Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Sheffield Dermatology Research

The University Of Sheffield.

Skin bioMARkers for atopic eczema Therapy evaluation

Study Title	Validation of a novel composite of skin biomarkers as a primary outcome measure for evaluating the safety of treatments for atopic dermatitis: a randomized controlled trial (phase 2) comparing the effects of crisaborole 2% ointment to betamethasone valerate 0.1% cream on skin structure and function in participants with atopic dermatitis.
Short Title	Skin bioMARkers for atopic eczema Therapy evaluation
Acronym	SMART
Date and Version	27 Jan 2021, Version 5.0
IRAS#	269415
NCT#	NCT04194814
EudraCT#	2019-002643-23
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Co-Investigators	Dr Simon Danby, Research Fellow (Trial Manager) Prof Stephen Matcher, Professor of Biophotonics, Miss Rosie Taylor, Principal Consultant, Dr Rob Byers, Research Associate
Study Site	The Royal Hallmshire 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	STH19966
Research Ethics Committee (REC)	East Midlands - Derby Research Ethics Committee
REC Reference	20/EM/0006
Funder	Pfizer, Inc.
Funder Reference	WI242083-UK-University of Sheffield-Eucrisa

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
1.0	2.0	11Feb20	SD	The following changes were implemented in response to feedback from the REC: Section 1 has been updated with a new lay summary Section 3 has been modified slightly to clarify the primary objective of this study New stop criteria have been added to the new sub-section 7.11.1 Section 11.6 was updated to specify the EDC system
2.0	3.0	25Jun20	SD	Section 9.3 updated to remove ambiguity over the expectedness assessment, which will only be performed for AE's that are serious. Section 11.5 updated to refer to the data management plan, where more detailed information on the capture of electronic source data will be provided. Updates to the tape-strip sample collection sections (7.8 and 11.4) to remove restrictions on which tape-strips will be used for the NMF metabolite analysis. The Lab Manual provide specific information on which tape-strips will be analysed Appendix B updated to remove the CRF names as some of these names have changed. Collection of TEWL measurements during tape-stripping as well as afterwards has been added in.

				This is to ensure the data collected can be compared with recently published data. TEWL measurements adjusted from 3 to 2 repeats per test area to compensate for the additional measurements during tape-stripping. This still provides a robust 4 repeats per treatment area in total. FTIR repeats reduced to a single measurement (down from 2) comprising a greater number of scans per measurement. This will greatly simplify data management and enable the TEWL measurements indicated above to be collected. Note that baseline measurements will still involve the collection of two measurements from the 2 test areas per treatment area. Internal data demonstrates that repeats provide only marginal reductions in error because each measurement consists of multiple (repeat) scans anyway.
3.0	4.0	21Oct20	SD	Updated to permit certain elements of the trial to be conducted in unspecified clinical areas within the Royal Hallamshire Hospital, those areas to be agreed as suitable by the study sponsor. This also corrects the erroneous use of two versions of the 'CRF' abbreviation, with it now only referring to case report form.
4.0	5.0	27Jan21	SD	Expected weights of investigational product used have been updated to reflect actual weights used, which are higher than originally quoted due to the larger nozzle size of the betamethasone valerate dispensed (100g tube with 7mm nozzle instead of 5mm). The dosage (in fingertip units) has not been changed and both products are still being used in line with their respective marketing authorisations. A new compliance visit has been added in, Visit 6a, with no changes to the objectives or endpoints. The additional compliance visit has been added to encourage consistent topical product usage throughout the study. The large 2-week gap between visits 6 and 7 has led to a

				gradual tapering down of product usage. The new visit will help encourage good compliance without materially changing the study design. Minor inconsistency in the days quoted before re-consent is required between sections 7.1 (28 days), 7.3 (28 days) and 7.4.3 (21 days) corrected. Now consistently quoted as 28 days.

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: .....

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SYNOPSIS

Title	Validation of a novel composite of skin biomarkers as a primary outcome measure for evaluating the safety of treatments for atopic dermatitis: a randomized controlled trial (phase 2) comparing the effects of crisaborole 2% ointment to betamethasone valerate 0.1% cream on skin structure and function in participants with atopic dermatitis.	
Acronym	SMART	
Logo		
Clinical Phase	Phase 2	
	Objectives:	**Outcome measures:**
Primary	• To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, for up to 4 weeks is a cause of skin atrophy in patients with atopic dermatitis.	• The difference in the change in epidermal thickness (day 29 – day 1), measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.1%) cream.
Secondary	• To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.1%) cream • To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream	• The difference in the change in epidermal thickness measured by structural OCT during and after 28 days treatment. • The difference in the change in visual redness/erythema score during and after 28 days treatment. • The difference in the change in objective redness assessed with the Mexameter during and after 28 days treatment.

	• To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, on skin barrier function	• The difference in the change in Trans-Epidermal Water Loss (TEWL) during and after 28 days treatment. • The difference in skin barrier integrity ($TEWL_{ts20}$) after 28 days treatment.
	• To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, on skin dryness and the levels of natural moisturising factor (NMF) in the skin	• The difference in the change in visual skin dryness during and after 28 days treatment • The difference in Natural Moisturising Factor (NMF, filaggrin breakdown products) levels at the end of treatment.
Exploratory	• To investigate whether OCT-derived biomarkers (other than the established structural OCT biomarker) enable the accurate quantification of tissue changes (vascular and matrix) associated with epidermal atrophy in response to TCS treatment	• The difference in the change in superficial plexus depth (µm) measured by angiographic OCT • The difference in the change in mean blood vessel diameter (µm) measured by angiographic OCT • The difference in the change in blood vessel density (segments/mm^2) measured by angiographic OCT • The difference in the change in collagen matrix index (an index derived from birefringence images of collagen density and arrangement) measured by polarisation sensitive (PS)-OCT
	• To investigate whether FTIR spectroscopy-derived biomarkers enable the accurate quantification of skin barrier	• The difference in the change in carboxylate levels (indirect measure of natural moisturising factor levels) in the stratum

	condition and function in response to TCS treatment	corneum measured by FTIR spectroscopy • The difference in stratum corneum lipid structure measured by FTIR spectroscopy in conjunction with tape-stripping
	• 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 and epidermal thickness by mutation status, if sufficient participants with mutation are detected.
	• To identify (by completing the objectives above) a panel of biomarkers that best characterises epidermal atrophy. The biomarkers identified will be taken forward into the next stage of the research program.	• All of the above
Trial Design	An observer-blind forearm-controlled clinical trial in 37 AD patients, wherein each participant will undergo 4 weeks treatment with crisaborole (2%) ointment on one forearm and betamethasone valerate (0.1%) cream on the other (twice daily application in each case and randomised site allocation). At the start of the study the skin of the test sites (forearms) 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.	
Trial Participants	Participants 18-65 years old with AD not currently undergoing, or requiring, active drug treatment.	
Planned Sample Size	A single cohort of 37 participants will be recruited with a target of 33 for completion (allowing for a 10% drop out rate)	

Key eligibility criteria	**Inclusion criteria** 1. Volunteers with AD defined according to the UK working party diagnostic criteria 2. Male or female aged 18-65 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** 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 treatment (anatomical) sites is almost clear or greater (score ≥1) based on the Investigators static global assessment scale at screening and baseline. At the start of the study the skin of the test sites (forearms) will therefore be clear (0) of the signs of eczema 4. Participants with a condition that in the opinion of the investigator contradicts participation in the study. 5. 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. *6.* 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.* *7.* 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.*

	8. 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.* 9. Participants currently participating in another interventional clinical trial. 10. Volunteer is incapable of giving fully informed consent. 11. Participants judged by the PI to be inappropriate for the trial.
Investigational medicinal product(s)	• Crisaborole (2%) ointment • Betamethasone valerate (0.1%) cream
Formulation, Dose, Route of Administration	4 weeks twice-daily self-administered application of 1 finger-tip units of either crisaborole (2%) ointment or betamethasone valerate (0.1%) cream to the volar side of the forearm.
Treatment duration	28 days
Follow-up duration	28 days
Planned Trial Period	The duration participants will participate in this study is approximately 9 weeks, including the 1-week run-in period (no topical product use on test 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
CA	Competent Authority
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
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
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
RCT	Randomised Control Trial
REC	Research Ethics Committee
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
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
TSC	Trial Steering Committee
USPI	United States prescribing information

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. Julia Carpenedo Clinical and Research Collaborations Liaison External Medical Communications Pfizer Inc. Office: 212 733 9490 Mobile: 347 413 1769 Email: julia.carpenedo@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 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
CHIEF INVESTIGATOR: (Medical Expert)	**Prof Michael J Cork** Professor of Dermatology at the University of Sheffield Consultant Pediatric Dermatologist at the Sheffield Children's Hospital NHS Trust Consultant Dermatologist at the Sheffield Teaching Hospitals NHS Foundation Trust Sheffield Dermatology Research The University of Sheffield Medical School (K125), Beech Hill Road, Sheffield S10 2RX Tel: 0114 2712933 Email: m.j.cork@sheffield.ac.uk
SUB-INVESTIGATORS:	**Dr Simon G Danby** Independent Research Fellow Sheffield Dermatology Research The University of Sheffield Medical School (K123), Beech Hill Road, Sheffield S10 2RX Tel: 0114 2159563 Email: s.danby@sheffield.ac.uk **Prof Stephen Matcher** Chair in Biophotonics Dept of Electronic & Electrical Engineering, The University of Sheffield Tel: 0114 222 5994 Email: s.j.matcher@sheffield.ac.uk **Rosie Taylor** Principal Consultant The Statistical Services Unit, The University of Sheffield, Sheffield Tel: +44 (0)114 222 3900 Email: r.n.taylor@sheffield.ac.uk

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. Therefore, the aim of this study is to conduct a trial in eczema patients involving treatment of separate areas of their skin with either crisaborole ointment or betamethasone valerate cream. The effects of the treatments will be assessed using the non-invasive skin imaging techniques.

Both treatments 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 causes the same unwarranted skin thinning caused by betamethasone valerate in a direct comparison. 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].

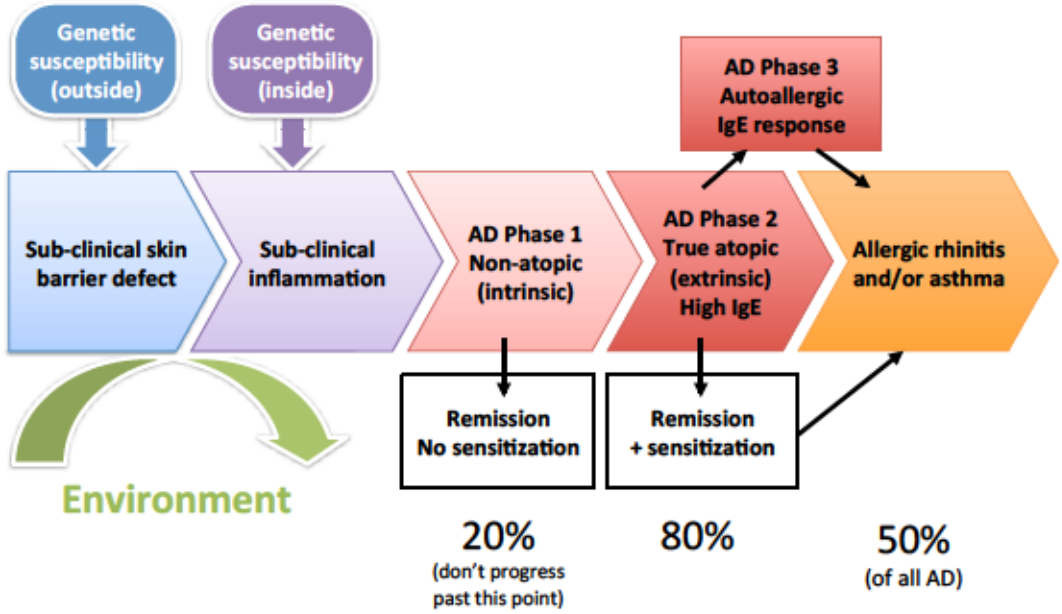


Fig 2.1: The development and progression of AD

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.

