Provide re-training/guidance to improve on application technique and/or dosing 'as required'
- Arrange taxi transfer for next session
- Remind participants not to apply products in the morning before study visits and schedule a reminder.

7.4.3 Visit 2 – Compliance Check, Day 2 +3 (days 2-5, approx. 20 min each)

- Re-confirm verbal ascent to continue the study
- Capture AE and new concomitant medications
- Check compliance with dosing conditions
- Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage.
- Copy diary for records and check all entries for missing applications and AE (skin reactions reported at the time of application).
- Ask the participant to perform a supervised product application (after measurements).
- Provide re-training/guidance to improve on application technique and/or dosing 'as required'
- Arrange taxi transfer for next session
- Remind participants not to apply products in the morning before study visits and schedule a reminder.

7.4.4 Visit 3 – Compliance Check, Day 5 +/- 3 days (days 2-8, approx. 20 min each)

Same procedure as Visit 2.

7.4.5 Visit 4, Day 8 +/- 2 days (days 6-10, approx. 2 hours)

- Re-confirm verbal ascent to continue the study
- Capture AE and new concomitant medications
- Check that an appropriate method of contraception is in use
- Check compliance with dosing conditions

Transfer to the skin barrier lab for skin assessments by the blinded investigators

- Participants will need to acclimatize the skin on their volar forearms to the room conditions for 20 minutes.
- The measurement sites should be marked out on each arm using a marker pen (as discreetly as possible). The marker is easily removed by washing the skin at the end of the study visit. Hair interferes with the measurements, so where present we may need to trim it.
- Once acclimatised the condition of the skin should be assessed:
    - Visually score eczema severity, skin dryness and redness/erythema at each measurement site
    - Capture 2D skin images of each measurement site
    - Perform Mexameter measurements of objective skin redness at each measurement site

- Take OCT (structural & angiographic) images/scans with the Vivosight at each measurement site
- **Safety measure:** Determine the percentage thinning observed at the cheek sites (RCH and LCH) from baseline (Visit 1). Treatment on the cheeks (both left and right sites) should be discontinued if the thickness of the epidermis is ≤70% of the thickness at V1 (≥30% thinning) on either cheek (RCH or LCH). To determine the % thickness: (100/ V1_Epidermal_thickness)*V4_Epidermal_thickness. This does not apply to the other treatment areas. In each case the epidermal thickness will be the mean of the three repeat measurements for the given site.
- Measure TEWL at each measurement site

Transfer back to the clinical area designated for unblind assessments

- Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage.
- Copy diary for records and check all entries for missing applications and AE (skin reactions reported at the time of application).
- Ask the participant to perform a supervised product application (after measurements).
- Provide re-training/guidance to improve on application technique and/or dosing 'as required'
- Arrange taxi transfer for next session
- Remind participants not to apply products in the morning before study visits and schedule a reminder.

7.4.6 Visit 5 – Compliance Check, Day 11 +/- 3 days (days 8-15, approx. 20 min)
Same procedure as visit 2

7.4.7 Visit 6, Day 15 +/- 3 days (days 12-18, approx. 2.5 hours)

- Re-confirm verbal ascent to continue the study
- Capture AE and new concomitant medications
- Check that an appropriate method of contraception is in use
- Check compliance with dosing conditions

Transfer to the skin barrier lab for skin assessments by the blinded investigators

- Participants will need to acclimatize the skin on their volar forearms to the room conditions for 20 minutes.
- The measurement sites should be marked out on each arm using a marker pen (as discreetly as possible). The marker is easily removed by washing the skin at the end of the study visit. Hair interferes with the measurements, so where present we may need to trim it.
- Once acclimatised the condition of the skin should be assessed:
  - Visually score eczema severity, skin dryness and redness/erythema at each measurement site
  - Capture 2D skin images of each measurement site
  - Perform Mexameter measurements of objective skin redness at each measurement site

- Take OCT (structural & angiographic) images/scans with the Vivosight at each measurement site
- Measure TEWL at each measurement site
- **Collect FTIR spectra at each measurement site**

Transfer back to the clinical area designated for unblind assessments

- Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage.
- Copy diary for records and check all entries for missing applications and AE (skin reactions reported at the time of application).
- Notify participants to stop treatment on the cheeks (except where treatment on the cheeks has already been terminated)
- Ask the participant to perform a supervised product application (after measurements).
- Provide re-training/guidance to improve on application technique and/or dosing 'as required'
- Arrange taxi transfer for next session.
- Remind participants not to apply products in the morning before study visits and schedule a reminder.

7.4.8 Visit 7 – Compliance Check, Day 22 +/- 4 days (days 18-26, approx. 20 min)
Same procedure as visit 2

7.4.9 Visit 8 – End of Treatment, Day 29 +/- 3 days (days 26-32, approx. 3 hours)

- Re-confirm verbal ascent to continue the study
- Capture AE and new concomitant medications
- **Female participants should be asked to take a urine pregnancy test**
- Check that an appropriate method of contraception is in use
- A physical examination of the participants skin should be performed by a suitably trained researcher to grade the severity of eczema overall. The researcher will need to see the signs of eczema to assess its severity.
  - Grade and record the severity of eczema using the EASI scoring system
  - Grade and record the severity of eczema according to the Investigators static global assessment
- Check compliance with dosing conditions

Transfer to the skin barrier lab for skin assessments by the blinded investigators

- Participants will need to acclimatize the skin on their volar forearms to the room conditions for 20 minutes.
- The measurement sites should be marked out on each arm using a marker pen (as discreetly as possible). The marker is easily removed by washing the skin at the end of the study visit. Hair interferes with the measurements, so where present we may need to trim it.
- Once acclimatised the condition of the skin should be assessed:

- Visually score eczema severity, skin dryness and redness/erythema at each measurement site
- Capture 2D skin images of each measurement site
- Perform Mexameter measurements of objective skin redness at each measurement site
- Take OCT (structural & angiographic) images/scans with the Vivosight at each measurement site
- **Take PS-OCT images/scans with the PS-OCT machine at each measurement site**
- Measure TEWL at each measurement site
- **Collect FTIR spectra at each measurement site**
- **Perform STS in conjunction with TEWL and FTIR assessments on FA2 (the second volar forearm site)**
- **Collect TS samples from FA2**
- **Perform STS (to 10 TS) in conjunction with TEWL on CF (includes FTIR measurement at the end of STS.**

Transfer to the dermatology department as applicable

- **Collect skin biopsies** (in a sub-group of participants only and subject to additional consent, can be undertaken at a sperate visit – see guidance below)

Transfer back to the clinical area designated for unblind assessments

- Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage.
- Check all entries in diary for missing applications and AE (skin reactions reported at the time of application).
- Retrieve diary and IMP's
- Arrange taxi transfer for next session

7.4.10 Visit 9, Day 57 +7 days (days 57-64, approx. 2.5 hours)*
*Must be arranged 28 days or more after cessation of treatment (Visit 8)

- Re-confirm verbal ascent to continue the study
- Capture AE and new concomitant medications

Transfer to the skin barrier lab for skin assessments by the blinded investigators

- Participants will need to acclimatize the skin on their volar forearms to the room conditions for 20 minutes.
- The measurement sites should be marked out on each arm using a marker pen (as discreetly as possible). The marker is easily removed by washing the skin at the end of the study visit. Hair interferes with the measurements, so where present we may need to trim it.
- Once acclimatised the condition of the skin should be assessed:
  - Visually score eczema severity, skin dryness and redness/erythema at each measurement site

- Capture 2D skin images of each measurement site
- Perform Mexameter measurements of objective skin redness at each measurement site
- Take OCT (structural & angiographic) images/scans with the Vivosight at each measurement site
- **Take PS-OCT images/scans with the PS-OCT machine at each measurement site**
- Measure TEWL at each measurement site
- **Collect FTIR spectra at each measurement site**

- Check the condition of the biopsy sites, where they were collected, to ensure satisfactory healthy – raise an AE where this is not the case and provide treatment as required.
- Ask participants to complete a claim form for the remuneration
- Document participant completion

7.5 Skin condition assessments

7.5.1 Assignment of Fitzpatrick skin type (background information form)
The Fitzpatrick skin type will be visually assessed by the investigator using the scale in the figure below.

Figure 7.1: Fitzpatrick skin type scale

7.5.2 The UK Working Party Diagnostic Criteria for eczema
According to the UK working party diagnostic criteria, eczema is defined as exhibiting an itchy skin condition 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

7.6 Grading the severity of AD

7.6.1 Grading the severity of AD using the EASI system

The EASI is a validated measure used in clinical practice and clinical trials to assess the severity and extent of AD. The EASI is a composite index with scores ranging from 0 to 72. Four AD disease characteristics (erythema, thickness [induration, papulation, oedema], scratching [excoriation], and lichenification) will each be assessed for severity by the investigator or designee on a scale of "0" (absent) through "3" (severe). In addition, the area of AD involvement will be assessed as a percentage by body area of head, trunk, upper limbs, and lower limbs, and converted to a score of 0 to 6. In each body region, the area is expressed as 0, 1 (1% to 9%), 2 (10% to 29%), 3 (30% to 49%), 4 (50% to 69%), 5 (70% to 89%), or 6 (90% to 100%). The determination of EASI will be supported by the use of the EASI scoring sheet. The EASI assessment should be performed every time an Admission form is completed.

7.6.2 The Investigators Static Global Assessment (ISGA)

The severity of eczema is assessed by an investigator according to the scale below:

Table 7.2: ISGA scale

Score	Grade	Definition
0	*Clear*	*Minor residual hypo/hyperpigmentation; no erythema or induration/papulation; no oozing/crusting*
1	*Almost Clear*	*Trace faint pink erythema, with barely perceptible induration/papulation and no oozing/crusting*
2	*Mild*	*Faint pink erythema with mild induration/papulation and no oozing/crusting*
3	*Moderate*	*Pink-red erythema with moderate induration/papulation with or without oozing/crusting*
4	*Severe*	*Deep or bright red erythema with severe induration/papulation and with oozing/crusting*

7.7 Pregnancy testing

For female participants of childbearing potential, a urine pregnancy test (beta-human chorionic gonadotropin (β-hCG), with sensitivity of at least 25 mIU/mL, will be performed at screening, prior to dosing with investigational product on Day 1 and at the end-of-treatment (Day 29) visit, to confirm the participant has not become pregnant during the study.

