Clinical Protocol
204516

An Exploratory Study to Assess Two Accelerated Models of Barrier Repair

GlaxoSmithKline Consumer Healthcare

Stockley Park West
1-3 Ironbridge Road
Uxbridge
UB11 1BT

Protocol approval date: 20-Feb-2015

Clinical Protocol
204516

Confidential

204516

An Exploratory Study to Assess Two Accelerated Models of Barrier Repair

GlaxoSmithKline Consumer Healthcare

Stockley Park West
1-3 Ironbridge Road
Uxbridge
UB11 1BT

Protocol Amendments **(Record details of amendment in table below, mark the sections/pages affected "See Amendment" and attach amendment to this protocol)**		
Number	**Date of Issue**	**Section(s) & Page(s) Amended**
1		
2		
3		
4		

Confidential

Summary Information

Title:	An Exploratory Study to Assess Two Accelerated Models of Barrier Repair
Protocol Number:	204516
Sponsor:	GlaxoSmithKline Consumer Healthcare (GSKCH), Stockley Park West, 1-3 Ironbridge Road, Uxbridge UB11 1BT, UK Telephone PPD
Protocol Author (Clinical):	PPD, BSc GSKCH, Weybridge
Protocol Author (Biostatistical):	PPD, BSc, MSc GSKCH, Weybridge
Protocol Author (Medical):	PPD, PhD GSKCH, Weybridge
PRIMARY CONTACT/ Study Manager:	PPD, BSc GSKCH St. Georges Avenue Weybridge Surrey KT13 0DE Telephone: PPD Facsimile: PPD
Director, Medical Affairs:	PPD, BSc, PhD GSKCH St. Georges Avenue Weybridge Surrey KT13 0DE Telephone: PPD

Expert medical advice outside normal office hours:	Weybridge Security desk: Telephone PPD
Study Site:	RCTS Incorporated 3207 Esters Road Irving TX 75062 United States
Principal Investigator	Barry Reece, MS, MBA.
Sub-Investigator	Gene Ream, MD (Board Certified Dermatologist)
Product Name:	Curel ® Ultra Healing Lotion for Extra Dry Skin
Phase of Study:	Not applicable

® Curel is a registered trademark of Kao Corporation

Protocol Agreement

An Exploratory Study to Assess Two Accelerated Models of Barrier Repair

204516

The signature of the investigator below constitutes his approval of this protocol and provides the necessary assurances that this study will be conducted according to Good Clinical Practice [ICH 1996] and to all stipulations, clinically and administratively, as stated in the protocol, including all statements as to confidentiality. It is agreed that the conduct and results of this study will be kept confidential and that the case report forms (CRFs) and his pertinent data will become the property of GlaxoSmithKline Consumer Healthcare (GSKCH).

It is agreed that the protocol contains all necessary information required to conduct the study and that the study will not be initiated without the approval of an appropriate Institutional Review Board (IRB). It is agreed that all participants in this study will provide written informed consent in accordance with the requirements specified in the Declaration of Helsinki [World Medical Association Declaration of Helsinki, 59th General Assembly, Seoul 2008]. All participants will also be informed that their medical records will be kept confidential except for review by representatives of GSKCH and/or appropriate IRB representatives and regulatory authorities.

In some instances, a summary of the protocol and study results, along with the names of the principal investigator from each study site, and details of the institutions with which the investigator is affiliated will be posted in one or more publicly accessible worldwide registers at any time after the commencement of the study. The signature of the investigator below constitutes his consent to have his name and institution disclosed should this study be made publicly available on a register. In addition, in order to avoid confusing or conflicting information, the signature of the investigator below constitutes his agreement not to post information about the study on any clinical trials registry without first obtaining the prior written consent of GSK.

	PPD	PPD
Barry Reece, MS, MBA.		
Principal Investigator	Signature	Date

Table of Contents

Study Synopsis

Title

An Exploratory Study to Assess Two Accelerated Models of Barrier Repair

Brief Summary

It is intended that this study will identify a method capable of measuring improvements in skin barrier function in no more than two weeks. This will be performed by exploring two potential clinical methods to assess barrier repair (1) product use before assessing barrier repair (following tape stripping) "pre-treatment" and 2) assessing product use after tape stripping "post-treatment", with observations occurring at various time points). In addition, a statistical method will be explored in the hope that results from it will be more sensitive to changes in barrier function.

Objective

Assess barrier repair in two exploratory clinical methods, using a marketed cosmetic moisturiser (Curel Ultra Healing Lotion for Extra Dry Skin).

Study Design and Methodology

This is a single-centre, split-body, single product study designed, to assess barrier repair in two different clinical methods in female subjects with dry skin.

Subjects will give their written consent prior to any study procedures taking place. Eligible subjects will undergo a 7-day washout period, during which only the washout soap provided will be used and also during the rest of the study to cleanse the lower legs.

Subjects must shave their legs 24 hrs before the Baseline visit. At the Baseline visit, only subjects with a trained examiner visual grading score of dryness ≥ 2 (moderate) on both lower legs will qualify for the study. In addition, there will be no greater than 0.5 point difference between the right and left leg, and the upper and lower areas on the lateral aspect of each leg.

Each subject's right lower leg and left lower leg's lateral (outer-side) aspect will be divided into two areas, an upper area (treated with moisturiser) and a lower area (untreated). The subject's right lower leg will adopt the "pre-treatment" method and the same subject's left lower leg will adopt the "post-treatment" method (methods are detailed later in this section). The test sites of both the right and left lower lateral leg's upper and lower area will be marked in a non permanent manner and defined as 10 centimetre (cm) by 12 cm in size (120 cm squared (cm^2) (as shown in Figure 1).

After the Baseline assessments (Day 0), subjects will be instructed to apply a 240 milligram (mg) sized amount (approximately 2 mg/ (cm^2)) of the moisturiser to each marked upper lower lateral area twice a day (morning (AM) and evening (PM)) approximately 8-12 hours (hr) apart for 14 days. There will be no moisturiser application prior to the morning visits, and will then be applied following Transepidermal Water Loss (TEWL) measurement. First on-site application of moisturiser will be supervised by the study site. Subjects must shave their legs 24 hrs before each subsequent site visit.

"Pre-treatment" method (Right lateral leg):

From the Baseline visit onwards, initial TEWL measurement at the designated skin sites (Figure 1) as specified in Table 1 will occur. Subsequently twenty tape strips will be taken following D-Squame disc challenge at these sites and time points as designated in Table 1. This will include TEWL measurement after every 5 tape strips (5, 10, 15 and 20 tape strips). Moisturiser application will occur 30 minutes (mins) following the last TEWL measurement on the upper area only. The last moisturiser application will be on the evening before the Day 14 visit.

Figure 1. Lateral aspect of right lower leg marked treatment areas and sites:

