Statistical Analysis Plan (SAP)

Validation of a novel composite of skin biomarkers as a primary outcome measure for evaluating the safety of treatments for atopic dermatitis study 2: a randomised controlled trial (phase 2) comparing the effects of crisaborole 2% ointment to betamethasone valerate 0.025% cream on skin structure and function in participants with atopic dermatitis.

Skin bioMARkers for atopic eczema Therapy evaluation study 2

Contents

Abbreviations

AD	Atopic dermatitis
ANCOVA	Analysis of Covariance
EASI	Eczema Area and Severity Index
FAS	Full Analysis Set
FTIR	Fourier Transform Infrared
FTU	Finger Tip Unit
ISGA	Investigators Static Global Assessment
NMF	Natural moisturizing factor
OCT	Optical Coherence Tomography
PPS	Per protocol set
PS-OCT	Polarization sensitive OCT
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCS	Topical corticosteroid
TEWL	Trans-Epidermal Water Loss

1. Introduction:

This study aims to directly compare the effects of crisaborole (2%) ointment to the moderately potent TCS betamethasone valerate (0.025%) cream on the properties of the skin at different anatomical locations (volar forearm, antecubital fossa and cheek) using an established model for quantifying the local adverse effects of TCS.

- 4 weeks twice daily applications to the antecubital fossa and volar forearm
- 2 weeks twice daily applications to the cheek

2. Study Methods

2.1 Study Design

This is an observer-blind, randomized within-subject controlled clinical trial. The participants will apply treatment (1 fingertip unit) on designated treatment areas on the arms (volar forearm and including the corresponding antecubital fossa) for 28 days. Additionally, 1/4 FTU of the respective treatment to be applied to the cheek on the same side of the body twice-daily 14 days .

Participants will attend 8 clinic visits during the study:

- Screening will be performed up to 28 days prior to visit 1
- Baseline (visit 1) will be performed on day 1*
- Visit 2 will be performed on day 2
- Visit 3 will be performed on day 5
- Visit 4 will be performed on day 8*
- Visit 5 will be performed on day 11
- Visit 6 will be performed on day 15*
- Visit 7 will be performed on day 22
- Visit 8 will be performed on day 29*
- Visit 9 will be performed on day 57* (after 28 days of washout)

*Skin assessments will be carried out at these visits.

There will be two treatments applied:
crisaborole (2%) ointment
betamethasone valerate (0.025%) cream

The primary objective is superiority: that crisaborole use will result in less epidermal thinning than the equivalent regimen with betamethasone valerate. The secondary objectives are: investigating the difference between the two treatments on tolerability, skin barrier function, dryness and natural moisturising factor and the ability to detect sub-clinical changes associated with adverse effects.

It is planned to recruit 40 adults (male and female, aged 18-65) with personal history of atopic dermatitis but no current eczema on the volar forearms and no possible allergy to the ingredients in the study medications (allowing for a 18% drop-out rate to meet the target of 33 subjects). They will not be permitted to use topical product on the test area for 7 days prior to baseline or to use a tanning bed for 28 days prior to baseline.

Subjects will remain in the study for 8-9 weeks.

At each study visit, concomitant medication and any AEs are noted in the Case Report Form (CRF). The study medication will be collected and weighed in order to estimate cream consumption.

Assessors will be blind to the treatment assigned to each site. Due the difference in consistencies between the two treatments (cream or ointment) subjects will not be blinded to the treatment assigned to each study site.

2.2 Randomisation

Screening will take place up to 28 days before baseline (day 1). Those eligible to take part will be randomised on day 1 with each subject testing both treatments – one on each of the left or right lower volar forearm, antecubital fossa and cheek. The randomisation list will be provided by the SSU and the SSU will unblind the database after database lock. The interim analysis has been designed to be performed on blinded data.

The pharmacy and a statistician (independent of the study statistician) will have access to the randomisation master list. Since only the research team assessing the study endpoints are blind to the treatment allocation and participants are all prescribed both treatments, no emergency unblinding arrangements are necessary.

2.3 Sample size:

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

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

2.4 Timelines

Planned milestones are as follows (these will be updated as information becomes available);

- First subject in: (est. Dec 2022, actual Feb 2022)
- Safety analysis of cheek epidermal thickness data after the first 5 participants complete visit 4*

- Safety analysis of cheek epidermal thickness data after the first 10 participants complete visit 4*
- Interim analysis: After 10th subject complete* (est. Aug 2023, actual Sep 2023)
- Last subject complete: (12 Dec 2023)
- Full database delivered to SSU: (est. 20 Dec 2023)
- Blind data review: SSU create tables: 6-8 weeks (est. 29 Feb 2024)
- Team review blind outputs (est. 30 Mar 2024)
- Data Base lock (est. 1 Apr 2024)
- Statistical analysis: 6-8 weeks (est. 27 May 2024)
- Statistical report: containing TFLs and brief text

*These analyses will take place on blinded raw data by the study team (no statistical analysis).