A negative pregnancy test result is required before the participant can receive investigational product. Pregnancy tests may also be repeated at the discretion of the investigator or his/her designee. In the

case of a positive urine β-hCG test during the treatment period, the participant will be withdrawn from administration of investigational product and from the study

7.8 Reminders between visits

Remind participants not to apply products in the morning before study visits 2-8. Reminders should be sent out the day before the appointment is due. Text messages are the preferred method; however, telephone calls or email can be used instead at the request of the participant.

7.9 Randomisation & blinding

The participant will be assigned a randomisation number at Visit 1. This will be the next available number on the Randomisation list. Allocation of the randomisation number is considered the point of entry onto the trial, which should be recorded on a Participant Screening, Enrolment & Completion Log.

7.9.1 Randomisation

Allocation of the treatments to the treatment areas (right/left) will be randomised (to avoid site position-dependent artefacts) 1:1 using a randomisation schedule (list) provided by the Statistical Services Unit. The list will be numbered using the unique participant randomisation number, such that participant 001 will be dispensed products according to the first anatomical site allocation on the list and so on. The Sheffield Teaching Hospitals Pharmacy will undertake and document the randomisation and label the IP's with the site of application upon issue to the site research facility team. Only the Pharmacy and SSU statistician will have access to the randomisation master list.

7.9.2 Blinding

The study will be conducted observer-blind. Because the products have distinct forms (ointment vs cream) and will be provided in clearly identifiable packaging study participants will be using the products open-label.

The IP's, labelled and released by the Pharmacy, will be issued by unblinded research staff within the site research facility, who will undertake all IP related duties, including compliance monitoring. Participants will be asked not to discuss/mention the IP identities with the study team members collecting the data (who will be blind – observer blinding).

The collection of study data will be conducted in a separate area (dedicated skin barrier research suite) by a separate team (comprising skilled dermatology researchers) who will be blind.

7.9.3 Emergency unblinding

Only the research team assessing the study endpoints are blind to the treatment allocation and participants are all prescribed both treatments. Therefore, no emergency unblinding arrangements are necessary.

7.10 Identification and demarcation of the treatment areas and measurement sites

There are 3 different anatomical regions of interest, the cheek (CH), antecubital fossa (CF) and the volar forearm. With the bilateral design of this study there are right and left sides for each anatomical location: treatment with one product will be made to the right CH, CF and FA together; and with the other product to the left CH, CF and FA (randomised left/right allocation). Figure 7.2 illustrates the locations of the areas. Below is a description of each as a guide.

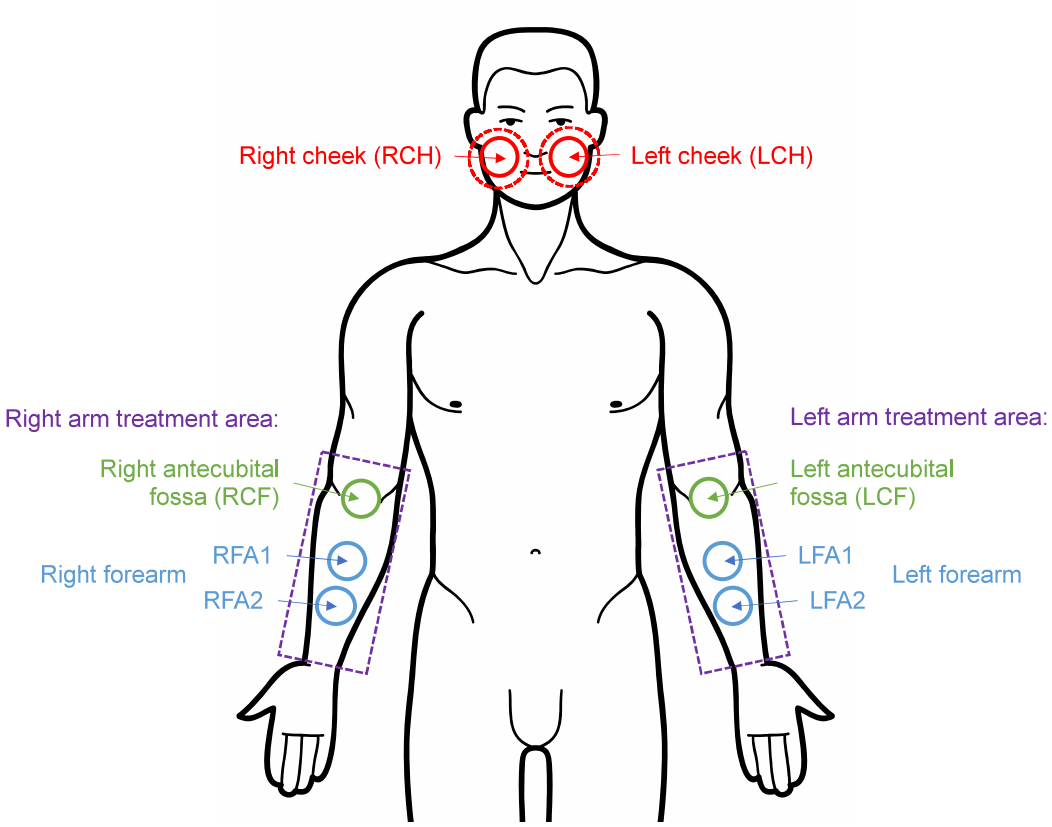

Figure 7.2: The location of the treatment areas and measurement sites

The exact location area within each anatomical location can be adapted to suit the skin of each participant as long at the 'approximate' areas of each are preserved, noting that dosing by fingertip unit and using 'hand' based areas of skin measurement is an imprecise measure (1 hand = 108 to 160 cm^2).

- The cheek (CH): Equivalent to ½ a hand and approx. 70 cm^2, a 9cm diameter circular area avoiding the eyes, nose and mouth. The diameter of the actual area treated should be recorded at V1.
    - The treatment area should be calculated as $A = \pi r^2$, where r is the diameter/2.
    - The measurement site is a 4cm diameter region within the treatment area (this should ideally be slightly distal, closer to the ear, where the skin is better supported and it is easier to take the measurements). The centre point of the measurement site between the nose and ear should be recorded in the participants notes for future reference.

- The arm (combined treatment area for CF and FA): Equivalent to 2 hands and approx. 280 cm^2, a region about 10 cm (w) x 28 cm located so that the area extends from the elbow crease up the bicep by 7 cm and down the volar forearm from the elbow crease by 21 cm. This equates to treatment areas of approx. 1 hand (140 cm^2) for the CF and 1 hand for the FA.
    - The measurements of the actual area treated should be recorded at V1: distance from the elbow crease to the edge of the treatment area up the bicep (height 1), distance from the elbow crease to the edge of the treatment area down the volar forearm (height 2), and the width of the treatment area at the elbow flexure (width). The treatment area will then be determined as (height 1 + height 2) *width.
    - The measurement site for CF is a 4cm diameter region positioned at the centre of the elbow crease so that the elbow crease intersects it horizontally.
    - There are 2 measurement sites on the volar forearm, each 4cm diameter approximately. They should ideally be located down the arm with FA1 located above FA2 centred vertically to ensure they fall within the treated area of skin.
    - Skin biopsies should be collected from the volar forearm at a location close to measurement site FA2, but not within the areas subject to STS.

Before performing any skin assessments, during the acclimatization period, the test areas should be marked out discretely using a ruler, and skin marking pen with the help of the guide below. A sketch with measurements should be documented in the participants notes.

7.11 Skin assessments

The skin assessments will be undertaken in the skin barrier suite at the Royal Hallamshire Hospital by experienced dermatology researchers.

The trial involves the use of a range of instruments to assess the biophysical properties of the skin. The instruments used on this study are listed in the table below. All the instruments are owned and maintained by the University of Sheffield Dermatology Research group. With the exception of the custom-built PS-OCT, all instruments are used as supplied by the manufacturer (no modification) and for their intended purposes. Each instrument will undergo annual Biomedical Engineering testing for electrical safety at the Royal Hallamshire Hospital.

Table 7.3: Instruments used in this study and the associated outcomes

Instrument	Manufacturer	Measurements
Vivosight OCT Scanner	Michelson Diagnostics Ltd.	Epidermal thickness (structural OCT) Superficial plexus depth (µm, angiographic OCT) Blood vessel diameter (µm, angiographic OCT) Blood vessel density (segments/mm^2, angiographic OCT)

AquaFlux AF200 TEWL machine	Biox Systems Ltd.	Skin barrier function (TEWL), and skin barrier integrity (when combined with STS; $TEWL_{10}$, $TEWL_{20}$)
PS-OCT machine	University of Sheffield custom build	Collagen index (an index derived from birefringence images of collagen density and arrangement) measured by PS-OCT – a putative marker of fibrosis[18]
4300 FTIR Spectrometer	Agilent Technologies Ltd.	Various biomarkers of skin barrier condition[19,20]: • Carboxylate levels (indirect measure of NMF levels) in the stratum corneum measured by FTIR spectroscopy • Stratum corneum lipid structure measured by FTIR spectroscopy
C-Cube camera	Pixience	Skin image documentation at 50x magnification. Erythema index is derived from the images as an indication of clinical inflammation (another marker for objective redness). Used here to assess tolerability.
Mexameter	C&K	Objective redness

We have no affiliation with the manufactures of the instruments used (with the exception of the PS-OCT machine), and the instruments themselves are not directly under investigation herein as tools to inform clinical decision making. Instead, the aim is to identify differences in the skin in response to treatment through research, not to directly inform clinical decision making. It is possible that the results of this trial suggest that one or more of the biomarkers measured using the instruments could be used clinically to monitor treatment safety. In said case, evaluating the instrument as a medical device will be the participant of a separate future study.

> ***Definition of a medical device:*** *any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: diagnosis, prevention, monitoring, treatment or alleviation of disease.*

The locations of the skin assessment sites are detailed in section 7.10.

7.11.1 Acclimatization

The participant should be asked to expose the skin of the test/treatment areas at least 20 minutes before taking the first skin measurements (including visual scores) so that the skin at these sites can

adjust to the room conditions. This may involve asking the participant to remove their jacket/jumper. The room temperature and humidity should be in the range 20±2ºC and 45±10% relative humidity.