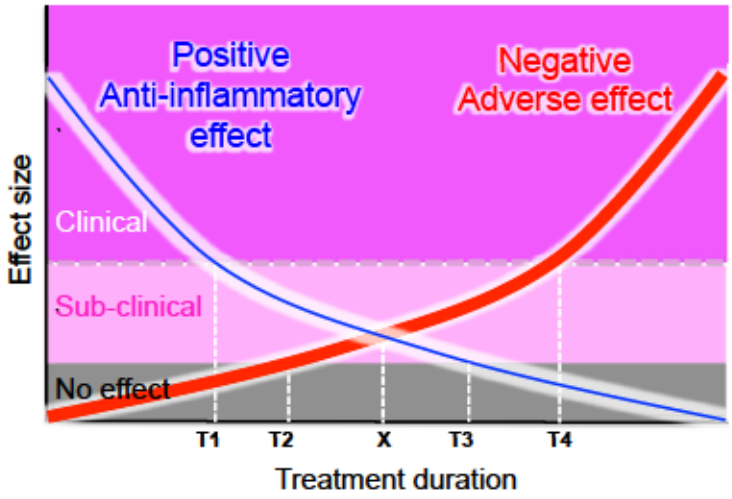


Fig 2.2: Positive and negative effects of TCS treatment on the skin (blue and red lines, respectively). Initially TCS exert positive effects by effectively reducing clinical/visible inflammation (T1). Clinically visible adverse effects (skin thinning) arise with prolonged, or inappropriate, use of TCS (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).

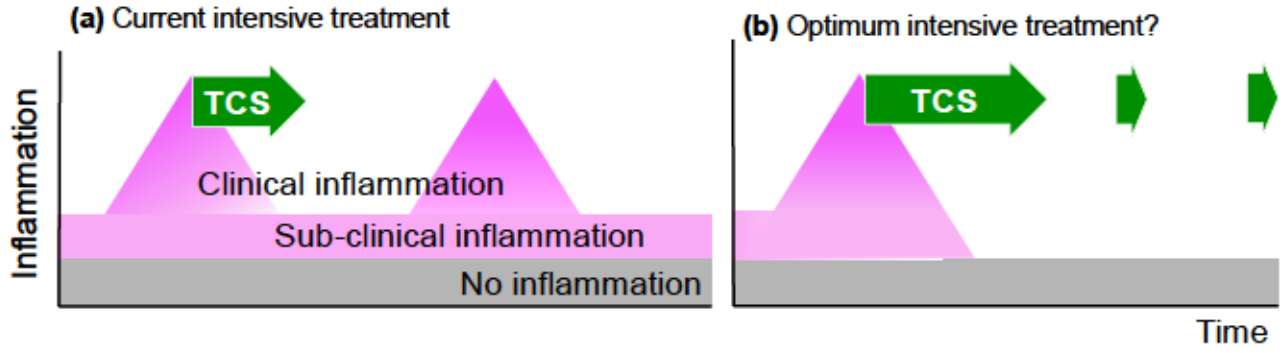


Fig 2.3: The effect of sub-clinical inflammation on the control of AD. The period of remission (flare-free) can be prolonged, by prolonged use of TCS to control clinical and sub-clinical inflammation early on.

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 Transepidermal 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).

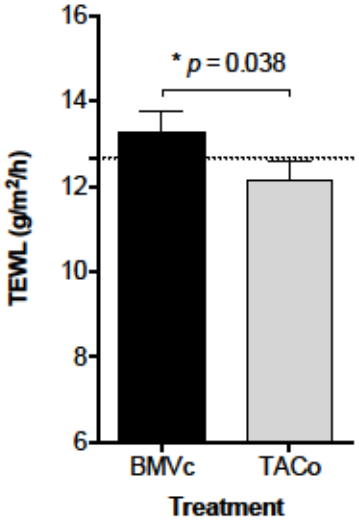


Fig 2.4: The effect of betamethasone valerate (0.1%) cream (BMVc) and Tacrolimus (0.1%) ointment (TACo) on skin barrier function in patients with quiescent AD. TEWL was significantly different post-treatment, accounting for baseline measurements (n = 25, one-way ANCOVA, $p = 0.038$). The dashed line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

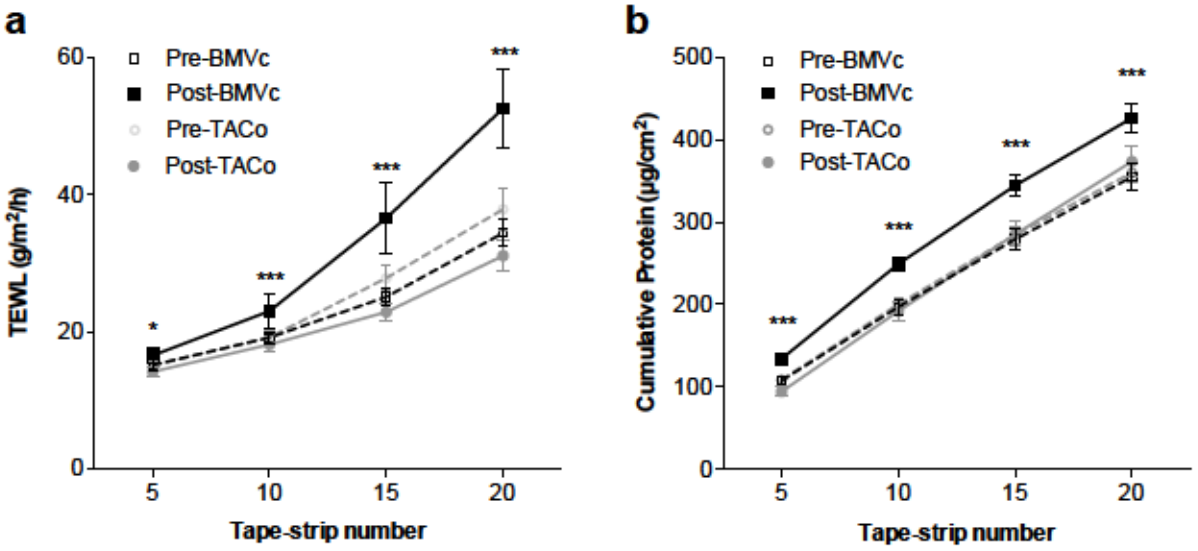


Fig 2.5: The effect of betamethasone valerate (0.1%) cream (BMVc) and Tacrolimus (0.1%) ointment (TACo) on skin barrier integrity and cohesion. (a) TEWL measured in conjunction with tape-stripping was significantly higher following treatment with BMVc compared with TACo (n = 25, $p = 0.0024$). (b) The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

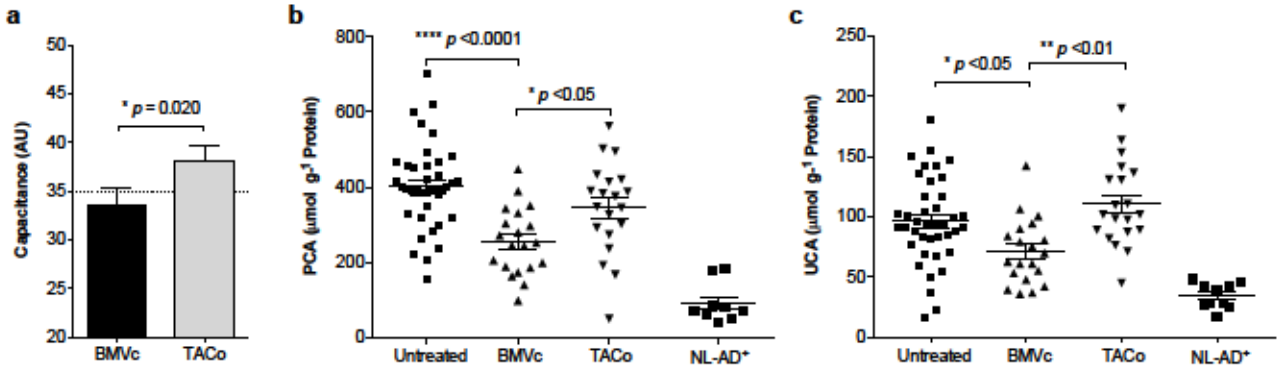


Fig 2.6: The effect of betamethasone valerate (0.1%) cream (BMVc) and Tacrolimus (0.1%) ointment (TACo) on the water holding capacity of the SC. (a) SC hydration measured using the capacitance method. A one-way ANCOVA revealed a significant difference between the two treatments (n = 25, $p = 0.020$). 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 (*$p < 0.05$).

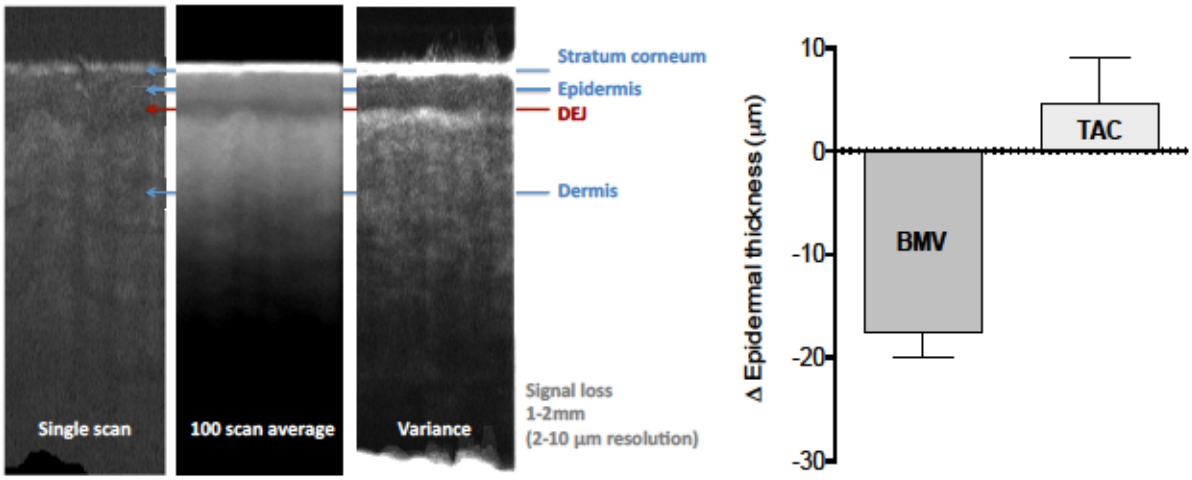


Fig 2.7: (Left) Representative raw B scan of epidermis (highlighted) determined by structural OCT. **(Right)** Change in epidermal thickness following a 4-week, twice-daily treatment regimen using betamethasone valerate (0.1%) cream (BMV) and Tacrolimus (0.1%) ointment (TAC).

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 topical corticosteroids, 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.

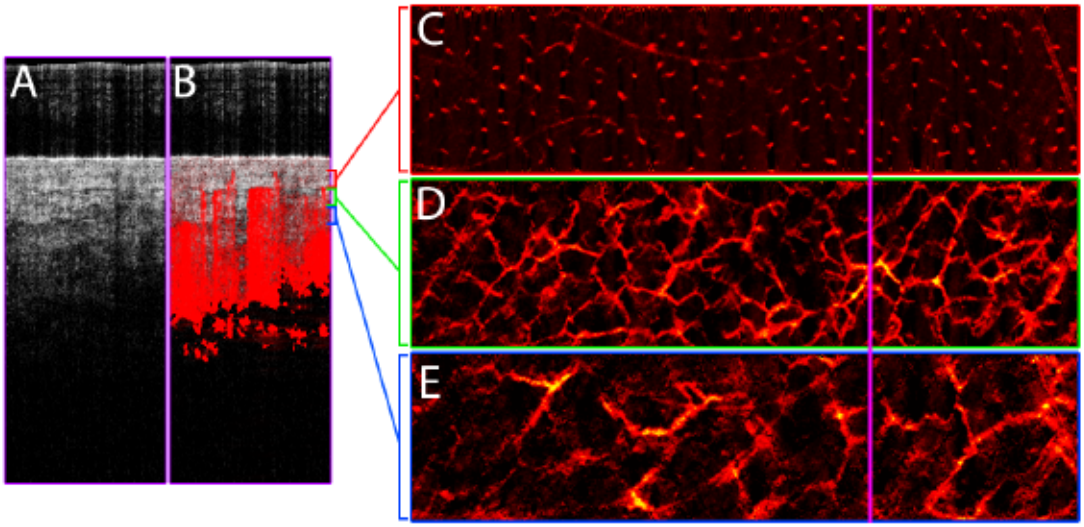


Fig 2.8: Angiographic analysis of OCT images

We have also used Fourier-Transform IR spectroscopy to quantify the molecular structure of the skin, including the levels of carboxylate groups (which relate to natural moisturising factor [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, transepidermal water loss 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.