Final analysis will take place once all participants have had their final assessment at day 57 or have discontinued and the data has been fully cleaned and the database locked.

Completion of the SAP, data cleaning and a blind review will take place prior to data base lock. During blind review all decisions about protocol deviations (ITT and PP populations- if applicable) and analyses (such as assumptions checking) will be made. Once all these activities have taken place then the database will be locked and unblinded.

3. Data Collection:

Data is to be collected in a custom electronic data capture system designed by the Clinical Trials Research Unit (CTRU) within the University of Sheffield. Adverse events will be coded to MedDRA preferred terms & system organ class within the database. Data will be provided to the SSU as clean csv or sas files.

Data transfers between the study team and Pfizer will be in csv format, the study team will be responsible for these data transfers.

4. Analysis Objectives:

4.1 Primary objective:

To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy on the volar forearm in patients with atopic dermatitis.

4.2 Secondary objectives:

- To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy (on the antecubital fossa) in patients with AD.

- To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 2 weeks is a cause of skin atrophy on the cheeks in patients with AD
- To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream at different anatomical sites
- To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream
- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on skin barrier function

4.3 Exploratory objectives:

- To visually confirm that structural OCT measurement of epidermal thickness (angiographic OCT-derived biomarkers, PS-OCT derived boiomarkers and FTIR carboxylate levels) provides an accurate indication of epidermal atrophy in response to TCS treatment
- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on epidermal vascular structure
- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on collagen matrix structure (fibrosis)
- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on stratum corneum NMF levels (key skin metabolites linked to skin homeostasis, skin microbiome, and skin moisturization.
- To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on stratum corneum lipid structure (a key determinant of permeability barrier function and general skin health that is adversely affected by TCS treatment)
- 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

5. Analysis Sets and Protocol Deviations

Full Analysis Set (FAS): All subjects who were randomised into the study.

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

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

All efficacy analyses and summaries will be performed on the FAS. If there are concerns about protocol deviations at the blind review, then a sensitivity analysis of the primary objective may be performed on the PPS.

Safety summaries will be performed on the safety set.

Prior to unblinding, a blind review of the data will be performed. The objective of the review is to identify protocol deviations and data queries and to make decisions regarding data analytical issues under blind conditions. Important violations of eligibility criteria and other deviations from the protocol will be assessed in cooperation with the study team. Important deviations from the protocol may lead to exclusion of a participant from the PPS. All deviations will be discussed and agreed prior to the unblinding of the data.

6. Endpoints and Covariates

The table below shows the endpoints for each objective:

	Objectives	Endpoints
	Primary Objective	
1.	To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy on the volar forearm in patients with atopic dermatitis.	Change in Epidermal thickness measured by structural OCT (day 29 - day 1) **Volar forearm**
	Secondary objectives:	
2.	To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 4 weeks is a cause of skin atrophy (on the antecubital fossa) in patients with AD.	Change in Epidermal thickness measured by structural OCT (day 29 - day 1) **Antecubital fossa**
3.	To determine whether twice daily treatment with crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, for up to 2 weeks is a cause of skin atrophy on the cheeks in patients with AD	Change in Epidermal thickness measured by structural OCT (day 15 - day 1) **cheek**
4.	To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream at different anatomical sites	Change in superficial plexus depth (µm) (days 1, 15, 29 and 57) **volar forearms and antecubital fossae.**
		Change in superficial plexus depth (µm) (days 1, 15 and 57 **cheeks**
		Change in Epidermal thickness measured by structural OCT (days 1, 15, 29 and 57) **volar forearms and antecubital fossae.**

		Change in Epidermal thickness measured by structural OCT (days 1, 15 and 57 **cheeks**
5.	To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream	Change in visual redness/erythema score (days 1, 15, 29 and 57) **volar forearms and antecubital fossae**
		Change in visual redness/erythema score (days 1, 15, and 57) **cheek**
		Change in erythema index from skin images (c-cube) (days 1, 15, 29 and 57) **volar forearms and antecubital fossae**
		Change in erythema index from skin images (c-cube) (days 1, 15, and 57) **cheek**
		Change in objective redness (Mexameter) (days 1, 15, 29 and 57) **volar forearms and antecubital fossae**
		Change in objective redness (Mexameter) (days 1, 15, and 57) **cheek**
6.	To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on skin barrier function	Change in TEWL measurements on day 1, 15, 29 and day 57 from the **volar forearm and antecubital fossa**
		Change in TEWL measurements on day 1, 15, and day 57 from the **cheeks.**
		Change in skin barrier integrity/STS (day 29 – day 1) **on the volar forearms** (TEWLts20)
		Skin barrier integrity/STS (day 29) on **antecubital fossae** (TEWLts10).
		Relationship between TEWL and TEWLts20 at day 29 (scatterplot) for the volar forearm
		Relationship between TEWL and TEWLts10 at day 29 (scatterplot) for the antecubital fossa
		Visual skin dryness scored on day 1, 15, 29 and day 57 from the **volar forearm and antecubital fossa**
		Visual skin dryness scored on day 1, 15, and day 57 from **cheeks**.