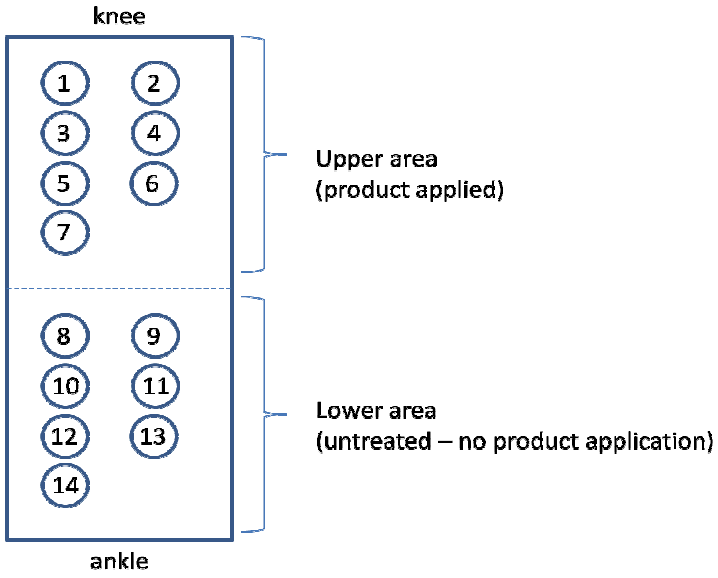


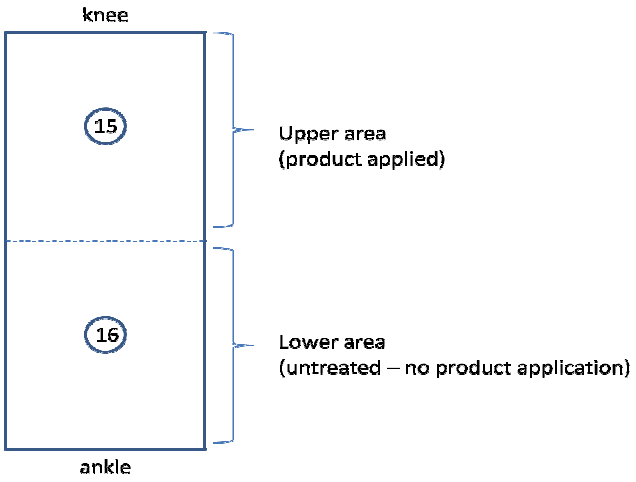
Table 1. Designated skin sites and time point for measurements

Study Day	Day 0	Day 3	Day 5	Day 7	Day 10	Day 12	Day 14
Designated skin sites	1, 8	2, 9	3, 10	4, 11	5, 12	6, 13	7, 14

"Post-treatment" method (Left lateral leg):

At the Baseline visit, initial TEWL measurement at the designated skin sites 15 and 16 (Figure 2) will occur. Subsequently twenty tape strips will then be taken following D-Squame disc challenge at these sites, followed by a second TEWL measurement. At all subsequent visits only TEWL will be measured at sites 15 and 16. Moisturiser application will occur 30 mins following the second (Baseline visit) or single (subsequent visits) TEWL measurement on the upper area only. The last moisturiser application will be on the evening before the Day 14 visit.

Figure 2. Lateral aspect of left lower leg marked treatment areas and sites:



Subjects will be acclimatised in a relatively stable environment (temperature 21 degrees centigrade (ºC) ± 2 ºC, room humidity 50 % ± 10 %) for a period of at least 30 mins before measurement of trained examiner visual grading of dryness (Baseline visit) and TEWL measurements.

For the D-Squame discs collected during the "pre-treatment" method, the amount of protein recovered will be measured (by Squamescan) [Lu *et al.*, 2014]. Further analyses of the "pre-treatment" method retained D-Squame discs may be performed within one year of the study report. If this analysis is not performed the tapes will be destroyed. Subjects will consent to this possible additional analysis at screening. Disc destruction will be authorised by GSKCH.

Planned Number of Subjects

Sufficient female volunteers will be screened in order that approximately 25 subjects will be treated to ensure that 20 female subjects complete the study.

Diagnosis and Main Criteria for Inclusion

Healthy female, Caucasian volunteers, aged ≥ 18 years, with a trained examiner visual grading score of dryness ≥ 2 on both lower legs will qualify for the study at the baseline visit. In addition, there will be no greater than 0.5 point difference between the right and left leg, and also between the upper and lower areas on each lateral leg.

Study Products, Dose and Mode of Administration

Study products will be supplied by the Clinical Trial Supplies Department at GSKCH:

- Curel Ultra Healing Lotion for Extra Dry Skin (moisturiser)

Subjects will be instructed to apply 240mg amount of the moisturiser to the marked upper area of the lower leg twice daily (in the morning and evening). First on-site application of moisturiser will be supervised by the study site.

Soap (Ivory® Original) will be provided for subject use from the screening visit until study completion.

Reference Product Dose and Mode of Administration

Not applicable

Duration of Product Use

Subjects will use one study product twice daily for 14 days on the marked lower lateral area for both legs.

Study Duration

The anticipated duration of participation for each subject in the study will be approximately 3 weeks including the washout period. To complete the study, each subject will be required to attend the study site on eight occasions. The total study duration at the site from the first subject's first visit through to the last subject's last visit will be approximately 5 weeks.

® Ivory Original soap is a registered trademark of Proctor & Gamble.

Criteria for Evaluation

For each method ("pre-treatment" and "post-treatment"), comparisons between the treated and untreated areas will be performed to characterise the speed and extent of barrier repair.

The results of this study will determine how barrier repair may be measured in future clinical studies.

Statistical Methods

Since this an exploratory method development study, a number of different parameters are being evaluated in various ways, in order to ascertain the most sensitive measure to use in future studies.

"Pre-Treat" Method:

Using the methodology described in Lu et al [Lu *et al.*, 2014], utilising Fick's first law of diffusion and the fact that the thickness of the stratum corneum (SC) is approximately proportional to the cumulative SC protein removed by tape stripping, a random coefficients model will be fitted with 1/TEWL as the response variable and fixed effect model terms for cumulative protein, treatment (treated or untreated) and the protein*treatment interaction, and random coefficients for subject*treatment and the subject*treatment*protein interaction. Cumulative protein (p) will be obtained at pre-stripping (where p will be set to 0), and after 5, 10, 15 and 20 strips have been removed. The relative barrier quality will be determined from the slopes of the 1/TEWL vs. p relationship. The relative SC thickness will be determined from extrapolation of the linear regression lines for each treatment group to where each intercepts the x-axis (cumulative protein axis). This analysis will be performed for each visit day (day 0, 3, 5, 7, 10, 12 and 14)**.** The day 0 analysis will give a measure of the baseline values of slope and x-axis intercept in each of the treated and untreated areas. The use of a simple linear regression model does have some drawbacks, including the overestimate of the SC thickness (or total SC protein) as 1/TEWL does not reach 0 when all the SC is removed from the skin. However, since the aim of the study is to determine the relative difference in SC thickness and barrier quality between treated and untreated skin, the simple linear model approach is sufficient (Lu et *al.,* 2014).

From each of these random coefficients models, individual subject estimates of slope and x-intercept will be obtained. The individual subject by treatment estimates for slope and x-intercept on each post-baseline visit day (day 3, 5, 7, 10, 12 and 14) will subsequently be analysed using a repeated measures model with factors for treatment, day and treatment*day (fixed effects) and subject (random effect), and a covariate for the corresponding baseline estimate of slope or x-intercept (obtained from the estimates at Day 0). Treatment differences will be

presented at each treatment day with 95% confidence intervals, together with p-values.

On each post baseline visit day (day 3, 5, 7, 10, 12 and 14), the change in TEWL value from pre-stripping to post-stripping (after 20 strips have been removed) will be calculated and compared between treated and untreated groups. A repeated measures model will be fitted with factors for treatment, day and treatment*day (fixed effects) and subject (random effect), and a covariate for the change from pre-stripped TEWL to post-stripped TEWL assessed at the baseline visit (Day 0). The change from pre-stripped TEWL to post-stripped TEWL at Day 0 will provide a baseline measure of barrier strength of the skin when it is challenged. Treatment differences will be presented at each day with 95% confidence intervals, together with p-values. A stronger barrier will result in a smaller increase in TEWL.

"Post-Treat" Method:

The change in TEWL value from post-stripping at baseline (pre-treatment) to day 3, 5, 7, 10, 12 and 14 will be calculated and compared between treated and untreated groups. A repeated measures model will be fitted with factors for treatment, day and treatment*day (fixed effects) and subject (random effect), and covariates for pre-stripped TEWL and post-stripped TEWL (both measured pre-treatment at the baseline visit). The pre-stripped value will be included as a covariate as this is a baseline measure of the unchallenged skin. Treatment differences will be presented at each day with 95% confidence intervals, together with p-values. A reduction in TEWL over time from the post-stripped value will indicate the barrier is repairing itself.