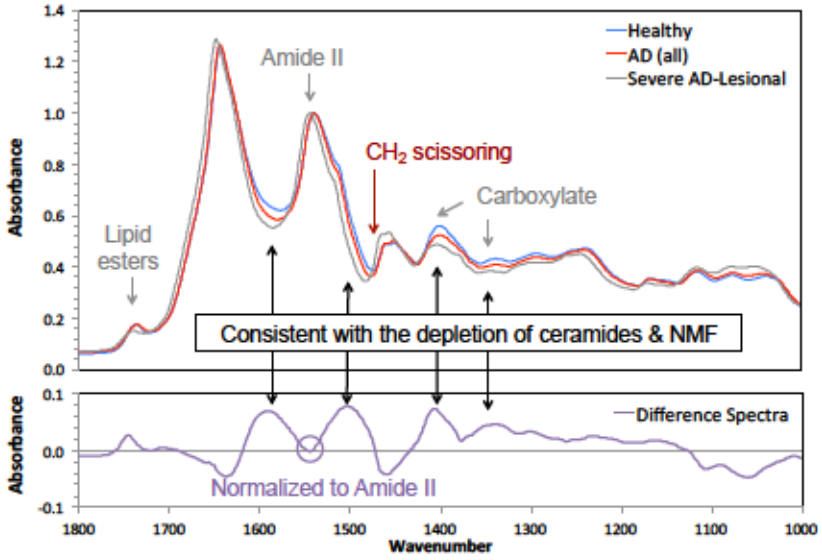


Fig 2.9: Top: Mean ATR-FTIR absorbance spectra collected at the cubital fossa for healthy participants (blue line), all AD patients (red line), and severe AD patients with clinical signs at the test sites (grey line). Bottom: Difference spectra (Healthy – all AD).

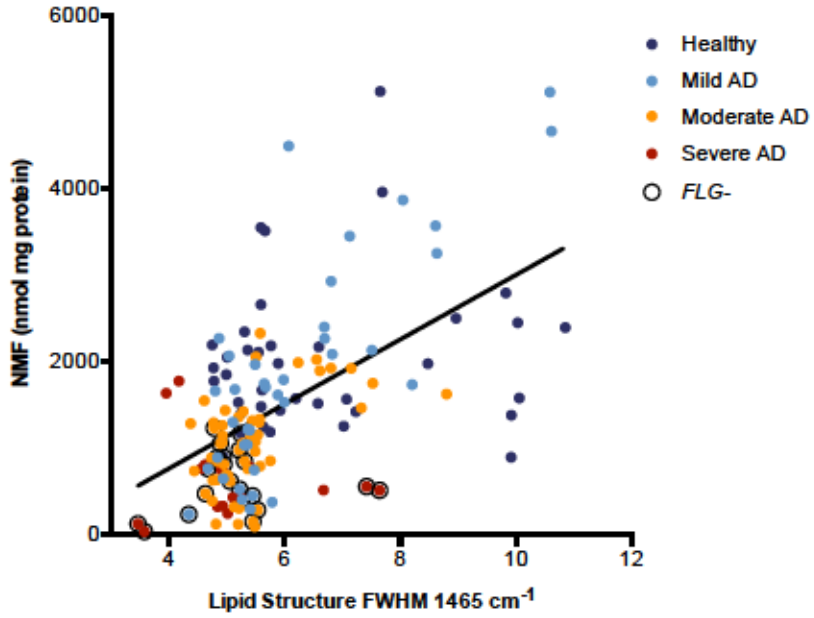


Fig 2.10: Correlation between stratum corneum NMF levels and lipid structure. Lipid structure was determined according to a previously published protocol. FWHM, full width at half maximum – a spectral feature associated with lipid membrane lateral chain packing. A highly ordered orthorhombic state (toward 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 topical corticosteroid 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 (0.25%) cream forms.

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 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. While 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. 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, randomized, double-blind, vehicle-controlled studies evaluated the efficacy and safety of crisaborole in patients ≥ 2 years of age with mild to moderate AD affecting at least 5% body surface area (BSA). 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 ≥ 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).

The majority of adverse events (AEs) with crisaborole were mild in severity; there were no treatment-related serious adverse events (SAEs) reported with crisaborole. There were no clinically relevant differences by age group in the incidence, types, or severity of AEs, relationship of AEs to study drug, or the incidence of AEs leading to premature withdrawal from the studies. The long-term safety of crisaborole was further evaluated in patients who continued into the open-label extension study.

The Phase 3, open-label, single-arm extension study confirmed the tolerability of crisaborole for treatment of mild-to-moderate AD up to 48 weeks. This study included children (2-11 years of age), adolescents (12-17 years of age) and adults (≥18 years of age). The majority of AEs were mild or moderate in severity. There was no clinically significant difference between age groups in the extent of exposure to crisaborole or the incidence of AEs.

There was no evidence of significant or long-term cutaneous adverse reactions at the application site such as atrophy, telangiectasia, or hypopigmentation during short-term or long-term intermittent use of crisaborole.

Based upon the efficacy and safety of crisaborole 2% ointment (trade name EUCRISA) demonstrated in these studies, the drug was approved for the treatment of mild to moderate AD in patients 2 years and older by the United States (US) Food and Drug Administration (FDA) on 14 December 2016. Crisaborole (2%) ointment is currently an approved therapy in the United States (US) and Canada (EUCRISA®), and Israel and Australia (STAQUIS®) as a topical treatment in patients 2 years of age and older with mild to moderate AD. It is currently being developed worldwide as a topical therapy for patients with mild to moderate AD. Further details on crisaborole can be found in the crisaborole Investigator's Brochure (IB).

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. Published literature further demonstrates that epidermal skin thinning induced during short courses of topical corticosteroid treatment is transient, with skin returning to pre-treatment thickness within a matter of weeks following cessation of treatment.

Moreover, it is important to recognise that the treatment regimen proposed conforms to the current marketing licence for Betnovate cream and is in line with standard treatment practice for atopic dermatitis, with betamethasone valerate creams being widely prescribed (1,053,999 prescription items in 2016). There is therefore a strong ethical argument to identify any potential harm using this product may have.

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 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 Potential risks associated with the IMP

The study involves two topically administered IMP's: crisaborole (2%) ointment and betamethasone valerate (0.1%) cream. Both IMP's will be provided to each participant for concurrent use on different skin sites (one on each forearm).

- Betamethasone valerate (0.122%) cream is a topical preparation of 0.122% (w/w) betamethasone valerate in a petrolatum base containing the following excipients: Chlorocresol BP, Cetomacrogol 1000 BP, Cetostearyl Alcohol BP, White Soft Paraffin BP, Liquid Paraffin BP, Sodium Acid Phosphate BP, Phosphoric Acid BP, Sodium Hydroxide BP Purified Water BP. with a MA in the UK (PL 10949/0014) under the brand name Betnovate cream. Classified as a potent topical

corticosteroid, the Betnovate cream will be used in accordance with this MA. The MAH is Glaxo Wellcome UK Ltd. A copy of the SmPC is included in the application.

- Crisaborole (2%) ointment is a topical preparation of 2% (w/w) crisaborole (phosphodiesterase 4 inhibitor) in a petrolatum base containing the following excipients: white petrolatum, propylene glycol, mono- and di-glycerides, paraffin, butylated hydroxytoluene, and edetate calcium disodium. Whilst not currently licenced in the UK or wider EU, crisaborole (2%) ointment has a USPI in the US (since December 2016), under the brand name Eucrisa, for treatment of mild to moderate atopic dermatitis in patients 2 years of age and older. In this study crisaborole (2%) ointment will be used in accordance with this market authorization. The MAH is Pfizer. A copy of the USPI is included in this application. Since the IMP does not have marketing authorization for the UK an Investigator Brochure is also included in the application and the trial will require authorization by MHRA, rather than notification to MHRA.

Twenty-five (25) clinical trials of topical formulations of crisaborole have been completed to date, with a total of 2227 participants exposed to one or more crisaborole formulations. Crisaborole has been well tolerated across completed clinical studies. 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. The FDA summarized that at this time the safety profile of crisaborole ointment appears more favourable than topical corticosteroids or calcineurin inhibitors but less favourable than device creams.

Given that the use of topical corticosteroids, including betamethasone valerate cream, and calcineurin inhibitors are standard medical care for atopic dermatitis patients in the UK, and that crisaborole ointment is presently marketed for this condition in the US and Canada but not yet marketed in the UK, the risks associated with this study have been classified as risk category B; somewhat higher than the risk of standard medical care. A full study-specific risk assessment is attached (see appendix). More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of crisaborole may be found in the IB, which is the single reference safety document (SRSD) for this study.

On the basis that this risk assessment, patient safety will be monitored locally by the PI and wider trial management group. A data monitoring committee will not be implemented, based upon the level of risk identified above, the inclusion of a single site only and the short duration of treatment. Safety data (AE reporting) will be collected in an ongoing manner and reviewed by the study monitor appointed by the Sponsor. Safety will be reviewed regularly at Trial Management Group meetings.

2.8.3 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

all located on the volar side of the forearm, and so participants will only be required to roll up their sleeves 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 (forearms only) 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.

2.8.4 Potential risks associated with the sample collection

Two types of samples will be collected in a non/minimally-invasive way: (1) superficial stratum corneum (skin) samples by tape-stripping and (2) saliva samples by buccal swabbing. Tape stripping is a painless procedure, that removes the dead cells (denucleated corneocytes) from the surface of the skin that will eventually be lost/shed as a result of normal desquamation. It can cause some discomfort and redness, but any redness usually dissipates in a matter of hours/days. No dressings are required. Tape-stripping 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 tape-stripped sites are fully healed.

2.8.5 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).

2.8.6 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) and skin images and spectra (as raw data files). To ensure skin measurements are accurately documented they will be directly entered into an electronic case record form (eCRF) developed by Epigenesis at the time of capture (no paper source for skin measurements). The system will utilise data field entry limits to immediately flag up to the researcher any value entered that does not conform to expected physiological ranges adding in a layer of data integrity checking not possible with paper records. To ensure the validity of these records as traceable source documents, electronic forms will be printed on the day of capture and signed by the researcher collecting the data. Access to the eCRF will be user controlled, with all entries and subsequent changes tracked to prevent data manipulation. The database will be manged by Epigenesis.

To preserve the integrity of skin images and spectra, the 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.

2.8.7 Retention of samples at the end of the study

No materials relevant under the Human Tissue Act will be kept beyond the end of this study.

The tape-strip samples are not considered relevant material under the Human Tissue Act as only enucleated non-viable corneocytes are collected. It is possible that viable cells are collected as tape-stripping nears complete removal of the stratum corneum skin layer (towards the 20th consecutive tape-strip). To mitigate any small risk of obtaining these cells, tape-strips 11-20 will be stored only after lysis in RLT buffer. Specific consent will be obtained from participants to store their superficial skin samples beyond the end of this study for use in future projects.

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.

2.8.8 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

3.1 The need for new biomarkers to evaluate the safety of treatments for atopic dermatitis

The first-line drug treatment for mild-moderate AD is currently a topical corticosteroid (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.

There is a need to further develop and validate new non-invasive technologies for the assessment of early sub-clinical skin changes associated with TCS adverse effects and to derive an optimum panel of safety biomarkers for use in future clinical trials of topical anti-inflammatory treatments.

- **Research Question 1:** Do OCT-derived biomarkers enable the accurate quantification of epidermal atrophy in response to TCS treatment
- **Research Question 2:** Do FTIR spectroscopy-derived biomarkers enable the accurate quantification of skin barrier condition and function in response to TCS treatment

In order to answer the research questions raised above a program of research has been developed as outlined in Fig 3.1.

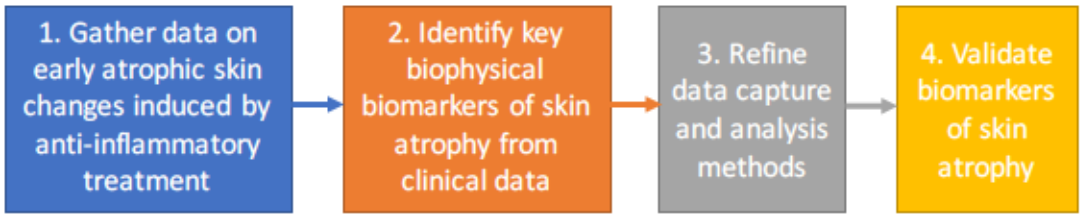


Figure 3.1: Overview of the research programme

Step 1 involves the collection of data on the early sub-clinical skin changes associated with TCS treatment using the non-invasive technologies; OCT and FTIR spectroscopy. This protocol details the clinical study designed to meet the needs of this first step. The safety of two topical anti-inflammatory treatments for AD will be compared, with a focus on early sub-clinical signs.