	Exploratory objectives:	
7a.	To investigate the kinetics of changes in epidermal thickness measured by structural OCT brought about by treatment with crisaborole (2%) ointment and betamethasone valerate (0.025%) cream at different anatomical sites	Change in superficial plexus depth (µm) (day 1 and day 8) **volar forearms, antecubital fossae and cheeks.**
		Change in Epidermal thickness measured by structural OCT (day 1 and day 8) **volar forearms, antecubital fossae and cheeks.**
7b.	To determine the tolerability of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream	Change in visual redness/erythema score (day 1 and day 8) **volar forearms, antecubital fossae and cheeks**
		Change in erythema index from skin images (c-cube) (day 1 and day 8) **volar forearms, antecubital fossae and cheeks**
		Change in objective redness (Mexameter) (day 1 and day 8) **volar forearms, antecubital fossae and cheeks**
7c.	To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on skin barrier function	Change in TEWL measurements (day 1 and day 8) from the **volar forearms, antecubital fossae and cheeks**
		Visual skin dryness scored (day 1 and day 8) from the **volar forearms, antecubital fossae and cheeks**
7d.	To visually confirm that structural OCT measurement of epidermal thickness provides an accurate indication of epidermal atrophy in response to TCS treatment	Skin biopsies collected from a subset of AD participants at day 29. Where image quality is acceptable, continuous scoring may be possible.
8.	To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on epidermal vascular structure	Change in blood vessel diameter (µm) and blood vessel density (segments/mm2) measured by angiographic OCT on **the volar forearms and antecubital fossae** on day 1, 8, 15, 29 and day 57
		Change in blood vessel diameter (µm) and blood vessel density (segments/mm2) measured by angiographic OCT on the **cheeks** on day 1, 8, 15 and day 57
8a.	To visually confirm that angiographic OCT-derived biomarkers provide an accurate indication of vascular changes associated with epidermal	Visualise epidermal tissue vascular structure by histological analysis of skin biopsies collected from a subset of AD participants at day 29*

	atrophy in response to TCS treatment.	
9.	To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on collagen matrix structure (fibrosis)	Change in collagen matrix index measured by PS-OCT on the **volar forearms** on day 1, 29 and day 57.
9a	To visually confirm that PS- OCT-derived biomarkers provide an accurate indication of collagen matrix changes (fibrosis) associated with epidermal atrophy in response to TCS treatment	Visualise collagen structure by second harmonic generation imaging of frozen tissue sections of skin biopsies collected from a subset of AD participants at day 29*
		Visualise dermal collagen staining by skin tissue sections of skin biopsies collected from a subset of AD participants at day 29*
10.	To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on stratum corneum NMF levels (key skin metabolites linked to skin homeostasis, skin microbiome, and skin moisturization.	Change in carboxylate levels (FTIR) at the **volar forearms and antecubital fossae** on day 1, 15, 29 and day 57 .
		Change in carboxylate levels (FTIR) at **cheeks** on day 1, 15 and day 57.
10a.	To confirm that FTIR carboxylate levels enable the accurate quantification of NMF changes associated with epidermal atrophy in response to TCS treatment, by describing the relationship between HPLC derived NMF levels and FTIR carboxylate levels	Correlation of FTIR spectra and superficial stratum corneum samples collected at days 1 and 29.
10b.	To visually confirm that FTIR carboxylate levels provide an accurate indication of changes in filaggrin expression (source of NMF) associated with epidermal atrophy in response to TCS treatment	Visualise skin tissue filaggrin staining by immunohistochemistry of skin biopsies collected from a subset of AD participants at day 29*
11.	To determine the effect of crisaborole (2%) ointment, compared to betamethasone valerate (0.025%) cream, on stratum corneum lipid structure (a key determinant of permeability barrier function and general skin health that is adversely affected by TCS treatment)	Change in stratum corneum lipid structure (day 29 - day 1) (FTIR spectra taken through the stratum corneum from the volar forearm (during STS) on the **volar forearm.**
11a.	To confirm that FTIR lipid structure provides an accurate indication of lipid changes associated with	Visualise skin tissue total lipid properties by histology of skin biopsies collected from a subset of AD participants at day 29*

	epidermal atrophy in response to TCS treatment.	
12.	To investigate the number of participants with FLG loss-off-function mutations and explore if there is any evidence of a relationship to treatment effects	Number of FLG loss-of- function mutation carriers Descriptive tabulations of TEWL, epidermal thickness, and carboxylate levels by mutation status.