To assess the importance of different covariates, the analysis described above will be repeated, but with a single covariate for the change from pre-stripped TEWL to post-stripped TEWL (assessed at the baseline visit).

In addition to the above, the change in TEWL from pre-stripping at baseline (pre-treatment) to day 3, 5, 7, 10, 12 and 14 will be calculated and summarised for the treated and untreated groups. A return of TEWL towards the pre-stripped value will indicate a recovery of the barrier function has returned to near pre-stripped levels.

Assumptions underlying all analyses will be checked, and if necessary, alternative non-parametric methods will be used.

List of Abbreviations

AE	Adverse Event
AM	Ante Meridiem
ºC	Degree Centigrade
cm	Centimetres
CRO	Contract Research Organisation
CRF	Case Report Form
fl	Fluid
g	Gram
GCP	Good Clinical Practice
GSKCH	GlaxoSmithKline Consumer Healthcare
hrs	Hours
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPL	Intense Pulsed Light
IRB	Institutional Review Board
ITT	Intention to Treat
MedDRA	Medical Dictionary for Regulatory Activities
m^2	Metre squared
min	Minutes
ml	Millilitres
mg	Milligrams
µg	microgram,
N	Number
nm	Nanometres
oz	Ounce
p	Cumulative protein
PM	Post Meridiem
PII	Personally Identifiable Information
PP	Per Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Stratum Corneum
SD	Standard Deviation
TEWL	Transepidermal Water Loss
UV	Ultraviolet

Study Schedule:

Event / Procedure				Visit 1 Day -7 (Screening)		Visit 2 Day 0 (Baseline)	Visit 3 Day 3	Visit 4 Day 5	Visit 5 Day 7	Visit 6 Day 10	Visit 7 Day 12	Visit 8 Day 14
Informed consent				X								
Demographics, medical history				X								
Inclusion/exclusion				X		X						
Current/concomitant medication				X		X	X	X	X	X	X	X
Study restrictions/compliance				X		X	X	X	X	X	X	X
Subject eligibility				X		X						
Dispense soap bar (to use during washout and treatment phase)				X								
Acclimatisation						X	X	X	X	X	X	X
Trained examiner visual grading of dryness of each leg[1]						X						
Dispense moisturiser and instructions						X						
Weigh moisturiser at start of study visit						X	X	X	X	X	X	X
On-site supervised moisturiser application [2]						X						
Leg Assessments						**Leg Assessments**						
Leg	Location	Designated Skin Site(s)	Measurements									
Both	Lower Lateral - Upper and Lower	Tape stripped site at specific visit	TEWL			X	X	X	X	X	X	X
Right ("Pre-Treatment" Method)	Upper Lateral (Treated)	1	Tape stripping site and site visit [3]			X						
		2					X					
		3						X				
		4							X			
		5								X		
		6									X	
		7										X
	Lower Lateral (Untreated)	8				X						
		9					X					
		10						X				
		11							X			
		12								X		
		13									X	
		14										X
Left ("Post Treatment" Method)	Upper Lateral (Treated)	15	Tape stripping site at Baseline visit [4]			X						
	Lower Lateral (Untreated)	16										
Adverse Events				X		X	X	X	X	X	X	X
Return soap bar and moisturiser												X
Study Conclusion												X

Wash out period 7 Days

[1] A dryness score of ≥ 2 is required at the Baseline visit to be eligible for each leg with no difference of greater than 0.5 between each leg and also between the upper and lower areas on each lateral leg.
[2] Product will be applied twice daily (morning and evening) over a 14 day period. At study visits, product application will occur 30 minutes after the final instrumental measurements.
[3] Tape stripping of designated site / time points will occur twenty times with TEWL measurement performed post each 5 tape strips (5, 10, 15 and 20 tape strips). Protein analysis of tape strips will also be performed for the "pre-treatment method).
[4] At the Baseline visit TEWL will be performed prior and post 20 tape stripping only.

1 Introduction

The primary focus of this work is to explore two potential methods to assess barrier repair (1) prior product use before assessing barrier repair or "pre-treatment" and 2) assessing barrier function after product use or "post-treatment"), with observations occurring at various time points over a 14 day period. Previously conducted barrier repair studies have been four weeks in duration. Barrier repair is a process that occurs over several weeks. It is intended that this work will identify a method capable of measuring improvements in barrier function in no more than two weeks. These methods are unlikely to assess full barrier repair, as that process takes longer than two weeks to complete; however, it is not necessary to observe complete repair, only the beginning of the repair. Since this is an exploratory method development study not intended to assess product performance, a marketed competitor product will be used.

2 Objective

Assess barrier repair in two exploratory methods, using a marketed cosmetic moisturiser (Curel Ultra Healing Lotion for Extra Dry Skin).

3 Investigational Plan

3.1 Study Design

This is a single centre, split-body, single product study designed to assess barrier in two different methods in female subjects with dry skin. Instrumental measurements of barrier function will be performed to assess impact of D-squame tape stripping on designated leg sites in pre-treatment and post-treatment methods to assess barrier repair. Protein analysis of D-squame discs will also be performed from the pre-treatment method.

3.2 Rationale for Study Design

The "pre-treatment" method will involve tape stripping at every visit with product application following the first set of stripping at the baseline visit and thereafter. This will provide understanding of the impact of treatment with a cosmetic moisturiser on the quality of the barrier.

The "post-treat" method will involve perturbation of the skin barrier by tape stripping prior to the first product application at baseline only. Barrier function (as assessed by TEWL) will be assessed on the perturbed site at each post baseline visit.

The primary focus of this work is to explore two potential methods to assess faster barrier repair (prior product use before assessing barrier repair "pre-treatment" and assessing barrier function after product use "post-treatment"), with observations occurring at various time points over a 14 day period. Previously conducted barrier repair studies have been four weeks in duration. Barrier repair is a process that occurs over several weeks. It is intended that this work will identify a method capable of measuring improvements in barrier function in no more than two weeks.

These methods are unlikely to assess full barrier repair, as that process takes longer than two weeks to complete; however, it is not necessary to observe complete repair, only the beginning of the repair. Since this is an exploratory method development study not intended to assess product performance, a marketed competitor product will be used.

4 Study Population

4.1 Source and Number of Subjects

Sufficient number of female, Caucasian volunteers will be screened in order that approximately 25 subjects will be treated to ensure that 20 female subjects complete the study.

The subjects will be recruited by RCTS Incorporated through advertising and from RCTS Incorporated database.

4.2 Inclusion Criteria

1. Consent
   Demonstrates understanding of the study and willingness to participate as evidenced by voluntary written informed consent and has received a signed and dated copy of the informed consent form.

2. Age
   $\geq$ 18years of age.

3. Skin Type
   Caucasian

4. Population
   Pre-menopausal female.

5. Compliance
   Understands and is willing, able and likely to comply with all study procedures and restrictions, including lifestyle restrictions.

6. General Health

   Good general and mental health with, in the opinion of the investigator or medically qualified designee:

   a) No clinically significant and relevant abnormalities in medical history (e.g. severe psoriasis, severe eczema) that may interfere with the study.

   b) Absence of any condition that could affect the subject's safety or wellbeing or their ability to understand and follow study procedures and requirements.

7. Diagnosis

    a) Subject has a minimum dryness grading of 2 (moderate) on both lower legs at the Baseline visit as assessed by a Trained Examiner (Section 8.1 Table I). No more than 0.5 point difference in dryness between the right and left leg corresponding quadrant (upper or lower) and between the upper and lower part of either leg.

4.3 Exclusion Criteria

1. Pregnancy
   Women who are known to be pregnant or who are intending to become pregnant over the duration of the study.

2. Breast-feeding
   Women who are breast–feeding.

3. Menopausal and perimenopausal women or women experiencing signs and effects of the menopause (e.g. facial flushing).

4. Allergy/Intolerance
   Known or suspected intolerance or hypersensitivity to the study materials (or closely related compounds) or any of their stated ingredients.