The data from this study will then be used to identify and refine biophysical biomarkers of skin atrophy and skin barrier disruption in steps 2 and 3. In a second clinical study the validity of the newly identified biomarkers will be demonstrated (outside the scope of this protocol).

3.2 Rationale for selecting betamethasone valerate 0.1% cream

The TCS betamethasone valerate is the chosen comparator based upon UK prescribing data (see section 1.5), and because we have established that this potency of TCS induces early 'sub-clinical' skin changes in the test system.

Comparing against the cream preparation of betamethasone valerate keeps parameters consistent with our previously tested model. Betamethasone valerate 0.1% cream is the most frequently prescribed preparation of TCS in the UK (excluding the mild TCS hydrocortisone), making it the pragmatic first choice.

3.3 Rationale for selecting 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 atopic dermatitis/eczema (AD) when compared to no treatment. To aid clinical decision-making in the treatment of mild-moderate AD a comparison between the local effects of crisaborole (2%) ointment and a potent TCS on the skin is required. As a new topical anti-inflammatory and nonsteroidal treatment for AD, with no reported TCS-like local adverse effects, crisaborole is the ideal comparator for TCS in the development of new safety biomarkers.

Aim of this study: To determine the relative local skin effects of crisaborole (2%) ointment compared to a potent and moderately potent TCS in participants with AD. The focus is on 'early biomarkers' of 'local skin changes', and not clinical efficacy, which has been established in previous trials.

- **Research Question 3:** Does crisaborole (2%) ointment, cause skin barrier damage and epidermal atrophy, and if so how does it compare with a potent and moderately potent TCS?

Crisaborole (2%) ointment will be compared to the potent TCS, Betamethasone valerate 0.1% cream, in this study and to the moderately potent TCS Betamethasone valerate 0.025% cream in a subsequent study.

There is the potential that in the absence of atrophic effects crisaborole (2%) ointment will exert a positive additional benefit on the skin (promotes healthy skin barrier) associated with the specific topical formulation being tested. The base (vehicle) contains ingredients other than the active, including but not limited to petrolatum and butylated hydroxytoluene (BHT), that have the potential to independently (from the active) promote a healthy skin barrier. As such any effect reported could only be associated with the complete preparation under investigation (i.e. active combined with base) and could not be generalized to any other topical preparation containing crisaborole. The biomarkers of skin barrier structure (FTIR-based) may therefore provide additional information on AD related positive treatment effects, in addition to the presence or absence of negative effects, that can be explored within the remit of this study (as part of research question 2).

3.4 Study Aim

- To directly compare the effects of crisaborole (2%) ointment to the potent TCS betamethasone valerate (0.1%) cream on the properties of the skin using an established model for quantifying the local adverse effects of TCS.

By achieving this aim, we will provide the data needed to identify the most informative biomarkers of epidermal atrophy/local adverse effects of TCS treatment using multivariate analysis techniques and develop a preliminary panel for further validation in a subsequent study.

3.5 Hypothesis

- Treatment with a potent TCS induces atrophic changes (reduced epidermal thickness determined by structural OCT) not observed with comparable crisaborole (2%) ointment treatment.
- Angiographic OCT measurements provide a robust and accurate biomarker of epidermal thickness/atrophy.
- FTIR spectra of the skin provide a biomarker of skin barrier structure that relates to skin barrier function and dysfunction, making it suitable for assessing both the negative effects of TCS and the positive effects of treatment bases/emollients.

4. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

4.1 Primary objective

To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, for up to 4 weeks is a cause of skin atrophy in patients with atopic dermatitis. This objective relates to research question 3.

- H_1: Treatment with crisaborole (2%) ointment twice daily for 28 days results in less (by ≥80%) pathologic epidermal thinning than the equivalent treatment regimen with betamethasone valerate (0.1%) cream.
- H_0: Treatment with crisaborole (2%) ointment twice daily for 28 days does not result in less pathologic epidermal thinning than the equivalent treatment regimen with betamethasone valerate (0.1%) cream.

4.2 Secondary Objectives

The following secondary objectives relate to research question 3:

- To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.1%) cream

- To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream

- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, on skin barrier function

- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, on skin dryness and the levels of natural moisturising factor in the skin

4.3 Exploratory objectives

The following exploratory objectives relate to research questions 1 and 2:

- To investigate whether OCT-derived biomarkers (other than the established structural OCT biomarker) enable the accurate quantification of tissue changes (vascular and matrix) associated with epidermal atrophy in response to TCS treatment. Biomarker measurements from the images will include:
    - Superficial plexus depth (µm) from angiographic OCT images[10]
    - Mean vessel diameter (µm) from angiographic OCT images[10]
    - Vessel density (segments/mm^2) from angiographic OCT images[10]
    - Collagen matrix index (an index derived from birefringence images of collagen density and arrangement) from polarisation sensitive OCT images[11,12]

- To investigate whether FTIR spectroscopy-derived biomarkers enable the accurate quantification of skin barrier condition and function in response to TCS treatment. Biomarker measurements from the spectra will include:
    - NMF levels in the skin[13,14]
    - Lipid Structure[15,16]

- 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

- To identify (by completing the objectives above) a panel of biomarkers that best characterises epidermal atrophy. The biomarkers identified will be taken forward into the next stage of the research program.

4.4 Primary endpoint/outcome

- The difference in the change in epidermal thickness (day 29 – day 1), measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.1%) cream.

4.5 Secondary endpoint/outcome

- The difference in the change in epidermal thickness measured by structural OCT during and after 28 days treatment. OCT images of epidermal thickness taken on day 1, day 15, day 29 and day 57.
- The difference in the change in skin redness/erythema (relating to tolerability) during and after 28 days treatment determined by:
    - Visual redness/erythema score determined on day 1, day 15, day 29 and day 57
    - Objective redness assessed with the Mexameter measured on day 1, day 15, day 29 and day 57
- The difference in the change in Trans-Epidermal Water Loss (TEWL, relates to skin barrier function) during and after treatment.[17,18] TEWL measurements on day 1, day 15, day 29 and day 57.
- The difference in skin barrier integrity ($TEWL_{ts20}$) after 28 days treatment. TEWL measurements after tape-stripping ($TEWL_{ts20}$) on day 29
- The difference in the change in visual skin dryness during and after treatment. Visual skin dryness scored on day 1, day 15, day 29 and day 57
- The difference in Natural Moisturising Factor (NMF, filaggrin breakdown products) levels at the end of treatment[19] NMF will be quantified from superficial stratum corneum samples collected on day 29 using HPLC.

4.6 Exploratory endpoint/outcome

Details pertaining to the exploratory endpoints/outcome measures can be found in section 4.7.

4.7 Table of endpoints/outcomes

Each study endpoint/outcome is listed in the following table by study objective.

Objectives	Outcome Measures	Timepoint(s) of evaluation
Primary Objective: To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, for up to 4 weeks is a cause of skin atrophy in patients with atopic dermatitis.	**Primary Outcomes:** The difference in the change in epidermal thickness (day 29 – day 1), measured by structural OCT, between the sites treated with crisaborole (2%) ointment and betamethasone valerate (0.1%) cream.	Structural OCT images of epidermal thickness taken on day 1 and day 29
Secondary Objectives: To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.1%) cream	**Secondary Outcomes:** The difference in the change in epidermal thickness measured by structural OCT during and after 28 days treatment.	Structural OCT images of epidermal thickness taken on day 1, day 15, day 29 and day 57.
To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream	The difference in the change in visual redness/erythema score during and after 28 days treatment.	Visual redness/erythema score determined on day 1, day 15, day 29 and day 57
	The difference in the change in objective redness assessed with the Mexameter during and after 28 days treatment.	Objective redness assessed with the Mexameter measured on day 1, day 15, day 29 and day 57
To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, on skin barrier function	The difference in the change in Trans-Epidermal Water Loss (TEWL) during and after 28 days treatment.	TEWL measurements on day 1, day 15, day 29 and day 57.
	The difference in skin barrier integrity ($TEWL_{ts20}$) after 28 days treatment.	TEWL measurements after tape-stripping ($TEWL_{ts20}$) on day 29
To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.1%) cream, on skin dryness and the levels of natural moisturising factor (NMF) in the skin	The difference in the change in visual skin dryness during and after 28 days treatment.	Visual skin dryness scored on day 1, day 15, day 29 and day 57
	The difference in Natural Moisturising Factor (NMF,	NMF will be quantified from superficial stratum corneum

	filaggrin breakdown products) levels at the end of treatment	samples collected on day 29 using HPLC.
Exploratory objectives: To investigate whether OCT-derived biomarkers (other than the established structural OCT biomarker) enable the accurate quantification of tissue changes (vascular and matrix) associated with epidermal atrophy in response to TCS treatment	**Exploratory outcomes:** The difference in the change in superficial plexus depth (µm) measured by angiographic OCT	Angiographic OCT images taken on day 1, day 15, day 29 and day 57
	The difference in the change in mean blood vessel diameter (µm) measured by angiographic OCT	
	The difference in the change in blood vessel density (segments/mm^2) measured by angiographic OCT	
	The difference in the change in collagen matrix index (an index derived from birefringence images of collagen density and arrangement) measured by polarisation sensitive (PS)-OCT	Polarisation sensitive (PS)-OCT images taken on day 1 and day 29.
To investigate whether FTIR spectroscopy-derived biomarkers enable the accurate quantification of skin barrier condition and function in response to TCS treatment	The difference in the change in carboxylate levels (indirect measure of NMF levels, not to be confused with direct quantification from stratum corneum samples by HPLC) in the stratum corneum measured by FTIR spectroscopy	FTIR spectrum of the skin surface taken on day 1 day 15, day 29 and day 57
	The difference in stratum corneum lipid structure measured by FTIR spectroscopy in conjunction with tape-stripping	FTIR spectra taken through the stratum corneum (during tape-stripping) on day 29
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	Saliva sample at visit 1 for *FLG* genotyping
	Descriptive tabulations of TEWL and epidermal thickness by mutation status, if sufficient participants with mutation are detected.	TEWL and structural OCT derived epidermal thickness measured at day 1, day 15 and day 29

To identify (by completing the objectives above) a panel of biomarkers that best characterises epidermal atrophy. The biomarkers identified will be taken forward into the next stage of the research program.	All the above	Not applicable

5. TRIAL DESIGN

An observer-blind randomized within-subject controlled clinical trial in 33 AD patients is proposed, wherein each participant will undergo 4 weeks treatment with crisaborole (2%) ointment on one forearm and betamethasone valerate (0.1%) cream on the other (twice daily application in each case and randomised site allocation). At the start of the study the skin of the test sites (forearms) 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 4.1). A post-treatment washout period of 4 weeks post-treatment is included to establish how quickly skin changes re-adjust to baseline.

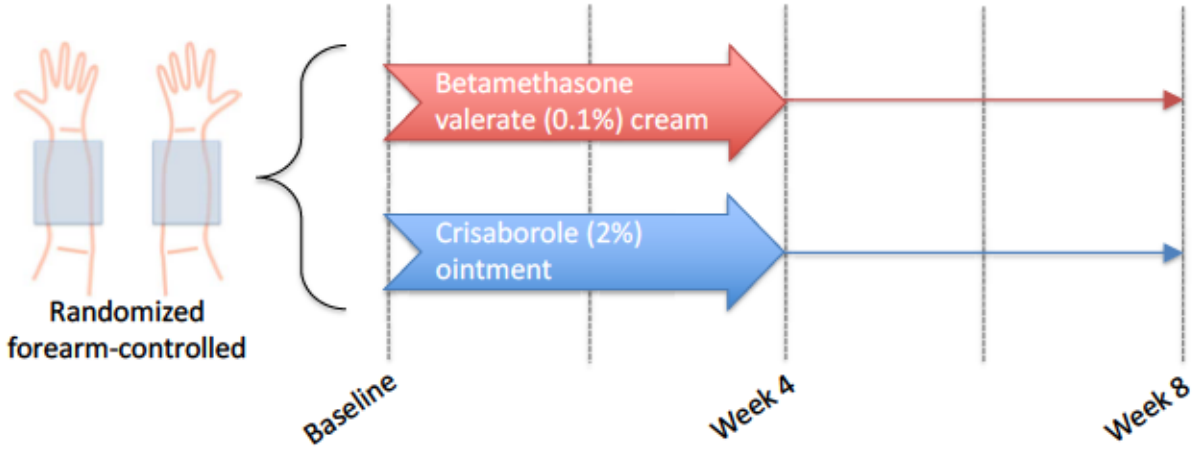


Fig 4.1: study design

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 test sites).