* Note: where continuous scoring is not possible, visualisations will be described qualitatively by the study team – not by SSU.

6.2 Variables

Epidermal Thickness

Triplicate measurements will be performed at each test site (upper part of the right and left volar, the right and left antecubital fossa and the right and left cheek at baseline (day 1), after 1 week treatment (day 8), after 2 weeks treatment (day 15), after 4 weeks treatment (forearm and fossa only, day 29) and 4 weeks after treatment cessation (day 57) using Structural OCT image capture at depth of focus 1.0mm and optical resolution of 7.5x5.0 µm. The average (mean) reading for each patient at each time point at each site will be used in the analysis.

TEWL

Triplicate measurements will be performed at each test site at baseline (day 1), after 1 week treatment (day 8), after 2 weeks treatment (day 15), after 4 weeks treatment (day 29) and 4 weeks after treatment cessation (day 57) using an AquaFlux condensing chamber probe (For the volar forearm this will be the lower measurement site throughout, designated FA2 in the protocol). A fourth measurement may be taken if the technician feels that one of the previous readings was abnormal. The average reading for each patient at each time point at each site will be used in the analysis.

On the volar forearms, TEWL measurements will also be performed in conjunction with tape-stripping as a measure of skin barrier integrity. Because of the disruptive nature of tape-stripping the procedure will be performed at the higher measurement sites at baseline (designated FA1 in the protocol) and at the separate lower sites after 4 weeks (designated FA2 in the protocol). TEWL values will be plotted against tape-strip number (in increments of 5 strips), including baseline TEWL measurements (i.e. before tape-stripping) made at the specific site subject to tape-stripping (either FA1 at baseline or FA2 after 4 weeks). Statistical analysis will be restricted to TEWL values made after 20 tape-strips (this outcome is known as $TEWL_{ts20}$).

On the right and left antecubital fossae, tape-stripping will only be performed after treatment (no baseline) and only after 10 tape-strips (no 5, 15 or 20). Accordingly, the outcome for analysis will be known as $TEWL_{ts10}$ and will be recorded at day 29 only.

Redness

Visual scoring of the redness (erythema) of the subject's skin by an expert will take place at baseline (day 1), after 1 week treatment (day 8), after 2 weeks treatment (day 15), after 4

weeks treatment (forearm and fossa only, day 29) and 4 weeks after treatment cessation (day 57). Scores will be on a scale from 0 to 3 and will be graded by a single expert grader. The lower volar forearms on each side will be used to give one overall result for the right forearm and one overall result for the left forearm (data from the upper forearm [FA1] to be excluded from the analysis because this is only collected at baseline).

Objective scoring of the sites will also be measured using a mexameter and c-cube image documentation system. For the Mexameter, four repeats per site will be made; the average reading for each patient at each time point at each site (right and left, upper and lower part of the volar forearm, the antecubital fossa and the cheek) will be used in the analysis. A fifth measurement may be taken if the technician feels that one of the previous readings was abnormal. For the c-cube, a single image will be captured for each site, generating a single value for erythema index.

Dryness

Visual scoring of the dryness of the subject's skin by an expert will take place at baseline (day 1), after 1 week treatment (day 8), after 2 weeks treatment (day 15), after 4 weeks treatment (day 29) and 4 weeks after treatment cessation (day 57). Scores will be on a scale from 0 to 4 and will be graded by a single expert grader. The lower volar forearms on each side will be used to give one overall result for the right forearm and one overall result for the left forearm (data from the upper forearm [FA1] to be excluded from the analysis because this is only collected at baseline). There will also be right and left antecubital fossae and cheek results.

Blood Vessel Diameter/Density

Angiographic OCT will be used to derive the blood vessel diameter (μm) and blood vessel density (segments/mm^2) at baseline (day 1), after 1 week treatment (day 8), after 2 weeks treatment (day 15), after 4 weeks treatment (forearm and fossa only, day 29) and 4 weeks after treatment cessation (day 57).

Collagen Matrix Structure (Fibrosis)

PS-OCT images will be taken of the volar forearms at baseline (day 1), end of treatment (day 29) and four weeks after cessation of treatment (day 57). The collagen matrix will be derived from these images.