5. Clinical Study/Experimental Medication

    a) Participation in another clinical study or receipt of an investigational drug within 30 days of the screening visit or who are scheduled to receive an investigational drug other than the study products during the study.

    b) Previous participation in this study.

6. Medication

    a) Used contraindicated prescription or non-prescription drugs or medicines within 30 days of first using study product. Currently using any medication which, in the opinion of the Investigator, may affect the evaluation of the study products or place the subject at undue risk.

    b) Subjects taking medication for any skin condition, specifically rosacea, acne or atopic dermatitis/eczema, in the test area.

    c) Subjects currently using:-

        i. Topical or systemic steroids; occasional use of inhaled steroids is permitted.

ii. Topical or systemic antihistamines (occasional use of topical antihistamines permitted, except to area of skin to be studied).

iii. Retinoids (e.g. retinoic acid or retinol).

iv. Immunosuppressive drugs

v. Anti-inflammatory drugs

vi. Aesthetic or dermatological treatment involving the area of skin to be studied.

7. Substance abuse
   Recent history (within the last 1 year) of alcohol or other substance abuse.

8. Medical Conditions
   Subject has a skin condition in the test area e.g. rosacea, atopic dermatitis/eczema or acne.

9. Test area

   a) Moles, tattoos, scars, hairs, etc at the test areas if it is likely that they could affect the assessments.

   b) Use of self-tanning products on the test areas within 2 weeks prior to the screening visit.

   c) Subject has had prolonged, unprotected sun exposure on the legs within the last 1 week.

   d) Subject has visible sunburn on the lower legs at the baseline visit.

10. Personnel
    An employee of the sponsor or the study site or members of their immediate family.

4.4 Subject Withdrawal Criteria

Subjects have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw subjects from the study in the event of intercurrent illness, AEs or product failure after a prescribed procedure, protocol deviations, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. Whatever the reason for withdrawal, a complete final evaluation at the time of the subject's withdrawal should be made with an explanation of why the subject is withdrawing from the study.

If the reason for removal of a subject from the study is an AE or an abnormal laboratory test result, the principal specific event or test will also be recorded on the case report form (CRF). If a subject is withdrawn from the study because of a product-limiting AE, thorough efforts should be clearly made to document the outcome. Any AEs ongoing at the final visit will be followed up until resolved, the condition stabilises, is otherwise explained, or the subject is lost to follow-up.

4.5 Subject Replacement

Subjects who withdraw from the study post-randomisation will not be replaced.

4.6 Subject Restrictions

4.6.1 Lifestyle

- Subjects will not be permitted to use any of the following skin care products;-moisturisers, lotions, creams, sunscreen, soaps, cleansing, exfoliation products, moisturising products etc on their legs from the screening visit and for the duration of the study, other than the washout soap bar and moisturising product provided.

- No application of cleansers or water (including no showering/bathing permitted with soaps/shampoo) on the leg sites, within 2 hrs of all instrumental measurements on visit days within this period).

- Subjects must shave their legs 24 hrs before each visit. If desired, the provided cleansing bar can be used as a shaving aid. No other chemical or physical hair removal methods are permitted during the course of the study.

- Subjects may not take any of the medications prohibited in the exclusion criteria throughout the course of the study.

- At all subsequent study visits, subjects must apply the moisturiser provided approximately 12 hrs before the study appointment and attend the study site with cleansed legs by use of soap provided at least 2 hours before instrumental measurements. On study days the AM product application will be performed at the site after all assessments have been completed.

- Subjects must not smoke or have coffee or any product containing caffeine or alcohol within 4 hrs before the clinic visits and until after the instrumental measurements are completed.

- No hard physical exercises (with heavy sweating), sauna or swimming, 24 hrs before the clinic visit.

- No exposure to artificial ultraviolet (UV) light or cosmetic procedures (includes tanning beds, Intense Pulsed Light (IPL), etc) on the test area for the duration of the study.

5 Study Products, Assignment, and Supply Management

5.1 Study Products

5.1.1 Identity of study products

The following study test preparations will be supplied by the Skin Health Clinical Supplies Department, GSKCH:

- Curel Ultra Healing Lotion for Extra Dry Skin

Other items to be supplied by the Clinical Supplies Department, GSKCH:

- Washout soap bar: Ivory Original soap will be provided to all subjects for use during the entire study duration.

5.1.2 Selection of doses

The dose of the moisturiser has been selected to reflect typical consumer usage of this product and also reflects the standard application rate used in clinical studies.

5.1.3 Administration

The test sites of both the right and left lower leg's upper and lower area will be marked with a non permanent pen and defined as 10 cm by 12 cm in size (120 cm^2)(as shown in Figure 1). Subjects will be instructed to apply 240 mg of the moisturiser to the lower upper area only (approximately 2 mg cm^{-2}) on both legs twice daily (in the morning and evening).

The first use of the moisturiser will be under supervision at the study site at Baseline (Day 0). Subsequent use of study products will be applied by the subject at home, as instructed by site staff, or if at a site visit, 30 mins after TEWL measurement. The last moisturiser application will be on the evening before the Day 14 visit.

Subjects will bring their allocated product with them to each post-baseline visit, having applied product 12-16 hrs prior to their appointment time. Subjects will arrive at the study site with cleansed legs 2 hrs before the visit.

5.1.4 Dose schedule

Subjects will be required to apply their allocated test product twice a day (morning and evening).

Subjects will need to apply the study product on Day 0, 3, 5, 7, 10 and 12, 30 minutes post TEWL measurement. The first application on Day 0 following all instrumental assessments will be supervised.

Subjects will need to be reminded to bring their study products with them to the study site for the Day 14 visit.

5.1.5 Dose modification

No dose modifications are permitted during the study.

5.1.6 Product compliance

Subjects will be supervised when using the study product at the Baseline (Day 0) visit, a record of the dispensing and administration of the study products will be kept using the dispensing and administration log and the CRF.

5.1.7 Precautions

No special precautions, are necessary provided the study is carried out in accordance with this protocol.

5.1.8 Overdosage

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than specified in the protocol.

Overdose is not likely to occur in this study. Limited quantities of the product will be supplied, and closely monitored by the site for each subject.

5.2 Study Product Assignment

5.2.1 Randomisation procedure

Not applicable. All subjects will apply the moisturiser provided to each lateral upper leg.

5.2.2 Blinding procedure and code breaks

Not applicable. All subjects will apply the moisturiser provided to each lateral upper leg.

5.3 Study Product Supplies Management

5.3.1 Packaging and labelling

Packaging and labelling of all test products will be carried out according to ICH GCP guidelines and will be the responsibility of the Clinical Supplies Department GSKCH.

- Curel Ultra Healing Lotion for Extra Dry Skin in 6 fluid (fl) ounce (oz) (177 ml) containers.

Each study label will contain, but not be limited to, protocol number, directions for storage, emergency contact number. Ivory Original soap bar will be supplied in commercial containers, with labels attached.

All products will be supplied to site and details will be outlined in the shipping manifest. Care should be taken with the supplied study products and their labels so that they are maintained in good condition. It is important that all labels remain intact and legible for the duration of the study. Subjects should be instructed to not remove or deface any part of the study label.

5.3.2 Accountability of study supplies

All material supplied is for use only in this clinical study and should not be used for any other purpose.

The investigator or designee will maintain a full record of study product accountability. An Investigational Product Dispensing Log must be kept current and will contain the following information:

- The identification of the subject to whom the study product was dispensed.
- The date(s) and quantity of the study product dispensed to the subject.
- The date(s) and quantity of the study product returned by the subject (if applicable).

The inventory must be available for inspection by the study monitor during the study. At the end of the study, study product supplies will be verified by the monitor. Study product supplies will then be either collected by the study monitor or returned by the investigator or designee after study conduct to the GSK Clinical Supplies Department or designated vendor.