7.11.2 Visual scoring of skin condition (eczema severity, dryness and redness/erythema)
Visual dryness and redness/erythema of each anatomical area (volar forearm, cubital fossa and cheek) will be scored by an experienced grader using the scales below at the visits indicated in the schedule of events. The severity of eczema will be scored for each anatomical area (volar forearm, cubital fossa and cheek) using the ISGA scale by an experienced grader (Table 7.2).

Table 7.4: Visual assessment scale for dryness – *the overall dry skin score (ODS)*

Score	Description
0	Absent
1	Faint scaling, faint roughness and dull appearance
2	Small scales in combination with a few larger scales, slight roughness, whitish appearance
3	Small and larger scales uniformly distributed, definite roughness, possibly slight redness and possibly a few superficial cracks
4	Dominated by large scales, advanced roughness, redness present, eczematous changes and cracks

Table 7.5: Visual assessment scale for erythema

Score	Description
0	No redness.
0.5 / +	Slight, patchy erythema – barely perceptible
1	Slight uniform erythema – mild erythema
1.5	
2	Moderate, uniform erythema – Moderate erythema
2.5	
3	Strong erythema – Marked erythema

7.11.3 2D image capture with the c-cube camera

A close-up image of each test area will be captured using the c-cube (Pixience, France) to document the skin condition. A 16x12mm area of the measurement site skin is imaged therefore there are no data confidentiality concerns. 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. Images will be analysed to derive the erythema index; an objective measure of skin erythema/redness. Further details on taking skin images with the c-cube can be found in SOP-050.

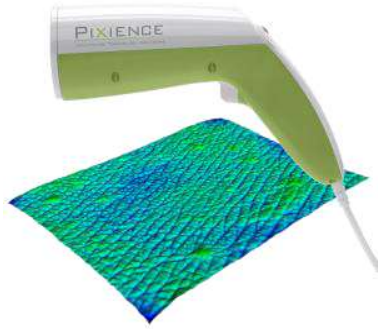

Figure 7.3: The Pixience c-cube

7.11.4 Mexameter measurements of objective skin redness/erythema (tolerability)

Objective skin redness will be measured 4 times within each measurement site using a C&K Mexameter probe. After use in each participant the probe will be decontaminated with a sanitation/detergent wipe. Further details on how to take skin measurements with the Mexameter can be found in SOP-023.

Figure 7.4: The Mexameter probe

7.11.5 Structural & Angiographic OCT scans using the Vivosight OCT machine

OCT is a non-invasive imaging modality conceptually similar to ultrasound (US) but uses near-infrared radiation rather than sound. It has a 2-10 micron depth resolution compared with 100-1,000 micron typical for clinical US; and 1-2 mm imaging depth *vs*. 10-100 mm for clinical US. It is thus ideal for imaging the surface layers of accessible tissues such as the skin. It is attracting interest

throughout the medical community as a scanning tool and for diagnosis of illnesses such as epithelial cancer, connective tissue disorders, and atherosclerosis.

Here we will use the Vivosight OCT machine, running bespoke software developed by the University of Sheffield, to take volumetric scans (images) of the skin. The Vivosight is a CE marked clinical OCT device (Michelson Diagnostics Ltd) now routinely used worldwide to identify various skin cancers. The scans captured on this device using our software comprise both structural and angiographic information, meaning that a single scan provides the information for both the structural OCT and angiographic OCT outcomes.

1. *Structural OCT:* With a depth focus of 1.0mm and optical resolution of 7.5x5.0µm the Vivosight provides structural images of the skin comparable to histology sections. From these images the thickness of the epidermis, the suprapapilary epidermis and the papillary region can be extrapolated. Epidermal thickness has been identified using histology, as a useful biomarker of disease activity and treatment effects in AD. We have previously utilized OCT to quantify epidermal thickness *in vivo* and assess the atrophogenic effects of TCS.[21] We have also studied the effect of TCSs on the skin barrier, the disruption of which is mechanistically associated with epidermal atrophy.[22,23]
2. *Angiographic OCT:* Structural OCT performed at high image frame rates can map areas of temporal decorrelation due to moving red blood cells. Based on this, we have demonstrated that the Vivosight can acquire high-quality maps of the superficial vasculature in living participants and is an ideal tool to monitor and quantify erythema, hyperplasia and TCS-induced tissue remodelling.

Figure 7.5: The VivoSight OCT scanner

Three scans will be taken spread uniformly across each measurement site (can be neighbouring, but not overlapping positions) at each designated visit (see schedule of events). All images will be checked for quality upon collection, and images with visible imaging artefacts will be re-captured.

Any part of the device in contact with human skin (the stand-off) will be cleaned between uses. Further details on how to collect OCT scans with the Vivosight can be found in SOP-047.

7.11.6 PS-OCT scan using the custom PS-OCT machine

Combining polarimetry with OCT leads to a new technique called PS-OCT. PS-OCT can detect areas of enhanced or reduced birefringence in cartilage. This can be associated with a repair mechanism, where degenerated hyaline cartilage (chiefly type-II collagen) is replaced by fibrocartilage (predominantly type-I collagen). During inflammation epidermal and dermal fibrosis is seen and contributes to tissue hyperplasia. This fibrosis occurs as collagen synthesis is elevated abnormally, and so we propose using PS-OCT to monitor epidermal fibrosis. Prolonged use of TCS's causes abnormal lowering of collagen levels, a factor contributing to skin atrophy. Determining at what point collagen levels return to normal, following hyperplasia, before becoming depressed as a TCS side effect will enable assessment of the TCS risk-benefit ratio. A PS-OCT device has already been developed by us and applied to the analysis of collagen levels in cartilage.[24] Having been assessed by the Sheffield Teaching Hospitals Biomedical Engineering Department it has been identified as suitable for use on patients.

PS-OCT images will be captured using a custom device developed by Prof Matcher's team. The device has been evaluated for safe use in humans by the STH Biomedical Engineering Department. Three scans will be taken spread uniformly across each measurement site (can be neighbouring, but not overlapping positions) at each designated visit (see schedule of events). All images will be checked for quality upon collection, and images with visible imaging artifacts will be re-captured. Any part of the device in contact with human skin (the stand-off) will be decontaminated between uses. Further details on how to collect PS-OCT scans can be found in SOP-048.

7.11.7 TEWL measurements

This is a well-documented, standardised dermatological procedure for measuring skin barrier function.[25] The study team have extensive experience in measuring TEWL using a CE marked, AquaFlux AF200 condensing chamber probe (Biox Systems Ltd, UK) in both adult and baby cohorts.[23,26-30] The TEWL machine will be calibrated in accordance with the manufacturers specified recommendations before each use. No specific skin preparation is required prior to TEWL measurements, but visible contaminants will be removed from the measurement sites using a dry wipe if present. Skin sites will be acclimatised (exposed to the open air) for 20 minutes prior to starting TEWL measurements. Further details on how to take skin measurements with the Aquaflux TEWL machine can be found in SOP-017.

Figure 7.6: The Aquaflux TEWL machine

Three (triplicate) repeat surface (without STS) TEWL measurements will be collected from each measurement site at the visits indicated in the schedule of events (at neighbouring positions, i.e. not in the exact same place). The measurement will be repeated if an anomaly occurs during measurement (i.e. deviation from a typical bell shaped TEWL curve **or significant deviation [StDev >3.5] from other measurements within the same treatment area or measurement site**), which can occur if a participant becomes uncomfortable. In such an event the measurement will not be retaken until at least 4 minutes has passed from the collection of the first measurement to permit restoration of normal TEWL at sites occluded by the probe. If TEWL is retaken due to a measurement anomaly the first, anomalous, reading will be discarded (only 3 repeat measurements to be collected for each measurement site). Where TEWL measurements are found to be anomalous, as defined here, after the visit has completed a protocol-non-compliance report will be raised.

7.11.8 FTIR spectroscopy measurements of the skin surface

Attenuated Total Reflectance (ATR) FTIR spectroscopy is a form of molecular spectroscopy useful for the analysis of surfaces, including skin. It has proved valuable for quantifying the lipid, water, and carboxylate (indirect measure of NMF, which is comprised of compounds with characteristic carboxylate functional groups) content of the skin barrier and for analysing lipid arrangement/structure.[31-35] Using a fixed bench top device, this technique has recently been utilised by members of the study team to assess the molecular composition of baby and adult skin in combination with STS.[36] More recently the 4300 handheld, portable ATR-FTIR was used by our team to assess the skin of 120 newborn babies in the STAR study (currently recruiting). It is this portable device that will be used in this study.

Figure 7.7: The Agilent 4300 ATR-FTIR spectrometer

Measurements will be performed by gently but firmly placing the probe in contact with the skin. Duplicate surface measurements (without STS) will be collected from each measurement site at the visits indicated in the schedule of events. The quality of spectra will be assessed at the point of collection, and any that do not meet the established quality parameters (signal to noise ratio, described in the lab manual) will be replaced. After use in each participant the ATR (probe head) will be decontaminated with a 70% alcohol wipe. Further details on how to collect skin spectra with the 4300 spectrometer can be found in SOP-039.

7.11.9 STS (skin tape-stripping) in conjunction with FTIR and TEWL measurements

STS, to study deeper layers of the stratum corneum, involves the repeated application and removal of D-Squame cutaneous stripping discs (CuDerm cooperation, Dallas, USA) to/from the skin in a standardised manner.[33,37] 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 volunteer and the treatment conditions. This enables measurements of deeper layers of the skin to be collected by TEWL machine or FTIR spectroscopy. The depth through the stratum corneum is determined by measuring the amount of corneocytes removed by each disc using the SquameScan Device. Squamescan readings of each TS should be collected in triplicate.

STS will be performed during visits 1 and 8 only, so that subsequent assessment of the skin is not conducted at these sites during the study. STS disrupts the stratum corneum and therefore alters the biophysical properties of the skin. For this reason, STS sites must be carefully selected so that they are not re-tested during the remainder of the study. In order to repeat the STS procedure at baseline and again at the end of treatment, 2 separate sites must therefore be identified:

- Once at FA1 at Visit 1
- Once at FA2 at Visit 8
- Once at CF at Visit 8

The procedure is different for the different anatomical sites due to the differences in skin thickness:

- STS at FA1 and FA2: 20 consecutive tape-stripping's will be performed. In conjunction with STS, (1) TEWL measurements (single repeat) and then FTIR measurements (single repeat) will be collected from the STS site after every 5 consecutives TS as described above. The

FTIR data is used to determine the lipid structure throughout the stratum corneum (depth). The TEWL data is used to stratum corneum integrity (skin barrier function following experimental barrier perturbation). The parameter is referred to here as $TEWL_{ts20}$. The TS from this site, containing a sample of the stratum corneum, will be collected as outline below.