The study is expected to last 12 months in total, with recruitment taking 6 months.

5.2 Trial setting

This single-centre clnical 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

Participants with AD not currently undergoing, or requiring, active drug treatment at baseline (visit 1).

6.2 Inclusion criteria

1. Volunteers with AD defined according to the UK working party diagnostic criteria
2. Male or female aged 18-65 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 treatment (anatomical) sites is almost clear or greater (score ≥1) based on the Investigators static global assessment scale at screening and baseline. At the start of the study the skin of the test sites (forearms) will therefore be clear (0) of the signs of eczema
4. Participants with a condition that in the opinion of the investigator contradicts participation in the study.
5. 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.
6. 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.*
7. 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.*

8. 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.*
9. Participants currently participating in another interventional clinical trial.
10. Volunteer is incapable of giving fully informed consent.
11. 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 condom or female condom used WITH a spermicide product (ie, foam, gel, film, cream, or suppository).
4. Male sterilization with absence of sperm in the post vasectomy ejaculate.
5. 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**	**Compliance monitoring** [b]				**Follow-up**			**Wash-out**
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 6a	Visit 7	Visit 8
Study Week:		-3 to 1	1	1	1	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 + 4	3 + 5	4 + 6	5 + 7	15 ± 3	22 ±3	29 ± 3	57 + 4[s]
Duration (minutes) - 20 h total:		20 - 60 min	2.5-3 h	20 min	20 min	20 min	20 min	2.5 h	20 min	3 h	2.5 h
Screening, consent and enrolment in a clinical area designated for unblinded assessments											
1	Receive volunteer referrals [c]	X									
2	Schedule the study visits according to this schedule	X									
3	Informed Consent (Informed Consent sheet) [d]	X									
4	Re-confirm consent verbally		X	X	X	X	X	X	X	X	X
5	Confirm suitability and complete Admission Form (includes collection of demography & medical history) [e]	X									
6	Complete a follow-up Admission Form IF enrolment was subject to a washout period or more than 28 days has lapsed since screening [e]		(X)								
7	Capture of AE and concomitant medication using the AE Form and Con Med Form [f]	X	X	X	X	X	X	X	X	X	X
8	Carry out EASI and ISGA assessment of eczema severity	X	(X)[q]								
9	Conduct a urine pregnancy test (female participants only) [g, h]	X	X							X	
10	Check that an appropriate method of contraception is in use [g, i]	X	X					X		X	

Study Procedures: [a]		Consent & screening	Baseline	Compliance monitoring [b]				Follow-up			Wash-out
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 6a	Visit 7	Visit 8
Study Week:		-3 to 1	1	1	1	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 + 4	3 + 5	4 + 6	5 + 7	15 ± 3	22 ±3	29 ± 3	57 + 4[s]
Duration (minutes) - 20 h total:		20 - 60 min	2.5-3 h	20 min	20 min	20 min	20 min	2.5 h	20 min	3 h	2.5 h
11	Completion of Screening, Enrolment & Completion Log as appropriate	X	X								
12	Issue of a Randomization number		X								
13	Remind participants not to apply products in the morning before study visits. Reminders should be sent out the day before the appointment is due.[r]			X	X	X	X	X[r]	X	X[r]	
14	Check compliance with dosing conditions on the study days involving skin assessments [n]			X	X	X	X	X[r]	X	X[r]	
15	Access and update patient notes with study details. If the participant has no STH notes already, a new set of notes should be prepared.[p]	X									
16	Transfer participant to skin barrier suite		X					X		X	X
Skin assessments and sample collection at the skin barrier suite [j]											
17	Acclimatise test sites for 20 minutes		X					X		X	X
18	Identify and demarcate the test sites		X					X		X	X
19	Visually score skin dryness and redness/erythema at each treatment area		X					X		X	X
20	Capture the 2D skin images		X					X		X	X
21	Perform Mexameter measurements at each site (skin redness, 4 repeats per site)		X					X		X	X
22	Take OCT (structural & angiographic) images/scans with the Vivosight at each test site (in triplicate)		X					X		X	X
23	Take PS-OCT images/scans with the PS-OCT machine at each tests site (in triplicate)		X							X	
24	Measure TEWL at each test site (in triplicate)		X					X		X	X
25	Collect FTIR spectra at each test site		X					X		X	X

Study Procedures: [a]		Consent & screening	Baseline	Compliance monitoring [b]				Follow-up			Wash-out
Visit:		Consent & screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 6a	Visit 7	Visit 8
Study Week:		-3 to 1	1	1	1	1	1	2	3	4	8
Study Day (incl. window):		-28 to 1	1	2 + 4	3 + 5	4 + 6	5 + 7	15 ± 3	22 ±3	29 ± 3	57 + 4[g]
Duration (minutes) - 20 h total:		20 - 60 min	2.5-3 h	20 min	20 min	20 min	20 min	2.5 h	20 min	3 h	2.5 h
26	Perform tape-stripping in conjunction with TEWL and FTIR assessments									X	
27	Collect tape-strip samples (including NMF sample collection)									X	
28	Collect buccal swab sample for *FLG* genotyping [m]		X								
29	Transfer participant to CRF		X					X		X	X
Treatment and compliance [k]											
30	Issue IMP's (weighed) and provide demonstration of dosing by fingertip unit		X								
31	Issue treatment diary, complete the relevant sections, and provide training on its completion		X								
32	Weigh IMP's, record weights in the CRF and treatment diary, and inform participants of their usage in the context of expected usage.[l]			X	X	X	X	X	X	X	
33	Check all diary entries for missing applications and adverse events (skin reactions reported at the time of application).			X	X	X	X	X	X	X	
34	Supervised product application (after measurements).		X	X	X	X	X	X	X		
35	Provide re-training/guidance to improve on application technique and/or dosing 'as required'		(X)	(X)	(X)	(X)	(X)	(X)	(X)		
36	Retrieve diary and IMP's									X	
37	Arrange taxi transfer for next session		X	X	X	X	X	X	X	X	
38	Complete claim form for remuneration										X
39	Study completion (Screening, Enrolment & Completion Log)										X

a, A description of the procedures carried out at every visit is provided in section 7.4.

b, Supervised IP applications. The aim is for participants to undertake 5 supervised applications during the first 2 weeks of treatment to monitor compliance and encourage consistent adherence to the application regimen. To minimize the burden on participants sufficient variance has been included to allow gaps of up to 7 days between supervised

applications. 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 6 hours**. See section 8.11 "compliance monitoring" for details

c, refer to sub-section 7.2 "Recruitment". Referrals will be sent by the dermatology team following successful pre-screening.

d, refer to sub-section 7.3 "Screening & Consent"

e, 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 new admission form must be completed and signed off by a medically qualified study investigator prior to enrolment.

f, forms are listed in the Appendix and available separately. Details on reporting concomitant medication are provided in section 8.8 "Prior and concomitant medication". See section 9.0 "Pharmacovigilance" for details on AE reporting.

g, female participants of childbearing potential only

h, See sub-section 7.6 "pregnancy testing" for further details

i, see sub-section 6.5 "contraception" under the 6.0 "participant eligibility criteria" section for further details

j, see sub-section 7.7 "skin assessments" for details of the skin assessments and sub-section 7.8 "sample collection" for details of the sample collection procedures.

k, refer to subsection 7.9 "treatment and compliance" in this section and sub-sections 8.11 "compliance monitoring" and 8.12 "treatment dosing and compliance" in the "Trial Treatments" section (8.0) for further information.

l, 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.

m, See section 7.8.2 for further information.

n, for further details see sub-section 8.12.1 "IMP application during the morning of study visits"

o, see section 6.4 "instructions to participants and lifestyle changes" for details of the criteria. If participants have applied the test products in the morning before the study visit the appointment should be rescheduled.

p, see section 11.5.1 "research notes"

q, the EASI assessment should be repeated whenever the admission process is conducted (i.e. at screening, after a given washout period, and if more than 21 days has lapsed since screening)

r, 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. See special conditions in section 8.12.1 regarding action required on Visits 6 and 7.

***s*, must be ≥28 days following Visit 7**.

7.2 Recruitment

Recruitment will be conducted by the Sheffield Dermatology Research Group in several ways:

1. General advertisement using posters (see attached *Adverts*). The poster contains telephone and email contact details for the study team. Posters (see attached) will be displayed at/in:
    a. The University of Sheffield premises, including the Medical School
    b. Sheffield Hallam University (SHU) premises
    c. Sheffield Teaching Hospitals premises, including the Royal Hallamshire Hospital
    d. Online, on the University of Sheffield website, STH website, SHU website (student portal), Facebook, Twitter, including @Shef_Derm Twitter pages
    e. Printed media including local leaflets, magazine and newspapers.
    f. Local shops, libraries, community centres and practices with the permission of the owner/manager in each case.
2. Email lists. An email, containing the information in the Poster (and a copy of the *Participant Information leaflet, PIL*), 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, SHU and STH (via the media team) using the appropriate moderated email lists at each institution.
3. Local GP practices/Patient Identification Centres. Interested GP's at these sites will help identify patients and refer them to the study team for screening and enrolment. To support this activity we will provide:
    a. Posters to display in waiting areas
    b. *Recruitment letters.* Pre-drafted invitation letters for distribution to patients with AD
    c. *Participant Information Leaflets* (summary PIS) to be displayed in waiting areas, handed out at scheduled appointments and distributed with patient letters.

To support recruitment, we will set up a dedicated University of Sheffield CICS managed email account for the study.

We will also establish a web-page on the Sheffield Dermatology Research website (http://www.sheffield.ac.uk/iicd/dermatology) on the study, replicating information from the Poster and PIL. A link to a downloadable copy of the PIS will be provided. All poster adverts, the PIL and PIS will link to the website.

The study office (University) will handle all expressions of interest. The Pre-Screening Log will be used to track new approaches. Participants responding to our adverts/letters by phone/email will be asked to complete a *registration form* (this may be done by phone/email/post). *The registration form will make it clear that the next step will be a phonecall from a researcher on the study to conduct the pre-screening.*

A copy of the full *Participant Information Sheet (PIS)* will be sent by post/email to each volunteer completing the registration form.

7.2.1 Pre-screening

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

A member of the team will then 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. During the pre-screening session we will complete the study *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.

Completed Pre-Screening forms will be forwarded to the research team at the site research facility (by secure electronic delivery or in person, by a member of the study team) to arrange the study visits. 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 the on the skin of their test sites prior to visit 1/baseline. A screening number will be assigned using the *Participant Screening, Enrolment & Completion Log*.

7.3 Screening & Informed Consent

At the beginning of the consent study visit (at least 24 hours after Participant Information Sheet have 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 (using the Admission Form template). 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 provided participants will be assessed for eligibility. This will involve capturing a medical history and other background information pertinent to the study. The process is referred to here as the admission process, and will be documented using the *Admission Form*.

The collection of background information involves:

- Recording the participants date of birth
- Recording the participants sex at birth
- Recording the participants ethnicity
- Assessing and recording the participants Fitzpatrick skin type (see section 7.3.1)
- Assessing and recording the participants history of eczema according to the UK Work Party Diagnostic Criteria (see section 7.3.2)
- Grading and recording the severity of eczema using the EASI scoring sheet (see section 7.3.3)
- Grading and recording the severity of eczema according to the Investigators static global assessment (see section 7.3.4)
- Recording the time since the participants last flare

- Recording how many times the participant's condition has relapsed/flared up in the last 12 months

There are 3 possible outcomes of the admission process: (1) the participant is found not to be eligible; (2) the participant meets all of the criteria and is eligible to take part; or (3) the participant may become eligible after an appropriate wash-out period (defined in section 8.8). In the latter case, the required wash-out period should be documented in the Admission form and the admission process (including screening) repeated at the start of visit 1 This will involve completing another Admission Form (ticking the follow-up form option) to ensure that the participant is in fact eligible to take part before proceeding onto any study procedures, and that valid background information is captured. The severity of eczema will be reassessed as part of the follow-up admission process.