5.3.3 Storage of study product supplies

In the study centre, the study product supplies must be stored in compliance with the label requirements in a secure place with limited or controlled access. Study product should be stored at room temperature (15°C to 25°C) away from direct light. A 24 hr/ 7 day temperature monitoring device will be used to monitor the temperature of the study products. Only the Investigator, designated study nurse/ coordinator or product manager are to have access to the study products.

The subjects will be informed about the storage conditions when the product is dispensed on Day 0.

6 Study Schedule

Please refer to the study schedule for a complete listing of the assessments to be performed.

7 Screening and Baseline Methods, Measurements and Evaluations

7.1 Screening

7.1.1 Informed Consent

The investigator, or designee, must obtain written (signed and dated by the subject) informed consent from each subject participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study. The investigator, or designee, must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for documenting a written consent will be provided by the investigator or by GSK. The investigator, or designee, should sign and date the consent form to confirm that the consent process was completed correctly. The subject will be provided with a copy of their signed and dated consent form and any other written information which they should be instructed to retain. Subjects will give consent to the collection of the D-squame discs and the potential further analyses of the "pre-treatment" D-Squame discs within one year of the study report. If this analysis is not performed the tapes will be destroyed. Disc destruction will be authorised by GSKCH.

If, during a subject's participation in the study, any new information becomes available that may affect the subject's willingness to participate in the study, each ongoing subject should receive a copy of this new information and be re-consented into the study. Subjects should be provided with a copy of the signed and dated amended consent form.

7.1.2 Demographics

The Investigator, or designee, will record each subject's year of birth, age, gender and race.

7.1.3 Medical History

For each subject, the medical history will be taken and reviewed by the Investigator or medically qualified designee. Details of any relevant medical or surgical history, including allergies or drug sensitivity will be recorded. Any concomitant therapy taken in the 30 days prior to the Screening Visit and throughout the study will also be recorded.

7.1.4 Study Conclusion

Subjects will be evaluated to determine if they completed all study procedures or if they were discontinued from the study early. If the subject discontinued at any point during the study, the primary reason for withdrawal should be recorded on the Study Conclusion CRF by selecting one of the options below.

- Subject did not meet study criteria
- Adverse Event
- Lost to Follow Up
- Protocol Violation
- Withdrawal of Consent
- Other

8 Efficacy Measurements and Evaluations

Subject will be acclimatised in a controlled environment (temperature 20ºC ± 2ºC, room humidity 50% ± 10%) for a period of at least 30 min before visual assessments and TEWL are taken (at each time point).

8.1 Assessment of Leg Dryness

At the Baseline visit only subjects with a trained examiner visual grading score of dryness ≥ 2 on both lower legs will qualify for the study. In addition, there will be no greater than 0.5 point difference between the right and left leg, and also between the upper and lower areas on each lateral leg. The following static (without reference to any prior visits) scale and definitions will be used for examiner visual grading of dryness of each leg area (with half-point scores used as necessary to better describe the clinical condition):

Table I: Grading Scale for Assessment of Dryness*

Grade	Dryness Descriptor
0	No dryness
1	Slight flaking
2	Moderate flaking/scaling
3	Marked scaling/slight fissuring
4	Severe scaling/fissuring

*Half points may be used. Nine point scale in total.

8.2 Transepidermal Water Loss (TEWL)

TEWL assessments for barrier function will be performed by a trained technician, using the Tewameter® TM 300 (Courage+Khazaka electronic GmbH) device.

TEWL will be initially measured for each visit, prior to tape stripping (if applicable) for the designated skin site. TEWL will also be performed if tape stripping occurs for the designated skin site / time point after every five tape strips (5, 10, 15 and 20 tape strips) for the pre-treated sites. For the post treatment sites at baseline, TEWL will be repeated post all 20 tape strips.

TEWL is a non-invasive method to measure the integrity of stratum corneum barrier function. Briefly, the measuring principle is based on water vapour gradient determination between two pairs of sensors (temperature and relative humidity) placed at different distances perpendicularly to the skin. The probe will be held in place on the skin for one measurement, for approximately 40 seconds, to ensure that a stable value has been established. The probe should be placed keeping the same orientation of the probe on the subject's skin (relative to horizontal/vertical planes) for all measurements. The first part of the measurement belongs to the equilibration phase. The values of the last 20 seconds are averaged as the actual measurement values. TEWL values will be expressed in gram/square metre/hour ($g/m^2/hr$). An increase in TEWL values shows damage to the skin barrier function.

Subjects will be acclimatised in a relatively stable environment (temperature 21 ºC ± 2 ºC, room humidity 50 % ± 10 %) for a period of at least 30 minutes before each measurement session.

The measurements will be performed on the designated leg skin site for each specific time point. The measurements will be taken in duplicate, on different parts of the test site so the measured parts do not overlap, and then an average reading will be taken for each test site and time point. The same operator should be used throughout the study for any given measurement, in order to reduce the variability. If not possible, then it should be recorded which operator did which measurements.

8.3 D-Squame Tape Stripping

Tape stripping will be performed at the designated leg skin site for each specific time point by a trained technician using the D-Squame® discs (CuDerm corporation).

A series of D-Squame discs will be gently smoothed sequentially over the test sites applying uniform pressure for 5 seconds with the D500 D-Squame Pressure Instrument® (CuDerm corporation) supplied stamp, using constant pressure to ensure consistent adhesion to the skin. Gripping the white portion of the disc, each disc is then pulled off the skin with one fluent and

---

® Tewameter is a registered trademark of Courage+Khazaka electronic GmbH
® D-Squame disc is a registered trademark of CuDerm corporation
® D-Squame Pressure Instrument is a registered trademark of CuDerm corporation

decisive movement. Multiple strips can be done on the same site to move progressively deeper into the stratum corneum. The site being stripped should be accurately marked at the beginning of the process to ensure that subsequent strips are aligned with the first one.

The tape stripping will be performed on the designated leg skin site for each specifictime point. The same specific area will not be restripped within the study. A total of 20 D-squame discs will be taken from the designated leg skin site / time point.

8.4 Measurement of Protein from D-Squame Discs

For the pre-treatment method, D-Squame discs will be analysed at the study site on the same day as the stripping samples are taken, using the SquameScan® 850 (Heiland electronic GmbH). The strips will be stored in a fridge at 4°C between the samples being taken and analysed. Following the protein analysis, the D-squame discs will be stored in a freezer at -20°C.

SquameScan 850 is the instrument used to indirectly measure the stratum corneum protein content on D-Squame tape strips. Determination is performed by measuring the optical absorption of the strip at about 850 nanometres (nm) (infrared light). The value displayed in % is proportionally related to the protein content. The protein will be analysed for each of the 20 discs obtained from the designated leg skin site / time point.

Further analyses of the retained pre-treatment method, D-Squame discs may be performed within one year of the study report. If this analysis is not performed the tapes will be destroyed. Subjects will consent to this possible additional analysis at screening. The D-Squame discs will be classified as human biological. Disc destruction will be authorised by GSKCH.

9 Safety Measurements and Evaluations

9.1 Adverse Events and Serious Adverse Events

The investigator or site staff will be responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE.

9.1.1 Definitions

9.1.1.1 Adverse event

Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of an investigational product (or washout product), whether or not considered related to the investigational product (or washout product).

® SquameScan is a registered trademark of Heiland electronic GmbH

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of an investigational product (or washout product).

Events meeting the definition of an AE **include:**

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New condition(s) detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concomitant medication (overdose *per se* should not be reported as an AE/SAE).

Events that do not meet the definition of an AE **include**:

- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.

Clinical AEs will be described by diagnosis and not by symptoms when possible (e.g., upper respiratory tract infection, seasonal allergy, etc. instead of runny nose).

Reporting instructions are provided in Section 9.1.2.