- STS at CF: 10 consecutive tape-stripping's will be performed. In conjunction with STS, (1) TEWL measurements (single repeat) will be collected from the STS site after every 5 consecutives TS as described above. After STS a single FTIR measurement will be taken. The FTIR data is used to determine the lipid structure at a single stratum corneum depth. The TEWL data is used to stratum corneum integrity (skin barrier function following experimental barrier perturbation). The parameter is referred to here as $TEWL_{ts10}$. The TS from this site, containing a sample of the stratum corneum, will be collected as outline below.

Further details on performing STS can be found in SOP-021.

7.12 Sample collection

7.12.1 Superficial stratum corneum sample collection for metabolic (NMF) analysis
The superficial skin samples collected during STS will be retained for analysis:

- The first 10 TS (TS 1-10) will be stored individually in 2 ml tubes at -70ºC for determination of NMF levels by HPLC (3 TS required per analysis).
- The remaining TS (TS 11-20) will be discarded.

STS 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. STS will not be performed on broken skin. The only skin preparation required before STS is the removal of visible contaminants by dry wipe if necessary.

The metabolomic analysis will be performed as described previously, and involve quantitation of pyrrolidone carboxylic acid, urocanic acid and free amino acids, all constituents of NMF, in the superficial layers of the stratum corneum.[38] We have previously shown that TCS treatment reduces the level of these metabolites in the skin.[23] NMF is an important determinant of stratum corneum homeostasis, the skin microbiome and skin moisturization.[39,40] Low levels of NMF are a risk factor for AD and associate with more severe skin inflammation.[41]

The procedure for collecting, processing, storing and analysing the superficial stratum corneum samples in described in the study specific laboratory manual.

7.12.2 Saliva sampling (buccal swab) for FLG genotyping
A saliva sample will be collected (by buccal swab) from the inside of the participants cheek during visit 1 in order to obtain a sample of genomic DNA. Extracted genomic DNA will be genotyped for the 3 most common *FLG* gene mutations. The procedures for collecting, processing, storing the saliva and storing and analysing the genomic DNA are detailed in the study specific laboratory manual.

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 TCS treatment is therefore of interest.

7.12.3 Skin biopsies and histology

All participants will be asked to provide a biopsy sample on a voluntary basis (optional initially), with the aim of gathering exactly 20 biopsies (no more or less); 10 from each treatment site at the end of treatment (volar forearm sites only). Where 10 participants do not voluntarily opt to provide the skin biopsies, the collection of biopsies will become compulsory for the last 10 participants in order to reach the target. The two punch biopsies (ø4mm) per participant will be collected by staff at the Royal Hallamshire Hospital; 1 from close to RFA2 and 1 from close to LFA2 during visit 8. Each biopsy will be halved immediately upon collection so that half may be snap frozen and half paraffin embedded. The procedure for collection, processing, storing and analysis the biopsies in described in the study specific laboratory manual.

Biopsies should ideally be collected immediately after the biophysical skin assessments and within 48 hours of the start of visit 8, this means that a separate visit may be scheduled for the biopsy collection as necessary as long as it takes place within 48 hours of visit 8. Biopsy wound sites will be assessed at Visit 9 for satisfactory healing.

Histology performed on sections of the biopsy tissue will seek to verify the changes observed by OCT imaging and FTIR spectroscopy. This will include for example qualitative assessment of skin tissue expression of K16 as a marker of hypertrophy and atrophy (relating to structural OCT imaging of epidermal thickness)[42], collagen density (picrosirius red staining) and arrangement (second harmonic generation imaging)[43] as a marker of collagen remodelling in response to TCS treatment (and relating to PS-OCT imaging), lipid distribution (relating to FTIR skin analysis), vascular structure (vWF staining), and Filaggrin expression (relating to NMF/carboxylate levels in the stratum corneum determined by FTIR and HPLC)[38]. Images of the tissue will be documented electronically.

The purpose of the biopsy analysis is to support the results obtained using the biophysical techniques (OCT imaging and FTIR spectroscopy) visually, and there is no plan to derive quantitative outcomes pertaining to the effects of the treatments from them. For instance, if we see marked atrophy based on OCT derived epidermal thickness the biopsy tissue will be used to confirm whether a marked reduction in epidermal thickness is observed visually. Similarly, if PS-OCT images suggest a change in collagen matrix structure based on changes in birefringence this will be confirmed visually based on changes in collagen staining and second harmonic generation imaging of collagen alignment.

7.13 Treatment and compliance

During visit 1, after the skin assessments, participants will be issued with the study IMP's.

For doses to be administered at home, the participant or caregiver should be instructed to maintain the product in its original package provided throughout the course of dosing and return the product and its original package (including empty, partial used and unused tubes) to the site at the end of the treatment regimen. All previously dispensed investigational product tubes will be retained by the site.

Investigational product tubes will be weighed individually or collectively by the study site before dispensing and after return and the weights will be recorded. The recorded weights will be used to estimate usage (mg/day) by each participant. Note that the weight recorded on the investigational product label is a nominal weight and not an exact weight of the investigational product and tube.

The site staff will provide a demonstration of the dosing by fingertip unit.

A treatment diary will be issued, after completion of all relevant sections by the trial team. The participants should be trained in the completion of the diary.

The participant should then be asked to undertake a supervised application (both treatments). The site staff should observe the participant and where necessary provide guidance to encourage good technique.

At each subsequent visit the following tasks should be undertaken:

- Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage.
- Check all diary entries for missing applications and AE (skin reactions reported at the time of application).
- Supervised product application (after measurements).
- Provide re-training/guidance to improve on application technique and/or dosing as required

At the end of the 28-day treatment regimen, the diary and treatments, including all packaging and empty containers, should be retrieved. Further information can be found in section 8.

7.14 Participant completion/withdrawal

7.14.1 Participant completion

Participants are considered to have completed the study when all study procedures have been completed as designated by the protocol. Completion should be noted in a Participant Screening, Enrolment & Completion Log.

7.14.2 Participant withdrawal

When an individual who has signed the Informed Consent Form is not enrolled in the study or withdraws/is withdrawn prior to completing the study, the reason is to be documented. Reasons for participant withdrawal may include:

1. Participant is determined to be ineligible after enrolment
2. Participant's choice to withdraw
3. Exclusion criteria met
4. Non-compliance with the study protocol.
5. AE, including a relapse of AD (defined as an escalation in treatment, i.e. the need for TCS).
6. Loss-to-follow (participant cannot be contacted)
7. Other

Participants may withdraw from the study at any time at their request, or they may be withdrawn at any time at the discretion of the PI, or designee for safety, behavioural, or administrative reasons. If a participant does not return for a scheduled visit, at least 2 attempts will be made to contact the participant in order to establish the reason for withdrawal, and the outcome will be documented, if reasonably possible within the constraints of the study design. No more than 4 attempts will be made to contact participants; a voicemail to a personal mobile number may be left once but this will not detail any study or clinical information, it will be limited to a request to call back on a given number. The PI or designee should inquire about the reason for withdrawal, request that the participant return for a final visit, if applicable (if withdrawing from the study during the treatment period between study visits), and follow-up with the participant regarding any unresolved AE. In such circumstances, where the treatment regimen has not completed the final visit will be conducted solely to retrieve the products and capture any unreported AE's.

Additional participants may be enrolled in the study, up to the maximum recruitment target, to compensate for early participant withdrawal.

7.14.3 Remuneration

All participants enrolled onto the study will be remunerated for their time. Participants who fail to complete the study, for whatever reason, may claim a proportion of the remuneration commensurate with the extent of their participation. Taxi transfers will be arranged to/from the study site for all visits.

For participants who attend the consent/screening only, and are found to be ineligible a £5 voucher can be issued. Pre-screening by telephone will be conducted to limit the number of instances where participants attend the site and are subsequently not enrolled (failure to meet eligibility criteria). Travel expenses incurred in order to attend the consent/screening visit will be paid upon request, and require the provision of receipts and completion of a University of Sheffield claim form.

7.15 Definition of the end of trial

The end of the trial is defined as the point at which all lab analyses required for the study endpoints has been completed.

7.15.1 Early termination of the trial

The criteria for electively stopping the trial prematurely includes:

- New information becomes available on either of the study interventions or the study procedures that suggests that participants or researchers will be placed at unacceptable risk. This will be decided by the CI and Sponsor.
- The CI and Sponsor, with the support of the TMG, deems that nature and/or frequency of AE is inconsistent with expectations and suggests that participants are being placed at greater risk.

The decision to terminate may also occur because of a regulatory authority decision or a change in opinion of the REC.

8. TRIAL TREATMENTS

8.1 Name and description of investigational products

The treatments used in this study are listed in Table 8.1.

Table 8.1: Investigational products

Name, Strength & Form	Brand Name	Pack size and quantity per participant	Manufacturer	Formulation
Betamethasone Valerate 0.025% topical cream	Betnovate RD cream	2x 100g tube* (200g total) *with 7mm nozzle	GlaxoSmithKline UK	*Betamethasone Valerate BP 0.025% (w/w)*, Cetostearyl Alcohol, Cetomacrogol 1000, White Soft Paraffin, Liquid Paraffin, Chlorocresol, disodium hydrogen phosphate, citric acid monhydrate, Purified Water
Crisaborole 2% ointment	N/A	3x 60g tube* (180g total) *with 7mm nozzle	Pfizer	*Crisaborole 2%,* White Petrolatum, Propylene Glycol, Mono- And Di-Glycerides, Paraffin, Butylated Hydroxytoluene, And Edetate Calcium Disodium

8.2 Regulatory status of the drugs

8.2.1 Betamethasone Valerate (0.025%) cream

Betamethasone Valerate (0.025%) cream is currently licenced in the UK for the treatment of eczema and will be used in accordance with this licence in this study (see Betnovate RD [0.025%] cream SmPC). Betamethasone valerate creams are manufactured by a number of companies, however this trial will be performed using the Betnovate RD (GlaxoSmithKline UK) brand only to avoid the introduction of variability arising from the differences in topical formulation (known to affect bioavailability of the active).