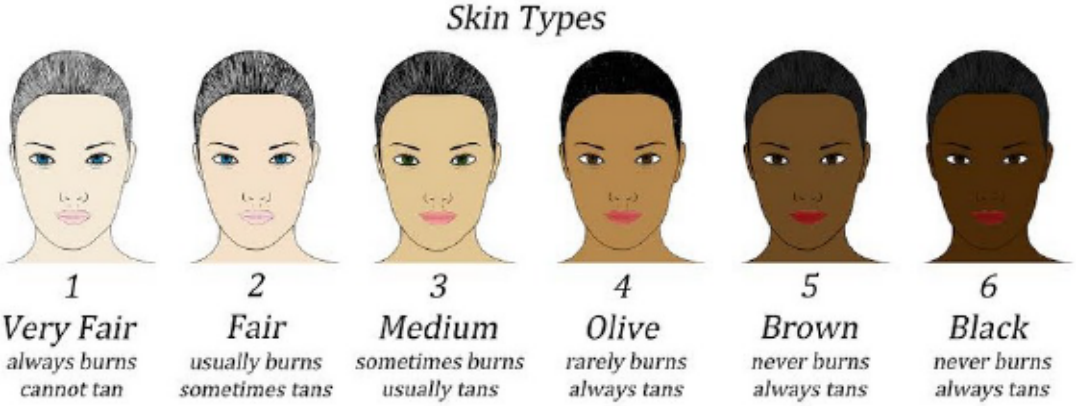
Informed consent will not be retaken where the participant is returning after a defined/scheduled washout period of up to 16 weeks (12 weeks + 28 days). Volunteers who reapply to take part after this time or where a washout period was not scheduled (and documented in the admission form), will be required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

Where the participant meets all of the eligibility criteria at the screening visit, Visit 1/baseline may be arranged within 28 days without the need to repeat the admission process. If more than 28 days lapses before the participant can attend visit 1 the admission process should be completed to ensure the participant is still eligible and that the background information is still valid (this will involve completing a follow-up admission form). Visit 1 may also be combined with the screening visit (same day) IF the participant is found to be eligible without the need for a washout period.

The *Participant Screening, Enrolment & Completion Log* will be completed and all screened participants assigned a unique study screening number.

7.3.1 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.



7.3.2 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

7.3.3 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, edema], 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.

7.3.4 The Investigators Static Global Assessment (ISGA)

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

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*

7.4 Study visits

Procedures conducted during the study visits will be recorded using paper CRFs first (and then transferred to the EDC) or directly using an electronic form in the EDC system (which double as task lists for each visit). Where direct electronic entry is used, at the end of each session the database entry

will be printed and signed by the researcher to verify the source data and stored in the site file. Further details can be found in section "Study Conduct".

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).

A description of the visits is provided below:

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 detailed in the Admission Form.
    - Collect basic demographic/background information about the participant
    - 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.
    - A physical examination of the participants skin should be performed by a study dermatologist to grade the severity of eczema overall. The dermatologist will need to see the signs of eczema to assess its severity.
    - Screening 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.
- If eligible to take part the site staff will then arrange the subsequent study visits with the participant. Where possible, dependent on the availability of the participant and the site staff and facilities, eligible participants may undertake Visit 1 on the same date as the screening and consent visit. If the participant may become eligible following a wash-out period from any current medication, Visit 1 can be arranged to take place after the wash-out period. In these cases, the washout period should be documented in the Admission Form and the admission process repeated at the start of Visit 1.

7.4.2 Wash-out

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.

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

- If a wash-out period was required, or if more than 28 days has lapsed since the screening visit, the admission process should be repeated by completing a new Admission Form and following the follow-up procedure:
    - Update basic demographic/background information about the participant if necessary
    - Update medical history
    - Update concomitant medication
    - 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 adverse events 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
- Issue a randomization/study ID number
- Transfer participant to skin barrier suite
- Participants will need to acclimatize the skin on their 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 skin dryness and redness/erythema at each treatment area
    - Capture 2D skin images of the test areas of skin
    - Perform Mexameter measurements of objective skin redness at each site
    - Take OCT (structural & angiographic) images/scans with the Vivosight at each test site
    - Take PS-OCT images/scans with the PS-OCT machine at each tests site
    - Measure TEWL at each test site
    - Collect FTIR spectra at each test site
- A mouth swab sample should be collected
- Transfer patients back to the site research facility (if different)
- 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).
- Arrange taxi transfer for next session

7.4.4 Reminders between visits

- Remind participants not to apply products in the morning before study visits 2-7. 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.

7.4.5 Visits 2-5, Days 1 to 12 (approx. 20 min each)

Treatment usage should be monitored on 4 occasions during the first 12 days of treatment. The scheduling of these visits is flexible within the constraints in the protocol schedule of events. At each visit the following activities should be carried out:

- Re-confirm verbal ascent to continue the study
- Capture adverse events 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.
- Check all diary entries for missing applications and adverse events (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

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

- Re-confirm verbal ascent to continue the study
- Capture adverse events and new concomitant medications
- Check that an appropriate method of contraception is in use
- Check compliance with dosing conditions
- Transfer participant to skin barrier suite
- Participants will need to acclimatize the skin on their 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 skin dryness and redness/erythema at each treatment area
    - Capture 2D skin images of the test areas of skin
    - Perform Mexameter measurements of objective skin redness at each site
    - Take OCT (structural & angiographic) images/scans with the Vivosight at each test site
    - Measure TEWL at each test site
    - Collect FTIR spectra at each test site
- Transfer patients back to the site research facility (if different)
- 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 adverse events (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

7.4.7 Visit 6a, Day 22 +/- 3 days (approx. 20 min)

Same procedure as visits 2-5

7.4.8 Visit 7, Day 29 +/- 3 days (approx. 3 hours)

- Re-confirm verbal ascent to continue the study
- Capture adverse events 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 compliance with dosing conditions
- Transfer participant to skin barrier suite
- Participants will need to acclimatize the skin on their 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 skin dryness and redness/erythema at each treatment area
    - Capture 2D skin images of the test areas of skin
    - Perform Mexameter measurements of objective skin redness at each site
    - Take OCT (structural & angiographic) images/scans with the Vivosight at each test site
    - Take PS-OCT images/scans with the PS-OCT machine at each tests site
    - Measure TEWL at each test site
    - Collect FTIR spectra at each test site
    - Perform tape-stripping in conjunction with TEWL and FTIR assessments
    - Collect tape-strip samples (including NMF sample collection)
- Transfer patients back to the site research facility (if different)
- 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 adverse events (skin reactions reported at the time of application).
- Retrieve diary and IMP's
- Arrange taxi transfer for next session

7.4.9 Visit 8, Day 57 +/- 4 days (approx. 2.5 hours)

- Re-confirm verbal ascent to continue the study
- Capture adverse events and new concomitant medications
- Transfer participant to skin barrier suite
- Participants will need to acclimatize the skin on their 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 skin dryness and redness/erythema at each treatment area
  - Capture 2D skin images of the test areas of skin
  - Perform Mexameter measurements of objective skin redness at each site
  - Take OCT (structural & angiographic) images/scans with the Vivosight at each test site
  - Measure TEWL at each test site
  - Collect FTIR spectra at each test site
- Ask participants to complete a claim form for the remuneration

7.5 Randomization & blinding

The participant will be assigned a randomization number (Trial Number) at Visit 1. This will be the next available number on the Randomization list. This number will be the principle identifier against which the study data will be stored. Allocation of the randomization number is considered the point of entry onto the trial, which should be recorded on the *Participant Screening, Enrollment & Completion Log*.

7.5.1 Randomization

Allocation of the treatments to the test sites (right/left forearm) will be randomised (to avoid site position-dependent artefacts) 1:1 using a randomization schedule (list) provided by the Statistical Services Unit. The list will be numbered using the unique participant randomization 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 randomization and label the IP's with the site of application upon issue to the site research facility team. Only the Pharmacy and study Statistician (SSU) will have access to the randomisation master list.

7.5.2 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.

7.5.3 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.

7.6 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

7.7 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 7.2: Instruments used in this study

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.	Trans-Epidermal Water Loss (TEWL)
PS-OCT machine	University of Sheffield custom build	Collagen index (an index derived from birefringence images of collagen density and arrangement) measured by polarisation sensitive OCT

4300 FTIR Spectrometer	Agilent Technologies Ltd.	Carboxylate levels (indirect measure of natural moisturising factor levels) in the stratum corneum measured by FTIR spectroscopy Stratum corneum lipid structure measured by FTIR spectroscopy
C-Cube camera	Pixience	Skin image documentation only
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 Figure 7.1, and the order in which they should be collected provided in Table 7.3.

7.7.1 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±2°C and 45±10% relative humidity.

7.7.2 Identification and demarcation of the test sites

The test/treatment sites are located on the volar side of the forearm (Figure 7.1). There are 2 treatment areas in total, one on each forearm. Within each treatment area there are 2 defined test or measurement areas. 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. The table lists the measurements performed at each test area in the order they should be collected.

Figure 7.1: The location of the treatment areas and test sites

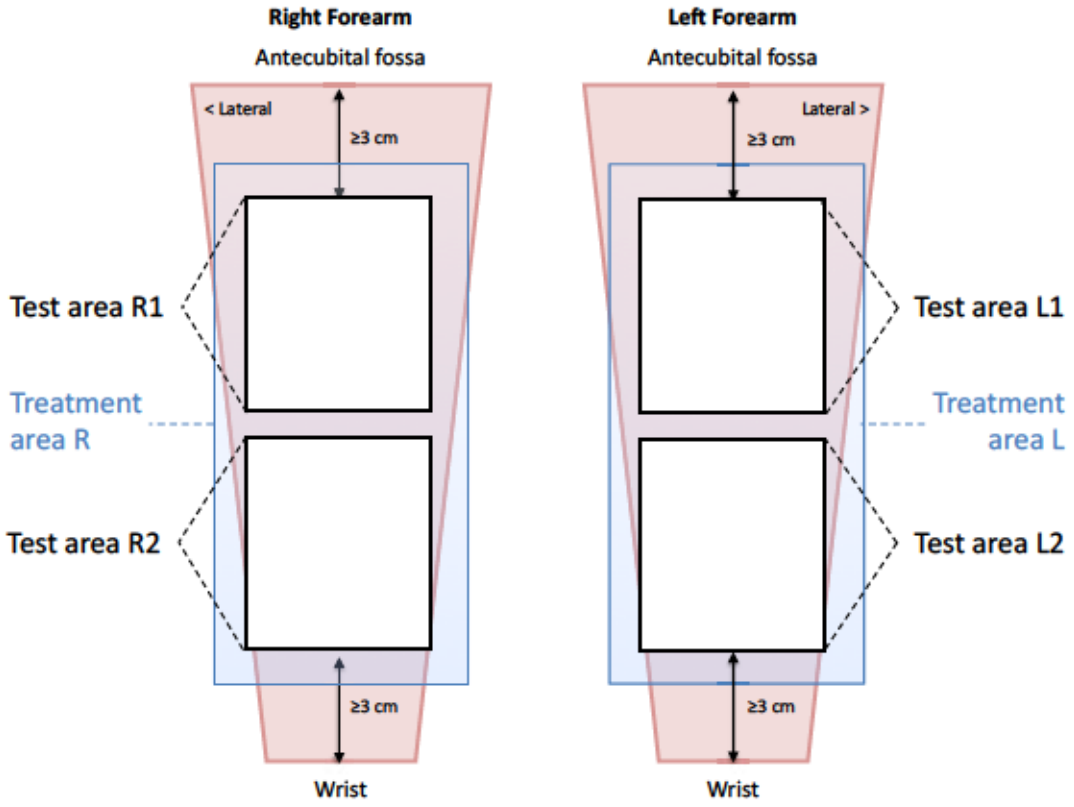


Table 7.3: The measurements to be performed at each test site

Test area	Measurement
R1/2 (combined), L1/2 (combined)	1. Visual scoring of skin dryness and redness at each test site
R1, L1	2. Capture of 2D skin image using the c-cube
	3. Mexameter measurement at each site (skin redness, 4 repeats)
	4. OCT (structural & angiographic) image capture at each site (in triplicate)
	5. PS-OCT image capture at each site (in triplicate)
	6. TEWL measurement at each site (in duplicate)
	7. FTIR spectrum from the skin surface collected at each site
R2, L2	2. Capture of 2D skin image using the c-cube
	3. Mexameter measurement at each site (skin redness, 4 repeats)
	4. TEWL measurement at each site (in duplicate)
	5. FTIR spectrum from the skin surface collected at each site
	6. Tape-stripping in conjunction with TEWL and FTIR (to achieve full stratum corneum depth)
	7. Tape-strip sample collection

7.7.3 Visual scoring of skin dryness and redness/erythema

Visual dryness and redness/erythema of the forearms (whole volar face/treatment area) will be scored by an experienced grader using the scales below at the visits indicated in the schedule of events.