NMF levels

Samples for analysis of the carboxylate levels in the stratum corneum will be measured by FTIR spectroscopy at baseline (day 1), after 2 weeks treatment (day 15), after four weeks treatment (volar forearms and antecubital fossae only, day 29) and four weeks post treatment cessation (day 57). There are 2 separate FTIR-derived metrics for carboxylate level; designated 1410 and 1340 based on their wavelength (cm-1). Carboxylate levels will also be presented in 2 ways; (1) skin surface levels based upon the mean of 2 repeat spectra collected from intact skin (without tape-stripping) and; (2) stratum corneum levels based upon the mean of single measurements taken after 5, 10, 15 and 20 tape-strips (based on the tape-strip 10 measurement only for the cubital fossa sites) where tape-stripping is performed.

In addition, superficial stratum corneum samples will be collected at baseline (day 1) and after four weeks treatment (day 29) to facilitate description of the relationship between HPLC derived NMF levels and FTIR carboxylate levels. The superficial stratum corneum samples will undergo HPLC analysis to quantify the levels of pyrrolidone carboxylic acid, urocanic acid and free amino acids. Each will be summarised and analysed separately and together as a combined total (total NMF levels)

Samples for analysis of stratum corneum NMF levels will be collected from the volar forearm only by tape stripping before (day 1) and after 4 weeks of treatment (day 29). One sample will be collected from each sampling site (tape-strips/discs 1-3 on lower right and left volar forearm). The samples will be analysed separately.

Lipid Structure

Lipid structure will be determined from FTIR spectra and analysed as per carboxylate levels above.

Product Consumption

Product consumption will be based on the difference in weight of the ointment and cream between the start (day 1) and end (day 29) of treatment phase.

Adverse Events

Participants will record AEs throughout the study in their diaries with the information transferred to the CRF at day 29 and day 57.

FLG loss-of-function mutations

Saliva samples will be collected at baseline (day 1) to obtain genomic DNA for determination of participant FLG gene status. Samples will undergo DNA extraction and genotyping at the University of Sheffield for the 3 common European loss-of-function FLG mutations that have been reported to confer increased AD risk.

7. Statistical Analyses

The primary and secondary analyses will investigate the difference between the two treatments in the change from baseline and the restoration of values after cessation of treatment. Exploratory analyses will investigate whether there are differences between treatments for the secondary endpoints within 1 week of treatment. Also, whether there are differences between treatments for other endpoints. Finally, image data will be explored to see how well it quantifies epidermal atrophy associated with treatment.

Outcome and demographic data will be summarised at each time point using appropriate descriptive statistics such as N, mean, standard deviation, minimum, lower quartile, median, upper quartile and maximum.

7.1 Primary Analysis

The primary analysis of change in epidermal thickness on volar forearm over 28 days treatment will be estimated using a repeated measures mixed model with change from day 1 as the outcome, treatment, timepoint as factors together with a treatment by timepoint interaction, subject as a random effect and baseline as a covariate. The estimate of change from baseline to day 29 from this model will be the primary outcome, the estimate of change from day 1 to day 15 and change from day 29 to 57 will be secondary as described by in section 7.2.

7.2 Secondary Analysis

The analysis of three of the secondary endpoints will use a repeated measures mixed model with change from day 1 (to day 15, day 29, day 57) as the outcome, treatment, timepoint as factors together with a treatment by timepoint interaction, subject as a random effect and baseline as a covariate.
The model will be used to estimate the difference between treatment arms for the change from baseline to day 29.

Summary statistics will be presented for all timepoints.

This applies to:

- Change in epidermal thickness in volar forearms (change from day 1 to day 15, and from day 29 to day 57 as secondary endpoints)
- Change in epidermal thickness in antecubital fossae (change from day 1 to day 29, from day 1 to day 15, and from day 29 to day 57 as secondary endpoints)
- Change in epidermal thickness in cheek (change from day 1 to day 15, and from day 15 to day 57 as secondary endpoints)
- Change in redness/erythema (day 1 to day 15 (all sites), day 15 to day 57 (cheek only) and day 1 to day 29, day 29 to day 57 (volar forearm and antecubital fossa only))
- Change in Trans-Epidermal Water Loss (TEWL) (day 1 to day 15 (all sites), day 15 to day 57 (cheek only) and day 1 to day 29, day 29 to day 57 (volar forearm and antecubital fossa only))
- Change in $TEWL_{ts20}$ (day 1 to day 29) (volar forearm only)
- Change in visual skin dryness (day 1 to day 15 (all sites), day 15 to day 57 (cheek only) and day 1 to day 29, day 29 to day 57 (volar forearm and antecubital fossa only))

The $TEWL_{ts10}$ (antecubital fossa) after 28 days treatment will use a paired t-test to compare treatments (no baseline measurement taken).