9.1.1.2 Serious adverse event

An SAE is any untoward medical occurrence that, at any dose:

a) Results in death.

b) Is life-threatening.

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject 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.

c) Requires hospitalisation or prolongation of existing hospitalisation.

NOTE: In general, hospitalisation signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or product that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalisation" occurred or was necessary, the AE should be considered serious.

Hospitalisation for elective product of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in disability/incapacity, or

NOTE: 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, diarrhoea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly/birth defect.

f) Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive product in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse or reports of spontaneous abortion.

9.1.2 Reporting adverse events and serious adverse events

9.1.2.1 Time period for reporting adverse events and serious adverse events

The investigator or site staff will be responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

AEs will be collected from the start of the investigational product (or washout product) until 5 days following last administration of the investigational product.

SAEs will be collected over the same time period as stated above for AEs. However, any SAEs assessed as **related** to study participation (e.g., investigational product, protocol mandated procedures, invasive tests, or change in existing therapy) or related to a GSK concomitant medication will be recorded from the time a subject consents to participate in the study up to and including any follow-up contact. All SAEs will be reported to GSK within 24 hrs of the investigator or designee becoming aware of the situation.

Medical conditions reported prior to the time period for reporting AEs/SAEs should be recorded as part of the subject's medical history.

9.1.2.2 Reporting and reviewing adverse events

AEs will be recorded in the AE section of the CRF.

Medical conditions recorded by the subject on a diary card or similar document that meet the definition of an AE must also be recorded in the AE section of the CRF, if not previously well-characterised by the investigator in the subject's medical history.

AEs elicited by the investigator in a standard manner at the study visits should also be recorded in the AE section of the CRF. The investigator or designee must ask the subject the following question during each visit including any follow-up visits: **"Have you felt unwell, experienced any symptoms or taken any medication *(since your last visit) (today) (since your last dose) (since the last session)?"***

The medically qualified investigator should review AEs in a timely manner; this review should be documented in writing in the source document or in the case report form. Photographs of skin irritation may be taken to document an AE.

9.1.2.3 Reporting serious adverse events

A copy of the SAE form provided in the investigator study master file should be completed as fully as possible.

It is essential to enter the following information:

- Protocol and subject identifiers
- Subject's demography
- Description of events, with diagnosis if available
- Investigator opinion of relationship to study product (see Section 9.1.3.2 below)
- Criterion for seriousness.

The following are desirable and are of particular relevance for investigator and GSK assessment of the SAE report:

- Date of onset of AE
- Date AE stopped, if relevant
- Study product start date
- Study product end date if relevant
- Action taken on study product
- Outcome if known

The SAE form, completed as fully as possible, and SAE fax cover sheet must be faxed or e-mailed to the Case Management Group Global Clinical Safety and Pharmacovigilance, as soon as possible, **but not later than 24 hrs** after study site personnel learn of the event. The GSK Study Manager should also be notified of the situation by telephone or email.

Fax Serious Adverse Events to PPD **(UK),**
E-mail: PPD

The GSK Study Manager will forward the SAE form to the Medical Director responsible for the study and other GSK personnel as directed/appropriate.

The initial report will be followed up with more information as relevant, or as requested by the GSK Study Manager. This may require the investigator to obtain copies of hospital case reports, autopsy reports and other documents as applicable.

9.1.2.4 Follow-up of adverse events and serious adverse events

After the initial report, the investigator is required to proactively follow up with each subject and provide further information on the subject's condition. All AEs/SAEs will be followed until resolution, until the condition stabilises, until the event is otherwise explained, or until the subject is lost to follow-up. The investigator may be required to obtain additional laboratory tests or investigations, and/or provide GSK with additional documentation, including autopsy reports.

Investigators are not obliged to actively seek AEs or SAEs in former subjects. However, if the investigator learns of any SAE, including the death, at any time after a subject has been discharged from the study, and considers the event reasonably related to the investigational product or study participation, the investigator will promptly notify GSKCH.

9.1.2.5 Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to the Case Management Group Global Clinical Safety and Pharmacovigilance, and the GSK study Manager within the designated reporting timeframes (within 24 hrs of learning of the event). GSK has a legal responsibility to notify, as appropriate, the local regulatory authority and other regulatory authorities about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to GSK is essential so that legal obligations and ethical responsibilities towards the safety of subjects are met.

GSK will comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, IRB and investigators.

Investigator safety reports are prepared according to GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to investigational product and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary of listing of SAEs) from GSK will file it with the Safety Statement and will notify the IRB, if appropriate according to local requirements.

9.1.3 Adverse event grading and assessments

9.1.3.1 Intensity grading

All AEs will be graded on a three-point scale and reported in detail as indicated on the CRF:

- Mild – easily tolerated, causing minimal discomfort and not interfering with normal everyday activities.
- Moderate – sufficiently discomforting to interfere with normal everyday activities.
- Severe – any event that prevents normal everyday activities.

9.1.3.2 Relationship assessment

The investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the Investigator Brochure and/or Product Information, for marketed products, in the determination of his/her assessment.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event prior to the initial transmission of the SAE data to GSK.** The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE data collection tool accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

The investigator will provide the assessment of causality as per the instructions for completion of the AE/SAE form.

9.2 Pregnancy

9.2.1 Time period for collecting pregnancy information

Pregnancy information will be collected on all pregnancies reported following the signature of informed consent. Information on pregnancy identified during the screening phase and prior to investigational product (or washout product) administration does not need to be collected.

9.2.2 Action to be taken if pregnancy occurs

The investigator will collect pregnancy information on any subject who becomes pregnant while participating in the study after administration of the investigational product (or washout product). The investigator will record pregnancy information on the appropriate form and submit it to GSK within 2 weeks of learning of the subject becoming pregnant. The subject will be followed to determine the outcome of the pregnancy. Information on the status of the mother and infant / neonate (including concomitant medications taken by the mother during the pregnancy) will be forwarded to GSKCH. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE, any pregnancy complication or elective termination for medical reasons will be recorded as an AE or SAE as defined in Section 9.1.1.

A spontaneous abortion is always considered to be an SAE and will be reported as such. An SAE occurring in association with a pregnancy, brought to the investigator's attention after the subject completed the study and considered by the investigator as possibly related to the investigational product, must be promptly forwarded to GSKCH. While the investigator is not obliged to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

There is no requirement for the subject to be withdrawn from the study as a result of the pregnancy. However if they are withdrawn, this should be recorded in the appropriate section of the CRF.

10 Data Analysis Methods

All statistical tests will be two-sided with a significance level of 0.05 unless stated otherwise.

10.1 Sample Size Determination

This is an exploratory method development study to determine the best approach to assess barrier repair. Based on Lu et al [Lu *et al*., 2014], in subjects with dry skin, the mean slope of 1/TEWL versus cumulative protein removed was -3.4×10^{-5} (standard deviation (SD) approx. 1.4×10^{-5}). The mean total protein removed from SC was 3985µg (SD approx. 1150). Since the proposed study is a split-body design, the variability of the paired differences is required. It will be assumed that the correlation between the treated and untreated responses within a subject is 0.5, so that the SD of the paired differences will be the same as the SD between subjects.

Assuming that the untreated sites in the proposed study behave the same as the dry skin cohort in Lu et al [Lu *et al*., 2014] with regards to response and variability of response, then a study with N=20 subjects will have 90% power to show a difference in slopes of 1/TEWL vs. Cumulative protein removal (treated versus untreated) of 1.1×10^{-5}, or approximately 32% of an untreated dry skin 1/TEWL vs. Cumulative protein removal slope, at the 2-sided 5% significance level.

Similarly, for the cumulative protein removed endpoint, N=20 subjects will have 90% power to show a difference (treated versus untreated) of 880 microgram (µg), or approximately 22%, at the 2-sided 5% significance level.

In summary, N=20 subjects will have 90% power to show a difference between treated and untreated groups of 32% in barrier quality (as defined by the slope) and 22% in SC thickness (as defined by the total protein intercept of the regression vs. 1/TEWL).