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
2	Moderate, uniform erythema – Moderate erythema
3	Strong erythema – Marked erythema

7.7.4 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 test 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. Further details on taking skin images with the c-cube can be found in SOP-050.

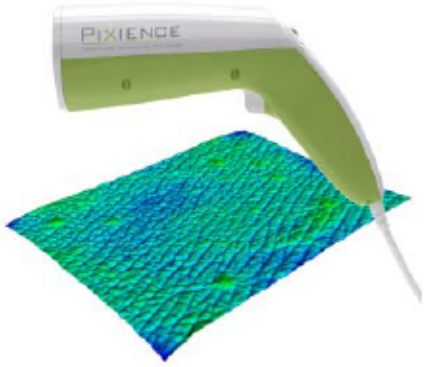


Figure 7.2: The Pixience c-cube

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

Skin redness will be measured 4 times within each test area (2 areas in total per treatment area) 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 7.3: The Mexameter probe

7.7.6 Structural & Angiographic OCT scans using the Vivosight OCT machine

Optical coherence tomography (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 topical corticosteroids.[20] We have also studied the effect of TCSs on the skin barrier, the disruption of which is mechanistically associated with epidermal atrophy.[17,21]
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 7.4: The VivoSight OCT scanner

Three scans will be taken from each test area 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.

7.7.7 Polarization sensitive (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.[22] 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 images will be taken from each test area 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.

7.7.8 TEWL measurements

This is a well-documented, standardised dermatological procedure for measuring skin barrier function.[23] 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.[21,24-28] 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 test 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 7.5: The Aquaflux TEWL machine

Two repeat TEWL measurement will be collected from each test area (= 4 per treatment area) at the visits indicated in the schedule of events. After use in each participant the probe will be decontaminated with a 70% alcohol wipe.

7.7.9 FTIR spectroscopy measurements of the skin surface

Attenuated Total Reflectance (ATR) Fourier-Transform Infrared (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 content of the skin barrier and for analysing lipid arrangement/structure.[16,29-32] 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 tape stripping.[33] 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 7.6: The Agilent 4300 ATR-FTIR spectrometer

Measurements will be performed by gently but firmly placing the probe in contact with the skin. Single measurements will be collected from each test 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) 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.

7.7.10 Tape-stripping in conjunction with FTIR and TEWL measurements

Tape-stripping, 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.[16,34] 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.

Tape-stripping will be performed on day 29 only, once within each treatment area (test sites R2 and L2). At each site 20 consecutive tape-strippings will be performed. The depth through the stratum corneum is determined by measuring the amount of corneotypes removed by each disc using the SquameScan Device. Squamescan readings of each tape should be collected in triplicate. The tape-strips, containing a sample of the stratum corneum, will be collected as outline below.

In conjunction with tape-stripping, FTIR spectra and TEWL measurements (single) will be collected from the tape-stripped site after every 5 consecutives tape-strips as described above. This data is used to determine the lipid structure throughout the stratum corneum.

After the 20th tape-strip and final FTIR measurement, a single TEWL measurement should be collected, as described above. This is taken to determine stratum corneum integrity (skin barrier function following experimental barrier perturbation). The parameter is referred to here as $TEWL_{ts20}$

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

7.8 Sample collection

7.8.1 Superficial stratum corneum sample collection

The superficial skin samples collected on the tape-strips used in tape-stripping (above) will be retained for analysis:

- The first 7 tape-strips (tape-strips 1-10) will be stored individually in 2 ml tubes at -70°C for determination of NMF levels by HPLC (3 tape-strips required per analysis) and future analysis of protein and metabolomic biomarkers (remaining tape-strips).
- The remaining tape-strips (tape-strips 11-20) will be transferred to RLT lysis buffer and stored -70°C for future analysis of gene expression.

Tape stripping is a painless procedure, that removes the dead cells (denucleated corneocytes) from the surface of the skin that will eventually be lost/shed as a result of normal desquamation. It can cause some discomfort and redness, but any redness usually dissipates in a matter of hours/days. No dressings are required. Tape-stripping will not be performed on broken skin. The only skin preparation required before tape stripping is the removal of visible contaminants by dry wipe if necessary.

7.8.2 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 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.

7.9 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/cm^2/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 (see Appendix) 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 adverse events (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.

7.10 Participant completion/withdrawal

7.10.1 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 on the *Participant Screening, Enrolment & Completion Log*.

7.10.2 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 on the *Participant Screening, Enrolment & Completion Log*. 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. Adverse event, including a relapse of AD (defined as an escalation in treatment, i.e. the need for topical corticosteroids).
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 adverse events. 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.

7.10.3 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 no voucher will be offered. 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 completion of a University of Sheffield claim form.

7.11 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.

7.11.1 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 adverse events 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 9.1.

Table 8.1: Investigational products

Name, Strength & Form	Brand Name	Pack size and quantity per participant	Manufacturer	Formulation
Betamethasone Valerate 0.1% topical cream	Betnovate (0.1%) cream	1x 100g tube*	Glaxo Wellcome UK Limited.	*Betamethasone Valerate BP 0.122%*, Chlorocresol BP, Cetomacrogol 1000 BP, Cetostearyl Alcohol BP, White Soft Paraffin BP, Liquid Paraffin BP, Sodium Acid Phosphate BP, Phosphoric Acid BP, Sodium Hydroxide BP, Purified Water
Crisaborole 2% ointment	N/A	2x 60g tube	Pfizer	*Crisaborole 2%,* White Petrolatum, Propylene Glycol, Mono- And Di-Glycerides, Paraffin, Butylated Hydroxytoluene, And Edetate Calcium Disodium

*Additional tube can be prescribed upon request where usage tracks consistently high (noting upper acceptable usage limit of 112g)

8.2 Regulatory status of the drugs

8.2.1 Betamethasone Valerate (0.1%) cream

Betamethasone Valerate (0.1%) 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 [0.1%] cream SmPC). Betamethasone valerate creams are manufactured by a number of companies, however this trial will be performed using the Betnovate (Glaxo Wellcome UK Ltd) 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 licenced in the UK; however, it is licenced by the FDA for use in the United States of America and will be used in accordance with this licence in this study.

8.3 Product characteristics

8.3.1 Betamethasone Valerate (0.1%) cream

Please refer to the attached SmPC

8.3.2 Crisaborole (2%) ointment

Please refer to the attached crisaborole Investigator's Brochure (IB)

8.4 Drug supply

8.4.1 Betamethasone Valerate (0.1%) cream

Betnovate (0.1%) 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 (0.1%) 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 (0.1%) 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 randomization 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 randomization).

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 forearm	Betamethasone Valerate (0.1%) cream
Right/left forearm	Crisaborole (2%) ointment

Twice-daily self-administered application of 1 finger-tip units of either crisaborole (2%) ointment or betamethasone valerate (0.1%) cream to the designated treatment areas on the volar side of the respective forearm for 28 days. The 2 daily applications should be conducted once in the morning and once in the evening, separated by at least 6 hours. When washing, the product should be applied after washing (not before). Randomized 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 test areas*.
- 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 test areas*.
  - Use of topical antihistamines *on the test areas*.
- 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 test areas*, 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 2-5 are conducted for the purposes of compliance monitoring and participant training only. The aim is for participants to undertake 5 supervised applications during the first 2 weeks of treatment (including at the end of visit 1) to monitor compliance and encourage consistent adherence to the application regimen. To minimize the burden on participants sufficient variance has been included to allow gaps of up to 7 days between supervised applications. It is anticipated that after 5 observed applications during the first 2 weeks, participants will be sufficiently trained to reliably carry out the treatment. 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 6 hours.

Treatment usage should be determined from the IMP 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 visit 1-5, 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 1-5 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. The dose for both investigational products in this study is 1 FTU applied to the volar face of one forearm each twice per day (Figure 8.1).

Despite the different size tubes for crisaborole ointment and betamethasone valerate cream, both have a nozzle size of approximately 7mm (38.48cm^2) ensuring consistent dosing between them.

This means the anticipated weight per FTU of each product is approximately 1.0g (based on established dosing of 0.5g from a 5mm nozzle, having a 19.63cm^2 area).

The treatment area on the volar face of the forearm from the elbow crease down to the wrist is the equivalent of 2 hands or 264 cm^2 (1.46% body surface area), making the dosage 3.8 (1.9-7.6) mg/cm^2.

With 2 applications per day, the expected usage is 2g per day of each product. Over the 28-day treatment regimen total usage of each product is estimated at 56g.

Due to the expected variability of the FTU participants are expected to apply between 28 and 112g of each product (assessed at the end of treatment) to be compliant with the protocol.

The per gram dosing is consistent with the manufacturer's recommendations for crisaborole ointment (and with is international marketing authorizations). Retraining should be provided at each visit where IMP usage falls outside the expected parameters provided here.

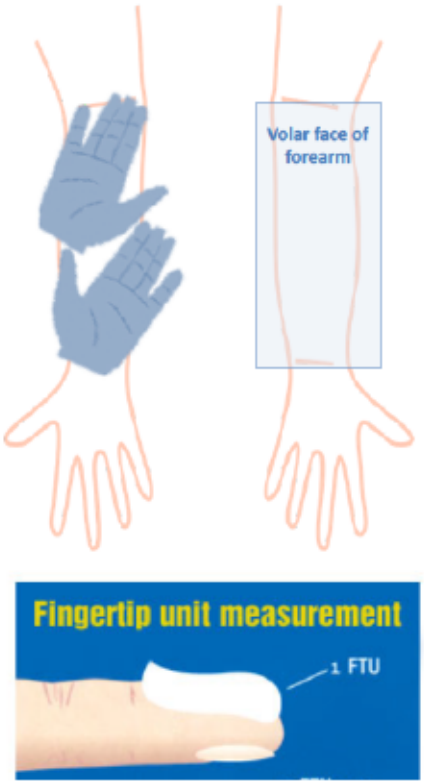


Figure 8.1: A guide to dosing topical creams

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 Visit 5

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. Participants deemed at Visit 5 to be incapable of meeting the usage requirements by the end of the study should be withdrawn.

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 6 and 7, 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 6 and 7 until after skin assessments have been conducted.**

Where the treatments have been applied on days not involving skin assessments, and 6 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 6 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 fulfills 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 preexisting condition that did not worsen from baseline is not considered an AE. • results in persistent or significant disability/incapacity

	○ The term disability means a substantial disruption of a person's ability to conduct normal life functions. ○ This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, 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 adverse events will be from the date of informed consent until approximately 28 days after the last administration of IMP (range from 25 days to 36 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 Adverse Event (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 adverse events will be recorded in a Case Report Form and assessed for seriousness, expectedness (serious AE's only), intensity and causality as outlined below.

9.3.1 Seriousness

A Serious Adverse Event (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 adverse event 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 Serious Adverse Event 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 adverse event 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 adverse events 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 fax (STH R&D 0114 2265937) or email (sae@sth.nhs.uk) 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 postmortem 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 28 days between treatments: betamethasone valerate cream vs crisaborole, utilising the within participant comparisons of left vs right forearm. Assuming that the change from baseline at day 28 in the betamethasone valerate group will be approximately -4.44µm and a standard deviation of 5 µm (for the change from baseline within each participant), to detect a reduction in this change of 80% to -0.88 µm in the crisaborole arm with 80% power, a parallel group study (one group for betamethasone valerate and one group for crisaborole) would require 33 participants in each group. The study described here is comparing within participants (left vs right arm) rather than a parallel group (between participants) design, however it is assumed that this within patient comparison will have greater power than the parallel group design. This assumption is necessary because no information is available in the literature as to the standard deviation of the difference between change from baseline on the TCS arm and change from baseline on the crisaborole arm. Assuming that the correlation between these two 'change from baseline' measures will be greater than zero then the within participant comparisons can be assumed to have greater power than a parallel group design.