8.2.2 Crisaborole (2%) ointment
Crisaborole (2%) ointment is not currently marketed in the UK; however, it is licenced by the FDA for use in the United States of America (USA) under the brand name Eucrisa, and will be used in accordance with this licence in this study.

8.3 Product characteristics

8.3.1 Betamethasone Valerate (0.025%) cream
Please refer to the attached SmPC

8.3.2 Crisaborole (2%) ointment
Please refer to the attached Investigator's Brochure (IB)

8.4 Drug supply

8.4.1 Betamethasone Valerate (0.025%) cream
Betnovate (0.025%) cream will be provided as supplied by the manufacturer (no re-packaging). Whilst this product is marketed in the UK the Pharmacy does not stock this item (instead supplying from a range of generic manufacturers with varying formulations). The Pharmacy will therefore order the product as (segregated) clinical trial stock. Annex 13 compliant labels will be applied prior to dispensing by the pharmacy (example attached).

8.4.2 Crisaborole (2%) ointment
Crisaborole (2%) ointment will be provided by Pfizer. The product will be imported by Pfizer and released onto this study by a suitable Qualified Person nominated by Pfizer. The product will be provided in plain packaging with Annex 13 compliant labels (example attached).

8.5 Drug storage

The study pharmacist or other appropriately trained personnel, will ensure that all investigational products are stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements.

Investigational products should be stored in their original containers and in accordance with the labels. The conditions for storing both products are:

- Store at 15–25°C (59–86°F) for crisaborole (2%) ointment and ≤25°C (77°F) for betamethasone valerate (0.025%) cream.

Site systems must be capable of measuring and documenting (for example, via a log), at a minimum, daily minimum and maximum temperatures for all site storage locations (as applicable, including frozen, refrigerated, and/or room-temperature products). This should be captured from the time of investigational product receipt throughout the study. Even for continuous-monitoring systems, a log or site procedure that ensures active evaluation for excursions should be available. The intent is to ensure that the minimum and maximum temperature is checked each business day to confirm that no excursion occurred since the last evaluation and to provide the site with the capability to store or view

the minimum/maximum temperature for all non-working days upon return to normal operations. The operation of the temperature monitoring device and storage unit (for example, refrigerator), as applicable, should be regularly inspected to ensure they are maintained in working order.

Any excursions from the product label storage conditions should be reported to the CI upon discovery. The site should actively pursue options for returning the product to the storage conditions described in the labelling, as soon as possible. Deviations from the storage requirements, including any actions taken, must be documented. Permitted excursions require no further action.

Once an excursion outside the permitted range is identified, the investigational product must be quarantined and not used until the CI provides permission to use the investigational product. For crisaborole (2%) ointment the CI will seek permission from Pfizer to use the investigational product OR request replacement stock. It will not be considered a protocol deviation if Pfizer approves the use of the investigational product after the temperature excursion. Use of the investigational product prior to Pfizer approval will be considered a protocol deviation. Specific details regarding information the site should report for each excursion will be provided to the site. For betamethasone valerate (0.025%) cream, replacement stock will be procured, with the permission of the CI and where necessary the Funder, in the event of an excursion outside the permitted range.

Receipt of materials, door opening and closing, and other routine handling operations where the products are briefly out of the temperature range described in the labelling are not considered excursions. Site staff will instruct participants and/or parents/legal guardians on the proper storage requirements for take home investigational products (details can be found in the treatment diary).

8.6 Preparation and dispensing

The investigational products will be prescribed by a medically qualified and appropriately delegated member of the research team.

The investigational products will be labelled (as required) and dispensed by the Pharmacist, according to the randomisation schedule, at the request of the site research facility. Annex 13 compliant labels will be used, containing study specific usage instructions including dose and application frequency, and capture the unique participant ID, the participant's initials and the 'application site' (according to the randomisation).

Unblinded researchers at the site research facility, as delegated by the PI, will issue the investigational products to the study participants during visit 1, provide the demonstration of product application and undertake all compliance monitoring. Unblinded researchers will not be involved in the collection of study data.

8.7 Treatment regimen

Every enrolled participant will undergo the same intervention, which involves the use of 2 investigational products according to a predefined regimen (Table 8.2).

Table 8.2: Treatment conditions

Treatment site	Investigational Product
Right/left volar forearm, antecubital fossa and cheek	Betamethasone Valerate (0.025%) cream
Right/left volar forearm. antecubital fossa and cheek	Crisaborole (2%) ointment

Twice-daily self-administered application of 1 finger-tip unit (FTU) of either crisaborole (2%) ointment or betamethasone valerate (0.025%) cream to the designated treatment areas on the arms (volar forearm and including the corresponding antecubital fossa) of the respective side of the body for 28 days. Additionally, ¼ FTU of the respective treatment to be applied to the cheek on the same side of the body twice-daily 14 days.

The 2 daily applications should be conducted once in the morning and once in the evening, separated by at least 8 hours.

When washing, the product should be applied after washing (not before). Randomised site allocation to avoid site dependent effects.

Participant diaries will be provided to record product usage (compliance) and provide usage instructions, including a visual guide to the IP allocation.

8.8 Prior and concomitant medication

All prior medications, including all medications, non-medication therapies, bland (non-medicated) emollients, over the counter drugs, vitamins, and antacids used within 28 days prior to Screening will be recorded. Any changes in concomitant medications or dosage will be recorded at Baseline/Day 1 and at each subsequent visit until completion (defined as completion of the last study visit). Medication entries should provide the correctly spelled drug or therapy name and the dose, units, frequency, route of administration, start and stop date, and reason for use. The use of any concomitant medication must relate to the participant's medical history or to an AE, except for vitamins/nutritional supplements and routine immunizations.

8.8.1 Prohibited Medications

Classes of medications and non-medication therapies that may alter the underlying (inflammatory) state of the skin and for which washout is required prior to Baseline/Day 1 are listed below. If a participant requires a washout, the investigator or his/her designee will provide instructions on discontinuing the prohibited medication(s) or non-medication therapy at the Screening Visit. The use of any excluded medications during the study will result in discontinuation of the participant.

- Medications Prohibited 12 weeks prior to Baseline/Day 1 and throughout the study
    - Biological drugs.
- Medications Prohibited 28 Days Prior to Baseline/Day 1 and throughout the study

  - Use of systemic (oral, parenteral) corticosteroids. 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.
  - Light therapy Ultraviolet (UV), Ultraviolet B (UV-B), psoralen–UV-A [PUVA]) anywhere on the body.
  - Use of TCS, or TCI *on the measurement sites*.
- Medications Prohibited 14 Days Prior to Baseline/Day 1 and throughout the study
  - Use of systemic antibiotics.
  - Use of TCS, or TCI, anywhere on the body.
  - Use of crisaborole ointment, 2%, anywhere on the body.
  - Use of topical retinoids or benzoyl peroxide *on the measurement sites*.
  - Use of topical antihistamines *on the measurement sites.*
- Medications Prohibited 7 Days Prior to Baseline/Day 1 and throughout the study
  - High doses of systemic sedating antihistamines (eg, hydroxyzine or diphenhydramine or other sedating antihistamines). Use of over-the-counter systemic sedating and non-sedating antihistamines is permitted at recommended doses for allergic rhinitis/hay fever
  - Use of bland (non-medicated) emollients, moisturisers or sunscreen *on the measurement sites*, within 7 days prior to Baseline/Day 1. Use of bland (non-medicated) emollient(s) and/or sunscreen is permitted during the study to manage dry skin and sun exposure in areas surrounding but not on or overlapping the test areas.
- Medications prohibited from Baseline/Day 1 and throughout the study
  - Use of nonsteroidal anti-inflammatory agents (NSAID's, excludes paracetamol), including ibuprofen, naproxen, diclofenac, celecoxib, mefenamic acid, etoricoxib, indomethacin, high-dose aspirin.
  - 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.

8.9 Investigational product accountability

The investigator site must maintain adequate records documenting the receipt, use, loss, or other disposition of the investigational product supplies. All investigational products will be accounted for using a drug accountability form/record.

Throughout the study, detailed investigational product accountability records, including tube weights, will be maintained for each participant by study staff.

The participants and/or caregivers will be asked to bring all dispensed investigational product (including empty, partial used and unused tubes) and the treatment diary to the clinic at every visit.

Detailed drug accountability records, including fortnightly tube weights measured in the site research facility, will be maintained by unblinded personnel for each participant.

The original investigational product accountability log, or equivalent document, must be accurately completed, and retained at the study site when the study is complete. The accountability log will be an unblinded document until study completion and therefore should only be accessed by unblinded site personnel.

8.10 Disposal of the study treatments

The sponsor or designee will provide guidance on the destruction of unused investigational product (eg, at the site). If destruction is authorized to take place at the investigator site, the investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and all destruction must be adequately documented.

For all investigational product returned to the investigator by participants and/or the parents/legal guardians, the investigator will maintain the returned supply until destruction is authorized by the Sponsor.

Only unblinded personnel should have access to or view any returned product. The sponsor or designee will provide instructions as to the disposition of any unused investigational product.

8.11 Compliance monitoring

The IP will be weighed at every visit and the diary reviewed. Both product weights and product application information will be captured in the EDC system.

Visits 2, 3, 5 and 7 are conducted for the purposes of compliance monitoring and participant training only. Compliance visits are not expected to take place on weekends. Only one application per day is to be supervised. The sessions should be arranged such that participants can fully adhere to the treatment regimen, requiring 2 applications per day, once in the morning and once in the afternoon/evening separated by at least 8 hours.

Treatment usage should be determined from the IP weights during every visit so that the information can be shared with the participant (and recorded in the treatment diary) in the context of expected usage, and any required changes in dosage or application technique suggested prior to the supervised application. Supervised applications should be performed at every study visit (after any scheduled skin assessments) to ensure consistent adherence to the dosing regimen. Re-training should be provided as required based on completion of the diary, product weights and application technique. The criteria for assessing usage is provided in the next section.