If any of the above models do not fit satisfactorily or appear to be over fitted the analysis may be changed to look at change from baseline to day 29 and change from day 29 to 57 separately.

Since these analyses are exploratory, no adjustment for multiplicity will be made.

Visual confirmation that structural OCT, angiographic OCT, PS-OCT derived biomarkers and FTIR carboxylate levels provide accurate quantification of related changes associated with epidermal atrophy in response to treatment will be assessed. There is no plan to derive quantitative values from the biopsies. Images of the skin tissue will be used to visually support the primary and secondary outcomes only. Where no quantitative values are derived, description of the findings will be qualitative only and provided by the study team (not SSU). Where quantitative values are available (7d: structural OCT measurement of epidermal thickness and 10a: HPLC derived NMF levels and FTIR carboxylate levels) then scatterplots will be produced by SSU along with correlation statistics.

The analysis of some of the exploratory endpoints will use a repeated measures mixed model with change from day 1 (to day 8) as the outcome, treatment, timepoint as factors together with a treatment by timepoint interaction, subject as a random effect and baseline as a covariate.

The model will be used to estimate the difference between treatment arms at each site for the change from baseline to day 8.

This applies to:

- Change in epidermal thickness (structural OCT and angiographic OCT) on all sites (change from day 1 to day 8)
- Change in redness/erythema (change day 1 to day 8)
- Change in Trans-Epidermal Water Loss (TEWL) (change day 1 to day 8)

The analysis of other exploratory endpoints will use a repeated measures mixed model with change from day 1 (to day 8, day 15, day 29 and day 57) as the outcome, treatment, timepoint as factors together with a treatment by timepoint interaction, subject as a random effect and baseline as a covariate.
The model will be used to estimate the difference between treatment arms at each site for the change from baseline to day 29 and the change from day 29 to day 57.

This applies to:

- Mean blood vessel diameter (µm) (day 1, day8, day 15, day 29 (volar forearm and antecubital fossa only), day 57)
- Blood vessel density (day 1, day 8, day 15, day 29 (volar forearm and antecubital fossa only), day 57)
- PS-OCT collagen matric index (volar forearm only) (day 1, day 29, day 57)
- Carboxylate levels (day 1, day 15, day 29 (volar forearms and antecubital fossa) and day 57)
- Stratum corneum (volar forearm only) (day 1, day 29)
- Lipid structure (day 1, day 15, day 29 (volar forearms and antecubital fossa) and day 57)

Furthermore, summaries of the key outcomes (TEWL and epidermal thickness) by visit (day 1, day 15, day 29) will be made split by FLG loss-of-function mutation carriers.

Exposure and Compliance
A summary of the number of treatment days and the used weight per treatment day by treatment will be provided.

7.3 Adverse event data:

All Adverse Events will be summarized for all subjects enrolled in the study – both the number of participants with an AE and the total number of AEs. AEs will be tabulation by system organ class and by preferred term, as well as severity and relationship to treatment. Finally, those AEs which are specifically recorded as related to one of the test sites, the AEs will be summarised by test site. A listing, by participant number, will be produced to show the AEs at the worst severity and worst relationship to treatment.

7.4 Multiplicity considerations

All analyses in this SAP will be carried out with a two sided 5% significance level.

Secondary analyses are considered to be exploratory and so no adjustment will be made for multiplicity.

7.5 Missing and unusual data

Missing data will not be replaced. If appropriate the drop out rate will be calculated and analysed.

During data review (prior to data base lock and unblinding), readings that are incorrect, i.e. outside the equipment measurement range, will be removed. However, all readings that are possible, even if they are unusually low or high, will be kept in the analysis.

