Statistical Analysis Plan (SAP)

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

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Date: _____

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Abbreviations

AD	Atopic Dermatitis
ANCOVA	Analysis of Covariance
FAS	Full Analysis Set
FLG	Filaggrin mutation
FTU	Finger Tip Unit
PPS	Per protocol Set
NMF	Natural Moisturising Factor
SLS	Sodium Lauryl Sulfate
TEWL	Trans Epidermal Water Loss

1. Introduction:

This study is designed in order to evaluate the skin barrier strengthening effects of Miniderm Novum and the two reference creams compared to untreated. It is a randomised, controlled, blinded, four parallel group study.

The participants will apply cream (1 FTU) twice daily on designated treatment areas on their volar forearms for 28 days. On day 5 and 15 the participants will visit the clinic where the test and reference products will be weighed and the diary reviewed. Skin imaging to analyse erythema will be performed on day 1, 15, 29 and 31. On the morning of day 29 the participants will wash their arms and not apply any product. They will visit the clinic and TEWL and skin capacitance will be measured. Small metal cups (Finn chambers) with SLS will be attached to the skin on the 4 treatment areas and the participants will be instructed to avoid showering or heavy exercise to avoid the patches getting wet. On day 30 the participants will remove the patch and leave the skin to recover for 24 hours. On day 31 the participants will visit the clinic and TEWL and skin capacitance will be measured again.

2. Study Methods

2.1 Study Design

It is planned to recruit 40 adults (male and female, aged 18 and above) 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.

Four treatment sites on the lower volar forearms (two on the right and two on the left) will be randomised for each participant with one treatment to be applied to each of three sites and the fourth remaining untreated as a control. The four treatment groups are;

- No treatment (control)
- Miniderm Novum (test cream)
- Miniderm 20% (reference cream)
- Diprobase (reference cream)

One Finger Tip Unit (FTU) of each cream will be applied twice daily on the designated study area. The participants will record details of cream application frequency, as well as participant tolerability in a diary.

Subjects will remain in the study for 4-5 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.

Participants will attend 5 clinic visits during the study:

- Visit 1 (screening) will be performed at baseline (Day 1)
- Visit 2 will be performed after 5 days of treatment
- Visit 3 will be performed after 15 days of treatment

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

Assessors will be blind to the treatment assigned to each site. Subjects will be blinded to the cream assigned to each study site – although the untreated site will remain unblinded.

2.2 Randomisation

Screening will take place either before or at the same visit as Day 1. Those eligible to take part will be randomised on Day 1 with each subject testing all four treatments – one on each site of the left or right lower volar forearm.

Miniderm Novum and Miniderm 20% will be packaged in identical 100mL plastic tubes by Pharmavize. Diprobase cream will be used in its original tube of 50 mg. The tube will be concealed with concealing tape and labelled as the other products.

The Investigator will keep the randomisation key and the numbering of the products in a secure, limited-access location to prevent inadvertent breaking of the blind. In case of emergency, the blind may be broken. All unblinding events will be recorded.

Full unblinding information will be provided to the statistician at the time of database lock.

2.3 Sample size:

The sample size for this study is based on the primary end point, change in Trans Epidermal Water Loss (TEWL) after induction of skin irritation (change from day 29 to day 31), with the primary comparison being between the test treatment and no treatment and the secondary comparison being between the test treatment and the two reference treatments.

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

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

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

If the assumptions of a paired t-test are not met (and a suitable transformation cannot be found) then a sign test (or a wilcoxon matched pairs test) may be used. A power calculation

can be carried out for the sign test[1] although this too makes some assumptions. This calculation shows that a sample size of N=40 would have >80% power to detect a difference such that the probability of X>0 (where X is the difference in change, as above) is approximately 0.7. This probability is not unrealistic given the data seen previously, and, if the clinically relevant difference detailed above is seen, the probability of X>0 may in fact be increased leading to higher power.

2.4 Timelines

Analysis will take place once all participants have had their final assessment at Day 31 or have discontinued.

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) 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 excel format on a secure drive available only to the necessary study staff. Data will be provided to the SSU as clean data files.

Adverse event data is being collected in a separate study database and will also be provided to SSU as an excel file. Coding of AEs will be carried out prior to delivery of the data to SSU.