The diary should be reviewed at every visit for completeness and any gaps in application discussed with the participant. If, at all study visits except visits 2, 3, 5 and 7, the participant is found to have missed more than 20% of the scheduled applications, and/or missed more than 4 consecutive applications, and/or missed either of the 2 applications immediately preceding a study visit involving skin assessments the participant should be withdrawn. During Visits 2, 3, 5 and 7 the study

Investigators may use their discretion to judge a participant's ability to adhere to the treatment regimen.

8.12 Treatment dosing and compliance

Dosing is by finger-tip unit (FTU) rather than by gram to permit at home treatment by the study participants in accordance with routine clinical practice (Figure 8.1).

Figure 8.1: A guide to dosing topical creams

With 2 applications per day, **the expected usage is 2.5g per day of each product for the first 2 weeks and then 2.0g per day for the remaining 2 weeks** (after discontinuation of treatment to the cheeks).

Over the 28-day treatment regimen total usage of each product is estimated at **63g** (Table 8.3).

Table 8.3: Treatment dosage

Treatment site	Treatment area	Dose per application	Frequency of application	Total usage
Right/left volar forearm and antecubital fossa	2 hands, 1.46% BSA, ~280 cm^2	1 FTU, ~1g	Twice daily for 28 days (56 application)	56g
Right/left cheek	½ hand, 0.37% BSA ~70 cm^2	¼ FTU, ~0.25g	Twice daily for 14 days (28 application)	7g
Total	2 ½ hands, 1.83% BSA, ~350 cm^2	1 ¼ FTU, ~1.25g	Various	63g (31.5-126g)

Due to the expected variability of the FTU participants are expected to apply between 31.5g (50%) and 126g (200%) of each product (assessed at the end of treatment) to be compliant with the protocol.

Retraining should be provided at each visit where IMP usage falls outside the ideal range of 85% to 130%.

Rather than excluding participants based on the amounts of treatment used, the focus should be on education so that participants can achieve satisfactory compliance by the end of the study. At Visits 2 and 3 the study Investigators may use their discretion to judge a participant's ability to meet the target usage based upon their application technique, the adherence to the application schedule and the amounts used together.

8.12.1 IMP application during the morning of study visits

Treatments should not be applied on the day of study visits where a supervised application is scheduled OR where skin assessments are scheduled (as per Table 7.1).

To remind participants not to apply the morning application reminders should be issued. Where possible this should be done by text message using a work mobile phone. Where this is not possible telephone or emails reminders can be sent according to the preference of the participant.

Where the treatments have been applied on the day of study visits 4, 6 and 8, the visit will need to be re-scheduled, because treatment residues will interfere with the assessments. Washing the skin is not an acceptable substitute. **Treatments should not be applied on the day of study visits 4, 6 and 8 until after skin assessments have been conducted.**

Where the treatments have been applied on days not involving skin assessments, and 8 hours has passed since the last application the visit can be conducted as normal AND the application recorded as the second daily application (no further application that day). Where 8 hours has not passed, the supervised application should not be performed to prevent overdosing. The deviation from the protocol should be logged on the protocol deviation form. In this situation the session will not require rescheduling and all other activities should be completed as normal.

On issuing the treatment diary, the relevant mornings should be highlighted to remind participants not to apply the product as described above.

8.13 Investigator termination of participant involvement

Examples of participant non-compliance that may arise are:

- The participant fails to satisfactorily follow the study schedule (appointments not attended within the given timeframes), as per the schedule of events and this affects collection of data pertaining to the primary outcome measures.
- The participant fails to apply the product at the specified times/days. Participants who miss more than 20% of the scheduled applications, and/or miss more than 4 consecutive applications, and/or miss either of the 2 applications immediately preceding a study appointment should be withdrawn.
- The participant fails to use the investigational products in acceptable quantities (see "treatment dosing and compliance"). At each session usage should be assessed. Where IP use is above or below expectations a new demonstration should be provided. Supervised applications are performed at every visit as required.

- The participant fails to bring the diary to any visit and cannot provide their usage history. Where a diary is not available, the participant should be asked to recite their application history for the given study period and bring the diary at the next visit. Every effort should be made to retrieve the diaries. Where completed diaries cannot be obtained *AND* a participant's application history cannot be documented the participant should be withdrawn.
- The participant fails to bring the IP to study visits. If the participant fails to bring the IP to any visit they should be asked to return as soon as possible with the IP for weighing. A weight post 4-weeks treatment is required for compliance purposes (it is desirable for all other visits).
- Participants who inadvertently use the IP in the morning before the study visit – In these cases a new appointment should be scheduled where possible – if this is not possible within the constraints of the study the participant should be withdrawn.

9. PHARMACOVIGILANCE

9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions. It is important to note that this is entirely separate to the known side effects listed in the SmPC. It is specifically a temporal relationship between taking the drug, the half-life, and the time of the event or any valid alternative aetiology that would explain the event.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation o In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. o Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

	• results in persistent or significant disability/incapacity o The term disability means a substantial disruption of a person's ability to conduct normal life functions. o This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption. • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the reference safety information: • in the case of a product with a marketing authorisation, this could be in the summary of product characteristics (SmPC) for that product, so long as it is being used within it's licence. If it is being used off label an assessment of the SmPCs suitability will need to be undertaken. • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question

9.2 Reporting Period

The reporting period for AE will be from the date of informed consent until approximately 28 days after the last administration of IMP (range from 28 days to 35 days follow-up). All AE's will be followed up until causality has been assigned. Once causality has been assigned, only SAEs that are ongoing at the close of the reporting period will be followed up by the study team until they are either

resolved or stabilised with properly assessed expectedness assessment for those SAEs which are considered causally related.

For participants who are screen failures, the active collection period ends when screen failure status is determined.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

9.3 Operational definitions for (S)AE's

An AE is defined as any untoward medical occurrence in a participant during the course of the trial. Pre-existing conditions, although they will be recorded, will not be regarded as AEs unless they worsen significantly.

Recognised side effects of the IMP are fully documented in the Investigator Brochure (IB) for crisaborole or the SmPC for betamethasone valerate.

Whilst the study aims to investigate the sub-clinical adverse effects of the study IP's, for the purposes of this study only clinically observable adverse effects will be monitored from a safety perspective in line with normal practice. This is justified by the fact that (1) sub-clinical changes will only become evaluable after analysis which is likely to occur after cessation of treatment, and (2) it is not clear at this stage what level of sub-clinical changes correspond with the risks of developing clinical adverse effects.

All AE will be recorded in a Case Report Form (CRF) and assessed for seriousness, expectedness (serious AE's only), intensity and causality as outlined below.

9.3.1 Seriousness

A SAE will be defined as an AE which either

1. Results in death
2. Is life-threatening
3. Requires hospitalisation or prolongation of existing hospitalisation
4. Results in persistent disability or incapacity
5. Consists of a congenital abnormality or birth defect

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above will also be considered serious.

The investigator will determine the seriousness of each AE as per these criteria.

All SAE are subject to expedited reporting to both the Sponsor AND Pfizer (as the manufacturer of crisaborole, even where the SAE is unrelated to crisaborole) as outlined below. SAE will be reported using both the STH SAE form and Pfizer Investigator-Initiated or Clinical Research Collaboration Interventional Study SAE Report Form. When completing the Sponsor SAE form the type of report (initial or follow-up) should be clearly indicated. When providing follow-up information to Pfizer, a new form should be used in each case that includes the data that are new or revised from the previous report. Follow-up information should never be added to a previously submitted report form. Further guidance on the completion of the Pfizer form can be found in the Pfizer-provided Safety Reporting Reference Manual and SAE Form Guide.

9.3.2 Causality

Causality assessment:

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the investigator's brochure (IB) and/or product information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Funder (this is not a requirement for initial reporting to the Sponsor).
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.
- If the investigator does not know whether or not the investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes, as defined by the sponsor. In addition, if the investigator determines that an SAE is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, and report such an assessment in the dedicated section of the CT SAE Report Form and in accordance with the SAE reporting requirements.

The investigator will determine the causality of each AE as defined here:

- Unrelated or unlikely: a clinical event including laboratory test abnormality with temporal relationship to trial treatment or IMP administration, that makes a causal relationship

incompatible or for which other drugs, chemicals or disease provide a plausible explanation. This will be counted as "unrelated" for notification purposes.

- Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment or IMP administration which makes a causal relationship a reasonable possibility, but which could also be explained by other drugs, chemicals or concurrent disease. This will be counted as "related" for notification purposes.
- Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment or IMP administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other drugs, chemicals or concurrent disease. This will be counted as "related" for notification purposes.
- Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment or IMP administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

A SAE whose causal relationship to a study IMP is assessed by the Chief Investigator as "possible", "probable" or "definite" will be considered 'related' and is a Serious Adverse Drug Reaction.

9.3.3 Expectedness

Expectedness will be assessed for SAE's only. A SUSAR is any event which qualifies as an SAE and meets the criteria of being judged as possibly, probably or definitely related to study IMP and has a nature and/or severity of which is not consistent with the information about the medicinal product in question as set out in the summary of product characteristics, investigator brochure or IMP dossier for that product (ie it is 'unexpected').

A SUSAR will require expedited reporting as per the Clinical Trials Regulations. All serious AE that fall or are suspected to fall within these criteria shall be treated as a SUSAR until deemed otherwise.

9.3.4 Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.

- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.

- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

9.4 Reporting time-frames

The time frame for reporting an SAE to the Sponsor AND Pfizer is:

- Immediately upon awareness, if the SAE is fatal or life-threatening (i.e., causes an immediate risk of death) —regardless of the extent of available information
  OR
- Within 24 hours of first awareness of the SAE, if the SAE is not fatal or life threatening

These timeframes are applicable to all reportable SAEs

9.5 Responsibilities

The Principal Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the study IMP as set out above
- Record all SAEs on standardised SAE forms and report them to the Sponsor by email (sth.sae1@nhs.net) within 24 hours of the becoming aware of the event.
- Complete the Pfizer-provided *SAE Report Form*, and submit it to Pfizer, with the *Reportable Events Fax Cover Sheet,* immediately for a death or life-threatening event, and within 24 hours for all other reportable SAEs. It should be noted that the CT SAE Report Form for reporting of SAE information is not the same as the AE page of the CRF. When the same data are collected, the forms must be completed in a consistent manner. AEs should be recorded using concise medical terminology and the same AE term should be used on both the CRF and the CT SAE Report Form for reporting of SAE information.
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action
- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor or Pfizer to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other healthcare professionals.
- If a participant dies during participation in the study or during a recognized follow up period, the investigator will provide Pfizer Safety with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.