7.6 Interim Analysis:

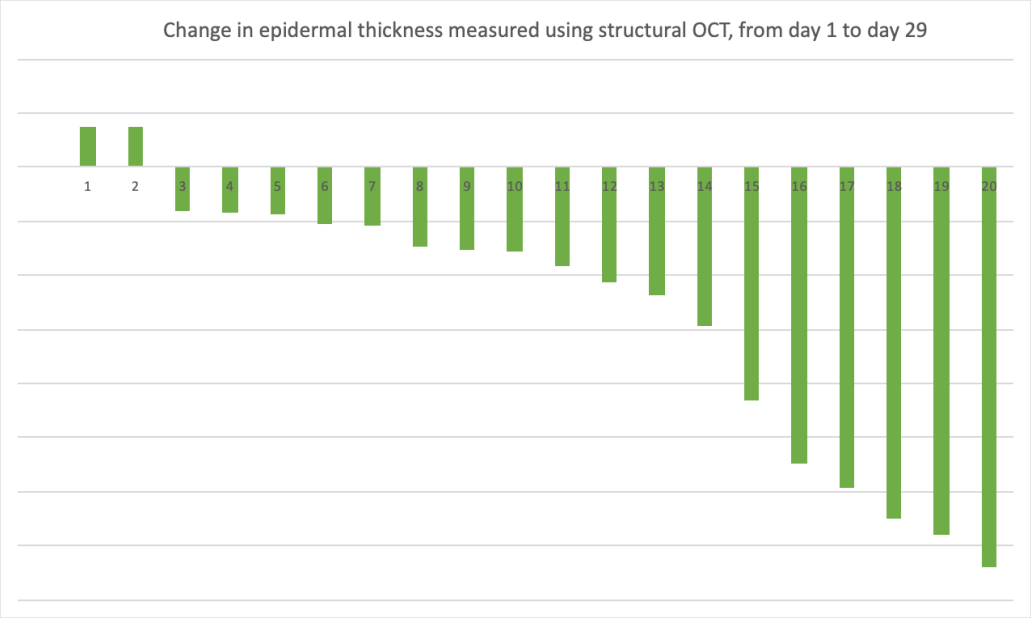
It is of interest to review the data at an interim point to assess the ability of key biomarkers (epidermal thickness and Carboxylate levels) to detect any thinning of the skin over the first 4 weeks of the study. In order to explore this data without breaking the blind or introducing unplanned statistical analysis the following graphical approach is proposed:

For each patient with complete data, the change from baseline to end of treatment will be calculated (day 29 for left and right volar forearms, day 29 for left and right antecubital fossae separately and day 15 for left and right cheek separately). All the available data per site will be displayed together and no attempt will be made to identify which observations are related to each treatment. For each endpoint of interest, the observations will be ordered according to size and plotted (see example below). This plot will allow clinical interpretation of the raw data to give an early indication of the magnitude of change in skin thickness that is being observed and the variation in that change.

A similar plot of the change from day 29 to day 57 (or day 15 to day 57 for cheek), with the observations ordered in the same way, will allow an early look at the recovery of the skin once treatment ends.

A further analogous plot showing skin barrier integrity (TEWLts20) on volar forearm after 28 days treatment will also be created. This will be repeated for the antecubital fossa ($TEWL_{ts10}$).

Each bar in the plot represents the observation from a single side, thus each patient will contribute two bars but these will not be linked in the plot.



7.7 Data Monitoring Committee (DMC):

No DMCs are planned for this trial.

7.8 Changes From Protocol

There are no planned changes from protocol for this study.

8. Transfer of documentation, data and reporting

Data, documentation and reporting will be shared between the Study team, CTRU, SSU and Pfizer as detailed below. The transfers will use a mutually agreeable electronic transmission method that is demonstrated to be compatible with uploading to the targeted Pfizer database or Pfizer systems and that protects the security and integrity of the data. All deliverables provided to Pfizer will be written in English.

Documentation

- Monthly status reports will be provided to Pfizer by **the study team** in Excel Format, SAS Format, or XPT Format to include:
    - monthly updates of first participant first visit, participant screened, and randomized to date, screen failures to date
    - Participants completed; and
    - any other information as may reasonably be requested by Pfizer
- At the time of study reporting **the SSU** will provide listings (pdf document) by Study subject, for all enrolled Study subjects (not including screen failures) for the following variables as a minimum:
    - subject no.,
    - enrollment date,
    - randomization date,
    - dosing date

- The **study team** will provide the following documents to Pfizer and the SSU in the form of one or more Portable Document Format ("**PDF**") files and as they become available:
    - annotated CRFs
    - an electronic "Data Dictionary" consisting of all versions of the CRFs used in the Study, annotated with variable names and corresponding datasets
    - documentation of dictionary coding versions used to code medications and SAEs/AEs
    - Any derivations used at the point of data entry or during data cleaning
    - TMF-associated documentation, including, but not limited to:
        - blank/template CRF,
        - CRF approval(s),
        - clinical data change report,
        - all versions of CRF completion guidelines,
        - completed CRFs,
        - database entry and database user acceptance testing documentation,
        - data management plan(s) (inclusive of edit checks),
        - data review plan(s) and database release documentation,
        - any other documentation as may reasonably be requested by Pfizer

- The **SSU** will provide SAP for review and at sign off (plus if any amendments are required)
    - Any derivations used for the analysis will be included in the SAP/added to an appendix of the SAP as appropriate
    - relevant statistical analysis assumptions or plans will be included in the SAP/added to an appendix of the SAP as appropriate

Clinical Datasets

A "**Clinical Dataset**" consists of all Study Data (with certain personal identifiers removed) available to Trust at the time of transfer. Clinical Datasets may include raw datasets and/or analysis datasets. Trust will transfer Clinical Datasets and in the form of SAS Export/Transport file format (XPT) files or CSV files as detailed below.