Table 10.1: Sample size calculations

Parameter	Anatomical location	AD Mean change from baseline	SD (change from baseline)	Clinically relevant difference (%)	Sample size per cohort	Power
Epidermal thickness (µm)	Forearm	-4.44	5	3.55 (80)	33	>80%

10.2 Planned recruitment rate

A single cohort of up to 37 participants will be recruited and treatment issued to meet the target of 33 for completion (allowing for a 10% drop out rate). Recruitment is estimated to take up to 6 months at a rate of ≥7 recruits per 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 intention to treat 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.

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 adverse events and serious adverse events will be summarised for all participants who signed the consent. All adverse events 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 (change in epidermal thickness measured by structural OCT at day 29, compared between crisaborole (2%) ointment and betamethasone valerate (0.1%) cream) 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
A this is an exploratory study investigating a number of possible biomarkers no formal adjustments for multiplicity will be made, however results will be presented alongside confidence intervals and will be interpreted cautiously. A future study is planned to confirm the selection of biomarkers from this study.

The analysis of the secondary endpoints:

- Change in structural OCT measurements of epidermal thickness
- Change in redness/erythema
- Change in Trans-Epidermal Water Loss (TEWL)
- Change in visual skin dryness
- Natural Moisturising Factor

will use paired analyses to compare the change over time between crisaborole (2%) ointment and betamethasone valerate (0.1%) cream.

10.4.4 Exploratory outcome analysis

The primary and secondary analyses will identify where there is evidence of a difference between crisaborole ointment and betamethasone valerate cream in the change from baseline and which biomarkers are able to identify this difference in the clinical trial setting.

The exploratory analysis will investigate whether a sub set of the OCT endpoints can be used to non-invasively identify skin changes. This analysis will use multiple logistic regression to determine which of the new biomarkers provide the most informative and sensitive data pertaining to the atrophic changes induced by TCS treatment. This will be accomplished by assessing the ability of each biomarker to differentiate the skin of participants before and after treatment with the potent TCS, which is known to induce sub-clinical atrophic skin changes within the treatment window.

The biomarker (or subset of biomarkers) which are identified by this analysis will also be recorded in a subsequent study comparing crisaborole to a moderately potent TCS. This will enable the confirmation of the ability of the biomarkers to identify skin changes in a second independent dataset.

11. STUDY CONDUCT

11.1 Study location

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

- Study enrolment, randomization 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 the 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 the Sample Tracking Log and all use documented on the 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)	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 tape strips 1-10	No (comprises enucleated corneocytes)	Storage at -70°C. Required for stratum corneum metabolite (NMF) quantification. Samples will be processed within 12 months following the last participant last visit. 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.
Superficial skin samples collected on tape strips 11-20	Possibly (as sampling depth increases there is an increasing risk of collecting viable keratinocytes from the skin layer below the stratum corneum)	Samples will be lysed immediately in RLT lysis buffer to render them enucleate. Storage at -70°C for future protein analysis. Specific consent will be sought from participants to retain these samples following completion of the study.

All samples will be stored at -70°C in a freezer with remote high temperature alarm system (24h monitoring for high temperature by mobile phone).

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:

- Using predesigned case record forms. Forms completed by hand during visits will be transferred to the custom electronic data capture system by data coordinators.
- Direct entry into a custom electronic data capture (EDC) system during visits. Completed electronic forms will be printed and signed to provide verifiable source data.
- Collection of raw data files

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

The custom electronic data capture system based on the Prospect clinical trial software will be designed by Epigenesys, a University of Sheffield company.

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 will be involved in the review of the participant information sheets to ensure 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.

Contact details captured on the registration form will be transferred to an electronic database and stored for a maximum of 5 years by the Sheffield Dermatology Research group to enable the group to notify participants about future studies. The consent form includes a line to specifically obtain consent for this (where a participant does not provide this specific consent, he/she will not be entered onto the electronic database). The database will be password protected and stored on the groups filestore (secure server) with access restricted to the PI and the study delegates only.

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 and electronic records stored on the secure Prospect (Epigenysis) server and/or the groups filestore (CICS managed secure server with access control). Pseudo-anonymised research data will be kept indefinitely under the custodianship of the PI for potential use in future research applications.

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.

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 adverse events 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.

15. REFERENCES

1 Odhiambo JA, Williams HC, Clayton TO *et al.* Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. *J Allergy Clin Immunol* 2009; **124**: 1251-8 e23.

2 Kerr OA, Tidman MJ, Walker JJ *et al.* The profile of dermatological problems in primary care. *Clin Exp Dermatol* 2010; **35**: 380-3.

3 Cork MJ, Danby SG, Vasilopoulos Y *et al.* Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009; **129**: 1892-908.

4 Punekar YS, Sheikh A. Establishing the sequential progression of multiple allergic diagnoses in a UK birth cohort using the General Practice Research Database. *Clin Exp Allergy* 2009; **39**: 1889-95.

5 Gupta R, Sheikh A, Strachan DP *et al.* Burden of allergic disease in the UK: secondary analyses of national databases. *Clin Exp Allergy* 2004; **34**: 520-6.

6 Lewis-Jones S, Cork MJ, Clark C *et al.* Atopic eczema in children - Guideline consultation: A systematic review of the treatments for atopic eczema and guideline for its management. In: National Institute for Clinical Excellence (NICE), Department of Health. 2007.

7 Hengge UR, Ruzicka T, Schwartz RA *et al.* Adverse effects of topical glucocorticosteroids. *J Am Acad Dermatol* 2006; **54**: 1-15; quiz 6-8.

8 Schmitt J, von Kobyletzki L, Svensson A *et al.* Efficacy and tolerability of proactive treatment with topical corticosteroids and calcineurin inhibitors for atopic eczema: systematic review and meta-analysis of randomized controlled trials. *Br J Dermatol* 2011; **164**: 415-28.

9 Batchelor JM, Ridd MJ, Clarke T *et al.* The eczema Priority Setting Partnership: A collaboration between patients, carers, clinicians and researchers to identify and prioritize important research questions for the treatment of Eczema. *British Journal of Dermatology* 2013; **168**: 577-82.

10 Byers R, Maiti R, Danby SG *et al.* Sub-clinical assessment of atopic dermatitis severity using angiographic optical coherence tomography *Biomedical optics express* 2018; **Under review**.

11 Mansfield JC, Winlove CP, Moger J *et al.* Collagen fiber arrangement in normal and diseased cartilage studied by polarization sensitive nonlinear microscopy. *J Biomed Opt* 2008; **13**: 044020.

12 Ugryumova N, Jacobs J, Bonesi M *et al.* Novel optical imaging technique to determine the 3-D orientation of collagen fibers in cartilage: variable-incidence angle polarization-sensitive optical coherence tomography. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society* 2009; **17**: 33-42.

13 Danby S, Brown K, Higgs-Bayliss T *et al.* The effect of a complex emollient cream containing 5% urea, ceramide 3 and lactic acid, compared with a simple emollient, on the skin barrier in older people with dry skin. *British Journal of Dermatology* 2014; **171**: 57.

14 Danby SG, Wan H, Chittock J *et al.* Characterisation of the skin barrier defect in atopic dermatitis using in vivo ATR-FTIR molecular spectroscopy. *J Invest Dermatol* 2016; **136**: S182.

15 Boncheva M, Damien F, Normand V. Molecular organization of the lipid matrix in intact Stratum corneum using ATR-FTIR spectroscopy. *Biochimica et biophysica acta* 2008; **1778**: 1344-55.

16 Damien F, Boncheva M. The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. *J Invest Dermatol* 2010; **130**: 611-4.

17 Chittock J, Brown K, Cork M *et al.* Comparing the Effect of a Twice-Weekly Tacrolimus and Betamethasone Valerate Dose on the Subclinical Epidermal Barrier Defect in Atopic Dermatitis. *Acta Dermato Venereologica* 2014: 0.

18 Danby S, Chittock J, Brown K *et al.* The Effect Of Tacrolimus Compared To Betamethasone Valerate On The Skin Barrier In Volunteers With Quiescent Atopic Dermatitis. *British Journal of Dermatology* 2014; **170**: 914-21.

19 Kezic S, O'Regan GM, Yau N *et al.* Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. *Allergy* 2011; **66**: 934-40.

20 Lu Z, Boadi J, Danby S *et al.* Optical coherence tomography demonstrates differential epidermal thinning of human forearm volar skin after 2 weeks application of a topical corticosteroid vs a non-steroidal anti-inflammatory alternative. *Proc. SPIE* 2013; **8565**: 85650C.

21 Danby SG, Chittock J, Brown K *et al.* The effect of tacrolimus compared with betamethasone valerate on the skin barrier in volunteers with quiescent atopic dermatitis. *Br J Dermatol* 2014; **170**: 914-21.

22 Lu Z, Kasaragod D, Matcher SJ. Conical scan polarization-sensitive optical coherence tomography. *Biomedical optics express* 2014; **5**: 752-62.

23 Imhof RE, De Jesus ME, Xiao P *et al.* Closed-chamber transepidermal water loss measurement: microclimate, calibration and performance. *Int J Cosmet Sci* 2009; **31**: 97-118.

24 Chittock J, Brown K, Cork MJ *et al.* Comparing the Effect of a Twice-weekly Tacrolimus and Betamethasone Valerate Dose on the Subclinical Epidermal Barrier Defect in Atopic Dermatitis. *Acta Derm Venereol* 2015; **95**: 653-8.

25 Chittock J, Cooke A, Lavender T *et al.* Development of stratum corneum chymotrypsin-like protease activity and natural moisturizing factors from birth to 4 weeks of age compared with adults. *Br J Dermatol* 2016.

26 Danby S, Al Enezi T, Sultan A *et al.* The effect of Aqueous cream BP on the skin barrier in volunteers with a previous history of atopic dermatitis. *Br J Dermatol* 2011; **165**: 329-34.

27 Danby SG, AlEnezi T, Sultan A *et al.* Effect of olive and sunflower seed oil on the adult skin barrier: implications for neonatal skin care. *Pediatric dermatology* 2013; **30**: 42-50.

28 Danby SG, Brown K, Higgs-Bayliss T *et al.* The Effect of an Emollient Containing Urea, Ceramide NP, and Lactate on Skin Barrier Structure and Function in Older People with Dry Skin. *Skin pharmacology and physiology* 2016; **29**: 135-47.

29 Boncheva M, Damien F, Normand V. Molecular organization of the lipid matrix in intact Stratum corneum using ATR-FTIR spectroscopy. *Biochim Biophys Acta* 2008; **1778**: 1344-55.

30 Brancaleon L, Bamberg MP, Sakamaki T *et al.* Attenuated total reflection-Fourier transform infrared spectroscopy as a possible method to investigate biophysical parameters of stratum corneum in vivo. *J Invest Dermatol* 2001; **116**: 380-6.

31 Ring A, Schreiner V, Wenck H *et al.* Mid-infrared spectroscopy on skin using a silver halide fibre probe in vivo. *Skin Res Technol* 2006; **12**: 18-23.

32 Takada S, Naito S, Sonoda J *et al.* Noninvasive In Vivo Measurement of Natural Moisturizing Factor Content in Stratum Corneum of Human Skin by Attenuated Total Reflection Infrared Spectroscopy. *Applied Spectroscopy* 2012; **66**: 26-32.

33 Cooke A, Cork MJ, Victor S *et al.* Olive Oil, Sunflower Oil or no Oil for Baby Dry Skin or Massage: A Pilot, Assessor-blinded, Randomized Controlled Trial (the Oil in Baby SkincaRE [OBSeRvE] Study). *Acta dermato-venereologica* 2016; **96**: 323-30.

34 Kao JS, Fluhr JW, Man MQ *et al.* Short-term glucocorticoid treatment compromises both permeability barrier homeostasis and stratum corneum integrity: inhibition of epidermal lipid synthesis accounts for functional abnormalities. *J Invest Dermatol* 2003; **120**: 456-64.

16. APPENDIX

Appendix A: Risk assessment

Appendix B: List of sperate documents relating to this trial

- SMART A4 Poster
- SMART Landscape Advert
- SMART Participant Information Leaflet (PIL)
- SMART Participant Information Sheet and Consent Form

Appendix C: List of IMP information available as separate documents

- Betnovate cream Product Information Leaflet
- Betnovate cream SmPC
- FDA summary review on Crisaborole (2%) ointment
- Crisaborole PF-06930164 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