The Sponsor will:

- Report SUSARs which are fatal and life-threatening to the Competent Authority within 7 days.
- Report SUSARs which are not fatal and not life-threatening to the Competent Authority within 15 days.
- Shall, within a further eight days send any follow-up information and reports to the MHRA.

- Make any amendments as required to the study protocol and inform the ethics and regulatory authorities as required

9.6 Pregnancy reporting

- All pregnancies within the trial (limited to the trial participant, with participants consent) should be reported to the Chief Investigator and the Sponsor using the *STH Pregnancy Reporting Form* AND Pfizer-provided *SAE Report Form* WITH an *Exposure During Pregnancy supplemental report form* within 24 hours of notification
    - The specific details that need to be included in the report of an Exposure During Pregnancy depend on whether the exposure was maternal or paternal, noting that only maternal instances will be reported in this study. Further guidance on the completion of the Pfizer forms can be found in the Pfizer-provided Safety Reporting Reference Manual and SAE Form Guide.
    - The anticipated date of delivery should be included in the Narrative section of the report.
- Whilst an SAE form is used for reporting, Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE and requires reporting as such.
- The Principal Investigator must then follow the participant throughout the pregnancy and is to notify the Sponsor and Pfizer of the outcome as a follow-up to the initial Exposure During Pregnancy report.

9.7 Safety updates

Information regarding unexpected clinical study SAEs that are causally related to crisaborole (a Pfizer product) will be provided to investigators via semi-annual line listings.

10. STATISTICS AND DATA ANALYSIS

10.1 Sample Size

The primary statistical analyses will compare the change in epidermal thickness from baseline to day 29 between treatments: betamethasone valerate cream vs crisaborole ointment, utilizing the within participant comparisons of left vs right volar forearm.

Assuming that a clinically relevant difference in the change from baseline to day 29 is 6 μm and a standard deviation of approximately 12 μm (for the change from baseline within each participant, taken from recent data), to detect this change with 80% power using a 2 sided 5% significance level, requires 33 participants in each group.

10.2 Planned recruitment rate

A single cohort of up to 40 participants will be recruited and treatment issued to meet the target of 33 for completion (allowing for a 18% drop out rate). Recruitment is estimated to take approximately 9 months at a rate of ≥4.5 recruits per calendar month.

10.3 Analysis population and protocol deviations

The analysis population will include all participants who provided informed consent AND were issued the treatments, this will be known as the modified intention to treat (ITT) population.

All protocol deviations, including missing or spurious data, will be recorded in the protocol deviations log. The protocol deviations log will be reviewed at the close of the study and (if appropriate) deviations will be listed in the report. Only important deviations, as defined by ICH will be listed. Important protocol deviations are defined by ICH as: a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a participant's rights, safety, or well-being.

Protocol deviations will be reviewed whilst the data is still blind and if there are concerns that a lack of compliance to the protocol may impact the analyses then a per protocol population may be defined and used for a sensitivity analysis. The ITT population will remain the primary analysis population.

In addition, summaries of each of the endpoints will clearly show the amount of missing data (if appropriate).

10.4 Statistical analyses plan

A statistical analysis plan will be produced before the start of the study.

In addition to the main analysis at the end of the study, the following analysis is planned:

- Epidermal thickness data collected from the cheek (blind) will be reviewed after the first 5 and 10 participants, and the degree of epidermal thinning from baseline (V1) calculated. The

TMG will review the data and consider the need for changes to the treatment regimen on safety grounds in an abundance of caution.

- An interim analysis of the primary outcome (blind) is planned after the first 10 participants have completed.

10.4.1 Summary of baseline data and flow of patients

Relevant baseline and demographic data (including Fitzpatrick skin type) will be summarised.

Treatment emergent AE and SAE will be summarised for all participants who received the treatment (at least 1 dose). All AE will be listed (including those which began before initiation of study treatment or after the follow up period).

10.4.2 Primary outcome analysis

Analysis of the primary endpoint will use a paired analysis to calculate the mean difference and a 95% confidence interval for that difference. The distribution of the differences will be reviewed and if the data requires it a non-parametric analysis may be utilised and a median may be presented.

10.4.3 Secondary outcome analysis

The analysis of the secondary endpoints will use paired analyses to compare the change over time between crisaborole (2%) ointment and betamethasone valerate (0.025%) cream. No formal adjustments for multiplicity will be made, however results will be presented alongside confidence intervals and will be interpreted cautiously.

10.4.4 Exploratory outcome analysis

Exploratory endpoints will be analysed utilizing appropriate tests and, where appropriate, descriptive analyses. There is no plan to derive quantitative values from the biopsies. Images of the skin tissue will be used to visually support the primary and secondary outcomes only. A detailed plan of the exploratory analysis will be included in the statistical analysis plan.

11. STUDY CONDUCT

11.1 Study location

The Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF

- Study enrolment, randomisation and IP related activities will be conducted in an appropriate location by suitably qualified members of the research team as agreed with the study sponsor
- Study procedures will be carried out on K-floor in the Sheffield dermatology research Skin Barrier Volunteer Suite, a dedicated space for human skin research with full climate-controlled conditions.

11.2 Study staff

All study team members are trained in good clinical practice. A record of training and delegation of duties will be kept in the site file.

11.3 Participant tracking

All participants enrolled into this study will be tracked using an excel Participant Tracking Log, located on the groups online Filestore.

11.4 Sample tracking and storage

All samples collected as part of this study will be logged in a Sample Tracking Log and all use documented in a Sample Tracking Log.

The number, type and destination of samples is indicated in the table below.

Sample	Relevant material under HTA?	Storage/Destination
Saliva sample (buccal swab) 1 per participant x40	Yes	Genomic DNA isolated for *FLG* genotyping. All samples will be processed within 12 months following the last participant last visit. Specific consent will be sought to keep gDNA beyond the end of this study in order to support future research projects.
Superficial skin samples collected on TS 1-10. 2 sets from	No (comprises enucleated corneocytes)	Storage at -70ºC. Required for stratum corneum metabolite (NMF) quantification. Samples will be processed within 12 months following the last participant last visit.

each participant at 2 timepoints.		Remaining *unused) samples retained for future protein and metabolomic analysis. Specific consent will be sought from participants to retain these samples following completion of the study.
Skin biopsies. 20 frozen and 20 paraffin-embedded.	Yes	Frozen tissue stored at -70°C. Paraffin-embedded tissue store at room temperature. Samples will be retained by the SDR group (University of Sheffield) at the end of the study for use in future eczema research (subject to additional REC approval).

11.5 Data collection tools and source document identification

All research data will be collected first hand by the study team and recorded in several ways to provide verifiable source:

- Paper Case Record Forms (CRFs): Most study information will be captured directly using a series of paper CRFs.
    - Captures demographic information, medical history, concomitant medication, adverse events, participant height and weight, eczema severity, pregnancy test results, visual scores, objective redness (Mexameter), TEWL, treatment weights.
    - CRFs will be stored in the study office within the site file.
- Skin images (2D): captured directly into a study specific Pixience c-cube clinical research database.
    - The database is stored on the university filestore in a secured study folder. The database includes an indexing file and separate .jpeg files for each image. Prescribed analysis (skin colour/redness) is conducted within the Pixience software and generates a single .csv file containing the full dataset.
- OCT images (.mat files) and accompanying image collection and analysis reports (containing the study variables)
    - The images and associated reports are stored in the university filestore in a secured study folder. The reports will be printed, signed and stored in the site file upon generation.
- PS-OCT images (.mat files) and accompanying image collection and analysis reports (containing the study variables)
    - The images and associated reports are stored in the university filestore in a secured study folder. The reports will be printed, signed and stored in the site file upon generation.
- FTIR spectra (.a2r and .spc files) and accompanying spectrum reports generated within the Microlab software environment.

  - The reports, which will be printed, signed (within 72 hours of collection) and stored in the site file upon generation, contain all the measurements needed for the FTIR-based endpoints.
  - The spectra and associated reports are stored in the university filestore in a secured study folder
  - EASI scoring worksheets (paper) stored in the study office within the site file.
- Squamescan data: captured directly using the DensPC software and pasted into an excel database file. The data is pasted directly into a printable template within the excel file, which will be printed and signed at the point of collection to provide verifiable source data.
  - The database file will be stored in the university filestore in a secured study folder
  - The signed source data will be stored in the site file.
- Images of skin tissue sections following histological analysis in an appropriate format such at .tif files.
  - The files will be stored in the university filestore in a secured study folder.
- Lab book(s): Bioanalysis of saliva, superficial stratum corneum, and biopsy samples will be documented within University of Sheffield lab books
  - Lab books are stored on site, secured in the study office or lab. Project specific lab books will be used where possible so that these can be archived with the study records. Where this is not possible copies will be made for archiving.

Source data will be transferred to a study database capturing all study endpoints required for analysis. The database will be designed and managed by the Sheffield Clinical Trials Research Unit (CTRU) of the University of Sheffield using the Prospect system.

A separate data management plan will be developed to provide details on how data will be managed in this study.

11.5.1 Research notes

Whilst research notes will not be used to gather source data, the notes will be accessed and updated with details of participation in this trial. This will include adding a Research Alert and a copy of the consent form to the hospital notes.

For participants not currently undergoing care at the hospital new notes will be created.

11.6 Data handling and record keeping

Research data will be stored in Prospect, CTRU's bespoke electronic data capture (EDC) system developed in collaboration with epiGenesys. Data entered directly into the EDC system will be qualified using pre-established parameter ranges. Printed records created at the time of data entry will be signed to provide source verification. Research data collected on paper CRF's and then transferred to the EDC system will be partially verified by checking 10% of all records against the original source.

11.7 Data Access

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections- in line with participant consent.