Test transfers

Prior to transfer of a given Clinical Dataset to Pfizer, **the study team** will transfer test data to support the effective transfer of such Clinical Dataset. A test data set will consist of complete "dummy" data for at least 5 hypothetical or actual Study subjects. Pfizer will perform certain checks on test data to determine if the transmission meets Pfizer requirements in content and process and if the data will load successfully into the target Pfizer database. Trust will work with Pfizer if changes are needed in the data formatting or transmission process to ensure data quality and usability. Trust will transfer additional sets of test data if needed after such changes are made, as well as if there are any changes in the Study variables or data collection tools during the Study. **Transfers from the study team will be of RAW data in csv format.**

When a test transfer is available it will be provided to the SSU by the **study team**.

Data transfers

The study team will transfer a Clinical Dataset to Pfizer: after ten (10) of subjects dosed and after study completion.

At the time of study reporting (when the final analysis is complete) the **SSU** will provide clinical datasets in SAS format. A test transfer can be done in advance, however, there may be small changes to variables up until the final analysis is agreed.

Data cleaning and validation

Pfizer, the **study team** and **the SSU** will alert each other promptly if they discover that any data within a Clinical Dataset fails to meet appropriate quality standards. This may include data queries from **Pfizer** or the **SSU** that **the study team** will investigate and resolve.

Results Summary

The **SSU** will provide tables and listings to the **study team** and **Pfizer** following the final analysis, the format of these will be agreed in advance and will include:

- subject population summaries
- efficacy results
- safety results
- the SSU will advise on statistical interpretation of primary results

In addition, the study team may provide commentary on the objectives and study design and clinical interpretation.
Timelines for the delivery of the tables and listings will be agreed between the study team, the SSU and Pfizer.

Study Report

- Whilst the **SSU** will input and advise with respect to the statistical design, methodology and interpretation the **study team** will be responsible for writing the study report.
- A draft of the written Study Report will be delivered to Pfizer in accordance with the information and formatting as agreed to by **the study team** and in accordance with ICH-E3 guidelines no later than eight (8) months after the last subject's last visit. The Study Report will reflect the results of the Study as a whole. **Study team** will provide Pfizer with an opportunity to review and comment on the draft Study Report for at least thirty (30) days after Pfizer's receipt of the draft Study Report, and Trust and Principal Investigator will consider in good faith any comments reasonably.
- Within eleven (11) months after the last subject's last visit or database lock or upon early termination of the study, whichever occurs first, **the study team** will provide Pfizer with the final Study Report. In case of early termination of the study (before Study Completion), the Study Report will include, at minimum, the Study Results through the date of termination. Unless otherwise agreed in writing, The Trust may submit the Study Report to applicable regulatory authorities.
- Unless otherwise agreed by Pfizer, the Study Report will address:
    - Ethics
    - Investigators and Study administration
    - Study objectives
    - investigational plan
    - Study subjects
    - efficacy evaluation
    - Drug compliance/adherence evaluation
    - safety evaluation
    - discussion and overall conclusions; supportive tables, figures and graphs;
    - biomarker findings; and
    - any other information as may reasonably be requested by Pfizer.

9. List of Tables, Figures and Listings

Tables

14.2.6.1	Change in TEWL (g/m2h) during and after treatment by site (day 8 – day 1, day 15 – day 1, day 29 – day 1 (FA & AF), day 57 – day 15 (CH), day 57 – day 29 (FA & AF)) (Full Analysis Set)
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14.2.10	Quantitative measure of epidermal atrophy (where image quality is suitable) (day 29) (Biopsy Analysis Set)
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14.2.13	Collagen matrix index on volar forearms (Full Analysis Set)
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14.2.14.3	Carboxylate 1410 levels within stratum corneum by site (Full Analysis Set) (mean of single measurements taken after 5, 10, 15 & 20 tape strips on FA and after 10 tape strips only for AF)

14.2.14.3.1	Change from baseline of carboxylate 1410 levels within stratum corneum (FTIR) (day 29 – day 1) (FA) (Full Analysis Set)
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† This will be calculated as the total product consumed divided by the total area covered over the whole study. The area will be computed as the area per site multiplied by the number of days applied. For example, the application area for each cheek is 64 cm2 and the product is applied twice per day for 15 days (= 30 * 64) whilst the product is applied twice daily for 28 days (56 instances) and the area on the FA and AF combined is calculated per person as (height1 + height2)*width.

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Listings

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