10.2 General Considerations

10.2.1 Analysis populations

10.2.1.1 Definition of Safety Population

The Safety population will include all subjects who receive at least one application of study product. The Safety Population will be used for the reporting of treatment emergent AEs.

10.2.1.2 Definition of Intent-to-treat Population

The 'Intent-to-treat' (ITT) population will include all subjects who receive at least one application of study product and have at least one post-baseline evaluation. The ITT population will be the primary population for all efficacy analyses.

10.2.1.3 Definition of Per-protocol Population

The 'Per-protocol' (PP) population will include all subjects in the ITT population who have no major protocol deviations. Major protocol deviations will be identified by the Statistician and Medical Affairs representative before starting the statistical analysis. A confirmatory analysis of the data will be performed based on the PP population only if more than 10 % of the subjects in the ITT population have major protocol deviations.

10.2.2 Criteria for evaluation

For each method ("pre-treatment" and "post-treatment"), comparisons between the treated and untreated areas will be performed to characterise the speed and extent of barrier repair.

10.2.3 Handling of dropouts and missing data

Only observed data will be analysed for the efficacy analysis. Subjects who withdraw from the study early will be included in the statistical analysis up to the point of when they withdraw. Since each subject acts as their own control for both the pre- and post-treatment methods, any bias that may usually be introduced by subject withdrawals should be minimal.

10.2.4 Interim analysis

An interim analysis is not planned for this study.

10.3 Statistical Methods and Analytical Plan

More details of the proposed statistical analysis will be documented in the statistical analysis plan (SAP), which will be written following finalisation of the protocol and prior to database lock and analysis.

10.3.1 Demographic and baseline characteristics

Continuous demographic and baseline parameters will be summarised by the number of non-missing observations, mean, standard deviation, median, minimum, and maximum. Categorical parameters will be summarised by frequencies and percentages.

10.3.2 Subject Disposition

Study completion status and reasons for discontinuation will be summarised by frequencies and percentages.

10.3.3 Efficacy

Since this an exploratory method development study, a number of different parameters are being evaluated in various ways, in order to ascertain the most sensitive measure to use in future studies.

"Pre-Treat" Method:

Using the methodology described in Lu et al [Lu *et al.*, 2014], utilising Fick's first law of diffusion and the fact that the thickness of the SC is approximately proportional to the cumulative SC protein removed by tape stripping, a random coefficients model will be fitted with 1/TEWL as the response variable and fixed effect model terms for cumulative protein, treatment (treated or untreated) and the protein*treatment interaction, and random coefficients for subject*treatment and the subject*treatment*protein interaction. Cumulative protein (p) will be obtained at pre-stripping (where p will be set to 0), and after 5, 10, 15 and 20 strips have been removed. The relative barrier quality will be determined from the slopes of the 1/TEWL vs. p relationship. The relative SC thickness will be determined from extrapolation of the linear regression lines for each treatment group to where each intercepts the x-axis (cumulative protein axis). This analysis will be performed for each visit day (day 0, 3, 5, 7, 10, 12 and 14)**.** The day 0 analysis will give a measure of the baseline values of slope and x-axis intercept in each of the treated and untreated areas. The use of a simple linear regression model does have some drawbacks, including the overestimate of the SC thickness (or total SC protein) as 1/TEWL does not reach 0 when all the SC is removed from the skin. However, since the aim of the study is to determine the relative difference in SC thickness and barrier quality between treated and untreated skin, the simple linear model approach is sufficient (Lu et *al.,* 2014).

From each of these random coefficients models, individual subject estimates of slope and x-intercept will be obtained. The individual subject by treatment estimates for slope and x-intercept on each post-baseline visit day (day 3, 5, 7, 10, 12 and 14) will subsequently be analysed using a repeated measures model with factors for treatment, day and treatment*day (fixed effects) and subject (random effect), and a covariate for the corresponding baseline estimate of slope or x-intercept (obtained from the estimates at Day 0). Treatment differences will be presented at each day with 95% confidence intervals, together with p-values.

On each post baseline visit day (day 3, 5, 7, 10, 12 and 14), the change in TEWL value from pre-stripping to post-stripping (after 20 strips have been removed) will be calculated and compared between treated and untreated groups. A repeated measures model will be fitted with factors for treatment, day and treatment*day (fixed effects) and subject (random effect), and a covariate for the change from pre-stripped TEWL to post-stripped TEWL assessed at the baseline visit (Day 0). The change from pre-stripped TEWL to post-stripped TEWL on Day 0 will provide a baseline measure of barrier strength of the skin when it is challenged. Treatment differences will be presented at each treatment day with 95% confidence intervals, together with p-values. A stronger barrier will result in a smaller increase in TEWL.

"Post-Treat" Method:

The change in TEWL value from post-stripping at baseline (pre-treatment) to day 3, 5, 7, 10, 12 and 14 will be calculated and compared between treated and untreated groups. A repeated measures model will be fitted with factors for treatment, day and treatment*day (fixed effects) and subject (random effect), and covariates for pre-stripped TEWL and post-stripped TEWL (both measured pre-treatment at the baseline visit). The pre-stripped value will be included as a covariate as this is a baseline measure of the unchallenged skin. Treatment differences will be presented at each day with 95% confidence intervals, together with p-values. A reduction in TEWL over time from the post-stripped value will indicate the barrier is repairing itself.

To assess the importance of different covariates, the analysis described above will be repeated, but with a single covariate for the change from pre-stripped TEWL to post-stripped TEWL (assessed at the baseline visit).

In addition to the above, the change in TEWL from pre-stripping at baseline (pre-treatment) to day 3, 5, 7, 10, 12 and 14 will be calculated and summarised for the treated and untreated groups. A return of TEWL towards the pre-stripped value will indicate the recovery of the barrier function has returned to near pre-stripped levels.

Assumptions underlying all analyses will be checked, and if necessary, alternative non-parametric methods will be used.

10.3.4 Safety

AEs will be tabulated according to the current applicable version of the Medical Dictionary for Regulatory Activities (MedDRA). Frequencies and percentages of subjects with treatment-emergent AEs will be presented by treatment group, for each system organ class, and for each preferred term. Summaries of treatment-emergent AEs, treatment-related AEsand serious AEs will be completed.

AEs will be regarded as treatment-emergent if they occur after first use of study treatment.

Any SAEs will be reported in an expedited timeframe to the sponsor.

11 Ethical and Regulatory Aspects

11.1 Local Regulations/Declaration of Helsinki

The Principal Investigator will ensure that this study is conducted in full conformance with the laws and regulations of the country in which the research is conducted and the Declaration of Helsinki.

11.2 Informed Consent

It is the responsibility of the investigator, or designee, to obtain written (signed and dated by the subject) informed consent from each individual participating in this study. Major/substantial amendments to the protocol that affect the scope of the study at the subject level and/or updates to the safety profile of the investigational product (Safety Statement) should be reflected in the consent form and active subjects re-consented.

11.3 Institutional Review Board

It is the understanding of GSK that this protocol (and any modifications) as well as appropriate consent procedures, will be reviewed and approved by an IRB. This body must operate in accordance with the current local requirements. A letter or certificate of approval will be sent by the investigator to the sponsor prior to initiation of the study, and also when subsequent modifications to the protocol are made.

12 Monitoring of the Study

In accordance with applicable regulations, ICH-GCP, and GSK procedures, monitors will contact the site prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy ethical, and GSK requirements. When reviewing data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.

GSK will monitor the study to ensure that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.

The extent and nature of monitoring will be described in a written monitoring plan on file at GSKCH. The monitor will inspect the CRFs, study files and subject records at regular intervals throughout the study to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered. The monitor will verify that each subject has consented in

writing to direct access to study records as well as to the study procedures. The monitor will also ensure that all required documents are present and up to date and that accountability and reconciliation of study product are performed according to GSK procedures. The investigator (or designee) agrees to co-operate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

13 Study Documentation, CRFs, and Record Keeping

Each subject will be assigned and identified by a unique Screening Number. Any reference made to an individual subject within the study must be done using the unique Screening Number.