11.8 Archiving

- The site will be responsible for archiving all study data
- Archiving will be authorised by the Sponsor following submission of the end of trial report
- All essential documents will be archived for a minimum of 15 years after completion of trial
- Destruction of essential documents will require authorisation from the Sponsor

12. MONITORING, AUDIT & INSPECTION

12.1 Monitoring

The study will undergo monitoring by the research Governance Sponsor, Sheffield Teaching Hospitals Trust (STH). STH will prepare a separate monitoring plan for this study. Monitoring will be conducted with the express purpose of ensuring the study meets all regulatory requirements.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Research Ethics Committee (REC) review and reports

- Before the start of the trial, approval will be sought from a REC and the HRA for the trial protocol, informed consent forms and other relevant documents e.g. Advertisements and GP information letters
- Amendments that require review by the REC and the HRA will not be implemented until a favourable opinion has been given (note that amendments may also need to be reviewed and accepted by the MHRA, and/or NHS R&D departments before they can be implemented in practice)
- All correspondence with the REC will be retained in the Trial Master File/Investigator Site File
- An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended
- It is the Chief Investigator's responsibility to produce the annual reports as required.
- The Chief Investigator will notify the REC of the end of the trial
- If the trial is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination
- Within one year after the end of the trial, the Chief Investigator will submit a final report with the results, including any publications/abstracts, to the REC

13.2 Peer review

The protocol has undergone peer review organised by Pfizer. A copy of the panels feedback is available.

13.3 Public and Patient Involvement

The study has been designed off the back of a public consultation to identify research priorities important to patients.[9] Further to this the public and patients were involved in the review of the participant information sheets used in the first SMART study, on which the documents for this study are closely based, ensuring they are easy to understand and informative.

13.4 Regulatory compliance

- The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA and a Favourable REC and HRA opinion has been given
- The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments

- Before the site can enrol patients into the study, the Chief Investigator/Principal Investigator or designee will ensure that appropriate approvals from the participating organisation is in place.
- For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body in order for them to issue approval for the amendment. The Chief Investigator or designee will work with the site (NHS R&D department as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

13.5 Protocol compliance

Protocol non-compliances are departures from the approved protocol.

Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used e.g. It is not acceptable to enrol a participant if they do not meet the eligibility criteria or restrictions specified in the trial protocol

Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Protocol deviations should be avoided whenever possible.

Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach.

When a protocol deviation occurs, it must be captured on a protocol deviation log and on the individual participant source documentation, as applicable.

Major protocol deviations, defined as deviations that compromise either the safety of participants or compliance with CT regulations, require expedited (within 24 hours of discovery) reporting to the Governance Sponsor.

13.6 Notification of serious breaches to GCP and/or the protocol

A “serious breach” is a breach which is likely to effect to a significant degree –

- The safety or physical or mental integrity of the participants of the trial; or
- The scientific value of the trial

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase

The sponsor will notify the licensing authority in writing of any serious breach of

- The conditions and principles of GCP in connection with that trial; or
- The protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach

13.7 Data protection and patient confidentiality

All investigators and trial site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

13.7.1 Patient Confidentiality

All investigators will follow ethical and legal practice and all information about the study participants will be kept strictly confidential. Any information about a participant that leaves the hospital (our site) will have the name and address deleted, so that they cannot be recognised by it. This includes data that are transmitted electronically.

Each participant will be allocated a unique study number, which will be used for recording demographic and study data.

All electronic data will be stored on a secure server, managed by the University of Sheffield, and will be identifiable only by the unique study number. Personal contact details will be recorded separately, and only be accessible by the direct study team. Any information included in written reports/presentations will not identify participants by name. Only the members of the research team will have access to personal identifiable data.

13.7.2 Data storage

Personal identifiable information (PII), captured on the paper Participant Screening, Enrolment & Completion Log and Registration Form, will be held in a study site file, kept in a locked cabinet within both the study office (locked and within a swipe-card restricted zone) and the site research facility. At the end of the study all PII stored on paper, except the information captured in the Participant Screening, Enrolment & Completion Log and on the consent forms, will be destroyed (within 1 year of the end of the trial). The Participant Screening, Enrolment & Completion Log and consent forms will be kept for 15 years after the end of the trial in line with current regulatory requirements.

Volunteer contact details will be recorded using an online database within the University of Sheffield Google Workspace. Access to this database is restricted to members of the SDR team. The account is password protected with multi-factor authentication. The University of Sheffield and SDR information security policies ensure that only appropriately trained and delegated individuals may access the volunteer contact details and only using secure computers (on campus or within the Cyber Essentials environment). To facilitate study management volunteers will be required to supply a contact email address and mobile number. Volunteer details will be held for up to 1 year from registration to allow study correspondence. Volunteers will be able to consent to the retention of contact details for a maximum of 5 years to enable the group to notify participants about future

studies. Upon registration of contact details volunteers are supplied with a privacy notice detailing their rights with respect to the personal data supplied.

All research data collected in the pursuit of this study (the study data, excluding any PII), will be collected in pseudo-anonymised form. For the purposes of this study, ***"pseudo-anonymised research data"*** is data that has had all PII (such as name, initials and date of birth) replaced by the subject identifier (***unique study number***), and so protects the identities of the participants whilst still enabling the information to be traced back by the direct study team (who will have access to the study number allocation). The study number allocation (contained within the enrolment log) will only be stored in paper form within a study Site File. All pseudo-anonymised research data will be collected in paper or electronic form, with paper records kept in a Site File in a locked cabinet in the study office and electronic records stored on the groups Filestore (UoS managed secure server with access control). Pseudo-anonymised research data will be kept indefinitely under the custodianship of the PI. Members of the Sheffield Dermatology Research team, who have been appropriately delegated activities on the study by the PI, will analyse the study data. Anonymised data may be shared with our research partners, including for example the study Funder, to make full use of the results – but we will never reveal the study number allocation.

13.8 Financial and other competing interests

MJC, has been/is a Clinical Trial Investigator for the following organisations: Atopix, Galapagos, Hyphens, Johnson & Johnson, Kymab, Leo, L'Oreal/LaRochePossay, Novartis, Pfizer, Regeneron, and Sanofi-Genzyme. He is an Advisory Board member, Consultant &/or invited lecturer for the following organisations: Abbvie, Amlar, Astellas, Atopix, Boots, Dermavant, Galapagos, Galderma, Hyphens, Johnson & Johnson, Kymab, Leo, L'Oreal/LaRochePossay, Menlo, Novartis, Oxagen, Pfizer, Procter & Gamble, Reckitt Benckiser, Regeneron, Sanofi-Genzyme.

SGD, has received fees for giving lectures and/or attending advisory boards and unrelated research funding from Almirall, Astellas Pharma, Bayer Dermatology, Leo Pharma, MSD, Pfizer, and Stiefel-GSK who manufacture topical anti-inflammatory treatments for eczema.

SS, None to declare

SJM, None to declare

RT, None to declare

RB, None to declare

13.9 Indemnity

The following indemnity protection is in place:

1. NHS indemnity protection is in place to meet the potential legal liability of the sponsor for harm to participants arising from the management of the research

2. NHS indemnity protection is in place to meet the potential legal liability of the sponsor for harm to participants arising from the design of the research

3. NHS and University of Sheffield insurance protection is in place to meet the legal liability of investigators/collaborators for negligent harm arising from the conduct of the study

4. Pfizer indemnity protection is in place to meet the potential legal liability of the manufacturer for harm to participants arising from an inherent manufacturing defect in the Pfizer Product crisaborole (2%) ointment

13.10 Amendments

Under the Medicines for Human Use (Clinical Trials) Regulations 2004, the sponsor may make a non-substantial amendment at any time during a trial. Non-substantial amendments to study documents included in the original Health Research Authority (HRA) submission package will require HRA approval before implementation. If the sponsor wishes to make a substantial amendment to the CTA or the documents that supported the original application for the CTA, the sponsor must submit a valid notice of amendment to the licencing authority (MHRA) for consideration. If the sponsor wishes to make a substantial amendment to the REC application or the supporting documents, the sponsor must submit a valid notice of amendment to the REC and the HRA for consideration. The MHRA and/or the REC will provide a response regarding the amendment within 35 days of receipt of the notice. It is the sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the MHRA and/or REC.

If applicable, other specialist review bodies (e.g. CAG) need to be notified about substantial amendments in case the amendment affects their opinion of the trial.

13.11 Post trial care

Participants are not enrolled onto this study because of their need for care (including the need for the intervention), and therefore there is no requirement to provide ongoing or post-trial care (with the exception of unresolved AE's).

13.12 Access to the final trial dataset

The following stake holders will have access to the final, anonymised, dataset:

- The Sponsor
- The Investigators at the Site
- The Funder, Pfizer Inc.

14. DISSEMINATION POLICY

14.1 Dissemination policy

- The data arising from this trial will be owned by the University of Sheffield
- on completion of the trial, the data will be analysed and tabulated and a Final Trial Report prepared
- The investigators have the right to publish any of the trial data subject to fair review by the study funder as defined in a separate agreement (30-day notice period)
- The Funder, Pfizer, will be acknowledged within the publications for their support
- A summary of the study findings will be shared with study participants after the Final Trial Report has been compiled and the results have been published

14.2 Final report

A draft report will be written and submitted to the Financial Sponsor for review and changes may be made to the draft report at the Financial Sponsor's request. Upon approval by the study team and the Financial Sponsor, the report will be finalized. The final report will include (but is not limited to) the following: study population demographics, statistical methodology, results, description of AE and deviations (if any), and conclusions. The report will not include patient level study data or patient identifiers.

14.3 Publication

Results from this study may be published in medical or scientific journals/books, publications, presentation materials, or advertising materials. Participant names and identifiable information will not be used in those materials or publications.

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16. APPENDIX

Appendix A: Risk assessment

Appendix B: List of sperate documents relating to this trial

- SMART2 A4 Poster
- SMART2 Landscape Advert
- SMART2 Social Media Advert
- SMART2 Participant Information Leaflet (PIL)
- SMART2 Participant Information Sheet and Consent Form
- SMART2 Treatment diary

Appendix C: List of IMP information available as separate documents

- Betnovate RD cream Product Information Leaflet
- Betnovate RD cream SmPC
- Crisaborole IB

Appendix D: Pfizer safety reporting material available as separate documents

- Investigator initiated research SAE form guide
- Safety Reporting Reference Manual
- Fax cover sheet
- Serious Adverse Event Report Form