4. Analysis Objectives:

4.1 Primary objective:

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

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

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

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

[1] Sample Size Determination for Some Common Nonparametric Tests Gottfried E. Noether Journal of the American Statistical Association, Vol. 82, No. 398. (Jun., 1987), pp. 645-647

4.2 Secondary objective:

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

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

4.3 Tertiary objective:

To investigate the number of participants with FLG loss-of-function mutations and to investigate any relationship with treatment effects.

5. Analysis Sets and Protocol Deviations

Full Analysis Set (FAS): All subjects who were diagnosed with a history of AD and were randomised into the study.
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.

The primary analysis outcome summaries will be performed on both the FAS and the PPS, however the FAS will be considered the primary analysis population . Secondary analyses will be performed on the FAS only.

Safety summaries will be performed on the safety set.

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

In addition the blind data review will consider if the location of observations and the adherence of SLS patches has any impact on the data and if so how to allow for this in the analysis.

6. Endpoints and Covariates

6.1 Primary endpoints

- Outcome relating to the strength of the skin barrier:
  - Change in TEWL post-induction of skin irritation after 4 weeks treatment (Day 31 – Day 29)
  - Change in objective skin redness (visual scores and mexameter) post-induction of skin irritation after 4 weeks treatment to (Day 31 – Day 29)

6.2 Secondary endpoints

- Outcome relating to the resting strength of the skin barrier:
  - TEWL after 4 weeks treatment (Day 29 – Day 1)
- Outcomes relating to the skin moisturisation:
  - Capacitance measurements (Day 29 – Day 1)
  - Skin surface dryness (3D skin images) (Day 29 – Day 1)
  - NMF levels (Day 29)
- Outcomes relating to tolerability:
  - Participant tolerability scores by VAS (Day1, Day 5, Day 29)
  - Investigator visual scores for redness (Day 15 and Day 29, adjusted for Day 1)
  - Objective erythema from 2D colour skin images (Day 15 and Day 29, adjusted for Day1)
- Outcome relating to cream consumption:
  - Cream consumption (g) (Day 1, Day 5, Day 15 & Day 29)
- Outcome relating to safety:
  - Number of adverse events (Screening, Day 1, Day 5, Day 15, Day 29 and Day 31)

6.3 Tertiary endpoints:

- Outcomes relating to FLG loss-of-function mutations: ▪
  - Number of FLG loss-of-function mutation carriers

6.4 Variables

TEWL

Triplicate measurements will be performed at each test site (upper part of the right and left lower volar forearm and lower part of the right and left lower volar forearm) at baseline (Day 1), after 4 weeks treatment (Day 29) and after skin irritation (Day 31) using an AquaFlux condensing chamber probe. 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.

Redness

Visual scoring of subject's skin by an expert will take place at 4 weeks (Day 29) and after skin irritation (Day 31). Scores will be on a scale from 0 to 3.

Objective scoring of the sites will also be measured using a mexameter. Triplicate recordings will be made; the average reading for each patient at each time point at each site will be used in the analysis.

Change will be calculated as post-irritation observation – 4 week observation (Day 31 – Day 29).

Secondary endpoint: Erythema index from 2d imaging will be measured at baseline and on days 15, 29 and 31.

Capacitance

Triplicate measurements will be performed at each test site at baseline (Day 1) and after 4 weeks of treatment (Day 29) using a corneometer. 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. Capacitance units are reported using an arbitrary scale from 0 to 130, where values below 30 indicate very dry skin and values above 45 indicate sufficiently moisturised skin.

Skin surface dryness (3D image)

A close-up image of each skin site will be captured using the c-cube (Pixience, France) at baseline (Day 1), after 2 weeks treatment (Day 15), after 4 weeks treatment (Day 29) and after induction of skin irritation (Day 31). Images can be analysed at the end of the study to obtain quantitative values for dryness (roughness).

2D Skin Imaging of Erythema

A single close-up image of each skin site will be captured using the c-cube camera with glass lens (Pixience, France). Captured 2D images are analysed to determine the skin erythema index (degree of redness, arbitrary numerical value) for the region of interest.

NMF levels

Samples for analysis of stratum corneum NMF levels will be collected by tape stripping after 4 weeks of treatment (single time point, Day 29). Two samples will be collected from each sampling site (tape-strips/discs 1-3 and 4-6), representing different depths through the stratum corneum. The samples will be analysed separately.