13.1 Investigator's Files/Retention of Documents

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two separate categories: (1) Investigator's study master file, and (2) study/subject clinical source documents.

The Investigator's study master file will contain the protocol/amendments, case report and query forms, IRB and governmental approval with correspondence, informed consent, study product records, staff curriculum vitae, authorisation forms, subject identification, screening and enrolment information and other appropriate documents/correspondence, etc.

Clinical source documents (defined in advance to record key efficacy/safety variables independent of the CRFs) would include patient/subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, and special assessment reports, consultant letters, screening and enrollment logs, etc. These two categories of documents must be kept on file by the Investigator according to local regulations or as specified by the supervisory regulatory agency (GSK recommends that documents be kept for 10 years). The Investigator is also required to keep subject identification codes on file for at least 15 years after completion or discontinuation of the study.

No study document should be destroyed without a prior written agreement between GSK and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, GSK must be notified in advance.

If the Investigator cannot guarantee this archiving requirement at the investigational site for any or all of the documents, special arrangements must be made between the Investigator and GSK to store these in a sealed container(s) outside of the site so that they can be returned sealed to the Investigator in case of a regulatory audit. Where source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the site.

The Investigator shall supply the sponsor, on request, with any required background data from the study documents or clinic records. In case of special problems and/or governmental queries, it is

also necessary to have access to the complete study records provided that subject confidentiality is protected.

13.2 Source Documents/Data

The source documents (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, subject files and records kept at the pharmacy, at the laboratory and at the medico-technical departments involved in the clinical study) which contain the source of data recorded in the CRF should be specified in the Source Document Designation Form. In some cases the CRF can be used as a source document.

13.3 Case Report Forms

A CRF is a printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor on each trial subject.

For each subject who has given informed consent/assent and has been screened, a CRF must be completed and signed by the Principal Investigator (or authorised designee) to certify that the data are complete and correct.

Management of clinical data will be performed in accordance with applicable GSK standards and data cleaning procedures to ensure the integrity of the data e.g. removing errors and inconsistencies in the data.

In order to protect the privacy of subjects, no Personally Identifiable Information (PII) including the subject's name or initials or birth date is to be recorded in the CRF or as part of the query text.

AEs and concomitant medications terms (if applicable) will be coded using the Medical MedDRA and an internal validated medication dictionary, GSKCH Drug.

All CRF pages should be completed during a subject assessment when the CRF has been designated as the source. Data that is sourced elsewhere should be entered into the CRF in an agreed upon timeframe between the Investigator and Sponsor.

The study monitor at the study site will review the CRFs in accordance with the monitoring plan, and any queries will be highlighted to the Investigator or designee enabling the errors to be addressed prior to collection of the CRF pages. Errors detected by subsequent in-house CRF review may necessitate clarification or correction.

13.4 Data Handling

Documentation of all data management activities should allow step-by-step retrospective assessment of data quality and study performance. This includes maintenance of documentation to support any changes or corrections applied to the CRFs.

13.4.1 Data queries

The study monitor at the study site will review the CRFs in accordance with the monitoring plan, and any queries will be generated to the Investigator or a designee enabling the errors to be addressed prior to Data Management review.

Queries arising during consistency checking will be provided to the site for clarification and approval by the Investigator or a designee. Data Management will run edit checks, reports and listings on the CRFs and raise queries if needed for site clarification or correction.

An audit trail will serve to provide a complete record of the changes and corrections endorsed by the Investigator.

13.5 External Data

External Data are subject data obtained external to the CRF. These data are transcribed into a format agreed upon by GSKCH to identify the subject and time point referenced in the CRF and/or protocol. An agreed upon quality control process is performed against the transcribed data to the source to ensure the accuracy of the transcription, since data from the external source will be used for analysis and reporting.

These data will be transmitted in an agreed upon format to GSKCH via secured web portal or CD/DVD via mail carrier with tracking capabilities**.**

14 Process for Amending the Protocol

Protocol modifications to ongoing studies which could potentially adversely affect the safety of subjects or which alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, duration of therapy, assessment variables, the number of subjects treated, or subject selection criteria are considered major/substantial amendments and must be made only after appropriate consultation between an appropriate representative of GSK and the Investigator.

Protocol modifications must be prepared by a representative of GSK. All changes must be justified in the Reason for Amendment section of the protocol amendment. Approval of amendments will be made by the original signatories to the protocol agreement, or their appropriate designees.

All major/substantial protocol modifications must be reviewed and approved by the appropriate IRB in accordance with local requirements, before the revised edition can be implemented.

Modifications which eliminate an apparent immediate hazard to subjects do not require pre-approval by the IRB but the IRB will be notified of these amendments. Non-substantial amendments do not require submission to the IRB and will be held on file by the sponsor and Investigator.

Amendments to protocols should be recorded on page 2 and attached to all copies of the protocol.

15 Conditions for Terminating the Study

Both GSK and the Investigator reserve the right to terminate the study at any time. Should this be necessary, the procedures will be arranged on an individual study basis after review and consultation by both parties. In terminating the study, GSK and the Investigator will assure that adequate consideration is given to the protection of the subject's interests.

If the study is terminated prematurely or suspended, the IRB will be informed and provided with the reason(s) for termination or suspension by the sponsor or by the Investigator/institution, as specified by the applicable regulatory requirement.

16 Confidentiality of Study Documents and Subject Records

The Investigator must assure that the subject's anonymity will be maintained. On CRFs or other documents submitted to GSK subjects should not be identified by their names or initials, but by an identification code.

The Investigator should keep a separate log of subjects' codes, names and addresses. Documents not for submission to GSK e.g. subjects' written consent forms, should be maintained by the Investigator in strict confidence.

17 Publication of Data and Protection of Trade Secrets

The Investigator will not seek to arrange for publication of any of the information or results from the study in any scientific journal or other publication or by way of lecture without prior written consent of GSK.

Any formal publication of the study in which input of GSK personnel exceeded that of conventional monitoring will be considered as a joint publication by the Investigator and the appropriate GSK personnel. Authorship will be determined by mutual agreement.

18 Audits/Inspections

The Investigator and study subjects should understand that source documents for this study should be made available to appropriately qualified personnel or designee(s) from the GSK Regulatory

Compliance Group or to regulatory authority inspectors. The verification of the CRF data may be by direct inspection of source documents (where permitted by law) or through an interview exchange.

The Regulatory Compliance Group or designee assesses the quality of data with regard to accuracy, adequacy and consistency. In addition, Regulatory Compliance assures that the sponsor's studies are in accordance with GCP and that relevant regulations/guidelines are being followed.

An inspection is the act by a regulatory authority of conducting an official review of documents, facilities, records and any other resources that are deemed by the authority to be related to the clinical trial and that may be located at the site of the trial, at the sponsor's and/or CRO's facilities, or at other establishments deemed appropriate by the regulatory authority (ICH GCP 1.29).

The focus of an inspection is compliance with GCP legislation, associated guidance documents, and company policies to ensure that data are accurate, complete and valid and to ensure subjects' welfare and safety are protected.

The sponsor will be available to help Investigators prepare for an inspection.

19 References

ICH Topic 6 Guideline for Good Clinical Practice CPMP/ICH/135/95 17th July 1996.

Lu N, Chandar P, Tempesta D, Vincent C, Bajor J and McGuiness H. Characteristic differences in barrier and hygroscopic properties between normal and cosmetic dry skin. Enhanced barrier analysis with sequential tape-stripping. *International Journal of Cosmetic Science,* 2014, 36, 167-174.

World Medical Association Declaration of Helsinki, 59th General Assembly, Seoul 2008.