Participant tolerability VAS

Participants will use a diary to record their VAS score of the degree of smarting at the start of treatment (Day 1), after 5 days (Day 15) and again after 4 weeks (Day 29).

Cream Consumption

Cream consumption will be based on the weight of the reference and test creams before (Day 1), during (Days 5 and 15) and after use (Day 29).

Adverse Events

Participants will record AEs throughout the study in their diaries with the information transferred to the CRF at Day 29 and Day 31.

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 5 common European loss-of-function FLG mutations that have been reported to confer increased AD risk.

7. Statistical Analyses

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

As noted in section 5 the data will be explored during the blind data review to assess if the proposed analysis methods needs to be updated to allow for the location or adherence of the SLS patches.

7.1 Primary Analysis

The primary analyses will compare the test treatment to no treatment and to the two reference creams in terms of change in TEWL and redness (visual scoring and mexameter) between days 31 and 29. This will be done using Analysis of Covariance (ANCOVA) with change (Day 31 - Day 29) as the outcome, treatment as a factor, subject as a random effect and day 29 incuded as a covariate. Key group comparisons (Miniderm Novum Vs Control, Miniderm Novum Vs Miniderm 20% and Miniderm Novum Vs Diprobase) will be calculated directly from the ANCOVA model.

These analyses will be carried out on both the FAS and the PPS, the analysis on the PPS will be considered supportive of the primary analysis carried out on the FAS.

In both analyses, if the assumptions of the test are not met, transformations of the raw data or alternative tests will be considered, such as the Wilcoxon matched pairs or sign test, and the most appropriate solution applied.

7.2 Secondary Analyses

For each secondary analysis key group comparisons (Miniderm Novum Vs Control, Miniderm Novum Vs Miniderm 20% and Miniderm Novum Vs Diprobase) will be presented. These analyses will be carried out on the FAS only.

In all analyses, if the assumptions of the test are not met, transformations of the raw data or alternative tests will be considered and the most appropriate solution applied.

Skin Barrier:
The secondary analyses will compare the test treatment to no treatment and to the two reference treatments in terms of change in TEWL from baseline (Day 1) to Day 29. This will be done using ANCOVA with change (Day 29 – Day 1) as the outcome, Day 1 TEWL as a covariate, treatment as a factor and subject as a random effect.

Skin Moisturisation:
Capacitance and skin surface dryness (Day 29-Day 1) will be analysed using an ANCOVA as described for TEWL above.

The test treatment will be compared to no treatment and to the two reference treatments in terms of skin moisturisation (NMF) at Day 29. This will be done using ANOVA with Day 29 NMF as the outcome, treatment as a factor and subject as a random effect. The samples representing different depths will be analysed separately.

Tolerability:
Patient tolerability scores will not be recorded prior to treatment and so will be summarised by time point (Day 1, Day 5 and Day 29) and the treatments compared using repeated measures ANOVA with score as the outcome, visit (Day 1, Day 15 or Day 29) and treatment as factors and subject as a random effect.

Tolerability scores as measured by investigator visual scores for redness and objective erythema from 2D colour skin images will be analysed including the observations from day 15 and 29, adjusting for baseline as necessary. This analysis will use a repeated measures ANOVA with change from baseline as the outcome, visit (Day 15 or Day 29) and treatment as factors, day 1 as a covariate and subject as a random effect.

Consumption:
The total consumption of the creams (test or reference cream), based on cream weight will be calculated and tabulated descriptively. The planned dose is 0.5g per application (2 applications per day), if consumption is twice or half that expected over the study this will be flagged. If appropriate, consideration will be given to including the cream consumption as a covariate in the model or in an exploratory analysis of the primary endpoints.

7.3 Tertiary Analysis

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

7.4 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 with the worst severity and worst relationship to treatment.

7.5 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.

7.6 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.

7.7 Interim Analysis:

No interim analyses are planned for this trial.

7.8 Data Monitoring Committee (DMC):

No DMCs are planned for this trial.

7.9 Changes From Protocol

An additional exploratory analysis has been proposed for the blind data review to investigate any impact of the location or adherence of the SLS patches on the observed data at day 31. This is due to investigators noting that the patches may not adhere so well in different locations along the forearm and also that the skin may react differently depending on distance from wrist/elbow.

8. List of Tables, Figures and Listings

Tables

Listings

Figures: